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President of the American Society of Sugar Beet Technologists for the biennium 1968-69 is Dr. George E. Rush. Dr. Rush is Director, Agricultural Research, The Amalgamated Sugar Company, Ogden, Utah.

Presidential Address

LLOYD T. JENSEN[1]

As president of the American Society of Sugar Beet Technologists, it is my privilege to welcome you to the 15th General Meeting of the Society. You are probably already aware that this is the largest meeting ever held by this society and we can give some credit for this to the location—a dramatic climatic difference from our last meeting in Minneapolis.

I would like to talk about technologists and their responsibilities and obligations in the beet sugar industry today. Webster states technology is, "A science of the application of knowledge through practical purpose." There has never been a time in our history when so much new technology has evolved in such a short period and one wonders if this rate of acceleration can continue. It took 56 years to really develop the telephone into a useful instrument of communication, whereas the transistor was developed completely in a span of 8 years. At this point we should ask ourselves if we are accelerating our rate of application of knowledge to practical purpose fast enough in our industry. We can point with pride to a great number of accomplishments that have kept the industry alive and progressive, but it is rather revealing to review the program for this meeting and note the number of subjects under discussion now that were also under discussion ten, twenty, or more years ago. We need to wipe more of these off the slate.

This industry has a double obligation in the areas of research and technological improvement. We, meaning the companies, have to provide the scientific disciplines necessary to acquire the knowledge the growers need to produce a better crop. We can't buy our raw material on a quality specification basis—we take what we can get. We therefore do have a mutual interest with the grower, but we also accept most of the research responsibility. The federal and state governments aid in this research effort, as do some of our institutions of higher learning. Our other obligation is to our own in-house research and development dedicated to the problems that are wholly under our control.

The production of the beet sugar industry has declined the past few years, much to our dismay. Many reasons have been given, but other than weather, most of the other difficulties that discouraged the growers might have been solved with an

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accelerated rate of technological improvement. The growers, on their part, should not resist changes that hold promise for improved results. The industry needs a level of crop production which will utilize all of the processing capability available.

The growers and the companies also need to jointly accelerate the mechanical improvements needed to plant the crop, take care of it, harvest it, and deliver it to the processing plant. Here plain ingenuity counts whether it be by the grower, the company, or the equipment manufacturers. Many technological improvements have been made in the processing plants, but the industry waited too long before it really applied itself to this effort. As we look back on these improvements, it is interesting to note that nearly all of them resulted from the necessity to reduce the unit material and labor costs in operating the plants and improve product quality. A number of small plants have been closed because they were inefficient and crop-production volume did not justify the capital expenditures to improve them. A number of processing plants have been enlarged and with crop volume and reasonable freight costs, they are more efficient. Most of the old "batch" processing steps have been replaced with continuous types—and if not continuous, the batch systems have been automated. Again, we must ask ourselves if the improvements to our processing plants have only kept pace with the economic problems? Are we lagging behind? Certainly, we have done very little in the way of developing new processes. We can't brag too much about our ability to increase the percent of sugar extracted from beets of a given purity and sugar content. We are now just beginning to seriously devote ourselves to some new processing methods that will produce more of the high quality crystalline product the market demands today.

Some of the technological improvements needed in this industry are the joint responsibility of the two areas of interest as we are constituted today—namely the agricultural department and the operating department. The solution to the problem of extraction should be brought about first by a substantial purity improvement in the sugar beets as they are grown; and then secondly, some new technique in the processing plant is needed that will eliminate more non-sugar constituents from the syrups before they reach the final crystallization stage. Since Mother Nature controls crystallization rates and yields, our only hope to increase extraction substantially is by reducing the soluble nonsugars in the beet and then removing more of the remaining ones in processing.

Another area demanding a great deal of attention is storage of sugar beets. Present practices indicate that for any given

harvest season, a processing plant operation of more than 130 days can lead to extremely high sugar losses through storage piles from respiration and spoilage. The cost of producing sugar could be noticeably reduced if the processing plants could extend their operating days on beets of harvest-time quality.

In relating these needs to you, I'm sure I have not said anything you are not already aware of. What we must do then is put our backs into these efforts to accomplish them in as short a time as possible. To do this, we are going to need more technical people, more scientists of all kinds, more people whose whole attention can be devoted to specific problems, and new, fresh outlooks from people who may be able to shortcut the path to success.

The accelerated effort on technology has created new problems in our society and will create new and different ones in our industry. An engineer described a human being; this way:

> "Man is a complete, self-contained, totally enclosed power plant, available in a variety of sizes, and reproducible in quantity. He is relatively long;-lived, has major components in duplicate, and science is rapidly making strides toward solving: the spare parts problem, He is water-proof, amphibious, operates on a wide variety of fuels: enjoys thermostatically-controlled temperature, circulating; fluid heat, evaporative cooling; has sealed, lubricated bearings, audio and optional direction and range finders, sound and sight recording, audio and visual communication, and is equipped with an automatic control called a brain."

If this was all there was to a human being, technological changes would mean nothing to this robot. The trouble is that humans have emotions. Max Ways, senior editor of Fortune Magazine makes this prediction:

> "Unless we change our thinking;, we won't be able to cope with the change that is taking place. Change, of course, has always been a part of the human condition. What's different about it now is the pace of change, and the prospect that it will come faster and faster, affecting every part of life, including personal values, morality, and religions, which seem most remote from technology.
>
> "So swift is the acceleration that trving; to make sense of change will come to be our basic industry. Aesthetic and ethical values will be evolving along with the choices to which they'll be applied. The question about progress will be 'how good?' rather than 'how much?' "

The more technologists we have working on technical mat-

ters, the more trouble we will have with their management and communications. Technologists have a tendency to group together under areas of common interest and may, knowingly or unknowingly, try to exclude outsiders. All areas of corporate life have become more technical, so we have more areas of possible exclusion. Data processing utilizing computors is now commonplace—the people running them are a new breed. We don't have accountants doing mere bookkeeping—we have people technically qualified to manage and control money. The labor relations field is not a public relations job—it now requires people knowledgeable in law, arbitration techniques, and psychology. Even our sales people must use technical devices such as market surveys and research to do their job effectively. Practically every area of endeavor now has its technical aspects.

This is what I see. The people with common interests and capabilities will band together with an all-absorbing interest in solving their technical problems. The managers of these individual technical groups will have to use all the basic management skills with a thorough understanding of psychological factors that affect creativity and productivity. The problem will be the sophisticated recognition of the interaction of people in groups. We can recognize some of this grouping in our societv today. At present teachers not only can and will strike for higher pay, but want the right to determine how much money should be spent for education and want more voice in how the schools are run. It is a grouping of people with common technical interests. The minority racial groups now want to determine for themselves the solution to the civil rights problem. Public employees now strike, in spite of laws against it. to prove they know what is best for the public. The Federal Government, state and local governments, have constant jurisdictional disputes over who should control man's environment and his money. Organized labor now has its own pension and hospitalization plans and strike funds so it can be an entity with its members. Government has agreed that the poverty program should be directed in part by the poverty stricken because they understand the problems better. The struggle is still on between the military and the executive branch of the government over who should conduct the war. We have all kinds of non profit organizations flourishing because of common interests of the members. Even the hippies feel that free love is their given right and the rest of us just don't understand them. It's no wonder our society is confused—anyone will be confused if he doesn't really know what is going on and why. Too many groups are trying to get the good out of our society at the expense of other groups.

This tendency to group is prevalent in our industrial society, also. The greater the technical effort, the greater the pressures on this grouping. People who direct these technical groups are so busy and preoccupied with group goals that they forget they must manage not only down, but across and up. We must get over the craft union concept which exercises exclusive rights to one kind of work, but shuns all responsibility for anything outside of this one area. As technologists, we have to consider the human's responsibility to himself and to others.

Our industry has been blessed with better than average communications between various areas of interest. As a matter of fact, the grapevine we have established operates at speeds greater than Alexander Graham Bell ever envisioned. Nonetheless, we are now specializing more and the grouping is becoming complex. As these groups get more self-centered and self-controlled, areas of distrust develop. The computer is a good example—everyone recognizes it is a very useful tool, yet a lot of people think it is a tool for someone else to use. Why? Maybe it is because the data processing people talk a language we don't understand, so we don't trust them. Maybe the growers, who are now exposed to a lot of technical information, don't fully understand it, and again, it could generate an element of mistrust.

The employees who work in the processing plants must manage automatic controls and other mechanical operations and we know they often feel that these devices are purely labor-saving. They fail to recognize that these devices improve operations and make their iobs more secure. The same is true of projects moving through research and development into the field or plant. If it doesn't work as prescribed, there is a tendency to give up. We must give up these ideas of mistrust based on misunderstanding. If we are to move forward more rapidly technically, we must attempt to understand what the other person is trying to do and help him if we can. A failure should prompt us to double our efforts to make a success.

As technologists, we now have new obligations. We must accelerate our accomplishments; we must integrate our group activities better with other groups; we must aspire to the same goals; and we must use all the new technical tools available. A major adjunct to all of this is better communiranon at all levels and across all boundaries. We must be good technicians, but to be good human beings, we must communicate.

The Future of the Sugarbeet Industry From a Grower's Viewpoint[1]

RICHARD W. BLAKE[2]

The title of this talk sums up what is on all growers minds at this time of year as they map out plans for the forthcoming crop season. What they believe the future holds is important as they decide what crops to plant this year.

Let me preface my further remarks with something of a disclaimer. I am, and have been for many years, a vigorous proponent of the sugarbeet industry because I know its values and its worth to our agriculture and national welfare. I believe in our industry, and if I have anything of a critical nature to say, it is intended to be constructive criticism. Only by honest, self-appraisal can we build an even stronger and prosperous industry serving the best interests of all concerned.

It is interesting, I think, that this meeting is being held in a region where commercial sugarbeet production has only recently begun. Our industry has grown and must continue to grow in order to remain competitively strong. Most established growers believe in bringing new blood into our industry, I think. At the same time, we believe that growth and expansion must be prudently accomplished to avoid having our production outstrip the market's ability to absorb added production in orderly and profitable fashion.

Spurts and cutbacks aren't good. In the late 50's and early 60's we witnessed rapid growth. Our production increased from 1,625,000 tons in 1955 to 3,073,000 tons in 1964. Then came a reversal. We estimate the sugar production from the 1967 crop to be in the neighborhood of 2,650,000 tons, or a decrease of nearly 15% in two years.

First we became burdened with large inventories as production far exceeded our marketing permissive, following which our production now has swung to the opposite extreme. We are deeply concerned with the drop-off in production today not only because it reflects loss of grower interest in the beet crop but also because it can have serious effects upon our quota status in the future, particularly when sugar legislation comes under review a couple of years hence.

With respect to sugar legislation, it is my sincere and candid

[1] Presented as part of a general symposium on looking into the future.

[2] Executive Vice President, National Sugarbeet Growers Federation, Greeley, Colorado.

opinion that we in the sugar industry face a fight for our lives in 1970 and 1971 when extension of the Sugar Act will be considered. While the opposition I foresee has not surfaced to any great extent to date, the danger signals are plain to read in various statements and other rumbles coming from opponents of the Sugar Act and even of the domestic sugar producing industry itself. We are not the only segment of agriculture facing opposition. Changing economic philosophies embraced by or recommended to the federal government promise to have effects upon wide segments of agriculture, if not all of our domestic agricultural industry.

Complicating the problem is the fact of dwindling rural political influence. Farmers are becoming fewer. Less than 6% of our people live on farms in this country today—a total of some three million farm families out of our total population of more than 200 million individuals. And by 1970 it is forecast that there will be only two and a half million farm families in the United States.

This confronts us with the political fact of life that no farm program can be approved without the support of urban-oriented members of Congress.

If the urban population—and especially their representatives in the Congress of the United States—has no understanding of and appreciation for agriculture, then our future is indeed bleak. Farmers have always understood and believed strongly that theirs is the most basic and important part of our national economy. Life cannot be sustained for the masses of our people without the food and fiber produced on our farms. But people take us and what we produce for granted. Little do most city people know of what is behind keeping those grocery shelves heaped with a multitude of different foods, always in ample supply. I sometimes think that because American farmers have done their jobs so well and our populace has become so accustomed to having unlimited supplies of food that the average American worries a lot more about the availability of color TV sets, electric carving knives and all the other goodies than about the availability of food. Yes, they are aware of food prices, I'll grant you, but not what makes those prices or the problems of the farmers who produce that food.

Therefore, agriculture in general, and the sugar industry in particular, must improve communications with the consuming public and build the consumer's awareness of the great role agriculture plays in our nation's standard of living and why it must be fostered and protected by whatever means can be devised.

I need not belabor the point for this knowledgeable group, but I do want to emphasize my belief that the future welfare of the sugarbeet industry probably will be more directly affected by what happens to other agricultural commodities than has been the case for many years up to now. Our fates are intertwined more than ever before.

Right now we are confronted with a very critical crossroad in our business. The economic status of farmers is lagging behind that of other segments of the economy. The earning rate of industrial labor, with a minimum of personal investment and responsibility, is pushing rapidly ahead of the earning rate of the farmer and the former way of life is growing more and more attractive as compared to farming. Young people today are weighing the risks, the demands, the responsibilities and the comparative earnings in industry versus farming and are casting their lots with industry. The ex-farm migration of the country's youth tells the story more clearly than any words.

The same considerations which influence a young man's decision when selecting his career are also affecting our future as an industry. The decision of a new grower to engage in sugarbeet production, or an established grower to continue with the crop, is reached only after careful weighing of costs versus potential return. The financial outlays required at today's prices for farm machinery, land, equipment and the investment in labor involved in producing a beet crop—plus water costs in an area such as this—demand careful assessment by the farmer before he plunges.

As I look down the road to the future of this industry, I am convinced that there are certain specific steps which must be taken if we are to insure that bright future toward which all of us are devoting our efforts. Some of these changes already are under way. Others are slower to come, but must be hastened.

It is my belief that we are beginning an era in which there must and will be change in the basic philosophy and methods of sugarbeet production, forced by economic and political factors beyond our control.

The number one economic question, so far as farmers are concerned, is the return they can expect from the beet crop. If this return is not fully competitive with returns from other crops, farmers will turn quickly to alternatives, as has happened in many, many cases since 1964, contributing greatly to recent production declines for the industry as a whole.

Times have changed in that farmers today are more sophisticated, better informed and much harder-headed businessmen than were most of their forebears. They recognize and will no

longer tolerate pressure to force down their throats the burdens of increased costs without commensurate increases in their returns. For too long a time, the grower has held his head above water in a situation of static returns and rising costs only by increasing his productive efficiency. Now he expects a more equitable sharing of the fruits of his investment and labors.

Along with a sharpening of his business acumen, the successful grower of today and the future must devote himself to improvements in farming methods and practices. Those who successfully survive in agriculture will be the ones who respect the principles of sound management policies, the wise use of land and labor, and who adapt themselves to modern concepts.

The outlook in our business is excellent for those who follow the principles about which I have been talking. The very nature of sugarbeet culture is that it encourages good management and wise use of the farmer's labor over a longer period of time than many other crops and it rewards the good farmer for his extra effort.

There are other changes taking place in the sugarbeet industry which affect our future. Once, ours was a way of life, for processor and grower alike. In the processing end were the "sugar tramps", many of whom came from the farms into the factories and climbed the ladder to supervisory and executive positions in the beet sugar companies. They spent their lifetimes in a field they loved and had a sort of blind loyalty to their jobs.

By the same token, many sugarbeet growers were brought up on beet farms and just naturally continued with the crop when they took over from their fathers. Theirs was an ingrained way of farming they did not question.

But times are changing. New business blood is coming into the companies as they grow larger, have more stockholders, diversify, and grow more impersonal in their business dealings.

On the farm, the son who takes over from his father is generally today a college graduate who has studied not just farm methods but has devoted a lot of his time to agricultural economics. He attacks his farmer job in an analytical manner—putting a great deal of head as well as heart into his decisions.

What I am saying is that there is no question that the day of the small operator is near an end. The producer on a small acreage simply cannot afford the equipment and machinery and other modern necessities for efficient, competitive production. He has to grow bigger or give up. I personally regret that this is true. I must recognize that progress won't let us live in the past, to cling to old values and still hope to survive.

We will see a continuing enlargement of beet acreage per

operation with an attendant decline in the number of farm operators. Hand labor will vanish to be replaced by mechanical and chemical alternatives. In this respect, we must look to you, the research people of our industry, for leadership and methods.

Farmers today are more research oriented than ever before and are willing to back up and support efforts to stimulate and perpetuate a strong overall research program. But in the final analysis, the positive gains and solutions to existing problems must come from the technical and scientific staffs of the processing companies and the Department of Agriculture.

The main roadblock to complete mechanization of the sugarbeet crop today is the lack of a satisfactory means for weed control. This is no revelation to you, so I state it only for emphasis. In seeking an answer, I would urge you to take fullest advantage of farmer cooperation and their practical knowledge which I am sure will continue to be freely and enthusiastically given. Working with the farmers will help to foreclose the possibility of a good theory becoming a practical flop.

Another problem is that of plant disease—such things as Rhizoctonia, blackroot and the viruses along with the spread of nematodes. Industry and government are making headway against some of these, but greater progress must be hastened to eliminate these costly crop spoilers.

Then there is the matter of production of sugar per acre. This actually might be coupled with what I said earlier about grower returns because it is the amount of sugar produced per acre, along with the rate of payment, which determines the grower's income.

I recognize that the grower has some control over the productivity of his beet acreage, depending upon the job he does. But more than this, I believe the seed varieties we are furnished hold the key to greater productivity. Frankly, I can report to you that growers are disappointed that seed varieties now in use are not producing up to expectations or to levels promised when these varieties were put into use a few years ago. Growers are determined to plant the most productive seed available and are willing to take whatever steps may be necessary to get it. The situation, I am convinced, calls for a concentrated effort to provide varieties which will result in increased yields and increased sugar content.

Now, if I may, let me depart from the farm level and return for a moment to the intermingled problems of production, quotas and sugar legislation.

As growers, we are worried about the future of the national sugar program. We believe we have two tough chores ahead;

One, to prevent destruction of the Sugar Act; and secondly, to preserve the present position of the sugarbeet industry in the domestic market. The closest, active cooperation of all segments of the domestic sugar industry—both beet and cane—is essential to our accomplishing these objectives.

But first, we must get our own house in order. Let me remind you that for three straight years we have failed to produce up to our quota level. We will probably fail again in 1968. There are a number of contributing factors, but the primary one is competition of other, financially more attractive crops. Under some of the new contracts we have been able to negotiate with processors, I think there will be some price improvement that will help to narrow the spread. Stronger sugar prices are helping, too. But these two things together may not be enough to provide incentive for farmers to resume beet production or to expand acreage to the extent needed.

If we do not get our production back into line with present quota levels, we may be pared back and part of our quota awarded to other producers. There are lots of aspirants hoping for bigger cuts on the market. There are both domestic and foreign producers who want a larger share and should any area—beet or cane—continue to fall short in its obligation, then Congress may be forced to make quota revisions to insure smooth operation of the sugar program.

The Act itself will, as always, come under attack from those whose primary concern is cheaper sugar. The spread between current world market and U.S. prices is cause for complaint among these interests and unless we get across to those who make the legislative decisions the fallacies involved in comparing world and U.S. prices, we could lose the ball game in 1970 and 1971.

It may seem that I have dealt over much with negatives in my view of the future. That was not my intention. The positive side of the picture we all know well. We are not here appraising the future just to tell each other happy stories. We know that we have a great and important industry. To make it even greater and more important, we must eliminate those weaknesses I have mentioned. I think the future is in our hands—the grower who lives with change and participates fully in progress, the processor who maintains high levels of efficiency and recognizes that the grower must receive a strongly competitive price for his commodity, and the research and technical people who take the practical approach and realize that only by successful application can their work bear the greatest rewards—all of us can guarantee our bright future together.

I congratulate all of you on your many and valuable contributions to sugarbeet technology and, in behalf of the growers I represent, I extend our thanks and appreciation for your great service to us.

The Agricultural Executive Looks at the Future of the Beet Sugar Industry[1]

B. E. EASTON[2]

I appreciate the great honor and opportunity to represent Agriculture during this General Session of our Society. The importance of this subject and scope of the material to be covered is so large and diversified that I felt it would be impossible for me to give an intelligent forecast of the future without some help. I, therefore, contacted some of my agricultural associates from each major beet-growing area and asked them to assist by expressing what they think the future of the beet sugar industry will be.

Before predicting what is going to happen, however, lets take a few moments and discuss some of the tremendous accomplishments that have been made by the sugarbeet industry in the last thirty years. Undoubtedly one of the most important discoveries in the first half of this century was monogerm seed. Its climb to almost 100 percent usage at this date has been spectacular. The way our scientists have bred into it increased tonnage, disease resistance, and at the same time maintaining sugar levels, indicates clearly that the calibre of our plant breeders is of the highest order.

When I started as a fieldman in 1937 it was quite common for the grower to advise you that he was putting one bag of 2-12-6 fertilizer to the acre. Although this was 125 pounds, you are all aware of the terrific change in the use of fertilizer today. Not only have the amounts per acre been increased but the analyses are more tailored to individual field requirements. The use of nitrogen has been a highly controversial subject. In general, it has helped produce more sugar per acre when properly used. Excessive use of it, however ,has been a factor in lowering quality. Fortunately this has been realized and seems to be in check in more recent years.

The tremendous and rapid growth of today's pesticides are well known by all. In regards to fungicides they appear to be well in hand. Insecticides are still a question mark, however, due to bothersome residues. We have some excellent herbicides,

[1] Presented as part of a general symposium on looking into the future.

[2] Agricultural Superintendent, Canada and Dominion Sugar Company, Limited, Chatham. Ontario.

but I will mention later what is required in the future in this regard.

In the old days when we sowed whole, multigerm seed at 15 pounds to the acre, some years every seed grew. The labor requirements and the job of blocking and thinning under those circumstances cannot even be visualized by some of our younger members today. The discovery of segmented seed, subsequent refinement of processing techniques, and, later, the advent of monogerm seed, has greatly reduced the amount of labor required. The job of thinning the beets is now much easier for everyone. Fewer people are thinning more beets than ever before, but people are still required.

The topic of machinery is difficult to cover properly in the time allotted. When one considers, however, the millions of dollars that specialized beet growers have invested in new drills, mechanical thinners, power cultivators, harvesters, special beet wagons and trucks, and many other items too numerous to mention, the change in beet machinery is quite impressive. When I started with our Company 31 years ago, every beet was pitched on to either wagons or trucks by hand and unloaded in the same way. Our first Silver piler was not purchased until 1942. Naturally we are and have been one hundred percent mechanized for receiving beets for many years. If a trucker, however, comes in today with a broken hoist and we have no other facility for unloading him other than pitching off, he stares at you in horror.

I only intend to touch briefly on the tremendous changes in our factories but they also are very significant. As fast as they can, companies are increasing their efficiency to lower the cost of processing. Some of these changes are storage silos for bulk handling, continuous diffusion, juice purification, liquid sugar, and great strides in automation with sophisticated instrumentation control.

The growth of our industry has been tremendous, particularly in certain areas. There have been several new factories opened in the last few years. More are planned and some under construction at the present time.

In times of low sugar prices, particularly in Canada, we, as an industry, have only been able to survive by increasing our efficiency both on the farm and in the factory.

In spite of this remarkable growth and record, we, as an industry, do have some immediate problems.

The first of these is to continue our never-ending struggle for the reduction and eventual elimination of hand labor in the spring. This can be accomplished by better emergence of seed and more effective herbicides. We probably require a stopgap

of improved electric-eye or other selective thinners. Secondly, although the quality of our beets has increased slightly in the last two to three years, due principally to better knowledge of how to use additional nitrogen, and improved factory performance, we still require help to further improve in this area. Thirdly, we must find some way to reduce our beet storage losses and reverse the trend of declining extraction. In the future these factors will be aggravated by rapid delivery and longer storage periods. Fourthly, by resolving the above problems we will automatically improve the net cash return to our growers in order to keep them interested in producing sugarbeets. Of course, the processor has to make money also, or the industry folds.

I now come to the point where my associates and I will try to predict the future of agriculture in relation to the beet industry. This has been broken down to several subtitles, which are as follows:

SEED—Almost unanimously it was predicted that future seed will be true hybrid monogerm varieties selected and adaptable to specific areas. They will carry genetic factors for even greater sugar per acre and stronger disease resistance. Our seed will be larger and emerge better. The rate of seeding will be reduced, and seed will be of a much more uniform size either due to refinements in processing, or coated (pelleted) for the same purpose. It is interesting to note that in Manitoba they have patented a process covering seed with a plastic cover for early sprouting in the spring. It will be planted the previous fall and may include pesticides, and nutrients. We will all watch this new technique with great interest.

FERTILIZER—Individual fields will be soil tested to determine specific requirements for each particular need. Leaf or petiole analyses will be common. There will be a greater use of minor elements. The use of nitrogen will still be common from several sources but the danger of over use and wastage will be guarded against. The fall application of fertilizer will continue to increase, followed by pop-up or starter fertilizer in the spring. In the irrigated areas, the application of fertilizer through sprinklers, as well as the use of more liquid fertilizer, will increase.

SOIL TILLAGE—Everyone recognizes the advantage of early planting and this will affect the method of tillage in the future. On the heavier ground the land will be plowed and partially worked in the fall. If you cannot fall plow, due to a season like 1967, some real heavy ground will not be plowed,

but just disked and planted. Minimum tillage, where practicable, will increase and surface tillage, particularly in some of the new areas, will become increasingly important. Every effort to preserve moisture will be exploited. It will be necessary to plow to dilute the effect of pesticides from the previous crop in concentrated cash crop areas. Improved and refined tillage implements will continue to play an important part in working the soil.

METHOD OF PLANTING—Everyone agrees that eventually our beets will be space planted and left untouched. This, of course, will be combined with the proper use of ideal herbicides, but it can and will be done. Row widths, wider than 24 inches, will decrease, and population of beets per acre will increase. With stronger seed it will be practicable to plant deeper to the moisture, although planting rates will become lighter. In both the east and west, scientists are now investigating the possibility of a brand new concept of planting. A single seed placed in a measured quantity of vermiculite and only working that small area rather than the whole field is their goal to completely eliminate thinning. Of course, again a tried and true herbicide will have to be part of this program.

IMPROVED DRILLS—Our present day drills are good and, if operated properly, do a creditable job. As our seed becomes larger and stronger and more exact in size, we will have more precision requirements. The possibility of power ejection of this seed and better depth control will come. Multiple rows, including 8 and 12 rows, will be common where practicable, and improved press wheels will prevent capping or crusting.

USE OF CHEMICALS—This is such a wide-ranging subject it is impossible to concentrate all facets of it into the time available to discuss it. There is a general increase of weeds on some farms due to poor culture, particularly in the previous crops. Growers will learn to control these weeds better and a more dependable broadleaf herbicide will make its appearance. We will have a herbicide as common and as good as the dramatic breakthrough of corn weed control with the use of 2,4-D, and today's atrazine. Postemergence weed control will be more common than preemergence treatments when the above chemical arrives. The price of these herbicides will be cheaper and the results more satisfactory. Soil testing for amount of chemical required will be used.

New insecticides and fungicides will appear fairly rapidly since there has been a ban on the use of several former good materials, due to chemical residue. More use of systemic ma-

terials will be common, and there probably will be a breakthrough in nematode control by chemicals.

CULTIVATION—With space planting and better weed control there will be less cultivation required. Some people predict there will eventually be no cultivation as the control of weeds is the prime function of it. In the meantime, as the planter grows in width, so will the cultivator, and there will be an increase in the use of rolling cultivators. Cultivation, when required, will be more precise. Until the miracle herbicide appears it will still be a very important part of our beet culture.

MECHANICAL THINNERS—When planting to a stand becomes common with better weed control, the use of thinners will probably be eliminated. One of my friends says they will be put in the museum. Until then, sophisticated selective thinners will appear, in spite of the high cost, and will become common. In the interim, the present day thinners will continue to slowly increase in use. However, when planting to a stand becomes a reality, all mechanical thinning operations will likely disappear. As in the case of many of our present day practices, there will be a breakthrough. In parts of the country where they have very large acreages the use of the spring-tine harrow, unique in its large area coverage and simple adjustment, will remain popular.

HARVESTING EQUIPMENT - HARVESTERS—Multi-row harvesters with efficient cleaning beds will be universal. Three-row machines will be common and with greater power 4- and 6-row machines will be used. As the sugarbeet will be more uniformly topped and cleaned in the field there will be less trash at the receiving stations. With individual sugar tests the growers will continue to be reluctant to start their harvest until the beet has a good sugar content; therefore, future harvesters will be geared to a short season. **TOP SAVERS**—The use of tops will grow. Their value in Europe is well known. More growers will make every effort to save the tops, particularly the average and below average growers who will require more net returns per acre. In some areas the natural drying and baling of tops will increase.

DELIVERY OF CROP—In most districts the beet has to be out of the ground before freeze-up. Aggravated by slow starting, wide-open delivery will be necessary. When the storage conditions are right the Company must be ready to receive them. Scales will be open 24 hours a day; trucks will get larger, receiving equipment will be updated with wider and larger cleaning rolls for faster unloading. The use of computers and electronic

scale equipment will be common. The number of receiving stations will diminish.

Ail possible means to reduce loss in storage will be tried. Eventually we may see large enclosures with controlled temperatures for proposed long-storage piles. Possible irradiation of beets to inhibit sprouting is not beyond our reach. Experiments are being done now towards this end.

GENERAL

Agricultural Research—Only by greater production and improved efficiency will the sugarbeet industry realize its full potential. As production and efficiency are by-products of research, it is essential that this area of work should be continued. It has been stated that "As a company's research program goes, so goes that Company." More emphasis and a greater challenge will be thrust upon agricultural research to bring about the above predictions. More money will be spent and there will even be closer co-operation with the United States Department of Agriculture, state universities, societies, foundations, and such institutions as the Sugar Research Advisory Committee. Special efforts will be geared to continue increasing sugar per acre. Better storage and extraction will be a reality. New varieties of seed will appear and beets will process better.

There is still too much time lag between research results and practical application in the field. Upon checking my report of two years ago, and even older papers, some of our same problems with suggested alternatives were mentioned as requiring immediate action.

We must step up and streamline research activities and diligently make valid interpretations of the results.

Future of Labor—There is a division of opinion on this subject. Some of our agriculturists feel that there will always be a need for some labor, although less common labor will be required. Others still feel that with the combination of space planting and a sure herbicide, hand labor will be completely eliminated. Some believe, that even if labor is available, growers will not be able to afford to pay it. I believe the answer is that we will go through a transition period of less and less labor while many growers each year will learn to become completely independent of expensive hand labor.

Irrigation—Although overhead irrigation is costly, this method will rapidly develop in areas that require water. The availability of water is still the key but there will be more efficient use and less waste as the supply becomes scarcer. Labor for irrigation will be more difficult to get. The combination

of fertilizer and irrigation will increase as in some areas it is a natural.

Where regular irrigation is now being used, greater use of concrete lined canals, aluminum supply lines, syphons, and gated pipes will be used. Fall irrigation to speed residue plow-down will become a common practice.

Future Trends—As farms get larger, so will beet contracts. Future beet growers will become highly specialized with key personnel responsible for production of the crop.

Company and Government information meetings will increase as will the demand for specialized publications and technical information.

Summary and Conclusion

There have been tremendous strides and changes accomplished in the agricultural production of sugarbeets during the last thirty years.

The future looks very promising. Some think we are just entering the new era of reduced labor requirements. We must not become complacent, however, because factories can close. Lack of acreage and lack of profit, both of which we personally have experienced recently, are the major culprits.

If I were asked today "What is required to ensure the future of this great industry?" I would say this. First, we must find the answer to complete elimination of hand labor. This can come with better emerging seed and surer herbicides. Secondly, we must strive for higher quality. In agriculture, this means improved varieties, better culture, and less storage losses. In the factory it means more efficient processing. Thirdly, both the grower and the processor must increase their profits or the future is in jeopardy.

Our industry has a remarkable history of survival. One crisis after another has failed to permanently suppress it.

In spite of our present experience recently of having our factory close, we still predict a great future and continued growth of this industry.

Companies should heed the warning signs, however, and put their own house in order so that similar catastrophes will not strike them. In Ontario our agricultural and factory performance were considered among the most efficient in North America. We could not survive, however, because of our economic environment largely due to extremely low refined sugar prices.

I believe the long-sought-for time of growing beets without hand labor is within our grasp and will be here soon.

This great Society can and must continue to give strong leadership to solve our immediate problems.

Agriculture is responsible to produce the raw product for our factories. The most modern up-to-date multi million dollar plant cannot run without beets. It is therefore very essential that agriculture and factory personnel should unite as a strong team to ensure the preservation of this wonderful Industry.

I know that I speak for all agricultural personnel when I say they are ready to do their part to help their companies stay healthy and make a fair profit. I am sure they all look forward to the new era and future with eagerness and confidence.

A Processing Executive Looks at the Future of the Beet Sugar Industry[1]

ROWLAND M. CANNON[2]

With the future of the beet sugar industry that has been spoken for by the beet grower and by the agricultural executive, I am not at all sure that there is anything left for processing the crop, but if there is to be something for processing the crop and after that for selling it and for taking care of general management needs like paying interest on borrowed money, paying taxes, and paying dividends to the owners of the business as a return on their investment, it must come through improved efficiency.

Our business is one of those in which the prices of finished products definitely have not kept pace with inflation. Besides that, such increases as we have had in the prices of sugar and by-products have not been used in processing the crop but have accrued to the benefit of other phases of the business. This means that the successful beet sugar processor has had to maintain or reduce his costs of processing the crop over a long period of time.

A careful look forward seems to suggest that in the future we cannot expect sugar prices to increase as fast as the general cost of living. It further suggests that the prudent processor must continue to find ways to offset the increases he is bound to face in unit material and labor costs. So perhaps the single most important prospect for the future facing the processing executive is that he cannot permit his total costs of processing to increase with rising costs of labor and material that he uses.

To meet this challenge, processing efficiency must be continually and dramatically improved.

In the years since World War II, and I suppose for some time before the war, increased factory capacities have probably contributed more to improved processing efficiency than any other single factor. These increases have not only resulted from higher slicing rates, but from longer campaigns, and have involved such innovations as staggered cropping systems, improved techniques for storing beets and the use of juice storage. Significant gains have been made in other ways also. Important among them are the use of automation techniques to reduce

[1] Presented as part of a general symposium on looking into the future.

[2] Executive Vice President, Utah-Idaho Sugar Company, Salt Lake City, Utah.

labor requirements, reductions in fuel requirements from more efficient generation and use of steam, reduced consumption of lime rock and other operating supplies and improved efficiency in maintaining processing facilities resulting from both better equipment design and maintenance techniques.

During this period we have had little or no improvement in rate of extraction, or sugar recovery from the beets sliced. In fact, many processors have been faced with a decreasing recovery of sugar over the past 15 to 20 years. Even though there has been improvement in purification systems and other processing techniques, they have been more than offset by declining beet purity and sugar content.

As we look to the future it seems pretty evident that major improvements in efficiency must come in quite different ways than they have during the past 20 years. For example, while factories will certainly be enlarged further, we are not likely to see a continuation of the dramatic increases in factory capacities of the past 20 years, and this will perhaps be a less important means of maintaining low processing costs than it has been in the past. And yet it will be extremely important in some areas.

Since it seems so evident that improved efficiency is the name of the game for the future, some analysis of ways and means for needed improvements should be significant. My crystal ball isn't much good and I don't use it well. Yet there are so many obvious areas for improvement—some proven and some yet to be worked out—that they must be reviewed.

This review will not be made just for the benefit of the process technicians in this audience. One of the real challenges for the future is for the industry to become less compartmentalized, for each of us to gain a better understanding of the problems of the industry, to see them in broader perspective, and to develop more comprehensive solutions to them. Too often we tend to solve problems by transferring them into someone else's area of responsibility, and these are not the kind of solutions we need. So I hope that my remarks will contain something for everyone in attendance.

It seems to me that the broad approach is extremely important for the younger people in the industry today. As time goes on vou will be dealing with larger and more complex problems. Factories will be bigger. Processing will be more precise. Volume requirements will be higher. Facilities will be much more expensive. In your game the stakes will be higher and the returns will depend on things being exactly right. But you will have better knowledge and information, better tools, and better facilities to work with. Many of the old hands don't understand

the approaches you have been taught to use. To make the best use of your modern approaches you must have a clear and comprehensive knowledge of the problems you are dealing with. If your analysis is based on misinformation or if it ignores basic facts, it isn't much good.

Now, as a processing executive looking to the future of the industry, let's head our list of needs with "better recovery of the sugar in the beets." For the industry in the United States as a whole, perhaps 86 or 87 percent of the sugar entering the factory ends up in the bag. If we back up a step, we would find that the recovery expressed as a percentage of the sugar delivered by the grower to the processor is lower—maybe only 82 or 83 percent recovery on the average. A loss of 13 or 14 percent of the sugar entering the factory, or 17 or 18 percent of the sugar delivered by the grower is a lot of sugar and we must find ways to reduce it. Many of the papers delivered in this convention touch upon things related to this problem, as have many papers delivered in past meetings. Our efforts in growing the crop, harvesting and receiving it, storing it and processing it must all be intensively directed toward achieving better extraction.

In Europe great strides have been made toward better recovery of sugar from the beets. One of the most important developments has come in the field through achieving material improvement in the quality of the beets. Process technicians from this country are always envious of the high sugar content and high purity of beets they see being processed in European factories. When measured in terms of our technology the quality of the sugar beets processed in Western Europe must certainly be worth two or three or four extra percentage points of extraction recovery as compared to the beets normally processed in this country. It is important that all of us connected with the industry—grower, agricultural technician and process technician alike—have a full understanding of the importance of high purity and high sugar content to efficient processing operations.

Losses in the handling and storage of the beet crop before it is processed are important. Climatic conditions vary widely over the country, and certainly the problem of sugar losses between receiving and processing the crop is vastly different in The Imperial Valley of California or this beautiful valley we are meeting in (Salt River Valley of Arizona), where harvest and storage temperatures are often above 100 degrees, than they are in The Red River Valley or in Montana where harvest temperatures are commonly below zero. And in some of the

other areas where alternate periods of heavy freezing and warm thawing temperatures are common during the storage period the problem is again quite a different one. Under all of these conditions sugar is being lost between the harvest and the time the crop is processed. Our interest must go beyond merely delivering sound beets to the factory for processing, and must carefully consider any changes in sugar content and purity that occur. This problem is of vital importance to both the growers and the processors and in the long range the interests of both will certainly be better served through both parties going all out to effectively minimize these storage losses.

We all know that sugar losses in storage vary widely, sometimes reaching a total loss where beets have deteriorated so badly that they are not worth processing. As a rule of thumb this wide magnitude of storage losses is sometimes evaluated as one-half pound of sugar per ton of beets per day of storage. This loss may show up in more than one way—a loss of weight of the beets, a loss of sugar content of the beets, or a combination of the two. In any case, it amounts to big money. For a typical factory located in a northern area of the country with a typical campaign the sugar that is lost between harvest and processing might have a market value of something like one-half million dollars if it could all be recovered in the form of refined sugar.

Factory losses have received lots of attention over a long period of time, and yet there remains much opportunity for improvement. For the average factory referred to earlier, if the sugar lost in molasses, pulp, lime sewer and other factory losses could all be recovered in the form of refined sugar, it would have a market value approaching two million dollars. Of course, if it were to cost something over two million dollars to recover this sugar, it would be no bargain, and this is the reason these losses are as big as they now are. But here is a great opportunity for improved techniques. And whether it be through the application of ion exchange, better carbonation, more precise sugar boiling, further application of Steffens process, or procedures and techniques not yet conceived, we must, and we will, find ways to achieve a higher recovery of sugar from the beets we buy.

In recognizing that both the sugar beet grower and the beet sugar processor need more money to meet future pressures, it seems fairly obvious that it cannot all come from the market place. It must then come from the only other places available, namely the production of more sugar per acre on the farm and the recovery of more of the sugar that is in the beets in the processing operation.

Our business is a seasonal business and with it come all of

the problems of seasonal workers. Great strides have been made in many factories both in Europe and this country toward reducing labor requirements through automation. With automation comes more precise process control, and this leads to more efficient operations. While the technology of automated process control is rapidly being worked out, its widespread application remains far behind. The future demands that we move ahead rapidly toward complete automation of our processing operations.

As process automation is achieved, it will undoubtedly be guided by automated decision making. I suppose that within the industry computors are now being used extensively to determine the most efficient level of operation for a given set of conditions, but this is an area where great strides will certainly be made in future years. And the change from operating control by instinct to computerized operating control will eventually result in further operating efficiencies.

The future is bound to bring further consolidation of processing operations and further enlargement of factories. This may take a different form than it has in the past. The very successful use of thick juice storage pioneered by the Holly people seems certain to find greater use in the future and be a part of the expansion of many factories. We may see factories in the future where the sugar end is in operation most of the year, and this will certainly change many of the concepts of sugar handling and sugar storage.

As juice storage is expanded and some factories become more intensive refining centers, other factories may become sattelites to them. The Tirlemont people in Belgium have developed this concept to the point that in order to make the best use of factory facilities they make raw sugar in some beet factories, while in others they are utilizing their sugar end equipment for white and high raw sugar. The raw sugar from some factories and the high raw machine syrup from others are then being transported to their refinery at Tirlemont. The possibilities of combining thick juice storage with the Tirlemont concept seem unlimited and they are certainly intriguing.

The highest cost operating materials, exclusive of sugar beets, are fuel and lime rock, and there seems to be opportunity for improving cost of both of them for many factories. We have the know-how to operate with much better fuel economy than is being accomplished in many factories. The guidelines for improving fuel economy are pretty clear cut. The facilities required cost a lot of money and the problem is one of achieving a satisfactory return on the investment required. This obviously

is affected by the volume handled by the factory, the stability of the volume, and other such pertinent factors. Factories of the future must have extremely efficient steam generators and heat consumption for maximum fuel economy.

There are now, two or three factories in the country equipped to reburn waste lime. I think that all of them are located in California where limerock is very expensive. While the cost of such facilities is very high, they must certainly be producing significant economies. The cost of such facilities seems to make them less attractive in most areas of the country where limerock is cheaper. It seems like an unnecessary waste to continually pay the cost of quarrying and transporting new limerock to our factories and at the same time to find suitable places to dispose of waste lime when it is theoretically possible to reburn at least a large part of the lime we are discarding. The beet sugar factories in the United States probably use something like one million tons of limerock annually. I suppose that the average mine cost of this rock is at least $2.50 per ton and the cost of transporting it from the quarry to the sugar factory ranges up to as much as $4.00 or $5.00 per ton depending on the distance involved. The industry must be spending something like $5 million annually for limerock laid in at the factories. The need for lower cost methods of reburning waste lime are great and such development seems almost certain in the future.

The cost of maintaining our factories is a major expenditure. Modern equipment and modern design often require less maintenance than those of former times, but maintenance procedures are often more precise and difficult, requiring more skilled technicians. In the future there will be increased emphasis toward maintenance-free equipment and design arrangements. It seems certain that we will use maintenance schedules that permit maximum use of equipment between overhauls, with maintenance schedules for some of the equipment perhaps following cycles that are different from normal inter-campaign periods.

In the framework of the traditional relationship between processors and growers there are several places in the operation where efficiency can be improved to the benefit of both the growers and processors. Reference has been made to beet storage as being in this category. Sugar packaging, handling and storage is another area where both parties are affected. Mechanization and automation of these operations offers considerable opportunity for savings but also involves substantial capital outlay. Thick juice storage facilities likewise are beneficial to both parties. The savings, that can be made from such improvements,

are so great that the interests of the individual parties must be coordinated to achieve maximum overall efficiency.

This review of ways and means, that might be used in the future to improve the efficiency of processing operations in the industry, is by no means exhaustive, nor is it intended to be. I do hope, however, that it has stimulated the idea that we have many opportunities for continued improvement in processing operations and many paths to explore for the purpose of maintaining processing costs at levels lower than would result from normal inflationary pressures.

It is certainly obvious by now that we have not discussed one very important item in connection with these points, and that is how much money it is going to cost to make such improvements and where the money is going to come from. The capital cost of many of the improved techniques discussed here is extremely high. One of my early experiences with my company —shortly after I was out of college—was on the design and construction of a new factory. It was modern and efficient with a slicing capacity of about 1500 tons per day, and I well remember that it cost about a million dollars. That is a far cry from the $20 to $30 million dollars that a new factory costs today. The cost of modernizing old factories has likewise increased a great deal. In some cases it almost involves the erection of new modern facilities along side the old.

If such ventures are to be made there must be an adequate return on the money invested to justify it. And there is little room for mistakes, because at present costs, mistakes are much more expensive than in former times. This means that procedures to be used must be well conceived and demonstrated and planning must be sound. Today's costs, and more particularly, those of tomorrow, leave no room for trial and error procedures.

Other requirements are equally important. The high cost beet sugar factory must have adequate volume to justify it, and this volume must be consistent to maintain it. Success in the future demands that the total industry—growing the crop, processing it and marketing it—operate at maximum efficiency, and one of the most important factors to this is volume. Inadequate and erratic volume does extensive and irrepairable damage to the industry as a whole and everyone who is a part of it.

If we are to move forward in a positive and aggressive way we must all have full confidence in the future and the things it holds for us. We have all seen the detrimental effects of lack of confidence. We have seen growers who have not modernized their equipment, facilities and production methods because of

a lack of confidence in the future, and very often this is the beginning of the end, because their very lack of moving ahead compounds the troubles that have undermined their confidence to begin with. We have seen processors who have not modernized their factories and facilities because of their lack of confidence in the future of a particular operation and this too is very often the beginning of the end. And we have seen areas that were down, where both the growers and the processors were discouraged, and new stimulations and new points of view have brought about a reawakening. New enthusiasm has developed, and suddenly their venture has developed new prosperity for growers and processors alike. This is a business where the participants—both growers and processors—rise together or fall together.

So as we look forward to the future of this business, we have much to be enthusiastic about. We have many challenges and we have many opportunities for success. But most of all we have in groups like this one assembled here this week the technical abilities and the determination to evaluate and find solutions to our problems, and to find new and better ways to do things, and we have people in the industry—growers and processors alike—who are willing and determined to evaluate new methods and confidently put them to practical use to make a better future for all of us.

The Sugar Sales Executive Looks at the Future of the Beet Sugar Industry[1]

B. A. OXNARD[2]

I am in complete agreement with the thoughts of other speakers who have participated in a discussion of the optimistic future of our beet sugar industry.

Not being bold enough to let my fancies fly into the future, my rather brief talk is on what the U. S. Department of Agriculture figures on sugar deliveries by various categories for the last 10 years may indicate for conditions in 1971.

First, the quality of our industry product is very high due to the efforts of many of you in attendance at this meeting. Please be sure to keep up this good work as it not only means much to your individual company but it also works for the benefit of the entire beet sugar industry.

Second, the yearly per capita consumption in the United States in 1971 may not be very much under what it is currently; i.e., 98 lbs of refined sugar. The current total U. S. deliveries is 10,300,000 short tons raw value, and this should go to about 10,800,000 in 1971.

Third, the distribution of household sugar packages is decreasing each year, both in total hundredweight sold and the percentage of total U. S. deliveries, beet and cane. 10 years ago, household sugars were 58 million hundredweight or 35% of the total U. S. deliveries of beet and cane. Currently, household sugars are 53 million hundredweight or 28% of the total U. S. deliveries of beet and cane. The estimate for 1971 household sugars is 50 million hundredweight or 25% of the total U. S. deliveries of beet and cane. The total hundredweight that went to households hit a peak in 1958, and since then has declined about 1 million hundredweight per year. At some point, it will level off, but exactly when, cannot now be estimated. In all this time the total U. S. deliveries were increasing, so it is evident how steep is the decrease in the percentage of total sugar that goes into households.

Fourth, as the per capita consumption is about steady and our population is increasing each year, it is evident that deliveries to industrial users are increasing both in absolute units and in

[1] Presented as part of a general symposium on looking: into the future.

[2] Senior Vice President, The Great Western Sugar Company, Denver, Colorado.

These accelerated expenditures are not entirely over, as one new plant is now under construction and a second is well along in planning as well as one being widely talked about but not yet committed.

Now why did we have these sudden increases in capital expenditure during this period? What did management see in the future that prompted it to make these decisions? Probably a number of reasons led to these actions and they were probably different for each company.

One reason was the demand from growers to grow the crop. Prices of other crops were down and supplies were in surplus. So sugarbeets were a good crop to grow, because they were a stable cash crop that could return a reasonable investment to the farmer, as well as being an excellent rotation crop.

These pressures from growers were felt by many of us, and by many of our political representatives. We had never had this kind of encouragement before.

Another reason for the expansion was our belief that we could receive a greater amount of sugar sales within the restrictions of sugar legislation. When this legislation was passed it specifically provided for new facilities in new areas and gave some protection to them for minimal volumes during their first years. This was an innovation that certainly gave encouragement to our industry to build new plants, and it was with such assurance that we built the new plant here in Arizona.

Something else was also happening. Population in this country was shifting toward the West and more sugar consuming industries were locating their new plants away from the eastern coast. This gave our industry, which produces a substantial part of its sugar in the West, a base for broader markets and outlets nearer home.

There was also the temporary world shortage of sugar that sent prices soaring and brought more interest from growers and even encouragement from Government.

You can thus see from these events that the immediate future looked good and thus the rapid growth.

Now how does management look at the future of our industry after this intensive period of growth and the reversal we had experienced the past two years because of lack of beet supplies?

I should make it clear that as I say these things they are only my own thoughts and may differ widely from others in the industry, but this is the future as I look into a pretty murky crystal ball.

For the short range outlook it now appears that we have turned the corner on lack of beet supplies. The feed grains, which

suddenly strengthened in price while beet prices were declining, have now reversed themselves and at the same time prices paid for beets appear to be approaching new highs. With a predicted 15% increase in acreage in 1968 over 1967 and reasonable growing weather this industry should again produce or slightly exceed its quota level.

In light of our present lower than normal inventories such a crop is badly needed to maintain our marketing position and build up inventories to a better working level.

For the long range I believe the big expansion within the beet industry is now behind us. The next decade will be similar to the period in the 1950's with steady growth, some new plants, and some older ones ceasing to operate because of shifting beet supplies or consolidation of equipment into larger more economical units.

Now this may not sound like a very optimistic forecast, but I believe that this type of growth is more characteristic of our industry and the type of growth, that in the long pull, will keep this industry in the sound stable position with which it has long been identified.

To achieve this type of growth will not be automatic, nor will it necessarily be easy. One of the concerns shared by many in this industry is the lack of any significant improvement in yields. If you go back as far as 1950 and take any kind of average you desire, you will quickly see that production of sugar per acre has not significantly improved.

One saving factor to this lack of improvement in yield has been the reduction in man hours of field labor per bag of sugar produced. The figures show a reduction of about 33% in man hours over the past decade.

It appears to me that you gentlemen here in this room have a real challenge facing you. Improved sugar prices can not always be the answer, nor can the change in sharing of the sugar dollar between the processor and grower. Greater returns must come from improved yields and more efficient field and factory operations to keep our supplies of beets in adequate amounts.

I am not saying or even hinting that you have done a poor job because I appreciate the tremendous advances made in the past few years in agriculture. Unfortunately we had to run very hard just to stay even with the new problems encountered in the field as well as spiraling costs of production. Some of these problems are now behind us, and I can't help but feel optimistic about the results of your work in the future, but I want to emphasize that you people are one of the keys to our continuing success.

From the factory standpoint it is becoming more evident that we must keep these plants up to date or close them down. Low recovery rates of sugar and poor utilization of labor and materials can not be tolerated because like the farmer, the processor is caught in the same squeeze. The shortage of labor in many areas makes automation a necessity as well as increasing our efficiency. The special demands of sugar users as to quality as well as more varied types of sugar puts added burden on our operations. While it may be somewhat easier to define a factory problem over an agricultural one, continued work and alertness to the whole processing end is necessary so that you in this field also have your work cut out for you.

The sugar beet grower to remain healthy in this industry will undoubtedly have to increase the size of his operation as his investment in special equipment becomes greater. This is the same thing the processor is already doing. Also the producer must perform all of his operations in the most efficient and low-cost method and with a minimum of labor.

If the ownership of our companies can see that the farmer interest is good and that through better farming methods and better yields the return is satisfactory, then they can see the assurance of adequate volume of raw material and will make the continuing investment necessary to keep the industry an efficient one.

This places much of the burden of the future on the agricultural side of our industry, and this is rightfully so, as this is an agricultural industry. You people in agricultural research and extension work and the farmer himself have the real challenge, and the future of our industry is in your hands. Management can only make the plans, you people must produce our raw material in sufficient volume.

There will be a great many problems both old and new facing you. I have a great deal of confidence in your ability to meet these challenges.

To sum this all up, I firmly believe that this industry has a good future ahead of it of steady but not spectacular growth. We don't have the glamour of the electronics or space age industries, but as I have told many of our new employees, "I don't know of an industry where you can find more challenge, and I can guarantee you that in this industry you will never be bored. Each year is different with new problems and new challenges."

Soil Moisture Conditions, Nutrient Uptake and Growth of Sugarbeets as Related to Method of Irrigation of an Organic Soil

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Introduction

Production of sugarbeets on highly organic soils of the Delta region at the mouths of the Sacramento and San Joaquin Rivers of California poses some special problems not encountered on mineral valley soils. The water table is generally within 3 or 4 feet of the ground surface, the topography is very flat and both the moisture and nutrient-supplying characteristics are markedly different from those of mineral soils. The high permeability of the organic soils prevents furrow irrigation, and the most common practice is to subirrigate by controlling the water table level within narrow limits throughout the season.

Previous experiments and observations have pointed to the need for simultaneous improvements in both moisture and nutrient supply. In 1955, an experiment comparing subirrigation alone with subirrigation plus supplemental sprinkling was conducted. There was no response to supplemental sprinkling, but analysis of petioles taken at regular intervals indicated a severe phosphorus deficiency by mid-season. Field trials in 1956, 1959 and 1960 indicated that fertilizer phosphorus was readily taken up by sugarbeets under supplemental sprinkler irrigation. There were yield responses as well as increases in petiole phosphorus when phosphorus deficient soils were fertilized. A field experiment in 1961 demonstrated uptake of phosphorus placed 10 or 16 inches deep by plants grown with subirrigation only. Evaluation was by petiole analysis. In this trial, however, drought, nitrogen deficiency and virus yellows resulted in low yields for all treatments.

It had been observed that under subirrigation an appreciable depth of soil became quite dry and that beets lost older leaves and wilted occasionally on hot days. Because of virtual lack of detailed information on soil moisture conditions and soil characteristics important in supplying plants with water, a detailed evaluation was undertaken. The importance of this was further

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emphasized by the indicated interrelation with nutrient supply and in 1962 a field experiment was conducted involving both irrigation and fertilizer treatments. The experiment was given detailed evaluation for the greatest possible insight into the reasons for the responses obtained.

Experimental Procedures

Plan of the experiment Differential irrigation treatments consisted of (A) subirrigation only, with the water table maintained between 3 and 4 feet below the ground surface, and (B) subirrigation supplemented by sprinkling in a manner which would maintain an adequate soil moisture level in the top 2 feet.

The entire experimental area was fertilized with 100 pounds N per acre applied by airplane as urea on February 2. Differential fertilizer treatments consisted of: (A) no additional fertilizer; (B) 87 pounds per acre P (200 pounds P_2O_5) applied as treble super phosphate placed 13 to 16 inches deep in early November, 1961; and (C) 87 pounds P plus 80 pounds N per acre injected as ammonia just prior to planting.

The plots were laid out in a split-plot design with four replications. Irrigation treatments were applied to the main plots and fertilizer treatments to subplots which consisted of 16 30-inch rows 475 feet long. The beets were planted on March 20.

Evaluation of soil moisture conditions Exploratory evaluation of soil moisture conditions under subirrigation in 1961 showed that both tensiometers and soil moisture resistance blocks functioned in organic soils within their inherent limitations. However, soil moisture tensions exceeded the useful range of the tensiometers within a few weeks after planting. Resistance blocks lacked sensitivity in wetter soil near the water table, but functioned very well under drier conditions at shallower depths. Gravimetric sampling for moisture content gave useful information, although sample variability was greater than that in mineral soils.

Because of the wide range of soil moisture conditions expected, both tensiometers and resistance blocks were used for measurement of soil moisture tension. Two subplots in each irrigation treatment receiving both P and N fertilizers were selected for soil moisture evaluations, and instruments were placed in two locations in each plot. At each location, wells were installed to a depth of 4.5 feet for measurement of water table depth and tensiometers were installed at depths of 12, 24 and 36 inches. Resistance blocks were placed at the 6, 12, 18, 24 and 30-inch depths in the subirrigation treatment and at 12, 18 **and** 24 inches in the **sprinkled** plots.

In the subirrigation treatment, samples for gravimetric determination of soil moisture content were taken near each instrument station in 6-inch increments to 3 feet. Twice during the growing season large core samples, for determination of bulk density, were taken from sprinkled plots where soil moisture conditions were favorable for core sampling.

Some moisture retention characteristics, obtained with the pressure membrane apparatus (3)[2] and by supplementary studies in tanks, are of value in interpretation of this experiment. These tests were conducted with similar soils, but the samples were not taken from the 1962 experimental area.

Evaluation of nutritional status The principal means of evaluation of nutritional status and nutrient uptake was petiole analysis. Each subplot was sampled at 2-week intervals by collecting about 40 petioles from the center four rows. Petioles were analysed for NO_3-N, $P0_4$-P and K by conventional analytical procedures (4).

Harvesting Small plots consisting of two rows 50 feet in length were hand harvested from each fertility subplot on August 13, September 10 and October 9. Data were collected on fresh weight of tops and roots and water and sugar content of roots. On November 1, four rows of the subplots were machine harvested, and root yields and sugar contents determined.

Results

Soil moisture conditions At the beginning of observations in early May the water table was 37 inches below ground surface. It dropped to 45 inches by early Tune and remained essentially at that depth until early August. This was followed by a gradual rise to the 34-inch depth by October. The rise after early August is attributed to a general rise in the area and to decreased water use by the beets, since by this time there had been considerable loss of older leaves, and foliar cover was sparse.

Moisture contents in 6-inch increments of depth down to 3 feet are reported in Table 1 at approximately monthly intervals throughout the season for the subirrigated treatment. They are expressed as volume or depth ratios (i.e. cubic feet of water per cubic foot of soil or feet of water per foot depth of soil). The total depth of water in the 0 to 36-inch depth at each date is given in inches. The difference in the totals between dates gives the net depletion of stored water.

The moisture content data in Table 1 show about 1.8 inches of moisture depletion between mid-April and the middle of May when the plants were small and the soil comparatively moist.

[2] Numbers in parentheses refer to literature cited.

Table 1.—Soil moisture content (volume ratio) at approximately monthly intervals during the growing season for subirrigated plots.

Depth	Sampling Date 4/19	5/16	6/15	7/13	8/23	9/25
0-6	0.38	0.34	0.30	0.24	0.20	0.20
6-12	0.55	0.45	0.37	0.34	0.32	0.31
12-18	0.50	0.41	0.33	0.34	0.34	0.32
18-24	0.47	0.43	0.34	0.30	0.31	0.31
24-30	0.55	0.52	0.41	0.37	0.40	0.45
30-36	0.63	0.61	0.48	0.50	0.49	0.60
Total*	18.4	16.6	13.3	12.5	12.4	13.1

* Expressed as inches of water in the 0-36" depth of soil.

Between mid-May and June 15 depletion was 3.3 inches, but the decrease between mid-June and late August was less than 1 inch. Late in the season there were increases in moisture content in the 2 to 3-foot depth associated with the rise in the water table, but the overall increase in equivalent depth of water in the soil was small.

Soil moisture tensions as interpreted from resistance block readings are presented in Figures 1 and 2 for the subirrigated and sprinkled treatments, respectively. In the subirrigated treatment there were rapid increases in soil moisture tension to the 18-inch depth as the beets developed; tensions throughout this zone exceeded 12 bars by mid-August. Tensions at lower depths lagged but attained values of $5^1/_2$ and $2^1/_2$ *bars* at the 24 and 30-inch depths, respectively. Tensiometers at the 36-inch depth (less than one foot above the water table) reached the top of their functional range (0.8 bar) by mid-June.

After mid-August, soil moisture tensions declined at depths of 18 inches and below, with the decrease greatest at the lower depths. The drop in tension at the 30-inch depth corresponds to the period of water table rise; those at shallower depths lag somewhat but can also be attributed to rise of the water table.

The combined data of Table 1 and Figure 1 show that the soil became very dry throughout most of the top 2 feet of the subirrigated treatment. Much of the water used by the crop early in the season came from stored moisture in the soil. When this was largely depleted, nearly all of the water was supplied by upward rise from the water table. There is no direct means of measuring the relative amounts of water absorbed from various depths of soil. However, dry soil conducts water very slowly and it is probable that most of the late season water was absorbed within a short distance of the water table.

As shown in Figure 2, soil moisture tensions in the sprinkled plots were lower throughout much of the growing season, in-

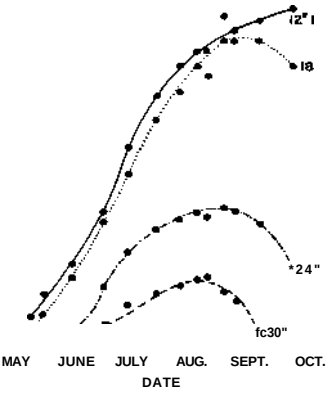

Figure 1.—Soil moisture tension conditions in the subirrigation treatment.

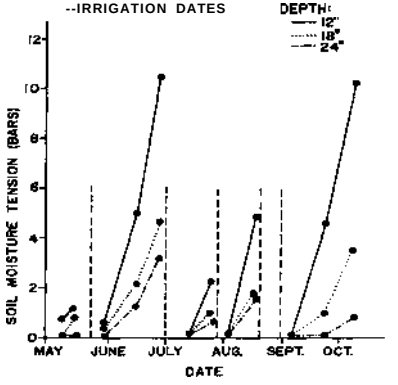

Figure 2.—Soil moisture tension in the treatment receiving supplemental sprinkler irrigation.

dicating that the plants were more adequately supplied with water. However, twice during the season, tensions exceeded 10 bars at the 12-inch depth and reached appreciable values at lower depths. Within their operating range, the tensiometers confirmed the data obtained with resistance blocks.

Nutrient uptake Nitrate-nitrogen contents of petioles at intervals throughout the season are given in Tables 2 and 3. All treatments started at high, uniform levels which were maintained until late May. Differences began to appear following the first irrigation, with both nitrogen fertilization and sprinkling increasing nitrogen uptake. These differences persisted throughout the season until the last sampling date on October 31, which was preceded by heavy rains on all plots on October 10. This rain caused a marked increase in the NO_3N levels of the subirrigated plots but had no effect on the nitrogen status of the sprinkled plots.

In the sprinkled treatment, petiole NO_3-N did not drop below the 1000 ppm critical level until very late *in* the season regardless of the nitrogen treatment. At such levels, the beets were adequately supplied with nitrogen (4). However, in the treatments receiving only subirrigation, petiole nitrate was at deficient levels after early July in the N_0P_{87} treatment and after mid-July in the $N_{80}P_{87}$ treatment.

It is interesting to note that sprinkler irrigation without nitrogen was more effective in supplying nitrogen than was the application of 80 pounds N per acre to unsprinkled sugar beets.

The same general effect of both fertilizer and irrigation treatments on phosphorus uptake is apparent (Table 2), but levels in all treatments were adequate to prevent deficiency. As with nitrogen, there was a large increase in petiole phosphorus of subirrigated plots following heavy rain in early October, but only small increases in the sprinkled treatments.

Potassium levels in petioles were not influenced by irrigation or fertilizer treatment, and K levels in petioles were adequate throughout the season (Table 2).

Disease conditions On June 13 from 38 to 52% of the plants showed symptoms of the yellows viruses. By July 23, 80 to 100% of the plants were infected and the viruses may have limited growth sufficiently to reduce the differences between treatments.

Harvest data Harvest data are presented in Table 4.

Supplemental sprinkler irrigation increased root yields an average 2.7 tons per acre by August 13. The difference increased to 7.5 tons by October 9 because of lack of root growth in the subirrigated treatment after September 10. In the subirrigated treatment there was a yield response to nitrogen application,

Table 2.—Nutrient content of petioles of recently matured leaves of plants of the machine harvested plots. Values (dry weight basis) are means of four replications. Dates of sprinkler irrigation: 5/25, 6/29 7/26, 8/17 and 9/13. Heavy rain on 10/10.

Irrigation	Fertilizer, lb/acre N	Fertilizer, lb/acre P	Sampling dates: Apr. 30	May 21	June 18	July 9	July 23	Aug. 17	Sept. 10	Oct. 31
					ppm, NO_3-N					
Sub	0	0	12400	14400	3020	560	260	270	340	2700
	0	87	12600	13800	2070	740	280	350	330	2390
	80	87	12500	14300	4080	1060	650	370	340	2520
Sprinkle	0	0	11800	14900	6910	1510	1360	840	610	640
	0	87	12400	14700	5910	1320	1370	740	560	640
	80	87	12400	15200	8090	2030	1850	930	610	720
F values: [1]	Irrigation		1.35	31.68*	82.57**	2.52	2.43	32.37**	19.78*	229.4**
	Fertilizers		0.40	5.43*	12.35**	3.88	3.76	0.67	0.03	1.63
	I × F		0.09	0.88	0.02	0.38	2.04	0.57	0.01	2.08
					ppm, PO_4-P					
Sub	0	0	2040	1350	1060	1580	640	1200	580	1620
	0	87	2300	1580	1450	2080	790	1520	910	1600
	80	87	2260	1590	1600	2050	760	1450	860	1750
Sprinkle	0	0	2160	1440	1480	1950	790	1680	1280	1400
	0	87	2040	1640	1850	2400	980	1920	1220	1400
	80	87	2050	1490	1620	2320	1060	2120	1190	1420
F values: [1]	Irrigation		5.95	0.66	2.94	19.12*	4.94	18.36*	13.41*	2.69
	Fertilizers		0.38	1.28	5.22*	86.35^{**}	3.23	2.67	1.51	0.40
	I × F		3.18	0.28	1.89	0.27	0.42	0.39	3.02*	0.20
					% K					
Sub	0	0			3.7		3.2	3.5		
	0	87			3.0		2.8	3.1		
	80	87			3.2		2.6	2.6		
Sprinkle	0	0			4.0		3.1	3.1		
	0	87			3.7		2.6	3.0		
	80	87			3.3		2.2	2.7		
F values:	Irrigation				27.84*		6.95	1.01		
	Fertilizers				1.08		15.01**	12.97**		
	I × F				0.19		0.62	2.29		

[1] F values required for significance at the 5% and 1% level respectively: Irrigation: 10.13, 34.12. Fertilizers or I × F: 5.88, 6.93.

Table 3.—Nutrient content **of** petioles of recently matured leaves of plants of hand **harvested plots.** Values **(dry** weight basis) are means of four replications. Dates of **sprinkler irrigations:** 5/25, **6/29, 7/26, 8/17 and 9/ 13.**

Irrigation	Fertilizer, lb/acre N	Fertilizer, lb/acre p	Sampling (and harvest d;ates) Aug. 13	Sept. 10	Oct. 9
				ppm, NOs-N	
Sub	0	0	280	360	250
	0	87	370	440	300
	80	87	310	460	280
Sprinkle	0	0	920	660	370
	0	87	870	730	340
	80	87	1250	920	350
F values: * Irrigation			15.29*	45.57**	4.75
Fertilizers			1.59	2.96	0.05
I x F			2.04	0.77	1.04
				ppm, P0⁴P	
Sub	0	0	1210	740	1220
	0	87	1390	900	1950
	80	87	1280	1120	1700
Sprinkle	0	0	1840	1790	2210
	0	87	1890	1900	1800
	80	87	1900	1710	1920
F values: [1] Irrigation			16.17*	262.61**	2.28
Fertilizers			0.31	1.19	0.18
I X F			0.14	2.58	2.40

[1]F values required for significance at the 5% and \% levels, respectively. Irrigation: 10.13, 34.12. Fertilizers or **I v** F: 3-88, 6.93.

but there was no effect in the sprinkled treatment. There was no yield response to phosphorus in either treatment. These responses are in accord with the nutritional status shown by petiole analysis.

On the fresh-weight basis, sucrose concentration in roots was appreciably lower in the sprinkled plots except for the last harvest date after the heavy rain; irrigation treatment averages differed by 2.2, 1.6, and 1.0 percent respectively on August 13, September 10 and October 9. While the data are inadequate for firm conclusions, they suggest that in the sprinkled treatment the sugar concentration was influenced by soil moisture conditions just prior to harvest. The September 10 harvest date was preceded by two closely spaced irrigations which kept soil moisture tension at low levels. The average sucrose concentration for all sprinkled treatments was 14.0 on September 10 as compared to 14.9 and 14.8 on August 13 and October 9, respectively. Sugar concentration dropped markedly at the November 1 harvest, which followed heavy rains by about 2 weeks. The decline was greatest in the subirrigated plots.

Nitrogen fertilization also tended to cause a lower sucrose concentration although the differences were small in the subirrigated treatment after the first harvest.

Table 4.—Top and root yield and root composition at different harvest dates. Values are means of four replications. Dates of sprinkler irrigation: 5/25, 6/29, 7/26, 8/17 and 9/13. Heavy rain on 10/10.

Treatments						Sucrose		
	Fertilizer, lb/acre		Fresh weight, tons/acre		Root, % dry matter	% fresh weight	% dry weight[6]	Tons/acre
Irrigation	~N~	P	Tops	Roots				
			August 13 (Hand harvest)					
Sub	0	0	10.3	13.4	26.1	17.4	66.7	2.33
Sub	0	87«	9.9	12.9	26.1	17.4	66.7	2.24
Sub	80	87	14.8	15.2	25.0	16.5	66.0	2.52
Sprinkle	0	0	22.4	16.7	23.3	15.0	64.4	2.50
Sprinkle	0	87	21.7	15.9	23.5	15.1	64.2	2.39
Sprinkle	80	87	28.0	16.9	22.8	14.6	64.0	2.46
			September 10 (Hand harvest)					
Sub	0	0	7.9	16.3	25.0	16.6	66.4	2.71
Sub	0	87	7.2	16.1	25.2	16.7	66.3	2.68
Sub	80	87	8.9	18.2	24.6	16.4	66.7	2.98
Sprinkle	0	0	19.5	21.3	22.7	14.0	61.7	2.99
Sprinkle	0	87	19.1	21.5	23.2	14.3	61.6	3.08
Sprinkle	80	87	24.6	21.4	22.5	13.7	60.9	2.93
			October 9 (Hand harvest)					
Sub	0	0	8.1	16.0	24.5	16.0	65.3	2.56
Sub	0	87	7.1	16.0	24.2	15.9	65.7	2.54
Sub	80	87	8.5	18.5	24.0	15.6	65.0	2.88
Sprinkle	0	0	20.8	24.4	23.1	15.0	64.9	3.66
Sprinkle	0	87	18.4	24.4	23.3	15.1	64.8	3.69
Sprinkle	80	87	20.1	23.3	22.7	14.2	62.6	3.30
LSD's for means of hand harvested plots, %5 level: [1]			3.0	2.4	1.0	0.7		0.39
			[2] 5-1	2.4	0.9	0.8		0.43
			November] (machine harvest)					
Sub	0	0		16.9		12.9		2.18
Sub	0	87		16.4		13.3		2.19
Sub	80	87		19.3		13.2		2.56
Sprinkle	0	0		23.3		13.0		3.03
Sprinkle	**0**	87		24.3		13.4		3.26
Sprinkle	**80**	87		24.6		13.2		3.24
LSD's for means of machine harvested plots, 5% level: [3]				2.2		N.S.		0.28
				2.3		N.S.		0.33

[1] Between fertilizers for the same irrigation treatment and date of harvest.
[2] Between fertilizers for different irrigation treatments and the same or different dates of harvest.
[3] Between fertilizers for the same irrigation treatment.
[4] Between fertilizers for different irrigation treatments.
[5] % sucrose, fresh weight x 1/% dry matter x *10~2*. Calculated from treatment means.
[6] 200 **lb** P2O5.

Dry matter contents of roots followed the same general trend as fresh-weight sugar concentrations, showing that both irrigation and nitrogen fertilization increased root water content. However, sucrose concentration on the dry-weight basis likewise was influenced by the treatments. Sprinkling decreased dry-weight

sugar concentration on all of the three harvest dates for which data are available.

Differences in sugar yield were small at the August 13 harvest date. By September 10, sugar production had increased by about one-half ton per acre with both irrigation treatments, and the difference between them increased slightly. A subsequent loss of sugar per acre in the subirrigated treatment and a further increase in the sprinkled treatment resulted in a difference of 0.88 ton per acre on October 9. While sugar yields of the large plots on November 1 were lower for both treatments, the 0.88 ton per acre increase by the sprinkled treatment persisted.

Discussion

Soil moisture conditions While general applicability of these data to other muck soils is not known, these soils have some special moisture characteristics that are important in evaluating their ability to supply moisture to plants, especially under subirrigation.

Organic matter contents average about 20 percent, and bulk densities range from near 1.0 in the surface foot to as low as 0.65 at lower depths. At saturation, the moisture content on a volume basis is typically about 65 percent. Typical 15-bar moisture contents average about 30 percent so that water-holding capacity between saturation and 15 bars is approximately 4 inches per foot depth of soil. However, both field and laboratory data show that one inch or more is drained at low tensions (on the order of 0.1 bar). Of the remaining 3 inches, nearly 2.5 are retained at 0.8 bar (the upper limit of tensiometers) and over 2 inches are held at 1.0 bar tension. The net result in the field is that soil moisture tensions tend to increase very rapidly through the range from about 0.1 to 0.8 bar as moisture is absorbed by plant roots. Tensiometers thus have limited value.

The effect on plant growth of having much of the available water held at comparatively high tensions is difficult to assess. If, as is frequently assumed, plant growth rates are inversely related to soil moisture tension, much of the available water would be better suited to plant survival than to rapid growth. On the other hand, if one looks at availability of soil moisture in the dynamic sense, higher moisture contents of muck soils could mean more rapid movement of water through the soil to plant roots. Consequently plants could be better supplied with water in muck than in mineral soils at the same soil moisture tension. Peters (2) concluded that rate of moisture movement was an important factor in water availability to plants in mineral soils of different texture.

In this experiment, there was an obvious top-growth response to the first irrigation which was apparent visually by early June. By this time soil moisture tension to a depth of 18 inches was about 4 bars in the subirrigated treatment (see Figure 1). Measurement would have shown reduced growth rate at still lower tension; the growth response was probably not related to nitrogen status since the NO_3-N content of petioles was still quite high at this time.

Soil moisture data show that this muck soil cannot transmit water upward rapidly enough under subirrigation to maintain high moisture levels near the surface under actively transpiring plants. In the early season, soil moisture depletion was probably sufficient to account for all the water used by the crop. From mid-May to mid-June soil moisture depletion was not sufficient to supply the water used so that water was obtained by a combination of net depletion and upward rise. After mid-July most of the water used was supplied by upward rise, but there are indications that most of the water was absorbed within about a foot of the water table, or below the 36-inch depth. This is confirmed by a tank study at Davis with sudangrass, in which a tracer was added to water applied by subirrigation. None of the tracer rose more than one foot above the water table.

Most crops in the area are grown with subirrigation only, and it is a common belief that only occasional supplemental sprinkling is required. Even discounting water supply to plants from the water table, muck soils have high water-holding capacities, suggesting the need for only infrequent irrigation. It is thus rather surprising that in spite of five thorough irrigations soil moisture tensions exceeded 10 bars twice at the 12-inch depth. In general, tension values were low for about 7 to 10 days following irrigation, then rose rapidly. This phenomenon is largely attributable to the relatively small amount of water retained between 0.1 and 1.0 bar tensions. However, the data likewise demonstrate the marked preferential absorption of water from near the surface even after roots have reached the water table. The water table in the sprinkled plots did not rise following any irrigation, precluding root injury by rising water.

Nutrition This experiment confirms the existence of interrelations between soil moisture conditions and nitrogen and phosphorus nutrition. There was a yield response to applied nitrogen in the subirrigated treatment but not in the sprinkled treatment. Thus the response to sprinkling was a combined nitrogen and soil moisture response. Increased petiole phosphorus under sprinkling indicates that a similar response would be obtained on phosphorus-deficient soil.

In these soils, a large part of the nitrogen supply, and perhaps phosphorus, is apparently derived from reactions involving organic matter. Low moisture conditions retard decomposition of organic materials and nitrification, and this effect is possibly the principal mechanism of the relationships noted. This is indicated by the very rapid increase in nitrogen and phosphorus uptake following October rains in the subirrigated treatment. The corresponding increase was very moderate in the sprinkled treatment. This is the pattern of events one would expect in release of nitrogen and phosphorus from organic matter, since the readily decomposable organic substances in the sprinkled treatment already would have been released because of more favorable moisture conditions and utilized by the plants.

On this basis, it seems probable that the interrelation between nitrogen and phosphorus nutrition and soil moisture conditions is more pronounced in muck soils than in soils low in organic matter. Subirrigation tends to accentuate the effect because of prolonged dry conditions in the upper soil, whereas with surface or sprinkler irrigation the upper soil is moistened intermittently. However, deep-rooted crops in mineral soils often experience long periods of dry surface soil without yield loss, indicating adequate levels of nitrogen nutrition. Frequently, nitrogen levels in droughted plants are higher than those grown under favorable moisture conditions (5).

Many experiments have been conducted on influence of soil moisture conditions on plant nutrition. The results with phosphorus in particular have been conflicting. It is difficult to segregate the effects of low soil moisture levels on the ability of soils to supply nutrients from the ability of plants to absorb them. The conflicting results of various experiments tend to indicate that the ability of dry soils to supply nutrients is the dominant factor, but this can not be confirmed from the present experiment. Some of the processes which affect nutrient availability may be markedly influenced by soil moisture levels whereas others may not—in this experiment, nitrogen and phosphorus uptake were influenced by soil moisture conditions, but the uptake of potassium apparently was not (Table 2).

Root yields and composition The data of this experiment clearly illustrate the effects of soil moisture conditions and nitrogen nutrition on sugar concentration and yield. They confirm the conclusion of Loomis and Worker (1) that drought and nitrogen deficiency both tend to reduce water content of root tissue and that this is a factor in increased fresh-weight sugar content. However, in this experiment similar conditions increased sugar content on a dry-weight basis as well.

The farmer growing beets on muck soils with high water table has a possible advantage in that moderate drought may be induced near maturity if desired without fear of extreme drought. At the same time, he effectively reduces the nitrogen supply. On the other hand, there is the disadvantage that the two variables cannot be controlled entirely independently. In any case, intelligent fertility and irrigation management are highly interrelated.

Summary

Soil moisture conditions, nutrient uptake, top growth and root and sugar yields of sugarbeets were evaluated under subirrigation alone (water table at 3 to 4 feet) and under subirrigation plus sprinkling. With subirrigation only, the upper soil was relatively dry for a major portion of the growing season. Sprinkler irrigation increased top growth, root and sugar yields and nitrogen and phosphorus content of petioles, but had no effect on petiole potassium content. Under subirrigation only, there was a response to applied nitrogen fertilizer but none with subirrigation plus sprinkling.

It is concluded that the response to sprinkler irrigation was caused by both higher soil moisture levels and more favorable conditions for nitrogen uptake. While petiole phosphorus contents for all treatments were above deficient levels, the increased P uptake with sprinkling points to the possibility of a similar response on phosphorus-deficient organic soils.

Acknowledgments

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The Evaluation of and the Use of the Top-Cross Test as a Method of Selecting Inbred Lines of Sugarbeets for General Combining Ability

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The performance of top-cross hybrids remains the primary initial method for screening inbreds for superior general combining ability in cross-pollinated crops. Plant breeders generally agree that no one tester strain allows perfect evaluation of inbred lines.

Part I of this paper presents the genetic evidence, collected by the plant breeders of The Great Western Sugar Company, used for choosing the German red beet as a top-cross tester variety for inbred lines of sugarbeets. This evidence is derived from a study of the association of results using different tester parents, an examination of the relative top-cross performance of inbreds from different open-pollinated sources and the most critical evidence, from the single-cross performance of inbreds of known top-cross performance.

Part II deals with the details of the use of the top-cross and the interpretation and use of the results.

Part I The German red beet as a top-cross tester variety

Literature Review

Plant breeders have generally chosen tester varieties with all or some of the following characteristics: (a) of different origin than the material being tested, (b) of relatively broad genetic base, whether it is a double cross hybrid, synthetic of inbreds or an open-pollinated variety, and (c) inherently poor in performance. Several investigators have reviewed the literature concerning the selection of tester varieties in corn (4, 5, 8, 9)[3].

Oldemeyer concluded that the German red beet variety was as reliable a top-cross tester as was a sugarbeet variety (6), and

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[3] Numbers in parentheses refer to literature cited.

the red beet was preferable because it had a dominant marker gene which made it useful for identifying hybrids in all psuedo-self fertile inbred lines of sugarbeets. Using a limited number of parents, represented by inbreds, heterozygous strains and tetraploid strains, Finkner et al. (3) concluded performance of parents *per se* and diallel data were more accurate measures of combining ability than performance of hybrids with the red beet.

Materials and Methods

The use of a tester variety with a dominant red color marker for testing lines of sugarbeets was outlined in a previous paper, as was the use of the red hypocotyl marker (7). All hybrids between inbred lines from which data were taken for this investigation were made using the red hypocotyl dominant character. Selective thinning to red hypocotyl plants produced homogeneous populations of hybrids.

Performance trials from which the data were collected comprised two-row plots, 18 feet long, generally in four replications. A control block (2) or a lattice experimental design was used.

A;fter machine harvest, all beets were uniformly crowned, washed, weighed and rasped. The sugar content was determined on the rasped brei by the standard Sachs-LeDocte cold digestion technique.

The results, using the German red beet, a sugarbeet variety, and a newly synthesized red beet, were compared. This new red beet was developed by J. O. Gaskill, U. S. Department of Agriculture, Fort Collins, Colorado; it was derived by introducing dark red color from the garden beet into the U. S. variety "Synthetic Check" by backcrossing. Yield of roots and sugar percentage of individual top-cross hybrids were converted to a percent of check which was GW359 X German red beet in case of German red beet hybrids and GW359 X Gaskill's red beet in the case of Gaskill's red beet hybrids; GW359 was the check variety in GW359 hybrids.

Results and Discussion

Tester Comparison

The association of the performance of the top-cross hybrids of a series of inbreds using three different top-cross hybrid tester parents was determined from performance trials conducted in 1958. Correlation coefficients were calculated from the data which were in percent of check, and these are summarized in Table 1.

The correlation coefficients, all significant at the 1% level, are within the range which might be expected from similar

Table 1.—Correlation coefficient of the performance of top-cross hybrids using (A) German red beet, (B) Gaskill's red beet and (C) GVV359.

	Number of pairs	Yield of roots	Sugar %
Tab	49	.58**	.70**
Tac	34	.64**	.68**
	34	.72**	.54**

** Significant at the 1% probability level.

tester parents, considering the precision of the trials from which the data were drawn. One could, by considering rather insignificant differences, deduce that tester C (GW359) gives a more accurate estimate of combining ability for weight. The GW359 tester correlates closer with both tester A (German red beet) .64 and tester B (Gaskill's red beet) .72 than A with B, .58. For sugar percent, A perhaps gives the best estimate, r_{ab} = .70, r_{ac}. == .68 while r_{be} = .54. The trends are not strong enough for a choice of testers to be made solely on the basis of these data, and there is the possibility that the closer relationships are meaningless.

The Great Western Sugar Company uses an arbitrary classification, based upon top-cross performance, for determining the disposition of inbred lines. Inbred lines are classified according to their top-cross performance as measured by gross sugar yield (root yield X sugar percentage) expressed in percent of check. With some exception for lines having extremely high sugar percentage or purity, lines having a top-cross below 105% of the check in total sugar are discontinued, those with 105-115% of the check are retested, and those having 115% or more of check are multiplied for more extensive and precise testing. Table 2 summarizes the possible disposition of a series of inbreds comparing the three different tester parents.

Table 2.—Comparison of the disposition of a series of inbred lines based upon top-crossing with (A) German red beet, (B) Gaskill's red beet and (C) GW359 sugarbeet and using total sugar as the base character.

n	n	n	n	n
A = B 88	A > B 4	B > A 4	A > > B 1	B > > A 2
A = C 25	A > C 5	C > A 7	A > > C 0	C > > A 2
B = C 22	B > C 4	C > B 7	B > > C 0	C > > B 1

N = M when both are 105% or less, both are 105-115% or when both are 115% or more of the check variety.
N > M when N is 105-115% while M is less than 105% or when N is 115+% and M is 105-115% of the check variety.
N >> M when N is more than 115% while M is less than 105% of the check variety.

None of the tester parents appears better than another when disposition of inbreds is considered. The proportion of lines classified differently is about the same for all three testers. (Table 2). A x^2 (Chi square) goodness of fit test did not indicate that the ratios deviated more than expected, p = .5-.7, and indicated that such distributions could be expected from random sampling.

Inbred Source Comparison

It was observed in the early years of testing, and continues to be observed, that the average top-cross performance in Colorado of inbred lines developed from varieties synthesized by Great Western is better than for inbreds developed from varieties obtained elsewhere. The proportion of superior lines identified and selected, after subsequent testing, from Great Western sources has also been greater.

The inbred lines which were tested in 1957, using the German red beet as a tester parent, were divided as to source and then as to performance of their top-cross hybrids (gross sugar yield) in 10% classes from 70 to 130% of check. These data are summarized in Table 3.

Table 3.—The top-cross performance of inbred lines divided in percent of check classes and as to having been developed from Great Western sources or other sources.

Check class	Other	GW
70-90	10	1
80-89	35	8
90-99	21	63
100-109	1*	105
110-119	1	43
120-129	0	9
Totals	82	229

Goodness of fit χ^2 = 118.92 from which P <.001.

It is clear that the level of performance is much lower for other sources, mode 80-89%, as compared to Great Western sources, mode 100-109%. The proportion of superior lines, above 110%, is far lower for other sources also.

When tested in Colorado, Great Western varieties always perform better than varieties developed in other areas of the world. Inasmuch as the top-cross performance of inbreds seems to be correlated with the performance of the varieties from which they are derived, it can be deduced that the top-cross hybrid performance of an inbred from within a source reflects the relative value of the inbred as **a** hybrid parent or its combining **ability.**

The Relationship Between Single-Cross and Top-Cross Performance

The true measure of whether a particular tester accurately measures the general combining ability of inbred lines is whether there is a positive relationship between the performance of the top-cross of a line and the average performance of specific hybrids involving that line. The single-cross trials of 1959 and 1960 contained hybrids of inbreds selected for their different levels of performance in top-crosses having the German red beet as the tester parent. Single-cross hybrid performance was the average performance in three trials in northern Colorado, each consisting of four replications of plots, two rows X 18 feet. The top-cross data were taken from only one trial of four replications of plots of two rows X 18 feet. Single-cross test A results are from an average of two years; while for test B, they are from one year. Test A contained 47 single-crosses involving 34 inbred parents, while test B contained 26 single-crosses involving 19 parents.

The relationship between single-cross performance and top-cross performance as expressed in correlation coefficients is summarized in Table 4. Columns one and two are multiple correlations in which the top-cross performance of p_1 and p_2 are compared to the performance of the single-cross p, X p- Only moderately high multiple correlations existed for tonnage and sugar percentage, while the correlation in test A for gross sugar was not significant. Using the average top-cross performance of the two parents in a simple correlation (third column[1]), a much higher correlation is obtained.

Table 4.—Correlation between top-cross and single-cross performance.

	Test A 1959-60	Test B 1960	Test B 1960
	$R_{P_1P_2.sc}$	$R_{P_1P_2.sc}$	$r_{\bar{R}\ P_1P_2.sc}$
Tonnage	.50*	.51**	.89**
% Sugar	.59**	.33*	.95**
Total sugar	.25 NS	.49*	.86**

*, ** Statistically significant at the 5% **and** 1% level of probability, respectively.

A clearer delineation of the relationship between top-cross and single-cross performance is obtained from a trivariate frequency table of the same data (Tables 5 and 6). Parental top-cross performances were arbitrarily divided into five classes, according to relationship to check variety, GW359 X German red beet. Plus signs indicate the inbred performed better than the check and minus signs poorer with double plus signs, indicating the best performing lines and the double minus signs

Table 5.—Comparison of top-cross performance, column and row headings, with single-cross performance, rank in variety trial in boxes, Test A, 1959-60. Or

Female / Male	Weight of roots: + +	+	=			Ratio[a]
+ +	(1) (3) (4) (12)	(28)	(6)(8)(17)(22) (23)(24)(26)(41)	(7)(10)(11) (16)(18)	(2)(5)(9) (14)	18:22
+			(35)(36)(37) (46)	(15)(32)		1:6
			(21)(25)(29)(40) (40)(42)(43)	(30)(38)(39) (44)(45)	(19)(36)	2:14
=			(20)(30)		(47)(48)	1:4
Ratio[11]	4:4	0:1	7:21	6:12	5:8	
			Sugar content (%)			
+ +	(5)	(1)(2)(4)(10) (14)	(12)(21)(42)	(3)(9)(15) (24)(39)		11:14
+		(6)(8)(17)(19) (23(25)(38)	(7)(28)(35) (40)(45)	(11)(13)(18)(22) (27)(33)(36)(43) (47)(48)		10:22
=			(20)(29)(32) (41)	(26)(30)(31) (44)(46)(49)		1:10
Ratio"	1:1	10:12	4:12	7:21		
			Total sugar (Weight of roots x sugar content)			
+ +	(22)	(3)(7)(18)(25)(28) (29)(37)(38)(46)	(1)(4)	(19)(21)(26)(27) (34)(40)		8:18
+		(13)(24)	(2)(5)(6)(30) (31)	(10)(17)(23)(29) (42)(45)(47)(48)		7:15
=			(16)	(8)(11)(12)(14) (15)(20)(35)		7:8
—				(33)(39)(41)(43) (44)		0:6
Ratio*	1:1	4:1]	6:8	11:26		

* Number of crosses which exceed the mean of the trial compared to the total in the class.

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Table 6.—Comparison of top-cross performance, column and row headings, with single-cross performance, rank in variety trial in boxes, Test B, 1960.

Weight of roots

Female / Male	++	+	=	−	Ratio[a]
++	(3)	(1)(2)(6)(7)	(4)(16)	(9)(19)(20)	6:10
+		(14)	(5)(10)(13) (25)(27)	(18)(22)(23) (24)	1:10
=			(12)		0:1
−			(11)(15)(17) (20)	(26)	0:5
Ratio[a]	1:1	4:5	4:12	1:8	

Sugar content (%)

++	(1)	(2)(24)	(13)	3:4
+	(3)(4)(5)(6) (9)(16)	(7)(11)(17) (19)(20)(26)	(8)(10)(12) (13)(24)	11:17
=		(18)(21) (23)	(22)(27)	0:5
Ratio[a]	6:7	3:11	5:8	

Total sugar (Weight of roots × sugar content)

++	(1)(3)(4)(6) (7)(10)(18)(27)	(8)(26)	(5)(16) (19)(24)	7:14
+	(2)(14)	(14)(17)	(12)(15)(20)(22)	1:8
−		(13)(21)(23)	(25)	0:4
Ratio[a]	6:10	1:7	1:9	

[a] Number of crosses which exceed the mean compared to the total in the class.

the poorest. The top-cross performance of the female is subdivided across the top row; of the male, along the left column. Numbers in the boxes are the rank in the trial of a single-cross having parents whose top-cross performance is indicated in the left of the row and top of the column. If the top-cross hybrid performance is related to single-cross performance, the low rank numbers should be on the top left and the high rank numbers in the lower right.

Yield of roots is predicted well, sugar content could not be expected to be predicted better, while total sugar was predicted less well than its two components, yield and sugar content. These data are consistent with the correlation coefficients.

Any one or all of four factors may account for the above associations not being closer:

First, the trials upon which the performances were determined were not very precise, i.e., four replicates of 36 feet of row for top crosses. This lack of precision, no doubt, is a major cause for the relationship appearing looser than it probably is.

Second, genotype X year interaction may have been reflected because the testing of the top crosses and single crosses was done in different years.

Third, specific combining ability could exist between the inbreds and the red beet testers. However, the comparison presented in Table 3 would indicate that it is not likely to be a significant factor unless Great Western lines are more likely to combine specifically with the red beet than others.

And fourth, specific combining ability between inbred lines in the single crosses used would result in the general combining ability measure (red beet hybrids) appearing erratic. In trials since 1960, our experience has been that the variation due to specific combining ability is very minor in comparison to general combining ability.

Conclusions

The evidence presented in this paper does not conflict with evidence presented before (7) as to the use of the German red beet as a top cross tester variety. The German red beet appears to be as reliable as Gaskill's red beet and a sugarbeet variety in assessing combining ability of inbred lines; as evidence for this, tester hybrids with it perform best when the inbred lines are from adapted material, and single-cross performance agrees reasonably well with top-cross performance using the red beet tester.

Part II Use of the Top-Cross Technique for Selecting Superior Lines

Inbred lines are initiated by self pollination of plants from an open-pollinated variety with or without selection of the parental roots for sugar content, and purity and/or disease resistance. Intense intraline and interline selection for agronomic type is made in the first and second selfed generations. A sugar content and purity (pressed juice) selection is made in the first selfed generation at which time only the very poor lines and roots are discarded.

Once an inbred has been established by self pollination as determined by its morphological uniformity, the S_2 generation or beyond, roots are selected for top-crossing with the German red beet. Some of the roots of each line are simultaneously selfed another generation. Top crossing is accomplished by planting rows of inbred lines alternately with rows of red beets. Seed is harvested from several roots of each inbred line.

To obtain a complete stand in top-cross hybrid trials, the following germination procedure is used:

Two grams of each top-cross lot of seed are germinated in a

ragdoll of paper toweling after standard washing. Strong light is shielded from the germination cabinet to suppress the development of pigment in the normally occurring red hypocotyl sprouts so that a clear distinction can be made between the red beet color and normal hypocotyl color.

Hybrids per two grams are counted during a 10-day period; the seeding rate is adjusted to 12 hybrids per foot which results in very satisfactory stands. A shortage of red beet hybrids occurs only with inbreds containing genes for self fertility, although, even then, many lines have enough hybrids for testing.

Trials, generally, consist of two 18-foot rows replicated four times arranged in a 7 X 7 simple lattice design. Top-cross seed lots, about which there is doubt as to whether they contain enough hybrids to establish a stand, are planted in separate trials which are smaller lattices or control block designs. Each trial contains three entries of a top-cross check. The check variety is different for each of the three different growing areas of The Great Western Sugar Company, being the standard open-pollinated variety crossed to the red beet for Colorado, Montana and Ohio areas. Components measured in the trials are calculated to percent of check for year to year and trial to trial comparison. Diseases caused by *Cercospora* and *Aphanomyces* cause such major reduction in productivity in all top crosses in Ohio except those involving highly resistant inbreds that a visual elimination of many lines can be made. In this case, only plots containing vigorous top crosses are harvested for comparison to the check top cross. Because only selected plots are harvested, a control block design has been used in Ohio. Further, one-row plots in eight replications are used because of the existence of high field variability.

Characters which are measured are yield of roots, sugar percentage, purity and bolting percent. In addition, *Cercospora* infection readings are made in Ohio. Yield of roots and sugar percentage are considered components of yield while apparent thin juice purity is the measure of processing quality. Apparent thin juice purity is determined on a pressed juice which has been purified by liming and phosphating followed by filtration in a technique outlined previously (1). Recoverable sugar is computed by multiplying yield of roots by sugar percentage and reducing the product by a factor which takes into account loss in 60 purity molasses and a constant processing loss of 0.3% on beets.

The averages for 1965 and 1966 of the statistical least significant differences for weight and sugar content and the coefficient of variation are summarized in Table 7. These statistics

Table 7.—Average coefficients of variability and least significant differences (% of check) for various characters in 1965 and 1966.

Year	No. trials	Wt. roots		Sugar %		Purity %		Recoverable sugar yield	
		CV	LSD*	CV	LSD*	CV	LSD*	CV	LSD*
1965	**9**	8.88	**14.71**	**5.31**	**7.78**	1.35	**2.00**		
1966	**10**	8.51	**13.04**	**4.08**	6.08	**1.46**	**2.17**	10.21	15.53

* Significant at the 5% probability level.

can be compared to the arbitrary limits which have been chosen for use in disposing of lines which have been top-crossed. As indicated in the section *Tester Comparison,* lines which have 105% or less total sugar than the check are discontinued, with 105-115% retested, and with more than 115% are considered for multiplication.

No least significant difference was calculated for recoverable sugar yield in 1965, but considering the greater variability of the components, weight of roots and sugar percentage, the LSD for sugar yield in 1965 would have been considerably greater than 15.5. Using an arbitrary figure of 15% greater than the check for selection, some lines selected as superior might actually be no better than the check. These lines will be eliminated on further testing with the only loss being testing effort. It is unlikely that many highly superior lines become discontinued. Results with lines in the 105-115% of check class indicate that only a few exceed 115% of the check on retesting; it would then be expected that only rarely would one of the lines with 105% or less have a retest of 115% or more.

To maintain the genetic purity of inbred lines of cross pollinated crops, the lines must be uniform enough for outcrossed plants and/or plants resulting from seed mixture to be identified and rogued. In practice, Great Western selects no line for multiplication unless it has reached a degree of uniformity equivalent to an inbred in the fourth selfed generation. (Visual uniformity, within lines and between sublines, is used rather than a stated number of selfings to determine when a line may be sib increased, because in the first and second selfed generation, roots arising from outcrossing are unavoidably selected for carrying the line.)

Early testing is practiced to eliminate poor combining lines and to allow concentration of testing effort on the better lines. Superior lines in the S_2 and S_H generations, as well as others, which are too variable to increase, are top crossed again along with the lines which have 105-115% of the check in total sugar. Two and sometimes three sublines are top crossed if a retest is indicated.

In recent years, fewer lines with only average performance are being found in the single cross trials. This increased reliability of selection of lines for general combining ability can be attributed to an increased amount of top-cross data being available for a line before it is multiplied. Data from several sublines and data from several years' trials allows a more critical selection.

Sugar content is generally used only as a yield factor in the selection of lines for multiplication. However, if a choice has to be made between lines of equal recoverable sugar yield, the line with the low sugar content will be discontinued because of the increased cost of harvest and delivery. Conversely, lines with high sugar content are favored.

The all inclusive character, apparent thin juice purity, is the quality selection criterion and is related to the proportion of total sugar that can be extracted in the factory process. Prior to 1961, thin juice apparent purity was not determined on top-cross hybrids because it was believed that high sugar lines also had high purity. As more experience was gained in testing, it became apparent that sugar content and purity were not necessarily related and, more often than not, lines with high root yields had lower than average purity. Lines having a purity significantly below the check are discontinued regardless of the yield of total sugar.

Complete pedigrees of the lines are at hand whenever a selection is made in order to minimize the selection of too many lines derived from the same "mother" root and same source. It has been necessary to accept from some sources lines with poorer agronomic characteristics and with lower top-cross performance than lines from other sources to insure that all sources are represented in the advanced testing program.

It is estimated that 75% of the lines are lost before top-cross testing due to self incompatibility. (Most occurs in selfing the original plant from the source.) Discarding of lines because of poor agronomic type, e.g. large crowns, bolting, sprangles and hairiness, accounts for another 50% loss. Top-cross testing eliminates at least 90% of the remaining lines so that fewer than 1.5% of all roots originally selfed result in lines being carried to the stage of advanced testing.

Top-cross testing, using the German red beet, is considered an efficient and accurate tool for selecting inbred lines of sugarbeets for superior general combining ability.

Summary

A comparison of top-cross results from a series of inbred lines, using the German red beet, Gaskill's red beet and GW359,

indicated little choice as to the tester which gives the most accurate estimate of general combining ability. Top-cross performance of lines from a source was found to be related to the performance of the source *per se*. Top-cross performance of a line, using the German red beet tester, was found to be related to single-cross performance. In the breeding program of The Great Western Sugar Company, more than 90% of the inbred lines which are top-cross tested are eliminated on the basis of the top-cross test.

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The Relation of Beet Molasses Composition to True Purity
Part I. Composition

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Molasses formation from beet sugar liquors has had the attention of technologists for more than 100 years. Its formation in the factory is dependent on many factors but probably the most important variables are the effect of some non-sugars in decreasing the rate of crystallization and others in increasing the solubility of sucrose in the molasses solution. Since equilibrium between crystallized sugar and sugar in solution is not reached in industrial molasses because of the time necessary to reach this equilibrium, factory molasses contains more sugar than a completely exhausted molasses. Molasses in this paper refers to factory discard molasses.

In an attempt to determine what chemical factors were related to variations in molasses purity a composition study was made of molasses produced in the different beet sugar areas of the United States. Samples were selected from straight house and Steffen factories and analyzed for a large number of compounds and groups of compounds. The analytical methods used and the data obtained are presented in Part I of the paper while the statistical evaluation of the data is presented in Part II.

Experimental

Molasses samples were obtained during the 1956 campaign from a cross section of the beet sugar producing areas of the United States. Straight house samples were obtained from Betteravia, Clarksburg and Manteca, California; Brighton, Eaton and Swink, Colorado; Rupert, Idaho; Carrollton, Michigan; Crookston and Moorhead, Minnesota; Sidney, Montana; Fremont, Ohio; Belle Fourche, South Dakota; West Jordan, Utah; Toppenish, Washington, and Green Bay, Wisconsin. Spring and fall campaign samples were obtained from Betteravia. Swink ordinarily operates as a Steffen house, but the sample included in this study was produced from Kansas beets while the factory was operating as a straight house. Steffen house samples were

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obtained from Alvarado, Oxnard, Spreckles, Tracy and Woodland, California; Loveland, Colorado; Twin Falls, Idaho; Mason City, Iowa; Grand Island and Scottsbluff, Nebraska; Nyssa, Oregon; and Torrington, Wyoming. A spring and fall campaign sample was obtained from Oxnard. A sample of molasses was obtained from Johnstown, Colorado as representative of the barium process and a sample of molasses produced by ion exchange in 1955 was obtained from Layton, Utah.

Samples were collected by factory personnel and shipped to this laboratory for analysis. An attempt was made to obtain composite samples representing at least a month's operation. After receipt of the samples (one to two gallons) they were heated, thoroughly mixed, subdivided into 200 g samples for analysis and stored at .34° F. Samples were removed later from cold storage, heated, stirred to insure uniformity, and a weighed sub-sample taken for analysis.

Total solids were determined in duplicate by taking aliquots from a diluted sample that would give a solid residue of 300-600 mg, placing them in tared glass weighing dishes, 50 mm diameter, and heating them in a forced draft oven at 60° C for about six hours to a thick syrup. The dishes were then placed in a vacuum oven and the pressure reduced until the syrup foamed and filled the dish. After 30 hours drying at 60° C the pressure was restored with air slowly bubbled through sulfuric acid. The dishes were placed in a desiccator and weighed when cool. Since these were beet molasses samples with relatively low reducing sugar content there was a negligible loss in weight from decomposition due to prolonged heating. Cane molasses decomposes to a much greater extent on heating than beet molasses (4,5)[2].

To obtain representative samples of molasses for sugar analyses 50 g were weighed in a beaker, washed into a liter volumetric flask with water and sufficient 95% alcohol added to make the final volume aproximately 80% alcohol. After heating and cooling, the volume was adjusted to the mark and the contents were allowed to stand overnight. A 100 ml aliquot of the clear solution was taken up in a pipet without filtration. Sucrose and reducing substances were determined by the Munson-Walker official method (1) (Sees 6.2 (b), 6.74 (a), 6.77, 6.78 (b) [2], 25.35, 29.39, 29.40 and 42.11). The cuprous oxide was collected and weighed in a porcelain filtering crucible. Sucrose was calculated as 0.95 times the total reducing sugar after invertase inversion, less reducing substances before inversion, less 0.72 times the raffinose content. Sucrose = 0.95 [Total Reducing

[8] Numbers in parentheses refer to literature cited.

Sugar—(Reducing Sugars +0.72 Raffinose)]. The factor 0.72 for the reducing power of anhydrous raffinose after invertase inversion was determined in this laboratory.

Raffinose was determined separately by paper chromatography using the method of Bevenue and Williams (2).

Milliequivalents of anions, total weight of anions and average equivalent weight of anions were determined by an ion exchange procedure (8). Only those compounds that pass through the cation exchanger and are adsorbed on the anion exchanger and subsequently eluted with ammonium hydroxide are included as anions. Amino acids and a few other amphoteric compounds are retained on the cation exchanger. Such compounds, although having acidic characteristics, are not included in calculations of anions.

Ash was determined on a diluted molasses sample charred in a platinum dish under an infrared heater and then heated in a muffle furnace at 550° C overnight, cooled and weighed.

Alkalinity of the ash was determined on the total sample of ash. The residue in the Pt dish was treated with 25 ml of 0.1 N hydrochloric acid, heated on a steam bath, cooled, and the excess acid titrated with 0.1 N sodium hydroxide to pH 4.0.

Total halides calculated as chloride was determined gravimetrically by the AOAC method (1) (Sees 6.5, 6.6).

Total nitrogen was determined by the AOAC Kjeldahl method (1) (Sec 2.23). The original molasses and ion exchange fractions containing nitrate were treated according to the improved procedure for samples containing nitrate (1) (Sec 2.24).

Determinations of other types of nitrogen were made as follows: nitrate according to the method of Johnson and Ulrich (6), ammonia by the AOAC method (1) (Sec 2.25) ; amide by the method of Winton and Winton (9) , amino nitrogen by the method of Peters and Van Slyke (7) , and betaine by the colorimetric method of Focht and Schmidt (3).

Potassium, sodium and calcium were determined by flame photometry with a Model 9200 Beckman[3] flame photometer attachment with a Model 4020, medium bore hydrogen-oxygen atomizer-burner for the Beckman Model DU Spectrophotometer. An attempt to determine magnesium by the method was made, but the values were very low and, because of the interference caused by potassium, sodium and calcium, the results were not considered reliable.

[8] Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

A Beckman Model H-2 pH meter was used for the pH determinations. They were made at 25° C on undiluted molasses and on samples diluted to 10% solids.

To obtain a better idea of nitrogen distribution in molasses, an ion exchange separation was made of the classes of nitrogen compounds according to the diagram in Figure 1 and the fractions analyzed for various groups of compounds. Fifty grams of molasses (A) was diluted to 200 ml and passed through a column of 400 ml of Dowex 50X8, 20-50 mesh, H^+ form[4]. After loading, the column was washed with distilled water (a) until the effluent pH was 3.2. The load and effluents were combined and made to 1,500 ml (B). Aliquots of B were taken for determinations of milliequivalents of anions. The results were not corrected for large molecules that were adsorbed on therein but not eluted. One liter of B was passed through a column of 400 ml of Duolite A-4, 10-50 mesh, base form[5]. The load was followed by two liters of water wash (b) . The combined load and wash effluent was evaporated to 500 ml and aliquots analyzed for "neutral nitrogen". Neutral nitrogen is defined as the nitrogen not retained on Dowex 50 or Duolite A-4 under the conditions of the fractionation.

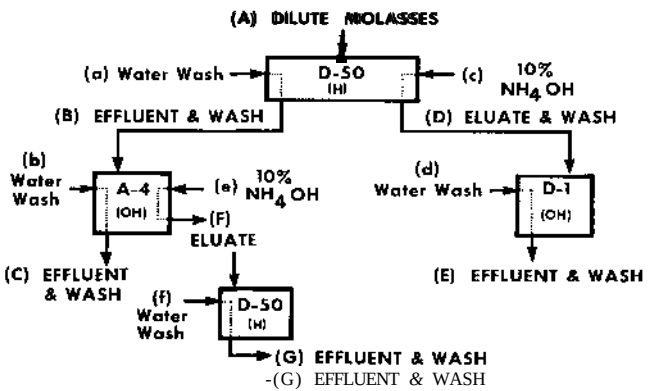

Figure 1.—Procedure for separating molasses nitrogen components.

After the water wash (b) the column of Duolite A-4 was eluted with 3 liters of 10% ammonium hydroxide (e). The ammonia was evaporated from the eluate (F) by boiling. After

* Dow Chemical Co,, Midland, Michigan.

[5] Diamond Alkali Co., Redwood City, California.

the ammonia was removed, the solution was treated with portions of dilute sodium hydroxide to displace ammonium ions. Boiling was continued until all ammonia was removed. This was shown by the absence of ammonia odor or drop in pH after further slight addition of sodium hydroxide and further boiling. The solution (F) was diluted to 200 ml and passed through a column containing 100 ml of Dowex 50, X-8, 50-100 mesh, H^+ form. The load was followed by a water wash (f) until the effluent volume (G) of load and wash was one liter. Aliquots of this acid solution were titrated in tared beakers with 0.1 N sodium hydroxide. The solution was evaporated and the beakers were weighed. The average equivalent weight of anions was calculated from the weight of sodium salt and equivalents of anions (8). Other aliquots of G were taken for the determination of acidic nitrogen.

The water wash (a) of the first Dowex 50 column was followed by 3 liters of 10% ammonium hydroxide (c). This procedure leaves nearly all metallic ions on the resin. Excess ammonia was evaporated from the eluate (D) and combined ammonia removed with sodium hydroxide as shown with F. The solution was made to two liters and aliquots analyzed for basic and amino nitrogen. A liter aliquot was concentrated to about 200 ml and passed through a column containing 100 ml of Dowex 1X8, 50-100 mesh, base form. The load was followed by a water wash (d) of 500 ml. The combined load and wash effluent (E) was concentrated to 250 ml and aliquots were taken for "betaine nitrogen" determinations. We assume that nearly all of this fraction is composed of betaine but slight amounts of other basic nitrogen compounds such as choline may be present.

Results and Discussion

Table 1 contains the results from straight house molasses samples. Table 2 shows the results from Steffen molasses samples and also for one sample obtained by the barium process and one by ion exchange.

Molasses purities varied from 59.01 to 67.45 for straight house molasses. The actual amount of sugar lost to molasses is greater for the higher purities than casual inspection would indicate. The ratio of sugar to non-sugar is 2.072 at 67.45 purity but only 1.440 at 59.01 purity. Thus the loss of sugar per unit of non-sugars is almost 44% greater in molasses of 67.45 purity than in molasses of 59.01 purity. The range of purity is less for Steffen molasses with a minimum of 61.23 and a maximum of 65.74. Average purity for both types of molasses is nearly the same.

Table 1.—Molasses composition (straight house)

	Belle Fourche	Betteravia spring	Betteravia fall	Brighton	Carlton	Carroll-ton	Clarks-burg	Crook-ston
Sucrose-g	61.57	60.97	6250	62.32	67.45	64.51	62.93	61.66
Raffinose-g	1.27	.49	.26	1.88	.23	.82	.66	1.85
Reducing sugars-g	.5	1.3	.8	.4	2.5	1.2	.5	.8
Potassium-g	7.31	4.85	4.19	6.18	3.62	5.47	4.80	4.90
Sodium-g	.89	1.14	1.86	1.77	1.47	.63	1.55	.95
Calcium-g	.11	.31	.21	.09	.11	.55	.36	.17
Ash-g	15.7	11.8	12.0	15.4	9.5	11.8	13.1	11.4
Ash alkalinity-meq	137.8	**111.3**	130.3	118.8	91.7	134.3	129.2	133.5
K+Na,meq	225.6	174.0	188.3	235.5	156.8	167.5	190.3	166.7
Ca-meq	5.5	15.4	10.5	4.5	5.5	27.4	18.2	8.6
Alkalinity-meq/g ash	8.78	9.43	10.86	7.71	9.65	11.38	9.86	11.71
Chloride-g	1.06	1.18	1.42	2.29	1.46	1.42	1.G0	.25
Nitrate-g	1.09	.44	.82	.98	.51	.58	.62	1.18
Anions-meq	192.0	175.0	177.8	203.8	146.7	172.5	185.8	157.2
Anions-eq wt	85.3	88.5	98.6	73.7	91.4	82.7	88.4	103.4
$Anion_s$-g	16.4	15.5	17.5	15.0	13.4	14.3	16.4	16.3
Total nitrogen-g	2.18	2.68	2.57	1.95	2.24	2.27	2.48	2.66
Amino nitrogen-g	.36	.59	.47	.37	.47	.37	.47	.54
Amide + ammonia-g	.05	.13	.12	.05	.09	.06	.10	.08
Basic nitrogen-g	1.32	**1.74**	1.53	1.32	1.55	1.51	1.56	1.61
Acidic nitrogen-g	.78	.83	.84	.61	.68	.67	.87	.94
Betaine fraction N-g	.75	.78	.79	.71	.71	.82	.80	.84
Neutral N-g	.02	.04	.04	.04	.09	.08	.05	.03
Betaine-g	6.10	.5.79	5.46	5.53	4.72	6.09	5.97	5.97
Basic solids-g	11.27	13.88	12.66	11.25	12.04	12.21	12.63	12.28
Petaine fraction-g	7.22	7.59	6.22	6.19	5.16	7.39	6.53	6.15
pH undiluted	8.90	6.98	6.88	9.21	6.81	6 75	7.38	~A\
pH diluted 1/10	8.72	6.55	6.68	9.24	6.70	6.49	7.10	*!.\2*

(g/IOO g solids unless shown)

Eaton	Fremont	Bay	Manteca	Moor-head	Rupert	Sidney	Swink	Toppen-ish	West Jordan	Avg
62.75	63.32	61.21	66.15	62.37	63.29	63.65	67.20	59.01	63.74	63.14
1.27	.47	1.20	1.00	.93	2.03	1.02	2.74	1.27	1.20	1.14
.4	.6	.5	.4	.8	.4	.4	.4	1.0	.4	.7
5.94	5.72	5.83	4.26	5.31	5.68	5.68	4.91	4.57	7.20	5.36
1.34	1.02	.52	2.24	.13	.61	1.44	1.63	1.18	1.06	1.22
.11	.23	.20	.13	.25	.14	.09	.14	.37	.13	.21
14.1	13.0	12.5	13.3	12.0	12.3	14.5	12.8	11.4	15.0	12.9
136.7	146.2	126.0	136.3	139.6	117.1	136.7	106.3	154.0	125.5	128.4
210.4	190.7	172.1	206.2	167.5	172.2	208.1	196.7	168.5	230.7	190.4
5.5	11.5	9.8	6.5	12.7	7.1	4.6	6.9	18.5	6.6	10.3
9.70	11.25	10.08	10.25	11.63	9.52	9.43	8.30	13.51	8.37	10.08
1.27	.91	.85	2.02	.40	.92	1.02	2.17	.37	1.96	1.25
1.29	.49	.70	1.17	1.02	.70	.91	1.06	1.02	1.33	.88
185.2	179.5	178.0	181.6	159.1	158.8	182.3	181.8	162.3	224.3	178.0
81.1	91.6	96.5	80.0	98.5	91.0	81.9	74.7	101.9	78.5	88.2
15.0	16.4	17.2	14.5	15.7	14.5	14.9	13.6	16.5	17.6	15.6
2.30	2.08	2.56	2.21	2.60	2.25	1.95	1.36	2.84	2.28	2.30
.43	.36	.43	.38	.42	.39	.36	.26	.46	.40	.42
.06	.07	.08	.07	.07	.07	.03	.09	.11	.05	.08
1.46	1.31	1.59	1.24	1.67	1.48	1.26	.93	1.63	1.50	1.46
.77	.68	.96	.78	.91	.66	.49	.37	1.10	.52	.75
.74	.74	.91	.62	.70	.69	.73	.45	.87	.79	.75
.02	.05	.03	.07	.12	.10	.11	.16	.17	.21	.08
5.87	5.43	6.73	4.74	7.12	6.22	5.75	3.35	5.88	5.65	5.69
12.85	10.49	13.13	10.45	14.41	12.95	10.52	7.64	13.61	12.00	12.02
6.11	5.88	7.06	5.09	5.73	5.53	5.91	3.59	6.72	6.13	6.12
9.06	8.09	6.79	9.11	7.48	8.15	9.13	8.43	8.12	8.93	
8.98	7.93	6.58	8.23	7.20	8.08	9.02	8.47	8.09	8.82	

Table 2.—Molasses composition

	Steffen house				
	Alvarado	Grand Island	Love-land	Mason City	Nyssa
Sucrose-g	01.55	63.28	62.10	63.66	65.10
Raffinose-g	2.02	1.79	2.46	1.80	2.44
Reducing sugars-g	.3	.5	.4	.5	.5
Potassium-g	3.78	5.71	5.21	4.23	4.51
Sodium-g	1.60	.53	1.33	.77	.91
Calcining	.41	.31	.22	.52	.15
Ash-g	11.2	11.5	13.1	10.5	10.5
Ash-alkalinity-nieq/100 g	138.1	128.9	140.7	125.1	113.4
K-hNa-meq/100 g	166.2	169.1	191.2	141.6	156.2
Ca-meq/100 g	20.6	15.2	11.0	26.1	7.4
Alkalinity-meq/g ash	12.33	11.21	10.74	11.91	10.80
Chloride-g	1.18	.76	.79	.46	.61
Nitrate-g	.55	1.09	1.09	1.00	.93
Anions-meq/100 g	167.6	158.6	180.0	156.9	139.0
Anions cq wt	94.8	99.6	94.0	104.9	100.4
Anions-g	15.9	15.8	16.9	16.5	14.0
Total nitrogen-g	1.91	2.15	1.89	2.35	2.20
Amino nitrogen-g	.39	.44	.36	.46	.44
Amide + ammonia N-g	.06	.07	.05	.11	.10
Basic nitrogen-g	1.28	1.17	1.22	1.36	1.28
Acidic nitrogen-g	.05	.68	.62	.79	.71
Betaine fraction N-g	.00	.68	.66	.63	.75
Neutral N-g	.06	.05	.04	.09	.12
Betaine-g	4.38	4.98	4.90	4.97	4.99
Basic solids-g	12.57	8.96	9.72	11.59	11.29
Betaine fraction-g	5.51	5.03	5.32	5.11	5.86
pH undiluted	8.25	8.21	8.35	7.11	6.91
pH diluted 1/10	8.12	8.12	8.39	7.27	6.73

(g/100 g solids unless shown)

Stcffen house									Barium process	Ion exchange
Oxnard spring	Oxnard fall	Scotts-bluK	Spreckels	Torring-ton	Tracy	Twin Falls	Wood-land	Average	Johns-town	Layton
65.29	63.23	61.23	63.02	61.75	63.83	64.12	65.74	63.38	71.96	60.10
1.05	1.11	2.76	2.27	4.15	2.26	3.16	1.62	2.22	16.00	2.62
1.3	.6	.4	2.4	*A*	.4	.7	1.0	.7	.4	6.2
2.92	3.22	6.32	3.29	5.41	3.80	4.65	4.18	4.40	.75	1.21
2.05	2.27	.67	1.74	1.13	2.02	.86	1.29	1.32	1.04	2.64
.23	.22	.18	.48	.11	.19	.18	.32	.27	.05	.30
10.6	11.7	13.7	11.2	12.8	11.8	10.7	10.2	11.5	3.8	8.5
110.9	122.3	130.2	118.2	117.1	115.8	100.9	121.1	121.7	56.4	127.0
164.0	181.4	191.3	160.0	187.7	185.3	156.3	163.2	170.3	64.6	145.8
11.7	11.0	9.2	24.0	5.3	9.3	9.1	16.0	13.5	2-7	15.0
10.46	10.45	9.50	10.55	9.14	9.81	9.43	11.87	10.63	14.84	14.94
1.08	1.09	1.18	1.66	1.26	2.08	.85	1.33	1.10	.19	.61
.43	.83	.73	1.14	.79	1.02	.65	.87	.86	.07	.20
155.2	179.4	179.1	166.9	172.0	172.7	155.7	192.0	167.3	58.8	161.7
96.1	99.4	87.5	85.3	82.2	82.4	93.4	87.0	92.8	101.1	102.0
14.9	14.8	15.7	14.2	14.1	14.2	14.5	16.7	15.2	5.9	16.5
1.65	1.72	1.78	1.86	1.58	1.98	1.97	1.98	1.93	.1	1.74
.28	.34	.36	.32	.30	.33	.39	.37	.37	.01	.15
.07	.07	.05	.12	.02	.04	.06	.07	.07	.01	.05
1.01	1.06	1.23	.91	.97	1.24	1.36	1.44	1.19	.15	1.16
.51	.59	.48	.58	.33	.61	.62	.67	.60	.04	.65
.46	.52	.70	.44	.65	.65	.71	.73	.63	.08	.78
.10	.12	.08	.12	.15	.20	.09	.20	.11	.03	.07
3.68	3.56	5.40	3.75	4.96	4.79	5.12	4.29	4.60	.96	5.57
8.96	9.26	10.99	7.52	9.82	10.08	11.50	11.96	10.32	1.62	9.68
4.13	3.85	5.73	3.60	5.47	4.76	5.49	5.56	5.03	.51	6.54
7.02	7.09	8.48	6.38	8.78	7.72	6.92	7.26		9.67	6.10
6.81	7.07	8.48	6.15	8.70	7.72	6.72	7.19		10.37	6.03

The raffinose content of straight house molasses averages about half that of Steffen molasses produced in the same area. There is a great overlap in the ranges, however, and eleven of the Steffen molasses had less raffinose than the maximum found in straight house samples. Many other constituents have a slightly higher average value in straight house molasses but no constituent measured in these experiments would enable one to distinguish between the two molasses types. A comparison of the analytical data for Manteca and Tracy molasses shows the close similarity of the two molasses types produced largely from comparable beets. The main difference is in the raffinose content which is concentrated by the Steffen process. Johnstown molasses (barium process) has a very high purity and raffinose content compared to other molasses. Consequently it has low values for other constituents calculated on the dry basis. Raffinose is accumulated in ion exchange molasses to about the same extent as in Steffen molasses. Purity of ion exchange molasses would appear to be lower than for most straight house or Steffen samples and the quantity per ton of beets is much less.

Reducing sugars average the same for both types of molasses but above pH 8 the amount of reducing sugars seems to be closely related to the pH of the molasses. Higher pH molasses have very little reducing sugar. This is probably due to the slow rate of formation of reducing sugars from sucrose at the higher pH values and to the more rapid destruction of glucose and fructose at these values. While sucrose is most stable near pH 9, glucose and fructose are most stable at pH 3-5. In the samples studied the pH varied from slightly acidic (6.38) to moderately basic (9.21). Generally the pH of a diluted sample is lower than that of the original but in some cases a higher value is obtained on dilution.

Potassium ions occur to the largest extent of any metallic ion present in molasses. In some cases part of the sodium present is from sodium carbonate added to maintain higher alkalinity. The calcium content is closely related to pH. Molasses with lower pH values contain more calcium. This would be expected since most calcium salts become more soluble as the pH decreases. Steffen molasses calcium content is about 50% higher than for straight house molasses. Part of this increase is due to recycling certain anions as calcium salts insoluble at the pH of the Steffen treatment but more soluble at the pH of first carbonation. Hence, these calcium salts will increase and the calcium will not be precipitated except by the addition of sodium hydroxide or carbonate.

Ash averages slightly higher for straight house molasses. Ash

alkalinity is a measure of organic acids that are converted to carbonates during ashing. Little difference is shown between the two molasses although there appears to be some preferential accumulation of organic anions over inorganic anions in Steffen molasses. This is demonstrated by the slightly higher ratio of milliequivalents of alkalinity to grams of ash.

Chlorides show the greatest quantitative variation for a major molasses constituent: eight-fold for straight house (0.25%-2.20%) and four-fold for Steffen molasses (0.4%-2.08%). Nitrate content varies about two-fold with little apparent difference between the two molasses types.

Milliequivalents of anions, equivalent weight of anions or weight of anions present show little variation between the two molasses types. Anions as a group average over 40% of the non-sugars.

Basic nitrogen compounds form the major portion of the total nitrogen. Betaine is usually half of this fraction with amino acids being most of the remainder. Pyrrolidone carboxylic acid is the principal acidic nitrogen constituent in most molasses with nitrate second. There is little apparent difference between the two types of molasses.

Johnstown molasses (barium process) with a very high purity has an extremely high raffinose content compared to other molasses and consequently shows low values for other constituents on the dry basis.

Ion exchange molasses has a higher sodium than potassium content. This is probably due to preferential adsorption of potassium on the ion exchanger. This difference appears to be a distinguishing characteristic from other molasses while nitrogen and anion composition are very similar.

Summary

Straight house and Steffen molasses cannot readily be distinguished on the basis of chemical analyses except that the average raffinose content of Steffen molasses is usually twice that for straight house molasses produced in the same area. However, unless this value is below 1% for straight or above 2.5% for Steffen it is difficult to distinguish between molasses samples.

Chloride shows an eight-fold variation, the greatest sample-to-sample variation of any major constitutent analyzed. Most constituents have a variation of less than two-fold with betaine showing the least variation for a major non-sugar.

The statistical relationship of sucrose and non-sugar is presented in Part II.

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The Relation of Beet Molasses Composition to True Purity
Part II. Statistical Evaluation

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Many studies have been made relating nonsugar composition to beet molasses purity. Most attention has probably been given to the proposal made by Dedek (2)[2] relating the equivalents of sucrose in beet molasses to the equivalents of sodium plus potassium. This ratio appears to be close to one for most beet molasses. Approximately the same ratio was confirmed by Wiklund (12) and extended by Carolan (1) to include moles of calcium. Wiklund also found that the ratio of moles of sucrose to moles of nitrogen was approximately one.

All these means of predicting the purity of molasses leave much to be desired because they imply that no difference exists in the melassigenic effect of various components. Pieck and Rens (6) attempt to overcome this difficulty by proposing that cations equivalent to certain antimelassigenic anions be subtracted from the sum of potassium and sodium. Their selection of sodium chloride as an example was particularly unfortunate since potassium and sodium chlorides seem to be among the most melassigenic compounds.

While the terms melassigenic and antimelassigenic are widely used, it must be understood that in reality all soluble nonsugars are melassigenic in the sense that they prevent complete recovery of sugar. For a given amount of sugar and water the addition of a particular nonsugar may increase the sugar recovery by decreasing sugar solubility, but in the absence of the impurity, further water may be evaporated and essentially all the sugar recovered. In this discussion the term melassigenic will be used for those substances which increase molasses purity with increasing concentration, and antimelassigenic will designate those substances which decrease molasses purity with increasing concentration. Some nonsugars increase the solubility of sucrose, others decrease it, while some may not affect it. While some substances may decrease sugar solubility, they may be deleterious

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[2] Numbrs in parentheses refer to literature cited.

because of difficulty in separating crystalline sugar from the nonsugar. Even though some nonsugars decrease the solubility of sugar, molasses would still be formed. It will be shown later in this paper that removal of some antimelassigenic compounds without removal of melassigenic ones could result in molasses of sufficiently higher purity to give a greater loss of sugar. A further difficulty in referring to specific compounds as melassigenic or antimelassigenic is that their effect on sugar solubility may vary depending on their concentration in the solution. At some concentrations, generally low, they may decrease sugar solubility while at other concentrations sugar solubility increases (5).

The analytical data presented in Part I of this study (10) were subjected to correlation analysis to determine their relationship to each other and to molasses purity. Data from the Carlton, California, sample shown in Part I (10) were excluded from this statistical study since they appeared atypical in some respects; the high reducing sugars, for example, would exert an extreme effect on some purity-composition relationships.

After data collection the important question must be answered as to the best method of determining the effect of various nonsugars on purity. Nonsugar concentrations may be expressed on the wet basis (g/100 g molasses as received), dry basis (g/100 g molasses solids), or on the impurities basis (g impurity/100 g total impurities). Table 1 shows the correlations of each variable studied against purity when the variables are expressed on each of the three bases. It is readily apparent that each method yields different results. Some variables, such as milliequivalents of anions, showing no significant correlation on the wet or dry basis have a significant correlation on the impurities basis.

The actual melassigenic properties of an impurity do not depend on the method of calculation so that the problem arises of choosing the correct procedure for evaluating the data. Useful relationships are calculated on the impurities basis, the quantity of the variable per unit of nonsugars against purity. Correlations of purity against specific impurities are invalid if calculated on the wet or dry basis because they contain a built-in spurious negative correlation. The following example will demonstrate this conclusion.

Using variable 7, milliequivalents of anions on the solids basis with average purity of 62.9059, impurities of 37.0941 and meq anions of 181.941, we can calculate the values of meq anions, (postulating no change in the ratio of meq anions to other impurities) as sugar is added or subtracted (increasing or decreas-

ing the purity). When the impurity basis is used for calculation there is no change in the value of the specific impurity as sugar is added or subtracted from a sample. However, if we add sugar to a molasses of average composition and purity to give 64 purity the meq of anions on the solids basis is 176.575 or remove sugar to give 61 purity the value for meq of anions is 191.289. The slope for this regression line is:

$$m = \frac{64 - 61}{176.575 - 191.289} = -0.20389$$

The constant for the line is calculated as follows:

$$62.9059 = (-0.20389)(181.941) + C$$

$C = 100.002$ and the regression equation becomes

$$\text{Purity} = (-0.20389)(\text{meq anions}) + 100.002$$

It is worthwhile to calculate the change due to the spurious relationship and that due to melassigenic activity. The spurious change is calculated as the difference between meq of anions at purities 64 and 61. This is

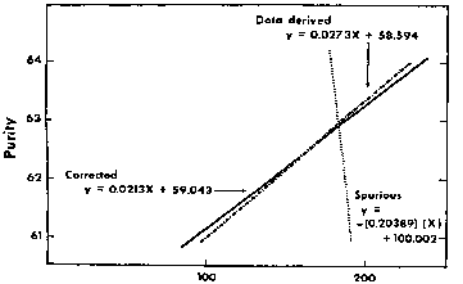
176.575 — 191.289 or —14.714

The actual change in meq anions determined from the calculated regression line on the solids basis: Purity = (0.02370) (meq anions) + 58.5939 or 228.105 at 64 purity and 101.523 at 61 purity. The difference is 126.582. This difference less the spurious change 126.582 — (—14.714) or 141.296 is the change in meq anions that would be expected if there were no spurious relationship. The spurious relationship for this variable is 10.4% of the total expected change. Note that the change in meq anion values or other variables are altered more at the extreme purity values than near the average purity.

A correlation of 0.64 is obtained by correcting meq anions on the solids basis for each factory sample for variation from the average value caused by the spurious relationship and determining the correlation between purity and the corrected value. This agrees very closely with the value of 0.65 obtained when the correlation is calculated on the impurities basis. Figure 1 shows the regression lines and equations for the experimental data, built in, and corrected regression lines for meq anions against purity. In a similar manner the built-in regression can be shown for data calculated on the wet basis.

Straight House Molasses

The following variables listed in Table I (impurities basis) show positive correlations with purity at the 5% or lower significance level: percent ash, meq sodium plus potassium, meq anions, meq potassium plus sodium less calcium and percent



X_7 meq. anions (solids basis)

Figure 1.—Correlation of molasses purity and meq anions.

chloride. A further related variable considered here is the average equivalent weight of anions (11) which has a significant negative correlation with purity. These variables are also all highly cor-

Table 1.—Correlations of beet molasses purity and composition variable (wet, dry and impurities bases).

Variable no.	Variable identity and units	Straight house molasses[1]			Stel"fen molasses-		
		Wet	Dry	Impurities	"Wet	Dry	Impurities
1	Reducing sugars, %	—.46	—.42	—.33	.75	.74	.76
2	Raffinose, %	.23	.28	.39	—.47	—.16	—.37
3	Ash, %	.08	.18	.57	—.83	—.81	•—.57
4	Alkalinity of ash meq[3]	—.35	—.36	.21	—.61	—.61	—.20
5	Na + K, meq	.16	.27	.59	—.68	—.57	—.17
6	Na + K less Ca, meq	.18	.27	.54	—.53	—.45	—.15
7	Anions, meq	.05	.18	.65	—.79	—.69	—.14
8	Chloride, %	.62	.65	.71	— .13	—.09	—.01
9	Nitrate, %	.08	.13	.31	—.05	—.03	.10
10	Anions less (CI 4- NOs), meq	—.65	—.71	—.30	—.60	—.61	—.19
11	Avg equivalent wt of anions	—.64	—.64	—.64	.26	.26	.26
12	Total wt of acids, %	—.68	—.71	—.03	—.43	—.41	.19
13	Amino N, %	—.69	—.67	—.48	.10	.11	.37
14	Total N, %	—.78	—.77	•—.57	.25	.32	.57
15	Basic N, %	—.77	—.74	—.50	.09	.15	.44
16	Basic N less amino N, %	—.73	—.70	—.42	.06	.10	.36
17	Acidic N, %	—.68	—.67	—.50	.51	.56	.68
18	Acidic N less nitrate N, %	—.69	—.68	—.59	.58	.63	.72
19	Betaine, %	—.65	—.66	—.41	—.32	—.29	.01
20	Basic N less (betaine N + amino N), %	—.50	—.45	—.21	.30	.34	.43
21	Basic fraction solids, %	—.76	—.75	—.51	—.01	.07	.31
22	PH	.30	.30	.30	—.83	—.83	—.83

[1] 17 factories

[2] 13 factories

[8] milliequivalents

Table 2.—Correlation of straight house molasses variables - impurities basis[1]

Variable no.	Variable identity	Variable number																		
		3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Purity
1	Reducing sugars	—.75	—.70	—.76	—.51		—.50		.48		.51	.52	.58	.52				.65		—.73	
2	Raffinose						.48	—.54		—.59	—.51	—.64	—.57	—.51		—.58			—.52		
3	Ash		.97	.95	.91	.70	.50		—.87		—.61	—.70	—.64	—.55	—.55	—.73		—.60	—.60	.79	—.57
4	Alkalinity of ash																				
5	Na + K			.99	.94	.80	.57		—.87		—.54	—.66	—.65	—.60		—.68		—.49	—.64	.79	.59
6	Na + K less Ca				.87	.73	.61		—.81		—.52	—.66	—.67	—.64		—.69		—.56	—.64	.83	.54
7	Anions					.88			—.90		—.49	—.60	—.52			—.60			—.56	.57	.65
8	Chloride								—.85			—.55	—.48			—.48	—.57		—.51		.71
9	Nitrate																			.66	
10	Anions less (Cl + NO_3)																.52				
11	Avg equivalent wt of anions										.52	.71	.56	.48	.63	.72			.54	.64	—.64
12	Total wt of acids																				
13	Amino N											.82	.79	.52	.55	.62		.55	.65	—.53	—.48
14	Total N												.90	.78	.77	.80	.56	.64	.83	—.56	—.57
15	Basic N													.94	.52	.63	.69	.74	.91	—.64	—.50
16	Basic N less amino N															.51	.80	.71	.89	—.59	
17	Acidic N															.89		.49			—.50
18	Acidic N less nitrate N																	.56	.51	—.63	—.59
19	Betaine																		.77		
20	Basic N less (betaine N + amino N)																		.57	—.60	
21	Basic fraction solids																			.53	—.51
22	pH																				

Correlation significance
1% 0.605 2% 0.557 5% 0.482

[1] All correlation coefficients were calculated but only the statistically significant ones are shown.

related with chloride (Table 2). Is the correlation of these variables with purity significant if the extent of their dependence on chloride were removed? Meq of anions less meq of chloride have a nonsignificant correlation [—0.16] with purity. The significant correlation for meq of anions (variable 7) thus appears to be due to its chloride component. The correlation of meq of sodium plus potassium with purity is 0.59. Assume chloride to be present in molasses as sodium and potassium chlorides and subtract the meq of chloride in each sample from the total meq of sodium plus potassium. The correlation of the remaining sodium plus potassium with purity is then a non-significant 0.17. This demonstrates that the correlation of meq of sodium plus potassium with purity is mainly dependent on changes in these cations to compensate for variability in chloride. The same observation is true of percent ash since the correlation between ash (variable 3) and meq sodium plus potassium (Table 2) is 0.97. The average equivalent weight of anions has a negative correlation with purity of —0.64. This variable also has a high negative correlation of —0.85 with chloride showing that over 70% of the variability in average equivalent weight of anions is due to variability in chloride. Without the chloride component average equivalent weight of anions does not show a significant correlation with purity (r = —.16). The factors, meq anions, percent ash, sodium plus potassium, meq sodium plus potassium less calcium and average equivalent weight of anions are only correlated with purity because of their chloride component or high correlation with the strongly melassigenic component chloride.

The magnitude of the chloride effect is shown by the following calculation. Using the regression equation for purity (Y) and chloride (X) on the impurities basis: $Y = 0.75 (X) + 60.344$ we can calculate the effect of one pound of chloride on carrying sugar into molasses. Taking the average molasses of 62..906 purity, 37.094 impurity and chloride oi 3.415% we can see from the regression equation that removal of all chloride (chloride of 0.00%) would yield a molasses of 60.344 purity and impurity of 39.656. One hundred pounds of average impurities at 62.906 purity would carry along 169.585 pounds of sugar or about 1.7 pounds of sugar per pound of impurities. Removal of the 3.415 pounds of chloride would leave 96.585 pounds of impurities with molasses at 60.344 purity. This amount of impurities at 60.344 purity would carry 146.972 pounds of sugar into molasses. The removal of the chloride shows 169.585 — 146.972 or 22.613 pounds less sugar carried into molasses or 6.62 pounds of sugar per pound of chloride. This value is slightlv higher than that reported by other investigators who obtained

their values by adding the specific impurity and sugar to a factory molasses. P. M. Silin (9) reporting the results of Z. A. Silina shows a value of 2.48 pounds of sugar removed for one pound of KC1. This is equivalent to 5.5 pounds of sugar for one pound of chloride. Rorabaugh and Norman (7) find 5 pounds of sugar lost per pound of chloride as KC1.

Factories using water or processing beets with a high chloride content that yields molasses containing six pounds of chloride per hundred pounds of impurities appear to be losing nearly 40 pounds of sugar more than if chloride were absent. Removal of this chloride should produce a gain of 40 pounds of sugar.

Chloride determinations can be used to predict molasses purity and amount of molasses produced. It will be shown in another paper that molasses purities may be predicted from the chloride content of thin or thick juices. Another way of showing the importance of this effect of chloride on molasses purity is to examine the amount of sugar carried into molasses per pound of impurity at 60 and 65 purity. Nearly 24% more sugar is carried into molasses at 65 purity than at 60 purity. The data from the present study show that 50% of the variability in molasses purity over the purity range studied is due to variability in chloride content.

The following variables have a significant (5% level or lower) negative correlation with purity: average equivalent weight of anions, amino nitrogen, total nitrogen, basic nitrogen, acidic nitrogen, acidic nitrogen less nitrate, nitrogen and basic solids. As stated previously the negative correlation of average equivalent weight of anions with purity is due to changes in chloride content. The remainder of the variables showing negative correlations are also complex mixtures but have one type of component in common: nitrogen containing compounds. Such compounds generally decrease the solubility of sugar and yield a lower purity molasses (7 ,8).

Since total nitrogen is negatively correlated with purity it might be thought that a multiple regression equation involving total nitrogen and chloride would give a much better estimate of purity than chloride alone. Unfortunately this is not the case. The multiple regression equation for these two variables is:

$$\text{Purity} = 0.60669\ (\%C1) - 0.61093\ (\%N) + 64.6206$$

The correlation coefficient is 0.743 and the standard error of estimate is 0.716. This standard error of estimate is little better than the 0.723 obtained using only percent chloride for purity estimates.

The negative correlation of nitrogen content and purity is shown not only by this work but also by some Belgian results.

Several factory campaign averages in Belgium for the years 1957-1961 are plotted in Figure 2, (3,4). The regression equation and slopes for the Belgian results are compared with the data presented here. Figure 2 shows that the slopes of the two equations are very similar, and that nitrogen content is related to purity in both cases.

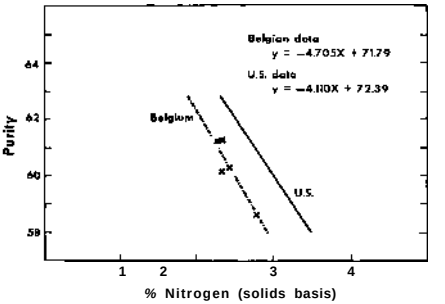


Figure 2.—Molasses purity and total nitrogen—comparison of Belgian and U. S. Data.

It is more difficult to determine the antimelassigenic effect of the nitrogen containing fractions since all are comprised of more than one compound. The nearest we can come to estimating this effect is to use variable 18 (acidic nitrogen less nitrate nitrogen). After removal of nitrate nitrogen most of the remaining acidic nitrogen is pyrrolidone carboxylic acid (PCA). Assuming that it is all PCA, we can develop the following relationship using the regression equation for variable 18: Y = —2.8954 (X) + 66.694. Converting from acidic nitrogen to PCA the equation becomes: Y = —0.314 (X) + 66.694. The values for the average molasses are Y = 62.906, X = 12.06 with impurities of 37.094. The value for X is estimated on the basis that all the acidic nitrogen less nitrate nitrogen is present as PCA. Actually this is a high value since approximately only 85% of this nitrogen is present as PCA. If we remove one pound of PCA from 100 pounds of molasses impurities, we have remaining 99 pounds of impurities with a PCA content of 11.17%. The purity of this molasses would be: Y = [—0.314] [11.171 + 66.694 or Y = 63.187. The 99 pounds of impurities at 63.187 purity would carry 169.927 pounds of sugar into molasses. The original 100 pounds of impurities carried 169.585 pounds of sugar into mo-

lasses. The removal of one pound of PCA shows 0.34 pound more sugar going to molasses even though there is one pound less of impurities. Unfortunately, no reliable data are available on the solubility of sucrose in the presence of PCA salts to confirm or deny the antimelassigenic character of PCA shown in this study. A paper by Rorabaugh and Norman (7) does show that salts of PCA appear less melassigenic than amino acids and that the latter decreased the solubility of sucrose.

Most basic nitrogen fractions have a negative correlation with purity. However, the correlation of betaine with purity is not significant. While betaine should appear antimelassigenic since it decreases the solubility of sucrose (10) it is likely that the variability in betaine content for the molasses in this study is so low that other factors obscure the real effect. For example, half of the betaine values are within 5% of the median value while over 70% are within 10% of the median. The three molasses having betaine values higher than 10% of the median have purities ranging from 61.21 to 59.01 while the two samples with the lowest betaine values have purities of 66.15 and 67.20.

Correlations of the variables with each other are shown in Table 2. It is extremely difficult to state in many cases if these relationships are valid or spurious. As we have shown earlier, the correlation of a number of variables with purity are dependent on their chloride content (meq of anions) or high correlation with chloride (meq of sodium plus potassium) and hence should be considered as spurious correlations. Of equal interest is the lack of correlation of certain variables with others: alkalinity of ash, meq of anions less chloride and nitrate and total weight of acids.

Dedek's (2) value of one for the ratio of sucrose over sodium plus potassium applies to the molasses samples discussed in this paper. The average value is 0.966 with a standard deviation of 0.106. A new ratio is presented here for consideration. The ratio of equivalents of sucrose to equivalents of anions is also close to one. Table 3 compares this ratio with Dedek's. Sucrose to anions has an average value of 1.029 with a standard deviation of 0.0876. The ratios found for Steffen molasses will be discussed later.

Steffen Molasses

Steffen molasses is defined as the molasses discard obtained from factory using the Steffen process. Usually foreign molasses from one or more straight houses is also processed with the factory molasses. A small portion of molasses may be discarded continuously or batches of molasses may be discarded when the

Table 3.—Comparison of the ratios of sucrose with anions and sodium plus potassiun

Straight house molasses			Steffen molasses		
Factory no.	meq sucrose/ meq anions	meq sucrose/ meq Na + K	Factory no.	meq sucrose/ meq anions	meq sucrose/ meq Na + K
1	1.017	1.023	21	1.074	1.083
2	1.029	.972	22	1.166	1.094
3	.938	.798	23	1.011	.952
4	.895	.775	24	1.185	1.514
6	1.078	1.110	25	1.367	1.216
7	.980	.967	26	1.231	1.165
8	1.145	1.080	27	1.031	1.020
9	.994	.875	28	.999	.986
10	1.031	.970	29	1.102	1.130
11	1.005	1.040	30	1.201	1.196
12	1.068	.936	31	1.052	.964
13	1.144	1.087	32	1.083	1.109
14	1.165	1.074	33	.995	1.170
15	1.020	.894	avg	1.1152	1.0976
16	1.078	.996	std. dev.	0.1097	0.1164
17	1.066	1.027			
18	.829	.806			
avg	1.0286	.9664			
std. dev.	0.0876	0.106			

purity of the returned calcium precipitate decreases to a particular value.

When we examine the correlations of the variables with Steffen molasses purity, shown in Table 1, we find a very unexpected situation. The correlations are very different from those found with straight house molasses. Variables that had a negative correlation with purity of straight house molasses may show a significant positive correlation with Steffen molasses purity. Variables showing a positive correlation with straight house molasses purity usually have a negative correlation with Steffen molasses purity. A substance that is melassigenic for one type of molasses should also be melassigenic for another type. The differences found cannot be due to a change in melassigenic properties but must be due to some other cause. The most probable cause is some change in the manufacturing process. Except for minor variations such as impurities in processing water, the impurities in straight house molasses come directly from the beets. This is not the case with Steffen molasses. Steffen molasses impurities arise from two major sources: (a) impurities from the new beets being added to the process and (b) impurities carried back by the calcium precipitate formed when dilute molasses is treated with calcium oxide. The discarded filtrate from the calcium precipitate will contain most of the soluble salts so the ratio of impurities to each other will be much different in this case than with fresh beets.

The impurities in the beets and calcium precipitate are

essentially the same in kind but are different in relative amounts. Hence, in the manufacture of sugar by the Steffen process we are mixing two different raw materials in varying amounts.

Because of these changes in operating procedures we do not obtain valid correlations relating the melassigenic properties of the variables to purity.

Since there are two sources of impurities, far fewer of the variables investigated show a significant correlation with each other than in straight house molasses. The correlations are generally of lesser significance.

The ratio of sucrose to meq of anions or sodium plus potassium is about 10% higher than for straight house molasses. During the calcium oxide precipitation of sucrose, reducing sugars and some sucrose are decomposed to acids. These are returned to the process as calcium salts. Generally sodium hydroxide or sodium carbonate is added early in processing to preserve alkalinity and remove excess calcium. Part I of this study shows that the sodium to potassium ratio is generally greater for Steffen molasses.

The best correlation is shown by purity and pH. The high pH samples have a significantly lower purity. Further study of this variable is desirable since no direct cause and effect relationship seems apparent to the authors. Reducing sugars are positively correlated with purity as would be expected because of their high correlation with pH.

Summary

Correlations of the non-sugars with purity have been calculated using percent of specific impurity in the impurities against molasses true purity. This method of calculation avoids the built-in spurious negative correlation obtained when calculations are made on the wet or dry basis.

The most important finding is the positive relationship between chloride content and purity. The results indicate that one pound of chloride carries between six and seven pounds of sugar into molasses.

Variables such as ash, meq of potassium plus sodium and meq of anions show a positive correlation with purity only because of their chloride content or high correlation with chloride. Total meq of sodium plus potassium less the meq of these ions present as chloride do not show a significant correlation with purity.

Many of the fractions containing nitrogen compounds show a negative correlation with purity which is probably due to a decrease in sucrose solubility in the presence of these compounds. These results are confirmed by comparisons using Belgium data.

Dedek's observation of a ratio near one for meq of sucrose to those of sodium plus potassium has been confirmed. A new ratio of meq of sucrose to anions is shown to be as reliable.

Melassigenic impurities in Steffen molasses do not show correlations with purity since the impurities are derived from two sources: processed beets and the calcium precipitate formed from straight house discard molasses.

Acknowledgments

We wish to thank Miss Marian Sandomire for advice on statistics and for determining the correlations of molasses constituents with purity.

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Minutes of the Fifteenth General Meeting of the American Society of Sugar Beet Technologists

The Fifteenth General Meeting business session of the American Society of Sugar Beet Technologists was held on Wednesday morning, February 21, 1968 in the Thunderbird Room of the Hotel Westward Ho, Phoenix, Arizona. Mr. Lloyd T. Jensen, President of the Society, called the meeting to order at 11:00 a.m.

Mr. Jensen served as Chairman of the meeting and announced that the Fifteenth biennial business meeting of the Society was called for the purpose of hearing a report of the Secretary-Treasurer, a report on the plans for the Third Joint ASSBT-IIRB Meeting, other committee reports and to transact such other business appropriate to the Fifteenth General Meeting.

The Chairman called for the reading of the Minutes of the Fourteenth General session held in Minneapolis, Minnesota on February 23, 1966. Upon motion made, seconded and unanimously carried, reading of the Minutes was dispensed with and approved as recorded in Volume 14, Number 1, April 1966 Journal commencing on page 79.

The Chairman than asked that the report of the Secretary-Treasurer be presented. The Secretary-Treasurer briefly reported on the activities of the Society during the biennial period ending December 31, 1967. Following his report and upon motion made, seconded and unanimously carried, the report of the Secretary-Treasurer was adopted, ordered placed on file and made a part of these minutes. He was instructed to publish the above report in the first issue of Volume 15, Journal of the American Society of Sugar Beet Technologists. The report of the Secretary and report of the Treasurer are recorded herewith as a part of these minutes.

Report of **the** Secretary
December 31, 1967

The Society membership at the close of the 1966-67 biennium was 734 individual and company members. These members reside in 34 states, the District of Columbia, five provinces of Canada and 17 countries outside continental North America. The total number of members has increased from the previous biennium by 16. A list of the membership by states and countries follows and becomes a part of this report.

Membership by States and Countries—1966-67

Washington, D. C.	4
Arizona	10
California	147
Colorado	111
Connecticut	5
Delaware	4
Georgia	1
Hawaii	1
Idaho	17
Illinois	26
Indiana	2
Iowa	6
Kansas	3
Kentucky	1
Louisiana	2
Maine	1
Maryland	6
Massachusetts	4
Michigan	84
Minnesota	17
Missouri	3
Montana	9
Nebraska	15
New Jersey	1
New Mexico	2
New York	40
North Dakota	7
Ohio	19
Oregon	8
Pennsylvania	10
Texas	11
Utah	56
Washington	21
Wisconsin	3
Wyoming	13
FOREIGN COUNTRIES	
Belgium	3
Canada	40
Chile	2
Denmark	6
Egypt	1
England	10
France	7
Germany	6
Iran	2
Iraq	1
Ireland	1
Italy	3
Japan	1
Netherlands	5
Spain	1
Sweden	4
Uruguay	2
West Pakistan	2
Total	717
U. S. Company Members	10
Foreign Company Members	7
TOTAL	734

Slightly in excess of 1100 copies of the Journal of the American Society of Sugar Beet Technologists are mailed to the membership and to subscribers. The Journals purchased by subscription are mailed to 33 states and 41 foreign countries. It has been a continuing policy of the Office of the Secretary to print approximately 1400 copies of the Journal so that a reserve, sufficiently large to supply future needs, is available in the Office of the Secretary-Treasurer.

Arrangements have been made with University Microfilms, a Xerox Company at Ann Arbor, Michigan, to provide Xerox copies of Proceedings, now out of print, that were published prior to 1948. The price of these varies with the particular Proceedings.

The Society wishes to express its thanks to the Beet Sugar Development Foundation for donating space and the time of its personnel in conducting the day to day business affairs of the Society. The Society similarly acknowledges the annual grant of $1,000 from the Foundation earmarked to assist in the cost of publishing the Journal.

Respectfully submitted,

JAMES H. FISCHER
Secretary-Treasurer

Report of the Treasurer

Balance Sheet, December 31, 1967

Cash Balance, January 1, 1966		$ 3,687.64
Savings Account Balance, January 1, 1966		1,346.17
1966 Interest Earned on Savings Account		131.96
1966 Cash Receipts	$16,379.82	
Less change for meeting	200.00	16,179.82
1967 Interest Earned on Savings Account		235.10
1967 Cash Receipts	5,658.98	
Less transfer from Savings Account	1,500.00	4,158.98
		$25,739.67
1966 Cash Disbursements		
Office Expense	1,883.66	
Publication Expense	7,003.34	
Reprints	467.08	
Refunds	121.30	
Meeting Expense	4,069.86	
Miscellaneous	$4,221.00	
Less transfer to Savings Account	4,000.00	
Less change for Meeting	200.00	21.00
Postage used by BSDF	140.16	
ASSBT-IIRB Tour Expense	47.10	
		$13,753.50
1967 Cash Disbursements		
Office Expense	1,638.57	
Publication Expense	4,203.10	
Reprints	956.87	
Refunds	14.65	
Meeting Expense	4.90	
Miscellaneous		
Postage used by BSDF	225.83	
		7,043.92
Savings Account Balance, December 31, 1967		4,213.23
Cash Balance, December 31, 1967		729.02
		$25,739.67
January 1, 1966 Cash and Savings Balance		$ 5,033.81
January 1, 1968 Cash and Savings Balance		$ 4,942.25
Net gain (loss)		($91.56)

The Chairman then called for a report from the Chairman of the Steering Committee for the Third joint Meeting of the ASSBT-IIRB, Mr. P. B. Smith. Mr. Smith reported that at the time of this meeting approximately 50 people had declared their intent to participate in the Third Joint ASSBT-IIRB Meeting and that copies of the proposed itinerary were available to those wishing to sign up for the tour, scheduled for June 3-23, 1969. The tour will take the participants to Germany, Denmark, Sweden, France, Belgium, Holland and England. The tour will be separated into three segments, one for those interested in agricultural matters, another for those interested in processing-techniques and the third group for the ladies.

Following Mr. Smith's report, the Chairman introduced Mr. O. J. Kint, Secretary-Generale, Institut International de la Recherches Betteravieres, from Tirlemont, Belgium, who brought greetings from the IIRB and then repeated the kind invitation of the IIRB and the host countries to the Third Joint Meeting. He briefly described the tentative tour plans.

The Chairman then reported that the Executive Committee and Board of Directors at a meeting held February 18 in Phoenix, Arizona, had selected the site for the Sixteenth General Meeting of the Society. He stated that the group had elected Denver, Colorado as the site and that the newly elected President, his General Arrangements Chairman and the Secretary were to choose a meeting hotel that could accommodate the Society on a date near the end of February 1970.

The Chairman reported that the Tally Committee was in the process of counting ballots cast for nominees for each of the Society offices. The results of the tally were not made known until the night of the banquet, February 21, but are included herewith for the purpose of record:

Executive Committee

President	George E. Rush
Immediate Past President	Floyd T. Jensen
Vice President	M. A. Woods
Secretary-Treasurer	James H. Fischer

Board of Directors

Pacific Coast Region	D. D. Dickenson
Intermountain Region	Ronald C. Johnson
Eastern Rocky Mountain Region	Whitney Newton II
North Central and Great Lakes Region	W. D. Foley
Canada	J. W. Hall
Processing at Large	C. W. Hogge
Agriculture at Large	D. L. Sunderland

There being no further business the Chairman acknowledged with thanks the numerous Society members and contributors of papers who devoted their personal time and effort to the outstanding success of the Fifteenth General Meeting. He particularly acknowledged and thanked the Program Chairman, Robert S. Caddie, along with his Agricultural Program Chairman, Ronald C. Johnson and Operations Program Chairman, Hugh G. Rounds. He further expressed his appreciation to the Section Chairmen, Charles E. Broadwell, Kent Nielson, David L. Mumford, Lloyd W. Norman, Varon Jensen and Eugene F. Trojan. He acknowledged with thanks the General Arrangements Committee, Arnold Mast, Chairman, assisted by William B. Morrow, Ralph Lambdin, Orin Hills and Earl Rupple. For the development of the slate of candidates to hold office during the Sixteenth biennium he thanked B. E. Easton, Chairman, assisted by William W. Barr, Frank H. Peto, John S. McFarlane and R. J. Tingley. He expressed appreciation for the efforts by the Publications Committee consisting of R. R. Wood, F. W. Snyder, Whitney Newton IT, Kent Nielson and James H. Fischer. He acknowledged with appreciation the members of the Awards Committee, R. K. Oldemeyer, Chairman, Fred G. Eis, John W. Hall and Sam C. Campbell for selection of candidates to receive the Meritorious Service Awards and Forty-Year Veteran Awards. Further expressions of appreciation were extended to the Board of Directors, the Tally Committee and to each one either presenting a paper at the meeting or participating in formal discussions. He particularly thanked the speakers at the afternoon General Sessions who represented the various segments of the industry in projecting a "Look into the Future." He thanked the management and staff of the Westward Ho Hotel and the staff of the Valley of the Sun Convention Bureau who were so helpful in making the Fifteenth General Meeting a Success. The Silver Engineering Works, Inc., Spreckels Sugar Company and the Western Seed Production Corporation were thanked for their assistance in providing refreshments and entertainment for the meeting registrants and the wives of members accompanying their husbands to the Phoenix meeting.

There being no further business, the meeting was declared adjourned at 11:50 a.m.

Meritorious Service
Award Presented
to
HERBERT L. BUSH

Mr. Bush has not only been a member of the Society since its beginning, but attended the meetings of the Sugar Beet Round Table, the parent meetings from which the ASSBT developed. For as long as can be remembered he has served as the faithful registrar at each of the biennial meetings of the Society. He has served on the Standardization of Experimental Methods Committee. The index to our Society publications lists his name as an author or coauthor on twenty-seven published papers. Herb was born September 27, 1903 at Fort Collins, Colorado and spent his youth on a ranch at Livermore, Colorado. He received his B.S. in chemistry at what is now Colorado State University in 1928 and has had postgraduate study at Colorado State University and North Carolina State. His work with sugar beets dates from 1930 when he became a clerk with the USDA at Fort Collins. In 1934 he joined the National Seed Company, a subsidiary of Rabbethge and Giesecke in Germany, at Brush, Colorado as research manager. In 1939 he was employed by The Great Western Sugar Company as statistician-agronomist at Longmont, Colorado, the position he presently holds. He is a member of the American Statistical Association, Biometric Society and Sigma Xi Research Fraternity. During his long residence in Longmont he has been a participant in numerous community activities.

Meritorious Service
Award Presented
to
ROBERT S. GADDIE

Mr. Gaddie has been a member of the Society since the first general meeting in 1938. He was elected to the Advisory Council in 1952 and was Chairman of the Chemistry and Factory Operations Section for the Eighth General Meeting and again for the Thirteenth General Meeting. He was Chairman of the Nominating Committee during the 1962-63 biennium and repeated as a member of the committee during the 1964-65 biennium. He has been General Program Chairman for the Fifteenth General Meeting. Bob was born November 4, 1909 in Sugar City, Idaho. He graduated from the University of Utah in 1932 with a B.S. degree in chemistry. He began his employment with the Utah-Idaho Sugar Company in 1932 where he served as End Foreman, General Foreman, Chief Plant Chemist, Head Chemist at the general laboratory, Assistant General Chemist, General Chemist and Assistant General Superintendent. He has been Associate Referee for both the International Commission on Uniform Methods of Sugar Analysis and the U. S. National Committee. He is a member of the American Chemical Society, American Association for Advancement of Science and the Institute of Food Technologists. The index of ASSBT publications lists him as an author or coauthor of sixteen papers. He also has published in official seed analysts, Northwest Industrial Waste Council and various trade publications. He is listed in American Men of Science and Who's Who In The West.

Meritorious Service
Award Presented
to
FRANK H. PETO

Dr. Peto has been a member of the Society since the first general meeting, was elected to the Advisory Council during the 1952-53 biennium and served as a member of the Nominating Committee in 1966-67. His interest in international cooperation was displayed by his participation in the first joint ASSBT-IIRB meeting at London in 1961. He has attended the Winter Congress of the IIRB and served as Official Representative of our Society at such meetings. He is author or coauthor of eight papers published in the ASSBT Proceedings and journals. Frank received his B.S. degree from Manitoba in 1928 and was awarded the Governor General's Gold Medal. He earned the M.S. degree from Alberta in 1930 and the Ph.D. degree from Wales in 1932. From 1933-40 he was a cytogeneticist with the National Research Council in Ottawa and between 1940 and 1944 was Director of Agricultural Research at Buckerfields. He became Director of Agricultural Research for the British Columbia Sugar Refining Company, Limited, in 1944, the position he presently holds. During his work with the National Research Council, he performed the feat of chromosome doubling in a wide variety of flowers, trees and sugar beets, thus leading to the first commercial trials in the world of triploid and tetraploid sugar beets.

Meritorious Service
Award Presented
to
ALBERT ULRICH

Dr. Ulrich has been a member of the Society since 1940. He was elected to the Advisory Council in 1950-51, was appointed Chairman of Physiology Section for the Ninth General Meeting and again for the Fourteenth General Meeting. He was a member of the Editorial Board in 1960-61. The index of ASSBT publications lists him as an author or coauthor of 22 papers. Albert was born in New York, New York, on September 7, 1907. He received the B.S. degree in 1930, and the Ph.D. in 1939, both from the University of California. He has been associated with the University of California throughout his professional career. During 1949-53 he was a Research Fellow at the California Institute of Technology at the Earhart Plant Research Laboratory. He is one of the countries leading authorities on the principles and practices of plant analyses in crop production. He served for three years as Chairman of the National Research Council Committee on Plant Composition and for more than fifteen years has been a lecturer on field plot and tissue testing techniques as aids in the systematic planning of fertilizer programs at the University of California. He also has lectured in New Zealand, Brazil, Argentina and Belgium. He is a member of and was elected a Fellow of the American Society of Agronomy in 1964.

FORTY YEAR VETERAN AWARDS

M. M. Afanasiev, Montana State University

T. William Cockayne, Utah-Idaho Sugar Company

J. Hunter Caddie, Utah-Idaho Sugar Company

George R. Hales, Utah-Idaho Sugar Company

H. B. Henderson, Holly Sugar Corporation

O. P. Kottwitz, Holly Sugar Corporation

George L. Lash, Utah-Idaho Sugar Company

V. E. Lott, Utah-Idaho Sugar Company

Albert M. Murphy, U. S. Department of Agriculture

Paul Douglas Scalley, Utah-Idaho Sugar Company

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Relative Damage of Cercospora Leaf Spot in Sugarbeet Varieties

GERALD E. COE[1]

Received for publication October 12, 1967

In 1948, Stewart (2)[2] reported the results of a spraying experiment to control leaf spot caused by *Cercospora beticola* Sacc. Under severe and prolonged exposure, the productivity of sprayed populations of the most resistant sugarbeet variety, US 216, was over twice that of the unsprayed diseased population. In 1965, an experiment was undertaken at the Plant Industry Station, Beltsville, Maryland, to reappraise losses caused by leaf spot, particularly the production loss, in the current resistant variety. Summer temperature and humidity at this station are favorable for the disease. A sprinkler system was used to promote infection during periods of drought.

Materials and Methods

Three varieties differing in leaf spot resistance were used. The experimental design was a randomized-block split plot with 6 replications, spray treatments being the main plots and varieties being subplots. A border of SP 6322-0, a resistant multigerm variety, surrounded each plot to minimize border effects of drifting spray and splashed spores. Each variety subplot was 4-rows wide and 20 feet long. The rows were 24 inches apart, and the plants were spaced about 12 inches apart in the rows.

Sugarbeets in the sprayed plots received 11 mist spray applications of copper oxychloride in oil emulsion at the rate of 2/3 lb per acre to control the disease. In 1964, Schneider (1) found copper oxychloride effective in controlling *Cercospora* leaf spot. The applications were made at weekly intervals except for more frequent applications during periods of rainfall. The rate of application was limited to 2/3 lb because of the danger of leaf damage from numerous applications of the fungicide. No leaf damage occurred from the treatments.

On June 8, leaf spot inoculum was applied to sugarbeet plants in the unsprayed halves of the split plots. The first symptoms of disease appeared about 10 days later. Fungicidal treatment of uninoculated plots was started on July 5. Fungicidal treatments were not applied to the inoculated populations.

[1] Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

[2] Numbers in parentheses refer to literature cited.

Five readings of leaf spot intensity were made on all plots between July 23 and September 4. The readings were based on a scale from 0 to 10—0 being no leaf spot and 10 being complete defoliation due to the disease. At the peak of the disease epidemic all mature leaves were blighted; only a few young immature center leaves of the foliar rosette remained free of spots in the susceptible variety. The disease epidemic continued until harvest, but declined in severity after August 31. Therefore, for a period of more than 60 days, the disease was severe and caused significant damage to the sugarbeets.

The center 2 rows of each subplot were harvested individually and weighed on October 6 and the beets taken for quality evaluation. Duplicate pulp samples were taken from each subplot, using all the beets in each individual row for sucrose analysis by polarization and for soluble substance determination by refractometer.

Results and Discussion

The fungicidal treatments were not fully effective in controlling the disease, as indicated by the leaf spot readings given in Table 1. Although oil emulsion of copper oxychloride is a standard fungicide for the control of Cercospora leaf spot of

Table 1.—Average leaf spot readings of sprayed and unsprayed populations of three sugarbeet varieties in 1965 fungicide spray test for Cercospora control.

Variety	Treatment	Average leaf spot reading				
		July 23	July 30	Aug. 6	Aug. 12	Sept. 4
SP 633269-0	No spray	4.50 a[1]	4.83 a	5.08 a	5.17 a	6.33 a
	Sprayed	1.42 d	2.00 cd	2.83 c	2.92 c	5.00 b
US 401	No spray	3.08 b	3.83 b	4.08 b	4.08 b	4.50 b
	Sprayed	1.00 e	1.83 d	1.92 d	2.17 d	3.75 c
SP 6322-0	No spray	2.08 c	2.25 c	3.00 c	3.00 c	3.42 c
	Sprayed	0.42 f	1.25 e	1.25 e	1.42 e	2.67 d
Average for both treatments:						
SP 633269-0		2.96 a	3.42 a	3.96 a	1.04 a	5.67 a
US 401		2.04 b	2.83 b	3.00 b	3.13 b	4.13 b
SP 6322-0		1.25 c	1.75 c	2.13 c	2.21 c	3.04 c
Average for 3 varieties:						
	No spray	3.22 a	3.64 a	4.06 a	4.08 a	4.75 a
	Sprayed	.94 b	1.69 b	2.00 b	2.17 b	3.81 b
Difference between treatments:						
SP 633269-0		3.08 a	2.83 a	2.25 a	2.25 a	1.33 a
US 401		2.08 b	2.00 b	2.16 a	1.91 ab	.75 a
SP 6322-0		1.66 b	1.00 c	1.75 a	1.58 b	.75 a

[1] Means in the same column which have the same letter are not significantly different at the 5% level.

sugarbeet, it was not as effective as desired in this experiment where disease development was intentionally intensified. However, the sprayed populations suffered strikingly less leaf damage through the middle of August and visibly less through the remainder of the growing season until harvest. This difference in treatments was highly significant each time leaf spot evaluations were made. After September 4, the difference in the amount of leaf spot between the treatments decreased considerably. The leaf spot readings of July 23, July 30 and August 12 indicate a significantly greater difference in the amount of disease between the sprayed and unsprayed populations of the more susceptible varieties, as compared to the differences between sprayed and unsprayed populations of the more resistant varieties. The differences between varieties in leaf spot readings were highly significant.

Harvest data are indicative only of productivity at different levels of disease intensity. The harvest results and laboratory analyses are presented in Table 2. Yields of beets and gross sugar for the sprayed populations undoubtedly would have been

Table 2.—Harvest data of three sugarbeet varieties in 1965 fungicide spray test for Cercospora control.

Variety	Treatment	Acre yield: Gross sugar	Acre yield: Roots	Sucrose	Raw juice apparent purity
		Pounds	Tons	Percent	Percent
SP 633269-0	Sprayed	4033 d[1]	17.86 c	11.29 c	75.25 c
	No spray	2395 e	12.63 d	9.48 d	72.59 d
US 401	Sprayed	6015 b	24.39 a	12.33 b	78.28 b
	No spray	4090 d	18.57 c	10.85 c	75.60 c
SP 6322-0	Sprayed	6620 a	25.21 a	13.13 a	80.18 a
	No spray	5444 c	21.67 b	12.56 b	79.36 ab
Average for both treatments:					
SP 633269-0		3214 c	15.25 c	10.39 c	73.92 c
US 401		5023 b	21.48 b	11.59 b	76.94 b
SP 6322-0		6032 a	23.44 a	12.85 a	79.77 a
Average for 3 varieties:					
	Sprayed	5556 a	22.49 a	12.25 a	77.90 a
	No spray	3956 b	17.62 b	10.96 b	75.85 b
Difference between treatments:					
SP 633269-0		1638 ab	5.23 a	1.81 a	2.66 a
US 401		1985 a	5.82 a	1.48 a	2.68 a
SP 6322-0		1176 b	3.54 a	.57 b	.82 a

[1] Means in the same column which have the same letter are not significantly different at the 5% level.

higher if leaf spot control had been more effective. Nevertheless many significant differences occurred.

SP 633269-0 was significantly lower than the other two varieties in root yield, and there were significant differences between treatments in root yields. However, there were no significant differences between varieties in the amount of loss of root yield caused by leaf spot.

Differences between varieties in percent sucrose were significant. The decrease in percent sucrose attributable to leaf spot in SP 6322-0 was significantly less than in the other two varieties.

Differences between varieties in gross sugar production were highly significant as were the differences between treatments. The decrease in gross sugar production attributable to leaf spot in SP 6322-0 was significantly less (1% level) than in US 401.

There were highly significant differences between varieties in raw juice apparent purity. There were highly significant differences between treatments in two of the varieties, SP 633269-0 and US 401, but no significant difference between treatments in raw juice apparent purity in the variety SP 6322-0. The difference between treatments in SP 6322-0 was smaller than in the other two varieties because of the greater leaf spot resistance of SP 6322-0.

There were no significant differences between varieties in the amount of decrease in raw juice apparent purity attributable to leaf spot.

There was essentially no difference between sprayed and unsprayed treatments in the amount of nonsucrose solutes in raw beet juice (Table 3). However, the differences between varieties in content of these nonsucrose solutes were significant and are the result of selection efforts.

It should be emphasized that even the resistant variety, SP 6322-0, suffered appreciable losses in tonnage and sugar percentage under the severe leaf spot conditions of this test and that more resistance is still to be desired. In trying to estimate whether application of fungicidal treatments to a variety with resistance equivalent to SP 6322-0 would be profitable on a commercial basis, two imponderables are present. First, in commercial sugarbeet districts, the intensity of leaf spot is almost never as severe as the epidemic induced in this test; consequently, the losses in commercial fields are not nearly so great as the losses under the conditions of this test. Second, the indicated losses realized in this test are not as great as they would have been if perfect leaf spot control had been achieved in the fungi-

Table 3.—Percentage nonsucrose solutes in press juice of sprayed and unsprayed populations of three varieties of sugarbeets in 1965 fungicide spray test for Cercospora control.

Variety	Treatment	Nonsucrose solutes
SP 633269-0	Sprayed	3.57 ab[1]
	No spray	3.70 a
US 401	Sprayed	3.50 b
	No spray	3.43 bc
SP 6322-0	Sprayed	3.27 c
	No sprav	3.25 c
Average for both treatments:		
SP 633269-0		3.64 a
US 401		3.46 b
SP 6322-0		3.26 c
Average for 3 varieties:		
	Sprayed	3.45 a
	No spray	3.46 a
Difference between treatments:		
SP 633269-0		.13 a
US 401		.07 a
SP 6322-0		.02 a

[1] Means in the same column which have the same letter are not significantly different at the 5% level.

cide-treated populations. Hence, there is no exact measure of the loss caused by leaf spot at the degree of disease severity experienced in this experiment.

Summary

Fungicidal treatments of three sugarbeet varieties differing in Cercospora leaf spot tolerance enhanced gross sugar yields, root weights, sucrose percentages, and percent purities under severe disease exposure. The productivity of untreated populations was associated with varietal resistance to the pathogen. SP 6322-0, the most resistant entry, had less decrease in percent sucrose due to leaf spot than the other varieties. The beets of SP 6322-0 had less nonsucrose solutes in both sprayed and unsprayed populations than the other 2 varieties. This was not attributable to the better leaf spot tolerance of SP 6322-0 but is inherent in the variety as a result of selection for this characteristic. A commercial variety with leaf spot resistance comparable to SP 6322-0 affords reasonably good protection against field epidemics of leaf spot, but even more resistance is needed if disease losses are to be avoided without fungicidal protection when leaf spot is severe.

Literature Cited

(1) SCHNEIDER, C. L. 1965. Control of Cercospora leaf spot of sugar beet with ultra-low-volume oil-based fungicidal mists. J. Am. Soc. Sugar Beet Technol. 13(7): 563-565.

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Effect of Low Colchicine Concentrations On Inducing Autotetraploidy In Sugarbeets

HELEN SAVITSKY[1]

Received for publication November 24, 1967

Introduction

The most effective method of inducing tetraploidy in sugarbeets involves treatment of seeds with colchicine solutions. The best results have been obtained by treatment of pregerminated seeds. These have produced more tetraploid plants than have treated dry, or germinated seeds (7)[2]. The colchicine concentrations usually applied ranged from 0.1% to 0.8% (1,2,3,4,5, 6, 7, 8, 9).

The higher doses (0.5%-0.8%) often produce malformed seedlings and decrease the number of tetraploid plants obtained. To induce autotetraploidy it is desirable to treat the seeds with the lowest colchicine concentrations possible. Effectiveness of treatment of sugarbeet seeds with the low colchicine concentrations is the subject of the present report.

Materials and Methods

Effectivenss of the low colchicine concentrations, 0.05% and 0.1% was compared with that of higher concentration of 0.4%, which is often used for inducing tetraploidy in sugarbeets. Sugarbeet seed rinsed in water during 3 hours was kept for 36-40 hours at 25° C in cuvettes lined with wet blotting paper. During this time the seed swelled, but the root tips did not emerge. Seeds of five self-sterile multigerm populations and of five self-fertile monogerm inbreds ready for germination (pregerminated seed) were treated for 16 hours with colchicine solutions. Treated seeds of each strain were planted in beds in the greenhouse in two replications. Two hundred seeds of each strain were planted (100 seeds per replication). Selection of tetraploid C_0 plants was based on the presence of diploid gametes, not on the leaf characteristics. Plants on which the main inflorescense carried exclusively large pollen grains (diploid gametes) were selected as tetraploids and intercrossed. The young C plants thus obtained have been checked for chromosome number and those

[1] Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Salinas, California.

[2] Numbers in parentheses refer to literature cited.

having 36 chromosomes were selected. Methods of seed treatment and of producing tetraploid plants, used in this experiment, have been previously reported (6, 7).

Experimental Results

The average effect of colchicine concentrations 0.05%, 0.1% and 0.4% by treatment of all strains is shown in the Table 1 (summarized data). Seed germination decreased from 80.30% to 57.00% with increased colchicine concentrations. In contrast to seed germination, percent of seedlings affected rose from 25.34 to 63.42 when higher concentrations of colchicine were applied. Only 33 tetraploid plants (8.38%) were obtained in all strains treated by the low concentration (0.05%). A concentration 0.1% increased the number of tetraploid plants to 104 (21.85%). A concentration 0.4% produced 344 tetraploid plants (52.92%).

Table 1.—Average effect of seed treatment with different colchicine concentrations on all 10 sugarbeet strains.

Colchicine concentration	Seedlings emerged		Seedlings affected		Plants examined for size of pollen	Tetraploids obtained	
%	No.	%	No.	%	No.	No.	%
0.05	1,606	80.30	407	25.34	394	33	8.38
0.1	1,490	74.50	509	34.16	476	104	21.85
0.4	1,140	57.00	723	63.42	650	344	52.92

In spite of the obviously more effective results from the treatment with the 0.4% concentration, the individual sugarbeet strains responded differently to colchicine concentrations (Table 2).

Seedling affection

Seedlings affected by colchicine are short with thickened hypocotyls and thickened, lobed leaves.

The lowest colchicine concentration (0.05%) affected seedlings in 7 of 10 strains. Percent of seedlings affected varied in these strains from 23.26 to 46.67.

The higher concentration (0.1%) increased percent of affected seedlings in the same strains. For strains 661, 772 and 19, both low concentrations (0.05% and 0.1%) were ineffective and did not produce affected seedlings.

The strongest concentration (0.4%) produced the highest percent (44.79 to 72.34) of seedlings affected in all strains, with the exception of strain 33, for which the 0.1% concentration was more effective.

Tetraploid plants obtained

Treatment of seed with a concentration 0.05% produced tetraploids in three strains only. In two of these strains (126 and 520) the percent of tetraploids was low—17.14 and 19.35, respectively. In the third strain (33), which responded better to the low concentration, the percent of tetraploids increased to 25.71.

Concentration 0.1% produced tetraploid plants in 7 of 10 strains. Percent of tetraploids in six of these strains was not high, ranging from 10.00 to 29.17. In the seventh strain 33, a high percent (54.29) of tetraploids was obtained after treatment with 0.1% concentration.

Table 2.—Effect of seed treatment of 10 individual sugarbeet strains with different colchicine concentrations.

Strain number	Colchicine concentration	Seedlings emerged		Seedlings affected		Plants examined for size of pollen	Tetraploids obtained	
	%	No.	%	No.	%	No.	No.	%
123 S.St. M_2	0.05	108	54.00	44	40.74	42	0	0
	0.1	104	52.00	46	44.23	46	6	13.04
	0.4	74	37.00	46	62.16	46	24	52.17
112 St.St. M_2	0.05	258	129.00	60	23.26	55	0	0
	0.1	267	133.50	111	41.57	92	14	15.22
	0.4	205	102.50	138	67.32	116	69	59.48
211 St.St. M_2	0.05	184	92.00	84	45.65	84	0	0
	0.1	192	96.00	99	51.56	90	9	10.00
	0.4	140	70.00	92	65.71	92	38	41.30
126 S.Fert. m_2	0.05	150	75.00	70	46.67	70	12	17.14
	0.1	136	68.00	84	61.76	84	18	21.43
	0.4	94	47.00	68	72.34	60	38	63.33
33 S. Fert. m_2	0.05	112	56.00	37	33.04	35	9	25.71
	0.1	92	46.00	70	76.09	70	38	54.29
	0.4	64	32.00	42	65.63	12	7	58.33
716 S. Fert. m_2	0.05	130	65.00	46	35.38	46	0	0
	0.1	98	49.00	45	45.92	40	5	12.50
	0.4	76	38.00	48	63.16	45	20	44.44
520 St.St. M_2	0.05	154	77.00	66	42.86	62	12	19.35
	0.1	98	49.00	48	48.98	48	14	29.17
	0.4	76	38.00	48	63.16	47	24	51.06
661 S.Fert. m_2	0.05	174	87.00	0	0	0	0	0
	0.1	166	83.00	0	0	0	0	0
	0.4	140	70.00	92	65.71	86	43	50.00
772 S.Fert. m_2	0.05	134	67.00	0	0	0	0	0
	0.1	124	62.00	0	0	0	0	0
	0.4	96	48.00	43	44.79	40	20	50.00
19 S. St. M_2	0.05	202	101.00	0	0	0	0	0
	0.1	213	106.50	0	0	0	0	0
	0.4	175	87.50	106	60.57	106	61	57.55

The strongest concentration (0.4%) produced the highest percent of tetraploids (41.30 to 63.33) in all strains. For three strains 661, 772 and 19, both low concentrations (0.05% and 0.1%) were ineffective. Tetraploid plants were obtained in these strains only after treatment with a concentration of 0.4%.

Colchicine concentration was the main factor influencing variability in polyploidy induction in these experiments (Table 3). The value of F calculated (275.92 and 1252.71) for seedling affection and tetraploid plants obtained, respectively, greatly exceeded the tabular value of F (3.55 at 5%, and 6.01 at 1% level). The next factor causing variability was the responsiveness of the individual strains to different colchicine concentrations. F calculated for seedling affection was 57.74 and for tetraploids obtained, 60.46, against a tabular F value of 2.46 at the 5% level, and 3.60 at the 1% level.

Table 3.—F test in analysis of variance for seedlings affected and tetraploid plants obtained.

Source of variance	Seedlings affected F calculated	Tetraploids obtained F calculated	F tabulated for seedlings affected and tetraploids obtained 5%	1%
Strains	57.74	60.46	2.46	3.60
Colchicine Conc.	275.92	1252.71	3.55	6.01
Replications	1.61	< 1	4.41	8.28
Replic. × Concentration	1.52	1.44	3.55	6.01
Strain × Replication	2.79	< 1	2.46	3.60
Strain × Concentration	13.61	20.49	2.25	3.19

Discussion and Conclusions

A colchicine concentration of 0.05% is too low to induce tetraploidy in the majority of sugarbeet strains. An insignificant number of tetraploids was obtained in only three strains after treatment with this concentration.

A concentration of 0.1% affected the majority of strains, but it did not produce a large number of tetraploids. For three strains, a concentration of 0.1% was ineffective.

The 0.4% concentration had a universal effect and produced the largest number of tetraploids in all strains.

In inducing tetraploidy, by seed treatment, not all morphologically affected seedlings produced tetraploid plants. In these experiments, low colchicine concentrations were sufficient in some cases to affect seedlings, but not strong enough to double the chromosomes in the sub-epidermal tissues from which the gametes develop.

In four strains treated with 0.05% concentration, 40.74%, 23.26%, 45.65%, and 35.38% of the seedlings were affected, but no tetraploid plants were obtained from them. Responsiveness of individual sugarbeet strains to different colchicine concentrations has not been previously reported. Although an indication of this was noted in Savitsky (7). Such responsiveness is, however, clearly demonstrated in these experiments. Three of 10 strains did not produce tetraploids at low concentrations of 0.05% or 0.1%, whereas, strain 33 responded very well to these concentrations. After seeds of the strain 33 were treated with concentration 0.05%, 25.71% of the plants were tetraploid. Concentrations 0.1% and 0.4% gave 54.29% and 58.33% tetraploids, respectively. Thus, for strain 33, a concentration of 0.1% was almost as effective as a concentration 0.4%.

Actually, every line or population should be treated with a particular colchicine concentration to obtain the best results. For the lines, which present some difficulties in being converted into tetraploids, the suitable concentrations should be found experimentally. However, for the majority of sugarbeet strains, treatment of pregerminated seed with concentrations from 0.2%, or 0.3 to 0.5%, as established in previous experiments (7), is highly effective.

Summary

The pregerminated seed of 10 sugarbeet strains were treated by 0.05%, 0.1%, and 0.4% colchine concentrations. A colchicine concentration 0.05% is too low to induce tetraploidy in the majority of strains. A higher concentration 0.1% affected seedlings in the majority of strains, but did not produce a large number of tetraploids. The concentration 0.4% produced the largest number of tetraploids in all strains. Individual sugarbeet strains demonstrated different responsiveness to colchicine concentrations. Three of 10 strains did not produce tetraploids at concentrations 0.05% and 0.1%, whereas 1 strain responded well to concentration 0.05%, and the concentration 0.1% was as effective for it as the concentration 0.4%. For the lines which present difficulties in being converted into tetraploids the suitable colchicine concentrations should be found experimentally.

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Breeding for Rhizoctonia Resistance in Sugarbeet[1]

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Introduction

Root and crown rot of sugarbeet {*Beta vulgaris* L.) of about middle age or older, caused by *Rhizoctonia solani* Kuehn, is a serious problem in all of the major sugarbeet-producing areas in the United States. Crop rotation gives only limited protection (10,13)[3]; no chemical treatments of soil or seed have proved to be commercially feasible; and no commercial varieties with appreciable resistance are known.

Breeding for resistance to *Rhizoctonia* has been hampered traditionally by the erratic behavior of the disease. It has long been recognized that artificial techniques are essential for creation of uniform *Rhizoctonia* exposure of acceptable levels of intensity (11). Results of methods studies, conducted at Fort Collins, Colorado, from 1957 through 1965, have been reported (1,2,3,4, 5,6,7,11). The information presented in those reports is too voluminous to be reviewed in this article. However, some of the more important conclusions are mentioned.

Concurrently with the research on disease exposure methods, selection and progeny evaluation for *Rhizoctonia* resistance were carried on at Fort Collins. Increments in resistance were small, individually, but the cumulative effects of repeated selection cycles were substantial. Results of the earlier years' selection work would serve no useful purpose in this article. Selection results are presented for 1965 and 1966, only.

Conclusions Regarding Exposure Techniques for Evaluation of Resistance of Lines or Progenies

Results of research on methods of exposing the sugarbeet to *Rhizoctonia,* for the purpose of evaluating the resistance of progenies or lines, have led to the following conclusions:

1. Residual inoculum (i.e. that remaining in the soil in the

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[3] Numbers in parentheses refer to Literature Cited.

field from one year to the next) is undependable and highly unsatisfactory. In our experience, exposure by this method has resulted in negligible, to almost complete, loss of stand in different years. Either of these extremes is unacceptable.

2. The degree of resistance achieved by selection at Fort Collins apparently is relatively ineffective in preventing stand losses in early seedling stages. This conclusion is based on a series of experiments and is in keeping with our observation that sugarbeet lines or progenies usually did not differ significantly in stand when inoculum (a dry, ground, barley-grain-culture preparation) was applied with the seed or by side dressing when the plants were small.
3. The placement of inoculum around, and in contact with, the tap root, approximately 1 inch below the soil surface, about 1 week after thinning, proved to be too severe and usually resulted in almost complete loss of stand.
4. The placement of inoculum in a semicircle, l 1/2 inches from the tap root and about 1 inch below the soil surface (the so-called "semicircle method") proved to be too severe if performed no later than 1 week after thinning. When performed 3 weeks after thinning, stand losses were less severe, and measurable differences in survival occurred between populations.
5. The application of inoculum in the center of the foliar rosette—the so-called "rosette method" (11)—also proved to be too severe when inoculation was performed 1 week after thinning. When inoculation by this method was performed 3 to 5 weeks after thinning, stand losses were less severe and, as with the semicircle method, measurable differences occurred between populations. On the basis of several years' results, it was concluded at the end of 1964 that the rosette method is the most dependable of the various inoculation techniques studied. It was used for all *Rhizoctonia* resistance evaluation work in 1965 and 1966, except where a comparison was made with the residual-inoculum method in one experiment in 1966.
6. Where the semicircle and rosette methods were compared, the interaction of sugarbeet strains X methods was not significant (2 years' results).
7. *Rhizoctonia* attack tended to be more severe where soil moisture was slightly deficient to moderate, during most of the postinoculation period, than where it was abundant. However, this tendency was not consistent and was considered inconclusive.

Methods of Plant Selection and Propagation

Breeding for resistance involved the selection of individual plants and the evaluation of their progenies under relatively severe *Rhizoctonia* conditions. The techniques employed to develop these conditions were changed from time to time as indicated by the results of the methods studies. Progenies showing little or no promise were dropped from year to year, and selections were made in the more promising progenies. The mass-selection technique—i.e. the production of seed by groups of plants selected from a given source—was used predominantly through 1964. During this period, seed was harvested and evaluated separately for individual "mother" plants only to a very limited extent. This latter practice was emphasized beginning with the 1965 seed crop.

Early attempts to select individual plants for resistance where inoculum was applied with the seed gave negative results. Likewise, attempts to pick resistant plants where inoculum had been applied in contact with the tap root also were disappointing. One instance of plant selection under residual-inoculum (i.e. field-overwintered inoculum) conditions gave encouraging results. SP 631001-0 is a product of selection under such conditions. In general, the most progress to date has been made by selecting where the semicircle or rosette methods were used.

Evaluation Tests and Results of Breeding Work

Screening tests, involving a total of 226 foreign introductions of *B. vulgaris*—mostly culinary types—and 18 of *B. maritima* L., failed to produce a single introduction with substantial *Rhizoctonia* resistance (2,4,12). Before 1965, only moderate progress was shown in the improvement of *Rhizoctonia* resistance of the sugarbeet by breeding (1,2,3,4,5,6). The results of 1965 were particularly encouraging in indicating that a new, higher level of resistance had been achieved (7,8). SP 631001-0, a product of two cycles of *Rhizoctonia* resistance selection from the commercial variety, GW 674-56C, was among the lines compared in 1965. In one experiment SP 631001-0 exceeded the parental variety by 66 and 77 percent in stand and root yield, respectively, at harvest. In another experiment the corresponding percentages for the same material were 39 and 51. The latter experiment also included SP 641004-(02), a product of three cycles of selection from the same source variety. SP 641004-(02) in turn surpassed SP 631001-0 in stand and root yield by 30 and 40 percent, respectively. All of these differences exceeded the level of significance designated as the 5-percent point, and all but one exceeded the 1-percent point.

In the second experiment, referred to in the preceding paragraph, there occurred a line designated SP 641005-(01), a product of three cycles of *Rhizoctonia* resistance selection from the variety, C817. SP 641005-(01) was quite attractive and actually was slightly higher in final stand than SP 641004-(02). In order to place this information in better perspective, a comparison may be made between the latter two lines and the two leaf spot-black root resistant commercial varieties occurring in this experiment as standards. The final stand (i.e. percentage of inoculated plants alive at harvest) for US 401 and SP 5822-0 was 58.7 and 46.7, respectively. The final stand for SP 641004-(02) and SP 641005-(01) was 92.8 and 97.1, respectively. The LSD at the 1-percent point was 21.3.

Plants selected from SP 641004-(02) and SP 641005-(01), in the inoculated plots in 1965, were brought to seed in two separate groups in the greenhouse in time for spring planting in 1966. The two seed lots were designated FC 701 and FC 702, respectively. These two seed lots were made available to the sugarbeet industry, through the Beet Sugar Development Foundation, in the fall of 1966, in quantities of 5 to 15 grams per company. Larger quantities, resulting from subsequent increases, were turned over to the industry, through the Foundation, in August 1967. Official release is being considered.

Field Tests, 1966

The purposes of this section are to give a rather detailed account of current techniques and to summarize the most recent results of *Rhizoctonia* resistance breeding work at Fort Collins.

Inoculum of a highly pathogenic isolate (B-6) of *R. solani* was prepared as follows: (a) Approximately 520 ml of dry, whole, barley grain and 300 to 305 ml of distilled water were placed in each 1-liter Erlenmeyer flask, stirred, and allowed to stand overnight; (b) the mixture was stirred again, and the flasks were plugged with cotton and autoclaved for 2 hours at 17 psi; (c) the grain in each flask was inoculated in two places, using mycelial agar chunks; (d) the cultures were incubated for 3 weeks on a laboratory bench without special temperature control (in the summer while the laboratory was quite warm); (e) the cultures were dried on trays in open air in the laboratory, with air movement augmented by fans; (f) the dried material was ground in a Wiley mill, passing through a 3-mm round-hole screen; and finally (g) the ground inoculum was blended, placed in paper bags, and stored in a refrigerator at about 2° to 4° C. As usual, inoculum used in the 1966 plots was stored for no more than two weeks. However, inoculum prepared and stored as described has remained viable and highly pathogenic for more than

a year. A more sophisticated method of inoculum preparation, involving precise temperature control for incubation and drying, has been described (11). The method used in 1966, and for several years immediately prior thereto, is described in some detail here because of its simplicity and low cost.

The eight varieties or lines listed in Table 1 were compared for *Rhizoclonia* resistance in Experiment R-l. All lines are multigerm and at least moderately resistant to *Cercospora* leaf spot. GW 674-56C is a commercial variety developed by the Great Western Sugar Company. C817, also known as Selection A54-1 Synthetic, was derived from another Great Western variety (GW 359) by Dr. LeRoy Powers[4] under conditions where disease exposure was negligible. US 401 and SP 5822-0, developed by the U.S. Department of Agriculture, are resistant to the type of black root caused by *Aphanomyces cochlioides* Drechs. The other lines in Experiment R-l were derived from GW 674-56C or G817 by selection for *Rhizoclonia* resistance as indicated in Table 1.

Experiment R-l consisted of two 8 X 8 Latin Squares in adjacent fields. In field "A" the plots were two rows (i.e. 40 inches) wide and 25 feet long. A 16-foot (2-row) section in each plot was inoculated on July 25, 1966, 4 weeks after thinning, using the dry, ground, barley grain inoculum described above, at the rate of i/6 teaspoon per plant. The inoculum was deposited by hand in the center of the foliar rosette—the so-called "rosette method" previously described (11).

In field "B", inoculum of isolate B-6 had been applied to the sugarbeet crop by the rosette method in 1965, and none was applied in 1966. Severe *Rhizoclonia* attack occurred in 1965, and the over-wintered or "residual" inoculum was the sole source of the fungus in 1966 plots. Plot size in field "B" in 1966 was the same as in field "A", but the portion of each plot considered as inoculated in field "B" conformed to that actually receiving inoculum in the preceding year—i.e. 2 rows X 14 feet.

Both sections of Experiment R-l were planted on May 25 and hand thinned at about the usual stage of plant development (6 true leaves, approximately), attempting to leave single-plant hills 10 to 12 inches apart. Planting rates were adequate to produce satisfactory thinned stands except as affected by disease in field "B". The soil (fine sandy loam) was high in fertility. Irrigation was performed by sprinkler. In order to avoid excessive drying of inoculum in field "A", during the first few days after inoculation, the sprinkling regime in that field included

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moderate to heavy applications immediately after inoculation (July 25) and on July 26 and 28. For reasons of comparability, field "B" received the same amount of sprinkling on those 3 days. At harvest (October 11-12), the roots of all living plants in the inoculated portion of each plot were trimmed as mother beets, washed and weighed. The criterion for classification of a plant as living was the presence of one or more turgid green leaves, regardless of size.

The results of Experiment R-1A, in which inoculation was performed by the rosette method after the plants had attained considerable size, confirmed the results of the preceding year in showing that *Rhizoctonia* resistance had been improved substantially by selection for resistance in both of the source varieties or lines (Table 1 and Figure 1) . A striking contrast between one of the *Rhizoctonia* resistance lines (FC 702) and a commercial check variety (US 401) is presented in Figure 2.

In Experiment R-1B, where *Rhizoctonia* and presumably other disease inocula were relatively abundant in the soil at planting time, much stand loss occurred before thinning, making it impossible to obtain full thinned stands in many plots. For this reason, it seemed advisable to consider actual stand at harvest, as well as percentage survival and root yield, as indications of performance. By all three of these criteria, the results of Experiment R-1B indicated that significant improvement in resistance had occurred as a result of selection in both source populations (Table 1). That these gains were less impressive than the gains shown by the results of Experiment R-1A, is attributed to several factors. In the first place, it is assumed that much of the early stand loss in R-1B occurred as a result of residual inoculum of species of *Pythium* and other damping-off pathogens—organisms to which the respective lines presumablv have little, if any, resistance. Secondly, most of the post-thinning stand losses in Experiment R-1B occurred soon after thinning. Results of earlier experiments had led to the tentative conclusion that the *Rhizoctonia* resistance then available (e.g. in lines such as entries 902 and 905) was relatively ineffective during the early stages of growth, up to about 2 weeks after thinning. The results of Experiment R-1B were in keeping with that conclusion. In this connection it should be pointed out that, although planting rates were high, variations in thinned stand in Experiment R-1B might have been due in part to variations in potential seedlings planted per unit of row. Consequently, the thinned-stand averages should be viewed with caution.

Progenies of 36 individual, open-pollinated plants were given preliminary *Rhizoctonia* resistance evaluation in 1966 in Experiment R-2. The three commercial varieties that occurred in

Experiment R-l (US 401, SP 5822-0, and GW 674-56C) were included in Experiment R-2 as standards. The *Rhizoctonia* resistant lines, FC 701 and FC 702, also were included. Plots were one row (20 inches) X 25 feet in size; a 16-foot section of each plot was inoculated; and a randomized complete block design was employed with four replications. Otherwise, this experiment was laid out and handled as described for Experiment R-lA.

Figure 1.—Comparison of sugarbeet lines in resistance to *Rhizoctonia,* Fort Collins, Colorado, 1966. Top—the inoculated portion of four 2-row plots, indicated by stakes, on October 4; from left to right: (a) FC 702 (derived from C817), (b) GW 674-56C, (c) FC 701 (derived from GW 674-56C), and (d) C817. Bottom—roots of all living plants in the inoculated area shown at top, as harvested on October 11 (same plot sequence, left to right); badly rotted roots in foreground.

Table 1.—Comparison of sugarbeet lines for *Rhizoctonia* resistance, Fort Collins, Colorado, 1966; results presented as 8-plot averages (Exp. R-1).

Description and/or source	Sel. for Rhizoc.res. No. of cycles	Sel. for Rhizoc.res. Method[a]	Current Ft. Collins seed no.	Other no.	Entry no.	Exp. R-1A (Rosette inoc. in 1966) Harvest results Survival[b]	Exp. R-1A (Rosette inoc. in 1966) Harvest results Root yield[c]	Exp. R-1B (Residual inoculum from 1965) Actual thinned stand[d]	Exp. R-1B (Residual inoculum from 1965) Harvest results Actual stand[d]	Exp. R-1B (Residual inoculum from 1965) Harvest results Survival[b]	Exp. R-1B (Residual inoculum from 1965) Harvest results Root yield[c]
						%	Lbs	No	No	%	Lbs
GW 674-56C	0	.....	Acc. 2168		901	23.66	11.55	20.38	3.13	14.55	4.80
do.	2	1,1	SP 631001-0		902	41.68*	21.11**	18.13	5.00	30.78*	11.18
do.	4	1,23,23,3	SP 661102-0	FC 701	903	73.44**	36.99**	26.00*	10.38**	38.74**	21.70**
C817	0	.....	SP 621220HO		904	35.39	18.95	18.75	4.13	22.10	9.73
do.	2	2,23	SP 621003-0		905	64.83**	33.15**	23.00	9.00*	35.81*	17.03*
do.	4	2,23,23,3	SP 661103-0	FC 702	906	73.18**	33.11**	27.13**	8.00*	29.26	13.86
US 401	0	.....	Acc. 2057		907	27.76	15.03	26.88	2.75	10.29	3.55
SP 5822-0	0	.....	Acc. 2591		908	27.26	13.41	26.25	6.38	23.75	11.30
General mean						45.30	22.91	23.31	6.09	25.66	11.65
LSD (.05)						13.64	7.02	5.24	3.75	12.81	6.72
LSD (.01)						18.24	9.39	7.01	4.98	17.13	8.98
Calculated F[f]						19.71	16.66	4.21	4.65	4.88	6.51

[a] Disease *(Rhizoctonia)* exposure techniques used in the respective cycles of root selection: 1—residual inoculum (i.e. inoculum surviving naturally in the field following inoculation of the sugarbeet crop in the preceding year); 2— inoculum applied in a semicircle about 1 1/2 inches from the tap root and approximately 1 inch below the soil surface, from 1 to several weeks after thinning of the current crop (i.e. the crop from which the root selections were made); and 3—inoculum applied to the center of the foliar rosette, from 1 to several weeks after thinning of the current crop (so-called "rosette" method).

[b] Percent of thinned stand alive at harvest.

[c] Total weight of roots of living plants per plot (32' of row).

[d] Actual no. of living plants per plot (28' of row).

[e] Total weight of roots of living plants per plot (28' of row).

[f] All F values shown are greater than the 1-percent point (3.10).

* Average significantly exceeds that of the source.

** Average exceeds that of the source by a highly significant amount—i.e. by a difference at least equal to LSD (.01).

Figure 2—Comparison of sugarbeet lines in resistance to *Rhizoctonia*, Fort Collins, Colorado, October 4, 1966; the inoculated portion of two 2-row plots, indicated by stakes, from left to right: (a) US 401, and (b) FC 702.

Of the three commercial checks or standards in Experiment R-2, GW 674-56C was highest in both percentage survival and root yield at harvest. FC 701 and FC 702 both exceeded GW 674-56C in percentage survival by highly significant differences. FC 701 and FC 702 also exceeded GW 674-56C in root yield. The difference was significant for FC 701, only.

The following progenies of individual, open-pollinated plants, in Experiment R-2, exceeded GW 674-56C in percentage survival by highly significant amounts: (a) three of five progenies, derived from GW 674-56C via SP 631001-0, each having a history of three cycles of selection for *Rhizoctonia* resistance; (b) seven of 11 progenies, derived from C817 via SP 621003-0, each having a history of three cycles of selection for *Rhizoctonia* resistance; (c) five of eight progenies, derived from monogerm material resistant to both *Cercospora* leaf spot and *Aphanomyces* type black root, each resulting from one to three cycles of selection for *Rhizoctonia.* resistance; and (d) three of 12 miscellaneous progenies, mostly products of a single cycle of selection for *Rhizoctonia* resistance. Of the progenies with high survival percentages, designated in (a), (b), *(c)* and (d), two, five, four and two, respectively, also were significantly above GW 674-56C in root yield (9). Various inoculation methods had been used to create the disease conditions where the selections represented in

Experiment R-2 were made. The results do not permit comparison of those inoculation methods.

Experiment R-3 of 1966 was conducted primarily to evaluate the *Rhizoctonia* resistance of 23 special lines or progenies (products of selection under *Rhizoctonia* exposure) furnished by the Great Western Sugar Company. Experimental design and techniques were the same as for Experiment R-2. Results for the 23 special company lines or progenies will not be reported here. Results for the five lines or varieties, included in Experiment R-3 as standards, are presented in Table 2. Insofar as EC 701, FC 702, and their respective sources are concerned, relative performance agreed rather closely with that reported for Experiments R-1A and R-2.

Table 2.—Comparison of sugarbeet lines for *Rhizoctonia* resistance, Fort Collins, Colorado, 1966; results presented as 4-plot averages (Exp. R-3).

Description and/or source	Seed no.	Harvest results		
		Survival[a]	Root yield[b]	Rhizoc. grade[c]
		%	Lbs	
GW 602 (com. var.)	Acc. 2664	19.2	4.53	8.8
GW 674-56C (com. var.)	Acc. 2168	16.1	4.63	8.8
FC 701 (from GW 674-56C)	SP 661102-0	74.6**	17.59**	4.5
C817	SP 621220HO	27.5	7.63	8.3
FC 702 (from C817)	SP 661103-0	72.0**	14.48*	4.8
LSD (.05)		20.2	5.70	
LSD (.01)		26.7	7.55	

[a] Percent of thinned stand alive at harvest.

[b] Total weight of roots of living plants per plot (16' of row).

[c] Visual preharvest estimate of *Rhizoctonia* irrurv based on depression of both stand and vigor: 0 = healthy; 10 = complete loss (all plants dead).

* Average significantly exceeds that of the source.

** Average exceeds that of the source by a highly significant amount—i.e. bv a difference at least equal to LSD (.01).

Discussion

The results presented in this report showed conclusively that the levels of *Rhizoctonia* resistance of the multigerm populations, GW 674-56C and C817, were raised substantially by several cycles of mass selection under artificial *Rhizoctonia.* exposure. The results also indicated that considerable improvement in resistance had been made by selection in other source material, including certain monogerm lines resistant to *Cercospora* leaf spot and *Aphanomyecs-type* black root.

Although these results are very encouraging, there are several reasons for tempered optimism. In the first place, the stand of resistant lines, such as FC 701 and FC 702, was rather severely damaged by *Rhizoctonia* in some individual plots inoculated by

the rosette method. Furthermore, the tap roots of many of the plants, classed as living in such lines at harvest, were in fact partially if not badly rotted (Figure 1). Some plants classed as living had lost their foliage before harvest, due to crown rot, and then had developed small tufts of new leaves. This tendency, though more pronounced in the susceptible lines (cf. Figure 1), also existed in the resistant lines. The resistance achieved in such lines as FC 701 and FC 702 apparently is relatively ineffective while the plants are small. Finally, the results presented in this report, in general, represent response to only one *Rhizoctonia* isolate and a narrow range of environmental conditions. Appraisal of resistance of such lines as FC 701 and 702 under a variety of environmental conditions, including a wide range of biotypes or strains of *Rhizoctonia,* obviously is needed.

It is evident that gains in *Rhizoctonia* resistance were made as a result of selecting plants under disease exposure created by: (a) residual inoculum (overwintered from the preceding year): (b) post-thinning inoculation (rosette or semicircle methods); and (c) residual inoculum and post-thinning inoculation in respective selection cycles. The data presented do not permit critical evaluation of the residual inoculum method and the semicircle and rosette inoculation methods for selection purposes. However, as brought out elsewhere in this report, the residual inoculum technique is not dependable. In considering the other two, it should be noted that the rosette method is effecive in differentiating between resistant and susceptible lines. Consequently, it may be assumed that it is suitable for evaluation of the resistance of individual plants. The simplicity and low cost of this method make it especially desirable.

FC 701 and FC 702 are not considered acceptable varieties for commercial use. They are multigerm and apparently lower in root yield than the vigorous source material from which they were derived. Very few plants were used in some of the reproduction steps in the development of both of these lines. Consequently, loss in root yield was to be expected. FC 701 and FC 702 are considered of value primarily as sources of genes for *Rhizoctonia* resistance.

Summary

Studies of breeding; sugarbeet for resistance to *Rhizoctonia* root and crown rot at Fort Collins, Colorado, from 1957 through 1966, included research on disease exposure techniques as well as the actual selection of plants and evaluation of progenies for resistance. High lights are as follows:

1. The application of dry, ground, barley-grain inoculum in

the center of the foliar rosette (the so-called "rosette method"), 3 to 5 weeks after thinning, is considered the most dependable of the various inoculation techniques studied. This method is quite simple and relatively inexpensive.

2. Substantial improvement in *Rhizoctonia* resistance has been achieved by selection in various sugarbeet populations. It is not known whether this improved resistance is effective against a wide range of *Rhizoctonia* races or biotypes. It apparently is relatively ineffective in early seedling stages.
3. Two *Rhizoctonia* resistant lines (FC 701 and FC 702), products of four cycles of mass selection for resistance, have been made available to the sugarbeet industry. They are not suitable for use as commercial varieties, and are considered valuable primarily as sources of genes for *Rhizoctonia* resistance.

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The Osmolality of Sugarbeet Press Juices

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In an investigation of the relationship between non-sugar chemicals and sugar in beets, we found that the calculated osmolality of press juice was remarkably constant (3)[2]. If there is a physiological limit to beet cell osmotic pressure one millimole of sodium chloride (59 mg) could displace about two millimoles of sucrose (648 mg). This would help to explain why the nutrition of beets is so important to the sugar content. It seemed worthwhile to examine other beet populations in an attempt to determine whether the osmolality of press juice samples is constant or is correlated in any way with the sugar content of beets.

Materials and Methods

Harvest Conditions—In this study no deliberate attempts were made to collect beet samples at the same time of day or under the same moisture stress.

Field Beets 1963—Twenty mature beets were selected from a field near Davis, California. Ten showed a positive reaction to Johnson's petiole nitrate test with diphenylamine-sulfuric acid, and ten showed a negative reaction (7).

Analysis of the 1963 field beets has been previously reported (3). Individual beets were reduced to brei and pressed to expell juice. Purified juice was prepared by the liming and phosphation of Carruthers and Oldfield (2), and sodium, potassium, total nitrogen and sugar by polarization (Pol) determined on the purified juice.

The total osmotic concentration of the 1963 beets was calculated from the N, K and Na concentrations expressed on the basis of press juice, and Pol measured on the press juice. We assumed that the non-sugar concentration is approximately the sum of the equivalents of nitrogen plus twice the equivalents of sodium and potassium.

Field Beets 1964—Thirty beets were collected from two fields near Clarksburg, California, representing three planting dates

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[2] Numbers in parentheses refer to literature cited.

and two levels of nitrogen fertilization. In Field A, three planting dates were represented in one small area, and beets were taken which had been planted about 8, 12 and 17 weeks earlier. Several weeks of cool weather following the two earlier plantings delayed the emergence and growth of the 17- and 12-week old beets. In Field B, the interior rows of beets gave a negative reaction for petiole nitrate but the end rows of beets had received additional applications of nitrogen and gave a positive reaction.

The 1964 field beets were ground to a brei in a vegetable chopper and pressed in a hydraulic press. Refractiometric dry solids (RDS) and sucrose by Pol were measured on a sample of the press juice. The remainder of the press juice was protected from evaporation, clarified by centrifugation at 33,000 g, frozen in small plastic tubes, and stored at —30° C.

Pot Beets—Brei from sixteen pot-grown beets representing three harvest dates was kindly supplied by Dr. Albert Ulrich, University of California, Berkeley. The beets were grown in pots, with adequate plant nutrients, at 20° C, with a 16-hour photoperiod at 3200 ft-c supplied by a combination of fluorescent and incandescent lighting. The beets were harvested after 9, 13 and 17 weeks. Sucrose was determined by Pol immedately, and 26-gm samples of brei were held frozen in plastic bags for approximately one year before receipt at this laboratory (8). Press juice was prepared from the quickly thawed brei in a hydraulic press. The press juice was clarified and frozen as described above.

Osmolality Measurements—The osmotic concentrations in the press juices from pot-grown beets and 1964 field-grown beets were measured with a Vapor Pressure Osmometer (Model 301A Mechrolab Inc., Mountain View, California). Measurements were made at 37.00° C, with sucrose solutions as standards. The osmolality values reported are the average of duplicate determinations on separately frozen samples of processed juice. The standard deviation of the observation was 12.8 milliosmoles per liter.

Results and Discussion

Table 1 is a comparison of press juice sucrose and calculated osmolality for the 1963 beets. There was no correlation between sucrose and calculated osmolality in this population. Furthermore the lower coefficient of varition for calculated milliosmoles indicates that the osmotic concentration is less variable than the sucrose concentration.

Table 2 and Figure 1 compare the press juice sucrose and actual osmotic concentration of the 1964 field beets. The meas-

Table 1.—Press juice sucrose and calculated osmolality of 1963 beets.

Sample	No. of beets	Sucrose average	v[1]	Milliosmoles average[2]	v[1]
High NO_3	10	13.45	12.7	777	4.9
Low NO_3	10	16.72	5.6	774	5.6
All	20	15.09	14.8	775	5.2

[1] Coefficient of variation as percent. $v = 100 \left(\frac{s}{\bar{x}}\right)$

[2] Calculated. Milliosmoles = (mM sugar + mM nitrogen + 2 (mM sodium + mM potassium))

Table 2.—Press juice sucrose and osmolality of 1964 beets.

Sample	No. of beets	Sucrose average	v[1]	Milliosmoles average	v[1]
Field A, 8 weeks	3	8.79	13.4	676	7.9
12 weeks	3	15.17	8.4	785	4.9
17 weeks	4	14.00	13.1	789	3.2
Field B, high NO_3	10	15.39	12.3	792	4.1
low NO_3	10	19.92	4.6	830	7.0
All	30	15.63	22.5	792	7.6
All except 8 weeks	27	16.59	17.0	805	5.7

[1] Coefficient of variation as percent. $v = 100 \left(\frac{s}{\bar{x}}\right)$

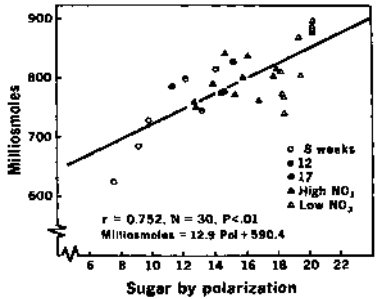

Figure 1.—Sugar content and osmolality of press juice from beets grown in 1964 at Clarksburg, California.

ured osmolality is very close to the calculated osmolality for the 1963 beets, but there is a highly significant correlation between sucrose and osmolality ($P < .01$). This correlation is due entirely to the beets with less than 10% or more than 19% sucrose in the press juice. Neglecting these beets, the correlation coefficient drops to 0.11, and the average milliosmoles is 787. Over

Table 3.—Beet sucrose and press juice osmolality for beets grown in pots.

Sample	No. of beets	Sucrose	v[1]	Millimoles average	v[1]
9 weeks	6	7.65	5.0	539	5.9
13 weeks	4	8.88	3.7	522	6.3
17 weeks	6	10.37	4.6	587	8.2
All	16	8.98	14.3	553	8.5

[1] Coefficient of variation as percent. $v = 100 \left(\frac{s}{\bar{x}} \right)$

a comparable range of sugar concentration, the results for the two years are in good agreement.

Table 3 compares beet sucrose and press juice osmolality for the pot-grown beets. They correlate significantly ($r = 0.583$, $p = 0.02$). The osmolality is much lower than for the field beets, even though the sugar content of the 13- and 17-week-old pot beets is higher than that of the 8-week-old field beets.

Plant physiologists have been studying the osmotic relations of plants for many years (1, 5, 6) and have developed much more refined methods than used in this study. The sap or press juice we obtained represents a mixture of contributions from all cells —active and non-active—in the beet root. At best, determination of osmotic pressure made on such juice represents no more than the average of the osmotic pressures of all the cells in the tissue. Even though the juices used were pooled fluids from different physiological structures, this study does show that the osmolality is less variable than the sugar content. It appears that there is no narrow physiological limit of osmolality of beet juices, as measured by the procedures used in these experiments.

It would be desirable to measure the osmotic pressure of the individual parenchyma cells that are involved in storage of sugar under the same moisture stress and without contamination of cell contents with free water from the vascular tissue and intercellular spaces. A new micro-technique has been reported that could determine the osmolality of beet cell sap with satisfactory precision (4). The preliminary results reported here indicate that the relation between osmolality and sugar content should be studied further.

Acknowledgments

We wish to thank Mr. Donald Griffin for making some of the osmotic pressure measurements, Dr. Albert Ulrich for the pot-grown sugar beet brei, and Mr. Norman Lawlor, American Crystal Sugar Company, Clarksburg, California, for aid in collecting beet samples.

Reference to a company or product name does not imply ap-

proval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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Biotreatment of Steffen House Waste

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Steffen waste is one of the most difficult to treat of the wastes produced in a beet sugar factory. The difficulty arises from its high BOD, heavy concentration of dissolved solids, pH in excess of 12.0 and high degree of alkalinity. It is distinguished by its heavy concentration of betaine, glutamine, amino acids and potassium. While the glutamine can be removed as monosodium glutamate, the market for the latter is very limited. Despite its possession of many characteristics adverse to biological activity, in time the waste becomes highly putrescible and malodorous through lowering of its pH level as a result of diffusion of atmospheric CO_2 into it.

In addition to those factors arising from the nature of the waste are those stemming from that of the beet sugar industry. One of the latter is the seasonal nature of the refining operation and the consequent inability to maintain a treatment facility at a constant capacity. Another is economic in nature. The profit margin in beet sugar manufacture usually is rather slim, and hence the industry must employ a treatment method in keeping with this limitation.

Biological treatment systems generally constitute the most reasonably priced of the various classes of waste treatment. Despite the highly unfavorable characteristics of the product as it leaves the beet sugar manufacturing plant, it must be amenable to some form of biological treatment since eventually it does putrefy when allowed to stand for a suitable length of time. The present investigation was made with the objective of developing a biological system for treating Steffen waste which could be applied with a minimum of assault on the environment and yet with a low financial expenditure.

Research Plan and Rationale

The overall plan of the research was to develop an aerobic biological system which could be adapted to lagooning, and

[1] Research reported in this paper was conducted at the Sanitary Engineering Research Laboratory of the University of California (Berkeley) by Dr. Ichikawa while on sabbatical leave from the Himeji Institute of Technology (Japan).

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possibly lead to the production of a feedstuff. The development of a process involving lagooning was made an objective because the capital and operating costs of lagooning systems generally are much lower than those of mechanical or of complex biological systems. The system was to be an aerobic one, primarily because aerobic processes generally are not characterized by the production of objectionable odors. The production of a feedstuff was made a goal since, aside from the conservation aspects, the sale of the feedstuff could be used to defray in part the cost of the treatment. So as to avoid overlooking a potentially desirable biological agent, the three principal classes of microbes, namely yeasts, algae, and bacteria, were studied with respect to the ability of each as a part of a mixed culture to serve in the decomposition of the waste, and to act to some extent as a symbiont.

The possibility of using yeasts in the treatment of Steffen waste is a very attractive one. Yeasts can and do serve as a proteinaceous feedstuff for livestock. They are efficient fermenters of carbohydrates, especially of sucrose. In addition to the sucrose normally present in Steffen wastes, yeasts can use the decomposition products of other microbes which take part in decomposition. Another important consideration is that many yeasts synthesize vitamins and nucleic acids which can serve as growth promoting substances for many forms of algae and bacteria. The CO_2 produced by yeasts in fermentation can assist in neutralizing raw Steffen waste. Since the process had to be an aerobic one involving lagooning, provision had to be made for the culture of algae. The function of the algae would be to supply the oxygen needed in the aerobic phase of the system. Hopefully, the algal concentration would be brought to a level exceeding 300 mg/1 or more, so that the algae could be harvested economically to serve as a feedstuff. There is no need to discuss the role of the bacteria. Suffice it to state that they would accomplish the major part of the decomposition and stabilization of the wastes.

The investigation was conducted in four major phases, in part sequential and in part concurrent. The principal concern of Phase I was the role of yeasts in the process. This phase of the investigation included making a comparison between the growth rates of 11 types of yeasts; determining the nature of the contribution of yeasts to the process, i.e., whether or not it is by way of the synthesis of some substance or substances which could be used to advantage by the algae and the bacteria; and finally, in evaluating the effectiveness of yeasts in decomposing the waste. In the second phase, factors related to the algae members of the mixed culture constituted the principal subject

for investigation. The experiments in this phase were concerned chiefly with deciding which of two available sources of algal inoculum was the more effective in treating Steffen waste. The third phase dealt with phosphorus nutrition. This phase involved the conduct of experiments dealing with the relation of PO_4 concentration to "seed" concentration, the effect PO_4 concentration itself and the comparative values of KH_2PO_4 and $Mg_3(PO_4)_2 \cdot 5H_2O$ as phosphorous sources. The fourth and last phase was concerned with the development of a system which could be used in a practical scale operation.

Materials and Methods

Ten species of yeasts obtained in pure culture and one in the form of "bakers' yeast" (Fleishmann's) were used in the study. The names of the species are listed in Table 2 in the section on Results. With the exception of the "bakers' yeast", cultures of the yeasts were obtained from Dr. Minoura, Department of Fermentation, Asaka University, Japan.

Pure cultures of algae and of bacteria were not used. Combined inoculums of algae and bacteria were obtained from samples removed from a two-thirds acre algae production pond located at the Richmond Field Station, and from an indoor culture of "high temperature" algae (33° - 38°C). The algal population of the pond at the time the experiments were run consisted almost entirely of *Scenedesmus* spp (approximately 95%). *Chlorella* spp constituted most of the remainder. No attempt was made to classify the bacteria, which, because of the influent to the pond, must have been those characteristic of sewage. *Chlorella* spp constituted approximately 95% of the population of the indoor culture, and Micrococcus, 5%.

In general, Erlenmeyer flasks (1500 to 2000 ml) were used as growth units. No provision was made for temperature control, hence the culture temperature was that of the ambient environment, viz. 25° to 29°C. In experiments involving the use of algae, light was supplied by a 30-watt daylight type fluorescent lamp. The intensity at the culture surface was approximately 350 f.c. Aeration was supplied the cultures by applying compressed air at 2000 ml/min at 15 min/hr intervals throughout an experimental run.

COD, BOD, biomass dry weight, nitrogen concentration, and algae cell count served as parameters. The COD and BOD values as given are those of the supernatant remaining after the algae had been removed by centrifugation. Unless otherwise specified, techniques described in Standard Methods[1] were used in making the analysis.

Naturally, Steffen waste was the major ingredient of the

media used in the investigation. In several of the experiments, carbon dioxide was bubbled through the raw waste prior to inoculation so as to precipitate the calcium in the waste and thereby lower the pH to a suitable level. The precipitate was removed by decanting and filtration. An analysis of a typical sample of Steffen waste before and after bubbling CO_2 through it is given in Table 1.

Table 1.—Analysis of Steffen waste.

Item	Raw (mg/l)	Treated with CO_2 (mg/l)
Solids (dry weight)		
Total	29,025	24,944
Volatile	16,664	16,566
COD	13,990	13,680
BOD	12,570	12,430
Nitrogen (total)	1,260	1,190
Sucrose		3,100
PO_4		0,024
pH	12.0	8.6

Procedures specific to the conduct of individual experiments or to particular sections of the study are described in the sections in which such experiments are discussed.

Results

Yeasts:

Growth Rate: In the experiments concerned with the comparative growth rates of several yeasts, the medium consisted of Steffen waste in which the calcium had been precipitated by bubbling with CO., and the precipitate ($CaCO_3$) removed by filtration. The pH of the waste after this treatment was 8.6. The yeasts were grown as stationary cultures in 30-ml test tubes (10 ml of medium per tube). Growth rate was determined by measuring the amount of biomass (dry wt) developed in 44 hrs and in 72 hrs. The names of the yeasts used in the experiment and the dry weights attained within the given times are listed in Table 2.

According to the information given in Table 2, *M. japonica* and S. *willians* surpassed the other yeasts in rate of growth. The highest total growth in the 72-hour period was attained by *S. carlsbergensis.* However, the concentrations of the yeasts *M. japonica, S. uvalis, S. willians* and bakers' yeast were fairly close to that of *S. carlsbergensis.* Apparently, *M. japonica, S. cerevisiae*

[4] Standard methods for the examination of water and wastewater. 1965. 12th Ed. Amer. Pub. Health Assoc. U. Y.

Table 2.—Growth rate of various yeasts on Steffen waste.

Yeast	Biomass (dry wt - mg/1)	
	44 hr	72 hr
Candida guilliermondi	94	146
C. tropica lis	22	89
Pichia membran aefaciens 1	25	67
P. membran aefaciens 2	56	73
Mycotorula japonica	252	253
Saccharomyces cerevisiae	199	200
S. carlsbergensis	163	293
S. fragilis	40	82
S. uvalum	110	248
S. ivillians	244	243
Bakers' yeast *{Saccharomyces* spp) (Fleischmann)	66	240

and s. w*illians* attained their full growth within 44 hours of inoculation.

Because of its rapid growth rate and of its proven value as a feedstuff, *AT. japonica* was selected as the yeast to be used in the remainder of the investigation.

Algae Growth Promoting Value of Yeasts: The assay of the algal growth promoting activity of yeasts was made by culturing algae in a dilute (1 part medium:5 parts distilled H_20) Molish's medium to which autolyzed yeast was added. Two types of autolyzed yeasts were tried. One was obtained commercially (Difco). The second was prepared by culturing *M. japonica* in Steffen waste (adjusted to pH 8.6 by $C0_2$ aeration) for 2 days, separating the yeast by centrifugation, washing the concentrate with distilled water and finally autolyzing the concentrate at 45° — 50°C. Ten-ml aliquots of medium were placed in 30-ml test tubes. Each tube was then inoculated with 1 ml of algal suspension obtained from the algae production pond. Casamino acids were added to one set of cultures to serve as a control.

The cultures were allowed to stand for a period of five days, at the end of which a visual estimate was made of the relative densities of the various cultures.

The results of the experiment are given in Table 3. According to the results, the autolyzed yeasts did have a substance or substances which promoted algae growth, since at all dosages above 0.05 mg/1 the growth in the presence of yeast autolysate was greater than that in its absence. That the substance was something in addition to casamino acids is attested by the fact that abundant growth was attained with the yeast concentration at 0.1 mg/1 of yeast B and at 0.2 mg/1 of yeast A, whereas the required concentration of casamino acids for equivalent growth

Table 3.—Algae growth promotion potential of autolyzed yeast and of casamino acids.

Amount of additive (mg/l)[2]	Relative algal growth[1]		
	Yeast A[3]	Yeast B[4]	Casamino acids
0	±	±	±
0.025	+	+	—
0.05	2+	+	+
0.1	3+	5+	+
0.2	5+	5+	+
0.4	—	—	5+

[1] From ±, which represents very slight growth to 5+, which represents abundant growth.
[2] i.e., additive in the form of Yeast A, Yeast B, or Casamino acids.
[3] Autolyzed *M. japonica.*
[4] Autolyzed yeast - commercial (Difco).

was 0.4 mg/1. The increased concentration of algae in the presence of yeast autolysates cannot be explained solely on the basis of availability of additional nitrogen, since the amount of added nitrogen would have been slight with respect to the total yield of algae.

Removal of COD by Yeasts: In the experiment on the effectiveness of yeasts in treating Steffen waste, CO, was bubbled through the Steffen waste until its pH level was lowered to 8.6. The waste was then filtered, and the filtrate was autoclaved at 25 psi for 15 min. Four-hundred-ml aliquots of the sterile filtrate were placed in sterilized 750-ml Erlenmeyer flasks. The contents of each flask were inoculated with one loop (2 mm in diameter) of a pure culture of *M. japonica.* The inoculated samples were aerated for 48 hours (2000 ml air/min.)

The average initial COD of the waste was 10,800 mg/1. At the end of the 48-hr period the average COD was reduced to 8,350 mg/1. Within the same period the biomass of the yeast increased until it reached an average of 700 mg/1.

Algae and Bacteria:

In the experiments conducted in this phase of the study, yeast *(M. japonica)* was grown in the medium prior to inoculation with algae and bacteria so as to allow the yeast to synthesize and release growth promoting substances into the medium.

Ratio of Medium to Inoculum and Type of Inoculums **In** the first experiment, $C0_2$ was bubbled through Steffen Avaste until the pH was lowered to 8.6. Precipitated $CaCO_3$ was removed by filtration. The filtrate (COD, 10,700 mg/1) was then inoculated with *M. japonica.* After two days, the yeast was removed by filtration, and the supernatant, its COD having been reduced to 8,350 mg/1 as a result of yeast fermentation, was inoculated with culture from the algae production pond at a ratio of 100:1.5.

The inoculated supernatant was aerated for a period of eight days, at the end of which the COD was down to 6,610 mg/1 (20% reduction). In an effort to hasten the stabilization process, additional inoculum was added at a ratio of 80:20. As a result of the dilution, the COD of the new mixture was 5,310 mg/1. After five days of aeration, the COD was reduced to 3,760 mg/1 (30% reduction).

Since the rate of COD reduction attained in Experiment 1 was too slow for practical application, and decreasing the ratio of medium to inoculum seemed to hasten it, the volume of inoculum was increased in the second experiment. The CO_L. bubbling and yeast culture applied in the first experiment was repeated in this one. The COD of the supernatant after these steps was reduced from an original of 10,700 mg/1 to 9,400 mg/1 (12% reduction). One half of the supernatant was inoculated with algae production pond culture at a ratio of 80 (medium):20 (inoculum). The resulting mixture had a COD of 7,500 mg/1. The second half received pond culture at a ratio of 60:40. The resulting COD was 5,700 mg/1. After five days of aeration, the COD of the first half was 4,300 mg/1 (24% reduction); and that of the second half, 3,100 mg/1 (28% reduction). Both samples were again inoculated, each at a ratio of 80:20 and were aerated for six days. The final COD of the first sample was 2,470 mg/1, and that of the second, 1,520 mg/1. This represented a 29% reduction for the former and a 43% reduction for the latter.

The third experiment was a repetition of Experiment 2, except that yeast (M. *japonica)* and seed were added to the filtrate simultaneously, i.e., without any pretreatment by culturing yeast in the waste. At the lower seed ratio (80 medium:20 seed), the COD reduction was 50% after the first seeding, and an additional 30% after the second. At the high seed ratio (60 medium:40 seed), the reduction at the end of the first step was 60%, and at the end of the second, 27%.

In the fourth experiment, the range of the ratios was broadened from 20 (medium):80 (seed) to 80:20. Equal volumes of culture from the algae production pond and from the indoor baths were used in each inoculum. With the exception of one, each culture was also inoculated with *M. japonica* and *S. cerevisiae.* As in the previous experiments, Steffen waste pretreated by bubbling CO_2 through it served as the medium. The results in terms of COD and total nitrogen reduction are given in Table 4.

The reduction in COD at the end of 3 days was almost the same at all dilutions, except that the reduction was much lower in run no. 5 (cf. Table 4) in which no yeast was added to the

Table 4.—Reduction in COD and total nitrogen at various ratios of volume of medium to volume of seed.

Exp. no.	Ratio Medium:Seed	Yeast	Days after Inoculation								
			0		3		6		12		
			COD	Tot.-N[1]	COD	Tot.-N	COD	Tot.-N[1]	COD	Tot.-N[1]	pH
1	20:80	+	2240	280	1060	—	670	150	600	50	9.3
2	40:60	+	4350	500	3140	—	1750	320	1650	200	9.1
3	60:40	+	6400	750	5540	—	3900	600	1920	450	9.3
4	80:20	+	8580	980	7400	—	6610	870	3000	610	9.2
5	80:20	—	8580	980	8030	—	6910	850	3620	710	9.3

[1] mg/l

culture. According to the table, after the third day the rate of COD reduction began to taper off quite rapidly at the lower dilutions, whereas it increased at the two higher dilutions. Except in run no. 2, percentage removal of initial COD declined progressively with decline in ratio of medium to seed, ranging from 73% in run 1 to 60% in run 5.

Type of Inoculum: A possible reason, other than that of dilution for the increase in COD reduction accompanying decrease in the ratio of waste to seed may have been the difficulty of pond algae to adapt to indoor conditions. This difficulty may have resulted in a slower microbial growth rate and a lessening of microbial activity. Doubling the volume of the inoculum resulted in doubling the number of cells introduced into the wastes, and hence of the total effect of their activity. Prior experience provides the basis for this conjecture. Throughout a decade of culturing algae under open conditions indoors and outdoors, *Scenedesmus quadricauda* and related species never appeared in appreciable numbers in indoor cultures; whereas they constituted from 85% to 98% of the algal population in the outdoor ponds. On the other hand, *Chlorella* spp and *Micrococcus* spp together always constituted from 85% to 99% of open cultures maintained indoors, and rarely more than a few percent of the algae in the outdoor pond. Since the present experiments were done under indoor conditions, it should be expected that an inoculum or seed of indoor-grown algae would respond more favorably than one from the high-rate pond. This concept was tested in an experiment in which seed obtained from algae production pond was compared with that consisting of "high temperature" *Chlorella* spp and *Micrococcus* spp grown in open baths in the laboratory.

The experiment was divided into two runs. In the first run *M. japonica* served as the yeast; whereas in the second run a mixture of *M. japonica* and *S. cerevisiae* were used. The waste to seed ratio with both types of algae inoculum was 80:20. Steffen waste pretreated by bubbling $C0_2$ through it served as the medium. The inoculated media were aerated for 6 days. At the end of the 6-day period, the cultures were re-inoculated (90:10 ratio), but the types were reversed; i.e., a culture originally seeded with algae from the production pond now received seed from the indoor algal culture.

The biomass yield in the wastes seeded with algae production pond algae was approximately 50% of that in wastes seeded with indoor bath culture. No appreciable difference could be attributed to type of yeast. The concentration of the culture seeded with pond culture and *M. japonica* was 510 mg/1; with pond

culture and M.. *japonica* and S. *cerevisiae*, 496 mg/1; with indoor bath culture and M. *japonica* and *S. cerevisiae*, 1,000 mg/1. Type of inoculum had no statistically significant effect on reduction in COD and nitrogen.

Phosphate Concentration:

Interaction of Factors: Because of the high pH of Steffens waste and the reactions involved in the process of which it is a product, the PO_4 content of Steffens waste is very low,—0.235 mg/1 in the material used in these studies. To determine the limiting effect, if any, on the growth and activity of the microbial population an experiment was conducted in which the programming was based on the "Orthogonal Square" method of experimental design. The factors which received attention are listed in Table 5. The program is shown in Table 6. Parameters used in determining the extent of the interaction of the factors were increase in biomass and decrease in nitrogen concentration and COD. The experiment was terminated at the end of twelve days.

Table 5.—Factors studied with respect to effect of phosphate concentration.

Factor	
Designation	**Description**
A_0	Yeast not added
A_1	Yeast added (*M. japonica, S. cerevisiae*)
B_0	Aerated (15 min/hr)
B_1	Not aerated
C_0	PO_4
C_1	PO_4 added (30 mg/l) (KH_2PO_4)
D_0	Inoculum from high-rate pond
D_1	Inoculum from indoor pond
F_0	Steffen waste to seed ratio, 8:2
F_1	Steffen waste to seed ratio, 9:1

The information gained in the studies is plotted in Figures 1, 2 and 3. The effects of the interaction of the various factors on combined biomass are indicated in Figure 1; on COD removal, in Figure 2; and on nitrogen removal, in Figure 3.

An analysis of the data as listed in the tables and plotted in the figures indicates that best results were obtained in those runs in which a combination of the following conditions were met: 1) PO, was added; 2) the cultures were aerated; 3) the cultures were inoculated with yeasts; and 4) the indoor algal culture served as the source of "seed."

PO_4 Concentration: Since the previous experiment demonstrated the importance of adding phosphorus to the Steffen waste, the next question was one of the optimum concentration of phos-

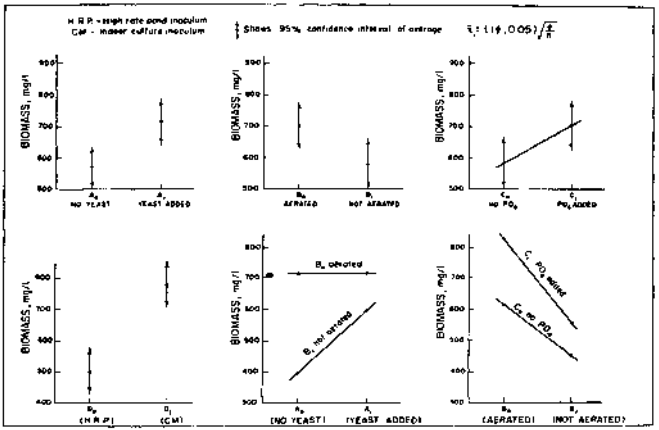

Figure 1.—Effects of interaction of various factors on combined biomass of bacteria, yeast and algae.

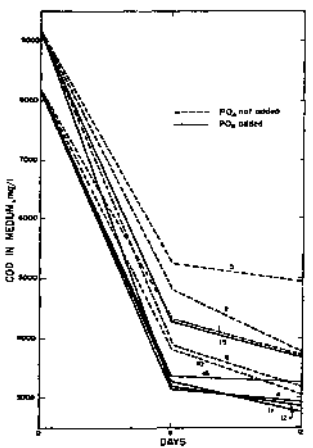

Figure 2.—Effect of phosphorus on COD removal.

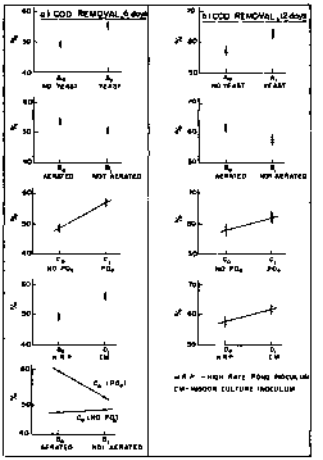

Figure 3.—Effect of various factors on COD removal.

phorus to be added. This determination was made in an experiment in which KH_2PO_4 served as the phosphorous source, and PO_4 concentrations ranged from 0 to 60 mg/1. The waste received the usual pre-treatment of bubbling CO_2 through it. It was then inoculated with *M. japonica* and S. *cerevisiae.* The algae-bacteria inoculum was obtained from the indoor algal culture and was added at a ratio of 90 (waste): 10 (seed). The cultures were aerated (15 min/hr), and were allowed to incubate for a total of 12 days. Analyses were made on the 6th and 12th days. (See Figures 4 and 5.)

The COD and the nitrogen concentrations of the cultures on the 6th and 12th days are listed in Tables 7 and 8, and the effect of PO_4 concentration of COD and nitrogen removal are shown in Figures 6 and 7. According to the data, the 6-day removal of COD increased approximately 0.44% for each mg/1 increase in concentration of PO_4 within the range 0 to 30 mg/1. Very little additional removal was attained at PO_4 concentrations above 40 mg/1. Apparently, PO_4 concentration had little, if any, effect on the short term (6-day) removal of nitrogen. However, on the long term (12-day), percent nitrogen removal increased 0.38% for each mg/1 increase in PO_4 concentration within the range 0 to 30 mg/1.

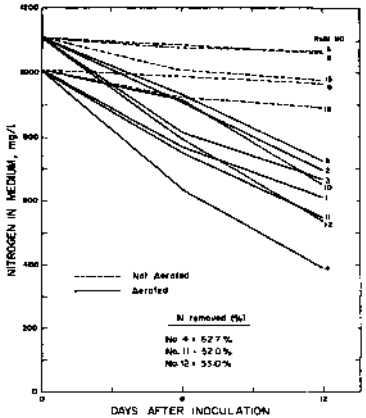

Figure 4.—Effect of phosphorus on nitrogen removal.

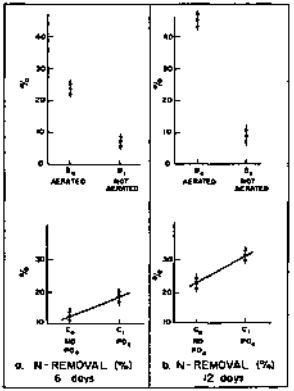

Figure 5.—Effect of various factors on nitrogen removal.

Table 6.—Experimental program for interaction of PO_4 and other factors.

Experiment no.	Column no.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	1	1	1	1			1	1
3	0	0	0			1	1	0	0	0	0			1	1
4	0	0	0			1	1	1	1	1	1			0	0
5	0	1				1	1	0	0	1	1			1	1
6	0	1				1	1	1	1	0	0			0	0
7	0	1				0	0	0	0	1	1			0	0
8	0	1				0	0	1	1	0	0			1	1
9		0				0	1	0	1	0	1			0	1
10		0				0	1	1	0	1	0			1	0
11		0				1	0	0	1	0	1			1	0
12		0				1	0	1	0	1	0			0	1
13		1	0			1	0	0	1	1	0			1	0
14		1	0			1	0	1	0	0	1		0	0	1
15		1	0		0	0	1	0	1	1	0		0	0	1
16		1	0		0	0	1	1	0	0	1	0	1	1	0
	A	B	A X B	C	A X C	B X C	A X B X C	D	A X D	B X D	A X B X D	C X D	A X C X D	B X C X D	A X B X C X D
	A_0 / A_1	B_0 / B_1	C_0 / C_1					D_0 / D_1							F_0 / F_1

Table 7.—Effect of PO_4 concentration on COD reduction.

PO_4-conc. (mg/l)	COD (mg/l)		
	0-day	6th day	12th day
0	9170	4230	3910
15	9170	3400	3020
30	9170	5890	3070
45	9170	6120	3020
60	9170	6150	2890

Table 8.—Effect of PO_4 concentration on nitrogen removal.

PO_4-conc. (mg/l)	Nitrogen (mg/l)		
	0-day	6th day	12th day
0	1140	780	599
15	1140	771	562
30	1140	769	468
45	1140	780	457
60	1140	778	457

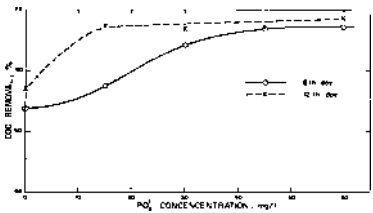

Figure 6.—Effect of $P0_4$-conc. on COD removal.

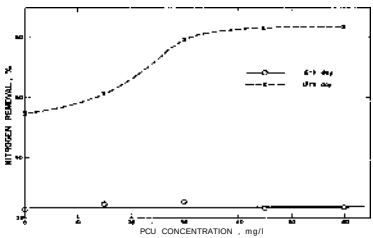

Figure 7.—Effect of $P0_4$-conc. on nitrogen removal.

$P0_4$ *Source:* Inasmuch as the addition of phosphate was shown to have a beneficial effect on the decomposition of Steffen waste, the question arose as to the utility of an inexpensive source of phosphate, i.e., an inexpensive phosphate compound which could be applied on a large scale without incurring an excessive economic penalty. To obtain an answer to this question, an experiment was run in which $Mg_3(P0_4) \cdot 5H_2O$ was compared with KH_2P0_4 as a phosphorous source. Although $Mg_3(P0_4)_2\ 5H_20$ is sparingly soluble in water, it is soluble in acidic solutions. Therefore the problem of high pH of Steffen waste and hence of poor solubility of the $Mg_3(P0_4)_2 \cdot 5H_20$ in it, can be lessened by lowering the pH of the waste by bubbling $C0_2$ or air through it and culturing yeasts and bacteria in it.

The conditions of the experiment were as follows: Steffen waste was pretreated by bubbling $C0_2$ through it and the resulting $CaC0_3$ precipitate was removed by filtration. A portion of the filtrate was enriched with the equivalent of 30 mg/l $P0_4$ in the form of **KH_2P0_4;** a second with $MgS0_4 \cdot 7H_20$ and 30 mg/l $P0_4$ in the form of KH_2P0_4; and a third with 30 mg/l $P0_4$ as $Mg_3(P0_4)_2 \cdot 5H_20$. The Mg in the second and third

portions was in a concentration of 10.9 mg/1. The portions were divided into 225-ml aliquots. Twenty-five ml of indoor algal culture were added to each aliquot, and each was inoculated with *At. japonica* and *S. cerevisiae.* The cultures were aerated for 15 min each hour.

The effect of $P0_4$ source on production of biomass and on COD and nitrogen removal are indicated by the data listed in Table 9. According to the data, yield of biomass in the presence of KH_2PO_4 and $MgS0_4$ was about 8.4% greater than that with KH.POj alone. The difference was greater when the algae were grown on $Mg_3(P0_4)_2 \cdot 5H_20$. Growth on the latter was 15% greater than that on the KH_2-PC),. The highest percentage of nitrogen removal (73.2%) was attained in the cultures provided with $Mg,(P0_4)_2 \cdot 5H_2O$ as the PO, source. Apparently, $P0_4$ source had but little effect on COD removal.

Development of a "Continuous" or "Series" System:
Series Systems: Having explored a number of important factors of concern in the biological treatment of Steffens wastes, an attempt was made to develop a system with which the information obtained in the study could be used in a practical application. An important characteristic of most large scale practical systems is continuity. Aside from the consideration of efficient usage of space and time, continuity ensures the maintenance of the microbial population at optimum age, and hence in peak condition. Because of the unavailability of suitable equipment for maintaining a continuous system in the strict sense of the term at the time of the investigation, a system embodying an approximation of continuity had to be followed. Because of its complexity the system which was developed and tested, is difficult to label. For lack of a better name, it was termed a "series" system.

The system as investigated in the laboratory study is perhaps best described with the use of the flow diagram shown in Figure 8. The experiment was begun by placing 500 ml of pretreated Steffen waste (i.e., bubbled with CO., until the pH level reached 8.6) in a 1500 ml Erlenmeyer flask, designated flask A in the following description. The waste was enriched with $Mg_3(P0_4)_2 . 5H_20$ at 30 mg/1 as $P0_4$. The enriched waste was seeded with 50 ml of indoor algae culture and inoculated with *At. japonica* and S. *cerevisiae.* The inoculated culture was aerated 15 min each hour at 2000 ml air/min. At the end of three days, 50 ml of culture were removed from flask A and used to "seed" another 500-ml aliquot of pretreated enriched Steffen waste contained in a second flask (1500-ml Erlenmeyer), designated flask B. The culture in flask B was aerated by passing through it the gaseous

discharge from flask A. The culture remaining in flask A was allowed to continue through a total period of 12 days (indicated by the dotted outlines of flasks in Figure 8 and entitled "Series I"). At the end of the sixth day (i.e., 3rd day for flask B), 50 ml were withdrawn from flask B and used to seed the pretreated enriched waste contained in a third flask, flask C, which was aerated in the manner indicated in Figure 8. The culture in flask B was allowed to continue through a total of 12 days, and is designated as Series II in Figure 8. At the end of the ninth day of the experiment (i.e., sixth day for flask B), an additional 50 ml of culture were removed from flask B and used to seed the enriched waste contained in a fourth flask,—flask D. The cultures in flasks C and D were aerated in the manner indicated in Figure 8. The two cultures also were allowed to continue throughout a 12-day period for each. They are entitled Series III and IV respectively. As the figure shows, the total duration of the experiment or combined series was 21 days, whereas the maximum per individual series was 12 days. Loss by evaporation was compensated by the addition of distilled water when needed. In Series I, II, and III, 90% of the culture remaining on the 6th day after the initiation of the culture was removed, centrifuged, and the supernatant returned to the flask. The same treatment was accorded the culture in flask D (Series IV) on the 9th day after its initiation.

Information gained during the course of the experiments with respect to COD and nitrogen removal are plotted in Figures 9 and 10. Judging from the slopes of the family of curves in Figure 9, the decline in COD was comparatively similar in the four series. No such similarity was noted in the removal of nitrogen, as is indicated by the relative slopes of the curves in Figure 10. The percentage removal of COD ranged from 72.4% to 74.9%, and of nitrogen, from 36.2% to 57.2%. The final biomass ranged from 1970 mg/1 to 2710 mg/1 (dry wt).

Secondary Treatment: An important step in a lagoon system of biotreatment is the treatment received by the wastes after they have been subjected to primary treatment. Hence an experiment was conducted in which the wastes were subjected to primary treatment for a period of 12 days. The primary treatment corresponded to that accorded Series I in the previous experiment, except that no biomass was removed during the twelve days. On the 12th day, the culture was divided into two aliquots, one of which was enriched with $Mg_3(P0_4)_2 \cdot 5H_20$ (equivalent to 30 mg/1 $P0_4$). Both aliquots were allowed to remain undisturbed, i.e., were not aerated, for a period of 185 days.

At the end of 35 days 16% of the COD of the wastes remain-

Table 9.—Effect of phosphorus source on yield of biomass and removal of COD and nitrogen.

Additive	Days after inoculation								
	0			6			12		
	Biomass (mg/l)	COD (mg/l)	Nitrogen (mg/l)	Biomass (mg/l)	COD (mg/l)	Nitrogen (mg/l)	Biomass (mg/l)	COD (mg/l)	Nitrogen (mg/l)
KH_2PO_4	–	11,080	1,054	1,310	3,920	727	1,380	3,580	422
KH_2PO_4 + $MgSO_4$	–	11,080	1,054	1,420	3,980	735	1,330	3,210	491
$Mg_3(PO_4)_2$ $5H_2O$	–	11,080	1,054	1,510	3,510	628	1,790	3,280	283

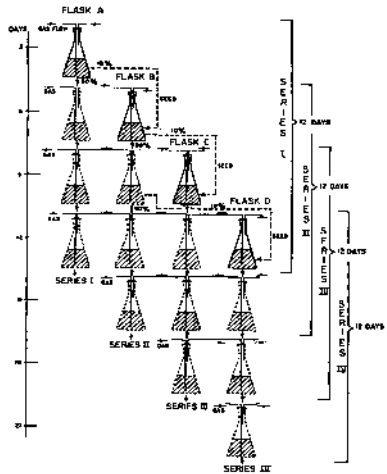

Figure 8.—Schematic diagram of biological system studied in the laboratory.

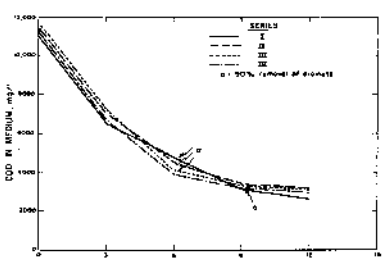

Figure 9.—Decline in COD concentration as a function of time.

ing after the 12-day primary treatment was removed from the aliquot not enriched with PO_4, whereas the removal from the enriched aliquot was 22%. The odor of NH_3 was quite pronounced in the unenriched aliquot; whereas that from the enriched aliquot was quite faint. At the end of 185 days the COD of the unenriched aliquot was reduced by 30.4%, and that of

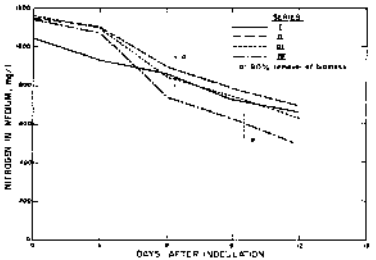

Figure 10.—Removal of N as function of time.

the enriched portion by 35.1%. The respective final CODs were 1650 mg/1 and 1540 mg/1. No ammonia odor was noted in the cultures.

Effect of Aeration: The rate of removal of COD during the period of the secondary treatment was very slow. To determine whether or not the rate could be increased by aeration, an experiment was conducted in which decline in COD and in nitrogen content was followed in an aerated and in a nonaerated culture.

As in the previous experiment, Steffen waste was subjected to primary treatment for a period of 12 days. At the end of that time, the COD of the medium was 2700 mg/1, and its nitrogen content, 600 mg/1. The medium was centrifuged and then divided into six aliquots. Four received $Mg_3(PO_1)_2 \cdot 5H_2O$, (PO_4, 30 mg/1). Two of the four aliquots were "seeded" with algae from an indoor culture, and two with algae from the algae production pond, and all four at a ratio of 90 (medium) : 10 ("seed"). The two unenriched aliquots were seeded with algae from the indoor culture, also at a ratio of 90 : 10. Three of the six aliquots (one with no added PO_4, one with $P0_4$ and indoor-culture "seed", and one with $P0_4$ and high-rate pond "seed") were aerated during 15 min each hour at 2000 ml air/min, while the remaining three received no aeration.

The extent of the COD and the nitrogen removal which took place during the 20-day period of the experiment is indicated by the data listed in Tables 10 (COD removal) and 11 (nitrogen removal). Removal of COD was from 27% to 67% greater in the aerated cultures than in the non-aerated cultures within the initial 10-day period, and from 22% to 40% at 20 days. Percentage removal of COD ranged from 42% to 73% greater in the aerated cultures enriched with PO_4 within the first ten days, and from 15% to 29% greater at 20 days. On the other hand,

the removal in phosphorous-enriched but non-aerated cultures was from 5% to 11% less than in the aerated cultures during the entire 20-day period. At the end of the first 10-day period, removal of nitrogen was from 84% to 90% greater in the aerated cultures than in those which were not aerated; and from 70% to 75% greater at the end of the 20-day period.

The relative degrees of stability of the supernatant of the various cultures is indicated by the data listed in Table 12, in which are shown the BOD to COD ratios of the various supernatants. As the data indicate, the BOD to COD ratios of the non-aerated cultures were from 3 to 5.5 times those of the aerated cultures; i.e., the carbon in the non-aerated cultures was in a far less stable form than was that in the aerated cultures.

Discussion

The research described in this paper brought out the importance of four requirements for the successful treatment of Steffen waste, viz. 1.) sizeable inoculum, i.e., a volume at least one-tenth that of the waste; 2.) an inoculum of organisms adapted to the environmental conditions encountered in the operation of the treatment system; 3.) enrichment with phosphorus; and 4.) suitable aeration. A large volume of inoculum not only provides an abundant microbial population, it also dilutes the waste and thereby somewhat diminishes the unfavorable environment constituted by the waste. The abundant initial population results in a shortening of the long lag period which occurs when a small inoculum is used. It also is instrumental in establishing a selected population, since the sheer number of inoculated organisms makes it difficult for competitors to establish themselves. The need for an adapted inoculum was shown by the heavier biomass production and greater reduction in nitrogen which took place when inoculums were used from cultures grown in the laboratory, as compared to those obtained when inoculum from the outdoor pond were used. Inasmuch as the requirement is one of adaptation, it follows that in large scale outdoor application, effluent from any satisfactorily operating facultative or algae production pond should be suitable.

The need for phosphate arises from the fact that the concentration normally present in Steffen waste (cf. Table 1) is lower than that needed for optimum growth of most microorganisms. Undoubtedly, as far as the algae are concerned, the required amount would be less than the 30 mg/1 applied in the experiments, since studies by others have shown the requirements for full growth to be a matter of a few mg/1. Even though

Table 10.—Effect of aeration on COD removal.

POi (mg/l)	Seed	Exp. no.	COD (mg/1) 10th day Medium (mg/1)	10th day Removal (%)	20th day Medium (mg/1)	20th day Removal (%)	Exp. no.	Not aerated 10 day Medium (mg/1)	10 day Removal (%)	20th day Medium (mg/1)	20th day Removal (%)
0	Indoor cult.	1	2300	14.9	1750	35.2	4	2410	10.8	1960	27.3
30	Indoor cult.	2	2130	21.1	1610	40.4	5	2410	10.8	2040	23.3
30	High rate pond	3	2000	25.9	1530	43.4	6	2531	6.3[1]	2000	25.9

[1]Foul odor

Table 11.—Effect of aeration on nitrogen removal.

Exp. no.	Nitrogen (mg/l) Aerated 10th day Medium (mg/1)	10th day Removal (%)	20th day Medium (mg/1)	20th day Removal (%)	pH	Exp. no.	Not aerated 10th day Medium (mg/1)	10th day Removal (%)	20th day Medium (mg/1)	20th day Removal (%)	pH
1	150	75.0	92	84.8	9.5	4	555	7.5	460	23.4	9.0
2	128	78.0	103	82.8	9.5	5	527	12.1	452	24.7	8.9
3	128	78.6	43	92.8	9.4	6	538	10.3	480	20.0	9.1

Table 12.—Effect of aeration on stability of supernatant[1].

Aerated				Not aerated			
Exp. no.	COD (mg/1)	BOD (mg/1)	BOD / COD	Exp. no.	COD (mg/1)	BOD (mg/1)	BOD / COD
1	1750	230	0.13	4	1960	1670	0.85
2	1610	270	0.17	5	2040	1370	0.67
3	1530	211	0.14	6	2000	1470	0.73

[1] Values given are those of medium on the 20th day after inoculation.

$Mg_3(P0_4)_2 \cdot 5H_20$ is difficulty soluble, its rate of solution is sufficiently rapid as to permit a rate and extent of growth equal to that obtained with KH_20_4.

Design For A Large Scale Application:

The discussion is closed with a suggested basic design for a large scale practical application of the system described in this paper, even though to venture any design at this time would *he* presumptious in view of the small scale of the experiments on which it is based. However, successful past experience with comparable systems in the treatment of other wastes furnishes some justification for making the attempt.

Before describing the design, it might be well to conjecture on the possible metabolic pathways that might be followed as decomposition of the waste takes place in the system so as to illustrate the functioning of the system. A diagrammatic sketch of possible pathways is presented in Figure 11. As the sketch shows, foul-smelling compounds are generated in the absence of aeration. On the other hand, the extent of the production of biomass under aerobic conditions exceeds that under anaerobic conditions.

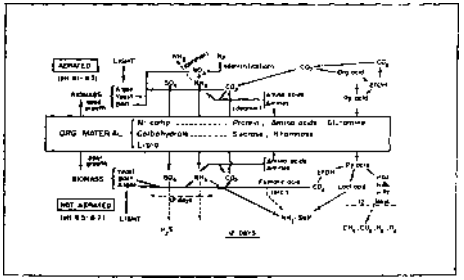

Figure 11.—Possible metabolic pathways in the biotreatment of Steffen waste.

A diagram of the proposed design is shown in Figure 12. In developing it, the assumption was that the waste would be pretreated before discharge into the biotreatment complex. The pretreatment would consist in applying sufficient aeration or in bubbling CO_2 to bring the pH of the medium to about 8.5 and

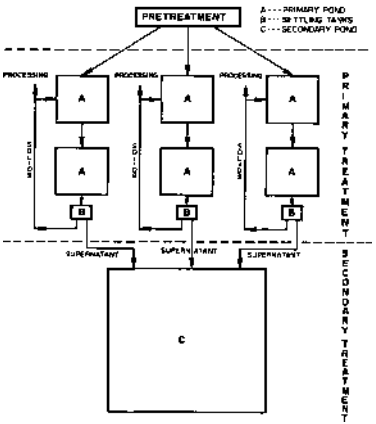

Figure 12.—Design for an application of a biotreatment process (units are not drawn to scale).

precipitate excess calcium. After pretreatment, the wastes are subjected to a 6-day period of primary treatment. The primary treatment complex consists of three sets of two-pond series, as is shown in the sketch in Figure 12. Each pond has a capacity equal to 1.1 day's output of Steffen waste, and each is equipped with an aeration device. Each pair of ponds is provided with a settling tank located at the distal end of the second pond.

In operation, in the initial run of a newly installed system, or at the start of a season, the first pond of each series is "seeded" with culture either from an algae pond or from a facultative pond having a fairly heavy algal population. The volume of the seed culture should be one-tenth that of the capacity of the pond. In each of the succeeding runs one-tenth of the pond volume is retained and serves as "seed" for the next run. Following the addition of the "seed", one day's output of Steffen waste is discharged into the second pond and retained there for another three days. At the end of this second 3-day period, it is passed

through the settling tank in which settable biomass would be removed. This settled biomass either may be returned to the first pond to augment the microbial population there, or may be processed for use as livestock feed. Passage through the settling tank marks the end of the primary treatment step.

The need for three series of ponds is obvious. While one day's output of waste is being detained in the first pond of the first series for 3 days, the second day's output can be discharged into the second series, and the third day's into the third series. Since by this time the first pond of the first series will have been emptied, the fourth day's output is discharged into it, and the cycle is repeated. The cycling continues until the end of the campaign.

Secondary treatment is accomplished in a single pond common to the three series of primary ponds. The settled effluent from the primary pond is discharged directly into the secondary pond. The volume or capacity of the latter should be large enough to provide a 60-dav detention period or to contain an entire season's production. Preferably, it should have no effluent, because even though the wastes are sufficiently stable to cause no odor problem in the pond, the COD of the effluent would be too high to meet public agency standards for discharge into a stream. However, stabilization could be hastened by providing some degree of aeration. If a lagooning system is already in use for treating flume water, one of the lagoons could serve as the secondary pond.

While many questions remain to be answered concerning details of the proposed design, the results obtained in the laboratory experiments demonstrate that the principles on which it is based are sound. The laboratory experiments showed that with pretreatment followed by six days of intensive primary treatment, decomposition of Steffen wastes can be advanced to a point at which the waste can be retained indefinitely in a lagoon without giving rise to an odor problem.

Summary

A description is given of the research involved in developing a biotreatment system for Steffen waste. The system incorporates the use of a mixed culture of yeast algae and bacteria grown on Steffen waste in lagoons. Yeasts were added to the usual algae-bacteria complex because yeasts are: 1.) efficient decomposers of sugars; 2.) synthesize vitamins and nucleic acids, which are growth promoters for algae and bacteria; 3.) produce $C0_2$ which is useful for lowering the pH of the Steffen waste and is a nutrient for the algae; and 4.), can be harvested for use as livestock feed.

Of eleven strains of yeasts tested in pure culture for rapidity of growth rate, *Saccharomyces cerevisiae, S. willian* and *Mycotorula japonica* had the highest growth rate,—reaching a concentration of 199 to 252 mg/1 in 44 hours. With the use of an autolysate of *M. japonica* and of a commercial autolysate, it was demonstrated that yeasts synthesize some substance (or substances) which contributes to the growth of algae. In a determination of the use of yeast as a primary treatment agent for Steffen waste, it was found that M.japonica inoculated into sterile Steffen waste reduced the COD of the waste by 23% in 48 hours, with an increase in concentration of biomass to 700 mg/1.

Results of experiments concerning ratios of inoculum to wastes and types of inoculums showed that for best results the ratio of the volume of inoculum to waste should be at least 1:9, and that it is essential for most efficient treatment to use as inoculum, algal-bacterial cultures adapted to the environmental conditions under which the waste treatment will take place.

The addition of PO_4 either as KH_2PO_4 or as $Mg_3(PO_4)_2 \cdot 5H_2O$ enhances the rate and extent of COD and nitrogen removal (the latter as high as 72%), and results in a significant increase in concentration of biomass.

A basic design was developed which was based on the principles investigated in the study. The design calls for three steps in the treatment of the waste: 1.) A pretreatment in which air or CO_2. is bubbled through the waste to lower the pH and precipitate calcium. 2.) Primary treatment in which the pretreated wastes are passed through a two-pond series with a detention of 3 days in each of the two ponds, and through a settling tank at the completion of the primary treatment. Biomass which settles out at this stage either can be recirculated as "seed" or can be processed for use as a feedstuff. It is important that the ponds be aerated. In the experiments, aeration was applied for 15 min each hour with good results. 3.) Secondary treatment, in which the discharge from the primary pond series is passed into a large facultative pond. This pond could be one of a lagoon complex in use for treating flume water wastes.

Acknowledgment

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Selection for Resistance and Chemical Control of Rhizoctonia Root Rot Disease of Sugarbeets[1]

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Most sugarbeets in Montana are grown under irrigation in heavy clay soils and are very often affected by *Rhizoctonia* root rot disease which causes considerable losses. Usually this disease becomes evident in the middle of the summer and gradually increases toward fall. Beets which become infected early with *Rhizoctonia* usually die quite rapidly. Beets infected later in the season may survive until harvest, however, their quality is poor and most of them become culls. It has been observed that infection of sugarbeets by *Rhizoctonia* in the seedling stage is not important in Montana. It is possible that cool temperatures early in the season are not conducive for the development of this disease (1,2,3)[3].

It appears that more *Rhizoctonia* root rot occurs in sub-irrigated or heavily watered soils than other soils. Short rotations with sugar beets also contribute to an accumulation of this disease. Introduction of longer rotations with cereals, corn and alfalfa are encouraged to help reduce the incidence of this disease.

In an attempt to control this disease various lines of sugarbeets were tested for resistance to this root rot. Numerous Montana selections of beets and selections supplied by various sugar beet companies and by agencies of the United States Department of Agriculture were tested. In addition, various chemical soil treatments were investigated in the field and in the greenhouse for control.

Testing Different Varieties of Beets for their Resistance to *Rhizoctonia* Root Rot Disease.

In recent years, four Montana selections of sugarbeets (A-339-1, 2, 3, 4) and lines received from Dr. R. K. Oldemeyer, Plant Breeder for the Great Western Sugar Company were tested for resistance to *Rhizoctonia* root rot disease. Originally Dr. Oldemeyer supplied us in 1962 with 48 varieties of beets to be

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[3] Numbers in parentheses refer to literature cited.

tested against this disease. Some of these lines were eliminated during 1963-65 tests because of their extreme susceptibility to *Rhizoctonia* or because of scarcity of seed. Only the following six varieties were tested during the 1966-67 period: 62108-8,10,16,26,27 and 39. In the spring of 1966, Dr. Oldemeyer supplied us with an additional 62 lines of beets for these tests. All 72 lines were tested for resistance to *Rhizoctonia* disease in highly infested soil at the Huntley Branch Station in 1966. The results of this test were rather disappointing because the degree of infection was rather low. It is possible, that cool temperatures of this summer prevented full development of this disease. Because of these circumstances it was decided to conduct further pathogenicity tests in the greenhouse in Bozeman.

In all these tests beets were planted in the greenhouse benches in steamed clay loam soil which was inoculated with several cultures of *Rhizoctonia* grown on steamed barley kernels. All seeds were disinfected with New Improved Ceresan. One row of beets $3^1/_2$ feet long was planted either with 40 whole or 80 segmented seeds which represented one replication of this test. In these tests Great Western Company variety GW 359 was used as a check. Beets were usually grown for 45-49 days and several readings were taken of healthy and diseased beets. At harvest time a final reading was made when each beet was examined for the presence of infection.

It was decided to submit the above mentioned 62 lines of beets which were received in 1966 to a screening test, and to test later more intensively those lines which showed a best resistance in the screening test. Each of these lines was planted in one row. The variation in percentage of healthy beets in these lines was considerable, and it varied from 1.1 to 68.1%. The check beets averaged 45.4% healthy beets. After analyzing these results it was decided to save for further tests only the 14 lines which had more than 50% of healthy beets in the screening test. These 14 lines of beets and also the above mentioned six Oldemeyer's and four Montana lines were submitted to an intensive pathogenicity test in which all these beets were planted in four replications. The results of these tests are presented in Table 1.

The results of this test show that there was some variation in the degree of resistance of these lines of beets to *Rhizoctonia* root rot disease. None of these lines showed an exceptionally high degree of resistance. However, the following seven lines 65102: 17-6, 27-3, 31-4, 31-5, 646: 9-1, 9-3 and 38-3 distinctly showed a higher degree of resistance to this disease than the remaining lines of beets. All lines except A339-1, had a higher percentage of healthy beets than the check (GW 359).

Table 1.—Testing sugar beet lines for resistance to *Rhizoctonia* root rot disease. Greenhouse 1967.

Sugarbeet lines	Sugarbeet plants	
Original No.	Healthy %	Diseased %
A 339-1	**10.2**	89.8
2	25.4	74.6
3	24.4	75.6
4	21.6	78.4
62108-8	24.6	75.4
10	22.4	77.6
16	21.0	79.0
26	22.5	77.5
27	27.2	72.8
39	31.6	68.4
6510217-6	43.7	56.3
27-1	35.4	64.6
27-3	40.8	59.2
31-4	41.8	58.2
31-5	38.8	61.2
646 0-4	34.4	65.6
9-1	39.9	60.1
9-3	39.6	60.4
17-4	30.2	69.8
38-2	32.3	67.7
38-3	43.2	56.8
39-3	30.3	69.7
41-2	32.8	67.2
41-3	26.4	73.6
GW 359	18.8	81.2

Control of *Rhizoctonia* Disease of Sugarbeets with Vitavax Treatments

It has been reported that Vitavax material (oxathiins) is especially effective against certain diseases of plants caused by *Basidiomycetous* fungi (4,5,6). This material was also suggested for treating sugarbeet seeds in controlling disease of young beets caused by *Rhizoctonia.* To investigate the effect of Vitavax on *Rhizoctonia* disease of sugarbeets, several tests were conducted in the greenhouse in Bozeman in which Vitavax was used for seed and soil treatment, and also as a spray.

These tests were conducted in greenhouse benches filled with clay-loam soil. The soil was steamed and inoculated with several strains of *Rhizoctonia* culture grown on autoclaved barley kernels. Each treatment was planted in three replications and each replication was represented by a 3.5 ft row of beets planted with 80 segmented sugarbeet seeds of GW 359.

First the maximum of adherence of Vitavax to sugarbeet seeds was established. It was found that 0.4 g of Vitavax would adhere to 240 segmented beet seeds which weighed 2.6 g. This proportion was used in establishing various rates of seed treatments.

First Test

In this test the following treatments were used:

1. Seeds were treated with $^1/_4$ of the maximum of adherence of Vitavax (0.1 g of Vitavax to 2.6 g of beet seeds or 62 oz for 100 lb of seed).

2. Seeds were treated with $^1/_8$ of the maximum of adherence of Vitavax (0.05 g of Vitavax to 2.6 g of beet seeds or 31 oz for 100 lb of seed).

3. Beet plants were sprayed three times with a solution of 250 ppm of Vitavax. The first spray was applied when beets had 2-4 leaves, the second time when they had 4-6 leaves, and the third time when they had 6-8 leaves.

4. Soil of each beet row (3.5 ft) was treated with 0.25 g of Vitavax. On the basis of row application, one acre of beets would receive 4.12 lb of 75% (or 3.09 of actual) Vitavax.

5. Check rows.

Beets were grown for 52 days. During this period readings of healthy and diseased plants were taken and the final readings were made at harvest time.

The results of this test are presented in Table 2. The number of healthy beets in both seed treatment tests was about the same and was equal to one fifth of the total number of beets. Beet plants showed some toxic effect from Vitavax which manifested itself in the form of a slight burning of leaves. Beets sprayed with Vitavax showed a slightly higher percentage of healthy beets than those where seeds were treated. In the soil treatment test practically no healthy beets survived. This was mainly due to toxicity of Vitavax and not because of the disease factor. Check beets showed only 14% healthy beets.

Table 2.—Seed and soil treatments and spraying of beet plants with Vitavex for controlling *Rhizoctonia* disease of sugarbeets.

Treaments applied	Percent of healthy and diseased plants			
	First Test		Second Test	
	Healthy %	Diseased %	Healthy %	Diseased %
1. Seed treatment $^1/_4$ of the maximum of adherence	21.0	79.0	—	—
2. Seed treatment $^1/_8$ of the maximum of adherence	21.9	78.1	28.8	71.2
3. Seed treatment 1/16 of the maximum of adherence			30.6	69.4
4. Spraying 250 ppm	28.4	71.6	13.0	87.0
5. Check	14.4	85.6	12.4	87.6

Second Test

Because of some toxicity to beets of Vitavax when it was used as a seed treatment and an extreme toxicity in the form of soil treatment, it was decided to conduct another test using lower amounts of Vitavax.

In this test seeds were treated with 1/8 and 1/16 of the maximum of adherence of Vitavax to seeds (equivalent to 31 and 15.5 oz of Vitavax respectively per 100 lb of seed). In the soil treatment only 0.125 g was used per 3.5 ft row of beets (about 2 lb of 75% Vitavax on an acre basis applied in rows). The same concentration of Vitavax was used for spraying plants in this test as in the first one. The same procedure was used here for planting, taking readings and harvesting beets as in the first test. Beets were grown for 33 days. The percent of healthy beets in both seed treatment tests was equal to about 30%. (Table 2). Plants in these tests again showed a slight toxicity from Vitavax. Beets sprayed with Vitavax and the check beets had about the same percentage of healthy beets (about 13%). Beets planted in treated soil again suffered a high degree of toxicity from Vitavax in spite of the fact that the amount of the chemical was reduced to half as compared with the first test. Because of this toxicity it was difficult to differentiate between symptoms of the disease and toxicity. For this reason no further discussion of soil treatments will be made in this paper.

Control of *Rhizoctonia* Disease with Terraclor and Potassium Azide Treatments

Field experiments were conducted in 1967 in an attempt to control *Rhizoctonia* root rot disease of sugarbeets by treatment of soil with Terraclor (PCNB) and potassium azide (KN_3) materials. The location of this test was near the town of Pompeys Pillar in South Central Montana. The sugarbeets were planted in 1967 in a field where beets were grown in 1966 and were severely infected with *Rhizoctonia* root rot. This grower usually follows a two-year rotation of beans and beets so in 1965 this field had been planted to beans.

The following materials were used as soil treatments. Terraclor granular 10%, Terraclor E. C. (emulsifiable concentrate), containing 2 lb of active per gallon and Terraclor Super X, E.C., containing 2 lb of active plus 0.5 lb of Terrazole (Olin compound 2424) per gallon. All the above materials were supplied by the Olin Company. Potassium azide (100% active) was furnished by the Pittsburg Glass Company.

Terraclor 10% granular was applied at the rate of 250, 500 and **1000 lb** per acre (25, 50 and 100 lb of actual, respectively).

Terraclor Super X, E.C. was used at a rate of 2, 4 and 6 gallons per acre and Terraclor E.C. in amounts of 4 and 8 gallons per acre. Potassium azide was used in amounts of 80 and 160 lb per acre. Granular Terraclor was broadcast and the azide, Terraclor Super X., E.C. and Terraclor E.C. were diluted with water and sprayed on the surface of the ground at the rate of 100 gallons of solution per acre. Chemicals were applied and the soil was rototilled, harrowed and planted on April 28, 1967. Unless otherwise stated, all treatments and the check plots were used in four replications. The size of Terraclor plots were 11 X 40 ft and azide plots 11 X 20 ft. Six rows of beets were planted in all these plots. Disease readings of beets were made twice during the summer and final readings were made at harvest time (September 19-20). At this time all beets in the four central rows of plots were pulled from the soil and examined for infection with *Rhizoctonia* root rot disease. The results of these tests are presented in Table 3. This table presents the average results on a percentage basis for four replications of all these treatments.

The greatest reduction in infection with *Rhizoctonia* was obtained in beets grown in plots treated with 1000 lb of granular Terraclor. The percentage of severely infected beets in this treatment was only about $^1/_3$ of that found in the check beets. Reduction of disease of beets grown in plots treated with 500 lb of Terraclor was quite comparable to the 1000 lb treatment.

Tabic 3.—Control of *Rhizoctonia* disease of sugarbeets with terracolor and potassium azide treatments.

Materials used	Per acre lb or gal	Healthy Beets %	Degree of infection *Rhizoctonia* with slight %	Degree of infection *Rhizoctonia* severe %
Terraclor 10% Gr.	250 1b	55.7	18.2	26.1
Terraclor 10% Gr.	500 1b	63.9	14.5	21.6
Terraclor 10% Gr.	1000 lb	66.0	15.1	18.9
Terraclor Super X, E.C.				
(2# + 0.5#/gal)	2 gal	45.2	16.7	38.1
(2# + 0.5#/gal)	4 gal	50.5	15.7	33.9
(2# + 0.5#/gal)	6 gal	45.4	17.8	36.8
Terraclor E.C.(2#/gal)	4 gal	55.0	22.1	22.9
Terraclor E.C.(2#/gal)	8 gal	56.4	15.6	28.0
Check		38.4	14.5	47.1
Potassium azide	80 1b	23.0	13.9	63.1
Potassium azide	160 1b	23.3	14.4	62.3
Check (only 2 rpl)		24.6	13.6	61.8

Beets with a silght degree of disease were considered to be those which had only a few superficial *Rhizoctonia* lesions. Severly infected beets were badly diseased and decayed and usually would not be accepted at the beet dumps.

Diseased beets in plots treated with 250 lb of Terraclor, 4 and 8 gallons of Terraclor E. C. ranged from 43.6 to 45.0% This amount of disease was slightly higher than for those treated with 500 and 1000 lb of Terraclor. Beets grown in plots treated with Terraclor X, E.C. had much more disease than beets grown in all the above mentioned plots. Check plots had the greatest amount of disease and about half of the beets in these plots were so decayed that they were of no commercial value.

The azide test was located in a different area of the field where the disease was very severe. For this reason a separate set of checks was arranged for this test. The results show that treatments with azide apparently did not have any effect on the control of *Rhizoctonia* root rot.

Conclusion

It is evident that some of the varieties of sugarbeets tested for resistance to *Rhizoctonia* root rot disease showed greater degree of resistance than the check beets, although none of them showed really an outstanding degree of resistance. Some inherent resistance apparently exists in sugarbeets for *Rhizoctonia* root rot, although multigenic inheritance probably decreases the possibility of obtaining highly resistant varieties among *Beta vulgaris* lines.

It appears that further development of sugarbeet varieties with resistance to this disease should be attempted either by concentrating resistant genes by recurrent mass selection or by searching for resistance to *Rhizoctonia* among wild species of *Beta* and incorporating this resistance into the commercial varieties of beets. This might result in the development of beet varieties with a higher degree of resistance to this root rot.

Existence of numerous pathogenic races in *Pellicularia filamentosa* greatly complicates breeding and selection work for resistance to *Rhizoctonia* disease of sugarbeets.

In an attempt to control the disease through the use of various chemicals which would suppress or destroy the causal organism, several materials were investigated in this study.

Experiments with Vitavax showed it to be generally ineffective in controlling *Rhizoctonia* disease of sugarbeets and to be toxic to the beets. It produced only a slight beneficial effect when it was used as a seed treatment and practically no effect when used as a spray. Vitavax was extremely toxic when used in the form of soil treatment.

Field test with Terraclor showed that PCNB used in amounts of 50 and 100 lb of actual material per acre produced a distinct beneficial effect in controlling *Rhizoctonia* disease of sugarbeets.

Other formulations of Terraclor alone or in combinations with Terrazole produced less beneficial effects probably because they contained smaller amounts of PCNB.

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Uptake of Phosphorus by Sugarbeets

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Introduction

Sugarbeets are an important cash crop in the Red River Valley of Minnesota and North Dakota. Proper fertilization and cultural practices are necessary to insure maximum net returns. Since the soils of the area are inherently low in phosphorus, the application of this element is essential for maximum yields. Any method which increases the uptake of phosphorus by sugarbeets and thereby increases the yield is of great interest to growers.

This experiment was initiated in 1964 to determine if sugarbeets take up more phosphorus from a mixture of ammonium nitrate and phosphate, or from phosphate fertilizer, and what effect these two fertilizer treatments may have on the yield, sucrose content, purity, total nitrogen and phosphorus content of the tops and roots.

Several workers (1,4,7,8)[2] have reported that corn and small grains absorb more fertilizer phosphorus when nitrogen in the ammonium form is mixed with the phosphate carrier than when fertilizer phosphate is applied.

Olsen et al. (6) reported that ammonium phosphate (11-48-0) and superphosphate were about equally available to sugarbeets. At the first sampling the plants absorbed less phosphorus from the ammonium phosphate than from the superphosphate, but thereafter about equal amounts were absorbed from the two fertilizers. Grunes et al. (3) found that the addition of ammonium nitrogen fertilizer orenerally increased the percent of total phosphorus absorbed by potatoes and sugarbeets from bands of concentrated superphosphate. Dubetz and Russel (2) reported that the early application of ammonium nitrate resulted in the highest dry matter and phosphorus content of seedling- roots and that time of nitrogen application had no effect on seedling beet tops.

Materials and Methods

In this experiment 0, 40, 80, and 120 Jb of phosphate (P as 0-46-0) alone and in combination with 0, 10 and 20 lb per acre

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[2] Numbers in parentheses refer to literature cited.

of nitrogen as ammonium nitrate (33-0-0) were applied in a narrow band at planting. A randomized block design on Bearden silt loam, a fine-textured soil retentive of moisture with a pH varying from 7.8 to 8.1, was used for the study. The plot area had been in black fallow the previous year, and no supplemental water was added during the growing season. Plots were six 22-inch rows wide and 50 feet in length, and the two middle rows were harvested for yield, percentage of sucrose, purity and total nitrogen and phosphorus content. May 13 was the average seeding date, and September 30 was the average date of harvest.

The roots and tops (petiole plus leaf blade) were sampled four times during the growing season for phosphorus determination. At each sampling, 12 plants were selected at random from each plot, and approximately six of the youngest leaves, both petiole and leaf blade, were taken. The average date for the first sampling was June 27 when the beets reached the eight-leaf stage and thereafter at monthly intervals.

Results and Discussion

Table 1 summarizes the data for the roots. The summer drought during 1964 reduced the yields and lowered the 3-year average yields given in Table 1. Since growing conditions during 1965 and 1966 were good, above average yields were obtained in those years.

Table 1.—Average sugarbeet yields and percentages of sucrose, purity, total nitrogen, and ppm of phosphorus in roots from the application of 10 fertilizer treatments made in 1964, 1965 and 1966.

Treatment rate/acre	Roots tons/acre	Percent sucrose	Percent purity	Percent total nitrogen	Ph^snhorus ppm
N P* K					
0-0-0	12.1	14.3	84.7	1.04	543
0-40-0	11.8	14.5	84.9	1.13	625
0-80-0	12.5	14.1	85.0	1.05	625
0-120-0	12.3	14.3	85.9	1.01	624
10-40-0	12.6	14.3	84.5	1.18	590
10-80-0	11.8	14.1	84.7	1.17	603
10-120-0	12.4	14.6	86.2	1.07	608
20-40-0	12.0	14,2	84.5	1.08	584
20-80-0	12.5	14.5	84.8	1.05	675
20-120-0	11.8	14.5	85.2	1.06	662
LSD (0.05)	N.S.	N.S.	N.S.	N.S.	72
LSD (0.01)	N.S.	N.S.	N.S.	N.S.	N.S.

* Rate in lb of P

There were no significant differences in the yield of roots as affected by the 10 fertilizer treatments. The three phosphate treatments average 12.2 tons per acre, which is 0.1 ton more than

the check plot. The addition of 10 and 20 lb of nitrogen to each of the phosphate treatments did not increase the yield, as stated in the literature (1,2) in experiments with sugarbeets and other crops. The fertilizer ratio of nitrogen to phosphate was not a critical factor as measured by the yield of beets.

The percent sucrose was very similar for all the treatments. The three phosphate treatments averaged 14.3% sucrose, which is identical to the check. The addition of 10 and 20 lb of nitrogen to the phosphate treatments did not bring about any significant change.

Purity is an important factor in the processing of sugar, and the additon of nitrogen fertilizer may influence this factor. The purity for the three phosphate treatments was higher than the check, but the differences were not significant. When 10 to 20 lb of nitrogen were added to the phosphate treatments, three of the purity values were above and three below the check, but the results were not significant.

The percentage of total nitrogen varied from a low of 1.01 to a high of 1.18, but this variation is not significant. Eight of the treatments were higher than the check. The percentage of total nitrogen decreased as the rate of phosphate increased both for the phosphate alone and with the addition of 10 and 20 lb of nitrogen.

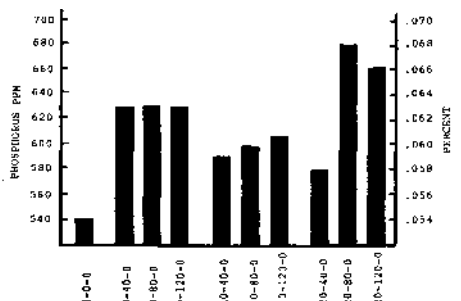

Figure 1.—Phosphorus content of sugarbeet roots as affected by 10 fertilizer treatments, Avg 1964 - 1966.

Figure 1 gives the phosphorus content of the roots in ppm and percentage for the 10 fertilizer treatments. The phosphorus content varied from a low of 543 in the check to a high of 675 ppm for the 20-80-0 treatment. The results from the three phosphate treatments were identical and were significantly different

from the check at the 5% level. The addition of 10 lb of nitrogen to each of the phosphate treatments lowered the phosphorus content, but they were not significantly different from the check or the three phosphate treatments. The 20-80-0 and 20-120-0 treatments were the highest in phosphorus content and were significantly higher than the check.

The average phosphorus content of the tops and roots for the three growing seasons at the four sampling dates is given in Tables 2 and 3, respectively. The greatest uptake of phosphorus by both tops and roots occurred in the early spring, and the amount decreased as the growing season progressed. The tops contained considerably more phosphorus than the roots. On June 27, the first sampling date, the percentage of phosphorus in the tops varied from a low of .591 for the check to a high of .697 for the 0-120-0 treatment. This treatment had the highest phosphorus content for three of the four sampling dates and also for the total average. There was no significant increase in the phosphorus content of the tops by increasing the rates of phosphate. The addition of 10 and 20 lb of nitrogen did not produce any consistent results for the different sampling dates.

When one considers the average data for the four sampling dates in Table 2, all the treatments, except 10-40-0, were significantly higher in phosphorus than the check. The amount of phosphorus varied from a low of .370% for the check to a high of .428% for the 0-120-0 treatment. This treatment did differ from the check and the other treatments except the 20-120-0 at the 5% level.

Table 2.—Phosphorus content of sugarbeet tops for four dates of sampling resulting from the application of 10 fertilizer treatments, Avg 1964 - 1966.

Treatment rate/acre	Date of sampling				Avg
	June 27	July~29	Aug. 31	Sept. 22	
N P K	Percent phosphorus				
0-0-0	.591	.346	.284	.258	.370
0-40-0	.627	.371	.287	.293	.395
0-80-0	.604	.365	.307	.304	.395
0-120-0	.697	.368	.341	.305	.428
10-40-0	.620	.348	.296	.281	.386
10-80-0	.647	.374	.300	.301	.406
10-120-0	.639	.384	.299	.296	.405
20-40-0	.648	.355	.309	.280	.398
20-80-0	.657	.358	.313	.297	.406
20-120-0	.671	.379	.296	.296	.411
LSD (0.05)	NS	NS	NS	NS	.020
LSD (0.01)	NS	NS	NS	NS	.026

When treatments were separated in the analysis of variance for the tops, the nitrogen and nitrogen-phosphorus interactions

were not significant. The phosphorus, however, was significantly different from the check.

The uptake of phosphorus by the roots as shown in Table 3, paralleled that of the tops. On the first sampling date, the phosphorus content varied from a low of .394% for the check to .484% for the 10-120-0 treatment, a variation of .090%. At the last sampling date, the phosphorus content of the roots was the lowest and varied from a low of .091% for the check to a high of .115% for 20-120-0 treatment. The increased rates of phosphate did not produce any consistent increase in phosphorus content. When 10 lb of nitrogen were added to each of the phosphate treatments, the results were variable and followed no definite pattern.

Tabic 3.—Phosphorus content of sugarbeet roots Or four dates of sampling resulting from the application of 10 fertilizer treatments, Avg 1964 - 1966.

Treatment rate/acre	Date of sampling				Avg
	June 27	July 29	Aug. 31	Sept. 22	
N P K		Percent phc•sphorus			
0-0-0	.394	.172	.125	.091	.196
0-40-0	.476	.191	.139	.097	.226
0-80-0	.475	.211	.142	.111	.235
0-120-0	.454	.209	.152	.113	.232
10-40-0	.451	.180	.137	.106	.219
10-80-0	.449	.188	.134	.099	.218
10-120-0	.484	.191	.152	.112	.235
20-40-0	.450	.175	.143	.102	.218
20-80-0	.466	.209	.146	.114	.234
20-120-0	.465	.233	.147	.115	.240
LSD (0.05)	NS	NS	NS	NS	.013
LSD (0.01)	NS	NS	NS	NS	.017

The addition of 20 lb of nitrogen to the three phosphate treatments did produce a slight successive increase in the phosphorus content of the roots at each sampling date.

Examining the average results for the four sampling dates in Table 3, we observed that all the treatments were significantly different from the check at the 1% level. The 20-120-0 treatment contained the highest amount of phosphorus, and the 10-80-0 and 20-40-0 had the lowest.

Figures 2 and 3 give the phosphorus content of the sugarbeet roots and tops, respectively, for the four sampling dates with an average for each treatment. The largest uptake of phosphorus was on the first sampling date and is indicated by the longest bar on the left hand side of each figure. The results for both roots and tops for all the sampling dates were rather uniform and exhibited no definite pattern as a result of the different rates of phosphate or the addition of 10 and 20 lb of nitrogen.

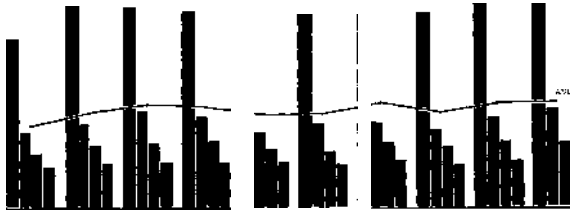

Figure 2.—Phosphorus content of sugarbeet roots for four dates of sampling as affected by 10 fertilizer treatments, Avg 1964 - 1966.

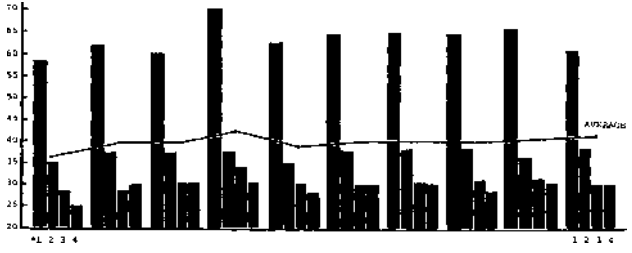

Figure 3.—Phosphorus content of sugarbeet tops for four dates of sampling as affected by 10 fertilizer treatments, Avg 1964 - 1966.

From previous work (5,9) high rates of nitrogen alone and in combinations with phosphate have lowered the percentage of sucrose and purity, and increased the total nitrogen content in sugarbeets. No adverse effects have been noted when lower rates of nitrogen (10-20 lb N) were used alone and in combinations with phosphate on this crop. In this trial there were no significant differences in the percent sucrose, purity and total nitrogen, and no great differences were anticipated.

The application of high rates of phosphate have not always increased the yield of beets in this area. With a pH of 7.8 to 8.5, some phosphate reverts to the insoluble tricalcium phosphate. The plot area in this trial tested medium in available phosphate and had been in black fallow the previous year. The phosphorus levels of both tops and roots on the check plots were high, indicating an available supply of this element was present in the soil. All of these factors may have influenced the yields and uptake of phosphorus by sugarbeets in this trial.

Summary

This experiment was undertaken to determine whether sugarbeets take up more phosphate from a mixture of ammonium nitrogen and phosphate or from phosphate fertilizer alone, and what effect this addition might have on the yield, sucrose content, purity, total nitrogen and phosphorus content of the tops and roots.

The addition of nitrogen (ammonium ion) to the three phosphate treatments did not significantly increase the yields of roots in this experiment. There were no significant differences among treatments in the percent sucrose, purity and total nitrogen content of the roots.

The greatest increase in the phosphorus content of the tops and roots took place at the first sampling date, and this amount decreased rapidly as the growng season progressed. There were no significant differences among treatments in the phosphorus content of the tops and roots for the individual sampling dates. However, the treatment averages for the tops, except 10-40-0 treament, were significant at the 5% level, and all treatment averages for the roots were significant at the *1%* level.

Acknowledgment

The author wishes to thank Dr. R. E. Finkner and Mrs. Libbie Eccles of the American Crystal Sugar Company for carrying out part of the chemical and statistical analyses, respectively, and Dr. J. M. MacGregor, University of Minnesota, for the chemical analyses of the tops and roots.

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The Use of Food-Grade Phosphoric Acid in Processing Sugarbeet Diffusion Juice to Obtain a By-Product Feed Supplement[1]

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The warm diffusion juice from sugarbeet cossettes is commonly processed by adding about 2% to 2.5% CaO (pH over 12 at 25C) to decompose invert sugars etc. and to remove impurities in the form of insoluble calcium salts. Addition of carbon dioxide to remove excess lime and adsorbed impurities occurs in two stages. Most of the impurities are removed after the first carbonation to a pH of about 11.2. A second carbonation of the relatively clear "first carbonation juice" brings the pH to about 9.2 with relatively slight precipitation. Filtration then gives a clear, somewhat amber-colored "clear, or thin" juice, which is ready for evaporation and further processing. The voluminous precipitate of $CaCO_s$ and organic matter that is discarded in the lime pond approximates 100 pounds of dry matter per ton of beets.

The availability of low cost, high purity, food-grade phosphoric acid, made via the electric furnace, has led to expanded use throughout the food industry and increased production of dicalcium phosphate for use as a feed supplement.

In the processing of sugar beets, it seemed of interest to attempt to replace the massive amounts of lime and carbonic acid with adequate quantities of lime and phosphoric acid, since the reaction product would have a commercial market. The present paper describes experiments to determine the minimum amounts of lime and acid needed to produce a clarified juice equal in purity to that presently produced by the factory, and to find out the quantity and composition of the resultant "mud" in terms of its value as a feed supplement. Rate of filtration etc. after different treatments was determined.

Literature Review

Sugar textbooks (5, 6)[3], patent literature and journal papers describe the infinite variations, difficulties and complications of

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the purification process, together with proposals for improvement. Among these reports are many that involve the addition of phosphates or phosphoric acid to remove calcium and other impurities. A patent search has shown at least eight reports since 1924 of patented methods by means of which phosphates have been claimed to give improved clarification, color, etc. (7). For the most part, cane and fruit juices have been involved, with primary emphasis on the means of facilitating removal of the precipitate. Journal articles, as Frankenhoff (3) and Cummings (2), are in agreement that "the use of calcium phosphate as a defecant in the refining of sugars is an old practice," which was rather generally discarded when pressure niters were introduced. 'Calcium phosphate plugs the pores and openings in the diatomaceous filter cake" and leads to slow filtration. It still has the advantage that its use promotes the production of a very clear juice, and it can be used in relatively minute amounts to assist in removing colloidal material by flotation, filtering or settling. The Carruthers-Oldfield (1) method of using phosphoric acid with massive amounts of lime to produce clear juice for purity determinations has shown clearly that this method will remove impurities from expressed juice fully as well as by carbonation of limed diffusion juice, and will produce a clear juice of good color by ordinary laboratory nitration.

Materials and Methods

Factory personnel took hourly samples of diffusion juice and thin juice during one shift each day throughout the campaigns of 1964-65 and 1965-66. These samples were frozen. At the end of the season they were thawed and composited, by weeks. These weekly composited samples, about 15 each year, were used in the individual experiments described. Using standard precision instruments, analyses for purity, lime salts, alpha amino nitrogen, potassium and sodium were made on juice purified with phosphate in the laboratory in comparison with that purified with carbonate in the factory. Rates of filtering, settling, etc. were determined with ordinary filter paper, graduated cylinders, etc. with no attempt to accurately simulate factory conditions.

Experimental Results

The 15 composited diffusion juices, for each year, were individually purified with CaO-H_3PO_4 in the laboratory and compared with those purified with CaO-$C0_2$ in the factory. Table 1 summarizes the data for the two seasons. Lime salts were determined by the company chemist.

During the 1964-65 campaign, treatment of the diffusion juice

Numbers in parentheses refer to literature cited.

by CaO_aP0_4 consistently gave a higher purity than that processed by the factory ($CaO\text{-}CO_2$). In either year, when correction was

Table 1.—The 15-week averages of analyses of juitc after purification with CaO-HsPOi (laboratory) versus $CaO\text{-}C0_2$ (factory), during the 1964-65 and 1965-66 campaigns.

	1964-1965		1965-1966	
	$CaO\text{-}H_3P04$	$CaO\text{-}CO_a$	$CaOH_3PO_4$	$CaO\text{-}C0_2$
Clear juice purity %	90.75	89.65	92.54	92.93
Mg. amino N/100 g sucrose	306	313	213	201
Mg. Potassium/100 g sucrose	1086	1187	1067	1131
Mg. Sodium/100 g sucrose	120	464*	130	243*
Lime salts,%				

* Sodium added by factory.

made for the sodium carbonate added to the juice in the factory, the juices were almost identical. No significant difference was found in the content of potassium or amino nitrogen in the juices processed with $CaO\text{-}H_3P0_4$ and $CaO\text{-}CO_2$. Although sodium carbonate was added to factory purified juices for the purpose (among others) of reducing the soluble calcium salts, the "lime salts" content of the factory juice was regularly about double that of the phosphated juice to which no soda was added. Obviously the additional sodium reduces purity and is avoided when adequate purification can be attained without it.

Minimum amount of lime to produce a satisfactory clear juice In about 60 separate trials, factory diffusion juices were brought to various pH values with lime or phosphoric acid, heated at various temperatures and filtered. Ease and clarity of filtration and color of filtered juice were noted. In the resultant clear juices, purity and content of sodium, potassium and amino nitrogen per lOOg sugar were determined.

Initial liming to a minimum of pH 11.8 (25C) was necessary to produce a juice as good as the factory juice. After adjusting the pH to 11.2 (25C) with acid, optimum purities were obtained by bringing the juice to a temperature of about 70C (during 8 minutes of heating) before filtration. After the first filtration, the addition of acid to pH 9.2 (25C) to juice of about 60C seemed to *give* optimum clarity but no improvement of purity over somewhat lower temperatures.

The minimum amount of lime required to bring 2400 pounds of diffusion juice (equivalent to about 1 ton of beets) to pH 11.8 was determined to be approximately 8.8 pounds of CaO. To bring this juice to pH 11.2 required about 5.5 pounds of 85% phosphoric acid. Obviously, the more lime is added, the more acid must be used to accomplish this. After the first filtra-

tion, about 10% additional acid was required to reach ph 9.2, or a total acid requirement of about 6 pounds per ton of beets. Following the second addition of acid, the precipitate was light and cloudy and was not saved for weighing.

Amount of precipitate formed in the first phosphatation mud The "mud" from 13.6 pounds of diffusion juice, treated with minimum lime as indicated above, was collected, oven dried, and found to weigh slightly over 20 pounds per ton of beets, varying somewhat with different juices. Since the phosphate is precipitated as (approximately) Ca_{10} $(OH)_2$ $(P0_4)_6$ (hydroxy-apatite), (Ca/P ^ 2.13) under these conditions, (4) the acid used could precipitate a maximum of 8 pounds of apatite in the first phosphatation mud, per ton of beets. The rest of the precipitate would be mainly in the form of organic matter, and calcium salts of acids, removed in increasing the purity from that of diffusion juice to that of clear juice.

Analyses of the mud Analyses by two independent laboratories gave the following figures (Table 2) on a dry matter basis of two lots of mud. From the analyses, it would appear that a little less than one-half the dry matter in the first phosphatation mud was organic matter, with a protein (Kjeldahl N X 6.25) content of about 20%. Since all of this organic matter was in solution or colloidal suspension, one would expect its digestibility to approach 100%. The proportion of calcium to phosphorus (about 2:1) indicates that considerably more phosphoric acid would need to be added to the mud to make the mud equal to the commercial dicalcium phosphate in calcium phosphorus ratio (Ca/P — 1.3:1). *(See discussion).*

Table 2.—Analyses of Mud Precipitated with CaO and H_3P0_4

	I	II	Commercial dicalcium phosphate
Phosphorus	5.7 %	5.8 %	19%
Protein	9.28%	9.22%	None
Calcium	10.9 %		22-23%
Ash		53.79%	
Moisture		3.88%	

Stability of phosphated clear juice during evaporation The quality of the factory diffusion juice was such that about 1.6 pounds of sodium carbonate was added per ton of juice the first year, on the average, and less than half this amount the second year in order to get proper defecation, lower lime salts, etc. To give some indication of the stability of thin juice prepared

with $CaO-H_3PO_4$ but without sodium carbonate, it was evaporated by heating under the vacuum of a water-aspirator to 65% solids. A drop in pH from 9.2 to 9.0 indicated a rather stable juice, without the addition of sodium carbonate.

Filtration of untreated first-phosphatation juice Because enormous amounts of processed juice must be filtered per minute in a sugarbeet factory, ease of filtration or other means of separation of insoluble solids is a primary necessity for operation. In the laboratory, we tried the ordinary practices used to get larger crystals or floccs as by "seeding", avoiding high degree of supersaturation, recirculation of crystals already formed, etc. In no case did we get substantial improvement in filtration rate. Use of extra CaO, in general, decreased filtration rate, both in Michigan and Monsanto experiments, but sometimes improved purity slightly.

Use of flocculants in the separation of mud by filtration, settling or centrifuging The use of several flocculants (Separan® AP-30, Kelco Gel® LV, Polyhall® 295)[4] in various concentrations produced pronounced improvement in the rates of filtration, settling or separation by centrifuging. Addition of the flocculant in low concentrations was more effective than addition in high concentration. Table 3 gives an idea of the effect of one of the flocculants used. Addition of flocculant to carbonated juice did not improve filtration or settling rate. Vacuum or pressure filtration of $CaO-H_3PO_4$ treated juices was more difficult than gravity filtration, primarily due to the small particle size and the compressible nature of the solids relative to the $CaO-CO_2$ precipitate. About twice as much flocculant was required to achieve clarity and rapidity of filtration in vacuum or pressure filtration as in gravity filtration. Rate of settling was enormously accelerated in laboratory tests by use of flocculants. Still lower levels of flocculants (0.01-0.02 pounds per ton of juice) appeared to be satisfactory if the solids were removed by centrifuging. This may be the easiest, and, in the long run, the cheapest way to remove waste solids from this juice.

No effort was made to approximate closely the practices that could be used in a factory, or to conduct an exhaustive survey of the efficacy of possible acceptable flocculants, either alone or in combination, or to estimate their effects on sugar quality or the acceptability of the mud as a feed supplement. However, Polyhall 295 is approved by the Food and Drug Administration for use in sugarbeet juice purifications. It seemed likely that

*Dow Chem. Co., Midland, Michigan, Kelco Co., Clark, New Jersey, and Stein Hall & Co., New York, New York respectively.

Table 3.—Comparative rates of gravity and pressure filtration of CaO-CC>2 and CaO-HsPCh treated beet juices, with various amounts and costs of flocculant (Polyhall 295) added.*•

Treatment of Juice, pH 10 (80 C) (pH 11.2 25C)	Conc, of Polyhall 295 solution, %.	Flocculant Treating Level		Filtration			Flocculant cost per ton juice
		cc/100	lbs;, active/ton juice	Time, 20 cc Gravity min.	Rates, cc/min. cumulative volume 20cc*75cc*		
2%CaO-C02	0	0	0	2:00-4:00			0
0.5% CaO-HaPOi	0.001	30	0.006	3:30			$0.0084
ditto	0.005	10	0.01	4:15			$0,014
ditto	0.005	20	0.02	2:35			$0,028
ditto	0.005	30	0.03	1:00			$0,042
2% CaO-$C0_2$	0	0	0		40	6	0
0.5% CaO-H_3P0_4			0.02		5.3	0.7	$0,028
ditto			0.03		11.4	2.8	$0,042
ditto			0.04		60.6	8.7	$0,056

* Pressure 26 psi. Filter area 9.6 cm^2, No. 1 filter paper

[1] C. E. Miles, Special Report No. 6921 "Use of Phosphoric Acid in Refining of Beet Sugar", Research and Development Dept., Inorganic Chemicals Division, Monsanto Company, St. Louis, Mo.

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the acceptability or the complications in industrial application could not be accurately inferred from small scale laboratory trials, no matter how comprehensive.

Whether separation was by gravity filtration, suction or pressure filtration, settling or centrifuging, an expense of from $0.01 to $0.05 for flocculant per ton of beets appeared likely to solve the problem of large scale separation of the precipitate from the juice.

Discussion

In the context of these trials, some discussion of the probable cost of materials, and value and uses of the product seems pertinent. The probable costs of materials seem relatively easy to establish. When the lime and acid were used at the minimum rate of produce a thin juice equal in purity, but superior in color, to the $CaO\text{-}C0_2$ thin juice, a total of about 9 pounds of CaO and 6 pounds of 85% H_3P0_4 was needed per ton of beets. This includes all acid used for purification, but not the acid required to convert the apatite and lime salts into feed-grade dicalcium phosphate ($CaHP0_4$. 2H..O). With CaO at about 1.4c and acid at 6.5c per pound, this would amount to about 52c per ton of beets. An additional cost for flocculant of about 3c per ton of beets would bring the processing cost to about 55c for materials.

The 6 pounds of 85% acid would precipitate about 22 pounds of total dry matter in the mud from a ton of beets, including the precipitate from the second addition of acid. Of this, about 9 pounds would be organic matter containing about 20% protein, 9 pounds would be hydroxyapatite and about 4 pounds would be CaO combined with weak organic acids. If the phosphorus and calcium from the beet roots were not considered, the rate of calcium to phosphorus would be about 4:1, on the basis of the purchased calcium oxide and phosphoric acid that was used. This ratio is somewhat high for most nutritional purposes. (Commercial "dical" has a Ca/P ratio of about 1.3 to 1.) Analyses of the mud, with a Ca/P ratio of about 2:1 shown in Table 2, indicate that the contributions from the roots may be of significant economic value. Although it would be expected that this contribution would vary widely in different regions, analyses of beets, pulp, molasses and mud indicate that the phosphorus recovered from a ton of beets (about 0.8 lb.) would have a value of about $0.20, if sold as dical. If the analyses shown in Table 2 are representative of the Ca/P ratios to be generally expected, no further addition of acid would be required to make the mud marketable for many nutritional purposes.

Nutritionists tell us that in many regions, where corn silage and urea are replacing legume hays (high in calcium) a Ca:P ratio in the supplement of 2 to 3:1 might be more generally acceptable for cattle than the 1.3:1 ratio in dical. As a supplement for hogs, where the ratio is high in grain, and thus in phosphorus, a still higher ratio of calcium might be advantageous. In Michigan, for example, in feeding swine on a standard corn-soybean meal ration, ground limestone as well as dical is needed in the supplement, on a 50-50 weight basis, to obtain the optimum Ca:P ratio of 3.6:1. If such a product could be furnished swine feeders at a competitive price, they would rather handle one material than two. In such a case, payment for the phosphorus content only would not be realistic, since the calcium also must be purchased.

Thus, it might not be desirable to add sufficient acid to convert all the CaO to the dical ratio of calcium to phosphorus. If this were done, however, and there were no contribution of phosphorus or calcium from the beets, an additional 12 pounds of 85% acid would be needed. This would be in the form of a cheaper "feed grade" acid. The total of 9 pounds of CaO and 18 pounds of acid would produce about 28 pounds of feed-grade dicalcium phosphate (Ca/P 1.3:1) mixed with about 9 pounds of organic matter with approximately 20% protein content. Each of these ingredients should be valued at going prices, — about $90 per ton for dical, and $60 for the protein supplement. The commercial value of feed-grade dical is roughly equivalent to that of the lime and acid used. In any case, any desired ratio of calcium to phosphorus could be provided at varying prices, regardless of whether the lime and phosphate are purchased as such, or come partly from the beets.

The operation at the sugar factory level might stop with the separation of the traditional wet mud, which could be sold to and processed further by a concern interested in the feed supplement. Although prices have been proposed, this seems a matter for inter-company bargaining.

From the standpoint of the beet sugar company, the operation of the lime kiln might be eliminated and the traditionally offensive lime pond rendered obsolete. The greatly reduced quantity of mud—perhaps one fifth—would be expected to affect filtration practices considerably and to lower the loss of sucrose in the mud. All lime and acid purchased (as well as phosphorus from the beets) could be sold as a feed supplement. The organic matter removed—about 0.5% on beets—would contain about 20% protein and its value might pay the processing costs of the mud. A thin juice equal to or better than $CaO\text{-}C0_2$ juice

in lime salts, purity, color and stability on boiling should be produced, possibly without the necessity of adding soda ash. Use of phosphoric acid, without the necessity of bubbling with carbon dioxide containing free oxygen, should reduce the alkaline oxidation of sucrose (6). The literature generally reports less trouble with evaporator scaling when phosphate is used (6). The lower color and the lack of bicarbonates should favorably affect the sulphitation process—or even eliminate it—and the use of a strong acid should reduce the problem of high viscosity due to potassium carbonate (6). Since we are not dealing with a volatile acid, —CO_2, and the alkalinity of the juice might be more stable on evaporation, the pH after completion of the second nitration might be reduced somewhat to reduce sucrose and amide hydrolysis due to —OH ions (5). In general, one might expect that the use of a non-volatile and highly heat-stable acid would enable a precision to be reached in processing diffusion juice that is difficult or impossible to attain with carbonic acid.

The yearly production of by-product from a factory processing about 200,000 tons of beets per year would approximate 3000 tons of dicalcium phosphate and 1000 tons of 20% protein supplement.

Summary

Slightly less than 0.5% CaO, on beets was sufficient to bring factory diffusion juice to pH 11.8 (25C) which was the minimum alkalinity required to produce a clarified juice as good or better than factory "thin" juice. About 0.3% of 85% $H_{3i}P0_4$, on beets, was needed to bring the limed juice to pH 11.2 (25C), (heat and filtration) and to a final pH 9.2. Purity of the juice thus treated was essentially identical with that of factory thin juice, with sodium, potassium and amino nitrogen almost the same when adjustment for added soda ash was made. Lime salts were about half as high in laboratory phosphated juice (without soda) as in the factory carbonated thin juice, to which soda ash had been added. Flocculants were required at the rate of about 0.01-0.03 pounds per ton to promote filtration or settling at a rate as rapid as that found with carbonated juice, with or without flocculant. With the quantities of chemicals specified above, slightly over 1% of dry matter on beets was precipitated. This dry matter contained over 40% organic matter, with a protein content of about 20%. The precipitate had a calcium/phosphorus ratio of about 2:1. The addition of about 12 pounds of additional acid (assuming no phosphorus from the beets) would bring the calcium/phosphorus ratio up to about 1.3:1, which is the ratio in commercial dicalcium phosphate feed supplement.

It is proposed that all of the lime and phosphoric acid purchased can be sold. The additional high protein supplement that is mixed with it should also have a considerable market value. The phosphorus from the beets, commonly discarded in the lime pond, would have a substantial value when recovered and sold as a feed supplement.

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The Influence of Planting Rate and Thinning Method On Sugarbeet Stand[1]

CLARENCE F. BECKER[2]

Received for publication February 7, 1968

The importance of sugar beet stand on the basis of number and spacing has been reported by several investigators. Friehauf, *et al.* (I)[3] reported that percent stand showed a positive effect on yield with maximum yields obtained from approximately 150 beets per 100 feet of row. Herron, *et al.* (3) indicated that the highest yield in Kansas occurred with approximately 25,000 sugarbeet plants per acre. Figure 1 shows the relationship between the number of beets harvested per 100 feet of 22-inch row and yield for each contract in the Worland-Riverton factory district of Wyoming during 1966. The linear regression line indicates an average increase in yield of 0.2 ton per acre for any increase in plant population of one beet per 100 feet of row, within the range of 30 to 120 beets per 100 feet of row.

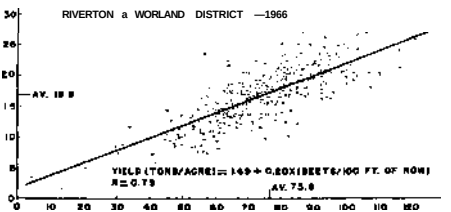

Figure 1.—Relationship between yield and number of beets harvested per 100 feet of row spaced 22 inches.

The average number of beets harvested was much lower than the recommended stand of 100 to 120 beets per 100 feet of row. Average yield could potentially have been greater with

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[3] Numbers in parentheses refer to literature cited.

increased stands. Even though growers who harvested 60 beets had yields as high or higher than others who harvested 100 beets per 100 feet of row, more than one half of the variation among yields for the Riverton-Worland factory district was explained by differences in the number of beets harvested. Variation in other important cultural and production factors such as tillage, fertility, irrigation, weed control, planting date, etc. accounted for the remainder of the yield differences.

It is common to plant excess sugarbeet seeds and then thin the emerged plants to the desired stand. Planting directly to stand has many advantages and will be practiced when good weed control and a relatively high and predictable emergence rate becomes the rule. Seedlings with increased vigor and reduction and control of hazards such as soil crusting, insect damage, toxicity from herbicides, etc. would allow planting directly to stand. Overplanting with subsequent plant thinning allows some insurance against unpredictable factors affecting emergence rate and stand reduction associated with mechanical weed control operations.

Plant Spacing

If for theoretical considerations we assume a uniform seed spacing of (s) inches in the row and seed that is 100% monogerm, plants spaced (s) inches will account for (e) portion of the total, where (e) is the emergence rate. Since the probability of a combination of independent events is the product of the independent probabilities, (1 - e)e of the plants will be spaced 2s, $(1 - e)^2$ of the plants 3s, etc., or:

$$\bar{x} = es + e(1 - e)2s \ldots e(1 - e)^{n-1} ns = \frac{s}{e} \qquad [1]$$

where: $\bar{x}$ = average plant spacing after emergence

Down the row random mechanical thinning has been practiced many years. Machines used for this operation cut out plants occupying fixed (but adjustable) portions of blocks of plants in the row. The theoretical portion of the total plants removed is equal to the ratio between the length of block cut out and the center distance between blocks.

If we assume that the remaining blocks after thinning contain plants in the same proportion that existed in the field prior to thinning and that the length of block skipped is small enough to contain one plant, the random thinner then leaves a minimum plant spacing of L. Applying the same theory used in equation [1]:

$$\bar{x}_r = pL + p(1 - p)2L \ldots p(1 - p)^{n-1} nL = \frac{L_s s}{L_s e} \qquad [2]$$

where: $\bar{x}_r$ = average spacing of plants after thinning
L = center distance between blocks
L_s = length of block skipped

$$p = c\frac{L_s}{s}$$

Selective thinning, whether it be the man with the hoe or a machine which cuts out portions of the row only after the presence of a plant has been detected, can be utilized. If the length of block skipped (L_s) after detection of the plant is small enough to contain one plant, the probability that a plant will exist in a block of L inches is p. Another group of plants will be spaced ($L + L_s$) with a probability of (1 -p)p etc. Utilizing an equation derived by Garret (2):

$$\bar{x}_s = pL + p(1-p)(L + L_s) + p(1-p)^2 (L + 2L_s) \ldots$$
$$p(1-p)^{n-1}[L + (n-1)L_s] = L + L_s\left[\frac{1-p}{p}\right] \quad [3]$$

where $\bar{x}_s$ = average spacing of plants after selective thinning.

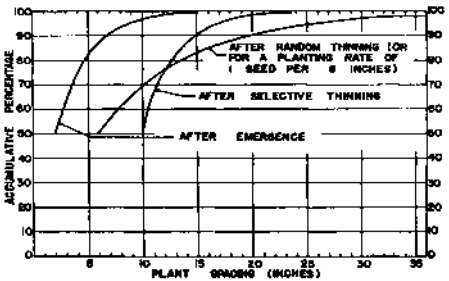

Figure 2.—Theoretical plant spacings before and after thinning for a seed spacing of 2 inches, emergence rate of 0.5 and a final plant population of one per foot of row.

Figure 2 shows the theoretical accumulative percentage of plants at various spacings before and after selective and random thinning for a uniform seed spacing of 2 inches, emergence rate of 0.5 and a final plant population of one per foot of row. Comparisons show that the use of a selective thinner should theoretically result in less variation of the plant spacing from the desired average when the emergence rate is less than 100%. Alternately, for a given minimum plant spacing the total plant population will be increased, i.e., if the minimum spacing or

block length (L) was 8 inches, seed spacing (s) 2 inches and the emergence rate (e) 0.5, the plant population will increase by 60%.

Field Evaluation

During spring, 1967, a field evaluation of sugar beet plant spacings before and after thinning by random mechanical thinners, hand labor and an electronic selective thinner was made on 27 different 100 foot lengths of row near Lovell and Powell, Wyoming.

The electronic selective thinner used was one of a limited production model of the Eversman Selectronic Row Crop Thinner. An electric eye senses the presence of a plant in the row. After the electric eye beam is interrupted by a plant, a knife actuated by a fast-action air cylinder removes plants ahead of the sensed plant for a pre-determined distance. This distance is adjusted by coordinating forward speed with the time the knife is held in the thinning position. The longitudinal position of the eye and forward speed will affect the distance ahead of the sensed plant at which the knife begins to cut.

Tall weeds, clods of soil and other debris in the beet row will also interrupt the beam from the electric eye and cause activation of the knives. Therefore, good seedbed preparation, weed control in the row, and relatively uniform plant height are prerequisites to proper operation of the electric eye selective thinner.

Three different commercially available random thinners were used in fields where stand evaluations were made.

A 10 foot length frame with marks 0.1 foot apart was placed adjacent to the beet row to determine the spacing between the individual beet plants to the nearest 0.1 of a foot. The determination was made on the same 100 foot interval before and after thinning in order to determine which plants were removed during the thinning operation.

Results

Table 1 shows the average number of sugar beet plants spaced at various intervals before and after different methods of thinning, i.e., hand hoeing, random mechanical and electronic selective thinning. The percentage of plants before and after thinning spaced at various spacings was calculated. The observed accumulative percentages could then be compared with the theoretical (or calculated) percentages using formulas [1], [2] and [3]. The calculated or theoretical plant spacings for selective thinning can be considered the best possible for the seed spacing (assumed uniform), emergence rate and final number of beets per 100 feet of row observed.

The results of these calculations are shown in Figures 3, 4 and 5 for hand, random and electronic selective thinning, respectively. Figure 5 shows the results secured with the electronic selective thinner after field adjustment.

Discussion of Results

Figures 3, 4 and 5 indicated that the observed and calculated or theoretical plant spacings after emergence compare favorably with spacing calculated using Formula [1]. However, plants spaced one inch or less are observed because some small percentage of the seed is multigerm and some seeds are spaced at intervals less than the average seed spacing due to non-uniform seed placement by the planter.

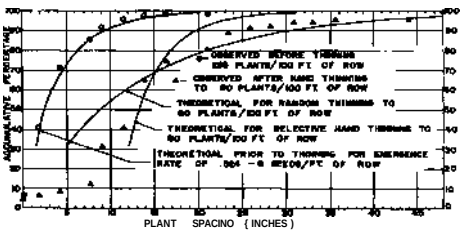

Figure 3.—Comparison between the observed and theoretical plant spacings before and after hand thinning.

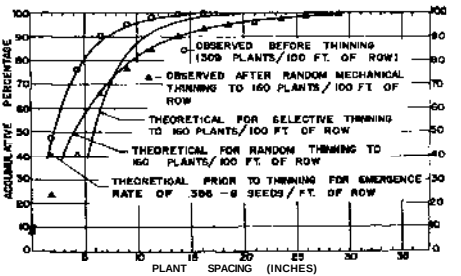

Figure 4.—Comparison between the observed and theoretical plant spacings before and after random mechanical thinning.

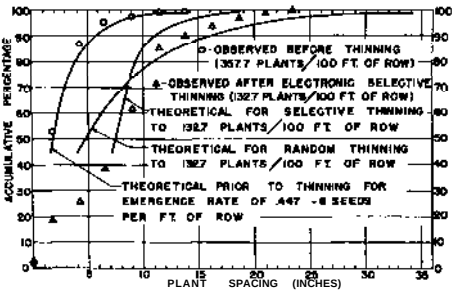

Figure 5.—Comparison between observed and theoretical plant spacings before and after selective electronic thinning. Average of three tests after field adjustment with beets at uniform height and rows relatively free of large weeds.

Table 1 and Figure 3 indicate that the average stand for seven observations after hand thinning was slightly higher than the average number of beets harvested per 100 feet of row in the Worland-Riverton factory district during 1966 (80 vs. 75.6). If the number of plants spaced less than 1 inch and some harvest loss is considered, the stands were probably very nearly equal. The results of hand thinning on the average indicated fewer and a lower percentage of plants spaced at small intervals (less than 1 or 5 inches) than for mechanical methods; however, this is probably due in part to the removal of a greater portion of the plants by hand thinning as indicated by final stands of 79 to 80 plants per 100 feet of row compared with 178 and 133 for random and selective thinning, respectively. One observation of hand thinning with a final stand of 100 beets per 100 feet of row indicates 11 plants at intervals of less than one inch and 18 less than 5 inches.

A comparison of the observed and theoretical accumulative percentage of plants at various spacings indicates that hand thinning resulted in a higher portion of spacing- at large intervals than theoretically possible with perfect selective thinning. The number of plant intervals of 40 inches or more was nearly equal to that which would theoretically result from random mechanical blocking to the same stand. The portion of the plants spaced 24 to 36 inches or more was less than would have resulted with random mechanical thinning but more than would have resulted with perfect selective thinning.

Figure 4 indicates that the observed number of plants spaced

Table 1.—Average number of sugarbeet plants spaced at various intervals before and after different methods of thinning—Theoretical planting rate of 8 seeds per foot of row.

Method		Number of observations	Average no. plants/100 ft	Less than 1	1 - 5	6 - 10	11 - 15	16 - 20	21 - 25	More than 25
Hoe	before	7	259	16	169	53	15	3	2	1
	after		79	8	8	18	28	13	5	8
Hoe (greatest stand)	before	1	247	33	131	57	21	1	3	1
	after		100	11	7	27	26	18	8	3
Hoe (least stand)	before	1	201	7	115	55	20	1	1	2
	after		46	....	....	5	8	11	3	19
Random thinner #1	before	2	309	29	206	60	12	2	....	....
	after		161	12	54	57	22	8	4	4
Random thinner #2	before	2	417	29	345	38	4	....	1	....
	after		174	9	72	63	19	5	3	3
Random thinner #3	before	2	366	28	253	45	10	....	....	....
	after		200	17	96	55	21	8	2	1
Electronic selective thinner	before	11	389	25	330	40	3	1	....	....
	after		164	8	64	50	29	8	3	2
Electronic selective thinner (after field adjustment)	before	3	358	7	304	40	6	1	....	....
	after		133	8	31	48	38	9	4	....

at various intervals alter random thinning can be calculated with a reasonable degree of accuracy if the final desired stand and planting and emergence rates are known. The figure shows the results for one of the three machines studied since the performance of all three were similar. However, as with the initial stand, plants spaced 1 inch or less are observed due to the non-uniform spacing of seed in the row and multi-germ seed.

The plant spacing after thinning with the electronic selective thinner for eleven different observations, two-thirds of which were taken while the machine was being adjusted, where large weeds were present in the row, and where the beets were not of uniform size, indicated intervals similar to those expected with a random mechanical thinner. However, after adjustment, field experience and operation in fields where the beets were relatively uniform in height and free of large weeds in the row, the resulting plant spacings approached the theoretical plant spacings for selective thinning (Figure 5 and Table 1). A higher percentage and number of plants were spaced less than 1 and 5 inches than for hand thinning. On the other hand, a larger number and percentage of the plants were spaced in the desirable range of 6 to 10 inches and the maximum observed spacing was 25 inches in 300 feet of row compared to an average of eight plants spaced more than 25 inches per 100 feet of row for hand thinning.

Summary and Conclusions

1. Observed and calculated or theoretical plant spacings compare favorably except for plants spaced at small intervals (1 to 2 inches or less) due to multi-germ seed and non-uniform seed placement in the row during the planting operation.

2. The number of plants spaced at various intervals after random thinning can be calculated with a reasonable degree of accuracy for a given final stand if the planting and emergence rate are known. Plants spaced at small intervals will be observed for the same reasons mentioned in the previous paragraph. The plant spacings after thinning will theoretically be the same as they would have been if the planting rates had been reduced to give the same final stand without thinning. Although studies were not made on weed control during mechanical thinning, it is assumed that the percentage of weeds removed from the beet row will be equal to the ratio between the length of block cut out and the center distance between blocks.

3. Observation of plant spacings after use of the electronic selective thinner while it was being adjusted, where large weeds are present in the sugarbeet row and where beet size was not

uniform, indicated spacings very much the same as would be expected with a random thinner. However, after adjustment and operation in a field with beets of relatively uniform height and free of large weeds, the plant spacings approached the theoretically best possible spacing for the emergence rate observed and the seed spacing used. A higher percentage and number of plants were spaced less than 1 and 5 inches, but a larger number and percentage were spaced in the desirable range of 6 to 10 inches when compared with hand thinning. The number and percentage of plants spaced 24 inches or more was less than for hand or random thinning. Theoretically, the selective thinner would remove all weeds in the row except those in the length of block skipped for sensed plants.

4. The number of plant intervals of 40 inches or more after hand thinning was nearly equal to that which would result after random mechanical blocking to the same stand and greater than observed after selective thinning.

Hand thinning resulted in a smaller number of plants spaced at small intervals (less than 1 or 5 inches) than for mechanical methods; however, this might not have been the case if the stands had been thinned to the recommended 120 beets per 100 feet of row instead of 80.

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A Method of Selecting Individual Sugarbeet Roots for Weight and Sucrose Percentage[1]

Received for publication February 2, 1968

JOHN O. GASKILL[2]

The selection of individual roots (so-called "mother beets") for weight and sucrose percentage, simultaneously, is a common practice in sugarbeet breeding. Powers (1)[3] has described a method of identifying mother beets genetically superior in both weight and sucrose. This method involves large populations of analyzed individuals. Quite frequently the breeder wishes to select from populations that are too small for the application of that technique. This article pertains to a method, applicable to populations of various sizes, which has been used with minor modifications in the disease resistance breeding program at Fort Collins, Colorado, for the past 10 years.

Where outstanding individuals are to be selected from a population of analyzed mother beets, the breeder frequently sets tentative minimum weight and sucrose percentage limits. Individual beets exceeding both these minimum standards are chosen. However, since weight and sucrose percentage tend to be negatively correlated, and for other reasons, the breeder may wish to make some allowance for especially high weight or especially high sucrose. For example, a beet that falls only slightly below the minimum standard for sucrose percentage may be considered desirable because of high weight. Conversely, an individual that is a little below the minimum standard for weight may be considered desirable because of high sucrose percentage. The use of gross sucrose per beet (i.e. weight X sucrose percentage) as the sole criterion for selection is unsatisfactory since, in actual practice, it tends to result in the selection of the largest individuals, giving too little "weight" to sucrose percentage.

If the weights and sucrose percentages of a group of comparable beets are plotted on a bivariate graph, a curve can be constructed to encompass the individuals to be selected. This curve can be so shaped as to give any desired weighting to each of the

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[2] Research Plant Pathologist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture.

[3] Numbers in parentheses refer to Literature Cited.

two variables at any given point. This procedure can be simulated, with much less time and expense, by the following steps: (a) adjust the weight and sucrose percentage for each beet in any given group to conform to predetermined means; (b) compute a root (e.g. square root, cube root, fourth root, etc.) of the adjusted weight; (c) compute the adjusted gross sucrose (AGS)—i.e. (root of the adjusted weight X adjusted sucrose percentage) —- 100; (d) rank the individual beets in descending order of the AGS values; and (e) choose the individuals ranking highest. After comparing curves simulated by this procedure, I decided to use the fourth root in step "b". Some breeders might prefer other mathematical roots. For example, if the cube root were used, the simulated curve would have less curvature, and use of the fifth root would result in greater curvature.

Adjustment of weights to conform to a predetermined mean is desirable for two reasons. First, if there are two or more groups of mother beets of any one genetic population, they will be made comparable for selection purposes; and second, the majority of the beets can be made to fall in an area where the simulated curve has a satisfactory slope. The first of these two reasons also is applicable to the adjustment of sucrose percentages.

Application of the Method

Table 1 shows the application of this technique to a group of 22 comparable beets of a rather heterogeneous, self-fertile, monogerm population. The plants were grown under severe *Cescospora* leaf spot exposure on the Hospital Farm at Fort Collins, Colorado, in 1966 with approximately 10-inch spacing: in 20-inch rows. They were selected for resistance to leaf spot, with some attention to size and conformation, within a relatively small area of the field, and all were competitive with respect to spacing. Other plants of this population, selected from a contiguous area in the field, were considered as belonging to a different group and are not included in this report.

As may be noted in Table 1, the beets were weighed in kilograms and their respective weights were adjusted to conform to a mean of 2.50 kg. Sucrose percentages were adjusted to conform to a mean of 15.00. The adjusted weights and adjusted sucrose percentages are shown graphically in Figure 1. If the beets had been weighed in pounds, the weights would have been adjusted in the same manner, resulting in a mean of 2.50 lb. Thus the word, "weight", as used on the graph, is applicable to anv scale of weights, and for this reason designation of the weight basis has been omitted.

Table 1.—Weights, sucrose percentages, adjusted gross sucrose values and intermediate computation steps for a group of 22 mother beets grown at Fort Collins, Colorado, in 1966.

no.	Weight		$\sqrt{\text{Adj. wt.}}$	Sucrose		AGS	Rank
	Actual	Adjusted		Actual	Adjusted		
	kg	kg	kg	%	%	kg	
711-25	0.50	2.16	1.212	14.5	15.67	0.190	9
-26	1.10	4.74	1.476	11.8	12.76	0.188	10
-28	0.45	1.94	1.180	15.2	16.43	0.194	7
-29	0.55	2.37	1.241	12.7	13.73	0.170	13
-30	0.35	1.51	1.109	14.8	16.00	0.177	12
-31	0.50	2.16	1.212	14.5	15.67	0.190	9
-32	0.45	1.94	1.180	12.2	13.19	0.156	19
-33	0.60	2.59	1.268	13.5	14.59	0.185	11
-35	0.50	2.16	1.212	12.4	13.40	0.162	17
-36	0.35	1.51	1.109	14.8	16.00	0.177	12
-37	0.90	3.88	1.404	13.6	14.70	0.206	4
-38	0.50	2.16	1.212	12.2	13.19	0.160	18
-39	0.45	1.94	1.180	15.6	16.86	0.199	6
-40	0.95	4.09	1.422	13.5	14.59	0.207	3
41	0.65	2.80	1-293	14.4	15.57	0.201	5
42	0.50	2.16	1.212	13.5	14.59	0.177	12
43	0.50	2.16	1.212	14.7	15.89	0.193	8
44	**1.10**	4.74	1.476	15.1	16.32	0.241	1
45	0.40	1.72	1.145	13.5	14.59	0.167	15
48	0.60	2.59	1.268	15.4	16.65	0.211	2
49	0.60	2.59	1.268	12.3	13.30	0.169	14
-50	0.25	1.08	1.019	15.0	16.22	0.165	16
Average	0.580	2-500		13.87	15.00	0.1857	

Computation methods:

For the entire group of 22 beets:

$$A_w = \frac{2.50}{\text{Actual average weight per beet}} = \frac{2.50}{0.580} =$$

$$A_s = \frac{15.00}{\text{Actual average \% sucrose}} = \frac{15.00}{13.87} = 1.08$$

For any one beet:

Adjusted weight = Actual weight $\times$ A_w

Adjusted % sucrose = Actual % sucrose $\times$ A_s

$$AGS = \frac{\sqrt{\text{Adjusted weight}} \times \text{adjusted \% sucrose}}{100}$$

The two curves in Figure 1 represent AGS values of 0.200 and 0.210, respectively. Beets ranking 1 and 2 in AGS values occur above the upper curve—i.e. they have AGS values higher than 0.210. Likewise, individuals ranking 1, 2, 3, 4 and 5, having AGS values higher than 0.200, occur above the lower curve. Just where the breeder would draw the imaginary AGS curve between the beets to be selected and those to be discarded would

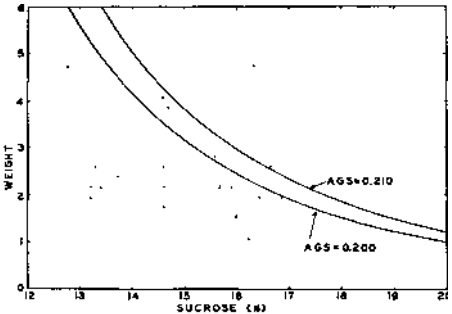

Figure 1.—Adjusted weights and adjusted sucrose percentages, for the 22 mother beets listed in Table 1, and adjusted gross sucrose curves for AGS — 0.200 and AGS = 0.210. Definition of weight unit is omitted since it is immaterial.

depend upon the number of beets available and the degree of selection pressure to be applied. In any case it should be noted that, in actual practice, there is no need to put the data in graph form. It is necessary, only, to construct a table, such as Table 1, and then select individuals on the basis of rank. If there are two or more groups of a given population, the AGS values also should be observed, or it may be more convenient to put the AGS values of all beets of all the groups of that population into a single list in the order of rank.

As may be observed in Figure 1 and Table 1, none of the adjusted weights were above 5.00 or below 1.00. The choice of 2.50 as the basis for the adjustment of weights has no special significance. However, if the basis for adjustment had been so low as to cause many of the adjusted weights to fall below 1.00, this would have been undesirable because AGS curves tend to be too nearly horizontal for weights below 1.00. Likewise, if the adjustment basis had been so high as to cause many of the adjusted weights to be above 6.00 or 7.00, this, also, would have been undesirable because, in my opinion, AGS curves in that region tend to be too nearly verticle. As a rule of thumb, I believe it is advisable to adjust weights in such a manner as to largely avoid adjusted weights below 1.00 or higher than 6.00 or 7.00.

Since the adjustment of sucrose percentages is desirable merely for the purpose of placing different groups of beets of a given genetic population on a comparable basis, the choice of a mean of 15.00 for adjustment purposes (Table 1) was an arbitrary one.

The choice of 14.00 or 16.00, for example, would have given the same results insofar as AGS rank is concerned.

The selection of the fourth root, in preference to other roots, for computation of AGS values, was merely a compromise between simulated curves of greater or lesser curvature. Actual research on the effectiveness of the method described in this article, with special emphasis on the use of other than the fourth root, would be desirable. At this point, the only advantages claimed for this technique are that: (A) it adds mathematical precision to a type of selection process that sugarbeet breeders frequently employ on a personal-judgment basis; and (B) it facilitates the delegation of selection duties to subordinates, thus freeing the breeder of much of the routine work involved. Presumably computerization of the mathematical procedures could be used to enhance the convenience of the method.

Summary

A mathematical technique has been devised by which individually analyzed mother beets may be selected with a curvilinear relationship between weight and sucrose percentage.

Acknowledgments

The assistance of Beverlie A. Nelsen and l.uther W. Lawson, Statistical Clerk and Agricultural Research Technician, respectively, Crops Research Division, Agricultural Research Service, is acknowledged. Mrs. Nelsen and George A. Milliken, Graduate Assistant, Department of Mathematics and Statistics, Colorado State University, have prepared a table of fourth roots, copies of which are available upon request.

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The Effect of Infection With Beet Yellows Virus On The Growth of Sugarbeet

R. HULL[1]

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Sugarbeet infected with beet yellows virus (BYV) are stunted. The leaves are yellow, brittle and necrotic and smaller than those of healthy plants. The yellowing and necrosis and the smaller leaf area doubtless all diminish photosynthesis and thus lead to smaller storage roots. The beard of fibrous roots close to the tap root of plants in the field with yellows suggests that the virus also affects the feeding roots. The extent to which these morphological differences between healthy plants and plants with BYV can account for the differences in yield was investigated in water culture in a glasshouse of the Soils and Nutrition Department, University of California, Berkeley.

Methods

The plants were grown in aerated Hoagland's solution in 20 litre containers following the procedure described by Kelley and Ulrich (2)[2]. Hybrid seed (F58-554H1-MS of NB1 X NB4) was sown in May 1967 and the seedlings transplanted into the culture solution *on* 15 May. On 18 May, 5 *Myzus persicae* from New Zealand spinach infected with Bennett's No. 2 strain of BYV (1) were caged for 48 hours on each plant to be infected, and then killed with insecticide. All plants developed typical symptoms of BYV in 10-14 days. Aphids from an old radish plant with beet western yellows virus (BWYV) were caged on other sugarbeet, none of which had developed BWYV symptoms by the end of the experiment. They yielded similarly to the control plants and it was concluded that they remained free from virus infection. Results from this treatment were included in the statistical analysis but are not recorded in this paper. They indicate that caging the plants for 2 days in hot sunshine did not influence yield. In the analysis of variance the F test for all attributes shows that the treatment effects are highly significant.

Each pot contained three plants and yields per pot are recorded. To investigate how much of the effect on yield was

[1] Broom's Barn Experimental Station, Higham, Burv St.Edmunds, Suffolk, Engalnd.
[2] Numbers in parentheses refer to literature cited.

caused by smaller leaf area, leaves were removed from uninfected plants to give them approximately the same leaf area as the infected plants. Six replicate pots of the following treatments were arranged in randomised blocks on the glasshouse benches: (A) control; (B) 33% defoliation; (C) 66% defoliation; (D) infected with BYV; and (E) attempted infection with BWYV.

On 28 May every third leaf was removed for the 33% and every second and third leaf for the 66% defoliation; on 1 and 8 June every third, or second and third, newly-developed leaf was removed. The leaf area of the plants of one block of all treatments was determined by tracing on each occasion and Figure 1 gives the results.

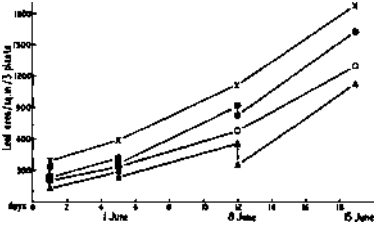

Figure 1.—Leaf area of the plants during the experiments. X control; • 33% defoliation; • 66% defoliation; O infected with BYV.

The plants were harvested on 15 June. Each plant was separated into leaf lamina, petioles and root crowns, tap root and fibrous roots, and fresh and dry weights of the parts are in Tables 1 and 2. The leaves from three blocks of all treatments were traced on brown paper and their area was determined by comparing the weight of the cut-out tracings with that of a known area of paper. These are the areas shown for 15 June in Figure 1. The graphs show that the leaf area of the BYV infected plants was conveniently bridged by that obtained with 33% and 66% defoliation. At harvest the BYV plants had the same number of live leaves as the control plants but only about $^{2}/_{3}$rds the leaf area. The fresh weight per unit area of BYV leaves was similar to the control, but the dry weight per unit area was greater. The defoliated plants had a greater fresh and dry Aveight of leaf per unit area than the control. The leaves on the defoliated plants were larger than those of the same age on control plants. The leaf area duration (D) was calculated from the area contained by each of the four graphs in Figure 1 between 28 May

and 15 June, and the base line for nil leaf area. They are: control 5,960 sq in/days (100%); 33% defoliation 4,660 (78.2% of control); 66% defoliation 2,840 (47.3%); and infected with BYV **(61.7%).**

Table 1.—Yields of fresh matter (gm per 3 plants).

	S.E. of treatment means	Control	33% defoliation	66% defoliation	Infected with BYV
Leaves	(± 11)	509	487	362	353
Crowns and petioles	(± 11)	573	453	305	358
Tap roots	(± 10)	302	309	260	156
Fibrous roots	(± 2)	85	81	68	47
Total	(± 29)	1,469	1,330	995	914

Table 2.—Yields of dry matter (gm per 3 plants).

	S.E. of treatment means	Control	33% defoliation	66% defoliation	Infected with BYV
Leaves	(± 1.21)	42.4	39.2	28.3	34.7
Crowns and petioles	(± 0.91)	31.2	25.0	17.5	19.2
Tap roots	(± 1.04)	25.8	24.1	19.7	14.1
Fibrous roots	(± 0.20)	5.5	5.0	4.2	3.2
Total	(± 3.06)	104.9	93.3	69.7	71.2

Yields

Defoliation decreased total yield of fresh matter (Table 1) by up to 32% and BYV by 38%. The effect of BYV was greater on the root system, yield decreased by 45-48%, than on the overground parts, yield decreased by 31-38%. Tap roots and fibrous roots of BYV plants weighed less than the 66% defoliated plants. The total yield of dry matter (Table 2) was similar for the 66% defoliation and for infection with BYV, which decreased yield 34% and 32% respectively. However, this similarity of average effect resulted from contrasting effects on root and leaves. Defoliation decreased leaf dry matter yield by 32%, compared with 18% for BYV, and root dry matter by 25%, compared with 45% for BYV. Presumably the leaves of BYV plants contained more carbohydrates than healthy leaves (4).

Defoliation decreased percentage dry matter content of the leaves, tap root and fibrous roots (Table 3), but slightly increased that of the petioles and crowns. On average, defoliation had negligible effect on the percentage dry matter content of the whole plant. Infection with BYV increased the percentage dry matter of the leaves, tap root and fibrous roots and had no effect on that of the petioles and crowns. BYV increased the dry matter content of the whole plant from 7.1 to 7.8%.

Table 3.—Percentage dry matter content.

	S.E. of treatment means	Control	33% defoliation	66% defoliation	Infected with BYV
Leaves	(± 0.13)	8.3	8.1	7.8	9.8
Petioles and crowns	(± 0.11)	5.4	5.5	5.7	5.4
Tap roots	(± 0.15)	8.5	7.8	7.5	9.1
Fibrous roots	(± 0.14)	6.5	6.2	6.1	6.8
Total	(± 0.10)	7.1	7.0	7.0	7.8

Table 4.—Yield of different parts as percentage of total dry matter.

	S.E. of treatment means	Control	33% defoliation	66% defoliation	Infected with BYV
Leaves	(± 0.5)	41	42	41	49
Crowns and petioles	(± 0.4)	30	27	25	27
Tap roots	(± 0.6)	25	26	28	20
Fibrous roots	(± 0.11)	5.3	5.3	6.0	4.5

Table 4 shows the effects of the treatments on the distribution of dry matter yield between the different parts of the plants. BYV[7] increased the proportion of the total dry matter yield in the over-ground parts and decreased it in the root system. In contrast, defoliation increased the proportion in the root system due, at least partly, to the removal of leaves and petioles at each defoliation.

Effect of Leaf Area on Yield

Figure 2 shows the relationship between leaf area duration and dry matter yield of infected and healthy plants. The yield of 71.1 gm of dry matter by BYV plants is only 88% of the estimated dry matter yield of healthy plants with the same D (80.5 gm). Of the total decrease in dry matter yield caused by BYV infection, 24.3 gm or 72% can be attributed to the direct effect of the smaller leaf area and 9.4 gm or 28% to the effect of the virus on net assimilation rate.

The dry weight of the roots of infected plants (14.1 gm) was 55% of the control (25.8 gm). By interpolation, as above, the root dry weight of a healthy plant with the same leaf area is estimated as 22.0 gm. Only 33% of the decrease in root dry weight can be attributed to smaller leaf area and 67% to the other effects of the virus.

Water and Potassium Uptake

During the 6 days, 10-15 June, the water required to bring the nutrient solution up to level each day in each pot was measured. The mean daily water usage of the plants of each

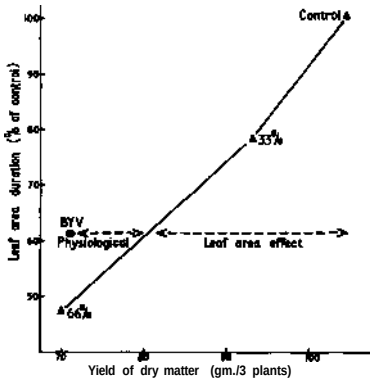

Figure 2.—Method of determining the proportion of the yield decrease caused by BYV due to smaller leaves or to other causes, by the displacement of the BYV yield from the line relating yield to leaf area duration for the healthy plants.

treatment is plotted against the mean leaf area of the plants during this period in Figure 3 and in Figure 4 against the fresh weight of the fibrous roots at the end of the period. The water used by the healthy plants was greater both with more leaf area and with greater weight of fibrous roots. The BYV plants used less water (approximately 10%) than healthy plants with a similar leaf area (Figure 3) but they had only about 70% of the weight of fibrous root system of healthy plants, so the water usage per unit weight of fibrous roots of the BYV plants was greater than for healthy plants. Taking all four treatments into account, water use parallels leaf area more closely than the size of the fibrous root system. The water use of plants in water culture is evidently determined by leaf area and not by the size of the fibrous root system.

On June 8 the nutrient solutions of the 66% defoliation and BYV treatments contained 50 and 55 ppm K respectively, the 33% defoliation treatment contained 4 ppm and the control nil. Fresh nutrients were added to give 177, 177, 215 and 223 ppm K, respectively, for the control, 33%, 66% defoliation and BYV treatments. By 15 June the first two contained no K, the 66% defoliation contained 32 ppm and the BYV 44 ppm K. The records are not detailed enough to determine how the size of the plant or root system influenced K uptake.

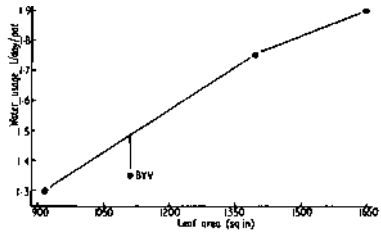

Figure 3.—Water usage of the plants in relation to leaf area

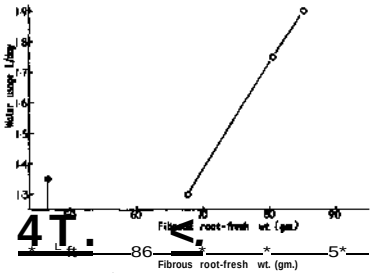

Figure 4.—Water usage of the plants in relation to weight of fibrous roots. O control, 33% and 66% defoliation respectively; • BYV.

Discussion

This experiment gives no evidence that the small, fibrous root system, or its efficiency for water or K uptake from water culture, is restricting yield of plants with BYV.

In aerated water culture the fibrous roots are bathed continually in nutrient solution and the limitations on plant growth are opportunity for photosynthesis and efficiency of the plant for photosynthesis. In soil in the field, plants depend at times on their roots exploiting fresh volumes of soil to obtain moisture and nutrients. The attenuated fibrous root systems of plants with BYV might then be an additional factor influencing yield. The brown appearance of roots of BYV plants in the field suggests that soil-borne pathogens are more prevalent on them than on the roots of healthy plants, and these pathogens may well restrict the efficiency of the roots in taking up moisture and nutrients.

The ratio, total d.m./D, gives an estimate of the mean net assimilation rate (E). This is an approximation because it takes

no account of the dry matter accumulated before the first defoliation on 28 May, but this was small compared with the final dry matter yield. Also, the 1.3 and 2.4 gm/pot of dry matter removed by the 33% and 66% defoliations respectively have not been included in the d.m. yield. From this ratio, mean E was 64, 72, 89 and 70 gm/m^2/week for the control, 33% defoliation, 66% defoliation and BYV treatments respectively. Although E for plants with BYV is less than for plants with the same D (see Figure 2), it is greater than for healthy plants with all their leaves. Removing 66% of the leaves of healthy plants has increased E by nearly a third. This might make the remaining leaves more efficient collectors of incident light by decreasing the extent to which leaves shade each other. Also, the larger plants depleted the culture solution of nutrients more quickly. The smaller dry matter content of the leaves of defoliated plants (Table 3), but greater dry matter content of the petioles and crowns, suggests that the concentration and flow of photosynthetic products may be different in the defoliated from the entire plants, but carbohydrates analyses, which were not made, would be needed to resolve this.

The smaller yield of the BYV plants in these tests is mainly due to their leaves being smaller than those of healthy plants: numbers of leaves were similar. The BYV plants partition their assimilate differently from healthy plants. They retain more in the leaves and less in the roots (Table 4), but approximately the same proportion in the crowns and petioles as healthy plants, whether defoliated or not. Watson & Watson (3) obtained very similar results on plants grown in the field over a period of 120 days, with a mean net assimilation rate of approximately 30 gm/m^2/week. In an earlier publication (4) they concluded that plants with BYV transport as much of their carbohydrate from their leaves at night as do healthy plants. The present experiment does not elucidate how the difference in partitioning of d.m. in healthy and BYV plants arises, but presumably similar factors to those which are restricting the growth of leaves of plants with BYV, although carbohydrates are there in excess, may also be restricting the growth of the root system.

Summary

The effect of infection with beet yellows virus (BYV) on sugar-beet grown in culture solution in the glasshouse was relatively greater on the tap root d.m. yield (45% decrease) than of the leaves (18%), crowns and petioles (38%) or the whole plant (32%). Comparable decreases for healthy plants defoliated to give the same leaf area duration as the BYV plants were 12%

for the whole plant d.m. and 15% for the tap root. Of the total decrease in yield of d.m. by BYV, 72% was attributed to smaller leaves and 28% to the effect of the virus on net assimilation rate; in contrast 33% of the decrease in tap root d.m. yield is attributable to smaller leaves and 67% to physiological effects. Water usage more nearly paralleled leaf area than size of the fibrous root system, which was significantly smaller for plants with BYV.

Acknowledgments

I am very grateful to Dr. A. Ulrich for advice on the planning of this experiment and providing the facilities; also to Mr. Clifford Carlson for growing the plants so efficiently and to other members of the Berkeley Soils and Nutrition Department for assistance with the harvest and analyses. The work was done during a temporary assignment in the Plant Pathology Department, University of California, Davis.

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Effect of Postemergence Applications of Herbicides On Sugarbeet Development and Weed Control In Central California

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The San Joaquin Valley of California is an important sugarbeet producing area where beets are planted from late October through March. Winter annual broadleaf weeds and grasses including volunteer barley are serious problems in the fall and winter planted fields. In addition to the winter annual weeds, summer annuals are abundant in the spring-planted fields.

S-propyl butylethylthio-carbamate (pebulate) and more recently N-ethyl-N-cyclohexythiolcarbamate (R-2063) are being used as preplant soil incorporated lierbicides. The ineffectiveness of the thiocarbamate herbicides on *Brassica* species is well known. Their performance in cold, moist soils, during the winter months, is poorer than in warm, dry soils.

In February and March planted fields, the performance of pebulate or R-2063 in controlling the summer annual weeds is much more effective.

7-oxabicyclo [2.2.1] heptane - 2,3 - dicarboxylic acid (endothal) and trichloroacetic acid (TCA) successfully used in other sugarbeet growing areas are not effective in central California where furrow irrigation is used after planting to establish a stand.

The introduction of 5 amino-4-chloro-2-phenyl-3 (2H - pyridazinone (pyrazon) created considerable interest in central California. Trials were conducted (1, 2)[2] using pyrazon alone and in combination with pebulate and TCA. Pyrazon incorporated prior to planting or at planting caused stand reductions ranging from 10 - 30%/

Pyrazon was successfully used in Canada in 1966 (3) for postemergence weed control in sugarbeets. Recently postemergence trials were conducted by the authors in central California, Kings and Fresno Counties, using pyrazon alone and in combination with 2,2-dichloropropionic acid (dalapon) and an adjuvant containing alkylaryl-polyoxyethylene glycols, free fatty acids and isopropanol (X-77). Excellent control of broadleaf weeds and grasses was obtained (4).

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[2] Numbers in parentheses refer to literature cited.

Two time series studies were conducted to evaluate the effectiveness of pyrazon, pyrazon plus dalapon and adjuvant mixtures in controlling winter and summer annual weeds. Tolerance of sugarbeets to postemergence application of these herbicides was also evaluated. This report presents a summary of these studies.

Methods and Materials

Early Spring Series. A trial area was selected in a commercial beet field planted on 30 inch beds January 18, 1967 on Chino loam soil.

The first series of treatments was applied March 1, when the beets were in the dicotyledon stage of growth. Treatment schedule, weeds present and the stage of weed and beet growth, are summarized in Table 1.

Table 1.—Schedule of postemergence applied herbicides in early spring time series studies.

Date of herbicide application	Stage of beet growth	Stage of weed growth	Weeds present
March 1	Dicot	Cotyledon to 1st leaf	Fiddleneck (*amsinckia Douglasiana*) Black Mustard (*Brassica nigra* (L.) Koch)
March 9	2 True lvs.	2-4 True lvs.	Shepherds Purse (*Capsella Bursapostoris* (L.) Medic) London Rocket (*Sisymbrium irio* (L.))
March 20	4-6 True lvs.	4-8 True lvs.	Knotweed (*Polygonum Aviculare* L. *prostrate*) Red Maids (*Calandrinia caulescens*)
April 13	8-10 True lvs.	12-14" Tall, Some in bloom.	Wild Oats (*Avena fatua* L.) Rabbitfootgrass (*Polypogon monspetiensis* (L.) Desf)

Each plot consisted of two rows 20 ft long replicated three times, in a randomized block design. The herbicides were applied in 850 cc water per plot with a CO_2 pressure sprayer equipped with two 8002 nozzle tips and operating at 30 psi.

The herbicides used were pyrazon 2 and 4 lb/A, pyrazon at 2 plus dalapon at 2 lb/A and pyrazon at 4 plus dalapon at 2 lb/A; all as active ingredient. An adjuvant (X-77) at *i/2%* of spray volume was added to all treatments.

Subsequent treatments scheduled for weekly application had to be modified because of inclement weather.

Periodic weed control and beet injury ratings were made. Following the April 24 evaluations, the check plots and the April 13 treated series were hand weeded and the beets in all plots were hand thinned.

The plots, with the exception of the April 13 treated series, were harvested September 13. Samples were taken from each plot for sucrose determinations.

Summer Series. To evaluate the effectiveness of pyrazon plus dalapon, 3-chlorohexyl-5,6-trimethyleneuracil (lenacil) and R-11914 on summer annual weeds a time series study was conducted June and July of 1967.

The sugarbeets were planted June 1, 1967 in dry soils. The field was furrow irrigated June 6 to obtain a stand.

Soil type, plot size and methods of herbicide application were the same as described in the early spring series. Pigweed *(Amaranthus retrojlexus* L. and *A. graecizans* L.) were planted in the trial area.

Treatment schedule, stage of beet and weed growth, temperature at time of treatment and weed population in the trial area are summarized in Table 2.

Table 2.—Schedule of postemergence applied herbicides in early summer time series studies.

Date of herbicide application	Stage of growth			Temperature at time of treatment	Weeds present
	Sugarbeets	Broadleaf weeds	Grasses		
June 22	Dicot	2 leaves	1-2 leaves	86°F	Pigweeds (*amaranthus retroflexus* L. and *A. graecizans* L.)
June 29	2 True lvs.	4-8 leaves	4 leaves	101°F	Lambsquarter (*Chenopidium album* L.) Goosefoot (*Chenopidium murale* L.)
July 5	4 True lvs.	3-9" 9-12 leaves	6 leaves	83°F	Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv)

The pyrazon 4 lb/A plus dalapon 2 lb/A combination that gave the most effective control in the early spring series along with pyrazon plus lenacil, R-11914 and the combination of R-11914 plus dalapon were the herbicides used in the summer series. Following the July 25 evaluations, the trial was terminated.

Results and Discussion

Winter Annual Weed Control. Pyrazon at 4 lb/A with $\frac{1}{2}$% adjuvant effectively controlled most of the winter annual broadleaf weeds infesting the trial area when sprayed in the seedling stage of growth. The addition of dalapon enhanced the effectiveness of pyrazon on broadleaf weeds. Dalapon was essential for controlling the winter annual grasses.

The effectiveness of weed control was somewhat enhanced by increasing the rate of dalapon to 4 lbs/A, but beet injury was also more severe as shown in Table 3.

Examination of Tables 3, 4, and 5 clearly demonstrates that the effectiveness of weed control was reduced as the weeds became older. It was important to note that even though the weed control was less effective when the weeds were sprayed in the more mature stages of growth, they were severely retarded and more readily removed by cultivation than the untreated areas. This may be an important consideration in commercial fields.

The weeds in the April 13 treated plots were large and with the exception of black mustard and London rocket they were not effectively controlled with any of the treatments, therefore, a summary of the April 13 series is omitted from this report.

Sugar Beet Injury. Sugarbeets in the dicotyledon and later stages of development tolerated pyrazon plus dalapon treatments but retardation in the growth and slight injury to the sugarbeets was evident in all plots.

Injury symptoms were expressed as thickening and slightly wrinkled appearance of the leaf blades. Marginal necrosis was evident, especially in plots where higher rates of pyrazon and pyrazon plus dalapon were used. The retardation in the growth of beets was shortlived as clearly indicated in Tables 3, 4 and 5.

Table 3.—Effect of postemergence foliar applied herbicides on weed control and sugarbeets in a spring time series[1].

Herbicide	Lb/A ai	Weed control[2] Dates evaluated 3/9	3/20	4/6	4/24	Beet injury[2] Dates evaluated 3/9	3/20	4/6	4/24	Clean beet yield T/A	Sugar percent
Pyrazon	2	5.3	5.6	6.0	5.6	1.0	1.0	1.0	1.0	24	10.1
X-77	.5%										
Pyrazon	4	6.6	8.6	8.0	7.3	1.0	1.0	0.6	0	22	12.0
X-77	.5%										
Pyrazon	2										
Dalapon	2	6.3	8.3	6.6	6.0	1.0	1.0	0.6	0	27	10.6
X-77	.5%										
Pyrazon	4										
Dalapon	2	8.3	9.0	9.0	9.0	1.0	2.0	1.0	0	26	10.3
X-77	.5%										
Pyrazon	4										
Dalapon	4	9.0	9.0	9.0	9.0	2.0	3.6	3.0	1.0	25	11.4
X-77	.5%										
Untreated	..	0	0	0	0	0	0	0	0	23	11.6

[1] Stage of Growth: Beets—Dicot stage
Weeds—Cotyledon to first true leaf

[2] Weed control and injury ratings based on a scale of 0- 10: 0— no control, injury. 10 = perfect control, or severe injury.

Table 4.—Effect of postemergence foliar applied herbicides on weed control and sugar-beets in a spring time series[1].

Herbicides	Lb/A ai	Weed control[2] Dates evaluated 3/20	4/6	4/24	Beet injury[2] Dates evaluated 3/20	4/6	4/24	Clean beet yield T/A	Sugar percent
Pyrazon	2	**3.6**	4.6	4.0	**0**	**.6**	**0**	**34**	10.4
X-77	.5%								
Pyrazon	4	**6.3**	7.0	6.3	**.6**	**2.0**	**1.0**	**34**	9.7
X-77	.5%								
Pyrazon	2								
Dalapon	2	**6.3**	5.6	4.0	**0**	**1.3**	**1.0**	**41**	**11.0**
X-77	.5%								
Pyrazon	4								
Dalapon	2	**8.0**	8.0	8.0	**1.0**	**3.0**	**1.0**	**36**	10.3
X-77	.5%								
Pyrazon	8	**7.3**	7.6	7.0	**0**	**2.3**	**2.0**	**38**	10.1
X-77	.5%								
Pyrazon	16	**8.5**	9.0	8.3	**3.0**	**4.5**	**3.0**	**38**	10.3
X-77	.5%								
Pyrazon	32	**9.0**	9.0	9.0	**5.0**	**3.0**	**3.0**	**36**	9.6
X-77	.5%								
Untreated		**0**	0	0	**0**	**0**	**0**	**32**	10.8

[1] Stage of Growth: Beets—2 true leaves
Weeds—2 to 4 true leaves

[2] Weed control injury based on a scale of 0 - 10: 0 = i control, or no injur; perfect control, or severe injury.

Table 5.—Effect of postemergence foliar applied herbicides on weed control and sugar-beets in a spring time series[1].

		Weed control[2] Dates evaluated		Beet injury[2] Dates evaluated		C'ean beet T/A	
Pyrazon		5.0	4.6	1.6	0		
Tronic		7.0	7.0	2.0	0		
Pyrazon	**2**						
Dalapon	**2**	**5.3**	**5.0**	**2.0**	**0**	**34**	**10.4**
X-77	**.5%**						
Pyrazon	**4**						
Dalapon	**1**	**7.0**	**7.0**	**2.0**	**1.0**	**35**	**10.0**
X-77	**.5%**						
Pyrazon	**4**						
Dalapon	**2**	**7.3**	**7.0**	**2.0**	**1.0**	**34**	**11.3**
X-77	**.5%**						
Untreated	....	**0**	**0**	**.6**	**0**	**34**	**11.3**

of Growth: Beets—4 to 6 true leaves
Weeds—4 to 8 true leaves
control and injury ratings based on a scale of 0 - 10: 0 = no control, = perfect control, **or** severe injury.

Effect on Sugarbeet Yield and Quality. The retardation of sugarbeet development was neglegible 30 days after treatment

and no visual evidence of injury was detectable 90 days following treatment even in plots treated with 32 lb/A of pyrazon.

Yield data were obtained by harvesting the plots with a mechanical digger. Samples for sucrose percentage determinations were drawn from each plot and analyzed by Spreckels Sugar Company. No significant differences in yield and sucrose percentage among treatments were obtained as shown in Tables 3, 4 and 5.

Summer Annual Weed Control. In the spring time series studies and in other trials conducted by the authors, it was demonstrated that the most effective control of annual broadleaf weeds and grasses can be obtained with the combination of 4 lb of pyrazon plus 2 lb dalapon per acre. Therefore, in the summer time series study, this was the only pyrazon dalapon combination included.

It was demonstrated in this series that pyrazon plus dalapon was less effective on barnyardgrass and pigweed once they were beyond the seedling stage of growth. This was also observed in other trials conducted by the authors at several locations in the central valley.

Lambsquarter was effectively controlled in the 4 - 6 leaf stage of development, but pigweeds became more tolerant to the herbicides once beyond the two true leaf stage of growth. It was noted that once the lateral buds at the base of the leaf petiole began to enlarge, the weeds became more tolerant to the herbicides.

Barnyardgrass was effectively controlled only when treated in the 1 to 11/, leaf stage of growth and growing vigorously. When treated later than the $1^1/_2$ leaf stage of growth or if the seedlings were stressed for moisture, effective control was not obtained.

Sugarbeet injury was more pronounced in the summer series. The beet injury and retardation in the growth was shortlived, three weeks, even though the beets were treated in the dicot stage of growth when the temperature was 86° F. The injury was much more severe when the herbicide application was made when the temperature was 101 °F even though the beets were older, as shown in Tables 6 and 7.

The beet injury was again less severe following the July 5 treatment when the temperature was 83°F as shown in Table 8.

Lenacil in combination with pyrazon did not enhance the control of the barnyardgrass at rates used. R-11914 had good herbicidal activity on broadleaf weeds, but it was weak on grasses and the tolerance of beets to this herbicide was narrow.

Table 6.—Effect of postemergence foliar applied herbicides on sugarbeet development and weed control in a summer time series[1].

		Weed control and beet injury evaluations[2] Dates of evaluation								
	Lb/A	Broadleaf weeds			Grasses			Beet injury		
Herbicides[3]	ai	7/5	7/13	7/25	7/5	7/13	7/25	7/5	7/13	7/25
Pyrazon	4	8.3	9.0	8.0	1.3	1.0	1.0	2.3	.7	0
Dalapon	2									
Pyrazon	1	7.5	8.0	7.0	.6	4.0	2.0	3.3	.7	0
Lenacil	1									
Pyrazon	2	8.3	8.0	7.0	3.0	4.0	2.0	3.3	2.0	1.0
Lenacil	2									
R-11914	1	9.5	9.0	8.0	2.6	1.0	0	4.6	3.0	3.0
R-11914	2	7.6	9.0	9.0	.6	1.0	0	6.6	4.0	4.0
R-11914	1	8.0	8.0	8.0	2.0	2.0	1.0	4.3	3.0	3.0
Dalapon	2									
Untreated	..	0	0	0	0	0	0	0	0	0

[1] Stage of Growth: Beets—Dicot
Weeds—Grasses 1 to 2 leaves
Pigweeds 2 leaves

[2] Weed control and beet injury ratings based on a scale of 0- 10: 0 = no control, or no injury. 10 = perfect control, or severe injury.

[3] An adjuvant (X-77) at *Vi%* of spray volume was added to all treatments.

Table 7.—Effect of postemergence foliar applied herbicides on sugarbeet development and weed control in a summer time series[1].

		Weed control and beet injury evaluations[2] Dates of evaluation								
	Lb/A	Broadleaf weeds			Grasses			Beet injury		
Herbicides[3]	ai	7/5	7/13	7/25	7/5	7/13	7/25	7/5	7/13	7/25
Pyrazon	4	9.6	9.0	8.0	4.3	3.0	2.6	8.0	8.0	7.6
Dalapon	2									
Pyrazon	1	7.6	6.0	5.0	4.0	2.0	2.0	6.3	5.0	5.0
Lenacil	1									
Pyrazon	2	9.0	8.0	7.3	3.0	4.0	3.0	7.3	5.0	5.0
Lenacil	2									
R-11914	1	9.6	9.0	9.0	4.0	2.0	2.0	8.6	7.0	7.0
R-11914	2	10.0	10.0	10.0	2.0	3.0	2.6	9.6	9.0	9.0
R-11914	1	10.0	9.0	9.0	3.3	2.0	2.0	9.6	9.0	9.0
Dalapon	2									
Untreated		0	0	0	0	0	0	0	0	0

[1] Stage of Growth: Beets—2 true leaves
Weeds—Grasses 4 leaves
Pigweeds 4 - 8 leaves

[2] Weed control and beet injury ratings based on a scale of 0 - 10: 0 = no control, or no injury. 10 = perfect control, or severe injury.

[8] An adjuvant (X-77) at *Vi%* of spray volume was added to all treatments.

It was observed that pyrazon did not control *Salsola kali* L. (Russian thistle) and Trifolium spp. (clovers). It was also noted that R-11914 effectively controlled Russian thistle.

Table 8.—Effect of postemergence foliar applied herbicides on sugarbeet development and weed control in a summer time series[1].

		Weed control and beet injury evaluations[2] Dates of evaluation					
	Lb/A	Broadleaf weeds		Grasses		Beet injury	
Herbicides*	ai	7/13	7/25	7/13	7/25	7/13	7/25
Pyrazon	4	5.0	5.0	4.0	2.0	3.0	2.0
Dalapon	2						
Pyrazon	1	2.0	0	0	0	2.0	1.0
Lenacil	1						
Pyrazon	2	3.0	2.0	1.0	0	3.0	2.0
Lenacil	2						
R-11914	1	3.0	1.0	1.0	0	3.0	2.0
R-11914	2	6.0	5.0	3.0	1.0	3.0	2.0
R-11914	1	7.0	6.0	3.0	1.0	4.0	3.0
Dalapon	2						
Untreated	....	0	0	0	0	0	0

[1] Stage of Growth: Beets—2 to 4 true leaves
Weeds—Grasses 6 leaves
Pigweeds 3 to 9 inches with 9 to 12 leaves

[2] Weed control and beet injury ratings based on a scale of 0- 10: 0 = no control or no injury. 10 = perfect control or severe injury.

[3] An adjuvant (X-77) at *Vz%* of spray volume was added to all treatments.

Conclusions

In central California, until very recently, winter annual broadleaf weeds have been the most difficult to control in winter and in early spring planted sugarbeet fields.

Pyrazon in combination with dalapon plus an adjuvant was the most promising chemical tool for the selective control of winter annual weeds in sugar beets.

From the numerous trials conducted in the central valley and in the two time series studies summarized in this report, the following conclusions can be drawn:

A. Winter annual weeds were more effectively controlled than the summer annuals with pyrazon plus dalapon.

B. Pyrazon at 4 lb/A with i/£% adjuvant effectively controlled the mustard species.

C. Pyrazon at 4 lb/A plus dalapon at 2 lb/A with V_2% adjuvant effectively controlled most of the winter annual grasses and broadleaf weeds commonly found in sugarbeet fields in central California.

D. The most effective control was obtained when the weeds were sprayed in the seedling stage of growth.

E. Sugarbeets tolerated pyrazon plus dalapon applications even in the cotyledon stage of growth.

F. Retardation in the growth of sugarbeets was evident, but shortlived.

G. As the rate of dalapon in combination with pyrazon was increased, the injury to the seedling sugarbeets was more severe.
H. To obtain summer annual weed control with pyrazon plus dalapon, the application of the herbicide relative to the weed growth was critical. Pigweed and barnyardgrass were the most difficult to control.
I. Pigweed beyond the 2-leaf stage, or when the axillary buds started growing, were not controlled satisfactorily.
J. To obtain good barnyardgrass control, the herbicides must be applied when the grass is in the 1 to li/£-leaf stage and growing vigorously. Grasses showing moisture stress at time of treatment, even in the seedling stage of development, were not killed.
K. Pyrazon plus dalapon and other herbicides, applied when the temperature was 101°F severely injured the beets and reduced the stand. The same herbicides applied when temperatures were in the 80's, did not cause severe injury.

Acknowledgment

The authors wish to express their appreciation to I,. M. Burtch, Agronomist and Wes Stroud, Research Assistant, Spreckels Sugar Company, for their assistance in conducting these trials.

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Standard Liquor Storage

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High density beet sugar liquors can be stored for indefinite periods of time under any weather conditions providing the conditions of storage are such that (1) yeasts and molds cannot flourish and (2) crystallization cannot proceed.

Yeasts and molds found in beet sugar juices will not develop and flourish in solutions that are above 8 pH and above 67 R.D.S. Dilute solutions of beet sugar juices, on the other hand, offer ideal media over a wide range of temperatures for the development and growth of many strains of yeasts and molds. It is paramount, therefore, in the storage of either standard liquor or evaporator thick juice that moisture from condensation on the walls of the storage tank or from other sources be completely eliminated at all times. This can be accomplished by establishing a vapor barrier at the surface of the liquor by covering it with a layer of suitable oil to a depth of approximately one-eighth of an inch. The oil acts to prevent moisture from escaping from the surface of the liquor into the vapor space of the tank. At the same time it coats the steel and prevents rust. The relative humidity can then be controlled at a point such that the tank walls never reach the dew point. This is done by circulating a sufficient amount of outside air through the vapor space of the storage tank to make sure that the relative humidity of the air inside the storage tank is equal to the relative humidity of the air outside the storage tank. Under such conditions condensation will not occur. Utah-Idaho Sugar Company's plant, at Moses Lake, Washington, has stored standard liquor during beet campaign and processed it during a summer juice campaign for the past five years without having any trouble with yeasts and molds.

For crystallization to occur in storage it is necessary for the liquor to be super-saturated and for a sufficient number of nuclei to be present to start the process. Crystallization can be controlled by adjusting the R.D.S. of the liquor going into storage so that it is only saturated or slightly supersaturated at the temperature stored. To prevent both yeast and molds and

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crystallization in 94 purity liquor the R.D.S. should be kept between 67.5 and 68.5. The amount of nuclei present in the juice can easily be controlled by proper filtration of the juice and by filtering all dust particles out of air that is circulated through the vapor space of the storage tank.

Pumping Juice Out To Storage

Whenever the amount of standard liquor flowing to the pan floor storage tanks exceeds the amount of standard liquor required for the white pan station operation, the excess is pumped to the outside standard liquor storage tank No. 7. This is done as follows: (Refer to Figure No. 1).

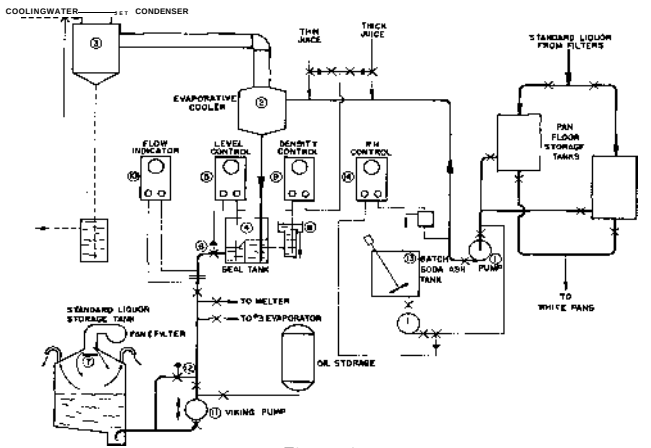

Figure 1.

Pump No. 1 is started which pumps liquor to evaporative cooler No. 2. Jet condenser No. 3, being in operation, maintains an absolute pressure of approximately 4" mercury on the evaporative cooler at all times. As liquor enters the cooler, evaporation takes place causing the temperature of the juice to lower to the temperature of evaporation corresponding to 4" mercury absolute or 125°F. Juice leaving the evaporative condenser flows to a seal tank No. 4 where the seal on the evaporative cooler is maintained. A level controller No. 5 measures the level in this seal tank and controls valve No. 6 to allow liquor to flow to the outside storage tank No. 7 without the entrainment of air. A sample of juice leaving the leg line in the seal tank is diverted

to a bubble tube sampler No. 8. Density control instrument No. 9 measures the density of this liquor and in turn regulates thin juice or thick juice flow to the evaporative cooler to control the density of the liquor or the R.D.S. of the liquor within 67.5 to 68.5 R.D.S. Flow meter No. 10 records the amount of liquor flow going to the outside storage tank. At storage tank No. 7 liquor bypasses pump No. 11 through remote controlled valve No. 12 and enters the storage sump underneath the oil which previously was placed in the tank. pH is controlled by drawing a sample of juice just after pump No. 1, measuring the pH and controlling a flow of dissolved soda ash from batch soda ash tank No. 13 by use of pH controller No. 14. The soda ash enters just upstream of pump No. 1 to assure quick response and thorough mixing.

Whenever the amount of standard liquor flowing to the pan floor becomes less than is required for the continuous operation of the white pan station, pump No. 11 is started and remote controlled valve No. 12 is closed to regulate the amount of liquor to be returned to the high melter for processing. From the high melter the liquor returns to the pan floor storage tank via the standard liquor filters.

Recovery of Standard Liquor from Storage During Juice Campaign

During juice campaign part of the factory evaporator station must be used to: (1) furnish water for the boiler and for process, (2) furnish steam to operate the vacuum pans and other process equipment and (3) reheat the liquor coming back from the standard liquor storage tank. Refer to Figure No. 2. Here well water is boiled in evaporator bodies No. 1 and No. 2 and the condensate, as needed, is returned to the boiler. Condensate remaining from evaporators, pans, heat exchanger, etc., is used for general hot water or process water and for centrifugal wash water. Vapor from evaporator body No. 1 is used for boiling white pans and preheating the water entering No. 1 body. Vapor from No. 2 body is used for boiling high raw and low raw pans and for reheating standard liquor in body No. 3. Body No. 3 acts as a condenser for the evaporator station while heating the juice. Number 4 and 5 evaporators are not used. To remove solids from the water feeding bodies No. 1 and No. 2 Nalco 75 balls are used and both bodies are provided with blow down lines.

Pump No. 11 is started to return liquor back to evaporator body No. 3. Remote valve No. 12 then is throttled to control the level in No. 3 evaporator. The temperature of the juice leaving No. 3 evaporator is controlled by steam control valve

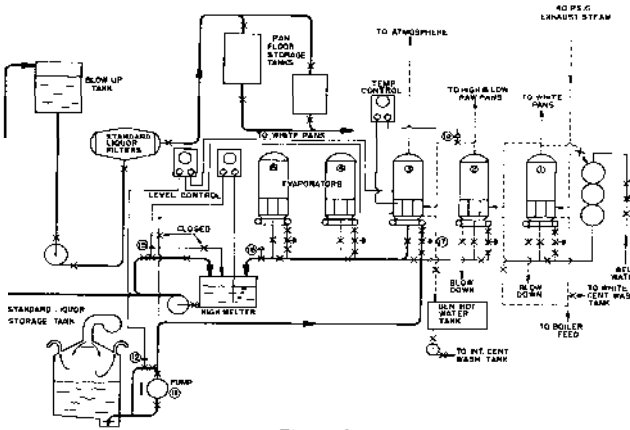

Figure 2.

No. 18. Valve No. 15 controls the level in the high melter. Here the high raw and low raw sugar is added and remelted. Then the liquor is pumped to the pan floor storage tanks by way of the blow up tank and standard liquor filters. The white pan operator controls remote valve No. 16 to regulate the amount of flow to the high melter and hence the flow to his pan floor standard liquor tanks. Pan operation and sugar end operation from here on are the same as for the beet campaign.

Sugar end management during juice campaign is the same as that of the beet campaign. Standard liquor purities are higher, due to the addition of the high and low raw sugars made during the juice campaign. This makes for increased white pan yields and better purity drops. High raw and low raw purities remain the same as for beet campaign. Molasses purities are as good or slightly better than for beet campaign.

Advantages of Liquor Storage

1. The sugar end and the beet end are less dependent upon each other. If the sugar end is shut down, the beet end can continue to operate, and the juice can be sent out to storage. If the beet end is shut down, juice can be pumped back from the storage tank, and the sugar end can continue to operate. This means that mechanical and operating delays are not so costly, and production levels are maintained at a higher rate.

2. Molasses purity can be maintained at a point consistent with good extraction without affecting the cutting capacity of the plant. The low raw purity can be lowered until the brown centrifugals are just keeping up. If the sugar end starts to get behind, standard liquor is put out to storage.
3. Juice storage provides additional sugar storage that is cheaper and safer than equivalent bulk granulated storage. This is true because: (1) the initial investment is less and (2) the beet end capacity can be increased without increasing the sugar end capacity.
4. Sugar made during juice campaign can be made to the specifications required by the market such as bottlers, grain size, and packaging. Sugar can often be packaged and loaded directly without being stored. This means the bags reach the market in better condition and costs of handling and breakage are reduced.
5. A schedule for packages for a complete sales year does not have to be decided upon before the end of the beet campaign. Large inventories of certain packages are not required at the end of campaign, and carry over of these packages from year to year is minimized.
6. Plant clean up at the end of campaign can be shortened since many of the remaining sugar end syrups can be adjusted for pH and R.D.S. and pumped into storage for processing during the juice campaign.
7. Juice storage can eliminate the cost of in and out charges for sugar stored in transit warehouses.
8. With sufficient juice storage capacity warehouse operations can be placed on a current basis and automated, thus requiring less labor and less warehouse space. Sugar is simply packaged as it is needed.

Comparison of the Effect on Beet Seed Production of Spring and Fall Infestations of Beet Leafhoppers Carrying Curly Top Virus'

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Introduction

The beet leafhopper, *Circulifer tenellus* (Baker), and the curly top virus that it transmits have been a major problem to producers of sugarbeet seed since the crop was first grown in the southwestern United States in the early 1930's. Observers have usually considered that the virus reduced yield. Also, since the crop is planted in late August and early September and is harvested the following June, some investigators have speculated about the comparative damage done by the fall and spring migrations of the insect. For example, in 1943, Romney (3)[3] described the injury to the crop in Arizona and New Mexico caused by the fall migrations from the surrounding semidesert areas, and stated that "Beet leafhopper populations often increase in Arizona seed beet fields during April and May, as a result of spring movement from winter annuals in the surrounding semidesert areas." He also stated that "These leafhoppers cause some damage, but not so much as that caused by an equal infestation in the fall when the beets are small." Hills et al, in 1948 (1), also reported that the major losses from curly top virus were attributable to fall movements of the beet leafhopper from desert breeding areas to seed beet fields and were manifested as reductions in seed yield; their further experiments (2) in 1960-61 showed that early spring (March 20-23) infestations of infective beet leafhoppers did not reduce yield or germination. Nevertheless, observers in the field continued to report, a reduction in germination and yield due to the spring migrations of infective leafhoppers. Experiments were therefore made in the Salt River Valley of Arizona from 1964 to 1967 to determine the comparative effects of the fall and spring infestations of curly top infective beet leafhoppers.

[1] In cooperation with the University of Arizona Agricultural Experiment Station.

[2] Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture.

[3] Numbers in parentheses refer to literature cited.

Materials and Methods

The field test plots[4] were 20 ft long and 2 rows wide (1 bed) with a 2-row buffer strip of untreated beets on each side and a 5-ft cleared alley at each end. Soon after the plants had bolted, the seed stalks in the buffer rows were cut off to prevent tangling with the plot rows; later, wires were stretched to prevent lodging. A monogerm curly top susceptible variety (GW-806) was used, and curly top inoculations were made by placing muslin cages over each plot and introducing infective beet leafhoppers from greenhouse colonies. Cages were of sufficient size to cover the plots (Figure 1). Thus when the plants were smaller, the cages were 24 in wide, 20 in tall and 20 ft long. Later, they were 30 in X 30 in X 20 ft. Finally, with the larger plants in April 1967, they were 36 in X 48 in X 20 ft.

The beet leafhoppers used to inoculate the test plots were either reared in the greenhouse on sugarbeets or gathered from weed hosts in the field. From 5 to 7 days before they were introduced into the field cages, they were caged on curly top infected sugarbeets. It was planned to use more leafhoppers per plot on larger plants in the spring than on smaller plants in the fall. However, numbers introduced were sometimes limited by the supply. The curly top inoculum was obtained by transplanting curly top infective beet plants from the field to the greenhouse. Virus strains used in 1964-65 were unknown, but the inoculum used in 1966-67 was identified by Dr. C. W. Bennett[5] as strain 11.

Each of the 3 years, the plots were arranged in a 6 X 6 Latin square. The test design called for two introductions of infective leafhoppers in the fall and three in the spring; one series of six plots was to remain uninfested as a check. However, because of inclement weather, irrigation, rapid plant growth, or a shortage of leafhoppers, it was not always possible to complete the full complement of infestations.

In 1964-65, the infective leafhoppers were introduced into the field cages by taking the colonies to the field, aspirating them from the colony cages, and blowing them through three small holes evenly spaced along the tops of the muslin covers. This same procedure was followed in October 1965, but infestations in April and March 1966 and all introductions of 1966-67 were made by anesthetizing the leafhoppers with CO_2,

[4] Field plots were provided by the Western Seed Production Corporation, Phoenix, Arizona.

[5] Plant pathologist, Crops Research Division, ARS, USDA, Salinas. California (retired April 1965).

Figure 1.—Plots of sugarbeets covered with muslin to confine curly top infective beet leafhoppers. October 24-31, 1966.

turning back the cage covers, sprinkling the anesthetized leafhoppers along the rows and quickly replacing the covers. After the desired exposure, the plants within the cages were dusted with 2% parathion by inserting the duster tube through the ends of the cages; after which the covers were removed.

At maturity the seed from each plot was harvested and cleaned in accordance with commercial practice. Criteria of damage was seed yield (pounds per acre) and quality (percentage germination). Also, in 1965-66, the number of seed balls per ounce was determined. Reduction in yield or germination was calculated against the means for all control plots for each year.

Results

1964-65 tests

Two fall and two spring infestations were made; thus 12 plots were uninfested. Table 1 shows a 16% reduction in yield in the plots infested in February. However, these insects were left on the plants 7 instead of 4 days because it was assumed that cold weather would partially inactivate the leafhoppers. This longer exposure may have been responsible for the greater reduction in yield. Also, the infestations in November caused a 6% reduction in yield. The reductions computed against the mean yield of the 12 untreated plots amounted to a loss of 513 and 198 pounds per acre, respectively, for the February and

Table 1.—Yield and germination of beet seed **from experimental plots** infested **at** various dates **with curly top infective beet leafhoppers (Phoenix, Arizona).**

Date of infestation	Plant development at time of infestation	Leafhoppers introduced	Seed yield (lb/acre[1])	Percentage germination[1]	Percentage reduction in yield	Percentage reduction in germination
1964-65 tests						
Uninfested			3179 ab	68.8		
October 9-12	4-8 leaf stage	200-300	3108 ab	66.2		
November 27-30	Plants 20 in tall; complete coverage	300	2981 b	62.8	6	
February 5-12	Spring growth just starting	300	2666 c	60.8	16	
March 5-9	Vegetative growth; no bolting	250	3220 ab	58.3		
1965-66 tests						
Uninfested			4133 a	69.5 a		
October 14-18	4-6 leaf stage	175	3997 a	66.3 ab		
March 1-14	Plants topped 2/15; new growth by 3/1	350	2427 c	62.3	41	10
April 5-11	Bolting; seed stalks 20-30 in tall	586	3037 b	51.8 c	27	25
1966-67 tests						
Uninfested			2465 a	53.5 a		
October 24-31	6-9 leaf stage	400	1763 c	40.8 b	29	24
November 7-14	10-12 in tall; 70% coverage	800	1376 e	41.2 b	44	23
Feb. 24-March 2	Spring growth just starting	400	2206 b	56.3 a	11	
March 20-27	16-20 in tall	600	1471 de	42.2 b	40	21
April 18-20	Seed stalks 4 ft tall; flower bud	900	1665 cd	29.2 c	33	45

[1] Values not followed by the same letter are significantly different at the 5% level of confidence by Duncan's multiple range test; 1964-6 germinations not significant by the F test.

November infestations. No significant differences in germination due to treatment occurred, but the plots infested in March had a tendency toward a lower percentage of germination.

1965-66 tests

Only one fall and two spring infestations were made because of shortage of leafhoppers; thus 18 plots were uninfested. Also, the numbers introduced were not as large as desired. However, Table 1 shows that March infestations reduced yield 41% (1,706 pounds per acre) and germination 10%, and the April infestations reduced yield 27% (1,096 pounds per acre) and germination 25%. The percentage reductions in yield were calculated against the mean of all 18 uninfested plots; however, percentage germination was obtained for only one series of six uninfested plots. In April, the plots were exposed to 586 leafhoppers for 6 days compared with 350 leafhoppers for 13 days in March. (Because of cold, rainy weather during the first half of March, the period of infestation was extended).

In February 1966, foliage on the plots was unusually heavy. Therefore, the beets in all plots were topped on February 15, a common practice among many growers at that time. By the time of the March 1 infestation, new growth had started, but it was lower than at the time of the April infestation. This difference and the longer infestation period should have and apparently did provide adequate exposure to the leaphoppers even though the actual number introduced was less than in April.

Also, during these tests, any possible effect of curly top virus on the size of the seed was checked by determining the number of seeds per ounce for each plot. The mean number per treatment ranged from 3,689 to 4,334, but the differences were not significant.

1966-67 tests

All five infestations were made as planned, and one series of six uninfested plots was the control. A more severe exposure was attempted by the use of more infective leafhoppers and a longer time. In October, the beets were comparatively small, and the 400 leafhoppers probably gave a good exposure. However, in February the foliage was heavy, and more leafhoppers would have been desirable but were not available.

The results are shown in Table 1. All infestations reduced yield, and all except the February infestation also reduced the percentage of germinating seed. Also, the infestation just before blooming (April 13-20) reduced the percentage of germinating seed significantly more than other treatments. Losses in yield ranged from 259 pounds per acre for the February infestation

to 1,089 pounds per acre for the November infestation. However, both yield and germination were much lower even in the uninfested plots than for the 2 previous years. Perhaps the native beet leafhoppers that were seen in the plots during the spring may account for at least some of this reduction.

Discussion and Conclusions

The heavy infestations of curly top infective beet leafhoppers in sugarbeets grown for seed caused reductions in yield that were sometimes accompanied by a lower percentage of germinating seed. Despite earlier reports that the early fall infestations cause the greatest reductions in yield, our test results did not consistently support this view. Sugarbeets grown for seed in southern Arizona are unthinned, and usually the test plots had more than 12 plants per foot of row. Many plants in the plots infested in the fall were so severely affected by curly top that they did not contribute to the seed yield, but the comparatively short exposure left enough healthy plants to produce a satisfactory yield. Under field conditions if leafhoppers are allowed to remain in the field for a longer time, many more plants become infected and much greater loss can be expected.

The effect of curly top virus on the yield of sugarbeet seed has been known for some time, but the effect on the viability of the seed was not proved. The data presented here show that the greatest reduction in the percentage of germinating seed occurs when curly top infective beet leafhoppers invade the fields just as the plants are approaching the bloom stage. Since the symptoms of the disease resulting from these late inoculations are not always easy to see, late season migrations of leafhoppers into the beet fields have been considered of comparatively little importance. However, results of these tests indicate that fields of seed beets should be watched for spring beet leafhopper infestations and control measures applied if necessary.

Summary

The effect of fall and spring infestations of curly top infective beet leafhoppers on sugarbeets grown for seed were compared in artificially infested field plots.

Infestation was accomplished by caging entire plots during the exposure period. Both fall and spring infestations reduced seed yields, and sometimes the germination of the seed was also affected. The greatest reductions in germination resulted from infestations that occurred just before the plants bloomed. Seed size was apparently unaffected by the virus.

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Chemical Control of Cercospora Leaf Spot of Sugarbeets in Nebraska, 1965[1]

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Cercospora leaf spot of sugarbeets is a major problem in Europe and North America. This disease caused by *Cercospora beticola* Sacc. is important in central and eastern Nebraska. Fungicide tests have been made in Europe and North America as referred to recently (1,2,3)[3].

The Cercospora leaf spot control tests of 1965 were a continuation of research begun in 1961 in Nebraska. Results of the 1964 leaf spot control tests indicated the need for more information on the efficiency of the newer fungicides in relation to the recommended products, plus additional data on rates and number of applications necessary to provide satisfactory disease control. The plot locations in Burt County, Nebraska were chosen on the basis of a relatively high incidence of disease in 1964, plus the intensive cropping practices employed by the growers. Because aircraft has been considered as a possible method of overcoming some of the problems of late application, a treatment was included with one fungicide using only 10 gallons of water per acre to simulate aerial application rates.

Materials and Methods

The following fungicides were incorporated in the tests conducted at both locations during 1965.

Fungicide	Supplier
Tri-basic copper sulfate	Tennessee Corporation College Park, Georgia
Daconil 2787 W-75*	Diamond Alkali Company Painesville, Ohio

[1] Published with the approval of the Director as paper No. 2297. Journal series, Nebraska Agricultural Experiment Station.

[2] Extension Plant Pathologist, Professor of Plant Pathology, former Director, Agr. Research American Crystal Sugar Company, and Extension Plant Pathologist, respectively, University of Nebraska, Lincoln.

[3] Numbers in parentheses refer to literature cited.

Dithane M-45[b]	Rohm and Haas Company Kansas City, Missouri
DU-TER 20% WP[c]	Thompson-Hayward Chemical Co. Kansas City, Missouri

[a]Active ingredient: Tetrachloroisophthalonitrile
[b]Active ingredient: Manganese ethylene bis-dithiocarbamate
[c]Active ingredient: Triphenyl tin hydroxide

The experimental design was a randomized complete block with three replications. The treatments were randomized within each replication at each location. Test plots were placed within beet fields away from extraneous influencing factors. Plots consisted of five 30-foot rows spaced 22 inches apart. Four of the five rows were treated, while the remaining row served as a buffer strip between plots. Replications were separated by a three foot alley.

Sprays were applied with a SOLO-PORT, a high air velocity engine driven mist blower. It was easily calibrated and gallonages were accurately controlled. Plyac, a spreader-sticker, was used with Daconil 2787, Dithane M-45, and tri-basic copper sulfate at the rate of one ounce per gallon of water. Spray application dates were as follows:

Cooperator	Number of applications	
	4	6
Englert	July 8	July 8
	July 22	July 22
	Aug. 5	Aug. 5
	Aug. 19	Aug. 19
		Sept. 2
		Sept. 15
Morrow	July 9	July 9
	July 22	July 22
	Aug. 5	Aug. 5
	Aug. 19	Aug. 19
		Sept. 2
		Sept. 15

Leaf spot data were gathered on whole plots August 19, September 2, and September 15, 1965. Disease incidence data consisted of a visual rating for leaf spot based on percentage of leaf surface infected, as listed below:

Rating	% of leaf Surface infected
1	0-10
2	11-25
3	26-50
4	51-75
5	76-100

Yield data and sugar analysis data were collected from the two center rows of each treated 4-row plot. Both tests were harvested October 8, 1965.

Results

Englert Plots: On July 8 at the time of first fungicidal application, disease incidence was 5 to 20 leaf spot lesions per plant. Two weeks later the amount had increased to a level greater than 100 leaf spots per plant. By August 19, heavy infection was observed in the untreated plots with somewhat less infection on foliage of plants in treated plots. Severely infected lower leaves had begun to dry and fall, giving rise to a slight "pineapple effect". Consequently, such leaves escaped detection and consideration in subsequent leaf spot ratings.

Results indicate several treatments effectively controlled disease development but had no significant effect on tons of beets per acre, percentage of sucrose, and pounds of sugar produced per acre on this farm. Only small differences could be attributed to four versus six applications of fungicides. Duncan's Multiple Range Test indicated better disease control was achieved with DU-TER than with Dithane M-45, Daconil 2787, or tribasic copper sulfate.

Morrow Plots: At the time the first application of fungicides were made (July 8), an insignificant amount of leaf spot was observed in this field (5-10 lesions per plant). Disease incidence increased rapidly during the next two weeks to well over 100 leaf spots per plant. The plants also were not developing as vigorously as those in the Englert plots. The stand was poorer, the leaves smaller, and the foliage less dense. By August 19, very heavy infection was noted throughout most of the plots, with severe infection on untreated beets between the experimental plots and a corn field north of the plots. While the present experimental plots were established in a field that was idle ground in 1964, the existing corn field had been in sugarbeets the previous year and sugarbeet residue could easily be collected from the corn field. Within the next two weeks severely infected lower leaves blackened and dropped, causing a moderate

to obvious "pineapple effect" of the crowns. Leaves killed in this manner escaped detection and were thus not reflected in subsequent leaf spot ratings.

Results of the fungicidal tests indicate that all chemical treatments provided better leaf spot control than did water alone (check plots) under high disease intensity. However, even with satisfactory control being achieved, there were little or no significant differences in tons of beets per acre, percentage sucrose, or pounds of sugar per acre. Moreover, no significant difference in control was noted with the addition of two fungicidal applications. Duncan's Multiple Range Test indicated DU-TER treatments resulted in belter control than did Daconil 2782, Dithane M-45, or tri-basic copper sulfate.

Composite date (Englert-Morrow plots): When the data for the two locations are combined (Table 1), significant differences occur between treatments and between locations for tons of beets per acre, percentage sucrose, and leaf spot ratings. There was a significant treatment by location interaction, indicating the degree of fungicidal control of leaf spot was influenced by the severity of the disease in 1965. The fact that the treatments and the locations of the plots had no effect on the pounds of sugar produced per acre is noteworthy.

Any treatment in which DU-TER was applied resulted in superior control of leaf spot as compared to the control achieved by Dithane M-45, Daconil 2787, or tri-basic copper sulfate.

To extract additional statistical information, treatment sums of squares for each of 3 dates-of-leaf-spot-incidence ratings at both locations were partitioned into 17 single degree of freedom orthoginal comparisons (Table 2). The following comparisons are of particular interest.

Number 9: The degree of control of Cercospora leaf spot achieved when Daconil 2787 was applied at the rate of 2 pounds of formulation in 100 gals/acre was no greater than when the same material was applied at the rate of 1 pound/100 gals/acre.

Number 13: DU-TER, when applied at the rate of 1.25 pounds of formulation in 10 gallons of water per acre, prevented disease development equal to or better than the same material applied at the same poundage in 100 gallons of water.

Number 14: No greater control of Cercospora leaf spot was achieved with DU-TER at the rate of 1.25 pounds of formulation per acre than that achieved by half that rate.

Number 15: DU-TER applied at the rate of 0.625 pounds in 10 gallons of water per acre was equal to or better than the same material in 100 gallons of water per acre, in terms of Cercospora leaf spot control.

Table I.—Cercospora leaf spot on sugarbeets, fungicidal trials, composite date (Englert • Morrow farms), 1965

Treatment	No. of appli	Rates/acres Lbs chem	Rates/acres Gals H_2O	Tons of beets per acre	Percent sucrose	Lbs sugar per acre	Average leaf spot rating	Duncan's mult range
Tribasics copper sulfate	4	4.0	100	24.5	11.50	5593	2.93	de
Tribasic copper sulfate	6	4.0	100	24.4	11.38	5507	3.07	e
Daconil 2787	4	1.0	100	23.2	11.18	5138	2.65	de
Daconil 2787	6	1.0	100	22.2	11.38	5004	2.88	de
Daconil 2787	4	2.0	100	23.4	11.40	5305	2.57	d
Daconil 2787	6	2.0	100	22.7	12.48	5599	2.57	d
Dithane M-45	4	2.0	100	22.7	12.44	5422	2.95	de
Dithane M-45	6	2.0	100	24.2	11.61	5588	2.97	de
Triphenyl tin hydroxide (DU-TER)	4	1.25	100	25.2	12.08	5984	1.90	bc
Triphenyl tin hydroxide (DU-TER)	6	1.25	100	21.4	12.00	5057	1.55	ab
Triphenyl tin hydroxide (DU-TER)	4	1.25	10	23.6	11.75	5413	1.38	ab
Triphenyl tin hydroxide (DU-TER)	6	1.25	10	22.6	11.83	5246	1.17	a
Triphenyl tin hydroxide (DU-TER)	4	0.625	100	23.9	12.27	5692	2.02	c
Triphenyl tin hydroxide (DU-TER)	6	0.625	100	22.8	11.62	5213	1.85	bc
Triphenyl tin hydroxide (DU-TER)	4	0.625	10	23.4	11.92	5449	1.15	a
Triphenyl tin hydroxide (DU-TER)	6	0.625	10	21.8	12.50	5326	1.27	a
Check (Water only)	4	0.0	...	20.2	12.22	4747	4.00	f
Check (Water only)	6	0.0	...	20.9	12.12	4923	3.83	f
Average				22.95	11.87	5344.8	2.37	

Source of variation			
Treatments (T)	8.57*	4.87*	N.S.	40.59*
Applications (A)	10.68*	N.S.	N.S.	N.S.
T X A	4.29*	3.67*	N.S.	N.S.
Location (L)	628.27*	1134.16*	N.S.	15.08*
T X L	22.74*	5.68*	N.S.	3.49*
Dates of rating (D)				N.S.
A X D				N.S.

* Denotes significance at the 5% level.

Table 2.—Sums of squares of 17 orthogonal comparisons between treatments. Rated for Cercospora leafspot of sugarbetts, 1965.

Orthogonal comparisons	August		September 2		September 15	
	Morrow	Englert	Morrow	Englert	Morrow	Englert
1. Tribasic copper sulfate, 4 vs. 6 appl.	1.500	1.500*	1.500*	.667	.000	.167
2. Daconil 1#/100 gal, 4 vs. 6 appl.	.667	.167	.167	.000	.000	.000
3. Daconil 2#/100 gal, 4 vs. 6 appl.	.167	.167	.167	.167	.000	.000
4. Dithane, 4 vs. 6 appl.	1.500	.167	1.500*	.667	.167	.167
5. DU-TER 1.25#/100 gal, 4 vs. 6 appl.	.667	.167	.167	.167	.667	.167
6. DU-TER 1.25#/10 gal, 4 vs. 6 appl.	.167	.000	.667	.167	.667	.000
7. DU-TER 0.625 #/100 gal, 4 vs. 6 appl.	.167	.667	.167	.167	1.500*	.167
8. DU-TER 0.625#/10 gal, 4 vs. 6 appl.	.167	.000	.167	.000	.000	.000
9. Daconil 1#/100 gal vs. 2#/100 gal	.083	.333	.000	.750	.333	.333
10. Tribasic copper sulfate vs. Dithane	.333	1.333*	.333	.333	.083	.333
11. Daconil vs. DU-TER	10.125**	18.013**	7.347**	13.347**	11.680**	3.556**
12. TCS & Dithane vs. Daconil & DU-TER	9.000**	12.840**	5.840**	14.694**	21.778**	3.361**
13. DU-TER, 125 #/100 gal vs. 1.25#/10 gal	.083	2.083**	.083	.333	1.33*	2.083*
14. DU-TER, 125 # vs. 0.625 #	.042	.042	.042	.375	.042	.167
15. DU-TER 0.625 #/100 gal vs. 0.625 #/10 gal	.333	3.000**	1.333*	.750	4.083**	.750
16. Check, 4 vs. 6 applications	.667	.167	.667	.167	.000	.167
17. Check vs. all chemical treatments	23.148**	13.021**	13.724**	28.009**	13.370**	8.898**
Error mean square	.66	.23	30	.27	.35	.24

* Denotes significance at the 5% level.
** Denotes significance at the 1% level.

Discussion

The results of the 1965 fungicidal trials were, in part, substantiated by research conducted in prior years in this project at other state experiment stations and locations. Carlson (1), Finkner, et al. (2), and Forsyth and Broadwell (3) found results similar to those reported herewith. Polyram was not used because it proved least effective in previous tests. DU-TER was used instead of Brestan (triphenyl tin acetate) because of its earlier possible registration, although Brestan was very effective in 1964.

High disease incidence apparently had a greater effect on tonnage than on percentage of sucrose in these experiments. However, the pounds of sugar per acre produced in the Morrow plots did not differ significantly from that produced in the Englert plots.

Summary

1. Significant control of Cercospora leaf spot was achieved in all fungicide treated plots over the water check plots.
2. There was generally no significant increase in disease control when chemicals were applied 6 times as compared to 4 times.
3. DU-TER gave significantly better disease control than did Daconil 2787, Dithant M-45, or tri-basic copper culfate.
4. Daconil 2787 at 1 pound was just as effective in controlling the disease as the higher 2 pound rate.
5. There was no significant increase in disease control when DU-TER was applied at the 1.25 pound rate as compared to half that rate.
6. DU-TER at 1.25 pounds per 10 gallons of water was equal to or better than the same amount of material in 100 gallons of water in terms of disease control.
7. DU-TER at 0.625 pounds per 10 gallons of water per acre was equal to or better than the same amount of material in 100 gallons of water in terms of disease control.

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Scintillation Counting Techniques In The Isotope Dilution Analysis For Sucrose

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Introduction

The Spreckels research laboratory currently uses an isotope dilution (ID) analytical technique to determine the true sucrose content of various materials. The ID method, as originally reported (3)[2], utilized a gas flow proportional counting system to detect radiation. This system was adequate for the analysis of a small number of samples where the counting time was unimportant. However, the limited sample capacity of the proportional counter did not permit the analysis of a large number of samples.

The ID method has been modified to use a liquid scintillation counting technique. The modification has expanded the general usefulness of the method with no loss in analytical accuracy.

This paper details the scintillation counting technique used in the ID method. Included are preparation of the scintillation solvent, the activity of standards, preparation of vials for counting and counting procedures. Factors that affect the accuracy of the method are also discussed.

Method and Materials

Extraction and purification of the sucrose from samples were reported earlier (3) and are essentially unchanged i.e., barium precipitation and carbonation, purification by ion exchange treatment and crystallization from methanol.

Standards: The radioactive standard sucrose is prepared by diluting 0.5 microcuries of sucrose-^{14}C with 1000 grams of nonradioactive sucrose. The sucrose-^{14}C and sucrose-^{12}C are dissolved in the minimum amount of distilled water, concentrated on a steam bath, and recrystallized from distilled methanol. A portion of this standard is added to the extraction sample so that the resultant mixture has about a 1:1 ratio of radioactive to nonradioactive sucrose.

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[2] Numbers in parentheses refer to literature cited.

A counting standard is prepared in the same manner as the sample. In this case however, the sample is an accurately weighed quantity of pure nonradioactive sucrose. The ratio of radioactive to nonradioactive sucrose is accurately determined and held at a 1:1 ratio.

Comparison of the activities of the counting standard and the pure sucrose recovered from the sample is the basis of the isotope dilution method. The ratio of activities is a direct measure of the sucrose originally present in the material being analyzed.

Preparation of Scintillation Mixture: A sample prepared for liquid scintillation counting includes at least three components: the material being counted, a solvent and a scintillator. Often a fourth component is added to facilitate sample preparation. Usually the physical or chemical nature of the sample, rather than the isotope in question, determines the solvents and additives used in sample preparation. We have found the system(l) described below to be well suited for sucrose analysis.

Components of the scintillation mixture include: Reagent grade p-dioxane; Naphthalene-recrystallized from alcohol; 2,5 diphenyloxazole (PPO). Normal mixture composition by weight is 100 p-dioxane, 4.5 naphthalene, 1.0 PPO.

The mixture is treated with 10% by weight activated carbon(2) and filtered through a 0.45 micron Millipore filter. It is advisable to prepare a fresh scintillation solution for each new group of samples to be counted. Scintillation solutions that have yellowed or set for a prolonged period of time are not reliable.

It should be noted that the scintillation solution described does not contain a secondary scintillator e.g., wavelength shifter. We have found, that for our particular scintillation counter, inclusion of a secondary scintillator does not significantly increase our counting efficiency. This situation will not be true for all counters. Maximum counting efficiency for any counter will result when the wavelength of the light emitted by the scintillator most nearly matches the counter's photomultiplier response. Use of a secondary scintillator with our scintillation mixture will, therefore, depend on the characteristics of the counter to be used.

Preparation of Samples for Counting: The choice of the amount of sucrose that can be used in scintillation counting is restricted by the limited solubility of sucrose in the organic scintillation solvent. To increase its solubility, sucrose is dissolved in water. Wa er is a good solubilizer, but it is also a strong chemical quencher i.e., interferes with the transfer of energy between the site of an event and a molecule of scintillator. Inclusion of

water in the scintillation mixture will naturally lower the counting efficiency. However, if a constant amount of water is used for all samples, and the counting rate remains sufficiently high to insure good statistics, the loss in counting efficiency is of little consequence.

We have found that 0.2000 grams of sucrose dissolved in 5.0 milliliters of deionized water will form a homogeneous mixture with the scintillation solvent and will remain in solution at reduced counting temperatures e.g., 50°F.

The sucrose is accurately weighed into glass vials for counting. Five milliliters of deionized water are pipetted into each vial. The vials are capped and warmed in a water bath until the sucrose is completely dissolved. After the vials have cooled, 15 milliliters of scintillation solution are pipetted into each vial. Each vial is capped, agitated for homogeneous mixing, and wiped with lens paper. The vials are then ready to count.

Extreme care should be taken to see that insoluble material does not enter the vial with the water. To prevent this the deionized water is filtered through a 0.45 micron Millipore filter before use.

The use of a constant amount of solution in all vials requires precise delivery of both water and scintillation solvent to the vials. Nonuniform delivery will result in greater than normal variation in average count rate between consecutive vials of the same sample. Automatic pipettes with good delivery precision are essential for dispensing scintillation solvent and water.

The choice of the number of standards and samples to be counted and the counting time required for any particular accuracy of sucrose determination is based, in theory, on the activity of the radioactive component. However, certain variables can limit the precision of the test. We have found that the physical and chemical nonuniformity of all commercially available glass vials can be one such limiting error. The magnitude of this error can be reduced by using vials that have been selected for their uniformity of counting. A simple, yet novel vial selection procedure is given below[7]. It should be remembered that vial selection is not a necessary part of the liquid scintillation technique. However, selection of vials will reduce the number of replications required and, therefore, the time necessary to achieve the desired analytical precision.

Vials can be selected for their uniformity of counting if each vial is counted with the same source of radioactivity. Variations in count rates between vials due only to the random nature of radioactive decay follow a known function and can be approximated. Therefore, it is possible to make a comparison of vials

where there is a reasonable probability that any detectable non-uniformity of counting is attributable to the physical or chemical nature of the vials.

If a weighed amount of a homogeneous radioactive scintillation solution is added to each vial, then all vials can be counted with the same source of radioactivity.

The random nature of radioactive decay is described by a Poisson distribution function and the expected variation due to this randomness is approximated by:

$$\text{Eq. [1]} \quad \sigma_p = \frac{\sqrt{\text{Total Counts}}}{\text{Total Time}}$$

The expected variation due to the random nature of radioactive decay can, therefore, be reduced by increasing the total counting time per vial.

The resultant counts of the vials will be distributed in a normal fashion. An example of such a distribution is given in Figure 1. The count interval in the distribution is twice the expected standard deviation of counts due to the randomness of radioactive decay calculated in terms of counts per minute per gram of scintillation solution. The 2σ value for the count interval allows a 95% assurance that the vials in any interval are uniform to 0.05% in counting. Vials in any one interval are considered sufficiently uniform for use.

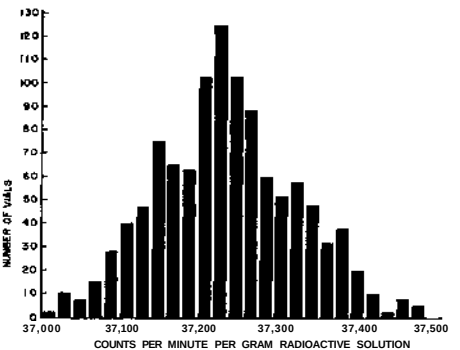

Figure 1.—Distribution of vials by count rate.

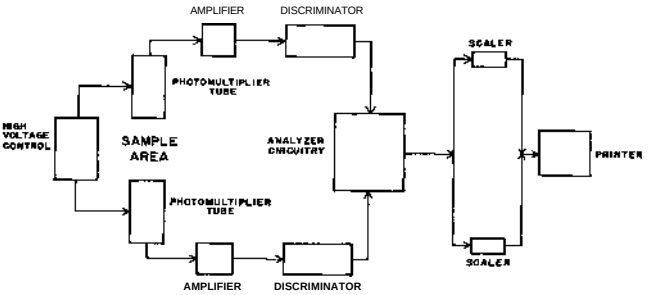

Figure 2.—A simplified schematic of a liquid scintillation counter.

Counting Procedure: Samples and standards are counted in a liquid scintillation counter. A simplified schematic of a modern scintillation counter is given in Figure 2.

It is generally accepted that a more efficient count is obtained if the sample temperature is lowered. This axiom was developed from early work in the field of liquid scintillation counting when counters were less sophisticated. Modern instruments have photomultiplier tubes that are less sensitive to thermal excitation, and ambient counting temperatures are now common. The choice of counting temperature should, therefore, be based on the particular scintillation counter.

The modern scintillation counters allow a choice of counting procedures. Two counting procedures, the channels ratio and balance point methods, are equally applicable to the ID method.

The channels ratio procedure does not give maximum counting efficiency, but it does provide a constant check on sample uniformity. This procedure is normally used for counting samples that vary in their degree of quench. If it is desired to prepare samples that are uniformly quenched, as in the ID procedure, then this counting method is a good check on sample preparation. Once sample preparation is standardized it is better to count the samples with the highest counting efficiency.

The balance point counting procedure insures maximum counting efficiency for samples of uniform composition. The energy discriminator settings used in this procedure will depend on the liquid scintillation counter to be used.

Calculation for percentage sucrose in the sample is the same as for proportional counting and has been described earlier (3). An example of typical counting data and calculations is given in Table 1.

Table 1.—Liquid scintillation counting data and percent sucrose calculation.

Sample	Molasses (beet)			Counting standard		
Vial No.	Wt Sample in vial gm	Total counts 10 min	Avg CPM 0.2000 gm	Wt std in vial gm	Total counts 10 min	Avg CPM 0.2000 gm
1	0.2003	1,682,300	167,978	0.2002	1,676,510	167,484
2	0.1998	1,685,720	168,741	0.2001	1,676,370	167,553
3	0.2000	1,683,970	168,397	0.2003	1,678,090	167,658
4	0.1999	1,686,300	168,714	0.2001	1,670,350	166,952
5	0.1998	1,686,610	168,829	0.2004	1,675,020	167,168
6	0.2002	1,687,130	168,544	0.2000	1,672,060	167,206
7	0.1999	1,682,580	168,387	0.2001	1,673,370	167,258
8	0.2000	1,686,120	168,612	0.1996	1,670,230	167,358
9	0.1998	1,685,500	168,719	0.2000	1,672,680	167,266
10	0.2000	1,681,930	168,193	0.2004	1,671,990	166,865

$W^{14C} = 6.0016$ $R_1 = 167,266$ $\sigma_{R_1} = \pm 74$ $a_1 = 0.00044$

$W_s = 12.0017$ $R_2 = 168,506$ $\sigma_{R_2} = \pm 87$ $a_2 = 0.00052$

$$\% \text{ Sucrose} = \frac{100\ W^{14C}}{W_s}\left(\gamma \frac{R_1}{R_2} - 1\right) \text{ and}$$

$$\sigma_{\% \text{ Sucrose}} = \frac{100\ W^{14C}}{W_s} \cdot \gamma \frac{R_1}{R_2} \cdot \sqrt{a_1^2 + a_2^2}$$

$$\% \text{ Sucrose} = \left(\frac{6.0016}{12.0017}\right)(100)(0.98528) = 49.27$$

$$\sigma_{\% \text{ Sucrose}} = (W^{14C})\ (100)\ (L985)\ <0.00068) = \pm 0.07$$

where:

W^{14C} = weight of standard sucrose added to the extraction sample
W = weight of the extraction sample
R = average counting rate(CPM) of the counting standard
R_2 = average counting rate(CPM) of the sample
a_1 = relative error of the counting standard
a_2 = relative error of the sample
γ = weight ratio $W^{12C} : W^{14C} = 2.0000$

A comparison of results by proportional and liquid scintillation counting techniques is given in Table 2. The data show no significant difference in percentage sucrose or analytical error between the two methods.

Table 2.—A comparison of results by proportional and liquid scintillation counting techniques in the ID determination of sucrose.

Beet sample	Proportional	Liquid scintillation
1	12.44 ± 0.02	12.44 ± 0.03
2	12.52 ± 0.03	12.54 ± 0.02
3	12.75 ± 0.06	12.75 ± 0.02
4	12.66 ± 0.02	12.69 ± 0.02

Summary

The use of a liquid scintillation counting technique in the isotope dilution analysis for sucrose is described. Included are

preparation of the scintillation mixture, activity of standards, preparation of vials for counting and counting procedures. Examples of typical counting data and analytical results are also given.

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Application of Sensitive Adiabatic Calorimetry to Beet Industry Problems

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Introduction

Over the years, the beet industry has experienced a number of costly fires in bulk storage of dried beet pulp. While some fires were the result of burning embers from the driers which were carried into storage, other fires were the result of spontaneous heating. Spontaneous heating may also degrade stored pulp and ultimately render it unfit for consumption, even when no ignition occurs.

Spontaneous heating occurs in many materials other than beet pulp and may be caused by the metabolism of microorganisms and by chemical reactions in the material [1,3,4,5][3]. Since microorganisms are not biologically active at the moisture levels and relative humidities encountered in normal pulp storage, spontaneous heating of dried pulp is largely due to oxidative reactions. It is reasonable to assume that oxidation of pulp constituents occurs at all practical storage temperatures. Since oxidation is an exothermic process, spontaneous heating of pulp cannot be prevented. The oxidative processes in beet pulp are not particularly harmful if the pulp temperature is kept reasonably low during storage. Elevated temperatures lead to accelerated heating rates and pulp degradation.

Spontaneous heating, degradation and ignition of dried pulp can be controlled through suitable storage practices which prevent harmful temperature increases. It is necessary to cool the dried pulp to a reasonable temperature before storage and to provide, during storage, for the removal of the heat generated through the spontaneous processes. Since dried pulp has a relatively low bulk density and 80% of its volume is air, it is a very efficient thermal insulator. It may thus be necessary to remove heat from a storage pile by artificial means. The design of facilities for heat removal requires a prior knowledge of the rates of spontaneous heat generation as a function of storage temperature and pulp composition. This knowledge may be acquired only through sensitive methods of calorimetry.

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[3] Numbers in brackets refer to literature cited.

Calorimetric Techniques

Figure 1 illustrates an elementary calorimeter. Here, the sample is contained in the inner calorimeter, C, which is enclosed in the calorimeter jacket, J. The inner calorimeter is separated from the jacket by an air space, A, which acts as a thermal insulator. The jacket is submersed in a water bath, B, which is stirred by stirrer, S, to maintain a uniform temperature throughout the bath. T_0 and Tj are temperature sensors.

When heat is generated in the inner calorimeter, its temperature rises above that of the jacket and heat flows through the air gap from the inner calorimeter to the jacket and bath. When the temperature of the jacket and water bath is held constant and heat is generated in the calorimeter at a constant rate, the temperature difference between the calorimeter and jacket, $T_c - T_J$, is also constant. The temperature difference is a function of the rate of heat evolution, dQ/dt, and the leakage modulus of the calorimeter, k. It is expressed by the equation: $dQ/dt = k(T_c - T_J)$. The leakage modulus k may be determined by electrical means, i.e., by passing a precisely known current through a resistor of precisely known value, located inside the inner calorimeter.

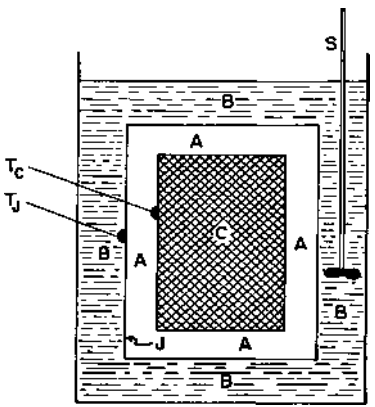

Figure 1.—Calorimeter.

The above procedure is generally referred to as the ordinary method of calorimetry. It is capable of high accuracy, subject to the ability of measuring extremely low temperature differences.

When calorimeter and jacket temperatures are exactly equal, no heat flows through the air gap. When heat is generated in the inner calorimeter while no heat flows through the air gap, the temperature of inner calorimeter and contents will rise. Obviously, it is necessary to raise the temperature of the jacket and bath at the rate of temperature increase occurring in the inner calorimeter in order to maintain the equality of temperatures necessary for zero heat flow through the air gap. This method of calorimetry, wherein all heat generated within the inner calorimeter is retained, is known as the adiabatic method. Here the rate of heat evolution, dQ/dt, is a function of the rate of temperature increase, dT/dt, and the heat capacity, C, of the inner calorimeter and its contents. This is expressed by the equation: $dQ/dt = C(dT/dt)$. As in the ordinary method, electrical means are employed for the determination of the heat capacities of the inner calorimeter and its contents.

Calorimeter Design

A comprehensive study of spontaneous heating in beet pulp must encompass a wide range of heating rates. At the lower rates of heating, the ordinary method of calorimetry may suffer from inaccuracies in the measurement of extremely small temperature differences. In the adiabatic method, where increases of temperature are measured, it is possible to extend the duration of the experiment until a precisely measurable temperature increase has occurred. In this method, the major problem is the continued maintenance of the adiabatic state, i.e., temperature equality of inner calorimeter and jacket.

In either method, the temperature of the bath cannot be held constant; at best, it oscillates about the control point by small deviations. In the ordinary method, the determination of the low temperature difference between calorimeter and jacket is obviously made difficult by the temperature oscillations of the bath. In the adiabatic method, the temperature of the inner calorimeter is unaffected by the temperature oscillations of the bath, and the temperature increase during the test can be made larger by extension of the test duration. The temperature oscillations of the bath do not preclude adiabatic operation; it is necessary, however, to maintain equality between the temperature of the inner calorimeter and the mean temperature of the bath. The adiabatic method is therefore preferable for the determination of spontaneous heating of beet pulp.

The Spreckels research group chose the adiabatic method for its investigations of spontaneous heating of beet pulp. Two calorimeters, of identical design, were constructed and placed into service in 1956. Details of calorimeter design, associated automatic controls, and operational procedures follow.

The inner calorimeter is fabricated from a section of copper tubing, 4" in diameter and 10l/£" long, with 1/16" wall thickness. Six copper fins extend from the wall inward and thereby provide for heat transfer between pulp and calorimeter wall. Figure 2 shows the construction of the inner calorimeter. The fins are soldered to the wall, together with twelve small copper tubes. These tubes contain twelve thermocouples for temperature control, three thermocouples for temperature measurement, a precision resistor for electrical calibration, and an additional resistance element used to bring the calorimeter to the desired starting temperatures for tests. The inner calorimeter is suspended from the lid of the calorimeter jacket by a structure of low thermal conductivity. Connections to bath thermocouples, reference thermocouples, controller and recorder leads, and for the resistance heaters are brought out through copper tubing attached to the lid.

Figure 2.—Inner calorimeter.

The calorimeter jacket is fabricated from 6" diameter copper tubing. A Neoprene gasket and twelve machine screws provide for watertight attachment of the jacket lid. A one-inch air gap separates the inner calorimeter from the jacket. The assembled calorimeter is shown in Figure 3.

It is desirable to draw atmospheric air through the pulp sample during the tests. The air must be adjusted to the temperature and to the equilibrium relative humidity of the pulp sample. This is accomplished by passing the air through the tubing coil at the base of the jacket and then over a suitable mixture of

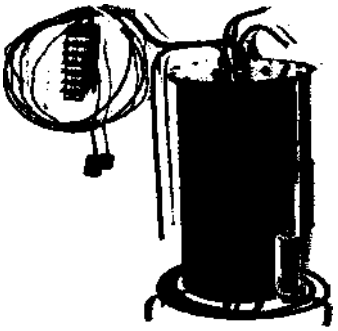

Figure 3.—Assembled calorimeter.

sulfuric acid and water in a test tube attached to the calorimeter. After passage through the pulp sample, the air is drawn through spiral absorbers [2] for subsequent analyses for the amount of CO_2 produced by the spontaneous reactions. The air pumps consist of hypodermic syringes driven through a scotch yoke by a constant-speed motor. The absorbers and pump mechanism are shown in Figure 4.

Figure 4.—Absorbers and air pumps.

During operation, the assembled calorimeter is submerged in a water bath, which serves as a temperature controlled environment. The two calorimeters employed in our tests are shown in Figure 5 with their associated recorders, controllers, and auxiliaries. The calorimetric equipment operates from the 115 volt laboratory lines; an auxiliary generator, located outside the laboratory, provides standby power in the event of power outages. The air temperature in the calorimetric laboratory is maintained at 75 ± 1°F.

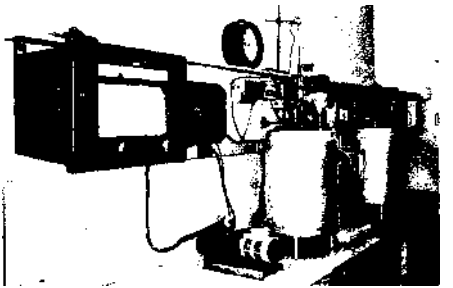

Figure 5.—Adiabatic calorimeters and controls.

The two large tanks are the calorimeter water baths, which are heated electrically by the output from variable-voltage transformers, one of which is shown in the foreground on the bench. Pulp temperatures are recorded on two stripchart recorders located at the extreme ends of the bench. The black enclosures adjacent to the recorders contain temperature control amplifiers which operate the variable-voltage transformers through servo motors and gear trains.

For adiabatic operation it is necessary that the temperatures of the inner calorimeter and the jacket be exactly equal. Since the jacket is in intimate contact with the water in the calorimeter bath, the required condition of temperature equality is achieved when the inner calorimeter and the bath are in thermal equilibrium. A string of 24 copper-constantan thermocouples senses any temperature differences and, through a Honeywell servo amplifier and motor, controls the electric input to the heaters in the shell of the bath. The thermocouple junctions are alternately located in the inner calorimeter and in the temperature probe in the bath. In this manner a temperature difference of 1°C produces an electric output of about 0.5 millivolts from

the string. Since the amplifier initiates motor action with inputs of 0.003 millivolts, a temperature difference of 0.006°C results in correction of the heat input to the bath. Sensing lags and the capacity of the bath produce a state of steady hunting of the bath temperature about the temperature of the inner calorimeter, with a period of about two minutes and a maximum deviation of less than ±0.01 °C from the mean. Without this steady hunting, the jacket temperature could remain about 0.01 °C above or below that of the inner calorimeter, due to the dead-zone of the servo amplifier. This would lead to significant heat transfer to or from the inner calorimeter. With steady hunting, the mean temperature difference is effectively zero and the calorimeter operates in the adiabatic mode.

The temperature of the water bath is controlled by the voltage applied to the heater windings. This voltage is adjusted by a variable transformer which is driven by a servo motor through a gear reducer with a ratio of 150:1. The control is shown in Figure 6. A resistor, placed in series with the heater, is bridged by

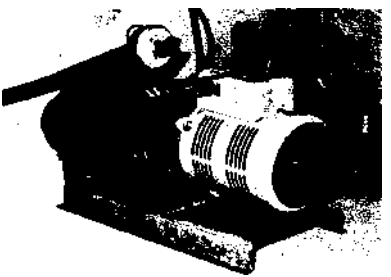

Figure 6.—Voltage control unit.

a mercury switch which is actuated by rotation of the servo motor shaft. When additional heat is required, initial rotation of the shaft in one direction causes the closing of the mercury switch and thereby increases instantly the heat input to the bath by a discrete amount without change of the voltage setting of the variable transformer. When less heat is required, the motor shaft begins to turn in the opposite direction and causes the mercury switch to open, thereby instantly reducing the heat input to the bath. With a heater resistance of 100 ohms and a series resistor of 50 ohms, the heat input to the bath varies between +40% and -40% of the mean, due to the action of

the switch. When the temperature of the inner calorimeter is constant, the output voltage of the variable transformer also remains constant. As the calorimeter temperature increases, due to spontaneous heating of the sample, the sorvo motor adjusts the output voltage gradually upward to maintain adiabatic operation of the calorimeter.

All servo amplifiers display a small amount of offset, i.e., an output signal is produced in the absence of any input signal. For adiabatic operation of the calorimeters, it is necessary to compensate for this offset. In our calorimeter controls, compensation is provided by insertion of an opposing voltage in the thermocouple leads. The magnitude and polarity of the compensating voltage is determined through calorimetric tests on the empty calorimeter. Aging of components causes a gradual change of the amplifier offset voltage. It is thus necessary to redetermine this offset at regular intervals.

The temperature of the inner calorimeter is sensed by three thermocouples and recorded on a strip chart recorder having a span of 1 millivolt, full scale. The recorder has provisions for variable suppression. It is thus possible to zero the recorder at any calorimeter temperature. The thermocouple reference junctions are maintained at 50 ± 0.005°C in a reference oil bath, shown in Figure 7.

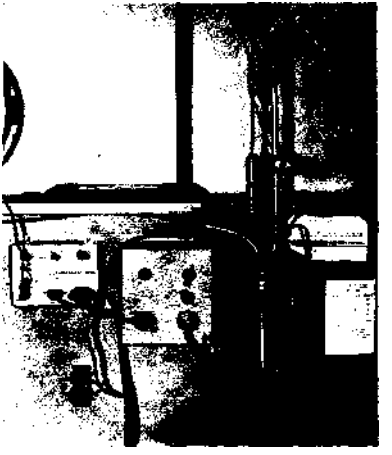

Figure 7.—Reference oil bath.

The oil bath consists of a 1-liter Dewar flask with an internal electric heater and a stirrer. The temperature is controlled by an adjustable mercury-in-glass contacting thermometer and a transistorized relay. Instead of the customary on-off control, we employ a two-position control wherein the electrical heat input is alternately slightly above and below that required to maintain the desired temperature. Bath temperature is maintained within ± 0.005 °C as indicated on a Beckman differential thermometer.

The quantity recorded on the strip chart recorders is the voltage produced by the thermocouples due to the temperature difference between the inner calorimeter and the reference bath. This quantity is converted to temperature, in °C, with the aid of conversion tables, calculated for three pairs of copper-constantan junctions and a reference temperature of 50°C. Recorder output may be read within ± 0.001 millivolt and the temperature may be determined within + 0.008°C. Thus, a temperature increase of 1°C may be measured with a maximum error of ± 1.6%. Duplicate tests with pulp samples of very low spontaneous heating rates and resultant temperature increases of about 0.5°C in a 27 day period have shown agreement within 3%. It is thus seen that calorimetric accuracy is limited mainly by the accuracy of temperature determinations.

Test Procedure

The pulp to be tested is mixed thoroughly and a sample is taken for moisture analysis by oven drying. While awaiting the results of the moisture analysis, the pulp is stored in a plastic bag to prevent changes in moisture content. From the moisture analyses and applicable equations, the specific heat of the pulp is calculated. With the aid of graphs, which relate pulp moisture to equilibrium relative humidity, the concentration of the dilute sulfuric acid charge for the air humidifying test tube in the calorimeter is determined. The inner calorimeter is charged with the sample, packing it firmly, and the weight of the pulp charge is determined.

The calorimeter is then assembled, placed into the water bath, and the electrical connections are made to the control and recording units. The air pump is connected to the calorimeter and is started. The suppression of the temperature recorder is adjusted to record zero on the scale, and the bath agitator is turned on. Next, the electrical heater in the inner calorimeter is energized, and the calorimeter temperature begins to increase. The resulting temperature difference between inner calorimeter and bath is sensed by the thermocouples, and corrective action is initiated by the amplifier and servo motor to

increase the heat input to the calorimeter bath. While the inner calorimeter temperature approaches the desired starting temperature of the test, the bath temperature follows within a small fraction of a degree.

When the starting temperature is reached, the heater in the inner calorimeter is de-energized and the suppression of the recorder is adjusted to match the starting temperature. The calorimeter is left on automatic control for about 20 hours during which the pulp charge and the inner calorimeter reach thermal equilibrium. This is followed by the calorimetric test run. During the test run, calorimeter temperatures are determined at intervals of 24 hours and recorded. Ventilating air flow through the pulp sample is maintained at constant rate. The incoming air is passed through a soda lime tube for the removal of atmospheric CO_2. The exhaust air passes through spiral absorbers filled with a solution of barium hydroxide for the determination, by titration, of the quantity of CO_2 generated by the spontaneous processes [5].

The duration of each test is governed by the rate of spontaneous heating of the sample. For accuracy, a minimum temperature increase of 0.5°C should occur during the test; an increase of about 1°C is generally employed in our tests.

Test Evaluation

Spontaneous heating rates determined in our calorimeters are expressed in units of gram-calories developed in 1 kg of pulp during a 24 hour period, i.e., g-cals/(kg) (day). This is calculated from the temperature increase, the test duration and the heat capacity of inner calorimeter and pulp sample. The following example may illustrate the procedure:

Data:		
Heat capacity of empty calorimeter,	C_c =	408 g-cals/°C
Heat capacity of pulp sample,	C_p =	166 g-cals/°C
	Total C =	574 g-cals/°C
Starting temperature		40.02°C
Final temperature		41.20°C
Temperature increase, ΔT =		1.18°C
Test duration, Δt =		7 days
Sample weight, W =		.439 kg

Calculations

The spontaneous heating rate, R, in g-cals/(kg (day), is calculated from the equation: $R = \frac{C\ \Delta T/\Delta t}{W}$. For the above data: R = (574 × 1.18) / (7.00 × .439) = 220 g-cals/(kg) (day). Stated in

more familiar terms, a ton of the pulp tested will generate spontaneously about 793 BTU per day when stored at 40-41°C. Under these conditions, a storage pile of 10,000 tons of this pulp will generate spontaneously nearly 8 million BTU/day. It is thus necessary to remove heat from the storage pile at a rate of 8 million BTU/day, through radiation, conduction, convection, and other means. If the rate of heat generation exceeds the rate of removal, the temperature of the pile will rise continuously, and it may eventually ignite spontaneously.

Factors in Spontaneous Heating

Since 1956 the research group of Spreckels Sugar Company has directed its attention to the determination of factors which govern spontaneous heating in dried pulp storage through adiabatic calorimetry and chemical analyses. Special emphasis was placed on the effects of factors which can be controlled in manufacture and storage. The data indicate clearly that spontaneous heating rates increase exponentially with temperature, moisture content and levels of additives. It was also determined that the type of additive dried onto the pulp has a significant bearing on spontaneous heating rates.

Adiabatic tests over a span of twelve years have shown that spontaneous heating rates of beet pulp are governed not only by the type and level of additives, moisture content and storage temperature, but also by seasonal factors. The heating rate of pulp produced one year differed from that produced in another year by a factor of 2.5 at comparable levels of molasses addition, moisture, and storage temperature. To insure storage safety, it is thus necessary to determine periodically the heating rates of production pulp and to adjust pulp composition or storage practices accordingly.

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The Use of Systemic Insecticides to Reduce the Incidence of Curly Top Virus Disease in Sugarbeets[1]

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Curly top virus of sugarbeets, named for the extreme curling and distortion it causes to the leaves of infected plants, severely restricted the sugar beet industry in several western states from 1916 to 1934. This disease is transmitted by the beet leafhopper, *Circuitfer tenellus* (Baker), and is characterized by sporadic and destructive outbreaks varying in intensity from year to year. The leafhopper migrates from adjacent and distant semi-desert flora to agricultural areas.

Sugarbeet varieties highly resistant to curly top have been developed, that yield reasonably well, despite losses in the worst years. However, when these resistant strains are hybridized with leaf spot resistant strains, the resulting hybrids are intermediate in resistance, and severe losses may occur. Eastern New Mexico and western Texas are ecologically susceptible to both diseases, and a higher degree of protection from these diseases is desirable.

The curly top virus can be transmitted in only a few minutes of feeding, so the leafhopper would theoretically have to be controlled before the insect completes a transmission feeding. Such drastic control was not feasible before the advent of modern systemic insecticides.

Control of certain insects on sugarbeets by seed or soil treatment has been reported by various workers. Hills et al. (4, 5)[3] and Dorst (1, 2) reported the effectiveness of phorate and Disyston applied to the seed for beet leafhopper control on sugarbeets grown for seed. Georghion et al. (3) showed a substantial reduction in the spread of curly top virus of sugarbeets in California by the application of phorate.

The objectives of this investigation were to determine: (a) if any of the insecticides would give protection; (b) which of four systemic insecticides give the best protection; (c) which

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[3] Number in parentheses refer to literature cited.

of two rates was the most effective in reducing curly top; (d) the length of effectiveness of the insecticides; and (e) to measure the influence of insecticides on yield.

Materials and Methods

Before seeding, four systemic insecticides, each at one and two pounds of active material per acre, were banded into the soil at a depth of approximately eight inches. The insecticides used were NIA 10242, Timek, phorate, and Di-syston. The eight treatments plus an untreated check were randomized in a field test with four replications. The experiment was conducted for two years, 1966 and 1967. A curly top susceptible sugarbeet variety, HC 2, from the Holly Sugar Corporation, was planted both years. Plot sizes were single 40-inch beds (two rows per bed) 100 feet long. Plot harvest consisted of 100 feet of row harvested by hand. A sample of 15 roots was taken from each plot for sucrose and tare analysis. Curly top counts were started approximately 10 days after thinning, when plants were six weeks old, and taken weekly for a period of 13 weeks. A plant was considered as infected with curly top virus if the leaf curling and distortion could readily be observed. Plants were not prudently scrutinized for curly top disease symptoms. No insect records were taken, and the curly top counts Avere assumed to indicate beet leaf hopper control.

Curly top data were analyzed by Aveeks for the tAvo years combined since readings were made on approximately the same date each year and the plants Avere nearly the same in growth and biological development. The tAvo years of data Avere also combined for analyses of stand, yield and sugar content. Analyses of variance Avere computed to estimate the primary sources of variation and interactions and to test for their statistical significance. Duncan's multiple range test Avas applied, where applicable, to differentiate specific means.

Table 1 gives the comparison of the tAvo years for seAeral agronomic practices and dates.

Results and Discussion

The percentages of curly top differed significantly betAveen years in 11 out of 13 weeks (Table 2). Overall, the disease Avas three times more severe in 1966 than in 1967, but more plants were affected during the first four Aveeks in 1967 than in 1966. During the two weeks of June 18 and June 25, the severity of curly top was similar each year. Although the amount of curly top increased during the 13 Aveek season in both years, the increase occurred more rapidly after June 25 in 1966 than in 1967.

Table 1.—Dates and agronomic practices in the insecticide tests with sugarbeets, Plains Branch Station, Clovis, New Mexico, 1966 and 1967.

	1966	1967
Insecticide applied	5/15	5/22
Variety	HC 2	HC 2
Date planted	5/16	5/23
Emergence	5/30	4/3
Thinned	5/9	5/10
Number of Irrigations	7	7
Fertilizer applied	100-44-0	150-100-0-55S
Harvest	11/15	11/16
First curly top readings	5/23	5/20
Last curly top readings	8/15	8/12
Field design	Randomized complete block	3 × 3 lattice

Table 2.—Mean curly top percentage, all sugarbeet plots, for 13 weeks, Plains Branch Station, Clovis, New Mexico, 1966 and 1967.

Date	1966	1967	Differences
5/21	0.5	3.1	2.6**
5/28	1.5	4.5	3.2**
6/4	2.6	5.1	2.5**
6/11	5.2	7.2	2.0*
6/18	8.7	8.6	—0.1NS
6/25	12.3	10.2	—2.1NS
7/2	22.0	10.9	—11.1*
7/9	28.7	12.2	—16.5**
7/16	36.4	13.4	—23.0**
7/23	40.1	15.9	—24.2**
7/30	49.7	16.6	—33.1**
8/6	61.0	18.5	—42.5**
8/13	81.6	24.5	—57.1**

NS Non-significant
* Significant at the 5% level of probability
** Significant at the 1% level of probability

The mean responses (percentages of curly top) to insecticide treatments are shown in Table 3. The insecticides reduced the amount of curly top below that in the untreated checks at all periods except the first *two* where the disease obviously had not become well established. The final curly top counts showed that the means of the chemically treated plots and the checks were beginning to converge which suggests that the length of effectiveness of the chemicals was about 18 weeks.

Differential effects among the various insecticides were indicated with significant differences among the nine periods during June and July. Evidently, about three weeks were required for the curly top disease to become established, hence, the failure of mean separation among the treatments during the first two weeks. Much of the effectiveness of the insecticides seemed to

have been lost by August so that the differences in treatment responses were not significant in the last two periods. NIA 10242 was consistently most effective in preventing curly top, and Di-syston was the least effective. Responses from Timek and phorate were intermediate at all periods.

Differences between the one and two pound rates and the rates by insecticide treatment interactions were consistently non-significant. The only significant year by treatment interaction found in the June 25 data must have been a chance deviation. The consistent response of these four systemic insecticides in extremely different years suggests that these results could be applied over a wider range of conditions.

Figure 1 graphically compares the insecticides and the check for the average curly top percentage during the 13-week period. The means for the insecticides were the results of 16 estimates (4 replications, 2 rates and 2 years). The check mean contained only eight estimates (4 replications and 2 years).

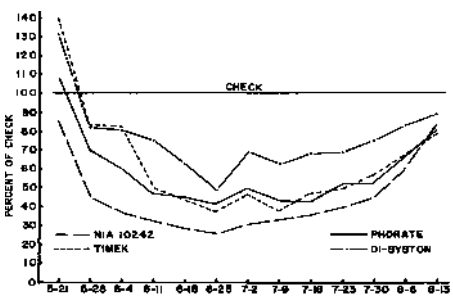

Figure 1.—Mean percentages of curly top in sugarbeets treated with four insecticides (averaged over rates and years). Plains Branch Station, Clovis, New Mexico, 1966-67.

Figure 2 shows the mean curly top counts for the four insecticides, given as percentages of the check. The insecticide lines slope downward for six weeks, indicating effective control of the leafhopper. However, after six weeks, the lines begin to slope upward, indicating the decreased effectiveness of the insecticide. Apparently, the systemic insecticides maintain some control of the leafhopper for about 18 weeks after application.

Table 4 shows a comparison of the two rates averaged over insecticides replications and years. In the first seven weeks there

Table 3.—Mean curly top percentage of sugarbeets during a 13-week period, when treated with four different systemic insecticides, Plains Branch Station, Clovis, New Mexico, 1966-1967.

Insecticides[1]	Date of curly top rating[2]												
	5/21	5/28	6/4	6/11	6/18	6/25	7/2	7/9	7/16	7/23	7/30	8/6	8/13
NIA 10242	1.38	1.81	2.12 a	3.56 a	4.88 a	6.44 a	9.25 a	13.56 a	15.94 a	19.12 a	24.00 a	33.94	52.12
Timek	1.75	2.75	3.44 ab	5.12 a	7.62 a	10.31 ab	14.92 a	17.56 ab	19.12 a	25.31 ab	27.94 a	34.31	50.56
Phorate	2.25	3.31	4.12 b	5.44 a	7.31 a	9.31 ab	14.00 a	15.19 a	21.06 ab	24.19 ab	30.19 ab	35.81	48.94
Di-syston	2.12	3.25	4.62 b	8.25 b	10.75 b	12.12 b	20.69 b	25.25 b	30.50 b	32.12 b	40.12 b	46.56	55.50
Sig. Level	NS	NS	*	**	**	*	**	*	*	*	*	NS	NS
Mean (Insect)	1.88	2.78	3.58	5.59	7.64	9.55	14.72	17.89	21.66	25.44	30.56	37.66	51.78
Check (Mean)	1.62	4.00	5.75	11.00	17.25	25.25	30.25	40.88	45.25	48.75	54.00	56.62	63.12
Ck. vs. insect.	NS	NS	**	**	**	**	**	**	**	**	**	**	*
Rates	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rates X Insect.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Yrs. X Treatments	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

[1] Average of 1 and 2 lbs/A. rates

[2] Duncan's multiple range test

NS Non-significant

* Significant at the 5% level of probability

** Significant at the 1% level of probability

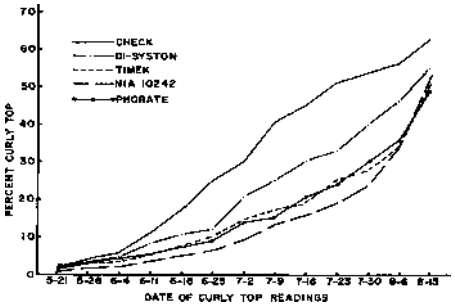

Figure 2.—Mean curly top count as a percentage of the check in sugarbeets treated with four insecticides (averaged over rates and years). Plains Branch Station, Clovis, New Mexico, 1966-67.

was little difference between the one and two pound rates. However, in the next four weeks, the plots treated at the one-pound rate had 7 to 15 percent more curly top than those treated at the two-pound rate. The curly top counts became similar again in the 12th and 13th weeks. Although the differences were not significant, the trend in the data suggests a longer residual effect from the higher rate of application.

Systemic insecticides are absorbed and translocated inside of a living plant. Plants and plant material are now scrutinized very carefully for pesticide residues by the Food and Drug Administration. Therefore, it would be desirable to have a systemic insecticide which would give good leafhopper control during that growing season but would be completely dissipated at harvest time. Some of the chemicals used in this test are approaching this ideal. Perhaps a split application of these insecticides, approximately ten weeks apart, at the one-pound rate would give better control of the disease for a longer time with no excess residue at harvest time. This would remain within the use limitations of the approved insecticides.[4]

Averages of stand and yield for the two years are shown in Table 5. Tonnage yield, sucrose percentages, stand and sugar per acre were all higher in 1967 than in 1966, and all differences

[4] NIA 10242 and Timek are not approved for sugarbeets. Recent correspondence (October 1, 1968) with Niagara Chemical Division of FMC Corporation indicated that they are hoping for registration of NIA 10242 (Furadan) for use on sugarbeets in the 1970 growing season.

Table 4.—Percent curly top of sugarbeets as affected by rates of insecticides (average of four insecticides), Plains Branch Station, Clovis, Nw Mexico, 1966-1967.

Dates	Pounds active/acre 1	2	Differences
5/21	1.88	1.88	**0**
5/28	2.84	2.72	**.12**
6/4	3.59	3.56	.03
6/11	5.69	5.50	**.19**
6/18	7.69	7.59	**.10**
6/25	9.59	9.50	**.09**
7/2	14.22	15.22	**— 1.00**
7/9	18.66	17.12	**1.54**
7/16	23.19	20.12	3.07
7/23	26.38	24.50	**1.88**
7/30	31.69	29.44	2.25
8/6	37.78	37.53	.25
8/13	51.81	51.75	.06

Table 5.—Yearly means for yield, stand, and percent sucrose of sugarbeets, insecticide tests, Plains Branch Station, Clovis, New Mexico, 1966 and 1967.

Years	1966	1967	Differences
No. of beets/100 ft.	82.5	97.5	15.0**
Tons per acre	17.11	26.76	9.65**
Percent sucrose	13.91	14.54	0.63NS
Pounds sugar/A	4781	7777	2996**

NS Nonsignificant
** Significant at the 1% level of probability

were significant except between the sucrose percentages. Curly top was more severe in 1966 (Table 2) and caused a greater reduction in yield and stand.

Table 6 summarizes results from the 1966-67 tests for the yield components. This analysis showed that a significant difference was obtained between the check mean and the mean of the insecticide-treated plots for tonnage and sugar per acre. Furthermore, mean yields in tons of beets and pounds of suear per acre were significantly higher for plots treated with NT A 10242 and phorate than those treated with Di-syston. Rate effects, rate by insecticide treatment interaction, and year by insecticide treatment interaction were all found to be nonsignificant.

Table 7 summarizes the results of insecticide rates for viHd and stand. Although no significant differences were found between rates, the two-pound rate gave consistently better yield and stand. A similar trend was noted in curly top percentage (Table 4).

The yield results appeared negatively associated with the curly top counts. For example, the check plots and the Di-syston-treated plots had the most curly top and the lowest yields. This

Table 6.—Years and rates combined analysis of yield, stand and level of significance from the application of insecticides on sugarbeets. Plains Branch Station, Clovis, New Mexico, 1966-1967.

Treatment	No. of beets per 100 ft	Tons per acre [1]	Percent sucrose	Pounds sugar per acre [1]
NIA 10242	87.1	23.30 a	14.35	6704 a
Timek	96.4	22.97 a	13.76	6352 ab
Phorate	91.2	24.24 a	14.29	6988 a
Di-syston	85.6	19.65 b	14.43	5721 b
Sign. level	NS	*	NS	*
Insecticide mean	90.1	22.54	14.21	6441
Check mean	89.4	17.12	14.38	4982
Check vs. insecticides	NS	**	NS	**
Rates	NS	NS	NS	NS
Rates × insecticides	NS	NS	NS	NS
Insecticides × years	NS	NS	NS	NS
C.V.	21.34%	18.61%	5.56%	20.48%

[1] Duncan's multiple range test
NS Non-significant
* Significant at the 5% level of probability
** Significant at the 1% level of probability

Table 7.—Effect of insecticide rates on yield, stand, and sucrose of sugarbeets, Plains Branch Station, Clovis, New Mexico, 1966-1967.

Insecticides	Rate/acre 1 lb	Rate/acre 2 lbs
No. of beets/100 ft.	88.8	91.3
Tons per acre	21.80	23.28
Percent sucrose	14.08	14.33
Pounds sugar/acre	6186	6696

was expected because effective systemic insecticides will control the leaf hopper which transmits the curly top virus. If the curly top disease can be controlled, higher production can be expected. Phorate and NIA 10242 showed the greatest promise of controlling the sugarbeet leafhopper.

Summary

Four systemic insecticides applied preplant to sugarbeet plots approximately eight inches below the soil surface at one and two pounds per acre for two years resulted in no important year X insecticide interactions. The insecticides were effective in reducing the amount of curly top infected plants and in increasing yield. Phorate and NIA 10242 gave the best control and the highest yield. Timek was intermediate for effectiveness, and Di-syston was the least effective.

Even though no significant differences were detected for rates, the two-pound rate consistently gave better results. The years differed significantly as shown by less curly top and greater production in 1967.

Acknowledgment

The authors wish to express appreciation to Holly Sugar Corporation for their cooperation in conducting these experiments.

Reference to a company or product name does not imply approval or recommendation of the product by the Agricultural Experiment Station, New Mexico State University, to the exclusion of others that may be suitable.

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Evaluating Soil Samples for Fungus Pathogens of Sugarbeet Seedlings[1]

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There are several fungi that attack sugarbeets during the early stages of seedling development. Although symptoms caused by an individual pathogen are sometimes characteristic enough for identification by an experienced observer, it is difficult, ordinarily, to determine the pathogen involved simply by observing diseased seedlings. Frequently, more than one pathogen infects a single seedling, thus increasing this difficulty.

Soilborne fungi infecting sugarbeet seedlings can be identified rapidly and with minimum effort simply by incubating all or part of the infected seedlings in water and examining them with low magnification. A combination of this procedure for identification and a plant infection test similar to that used for determining root rot potential of pea fields (l)[3] was used to evaluate soil samples from sugarbeet fields in Michigan.

Materials and Methods

Soil samples were obtained from 10 sugarbeet fields in the area of Bay City, Michigan. Another 10 samples were obtained from rotation plots at the Ferden Farm near Oakley, Michigan. Cropping sequences at this farm are carried out by the Soil Science Department of Michigan State University in cooperation with the Farmers and Manufacturers Beet Sugar Association. Two soil samples were taken from each of five rotations, one from a plot receiving high fertilization and one from a plot receiving low fertilization. In each instance the current crop of the rotation was sugarbeet. A key to the rotations is given in Table 1.

Two soil probes, 5 or 6 inches deep, were taken from each of 20 locations per field. The soil from 40 probes which constituted the sample from the field, was placed in a large polyethylene bag. One or 2 days after sampling, the soil from each

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[3] Numbers in parentheses refer to literature cited.

field was mixed thoroughly then transferred to five sterile 6-inch clay saucers. Two seeds of a susceptible sugarbeet variety that had been soaked in 1% sodium hypochlorite for 30 minutes were planted in each of seven locations in the soil of each saucer. Saucers were kept at a temperature of about 25 C and watered normally until emergence (4 days); then they were watered heavily until removal for examination.

Eight days after planting, five seedlings were removed from each saucer (one from each of five locations in the saucer). All seedlings were washed thoroughly and placed in petri dishes containing sterile tap water. The seedlings were examined microscopically for fungus pathogens 24 to 36 hours later.

Results and Conclusions

Aphanomyces cochlioides Drechs. and *Pythium ultimum* Trow, were the predominant pathogens in the soil samples tested. Both pathogens frequently infected the same seedling. This explains the occurrence of counts totaling more than 25—the total number of seedlings examined (Table 1). The absence of *Pythium aphanidermatum* (Edson) Fitz. and *Fusarium* sp., and the infrequency of *Rhizoctonia solani* Kuehn are not readily explainable. It had been repeatedly observed, however, that infection by *Fusarium* and *Rhizoctonia* are associated with seedling injury such as wind damage. The seedlings examined in this study received little or no injury during the time they were exposed to the fungi. McKeen (2) found *P. aphanidermatum* only in more sandy soils. This may account for its absence in these tests, where only clay soils were tested.

Data on samples from rotation plots seem to support earlier reports that losses due to *Aphanomyces* are severe following alfalfa. Only in soil samples taken from fields where the rotation included 2 years of alfalfa (Samples 11 and 12) were 100% of the seedlings examined infected with *Aphanomyces.* It is surprising that samples from a continuous beet rotation should be relatively low in amount of *Aphanomyces.* The high *Pythium.* rount in the sample obtained from a severely diseased area of th's plot may suggest that *Pythium* is more important as a seedling: pathogen of sugarbeets than previously recognized. No differences were indicated in infection level in samples from high nnd low fertilization plots.

The combination of procedures used here with the accompanving; illustrations (Figure 1) can be utilized by untrained personnel and requires little time. If only an identification of pathogens is desired, this can be accomplished, with reasonable accuracy, 24-36 hours after infected host tissue has been placed in water

at room temperature. This technique, although well known, is infrequently utilized. The data in Table 1 (Samples 1-10) indicate wide differences in abundance of pathogens in different fields. In areas where seedling diseases are serious, an evaluation of soil samples for disease potential would be helpful in choosing favorable fields for planting. Chemical seed and soil treatments differ depending upon type of pathogen involved. Determining the appropriate chemical treatment might be facilitated by a knowledge of the pathogens present.

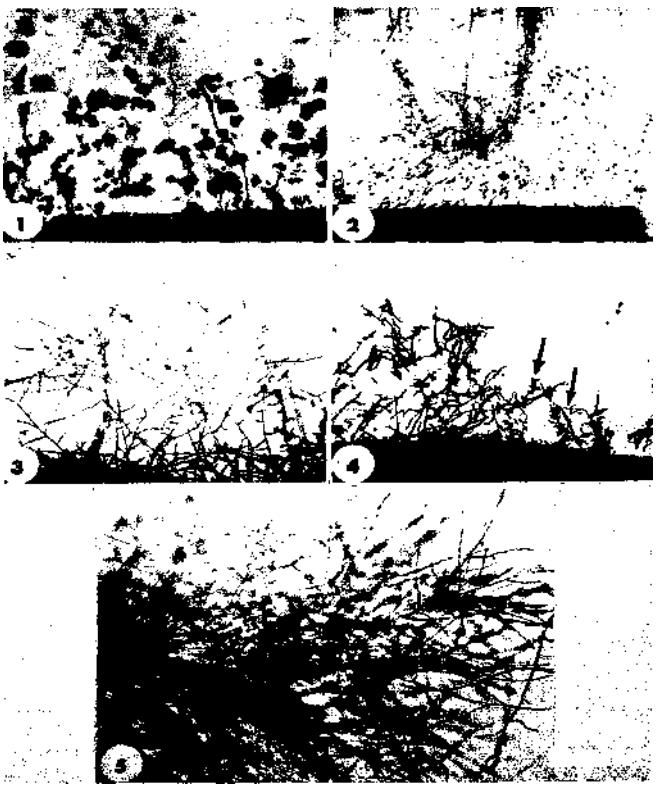

Figure 1.—Five fungus pathogens of sugarbeet seedlings growing from infected host tissue after 48 hours incubation in water. (1) *Aphanomyces cochlioides.* Note clusters of encysted zoospores at the end of evacuation tubes. (2) *Pythium tdtimum.* Note fine mycelial strands with numerous round sporangia. *(Pythium debaryanum* Hesse, which also is reported as a seedling pathogen of sugarbeet, could not be distinguished from this species at low magnification by the inexperienced observer). (3) *Rhizoctonia solani.* Note coarse mycelial strands and tendency for branches to arise at right angles. Mycelial strands commonly grow to the surface of the water. (4) *Pythium aphanidermatum.* Note irregular shaped, fingerlike sporangia (arrows) at ends of branches. (5) *Fusarium* sp. Note dense mycelial growth. Although not shown, this fungus often produces crescent-shaped spores.

Table 1.—Seedling infection test of soil samples for fungus pathogens of sugarbeet seedlings.

Soil sample	Preceding crop	Seedlings infected of 25 examined			SeedHngs uninfected
		A. cochlioides	*P. ultimum*	*R. solani*	
1	Beans	1	14	0	10
2	Wheat	6	9	0	11
3	Beans	7	14	1	6
4	Wheat	14	7	0	5
5	Beans	1	17	0	7
6	Wheat	16	12	0	2
7	Beans	12	11	1	2
8	Beets	1	7	0	17
9	Beans	2	20	0	3
10	Beans	5	17	0	5
11	*	25	2	0	0
12	*	25	5	1	0
13	*	19	16	0	1
14	*	17	6	0	5
15	*	20	9	0	5
16	*	12	2	0	12
17	*	14	7	0	6
18	*	16	16	0	2
19	*	13	13	0	5
20	*	11	19	0	1

* Rotation sequences for plots sampled from Ferden Farm.

Sample Number	Rotation
11 and 12	Barley, alfalfa, alfalfa, beans, beets
13 and 14	Corn, beans, wheat, sweet clover, beets
15 and 16	Soybeans, wheat, sweet clover, beans, beets
17 and 18	Barley, beans, wheat, corn, beets
19 and 20	Continuous beets. Sample 20 was taken from a local area showing severe seedling disease symptoms.

(Plots 11, 13, 15 and 17 received ¼ fertilizer rate of plots 12, 14, 16 and 18.)

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Changes in Concentration of Free Amino Acids by Selection From Yellows Infected Sugarbeet Leaves

J. M. FIFE[1]

Received for publication April 8, 1968

The concentrations of certain free amino acids are greatly altered in the leaves of sugar beet plants infected with beet yellows (2)[2] and with western yellows (1). The greatest changes occurred in newly matured leaves with chronic symptoms of the disease. In the leaves of some beet yellows-infected plants, the concentrations of aspartic and glutamic acids decreased as much as 70%. At the same time, glutamine increased and occasionally was more than double the concentration in the healthy controls. Progeny tests show that the concentrations of these amino acids are to a large extent under genetic control (3). This was demonstrated by simple mass selection for a high amino acid ratio (concentration: $\frac{\text{aspartic} + \text{glutamic}}{\text{glutamine}}$) from a population of plants infected with beet yellows. The concentrations of aspartic and glutamic acids increased significantly (while that of glutamine decreased) in infected leaves of progeny plants relative to the concentrations in infected leaves of parent plants; i.e., the shift in the concentrations of these amino acids was toward that in healthy plants. These changes in concentration resulted in a higher amino acid ratio for infected plants of the selections than for infected plants of the parent.

It was evident, from the papergrams, that the concentration of other amino acids had changed in plants of the progeny. The concentration of total free amino acids was, therefore, determined to evaluate the net change. The results are summarized in this report.

Methods and Results

The methods have been described (4) for growing the plants, inoculation with the virus, sampling, and the determination of the concentration of the three individual amino acids in the leaf extracts. The concentration of total free amino acids was

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[2] Numbers in parentheses refer to literature cited.

determined in these same leaf extracts. The ninhydrin color was developed on narrow strips of paper (3) under the same conditions used to develop the papergrams for the determination of the concentration of the individual amino acids. The concentrations of total amino acids are reported as mg% glutamic acid which is used as the standard.

Highly significant differences were found between the selections and the parent in both the first and the second successive cycle for the concentration of total free amino acids, (Table 1). In beet yellows-infected leaves, the concentration was higher in the selections than in leaves of the parent. In leaves of healthy plants, the reverse was true.

Table 1.—Concentration of total amino acids in leaves of healthy and beet yellows-infected plants of the parent and of first and second successive selections.

Sibs of 1st & 2nd suc. selections	Selection pressure applied for: Amino acid ratio	Selection pressure applied for: root wt	Concentration[1] total amino acids: healthy	Concentration[1] total amino acids: infected	Ratio infected to healthy
			Mg.%	Mg.%	
US 75	Parent		218	108	.50
First selections					
DS-3	$>\bar{x}$	$>\bar{x} + 2s$	217	118	.54
DS-22	$>\bar{x}$	$>\bar{x} + 2s$	189**	128**	.96
DS-23	$>\bar{x}$	$>\bar{x} + 2s$	149**	113	.76
DS-24	$>\bar{x}$	$>\bar{x} + 2s$	173**	168**	.73
DS-9	$>\bar{x}$	$>\bar{x} + 2s$	191**	156**	.82
DS-7	$>\bar{x}$	$>\bar{x} + 2s$	176**	167**	.95
Mean			174	142	.82
Second suc. selections					
DR-6	$>\bar{x} + 2s$	$>\bar{x}$	142**	124*	.87
RS-9	$>\bar{x} + 2s$	$>\bar{x} + 2s$	140**	121	.86
RS-3	$>\bar{x} + 2s$	$>\bar{x} + 2s$	144**	180**	1.25
RS-2	$>\bar{x} + 2s$	$>\bar{x} + 2s$	140**	186**	1.29
RS-5	$>\bar{x} + 2s$	$>\bar{x} + 2s$	145**	136**	.94
Mean			112	149	1.05

[1] Calculated as glumanic acid.

*,** Significantly greater or less than the parent at the 5% and 1% levels respectively.

Of the selections tested, the healthy plants produced greater top and root weights than healthy plants of the parent. The increased demand for amino acids, to maintain larger tops and produce greater root weights, may account for the lower concentration of total amino acids in the healthy selections.

The concentrations of certain amino acids, other than aspartic, glutamic and glutamine, appear to be changed in both healthy and infected plants of the progeny. The concentrations of each of these three amino acids have been reported (3) for the parent and for each of the selections. The percentage of the total

concentration of free amino acids, made up by the combined concentrations of the above three amino acids, has been calculated for the healthy and infected plants of the parent and of the selections, (Table 2). In healthy plants of the parent, aspartic, glutamic and glutamine made up 42% of the total; whereas, in healthy plants of the first and second successive selections they comprised 48% and 80% of the total concentration, respectively. In leaves of infected plants of the parent, these three amino acids represented 57% of the total as compared to only 37% in the second successive selections.

Table 2.—Percentage of the total concentration of free amino acids made up by aspartic, glutamic and glutamine in healthy and diseased leaves of the parent and of the selections.

Newly-matured leaves of	Healthy	Diseased
Parent	42a	57d
1st sel. (mean of 6 sibs)	48b	47e
2nd suc. (mean of 5 sibs)	80c	37f

c > b (P = 0.01), c > a (P = 0.01), b > a (P = 0.10)
d > e (P = 0.05), d > f (P = 0.01), e > f (P = 0.01)
d > a (P = 0.05), c > f (P = 0.01), a > f NS

Summary

The concentration of total free amino acids was changed in leaves of sugarbeet plants by simple mass selection for a high amino acid ratio (concentration: $\frac{\text{aspartic acid} + \text{glutamic acid})}{\text{glutamine}}$ from a population infected with beet yellows. The concentration of total amino acids was significantly higher in newly matured leaves of infected plants of first and second successive selections than in infected leaves of the parent. In healthy plants, the concentration was lower in leaves of the progeny than in leaves of the parent. The concentrations of amino acids other than aspartic, glutamic and glutamine were changed also.

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Herbicidal Control of Weeds in Sugarbeets[1]

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Annual cost of weed control in sugarbeets in the USA exceeds $20 million. In 1967 more than 1.1 million acres of sugarbeets were planted—approximately 30% of this acreage was planted in nonirrigated areas and 70% in irrigated areas. In the irrigated areas of the central High Plains and intermountain West the weeds most difficult to control in sugarbeets are kochia *[Kochia scoparia* (L) Schrad.] and Russian thistle *(Salsola kali* L.). Other major weeds such as barnyardgrass *[Echinochloa crusgalli* (L). Beauv.], lambsquarters *[Chenopodium album* L.], nightshade *[Solarium spp.*\, pigweed *[Arnaranthus spp.*\, and foxtail *[Setaria spp.]* also are troublesome, and hinder complete mechanization of sugarbeet production.

Use of herbicides for control of weeds in sugarbeets in the central High Plains and intermountain West has progressed appreciably since Deming (3)[3] first incorporated isopropyl A[r]-phenylcarbamate (IPC) into the soil as a preplant treatment in Colorado in 1947. The development of such herbicides as 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid (endothall) (9) and 2,2-dichloropropionic acid (dalapon) (13) in 1952; S-propyl butylethylthiocarbamate (pebulate) in 1959 (1); and 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone (pyrazon) in 1962 (5) has contributed significantly to the progress made in controlling weeds in this crop. Today most herbicides in the irrigated areas are applied as preplant treatments and are soil-incorporated. The principal herbicides used are S-2,3-dichloroallyl N,N-diisopropyl-thiolcarbamate (diallate), pebulate, pyrazon, and the mono *(N,N*-dimethyltridecylamine) salt of endothall (TD283).

In six states where these herbicides have been applied before planting, either singly or in mixtures, the control of annual weeds ranged from 27 to 89%, with the mean being 68% (4,7, 8,11,12,14). Major differences in soil, climate, cultural methods

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Colorado Agricultural Experiment Station. Published with the approval of the Director of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 1281.

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[3] Numbers in parentheses refer to literature cited.

and weed species contributed to these extreme variations. Although these preplant treatments control most grass weeds satisfactorily, 20 to 30% of the annual broadleaf weeds still escape and must be removed from sugarbeets by other means. Furthermore, kochia is controlled only 50 to 60% under ideal conditions. Therefore, field experiments were conducted to evaluate herbicides applied before planting (preplant) and after emergence (postemergence) of the crop for controlling weeds in sugarbeets. The objectives in these experiments were to compare several herbicides, applied as preplant or postemergence treatments and as a combination treatment (preplant plus postemergence), for controlling kochia and other annual weeds in sugarbeets; and to evaluate the performance of a mechanical weeder as a supplement to herbicide treatments.

Materials and Methods

Five experiments were conducted at Fort Collins between 1965 and 1967. The soil texture ranged from loam to clay loam (Table 1). Monogerm sugarbeet seed, size 2, was planted approximately $^3/_4$ inches deep each year at the rate of eight seed units per foot of row. The sugarbeet seed was planted simultaneously with the application of the herbicides. Information on dates of planting, evaluation and harvest for these experiments is shown in Table 2.

The plots were 4-rows wide and 45 to 50 feet long. The rows were spaced 22 inches apart. Randomized complete block designs with three replicates in 1965 and five replicates in 1966 and 1967 were used for all experiments.

Table 1.—Results of soil test.

	1965	1966	1967
Soil texture	clay loam	clay loam	loam
pH	7.8	8.0	7.8
Organic matter	2.6	2.5	3.1
Phosphorus (lb P_2O_5/acre 6 in)	124 H[a]	67 M	58 M
Potash (lb K_2O/acre 6 in)	960 H	867 H	1000 H

[a] Fertility level — L = low, M = medium, and H = high.

Table 2.—Dates of planting, evaluation and harvest for the five experiments.

Experiment No.	Year	Planting	Stand of sugarbeets and weeds counted	Harvest
1	1965	April 30	June 9 and August 2	
2	1966	April 4	May 19 and July 7	October 13
3	1966	April 5	May 21 and June 9	October 6
4	1967	April 4	May 9 and June 26	October 12
5	1967	April 3	May 8 and June 9	September 29

A mixture of weed seed which contained foxtail millet [*Setaria italica* (L.) Beauv.], kochia, redroot pigweed [*Amaranthus retroflexus* (L.)] and lambsquarters was sown on the experimental fields each year before planting. These species were present in sufficient numbers to evaluate each year. Weeds were counted twice—4 to 6 weeks and 12 to 14 weeks after planting. For the first evaluation, weeds were counted in four sites in each plot. Each site was 4 inches by 36 inches and centered on the sugarbeet row. For the second evaluation, weeds were counted in 4-inch bands, 45 feet long, on the inner two rows of each plot. All weeds were removed by hand from the treated plots after the second evaluation.

The herbicides—pebulate, 2-chloro-*N*-isopropylacetanilide (propachlor), pyrazon, *S*-ethyl cyclohexylethylthiocarbamate (cycloate), and TD283—were applied singly and as mixtures before planting. The herbicides were sprayed on a 7-inch band at a volume of 19.1 gallons (60 gpa broadcast) aqueous mixture per acre. All herbicides were incorporated 1½ inches deep with a front-mounted, hooded, 4-row, power-driven incorporator. The soil surface was dry and tilth was good. Each experiment was furrow irrigated within 3 to 7 days after planting. Natural moisture for April, May and June was 1.87 and 4.62 inches in 1965 and 1967, respectively, above the 70-year average and 3.19 inches below the long-time average in 1966.

A mixture of pyrazon plus dalapon was applied as a postemergence treatment in Experiments 3, 4, and 5 and benzamidooxyacetic acid (benzadox) in Experiment 5. The herbicides were sprayed on an 11-inch band over the row at a volume of 30 gallons (60 gpa broadcast) mixture per acre. An anionic surfactant, sodium alkyl naphthalene sulfonate, at 0.3% wt/v was included in the spray mixture of pyrazon plus dalapon.

In Experiment 3 the postemergence mixture of 3 lb/A each of pyrazon and dalapon was applied on May 27. Sugarbeets had four true leaves. Foxtail millet was ½ - 1 inches tall, kochia 1 - 5 inches tall, lambsquarters 1 - 2½ inches tall, and pigweed ½ - ¾ inches tall.

In Experiments 4 and 5 the postemergence mixture of 4 lb/A of pyrazon and 2.2 lb/A of dalapon or 2 lb/A of benzadox (Experiment 5) was applied on May 8. Sugarbeets had four true leaves, with the second pair of leaves pea size. Foxtail millet had 2 to 3 leaves and was ¼ - ½ inches tall; kochia was ¼ - ¾ inches in diameter and ½ - ¾ inches tall; and lambsquarters and pigweed had 1 to 2 true leaves and were ¼ - ½ inches tall. In Experiment 5 a mechanical weeder was used on May 8 and 12 in all plots before the postemergence treatments were applied.

At harvest, root yield, number of marketable sugarbeets and sucrose percentage were determined for each plot. Sugarbeets were harvested from 40 feet of each of the inner two rows.

Results

Preplant treatments. The results from the evaluation taken 12 to 14 weeks after planting are shown in Table 3. Results from the evaluation taken 4 to 6 weeks after planting for these same herbicides are not shown, but herbicides applied singly, controlled these weeds better at 4 to 6 weeks than at 12 to 14 weeks after planting in 1965 and 1966. Weed control from the herbicides applied as mixtures was also better 4 to 6 weeks after planting in 1966, but best 12 to 14 weeks after planting in 1967.

Kochia was not controlled satisfactorily by any herbicide treatment. However, the stand of kochia was reduced most by propachlor, TD283, or treatments which included one of these herbicides. The mixture of 3.75 lb/A of pyrazon plus 3 lb/A of propachlor reduced the stand of kochia the most, but this reduction averaged only 55% for the 2-year period. Kochia was very resistant to the thiocarbamate herbicides—pebulate and cycloate.

A mixed population of foxtail, lambsquarters, and pigweed was controlled best by 4 lb/A of cycloate applied singly or by any of the four herbicide mixtures. These treatments reduced the average stand of these weeds for the 2-year period by 77 to 91%.

The stand of sugarbeets over a 2-year period was reduced most by the mixture of 3.75 lb/A of pyrazon plus 3 lb/A of propachlor (18%) and 4 lb/A of propachlor (11%). In 1966 the relative retardation in growth of sugarbeets was greatest in those treatments which contained pebulate or cycloate. Sugarbeets treated with pebulate were retarded 60%, whereas sugarbeets treated with cycloate, singly or in a mixture, ranged between 41 and 45%.

Preplant and postemergence treatments. In 1967 the performance of six herbicide treatments applied preplant was very similar when weed control was assessed in June. However, the advantage of using a herbicide mixture as a postemergence treatment to supplement the control of weeds from herbicides applied before planting is shown in Figure 1. The average reduction in stand of kochia was 49% where herbicides were applied as combination treatments (preplant plus postemergence) compared to 19% where herbicides were applied only before planting. In contrast, the average reduction in stand of foxtail, lambsquarters and pigweed was 96% where herbicides were applied

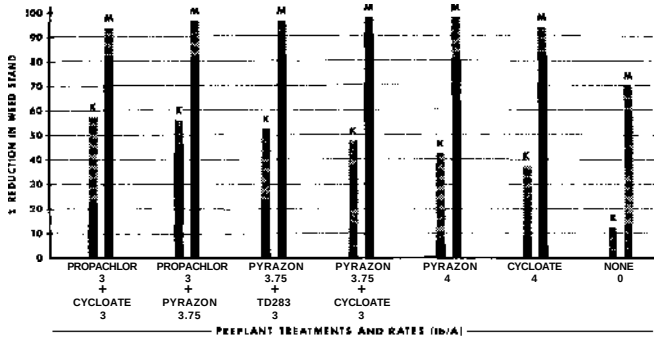

Figure 1.—Weed control from herbicides applied before planting versus a combination treatment of herbicides applied before planting and followed by a postemergence mixture of pyrazon at 4 lb/A plus dalapon at 2.2 lb/A. The solid portion of the bar shows control from the preplant treatment alone; the broken portion shows additional control from the postemergence treatment. K — kochia; M = a mixed population of foxtail, lambsquarters and pigweed.

as combination treatments compared to 84% where herbicides were applied only before planting. The postemergence mixture of 4 lb/A of pyrazon plus 2.2 lb/A of dalapon, applied alone, reduced the stand of kochia by only 13% and the other three weed species by 70%.

In 1966 a postemergence mixture of 3 lb/A each of pyrazon plus dalapon applied in combination with preplant treatments at Fort Collins and Rocky Ford, Colorado, also controlled more weeds than where only the preplant treatments were applied. At Rocky Ford the mixture of pyrazon (3.75 lb/A) plus cycloate (3 lb/A) or pebulate (5 lb/A) applied before planting and followed by a postemergence mixture of pyrazon plus dalapon, controlled 87% and 85%, respectively, of the foxtail, pigweed and Venice mallow (*Hibiscus trionum* L.) population (10). These two preplant treatments, applied singly, controlled only 53% of these weeds. Furthermore, the postemergence mixture, applied alone, controlled only foxtail satisfactorily at Fort Collins. Unsatisfactory control of broadleaf weeds at both locations and of foxtail at Rocky Ford, by this postemergence treatment, is attributed to applying it too late to control the larger weeds effectively.

Table 3.—Weed control 12 to 14 weeks after planting from several herbicides and herbicide mixtures applied preplant between 1965 and 1967.

Treatments		Percentage reduction in weed stand									
Herbicides	lb/A	Foxtail		Lambsquarters		Pigweed		Average		Kochia	
Single components											
pebulate	5	96	43	94	68	98	88			3	0
propachlor	4	94	22	74	30	84	0			67	14
cycloate	4	89	94	88	87	82	85			3	0
TD283	4.5(4)»	93	21	35	0	87	22			59	32
B. Mixtures		1966	1967	1966	1967	1966	1967	1966	1967	1966	1967
pyrazon + propachlor	3.75 + 3	60	75	98	91	91	77	83	81	65	46
cycloate + propachlor	3 + 3	90	94	97	75	84	80	90	83	50	23
pyrazon + TD283	3.75 + 3	51	83	96	85	95	81	84	83	44	24
pyrazon + cycloate	3.75 + 3	89	96	91	88	51	88	77	91	0	3

[a] Rate in parenthesis applied in 1966.

The application of a thiocarbamate herbicide as a preemergence treatment has increased the susceptibility of broadleaf weeds to several herbicides applied postemergence in soybeans (6). Application of some thiocarbamate herbicides as a preplant treatment seems also to precondition both weeds and sugarbeets to additional injury from a subsequent application of a postemergence treatment (2, 10). This phenomenon was apparent again in 1967, when a mixture of 4 lb/A of pyrazon and 2.2 lb/A of dalapon was applied as a postemergence treatment (Table 4). Retardation in growth of sugarbeets on May 18 was greater in all treatments where herbicides were applied both preplant and postemergence. Furthermore, sugarbeets grown in soil treated with cycloate and then sprayed with a postemergence mixture of pyrazon plus dalapon were retarded about 20% more than sugarbeets treated with the combination treatments which did not include cycloate. By July 14, however, sugarbeets previously repressed by herbicides appeared as large as the untreated controls (Table 4). This early repression had no significant effect on sucrose production, yield of roots or sucrose percentage. (Table 5).

Preplant, postemergence, and mechanical weeding treatments. There were 88 weeds per 100 feet of row in the untreated check where the sugarbeets were only mechanically thinned compared to 81 weeds (8% fewer) in the untreated check where sugarbeets were both mechanically weeded and thinned. Sugarbeets treated with 4 lb/A of cycloate had 20 weeds after being thinned mechanically and 17 weeds after being weeded and thinned mechanically. The number of weeds in the combination treatments that received 4 lb/A of cycloate followed by a postemergence treatment of 4 lb/A of pyrazon plus 2.2 lb/A of dalapon or 2 lb/A of benzadox was similar regardless of the mechanical operations performed. These treatments averaged 10 weeds or 0.1 weed per foot of row. Thus the use of a mechanical weeder did not supplement the control of weeds obtained with herbicides to a practical degree.

A combination treatment of 4 lb/A of cycloate applied before planting and 2 lb/A of benzadox applied postemergence on May 8 controlled kochia effectively (Table 6). Delaying the application of benzadox until May 25 reduced the effectiveness of the combination treatment to control kochia. Data from this experiment and others conducted in 1965 and 1966 confirm that the most effective control of kochia results when kochia is treated early (rosette-like stage), preferably before stem elongation begins. The reduction in the stand of foxtail, lambsquarters, and pigweed in soil treated with cycloate alone, or

with a postemergence mixture of pyrazon plus dalapon was similar to the results obtained in Experiment 4 (Figure 1). Also, date of application of the postemergence mixture of pyrazon plus dalapon did not appear to affect the control of foxtail, lambsquarters, and pigweed.

Cycloate applied at 4 lb/A had no effect on stand but had retarded the growth of sugarbeets 34% when evaluated on May 8. By June 9 the sugarbeets had completely recovered from the cycloate treatment (Table 6). In contrast, sugarbeets grown in soil treated with cycloate, and then sprayed with a postemergence treatment of either benzadox or a mixture of pyrazon plus dalapon were still retarded 37 to 51% on June 9. By July 17, these, too, had recovered and appeared identical to the untreated sugarbeets.

Neither sugar production, yield of roots or sucrose percentage was affected in sugarbeets treated with a combination of 4 lb/A of cycloate, applied before planting, and 2 lb/A of benzadox or a mixture of 4 lb/A of pyrazon plus 2.2 lb/A of dalapon, applied postemergence on May 25 (Table 7). However, sugarbeets treated with cycloate and a postemergence mixture of pyrazon plus dalapon on May 8 produced significantly less tonnage and sugar per acre. This same treatment also produced 1.8 tons of roots and 770 pounds of sugar per acre less than the hand-weeded check in Experiment 4 (Table 5), but this decrease was not statistically significant. Since the control of foxtail, lambsquarters, and pigweed was slightly better on June 9, when the postemergence treatment of pyrazon plus dalapon was delayed until May 25, and since neither sugar production, yield of roots or sucrose percentage was affected, it would seem that the postemergence mixture of pyrazon plus dalapon could be delayed several days, if an application of cycloate has stunted the growth of sugarbeets and was controlling the weeds satisfactorily.

Discussion

Although we were unable to control all weeds, these investigations indicate that practical control of many weeds is possible. Furthermore, several preplant and postemergence treatments were effective and the logical selection of which treatment to use depends on the infestation of weeds present. The advantages and limitations of the most effective treatments in these investigations will now be discussed.

Of the herbicides applied singly as preplant treatments, cycloate was the most effective for controlling a mixed population of foxtail, lambsquarters, and pigweed but least effective

Table 4.—Effects of herbicides applied preplant and postemergence on stand and relative top growth of sugarbeets in 1967.

Herbicides and method of application			Stand[b] reduction	Visual retardation[c]		
Preplant	lb/A	Postemergence[a]	May 9	May 18	June 9	July 14
pyrazon	4	pyrazon + dalapon	..	41	9	1
pyrazon	4	none	9	9	0	1
cycloate	4	pyrazon + dalapon	..	62	39	2
cycloate	4	none	0	29	0	4
pyrazon + propachlor	3.75 + 3	pyrazon + dalapon	..	44	18	1
pyrazon + propachlor	3.75 + 3	none	16	10	0	0
pyrazon + cycloate	3.75 + 3	pyrazon + dalapon	..	67	32	0
pyrazon + cycloate	3.75 + 3	none	8	24	0	2
pyrazon + TD283	3.75 + 3	pyrazon + dalapon	..	46	6	1
pyrazon + TD283	3.75 + 3	none	1	15	4	2
propachlor + cycloate	3 + 3	pyrazon + dalapon	..	64	27	0
propachlor + cycloate	3 + 3	none	1	30	5	2
none	0	pyrazon + dalapon	..	34	7	3
none	0	none	0	0	0	0

* A mixture of 4 lb/A of pyrazon, 2.2 lb/A of dalapon and 0.3% (wt/v) of sodium alkyl napthalene sulfonate was applied on May 8.

[b] Values are percentages of untreated sugarbeet stand.

[c] Injury scale: 0 = no retardation in top growth of sugarbeets and 100 = all plants killed.

Table 5.—Effect of herbicides applied preplant and postemergence on sugar production, root yield and sucrose percentage in 1966 and 1967.

Herbicides and method of application			Sugar (lb/A)		Roots (tons/A)		Sucrose (%)	
Preplant	lb/A	Postemergence[a]	1966	1967[b]	1966	1967[b]	1966	1967[b]
pyrazon	4	pyrazon + dalapon	....	7530	....	21.5	....	17.5
pyrazon	4	none	..	7670	....	21.6	....	17.7
cycloate	4	pyrazon + dalapon	..	7170	....	20.3	....	17.6
cycloate	4	none	....	7350	....	21.2	....	17.2
propachlor + cycloate	3 + 3	pyrazon + dalapon	6170	7200	21.0	20.6	14.7	17.4
propachlor + cycloate	3 + 3	none	6450	7060	21.7	20.3	14.9	17.4
pyrazon + propachlor	3.75 + 3	pyrazon + dalapon	6080	8110	20.6	22.5	14.8	18.0
pyrazon + propachlor	3.75 + 3	none	6530	7870	20.9	22.0	15.6	17.9
pyrazon + cycloate	3.75 + 3	pyrazon + dalapon	6090	7660	20.0	21.5	15.2	17.8
pyrazon + cycloate	3.75 + 3	none	6520	7600	20.6	21.7	15.3	17.9
pyrazon + TD285	3.75 + 3	pyrazon + dalapon	5960	7550	19.9	21.3	14.9	17.7
pyrazon + TD285	3.75 + 3	none	5660	7470	18.7	20.9	15.1	17.8
none	0	pyrazon + dalapon	620[c]	7250	2.0[c]	20.2	15.3	17.9
none (hand-weeded)	0	none	6180	7940	20.4	22.1	15.1	17.9
none (weedy)	0	none	470[c]	.....	1.5[c]		15.3	...
LSD (0.05)			730	860	2.1	1.8	0.7	0.7
C. V.			10.9%	9.0%	9.4%	6.8%	3.9%	3.0%

[a] In 1966 pyrazon and dalapon each applied at 3 lb/A. In 1967 pyrazon applied at 4 lb/A and dalapon at 2.2 lb/A.
[b] The F-test was nonsignificant at the 5% level for treatment means.
[c] Significant at the 5% level between treatment and untreated hand-weeded check.

H

Table 6.—Effect of herbicides applied preplant and postemergence on relative top growth of sugarbeets and on weeds in 1967.

Herbicides and method of application			Sugarbeets[a]		Percentage reduction in weed stand			
			Visual injury					
Preplant	Postemergence	lb/A	June 9	July 17	Foxtail	Lambs-quarters	Pigweed	Kochia
A. Evaluated May 8								
cycloate[b]	none	0	..	..	68	76	96	24
B. Evaluated June 9								
1. Mechanical thinning								
cycloate	none	0	2	2	98	89	89	18
cycloate	pyrazon + dalapon[c]	4 + 2.2	51	0	99	97	93	62
cycloate	benzadox[c]	2	27	1	99	97	92	84
2. Mechanical weeding and thinning								
cycloate	none	0	4	0	98	93	90	33
cycloate	pyrazon + dalapon[d]	4 + 2.2	37	0	100	99	99	37
cycloate	benzadox[d]	2	49	0	100	97	91	63

[a] Injury scale: 0 = no retardation in top growth of sugarbeets and 100 = all plants killed.
[b] Cycloate applied April 3 at 4 lb/A.
[c] Postemergence treatments applied May 8.
[d] Postemergence treatments applied May 25.

Table 7.—Effect of herbicides applied preplant and postemergence on sugar, root yield and percentage sucrose in 1967.

	Herbicides and method of application			Sugar	Roots	Sucrose
	Preplant	Postemergence	lb/A	(lb/A)	(tons/A)	(%)
A.	Mechanical thinning					
	cycloate[a]	none	0	6770	20.3	16.6
	cycloate	pyrazon + dalapon[b]	4 + 2.2	6190[c]	18.4[c]	16.6
	cycloate	benzadox[1,]	2	6640	19.8	16.8
	none	none	0	6780	20.4	16.6
	LSD (0.05)			440	1.7	0.4
	C. V.			5.0%	4.4%	2.0%
B.	Mechanical weeding and thinning					
	cycloate	none	0	6680	19.8	16.7
	cycloate	pyrazon -f dalapon[1,]	4 + 2.2	6410	19.5	16.4
	cycloate	benzadox[d]	2	6460	19.6	16.4
	none	none	0	6590	20.0	16.5
	LSD (0.05)			610	1.6	0.6
	C. V.			7.1%	5.9%	2.8%

[a] Cycloate applied April 3 at 4 lb/A.
[b] Postemergence treatments applied May 8.
[c] Significant at the 5% level between treatments and untreated hand-weeded check.
[d] Postemergence treatments applied May 25.

for controlling kochia. This herbicide did not reduce the stand of sugarbeets but did retard their growth during the cool, moist conditions of early spring. Later, the sugarbeets appeared to recover, and yield was not reduced. The residual activity of this herbicide is short. It will not persist in sufficient concentration to control weeds germinating late in the season.

Each of the four herbicide mixtures applied as preplant treatments effectively controlled a mixed population of foxtail, lambsquarters and pigweed. The mixture of 3.75 lb/A of pyrazon plus 3 lb/A of propachlor controlled kochia best, but this control averaged only 55%. This mixture also reduced the stand of sugarbeets the most, 18%. However, none of the four mixtures affected yield. These mixtures will persist longer in the soil and thus provide better control of late season weeds.

The most effective control of a mixed population of foxtail, lambsquarters and pigweed was obtained by applying herbicides, singly or as a mixture, as a preplant treatment and following with a postemergence mixture of pyrazon plus dalapon. Combination treatments which included pyrazon in both the preplant and postemergence treatments, however, may result in residues of pyrazon in the soil sufficient to injure succeeding crops. Also, combination treatments which include cycloate as a preplant treatment and a postemergence mixture of pyrazon plus dalapon may retard the growth of sugarbeets early in the season when weather conditions are cool and moist. Yield of roots and

sugar per acre also may be reduced if the postemergence treatment is applied too soon to sugarbeets that show symptoms of retardation from cycloate.

A major breakthrough in these investigations was the discovery that kochia could be controlled by benzadox. The stand of kochia was reduced 84% by a combination treatment which included 4 lb/A of cycloate applied preplant and 2 lb/A of benzadox applied postemergence. This combination treatment also controlled 96% of the stand of foxtail, lambsquarters and pigweed. Although both herbicides stunted the growth of sugarbeets for several weeks, yield was not reduced.

Summary

Kochia was controlled satisfactorily for the first time by 2 lb/A of benzadox applied as a postemergence spray to sugarbeets which were growing in soil treated with 4 lb/A of cycloate before planting. Cycloate does not control kochia.

The control of a mixed population of foxtail, lambsquarters and pigweed by herbicides applied preplant was supplemented by a postemergence mixture of 4 lb/A of pyrazon plus 2.2 lb/A of dalapon.

The application of cycloate, a thiocarbamate herbicide, as a preplant treatment, increased the susceptibility of both sugarbeets and weeds to a postemergence mixture of pyrazon plus dalapon. This combination treatment repressed the foliar growth of sugarbeets for 8 weeks. This early repression had no effect on sucrose percentage, but the combination of 4 lb/A of cycloate applied preplant and 4 lb/A of pyrazon plus 2.2 lb/A of dalapon applied postemergence significantly reduced the yield of roots and sugar per acre in one experiment.

The use of a mechanical weeder, in addition to mechanical thinning, did not supplement the herbicidal control of weeds to a practical degree.

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Decomposition of Molasses Pulp Pellets in Bulk Storage

JOHN D. JORGENSEN AND ROBERT GADDIE[1]

Received for publication March j, 1968

At the Idaho Falls plant of the Utah-Idaho Sugar Company most of the molasses dried beet pulp is pelletized into 14 inch pellets and stored in bulk in closed cylindrical tanks 85 feet in diameter by 64 feet high. The capacity of each tank is about 7500 tons of pellets. During the 1966-67 campaign, the pellets contained an average of 20.6 molasses solids and 9.0 StefFen nitrate solids percent on pellets.

In the latter part of April 1967, 3 months after the end of the campaign, the operators noticed a faint acrolein-like odor in the pellet recovery tunnel in one of the tanks. By this date the tank was about one-third unloaded. The odor was stronger in the head space above the pellets and was traced to an area about 20 feet in from the outside of the tank. Pellets were withdrawn as rapidly as possible from this area and it was soon evident that serious localized decomposition was taking place. A 16 foot square hole was cut in the tank and a large front-end loader was used to remove the pellets from the affected side. As the decomposed area was uncovered, large volumes of steam and gas evolved. The decomposition had started approximately 6 feet from the bottom and had progressed upward and outward covering an area nearly 15 feet square and about 20 feet high. The pellets at the bottom were a hard, black, glassy-looking mass. Above this, decomposition became progressively less and less until the pellets near the top were merely damp and sticky. No matter what the stage of decomposition, as soon as a fragment was broken off and exposed to the cool air, decomposition ceased. Luckily, the temperature in the affected area had not quite reached the ignition point so fire was not a problem and it was possible to make some rather detailed observations of the affected area in the tank. This led to the experimental work in the laboratory which is reported here in which the decomposition was duplicated and some of the causative factors delineated.

Equipment used in the tests consisted of two temperature controlled ovens and some wide mouth quart fruit jars with a small hole punched in the lid. Pennies were placed over these holes.

[1] Chief Chemist, Idaho Falls Plant and Assistant General Superintendent respectively, Utah-Idaho Sugar Company.

Test No. 1

One oven was controlled at 50° C, the other at 70°. A quart bottle full of clean pellets containing no fines was placed in each oven.

Pellet moisture 6.8%
Polarization 15.6%

No decomposition occurred at either temperature in 10 days. On the 11th day the temperature in the 50° C oven was raised to 100° C. At the end of 4 more days, decomposition was evident at this high temperature. However, at 14 days the pellets at the 70° C temperature still showed no decomposition.

Test No. 2

In each of two bottles a fine screen was fixed in the bottles about 1 inch from the bottom. Under this screen was placed 125 ml of water. The bottles were filled with clean pellets, sealed, one placed in the 50° oven, the other in the 70° oven.

Pellet moisture 6.8%
Polarization 15.6%

After 4 days the pellets at 50° had taken on enough moisture to appear wet, but even after 14 days little change was detectable. On the 3rd day the pellets in the 70° oven were giving off a faint odor. This increased in intensity by the 5th day to the typical pungent acrolein-type odor. The polarization by the 10th day was 4.4 and by the 14th day was minus 5.2. The decomposition seemed to reach a constant rate at about the 6th or 7th day but the generated heat radiated out of the bottle fast enough so that the pellets did not form the charred mass noted in the tank. The temperature of the reacting pellets did not get higher than 70° C.

It was noted that decomposition started and was most complete in the storage tank where there was a heavy concentration of fines. This would form an insulating barrier to the generated heat. To simulate this condition, the next four tests were made in bottles which had been completely covered with a y_A inch layer of asbestos.

A sample of pellets was obtained from the area in the tank where decomposition had started. This sample contained 42% fines finer than 10 mesh. Moisture on this sample was 7.2% and polarization 12.4%.

Test No. 3

Water (125 ml) was placed under the screen on the bottom of one of the insulated bottles, which was then filled with pellets containing 42% fines. The bottle was capped and placed in the 70° C oven. On the 3rd day the temperature was 85° C and rapid decomposition was evident. By the 6th day decomposi-

tion was practically complete and the mass had the appearance and brittleness of wood charcoal. Moisture was 28% and polarization minus 5.6. If the size of the sample had been greater and the insulation more perfect, there is every indication that ignition would have taken place.

Test No. 4

Another bottle was filled with pellets containing 42% fines but this time no water was added. It was also placed in the 70° C oven. In 3 days the temperature had reached 82° C and there was a faint odor showing that the initial stage of decomposition had started. However, by the 5th day the temperature was down to 70° C where it remained for 15 days. At 15 days (the end of the test) the pellets had browned slightly and there was a faint acrolein odor. Moisture was 2.5% and sufficient decomposition had taken place to drop polarization from 12.4% to 8.7%. It appeared that the moisture in the pellets was being driven off faster than the water of decomposition was formed and this greatly slowed the reaction.

Test No. 5

A 3rd aliquot of the pellet sample containing 42% fines was placed in an insulated bottle, 125 ml of water added underneath the screen and the bottle placed in the 50° C oven. After 3 days the temperature had reached 50° C and remained constant for the 15-day test. The typical odor was noted by the 10th day which persisted at about the same level to the 15th day. By the 15th day, moisture had increased from 7.2 to 18.0%; polarization had dropped from 12.4 to 6.7%.

Test No. 6

A sample of clean pellets, free of fines but containing 15.2% moisture, was placed in an insulated bottle in the 70° C oven. No water was added. Polarization was 15.6. At 5 days the typical odor was noted. At 10 days this became quite strong and persisted to the 15th day. The temperature did not rise above the 70° C, but it was evident that slow decomposition was taking place. At the end of 15 days the polarization was 5.2% and the moisture was 8.2%.

Because moisture concentration plays such an important part in initiating the decomposition reactions, an experiment was set up to determine moisture take-up by these pellets when exposed to environments of various relative humidities. At the same time, it was desired to determine at what moisture content these pellets would mold. Saturated solutions of various chemicals to produce relative humidities from 13% to 100% were placed under bell jars along with clean pellets containing 6.8% moisture. Analyses were made each week for 7 weeks. The temperature in the

laboratory varied from 20° C to 26° C so that the humidities inside the bell jars were not exactly constant during the 49-day period. Results are shown in Table 1.

No mold appeared on any of the samples except the one at 100% relative humidity and then only when the moisture content reached 25%. On the 35th day each sample was thoroughly seeded with moldy pelllets taken from the one which had molded. After 2 weeks there was no sign of further mold growth. In fact, the mold on the seeding pellets dried up.

Table 1.—Moisture uptake in pellets stored at different relative humidities—initial moisture 6.8%.

Chemical used	Relative humidity	Percent moisture—after days						
		7	14	21	28	35	42	49
Potassium Acetate	15	5.2	5.5	5.4	5.5	5.3	5.2	5.2
Calcium Chloride	32	5.8	5.6	5.6	5.4	5.4	5.5	5.8
Zinc Nitrate	42	7.0	8.4	8.4	8.3	8.0	8.0	8.0
Sodium Bisulfate	52	8.0	9.5	9.8	10.0	10.2	10.2	10.6
Sodium Nitrite	66	8.2	9.7	9.6	10.8	12.0	11.5	11.5
Sodium Thiosulfate	78	9.8	11.7	12.0	15.4	16.6	15.4	16.8
Water	100		22.8	23.6	25.6	(Mold)		

Summary

1) Pellets containing 7% moisture which are relatively free of fines show little tendency to decompose even in temperatures as high as 70° C.

2) Pellets free of fines but containing 15% moisture will decompose in temperatures as high as 70° C.

3) In the presence of 100% relative humidity, pellets of 7% moisture and free of fines show little tendency to decompose at 50° C. However, if the temperature gets as high as 70° C decomposition will take place fairly rapidly.

4) Pellets of 7% moisture but containing a high percentage of fines (30% to 40%) will decompose slowly at 70° C.

5) Pellets of 7% moisture but containing a high percentage of fines (30% to 40%) and in the presence of 100% relative humidity and 70° C will decompose very rapidly with liberation of sufficient heat to cause ignition.

6) Pellets of 7% moisture but containing a high percentage of fines and in the presence of 100% relative humidity will decompose at 50° C.

7) Pellets of the composition with which this experiment was concerned would not mold at moisture content below 25%. This leads to the conclusion that whenever pellets mold it is because water in form of condensate, rain, etc. has come in contact with them.

Conclusion

To prevent decomposition of pellets in storage, they should be produced at a moisture content of less than 10%; there should be no concentration of fines any place in the storage area; the pellets should be cooled below 50° C and held below this temperature even though supplemental ventilation through the pellet mass may be required. Pellets from production will continue to give off moisture for a time in storage. To prevent condensate dripping on the pellets, the storage facility should have enough ventilation and/or roof and wall insulation so that the walls and roof are not at the dew point for the relative humidity inside the tank. Such condensate produces mold with attendent heating, further production of water and decomposition.

Treatment of Sugarbeet Flume Waste Water by Lagooning, A Pilot Study[1]

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Introduction

Background

Fluming and washing of sugarbeets gives rise to a waste water rich in inorganic and putrescible organic substances and hence, often unacceptable for direct discharge to the environment. Treatment and recycle of flume water is feasible but excess flume water is usually produced and management of such water must be provided. Direct release of flume water to receiving streams which provided dilution and transport was once widely practiced, but under new federal and state laws, is generally no longer permissible. Irrigation has been a successful outlet for excess flume water but this can only be applied during the growing season or at least when the soil is not frozen. Impoundment of excess flume water has been the essential alternative to irrigation or release.

Excess flume water impoundments have usually been designed as holding basins rather than as treatment devices with consequences which have sometimes bordered on the calamitous. The major problem resulting from impoundment of flume water has been the production of vile odors followed by real or fancied damage to adjacent property and strident, if not violent, public dissent. As one result of the recurrence of these problems, several questions repeatedly arise, "Can excess flume water be impounded without odor under any circumstances?" What degree of waste treatment is accomplished when impoundment is applied to excess flume water?" Can economical design criteria be developed for impoundment of excess flume water?"

With regard to odor, laboratory studies had indicated repeatedly that aeration is a requirement of odor-free disposal, but the minimum degree of aeration and the method of applica-

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tion which would be most economical could not be determined in the laboratory. Moreover, studies of impoundment of domestic sewage and meat processing wastes by the authors of this paper had indicated that series ponding was an effective method of reducing BOD of domestic sewage and meat processing wastes without excessive odor. It had been found that large BOD reductions could be attained in ponds designed to emphasize anaerobic fermentation and that polished effluents could be produced for discharge or for use in dilution of incoming wastes in ponds designed to enhance algal growth and production of oxygen. The possibility of applying systems of ponds to beet wastes becomes apparent and pilot scale studies were thus deemed essential. Such pilot studies in a plant of prototype size would also make available data on the treatment accomplished and on fundamental design criteria.

The site selected for construction of the pilot plant was at the Holly Sugar Corporation, Tracy, California. Selection of this site was based upon the concept that the mild climate of California would minimize the influence of reduced temperature on odors; enhance the biological performance of the impounding system; extend the processing season sufficiently to permit step variations in loading, detention time, recirculation and mixing; and permit reasonable ease in sampling and observation. None of these conditions would have been as favorable in a colder climate.

Objectives

The overall objective was to demonstrate the possibility of reducing and eliminating pollution and odor problems in excess beet sugar factory flume water through the application of an anaerobic-facultative-aerobic lagooning system. Specifically, study was to be directed towards the establishment of the conditions under which the full efficiency of several components of a waste impoundment system could be realized. The experimental work was designed to determine the effects of loading, recirculation and series operation on the performance of each component in a pond system.

Theory and Design

The experimental plant was designed to consist of three ponds, — anaerobic, facultative and aerobic, — to be operated in series. The first or anaerobic pond was designed to operate at a depth of 15 feet and to receive an organic load such as to provide a potentially intense anaerobic environment throughout. The system was designed such that warm flume water from the factory would be introduced at the bottom of this pond to enhance

anaerobic decomposition and methane fermentation. The second pond (the facultative pond) was designed to be 7 feet deep and to be loaded in such a way that its surface could be aerobic and its bottom anaerobic, and thus supply the facultative phase. The effluent from the anaerobic pond was introduced at the bottom of the facultative pond. Finally, the aerobic, (algae) pond, was designed to operate at a depth of 1 to 3 feet to provide a suitable environment for algae growth, and thereby have the potential of being aerobic throughout its depth. This pond received effluent from the facultative pond.

Algae, being photosynthetic organisms, release oxygen into the water and, as do all microorganisms, incorporate carbon, nitrogen and phosphorus into their cells. The amount produced photosynthetically may be great enough to supersaturate the pond. In the system as designed, this highly oxygenated water would be recycled to the anaerobic pond surfare to satisfy the surface BOD and thereby reduce any objectionable odor present. Algal cells contained in the recycled water would settle to the bottom of the anaerobic pond where through decomposition their nitrogen and phosphorus would be made available for the organisms carrying out the process of methane fermentation.

Methods and Procedures

Although in the conduct of the experiments it would have been desirable to have allowed the ponds to reach "steady state" before concluding a run, a compromise had to be made because of limitations of time imposed by the seasonal nature of beet sugar refining operations. The compromise consisted in continuing each run until a trend could be established in the various parameters and a valid extrapolation made. Seven runs were made; the experimental conditions for each run are listed in Table 1. The full-scale runs were paralleled by smaller-scale experiments involving the use of small pond and laboratory bench investigations to explore in more detail, certain factors which would lend themselves to such study, and which could not be studied on a large scale because of time and budgetary limitations.

Physical Description

In the operation of the pilot plant a 300 GPM pump is employed to lift a portion of the Tracy factory waste water to the top of a settling tank where the water is passed through a Dorr-Oliver DSM screen. This screen removes all particles larger than about 0.06 inch from the waste. The screened water then enters a vertical tank tangentially creating a slow cyclonic action that brings about the settling out of heavy small inorganic

Table 1.—Experimental conditions.

Run	Feed rate (gpm)	Recycle rate (gpm)	Pond	Detention time (days)	Depth (ft)
1	100	100	Anaerobic	12	15
			Facultative	14	7
			Aerobic	10	3
			System	35	
2	100	50	Anaerobic	15	15
			Facultative	18	7
			Aerobic	13	3
			System	46	
3	100	0	Anaerobic	23	15
			Facultative	27	7
			Aerobic	20	3
			System	70	
4	150	0	Anaerobic	15	15
			Facultative	18	7
			Aerobic	13	3
			System	46	
5	50	50	Anaerobic	23	15
			Facultative	27	7
			Aerobic	20	3
			System	70	
6	260	200	Anaerobic	5	15
			Facultative	6	7
			Aerobic	4	3
			System	15	
7	260	0	Anaerobic	9	15
			Facultative	11	7
			Aerobic	8	3
			System	28	

particles. These solids are collected in a conical hopper located at the bottom of the tank and are discharged daily into the plant's treatment system. The effluent from the settling tank flows by gravity through a Parshall flume box for continuous metering and into the anaerobic pond. The water flows from the anaerobic pond through an adjustable effluent line by way of a depth control and metering weir to the bottom of the facultative pond. Discharge from the facultative pond flows through another depth control and metering weir into the aerobic pond. At the northeast corner of the aerobic pond, water flows through another depth control weir and into a pump sump. An automatic 250 GPM pump discharges the final effluent through a Parshall flume where it is again metered. A flow diagram of the pilot plant is given in Figure 1.

A recirculation line draws surface water from the aerobic pond and discharges it into a central sump which has three pumps - rated at 200 GPM, 300 GPM, and 500 GPM, respectively. The recirculant is pumped through a Parshall flume and into a junction box where it can be mixed with the influent, or be discharged at the surface of the anaerobic pond. The system can recycle from a minimum of 25 GPM to 1400 GPM.

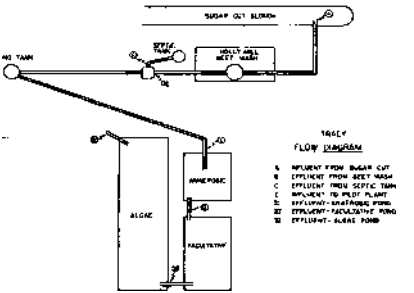

Figure 1.—Schematic flow diagram showing seven sampling points.

Sampling

During experimental runs samples of the influent and of the effluents from the anaerobic, facultative and aerobic ponds were taken daily. The influent sample was a composite of samples collected hourly over a 24-hour period by means of a solenoid valve and a reset timing device.

Observations and Analytical Procedures

Types of observations and analyses included the following:

1. *Organoleptic*
   a. Color: The following color index was used: Black, grey, red-brown, blue-green and green. The color intensity code based on degrees of intensity ranged from 1 for designating only a faint color to 5 for designating a heavy or deep color.
   b. Odor: Odor was coded as follows: 6 for foul, 5 for H_2S, 4 for "cow dung", 3 for sugarbeets, 2 for others, and I for no odor. The intensity of the odor was indicated by numbering it from 1 to 5, 1 designating no odor and 5 - a very strong odor. The combination of intensity and quality was expressed as the odor product. The odor product was obtained by multiplying the number for quality by that for intensity. Thus 1 would mean no odor, whereas 30 would imply a strong and disgusting odor.
2. *Biological*

Determinations were made of algae type and number, of the identity and number of algae grazers, of coliform, fecal streptococci, and total bacteria counts, and the total number of purple-sulfur bacteria.

3. *Physical*

Air temperature and relative humidity were recorded by means of a hygrothermograph. Sunlight energy was recorded by means of a Yellott Sol-A-Meter. Observations were made of the wind velocity and direction, of evaporation, and of precipitation at the site. The records of similar observations made by the U.S. Weather Bureau at the Tracy Pumping Station and Stockton Airport were also available. A copy of the latter data was received monthly from the weather bureau. Influent and effluent rates of the system were automatically recorded and the recirculation rate was controlled by valves. At each sampling station, the water temperature, color, and odor were observed daily. The water temperatures at the bottom of the ponds and at the top were taken by means of a telethermometer. Periodically, vertical temperature profiles in the ponds were determined through use of a telethermometer.

4. *Chemical*

Unless otherwise specified, chemical determinations were made according to Standard Methods (1). Solids were determined according to the Millipore method (2). The volumetric solid determination of algal biomass was made according to the centrifugal packed solids method described by Oswald (3). Algae counts were made with the use of a hemacytometer. An Orsat type gas analyzer was used for making the gas analysis. The LaMotte-Pomeroy kit was used for making the sulfide determinations.

Data Processing and Statistical Analysis

Items of data collected during the research were placed on IBM cards for storage and processing. Two programs for data processing were followed. In the first program two different parameters, such as temperature and algae growth, were correlated. The second program was designed to determine the formability percentiles of the various parameters.

Ancillary Studies

A part of the research effort was spent in the conduct of bench scale laboratory and small scale studies concerned with obtaining certain information needed in directing the course of the large-scale experiments. For these investigations, a small wooden tank (8 ft X 16 ft X 1 ft) lined with black plastic film was constructed on the levee of the anaerobic pond. At the beginning of each run the tank was filled with water from a nearby slough; at the end of a run the tank was emptied and cleaned out. The initial algae population of the water was approximately 5000 cells/ml. The detention time for all runs

was 10 days. A run was terminated when the algal population reached "steady state." The studies were programmed to demonstrate the importance of mixing and of the addition of nutrients with respect to algae growth in beet sugar flume waste water. In addition, a study was made of the use of pesticides for control of algae grazers.

In another ancillary study, a determination was made of the diurnal variation in the dissolved oxygen concentration of the aerobic pond. Observations of the dissolved oxygen content and water temperature were made at 2-hour intervals during the day and at 4-hour intervals during the night.

Results

BOD Removal

The BOD loading on the system was varied from 450 to 2250 lb/acre/day. Results concerned with BOD and COD removed are listed in Table 2. According to the data in Table 2, except during run No. 2 in which the removal was 83%, BOD removal ranged from 94% to 99% of the incoming BOD. At an input rate of 260 GPM and detention time of 28 days, the total BOD removal amounted to 2200 lb/acre/day.

As shown by the curve in Figure 2, BOD removal in the anaerobic pond increased in direct proportion to extent of loading. With the BOD loading at 2250 lb/acre/day, 90% of the loading was removed; at 1400 lb/acre/day, 82% of the loading was removed. However, the temperature of the water at the bottom of the pond at the time of the higher loading was 20°C and the detention period 9 days; whereas at the time of the lower loading, the temperature was 17°C and the detention period 28 days.

Except for a 3-week period in the spring, the facultative pond remained completely anaerobic. During the brief period in which the pond was not anaerobic, 83% of the incoming BOD was removed in the pond. When the loading was increased beyond 48 lb/acre/day the pond immediately turned anaerobic again. The BOD removal remained approximately 80% of the applied load under all conditions.

During this period, the aerobic pond removed an average of 62% of the incoming BOD. The lowest removal during this period occurred in run No. 5 in which the incoming BOD loading was extremely light, viz. 8 lb/acre/day.

COD Removal

The COD loading on the system varied from 850 to 4450 lb/acre/day. In general, removal of COD throughout the system followed the same pattern as that of BOD removal, COD re-

Table 2.—BOD-COD loading and removal.

Run	Pond	BOD loading (lbs/acre/day)	BOD removal (lbs)	% BOD removal (of incoming load)	% BOD removal (of total load)	COD loading (lbs/acre/day)	COD loading (lbs)	% COD removal (of incoming load)	% COD removal (of total load)
1	Anaerobic	1250	760	61%	61%	2980	1488	50%	50%
	Facultative	490	407	83	33	1492	991	66	33
	Aerobic	65	18	28	1	501	26	5	1
	Anaerobic	1020			63%	2249	1578	70%	70%
	Facultative	381			19	671	356	53	16
	Aerobic	190			2	335	33	10	2
	Anaerobic	1390	1141	82%	82%	1920	1561	81%	81%
	Facultative	249	119	48	9	359	161	45	8
	Aerobic	130	45	35	3	198	55	28	3
	System				94%				92%
	Anaerobic	1240	1091	88%	88%	2430	2140	88%	88%
	Facultative	149	116	78	9	290	223	77	9
	Aerobic	33	21	64	2	67	67	86	1
	System				99%				98%
5	Anaerobic	413	365	88%	88%	845	737	87%	87%
	Facultative	48	40	83	10	108	75	70	9
	Aerobic	8	2	25	1	33	5	15	1
	Anaerobic	2180	1760	81%	81%	4450	3654	82%	82%
	Facultative	420	298	71	14	798	511	64	12
	Aerobic	122	76	62	4	287	71	25	2
	System				99%				96%
	Anaerobic	2240	2015	90%	90%	4160	3605	87%	87%
	Facultative	225	175	78	8	555	408	73	10
	Aerobic	50	31	62	1	147	50	34	1
	System				99%				98%

moval of the system ranged from 84% to 98%. At the maximum input rate, 260 GPM, and with the detention time of 28 days, the total COD removal was 4200 lb/acre/day. From 70% to 87% of the COD loading was removed in the anaerobic pond.

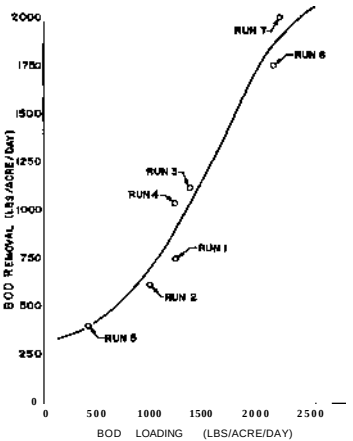

Figure 2.—Influence of loading on BOD removal in the anaerobic pond.

COD/BOD Ratio

The higher the COD/BOD (5-day) ratio the greater the stability of organic matter. As is shown by the curves in Figure 3, the COD/BOD ratio of the organic matter in the waste water increased as it passed through the pond series. The COD/BOD ratio of the raw flume water was 1.6, which indicates that 63% of the COD was biologically available within 5 days. In the anaerobic pond the ratio changed from 1.6 to 2.0. This change evidently occurred independently of detention period and loading. In the facultative pond the ratio approached 3.0 at the longer detention periods. During the spring, the COD/BOD ratio in the aerobic pond ranged from 3.5 to 5.4, the higher value occurring during a period of algal growth in the pond.

Total Nitrogen Removal

Changes in total nitrogen concentration may reflect variations in water quality, denitrification and biological assimilation. Nitrogen removals on the order of 75% were attained in the pond series. Nitrogen content decreased drastically at the lower loadings. Most of the removal was accomplished in the anaerobic pond. A slight incremental removal took place in the facultative pond. Increase or decrease in removal between the facultative and aerobic ponds was only minor.

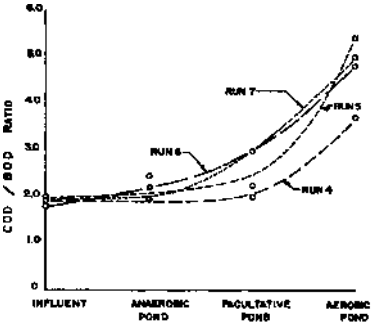

Figure 3.—Effect of anaerobic, facultative and aerobic series treatment on the COD:BOD ratio.

Volatile Solids Removal

The results indicate that the volatile solids removal by the system increased as the load increased. Thus, at an input rate of 260 GPM (run No. 6) and a recycling rate of 260 GPM, the removal exceeded 4000 lb/acre/day; whereas, in other runs in which the input was less, the removal ranged from 500 to 1800 lb/acre/day. The major portion of the removal occurred in the anaerobic pond. During run No. 6 the recirculant had a high algae content which settled out rapidly after introduction into the anaerobic pond. At this time, the detention time was only 5 days. At an input rate of 260 GPM and no recycling, the anaerobic pond removed approximately 1800 lb/acre/day which was less than half of that removed with the recycle rate at 260 GPM.

Indicator Microorganisms

Points at which samples were removed for the determination of numbers of indicator microorganisms (coliform, fecal coliform and fecal strep) are shown in the schematic diagram of the pilot plant in Figure 1. A summary of the coliform, fecal-coliform and fecal streptococci results are given in Table 3. The data presented in the table represent median values of the bacteria present in the system during the spring campaign. As shown in Figure 4, the number of coliforms, fecal-col iforms and fecal streptococci was rapidly reduced upon exposure to the anaerobic pond, and the same rate of destruction continued in the two following ponds. The percent reduction of coliforms and fecal streptococci within this treatment system was 99.99 percent in practically all cases.

Table 3.—Median values for coliform, fecal coliform and fecal streptococci, MPN—spring, 1967.

Station*	Coliform	Fecal coliform	Fecal streptococci
A	430,000	4,000	2,000
B	33,000,000	1,000,000	
			4,200,000
C	5,900,000	3,330,000	430,000
I	93,000,000	15,000,000	
			2,300,000
II	2,600,000	2,300,000	9,000
III	69,000	23,000	600
IV	2,000	400	20

*See Figure 1.

Odor Production

The odor product of the raw waste ranged from 1 to 5. The greatest odor production was in the anaerobic pond, the odor product ranging from 6 to 25. Increasing the loading to the facultative pond was accompanied by an increase in odor production. The odor product varied from 1 to 9 depending upon the amount of loading. With respect to the aerobic pond, the odor product was less than 5.

As shown in Figure 5, during run No. 6 (spring 1967), in which the input and recycling rates were 260 GPM and the algal population of the recirculant was over a million cells per ml, the odor product of the anaerobic pond was 9. The reason for the relatively low odor product was the fact that the surface water BOD was satisfied by recycled water supersaturated with dissolved oxygen as a result of algal activity in the aerobic pond. On the other hand, in run No. 7 in which the input rate was kept at 260 GPM, but in which recycling was discontinued, the odor product reached 25 within 24 hours and eventually reached a high of 30 during the run. The increase in odor product with recirculation found in the fall of 1966 resulted from inadvertant filling of the experimental ponds with some flume water from the factory ponds causing a severe overload in all ponds and death of all algae.

Ancillary Studies

Results of certain experiments conducted using the small pond are shown in Table 4. Anaerobic pond water was used in the second study, and factory effluent in the third. In both studies the algae population increased from low values to 2.2 million cells per ml within three weeks, — in spite of an algae grazer (Daphnia species) which attained a population of 5000 per ml.

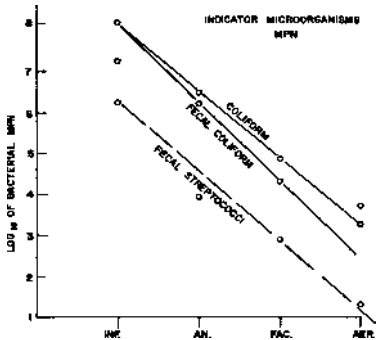

Figure 4.—Reduction of bacterial MPN at the sampling stations, spring of 1967.

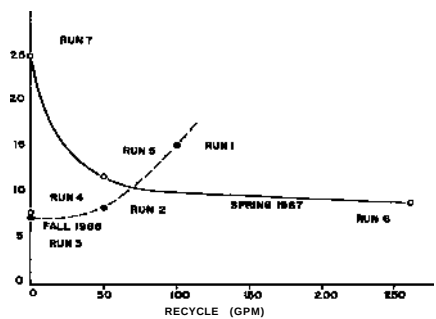

Figure 5.—Effect of recycle on the "odor product" in the anaerobic pond.

Later studies were concerned with effect of nutrient additions on algal growth. Factory effluent was used in these studies. Ammonium phosphate was tested as an additive nutrient in the fourth study and magnesium phosphate in the fifth. In both cases, the nutrient was added at a rate of 5 ppm per day. In both cases, the algae population reached a plateau of 1.8×10^6 cells per ml. Probably the light limited population for the depth of 10 inches. Algae grazer population ranged from 5000 to 25,000 per ml.

Table 4.—Effect of mixing on algal growth, operation log, 1967.

Parameters

Time:	10 days
Dimensions:	16' long, 8' wide, 1' deep
	Tank lined with black plastic film
Water source:	Nearby slough
Initial Algal Population:	Approximately 5000 cells per cc.
Volume	800 gallons
Detention time:	10 days
Feed Source: Run 1 - 3	Anaerobic pon effluent
Run 4 - 6	Factory effluent
Feed rate:	80 gal/day (except run 1)

Run	Date	Operation	Remarks
1.	Jan 10-Jan 23	No mixing; no feed	Algae count dropped to 0
2.	Jan 24-Feb 8	No mixing	Algae count dropped to 0
3.	Feb 9-Feb 28	Mixing twice a day	Algae count plateau: 2.2 X 10^6 cells per cc
4.	Mar 1-Mar 15	Mixing twice **a** day	Algae count plateau: 2.2 X 10^6 cells per cc
5.	Mar 23-April 27	Mixing twice a day 5 ppm ammonium phosphate added daily-	Algae count plateau: 1.5 x 10^6 cells per cc
6.	April 28-May	Mixing twice a day 5 ppm magnesium phosphate added daily	Algae count plateau: 1.8 x 10^6 cells per cc

The same small pond was also used to study the effect of the pesticides Malathion, Lindane and Baytex on algae grazers. In conducting the experiments grazers were transferred from the aerobic pond to the small pond. According to Table 5, no kill was effected by Malathion at a dosage of 0.5 mg/1, whereas a 100% kill took place at a dosage of 1.0 mg/1. Lindane and Baytex were 100% effective with 24 hours at dosages of 0.10 mg/1 and 0.25 mg/1, respectively. Baytex is said to have the advantageous characteristic of degrading to a non-toxic substance within 72 hours.

Diurnal Variation in Dissolved Oxygen

The determination of the diurnal variation in dissolved oxygen concentration of the aerobic pond was made during the month of April. To show the efficiency of oxygen production by algae even under unfavorable conditions, a rainy, overcast, cold day was selected. The variation is indicated by the data listed in Table 5. The lowest dissolved oxygen concentration

Table 5.—Diurnal variation in dissolved oxygen.

	temp. °C	Actual D.O.	*Saturation D.O.
10 a.m.	12.0	12.3	10.8
12 noon	12.8	15.0	10.6
2 p.m.	13.5	15.7	10.5
4 p.m.	14.0	16.5	10.4
8 p.m.	14.1	15.7	10.4
12 midnight	14.0	13.0	10.4
4 a.m.	11.2	11.1	11.1
8 a.m.	12.1	10.4	10.8
12 noon	13.0	12.8	10.6

during the run was 10.4 mg/1; and the highest, 16.7. It should be noted that the dissolved oxygen concentration was at or above saturation throughout the entire 26-hour duration of the study.

Discussion

The anaerobic-facultative-aerobic pond system as used in the study proved to be an extremely effective device for removing BOD, COD, volatile solids and nitrogen. The system removed 94% to 99% of the BOD, 88% to 98% of the COD, and 75% of the total nitrogen. By passage through the anaerobic pond, 82% to 90% of the incoming BOD, 70% to 87% of the incoming COD and approximately 75% of the incoming total nitrogen were removed. The heavy loss of nitrogen could have been a function of the anaerobic nature of the pond, since nitrogen loss by denitrification can be quite extensive under anaerobic conditions. Some of the initial removal of BOD, COD, volatile solids and nitrogen may have been due in part to precipitation and sedimentation of colloidal and suspended solids as normally occurs in any pond which is first in a series. However, the configuration of this anaerobic pond makes it the most efficient of the three types of ponds studied with respect to land use. Moreover, the great depth and volume of the anaerobic pond provides a buffer against sudden changes in waste quality.

Several problems were encountered in operating the anaerobic-facultative-aerobic pond system, — some of which can be explained in terms of shortcuts in pond design (i.e., of the aerobic pond), some in terms of the variations in method of operation necessitated in the conduct of research and some in terms of inhibitory substances in the influent flume water. For example, a mixing system and its required equipment and structural components were not installed in the aerobic pond in the period covered by this paper. Exigencies of time and budget as well as a desire to determine the feasibility of operating the pond

series as a whole without aerobic pond mixing led to the omission of the mixing system. However, the absence of mixing undoubtedly contributed to the failure of algae to grow to their potential abundance in the aerobic pond (cf. "Ancillary Studies") and hence to the lack of algae-produced oxygen in the pond contents. This in turn made recirculation ineffective as a method of oxygen input to the pond complex in those experiments in which an algal population either was not present or was present in low concentration, and hence ineffective as a method of controlling odor.

Only one type of series arrangement was explored in the study, viz. anaerobic-facultative-aerobic. An alternative in pond series arrangement would be the omission of the facultative pond. It is quite possible that the aerobic and the anaerobic ponds could adequately take over the function of the facultative pond, — in effect functioning as a kind of phase separation. Under such conditions in theory the aerobic pond would take over the function of the surface layer of the facultative pond and the anaerobic pond, its lower or anaerobic layer. The omission of the facultative pond would reduce the required land use by approximately one-third, but would probably also decrease the overall system BOD removal.

During the course of the study, the need for further investigation to find the answers to several questions became apparent. One of these questions pertains to natural and applied aeration. In this context, "applied" refers to aeration accomplished by physical means (mechanical aerators) and "natural", to that brought about by atmospheric diffusion and by algal activity. Preliminary studies indicated that decomposition can be hastened and odor considerably diminished by superimposing applied aeration upon natural aeration. One problem is that of determining the extent of the gap between the amount of natural aeration available and that needed for optimum treatment, i.e., the amount of applied aeration that must be used. Another is one of determining that the combination which is most economical for applying the required aeration. A third aspect of the aeration question is one of determining and exploring methods of enhancing natural aeration. Continuing studies are currently under way which should indicate the answers to these questions.

Conclusions

1. In an anaerobic-facultative-aerobic pond system, operated in the order named, the major part of the removal of BOD, **COD,** volatile solids, coliforms and odor causing substances takes place in the anaerobic **pond.**

2. **It** is **not** possible to operate an anaerobic pond without odor

production unless the surface of the pond is aerated either by way of recirculation from an aerobic pond or by mechanical aeration.

3. Odor production increased in the anaerobic pond with increase in BOD loading. It generally was low in the facultative and aerobic ponds at all of the loadings applied.

4. Increase in recirculation rate brought about a reduction in odor production in the anaerobic pond. No significant effect of recirculation rate on odor production by the facultative and algae ponds was noted.

5. Mixing is essential for production and maintenance of the algal population required for maximum efficiency of flume-water treatment in a pond series of the type used in the study.

6. In a 26-hour period of observation, the dissolved oxygen concentration of the aerobic pond remained at or above saturation, despite the fact that environmental conditions were extremely unfavorable for algal activity.

7. All of the nutrients needed for algal growth are present in the beet sugar flume water.

8. Algae grazers can be controlled through the use of commercial pesticides.

9. The number of coliforms, fecal-coliforms and fecal streptococci was reduced to satisfactory levels.

10. The following problems or questions will be explored in continuing studies.

a. Extent of gap between the amount of available natural aeration and that which must be applied mechanically to obtain optimum treatment.
b. The optimum method of supplying applied aeration.
c. Methods of bringing about and for enhancing natural aeration.
d. The need for the facultative pond in the system.

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The Effects of Potassium Carriers and Levels of Potassium and Nitrogen Fertilization On the Yield and Quality of Sugar Beets[1]

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High levels of potassium in the petioles of sugarbeets are conducive, if not essential, to the production of high yields (5).[3] High levels of potassium in the root at harvest are undesirable, however, as potassium along with amino-nitrogen account for a large proportion of the non-sucrose contaminants of the clear juice.

Considerable research on the effects of potassium carriers on the quality of crops other than sugarbeets has been reported. There is evidence that KC1 applied at high rates decreases the specific gravity of potatoes and lowers the quality of fruits and tobacco (4,6,7,8). One possible explanation of these observations is that the number of soil bacteria is reduced by chloride-containing potassium fertilizers. Yung (9) found that fertilizers containing chloride destroyed nitrifying and cellulose decomposing bacteria and increased the proportion of fungi in the microflora. Nearly all workers cited agree that the detrimental effects of KC1 on crop quality are not present when K_2SO_4 is used as the potassium source.

The primary objective of this work was to determine if KC1, KNO_3, K_2SO_4 or (K_2SO_4 + $MgSO_4$)[4] differentially affects the yield and/or quality[5] of sugarbeets. Special emphasis was placed on finding any detrimental effect on sugar beet juice quality when KC1 was applied. Additional objectives were to determine if rates of potassium or nitrogen fertilization or their interactions affect the yield and quality of beets.

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[3] Numbers in parentheses refer to literature cited.

[4] Sul-PO-Mag is available from International Minerals and Chemical Co., Skokie, 111., composed of a mixture of K and Mg sulfates containing 18% K and 11% Mg, and hereafter denoted as $KMgSO_4$.

[5] The term quality in this paper refers to the percent of sucrose in the beets, the percent clear juice purity, the amount of recoverable sugar from an acre and the relative proportion of the clear juice impurities, amino-nitrogen, potassium and sodium.

Materials and Methods

Experiments with potassium carriers were conducted at three locations in 1965 and were repeated at two of the locations in 1966. Two of these locations were on mineral soils typical of the sugarbeet growing area near the Saginaw Bay in Michigan. Location 1 was a Kawkawlin loam with a high test for potassium, and location 2 a Sims clay loam with a medium test for potassium. Location 3 was on an organic soil (Houghton muck).

Four potassium carriers, KC1, KNO_3, K_2S0_4 and $KMgS0_4$. were applied in replicated plots in randomized complete block designs at each location. At location 1, potassium was applied at rates of 100 and 200 pounds K_20 per acre, nitrogen at 80 and 60 pounds in 1965, and 30 and 150 pounds in 1966. Potassium was applied at the rate of 240 pounds K_20 per acre at location 2, and nitrogen was applied at the constant rate of 70 pounds per acre. At location 3, potassium was applied at rates of 200 and 600 pounds K_20 and 60 pounds nitrogen was applied. A 500-pound per acre NaCl application was made on one-half of the plots at location 3.

The treatment materials, along with 5 pounds per acre boron and 14 pounds manganese, were spread uniformly on the plot surface and mixed with the soil by means of a spring-tooth harrow just prior to planting. Phosphorus was banded at planting at the rates of 240 pounds P_20_5 per acre for locations 1 and 2 and 100 pounds per acre for location 3.

Petiole samples were taken from all plots in late July and in early October by randomly selecting 20 of the youngest mature petioles from each plot. The petioles were dried in a forced air oven at 60°C, ground and analyzed for potassium, sodium, magnesium and calcium.

At harvest, yield weights were recorded and ten beets were chosen from each plot in such a manner as to avoid selecting extra large or extra small roots and to obtain a uniformity of sampling. The ten beet samples were analyzed for percent sucrose and percent clear juice purity (1, 2). In addition, some of the samples were analyzed for the clear juice impurities, sodium and potassium (flame photometry) and amino nitrogen (3). Recoverable sugar values were calculated. Analyses of variance were employed in order to facilitate the interpretation of the data. Values of least significant difference were calculated for means judged significantly different at the *5%* level. The error possible in applying the least significant difference to more than two means is recognized.

Results

No significant differences in yield, percent sucrose, percent clear juice purity, or concentration of amino nitrogen, sodium, or potassium in the clear juice were noted at location 1 in 1965 or 1966. The possibility of a response to magnesium where $KMgSO_4$ was applied was checked by adding $MgSO_4$ to K_2SO_4 plots in amounts equivalent to the amounts of magnesium in the $KMgSO_4$ plots. No response to magnesium was found.

Neither sugarbeet yield nor quality was significantly different when 200 pounds of K_20 was applied than when 100 pounds was applied. Percent sucrose, percent clear juice purity and amount of sugar recoverable from an acre were reduced when 150 pounds of nitrogen was applied in 1966.

Table 1 shows that sugarbeets treated with KC1 had a higher percent potassium in their petioles in July than beets treated with KNO_3. When $KMgSO_4$ was the carrier of potassium, a higher percent potassium was obtained than when either KNO_3 or K_2SO_4 was the carrier. Sixty pounds of nitrogen per acre decreased the percent potassium in petioles in July in comparison to 30 pounds per acre. There were no differences in the percentages of potassium in the petioles in October. Beets treated with KC1 had a higher percent calcium in their petioles than beets treated with KNO_3 or K_2S0_4, and a higher percent magnesium than beets treated with KNO_3, K_20_4, or $KMgSO_4$. The application of 60 pounds of nitrogen increased the percent magnesium in petioles in October.

The data from location 1 for 1966 are presented in Table 2. When K01 served as the potassium source, sugarbeet petioles contained higher concentrations of potassium, sodium and magnesium than when any of the other carriers tested served as the potassium source. Petioles contained greater concentrations of potassium when either the higher rate of potassium or the lower rate of nitrogen was applied. The sodium concentration of petioles in October and the magnesium concentration in July and October were increased by the application of 150 pounds of nitrogen per acre.

At location 2 (Table 3), the yield and quality of sugarbeets were not affected by potassium carriers with the exception that plots treated with KC1 had a higher concentration of potassium in the clear juice as an impurity in 1965. Beets grown where KC1 was applied had higher percents potassium, calcium, and magnesium, and lower percent sodium in petioles than did beets on plots where other carriers were applied.

Table 1.—The effects of two rates of application of four potassium carriers and two nitrogen levels on the nutrient uptake of sugarbeets at location 1 (Kawkawlin loam) in 1965.

K carrier	Percent K in petioles		Percent Na in petioles		Percent Ca in petioles		Percent Mg in petioles	
	July	October	July	October	July	October	July	October
KCl	4.02	5.16	1.92	1.38	0.92	0.97	0.68	0.49
KNOa[1]	3.61	4.78	1.91	1.31	0.82	0.78	0.65	0.39
K_2S04	3.74	4.89	1.80	1.25	0.86	0.79	0.70	0.37
KMgS04	4.20	5.06	1.91	1.40	0.87	0.87	0.65	0.43
LSD 5%	0.34	NS	NS	NS	NS	0.13	NS	0.06
K2O Lbs/A								
100	3.83	4.95	1.92	1.32	0.87	0.81	0.66	0.41
200	3.95	5.00	1.85	1.35	0.87	0.88	0.67	0.44
	4.02*		1.87		0.87	0.85	0.66	0.40
60	3.76		1.90	1.32	0.87	0.84	0.67	0.44*

[1] KNO_3 plots where 200 lbs K_2O and 30 lbs N were applied received part of their K as K_2SO_4 in order to avoid applying over 300 lbs N.

* Significantly higher at 5% level.

Table 2.—The effects of two rates of application of four potassium carriers and two nitrogen levels on the nutrient uptake of sugarbeets at location 1 (Kawkawlin loam) in 1966.

K carrier	Percent K in petioles		Percent Na in petioles		Percent Ca in petioles		Percent Mg in petioles	
	July	October	July	October	July	October	July	October
KCl	4.64	4.71	1.91	1.02	0.99	0.88	0.75	0.51
KNO_3[1]	3.92	4.25	1.82	1.01	0.84	0.70	0.58	0.40
K2SO4	4.18	4.29	1.82	0.87	0.85	0.72	0.58	0.40
$KMgSO_4$	4.24	4.27	1.96	1.00	0.79	0.69	0.59	0.41
LSD 5%	0.35	0.33	NS	NS	0.10	0.11	0.07	0.07
K2O Lbs/A								
100	4.12	4.24	1.82	0.92	0.87	0.74	0.62	0.43
200	4.37*	4.57**	1.94	1.00	0.87	0.75	0.64	0.43
N Lbs/A								
30	4.45*	4.60**	1.86	0.92	0.90	0.73	0.60	0.38
150	4.03	4.21	1.90	1.03*	0.84	0.76	0.65*	0.48**

[1] KNOs plots where 200 lbs K2O and 30 lbs N were applied received part of their K as K_2SO_4 in order to avoid applying over 30 lbs N.

* Significantly higher at the 5% level.

** Significantly higher at the 1% level.

Table 3.—The effects of four potassium carriers on the yield, quality and nutrient uptake of sugarbeets at location 2 (Sims clay loam) in 1965 and 1966.

1965

K carrier	Yield-tons/A	Percent sucrose	Percent clear juice purity	Pounds sugar/A	Impurities in clear juice Mg/100 g sucrose			Percent K in petioles		Percent Na in petioles		Percent Ca in petioles		Percent Mg in petioles	
					Amino N	K	Na	July	October	July	October	July	October	July	October
O-K	20.9	15.4	94.3	4908	233	1188	51	6.14	5.91	0.57	0.44	0.84	0.47	0.58	0.54
KCl	22.4	13.2	94.1	5202	232	1414	45	6.84	6.66	0.48	0.33	0.95	0.55	0.57	0.42
KNO_3	23.7	13.5	94.1	5598	240	1183	47	6.28	5.78	0.53	0.40	0.82	0.44	0.52	0.51
K_2SO_4	22.3	13.9	94.6	5500	212	1055	40	6.47	6.20	0.52	0.36	0.81	0.44	0.54	0.50
$KMgSO_4$	22.4	13.5	94.6	5329	205	1159	48	6.47	6.11	0.49	0.42	0.74	0.57	0.47	0.30
LSD 5%	NS	NS	NS	NS	NS	228	NS	NS	0.58	NS	0.06	NS	0.11	0.07	NS

1966

K carrier	Yield-tons/A	Percent sucrose	Percent clear juice purity	Pounds sugar/A	Percent K in petioles		Percent Na in petioles		Percent Ca in petioles		Percent Mg in petioles	
					July	October	July	October	July	October	July	October
O-K	14.9	15.8	96.2	4330	3.82	4.37	0.81	0.51	0.68	0.83	0.52	0.44
KCl	14.5	16.1	95.8	4219	4.74	5.62	0.71	0.47	0.66	1.08	0.67	0.55
KNO_3	13.5	15.8	95.7	3875	3.97	5.26	0.71	0.63	0.64	0.98	0.48	0.47
K_2SO_4	14.5	16.1	95.0	4189	4.18	4.84	0.66	0.36	0.59	0.77	0.47	0.42
$KMgSO_4$	15.8	16.3	95.8	4694	4.81	5.06	0.84	0.60	0.57	0.85	0.46	0.49
LSD 5%	NS	NS	NS	NS	NS	0.59	NS	0.15	NS	NS	0.10	0.10

No differences in yield or quality of sugarbeets were found when two rates of potassium, two rates of sodium chloride and four potassium carriers were applied on the Houghton muck. Petiole analyses for sugarbeets grown on this organic soil are given in Table 4. The relative levels of potassium in the petioles are extremely high in July compared with those in other experiments and in October. This is perhaps an indication of luxury consumption of potassium.

Interactions noted at location 1 are given in Table 5. In 1965, significant interactions were found between rate of potassium fertilization and rate of nitrogen fertilization. A higher yield and more recoverable sugar were produced when 30 pounds of nitrogen per acre was applied with 100 pounds of K_20 than when 200 pounds of K_20 was applied. Applying 60 pounds of nitrogen per acre reduced the amount of recoverable sugar on areas where K_20 was applied at 100 pounds.

Table 4.—The effects of potassium carriers on the percents of potassium, sodium, calcium and magnesium in the petioles of sugarbeets from plots receiving no sodium chloride at location 3 (Houghton muck) in 1965.

K carrier	Percent K in petioles		Percent Na in petioles		Percent Ca in petioles		Percent Mg in petioles	
	July	October	July	October	July	October	J«iy	October
OK	13.22	6.67	1.53	1.45	0.36	0.31	0.52	0.31
KC1	12-91	8.78	1.52	1.98	0.38	0.34	0.54	0.30
KNO_3	13.75	8.18	1.62	1.08	0.47	0.30	0.59	0.28
K_2S0_4	12.79	8.13	1.56	1.09	0.40	0.31	0.51	0.28
$KMgS0_4$	13.89	8.82	1.57	1.00	0.44	0.35	0.67	0.29
LSD 5%	0.87	0.70	NS	0.26	NS	NS	NS	NS

Table 5.—Interactions in the yield and quality of sugarbeet data as affected by two rates of application of potassium carriers and two nitrogen levels at location 1 (Kawkawlin loam) in 1965 and 1966.

1965

K_20 Lbs/A	Yield - tons/A		LSD	Pounds sugar/A		LSD
	30 lbs N	60 lbs N	5% level	30 lbs N	60 lbs N	5% level
100	22.6	21.4	NS	6887	6366	454
200	21.2	22.0	NS	6414	6631	NS
LSD 5%	1.3	NS		454	NS	

1966

K carrier	Percent sugar			Pounds sugar/A		
	100 lbs K_2O	200 lbs K_2O	LSD 5% level	100 lbs **K2O**	200 lbs **K2O**	LSD 5% level
KC1	16.8	16.1	NS	7595	6710	620
KNOc	15.8	17.0	0.8	6988	7487	NS
K_2SO_4	16.9	16.3	NS	7330	7454	NS
$KMgSO_4$	17.1	16.7	NS	7493	7511	NS
LSD 5%	0.8	0.8		NS	NS	

In 1966, KNO_3 applied at the rate of 100 pounds K_2O per acre produced sugarbeets with a lower percent sucrose than did the other carriers. When 200 pounds per acre of K_2O was applied as KNO3, the beets produced had a higher percent sucrose than beets for which KC1 was the potassium source. The application of KNO3 at the rate of 200 pounds K_2O per acre resulted in a higher percent sucrose than its application at 100 pounds per acre. Recoverable sugar was reduced by applying 200 pounds of K_2O as KC1.

Discussion

The major portion of the research presented was done on a soil with a high potassium test (location 1 soil tested 240 pounds ammonium-acetate extractable potassium per acre). This soil was chosen because it was believed that effects injurious to the quality of sugar beets would be more likely to occur at higher soil potassium concentrations. The data indicate that the potassium carriers tested were all similar in their effect on the yield and quality of sugarbeets at given levels of potassium and nitrogen fertilization.

Decreases in the recoverable sugar per acre for sugarbeets when 200 pounds of K_2O as KC1 was applied may be due to factors discussed earlier in this paper and observed in potatoes, fruits, and tobacco. The reason beets treated with KC1 at the rate of 100 pounds K_2O per acre had a higher percent sucrose than those treated with KNO_3 may possibly be explained by the greater uptake of potassium from KC1.

The percent sucrose, percent clear juice purity and recoverable sugar of beets were markedly reduced by the application of 150 pounds of nitrogen in comparison to 30 pounds. This effect is consistent with many studies.

Inverse relationships between the relative amounts of potassium and sodium in sugarbeet petioles is apparent in the data from these experiments, indicating that there may be some substitution of sodium for potassium.

Summary

The yield and quality of sugarbeets grown on three soil types and in two successive years were affected similarly by the four potassium carriers: KC1, KNO_3, K_2SO_4 and $KMgSO_4$, For the experiments carried out, the rate of potassium applied to a soil with a high potassium test did not affect the yield or quality of beets. Sugarbeets supplied with 150 pounds of nitrogen were of lower quality than beets supplied with 30 pounds of nitrogen per acre. Some evidence is given to indicate that KC1 applied at a rate of 200 pounds K_2O per acre reduced the quality of sugarbeets in comparison to KC1 applied at 100 pounds K_2O per acre and to KNO_3 applied at a rate of 200 pounds per acre.

Highest concentrations of potassium generally occurred in petioles of sugarbeets to which **KC1** had been applied. When 150 pounds of nitrogen per acre was applied, sugarbeet petioles contained higher concentrations of magnesium and lower concentrations of potassium than did petioles of beets to which only 30 pounds nitrogen per acre was applied. Higher concentrations of potassium were found in petioles from beets which were supplied with 200 pounds of K_20 in comparison to beets to which 100 pounds of K_20 was applied. In general, the concentration of potassium in sugarbeet petioles was higher in October than in July, while the opposite trend was noted for the concentrations of sodium, calcium and magnesium at the two mineral soil locations. Sugarbeets grown on Houghton muck had high concentrations of potassium in their petioles. The relative concentrations were lower in October than in July.

The application of 500 pounds of NaCl had no effect on the yield or quality of sugarbeets grown on Houghton muck.

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Central Tare Laboratory Operator

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Introduction

The Utah-Idaho Sugar Company has established two semi-automatic central tare and individual sugar content laboratories to process samples from four of the company's five factory districts. The first was installed at Moses Lake, Washington in the fall of 1966 and receives beets from the Columbia Basin and Toppenish districts in Washington. The second laboratory was built at Garland, Utah a year later to handle samples from the Garland and Southern Utah districts. Overall operation of the new laboratories has been quite successful and marred only by the new process start-up "bugs".

Description of Equipment

The laboratories are equipped with an integrated system of sample cleaning, taring and weighing equipment. Extensive use has been made of electronic data gathering equipment and readout devices. Through an interlocked system of micro-switches and photo cells the beets themselves control the movement of samples through the cleaning and taring equipment. Some changes have been made based on experience and an improved washer-dryer design used in the second installation; however, the overall system design has not been changed from the original. In addition to the change in washer design, the Garland laboratory differs by having a mechanical second scale and one rather than two, Weibull sugar analysis lines.

The washer-dryer and taring stations designs were developed by Utah-Idaho Sugar Company in conjunction with Ogden Iron Works. Load cell scale systems and sugar content readout equipment were fabricated by Ormond Inc. of Los Angeles. Sugar content analysis equipment was furnished by Weibull A.B. of Malmo, Sweden, modified to the extent of using Bendix Automatic Polarimeters instead of the servo balance type used in Europe.

Description of Operation

Tare samples are taken from loads as received at the stations.

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They are placed in rubberized nylon bags and an identifying ticket is placed in a special pocket on the outside of the bag. The sample bags are equipped with a snap strap closure and have a large ring attached to the bottom. The bags are then placed in wooden tote bins each holding 25 to 30 samples for transport by truck to the laboratory. At the laboratory the bins are removed by fork lift and placed on the loading dock. The sample bags are taken from the bin and hung by the bottom ring on Chainveyor belts. The carriers are moved into the laboratory by a power chain and positioned over the washer feed hopper. The section of Chainveyor track over the hopper is part of a load cell scale linked to a printing calculator. After the sample is positioned the operator enters the total weight and empties the sample into the hopper. The operator takes the identity card from the pocket and places it in the printer. The weight of the empty bag and carrier is then entered. The calculator subtracts the second entry from the first and prints out the net weight of the sample as received. The card is then placed in the card conveyor which moves the identity card to the second scale position. The motion of this conveyor is linked to the washer and dryer so that the sample and card arrive at the second scale together. When the card is placed in the first position on the conveyor, it trips the hopper and moves the sample to the washer.

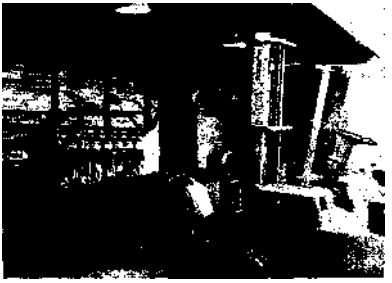

Figure 1.—Samples being loaded on Chainveyor for transport into the tare laboratory.

The washer is a horizontal eight-sided drum with a spiral flight welded into the inside. As it revolves (2-3 rpm) the beets remain in essentially the same position on its circumference, just past the bottom of the drum. In effect a series of compartments are formed which move through the drum as it revolves.

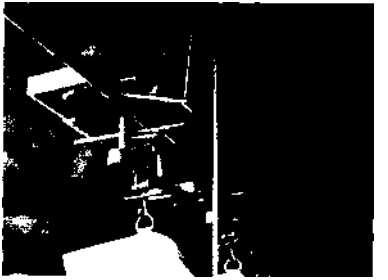

Figure 2.—Load cell scale portion of Chainveyor track with sample carrier in position for weighing.

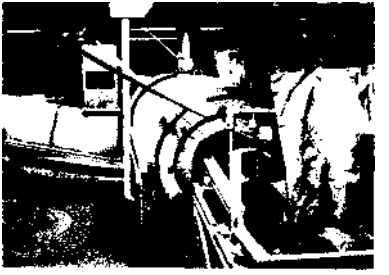

Figure 3.—Carver-Ogden sample washer showing sample feed belt and card conveyor.

A row of nozzles is fixed to spray the beets with a stream of water at 120-160 psi effectively removing all the soil and mud. From the washer the beets move to the dryer.

In the first installation the dryer is a compartment belt moving in a tunnel of warm air. In the Garland unit it is integrated with the washer and forms the last five compartments. By using this construction, operation is improved and maintenance greatly simplified. We feel that the washer-dryer design is a contributing factor to the overall success of the tare operation. The dryer discharges into a hopper convenient to the top tare operators.

The samples are crowned by hand using the standard two bladed rotary topping machine and placed in a scale feeding hopper. The second scale is a tilting pan load cell unit equipped with a card printer. At Garland the topped samples are placed

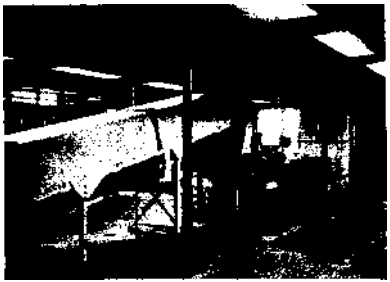

Figure 4.—Moses Lake tunnel dryer and top tare station. The housing for the Weibull sampling saws can be seen on the operator's right.

directly in the hopper mounted on a platform scale equipped with a card printer. After recording the weight the operator then dumps the sample onto a belt which feeds the Weibull sampling equipment. The top tare from the topping machines is also placed on this belt so that both are put through the sugar sampling equipment. By the addition of the tare material, the resultant sugar analysis more truly represents the sugar content of the beets as delivered to the pile.

Figure 5.—Operator top taring beet before second weighing.

The samples go into a slotted skip which moves through a pair of circular saws. These saws, 30 inches in diameter with 1 inch teeth, give a rather coarse, dry pulp that does not stick to handling equipment. The pulp is mixed by a pair of rotating rubber blades which also transfer it from one belt to a second. The mixed brei is then transferred to a third belt that is approxi-

mately 2 in wide. This 2 in belt then carries the brei to a tared metal cup on a proportioning balance. The balances are set up to take 80+- 10 gm of pulp and dispense proportionately 3x P[u]lp minus 10 gm of water. The water is dumped into the pulp cup which is then placed on the Weimix table.

This table consists of a circular set of eight individual mixers each with a four-bladed cutter rotating at 12,000 rpm. The table rotates at approximately 1/4 rpm. A sample cup is placed on each mixer as it passes the scale operator. As the cup moves around the table it activates a switch which turns the mixer on, and 2.5 minutes later a switch which turns the mixer off. Just as the mixer is turned off a 10 ml aliquot of 51 brix (Sp.Gr. 1.250) lead acetate solution is added and mixed by the cutter as it coasts to a stop. The balance operator then removes the cup and places it on a lower section of the table where it moves to the second operator at the filter station.

Figure 6.—Operator weighing sample for sugar analysis. (Weibull proportioning balance right; Weimix table, center; Lead acetate delivery apparatus, left).

The nitration and polarization are accomplished by means of a rather clever and elaborate system of solenoid valves and time switches. The sample cup is removed from the lower section of the Weimix table and placed under a filter apparatus. This consists of a frame with goose necks for three samples in sequence. The filter paper is a plug of compressed tissue paper which is placed in a thimble at the end of the gooseneck. This filter is lowered into the sample cup and an activating button pressed. The sequence stepping system then takes over. Suction is first applied for approximately 70 seconds. The sample is drawn into a chamber equipped with a float valve which stops flow when full. The lower portion of this chamber is isolated from the

upper in order to hold the initial filtrate which might be cloudy. At the end of 70 sec the valves reverse and air pressure is applied to blow the contents of the upper portion of the receiving chamber into a funnel from which it flows to the polarimeter

Figure 7.—Operator filtering and polarizing sample.

chamber. The lower valves then switch and the remainder of the sample and the filter are blown back into a sample cup. The cup is dumped and placed upside down on the lower section of the Weimix table where it moves through a washer and hot air dryed and is cooled to room temperature with cold air. The cup is now back to the balance operator where it can be removed and used again.

The sample, meanwhile, flows into the cell of the Bendix Automatic Polarimeter coupled to digital printing system. The identifying card which accompanied the sample through the Weimix equipment is placed in the printer and the sugar content recorded. The printed cards are collected and taken to the data handling centers where the dirty and clean weights, sugar content and identification information are transmitted to the computer in Salt Lake City. The average tare and sugar content for each grower are calculated and sent back to the corresponding receiving station by way of the respective factory offices.

Pan Scale

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The term "pan scale," as commonly used for non-sugar particles originating in the vacuum pan and showing up in sediment tests, may be divided into two main categories: 1) Inorganic scale which most often consists of oxides of metals and formed through corrosion of the pan parts and loosened through abrasion by the sugar. 2) Inorganic or organic scale formed by chemical deposition in the pan.

The first category may be largely eliminated by the use of corrosion resistant metals and the use of cleaning fluids or compounds with low corrosive action. It is not necessary to pursue the cause and prevention of this type of scale as the problem is a familiar one.

The second category is still one where causes are not fully known.

The following discussion describes a scaling problem which has been experienced at Spreckels factories and probably at the factories of other sugar companies.

In the late fall of 1959 and spring of 1960 a frequent occurrence of a white scale in sediment tests of sugars at the Woodland factory was noticed. Examination of the sugars at the Spreckels and Manteca factories did not show the presence of this type of scale. The scale was not visible on a white sediment pad, except for careful examination under magnification, but could be readily seen on a black pad due to the size of the thin particles.

The substance had some resemblance to tiny soap flakes having a waxy appearance with the thinnest scales being nearly transparent. Generally the flakes would show laminations suggesting that they were formed intermittently over a period of time. The flakes were readily soluble in dilute hydrochloric acid leaving a slight residue. They would burn leaving a white ash and were insoluble in organic solvents. They were slowly and partly soluble in boiling water. The inorganic constituents varied from time to time, except for calcium which remained at 14 to 16% in analyses of a number of samples.

Other elements would be present in varying quantities, such as silica from 1 to 17% as SiO_2, iron and aluminum from 1 to

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5%, magnesium from 0 to 1%. Analyses of a number of samples showed varying organic constituents. At this point the compound could be broadly classified as principally a calcium salt of one or more organic acids.

The white scale appeared later in the Spreckels and Manteca factories. However, by this time certain preventative measures had been adopted so that the presence of this type of scale was greatly minimized.

The rather sudden appearance of the white scale prompted us to investigate the possible causes. Changes in beet supply or factory processes were considered.

We came to the conclusion that there had not been drastic changes in such factors as climatic conditions, beet varieties, fertilization, retention time between harvesting and processing, etc. A gradual change from hand harvesting to mechanized harvesting was taking place resulting in an increase in tops and tails.

During the years since the first appearance of the white scale, the factory personnel have observed that a higher incidence occurs at the end of the Fall campaign and the beginning of the Spring campaign. The scale occurred in the "straight" house as well as in the factories using the Steffen process. The formation in the pans was increased with higher pH of the charge.

While a major change in the factory processing had occurred, namely, that of activated carbon treatment of the thick juice, we had occasion to test sugars which were manufactured prior to the installation, and found the presence of the white scale which had formerly not been noticed.

Other steps in factory processing were investigated and no other possible causes for the formation of the scale in the pans were found.

Due to the scale being principally a calcium salt, it is reasonable to assume that by keeping the lime salts low the scale formation would be minimized. When higher lime salts occurred, even though the alkalinity of second carbonation was kept as close as possible to the optimum, we did not find a correlation between higher lime salts and increase in formation of the white scale.

In a number of analyses by wet methods indications were found of the presence of more than one organic acid. One tentatively identified was malic acid. It was also indicated that the organic constituents were probably present in varying amounts at different times.

When the scale first appeared, analytical equipment was not available at the Spreckels laboratories for more precise determinations of the organic constituents. Only recently a sample of scale, which had been removed from the pan tubes during

the time of early appearance of the scale, was analyzed by the Spreckels Research Department.

The analytical scheme consisted of dissolving 0.6 grams of the scale by placing it in water and adjusting the pH to 4 with hydrochloric acid, filtering, and passing the solution first through a cation and then through an anion exchange column. The eluates from the two columns and the neutral effluent were evaporated to dryness and methylated by the method of Gehrke and Stalling. (I)[2] The fraction from the cation column and the neutral fraction were subjected to analyses by gas chromatography. Only small amounts of organic materials were found.

A gas chromatogram of the fraction from the anion column showed two major peaks. (Figure 1) Retention time data indicated that peak No. 1 was dimethyl malate and that peak No. 2 was trimethyl citrate. The identities were confirmed by comparison of their infrared spectra with known standards. (Figure 2 and Figure 3)

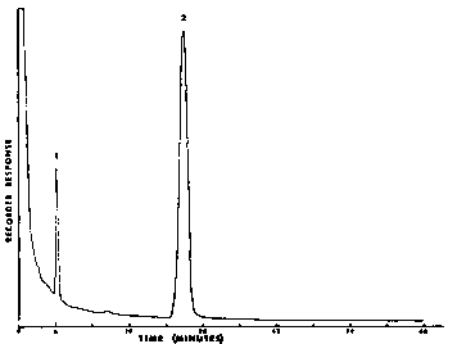

Figure 1.—Chromatogram of the methylated anion exchange column eluate from a sample of pan scale. Column 6' X i/s" O.D. packed with 20% DEGS on acid washed, silanized 60 - 80 mesh Chromosorb W; oven temperature 180° C; gas flow 40 cc/min.

The quantitative composition of this single sample is estimated to be

	% on solids
Inorganic	50
Citric acid	26
Malic acid	5

[a] Numbers in parentheses refer to literature cited.

Oxalic acid)	
Lactic acid)	2
Glycolic acid)	
Succinic acid)	
Not identified	17

It has been found that the natural organic acids present in sugarbeets are largely precipitated and eliminated during carbonation. However, the initial quantity, solubility and possible change in chemical composition may be factors in the amount passing through and precipitating when higher concentrations are reached in the pans.

While we have not found the initial cause or the conditions favoring the carry-through of the organic constituents to the pans, we have adopted certain measures which have greatly minimized the problem. These are: 1) Careful attention to minimizing soluble lime salts by keeping second carbonation alkalinity at the optimum. 2) The use of soda ash when the

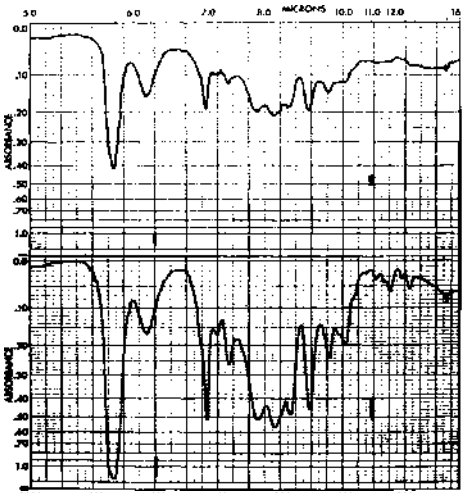

Figure 2.—Top, infrared spectrum of peak No. 1, Fig. 1, trapped on powdered KBr, 13 mm pellet. Bottom, infrared spectrum of 0.4μl dimethyl malate, 13 mm KBr pellet.

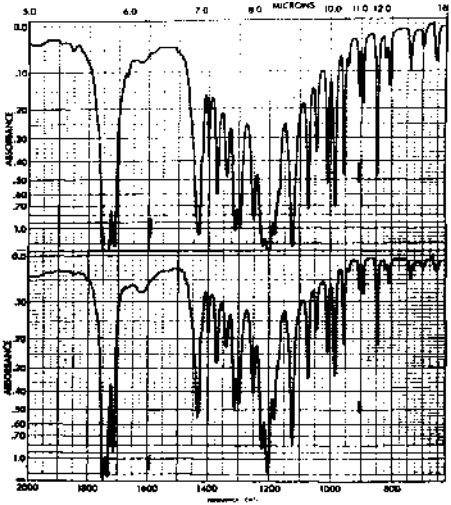

Figure 3.—Top, infrared spectrum of peak No. 2, Fig. 1, trapped on powdered KBr, 13 mm pellet. Bottom, infrared spectrum of 0.5μl trimethyl citrate, 13 mm KBr pellet.

Figure 3.—Top, infrared spectrum of peak No. 2, Fig. 1, trapped on powdered KBr, 13 mm pellet. Bottom, infrared spectrum of 0.5^.1 trimethyl citrate, 13 mm KBr pellet.

first measure is not fully satisfactory. 3) Boil-out of the pan when any sign of the scale shows up on sediment pads, which can be recognized by an experienced analyst when fractions of parts per million are present. For boil-out it is only necessary to use very slightly acidified water. Versene was found to be effective but more costly.

Summary

A white scale which has been occasionally troublesome in pans at the Spreckels factories was identified as calcium salts of organic acids such as citric, malic, oxalic, lactic, glycolic, succinic and possibly others.

The conditions causing formation of the scale in the pans were not established. Measures for preventing or greatly minimizing its presence were adopted. Such measures consist of controlling carbonation at optimum alkalinity, the use of soda ash when necessary, and boil-outs of the pans as necessary.

Acknowledgment

We appreciate and acknowledge the assistance of Gerda Madsen and T. S. Morrill, organic chemist and research assistant, respectively, of the Spreckels Research Department, in identifying the organic constituents of a sample of white pan scale.

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Selecting Sugarbeet for Yellows Resistance on the Relative Concentration of Three Amino Acids in Leaves of Infected Plants

J. M. FIFE[1]

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The beet yellows disease is capable of causing severe losses to the sugarbeet crop in Europe and in certain areas in the United States. In Europe, losses in yield of from 35 to 50%, and as high as 61%, have been reported (4,8,9)[2]. In areas in California, losses in yield ranged from 2.0 to 47.0%, with a reduction in the percentage sucrose ranging from 0.1 to 3.1 percentage points (1.2).

McFarlane and Bennett (10) reported that neither stunting, nor yellowing, nor necrosis of the tops will serve as a reliable selection criterion. In their field program greatest emphasis was, therefore, placed on making successive selections based primarily on superior root size. Superior yields of beets showed their third and fourth successive selections to be significantly more resistant to beet yellows than the parent variety, US 75. Percentage sucrose in the roots of these selections was similar to that in the parent variety, indicating that some criterion for resistance other than root size is needed if the percentage sucrose is to be improved.

Probably, the amino acids are involved in the most important biochemical changes that take place in the leaves of beet plants infected with beet yellows or with beet western yellows (5,6). Tests have shown that the concentrations of free aspartic acid and glutamic acid are frequently reduced as much as 70%, while in the same leaf, the concentration of glutamine sometimes was more than double that found in healthy control leaves. The amino acid ratio $\frac{\text{(concentration: aspartic acid + glutamic acid)}}{\text{glutamine}}$ varied from 0.34 to 0.67 in newly matured leaves of beet yellows-infected plants, grown in the greenhouse or in the field, as compared to a variation in the ratio of from 1.00 to 3.00 among healthy plants grown in the greenhouse under controlled nutritional conditions.

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[2] Numbers in parentheses refer to literature cited.

The concentrations of aspartic acid, glutamic acid and glutamine are, to a large extent, genetically controlled in the leaves of beet yellows-infected plants (7). Infected plants, selected on the basis of the magnitude of the above amino acid ratio, produced progenies having significantly higher concentrations of aspartic acid and glutamic acid and a significantly lower concentration of glutamine (consequently a higher amino acid ratio) than infected plants of the parent variety, US 75.

This highly significant shift (in the concentrations of these three amino acids in leaves of infected plants of the progenies toward that in healthy leaves) suggested that the magnitude of the amino acid ratio may be a reliable selection criterion for resistance to beet yellows.

This communication summarizes the results of 7 years of field testing of selections which were made on the basis of a combination of the amino acid ratio and root weight. It reports the correlations of the amino acid ratios of the selections with root yield and percentage sucrose.

Methods

The methods used in making the selections have been outlined (7). The selections (or lines) were tested along with the parent variety at Spence Field, Salinas, California, from 1960 through 1966. All agronomic and cultural practices were the same as those used by the plant breeders in testing their varieties. The plot design was either a 6, 7, or an 8 X 8 latin square with two-row plots 45 or 50 feet long. The planting dates were between April 8, and April 30, and with harvest dates between September 25, and October 21, resulting in a short growing season, varying from 160 to 178 days. The plants were inoculated 5 to 8 weeks after emergence with the same virus strain, as that used to inoculate the plant populations from which the selections were made, or with a more virulent strain. An effective spray program was carried out to control insects such as caterpillars and leaf miners. At harvest, two 20-beet samples were taken from each plot for sucrose determinations.

Experimental Results

Performance of Selections Made on the Basis of the Magnitude of the Amino Acid Ratio

Selections having amino acid ratios superior to that of the parent variety showed more tolerance to beet yellows than the parent variety (Table 1). Selection R-6 was tested for 4 years. In 3 of the years, the percentage sucrose and yield of beets was significantly greater ($P = 0.01$ and $P = 0.05$ respectively) than

Table 1.—Summary of five years of testing sugarbeet selections made on the basis of the magnitude of the amino acid ratio in the mature leaves of beet yellows-inoculated plants of variety, US 75.

Selection	Selection basis: Amino acid ratio[1]	Selection basis: Root weight	Amino acid ratio of progeny	Change relative to parent: Sucrose	Change relative to parent: Acre yield: Beets	Change relative to parent: Acre yield: Sugar
				%-Points	Tons	Pounds
US 75	(Parent)		1.20			
R-6	High	$\bar{x} \pm s$	3.50**	+ 1.25**	+ 1.7	+ 868**
DS-7	Low	$\bar{x} \pm s$	0.95*	− 0.28	+ 0.2	− 51
RS-C	High	$> \bar{x} + 2s$	3.00**	+ 0.80**	+ 2.0	+ 777**

[1] Concentration: $\frac{\text{aspartic acid + glutamic acid}}{\text{glutamine}}$

**, * Significantly greater, or less, than the parent at the 1% and 5% levels respectively.

the parent. The fourth test was inoculated late in the season with the BYV. In this test, the percentage sucrose and the yield of beets was greater than the parent but not significantly so. Of the 5 tests involving selection DS-7, the percentage sucrose was lower in 3 tests, in one it was equal to, and in the other test it was higher than that of the parent variety. In no test was the percentage sucrose or the yield significantly different from that of the parent variety. Selection pressure applied for both a high amino acid ratio and a high root weight (selection RS-C) caused only a slight increase in yield over that of selection R-6, but resulted in a significant decrease in the percentage sucrose.

Comparison of First and Second Successive Selections for Resistance to Beet Yellows

Thirteen first selection sibs, of the 17 tested in the field, had amino acid ratios significantly greater than the parent variety. The performance of these 13 selections are compared with the performance of 5 sibs of the second selection cycle and the parent variety. The greatest improvement in tolerance of sibs of the second selection cycle, over the sibs of the first selection cycle, is shown by the significant gain in the percentage sucrose (Table 2). This improvement in percentage sucrose was no doubt due to the increased selection pressure applied for the amino acid ratio in making the second successive selections.

Correlation Between Amino Acid Ratio and Percent Sucrose and Yield

A greenhouse test was conducted in 1959 to determine the amino acid ratios of the 17 first selections that were field tested and the parent variety, as follows. Twenty-five plants of each selection were grown under controlled nutritional conditions and inoculated with the same strain of the virus that was used in

making the selections. The concentrations of the amino acids were determined in a two-leaf sample taken from each plant. The amino acid ratio was calculated for each plant and the mean ratio for each selection computed. In 1961, the test was repeated with 6 sibs of the first selection (tested earlier) and 6 sibs of the second successive selection and the parent variety. Correlation coefficients were computed between the ratio of the amino acid ratios (selection to parent, S/P) and the percent sucrose ratio (S/P) and the yield ratio (S/P) from the field tests for each selection. The correlation coefficients between the amino acid ratio and the percent sucrose ratio are both positive and highly significant (Table 3). The correlation coefficients, between the amino acid ratio and the yield ratio, are positive and significant at the 10% level in the tests involving the 6 sibs of the second selection cycle.

Table 2.—Summary of seven years of field testing first and second selection cycles made on the basis of the magnitude of the amino acid ratio and root weight under severe beet yellows conditions.

Selection	Sel. pressure for A. acid ratio[1]	Sel. pressure for Root wt.	Selections Made	Selections Tested	Field tests	Sucrose % ratio sel./75	Acre yield Beets ratio sel./75	Acre yield Sugar ratio sel./75
			No	No	No	X 100	X 100	X 100
Parent US 75						100	100	100
1st	$>\bar{X}$	$>\bar{X} + 2s$	28	13	47	101.1	111.6	112.8
2nd Suc.	$>\bar{X} + 2s$	$>\bar{X} + 2s$	10	5	17	106.8	116.4	124.0

[1] Concentration: $\frac{\text{aspartic acid + glutamic acid}}{\text{glutamine}}$

Table 3.—Correlation between the ratio of the amino acid ratios, selection to parent, and the percent sucrose ratio, and the yield ratio of selection to parent.

Ratios correlated	Selections tested 1st	Selections tested 2nd Suc.	Selections tested Total	Field tests	Cor. coeff.
Amino acid ratios[1]	No	No	No	No	r
1959-S/P & % Suc. ratio: S/P	17	0	17	56	+ .459**
1961-S/P & % Suc. ratio: S/P	6	6	12	35	+ .501**
1959-S/P & Yield ratio: S/P	17	0	17	56	+ .188
1961-S/P & Yield ratio: S/P	6	6	12	55	+ .257*

[1] Ratio of amino acid ratios (concentration: $\frac{\text{aspartic acid + glutamic acid}}{\text{glutamine}}$) of selection to parent.

**, *Significant at the 1% and 10% levels respectively.

Summary and Conclusions

Seven years of field testing first and second successive selections, made on the basis of the magnitude of the amino acid ratio and root weight, has shown a progressive increase in resistance to beet yellows over that of the parent variety, US 75.

The second successive selections were significantly more resistant to beet yellows than the parent variety as shown by superior yields and also by a highly significant increase in the percentage sucrose.

The correlation between the amino acid ratio of the yellows-resistant selections and the percentage sucrose is positive and highly significant.

The correlation between the amino acid ratio and yield of beets is positive and significant at the 10% level in tests involving the second successive selections.

The superior performance of the selections over that of the parent variety, for a short growing season (160 to 180 days), suggests that early maturing varieties may be developed by selecting plants on the basis of the amino acid ratio.

Rapid progress may be made in breeding for resistance to beet yellows, and possibly to beet western yellows, by selecting plants having both a superior amino acid ratio and a superior root weight from populations (grown in the greenhouse) infected with a virulent strain of the virus.

Acknowledgments

I am indebted to Dr. C. W. Bennett for his helpful suggestions during this work, for supplying the aphids, and for his assistance in the inoculation of the plants.

I am also indebted to Dr. J. S McFarlane and Mr. I. O. Skoyen for supervising the plot work and for their assistance in the inoculation of the plants and in the harvest.

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Operation of the Hamilton City Experimental Ion Exchange Plant

S. E. BICHSEL AND D. A. HOOVER[1]

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Introduction

The ion exchange process was used by the American Beet Sugar Industry to deionize process juices and liquors from 1941 to 1949. A total of six ion exchange plants were described by Dickenson (4)[2]. All of these plants terminated operations, after several years, due to adverse operating economics. Various reasons were given for the plant failures. Chief among these were: short resin life, high regenerant costs, uneconomical white sugar to molasses price ratios, inadequate equipment design and general lack of basic knowledge concerning ion exchange.

In the past fifteen years the state of the art has improved significantly. Progress has been made in decreasing ion exchange resin sensitivity to oxidation (1, 6), and irreversible organic fouling (2). Physical and chemical stability of the more expensive weak anion exchangers has been enhanced by the development of the epoxy-amine and macroporous polystyrene resin matrix. Improvements in ion exchange basic hardware and design concept has also been noteworthy. Fixed bed systems (10) and continuous contactors (3) have been modified in design for greater efficiency. The beet sugar technologist has contributed pertinent basic knowledge concerning non-sugar constituents in ion exchange influent and effluent streams (8).

On the basis of this general improvement in the state of the art, coupled with increased practical knowledge of ion exchange operations (9), the Holly Sugar Corporation installed a commercial size fixed bed ion exchange system at their Hamilton City, California plant. This plant went on stream during the spring campaign of 1967. Plant operation during the spring and fall campaigns of 1967 proceeded with only minor operating problems.

Plant Description

The ion exchange plant is fed with a 30 brix 80 purity mixture of No. 2 green and thin juice. Plant capacity is approximately 20 to 24 tons of non-sugars eliminated per day. Under normal circumstances 50% to 60% of the non-sugars entering

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[2] Numbers in parentheses refer to literature cited.

the sugar end are eliminated. White sugar production increases as a direct function of non-sugar elimination, while amount of molasses produced is reduced accordingly.

The plant consists of three cation columns operating in service, regeneration and stand-by sequence. Cation regeneration is two stage in execution. The initial cation stripping is accomplished by counterflow of anion ammonium salt waste regenerant solution from the storage tank through the cation resin bed. After counterflow rinsing of the ammonia stripping from the cation, once-used acid, followed by 10% fresh sulfuric acid and rinsing, completes the cation resin regeneration.

The three anion cells operate independently of the cation cells in a merry-go-round manner. At all times two anion cells are in service, one in a primary position, and the other in a secondary position, with respect to juice flow. When the effluent pH of the anion cell in the secondary position indicates leakage, (pH 7.0), this partially exhausted cell is rotated to the primary position where total utilization of anion resin hydroxyl sites takes place. The secondary position is occupied by a freshly regenerated anion cell. The exhausted primary cell is sweetened off in preparation for regeneration with 6% aqua ammonia. Periodically the regular anion ammonia regeneration is combined with a 5% salt strip to eliminate residual color-body build-up within the anion resin matrix.

Effluent juice from the secondary anion is diverted automatically by a set point transmitter to either the sweet water or treated juice hold tanks. Figure 1 indicates the general plant flow. The juice from the sweet water and treated juice tanks is pumped through plate-type heat exchangers counterflow to hot incoming juice. Low brix sweet water is diverted to the evaporator supply tank, while higher brix treated juice is pumped to the high raw melter. By using counterflow heat exchangers, the hot incoming juice is cooled before refrigeration, and the cold juice returning to the factory is heated.

Decationized water for anion rinse and regeneration requirements is supplied from two small automatic cation columns. Decationized water supply from the large cation service cells is optional. Decationized water, 23% aqua ammonia, and 66 Be° sulfuric acid are supplied from near-by storage tanks. A water cooled anhydrous ammonia converter supplies the system with necessary 23% aqua ammonia for weak anion resin regeneration. The 23% aqua ammonia is diluted to 6% aqua ammonia just prior to weak anion regeneration. System material balance is accomplished by temperature-corrected volume meters paired with continuous samplers on all sugar bearing lines into and out of the system.

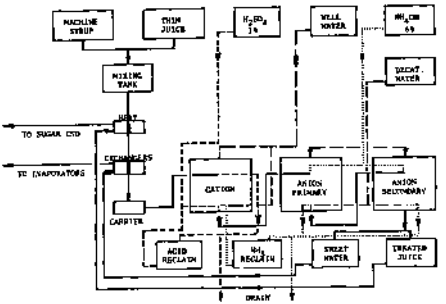


Figure 1.—Hamilton City experimental ion exchange plant.

All valve sequence operations and regenerations are automatic. Any operating sequence may be changed by a simple timer adjustment at the central control panel. In case of automation failure, column valve sequences may be operated from small individual switch panels located in front of each cell.

Operating Results

The following results were obtained from the Hamilton City ion exchange system under steady state operating conditions. Ion exchange feed varied from 30.0 to 29.5 brix while feed purity varied from 79.2 to 81.6 apparent purity. With reference to operation economics, the four most important operating results and the variables affecting these results will be examined in this paper:

1. Sugar losses.
   a. Physical
   b. Chemical (inversion)
2. Dilution.
   a. Sweet on
   b. Sweet off
3. Cation regenerant utilization.
4. Non-sugar elimination.

Referring to Figure 2, cation sweet off percent sugar ineffluent vs bed volumes of cation effluent, it is evident that the idealized plug displacement of sugar liquor is not in effect. McGarvey (7), in an excellent discussion, outlines the variables which affect the displacement dilution of sugar liquors. In practice, the cut-off point in sweet off and sweet on must be determined in each factory by comparing the value of sugar recovered with the cost of additional water evaporated.

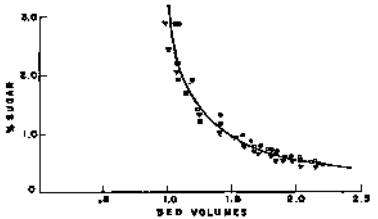


Figure 2.—Typical sweeten-off elution curve, % sugar in ion exchange column effluent vs. bed volumes of sweet-off water.

Figure 3 indicates a typical cation sweet on percent sugar in effluent vs effluent bed volumes. In sweet on, idealized plug displacement of column water is approached. Apparently, diffusion (5) of sucrose into the resin proceeds at a more rapid rate than the reverse situation in sweet off. The pronounced tailing effect in sweet off (Figure 2) is primarily due to slow diffusion of sucrose from the inner labyrinth-like channels of the resin bead. Measurements indicate that 90% of the total ion exchange dilution load is due to cation and anion sweeten off, while cation and anion sweeten on contribute only 10%.

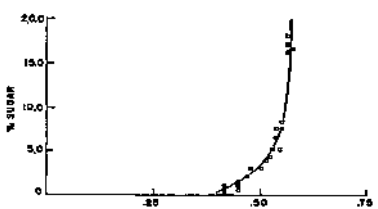


Figure 3.—Typical sweeten-on elution curve, % sugar in ion exchange column effluent vs. bed volumes on feed juice.

Figure 4 shows the graphic relationship between percent sugar in column effluent at cut-off point, and percent dilution calculated as tons-water in ion exchange system effluent divided by the tons of water in the ion exchange influent multiplied by 100. These two variables are related to percent total treated physical sugar loss. Both cation and anion sweet on and sweet off cycles are included in this graphic relationship. The graph, as such, gives the entire dilution, physical loss and effluent percent sugar cut-off relationship for the ion exchange system. As

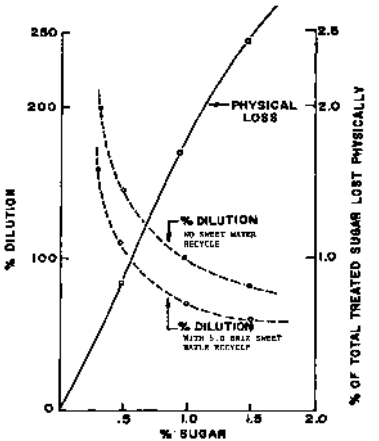


Figure 4.—Graphic relationship between the dependent variables of % dilution, % sugar in column effluent and % of total treated sugar lost physically.

an example, assume anion and cation cut-off points at 1.0% sugar in effluent; then a perpendicular line drawn from 1.0% sugar on the abscissa intersects the physical loss curve and the dilution curves at ordinates of 1.8% loss and, 100% and 65%, dilution respectively. The lower dilution curve assumes the judicious use of 5.0 brix sweet water recycle during the initial part of the cation and anion sweeten off cycle. The 5.0 brix sweet water is reclaimed from the latter part of the sweeten off cycle. In this way, total dilution may be substantially reduced.

Figure 5 portrays the relationship temperature and remaining cation capacity have upon total inversion loss in the cation exchanger (7, 8, 11). Contact time during these runs was 13.5 minutes as calculated from cation resin void volume. The magnitude of hetrogeneous catalysis is indicated by assuming that homogeneous catalysis is a fixed value represented by the flat part of the curve at 3.5 bed volumes. At this point column effluent pH of 1.3 has not varied significantly from the first bed volume effluent pH. However, total volume of regenerated cation resin has decreased from 80% to 20%. It is apparent that the decrease in invert formation is by and large due to the decrease in hetrogeneous catalysis.

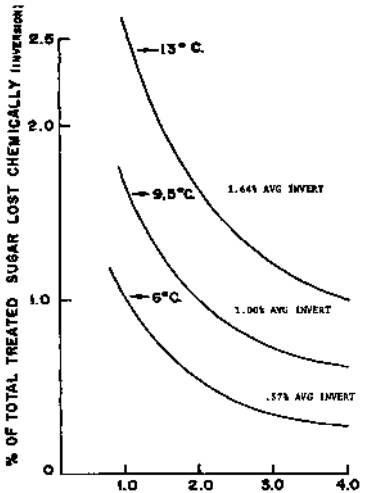
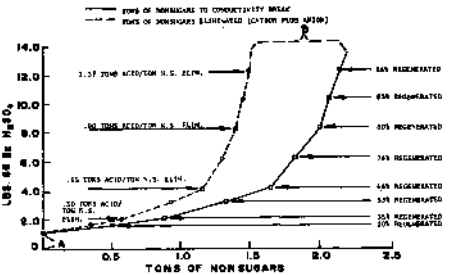


Figure 5.—Percent of total sugar lost through chemical inversion in cation columns vs. bed volumes of cation treated juice effluent.

Figure 6 compares cation column regeneration level (7), expressed as pounds 66 Be° sulfuric acid per cubic foot plus once used acid from the succeeding regeneration, with non-sugar loading to cation conductivity breakthrough capacity and actual cation plus anion total non-sugar elimination. The difference between the two curves (B) indicates the relationship between cation non-sugar capacity to conductivity break and total ion exchange system non-sugar elimination. In this case, 70% of the cation non-sugar breakthrough capacity was eliminated by the total ion exchange system. Intersection of the capacity curves at point (A) with the regeneration level ordinate indicates the minimum amount of total cation regenerated volume at which instantaneous conductivity breakthrough occurs. In this instance the regenerated volume corresponded to a cation resin depth of one foot.

The economic fallacy of total cation resin regeneration is much in evidence in Figure 6. Fresh acid usage far exceeding economical acid utilization would be necessary to obtain total resin regeneration. At the other extreme, lower acid regeneration rates severely limit column capacity as well as equipment



Fgure 6.—Graphic relationship between the dependent variables of lbs 66 Be° H_2S0_4/cu ft cation resin regeneration level and tons of non-sugars eliminated.

investment payout. The best compromise seems to be regeneration operation between 6.0 and 7.0 lbs of fresh 60 Be° sulfuric acid per cubic foot plus once used acid. At these points the ratio of fresh acid used to non-sugars eliminated is reasonable.

Weak anion exchangers pose few regeneration problems; 110% of theoretical equivalent requirements are usually adequate for complete regeneration.

Table 1 shows the non-sugar elimination obtained during three normal 8 hour operation periods. Unfortunately, due to the extremely poor quality of the beets processed during the 1967 fall campaign, this data may not be representative of normal northern California operations.

Table 1.—Ion exchange nonsugar elimination.

	Influent			Effluent			Percent nonsugars eliminated		
Run[1]	1	2	3	1	2	3	1	2	3
Purity A.P.	81.6	80.8	81.0	95.1	94.0	93.5	----	----	----
N.S./100 Sugar	22.54	23.76	23.38	5.15	6.38	6.85	77.2	73.2	70.8
Organic (N)/100 sugar	1.27	1.38	1.17	.29	.30	.29	77.2	78.2	75.2
Total amino (N)/100 sugar[2]	.51	.39	.24	.06	.08	.06	80.7	79.5	75.0
Betaine (N)/100 sugar	.36	.36	.39	.15	.16	.14	58.3	55.6	64.1
Unk. organic (N)/100 sugar	.60	.63	.54	.08	.06	.09	86.7	90.5	83.3
Calcium/100 sugar	.08	.08	.09	0	0	0	100.0	100.0	100.0
Sodium/100 sugar	.97	1.00	.89	.05	.04	.03	94.9	96.0	96.6
Potassium/100 sugar	1.49	1.55	1.59	.03	.04	.05	98.0	97.4	98.1
Chloride/100 sugar	1.01	.95	.90	.01	.01	.01	99.0	99.0	99.0
Color index[3]	2523	2420	2348	51	49	64	98	98	97
pH	8.9	8.7	8.8	7.2	7.1	7.3	—	—	----

[1] Eight hour composite.
[2] Includes amino acid(N) plus P.C.A. (N).
[3] Adjusted to ICUMSA tentative method No. 3.

Summary

Operating results pertaining to dilution, physical and chemical sucrose loss, regenerant utilization, and total non-sugar elimination have been presented. Emphasis was placed on variables affecting the operating results reported.

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Phosphorus Nutrition of Sugarbeet Seedlings

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Introduction

Sugarbeet plants in the field frequently require phosphorus fertilization for satisfactory growth (13)[2]. The need for phosphorus by sugarbeet plants can be determined by means of plant analysis, using the soluble phosphorus concentration of 750 ppm in the petioles of recently matured leaves as the reference value indicating a phosphorus deficiency. This value has been found to reflect the P status of the plant quite accurately from the time of thinning to harvest (17). Recently it has been necessary to estimate the P status of sugarbeet seedlings when the petioles are too small for convenient sampling, as at the cotyledon stage of development (13). To solve this problem a study was conducted with sugar beet seedlings in the greenhouse by the culture solution technique, to find, if possible, a more convenient part of the seedling to sample and to relate these results to growth and mineral composition of the plant tissue analyzed.

Materials and Methods

Plant culture

Sugarbeet seedlings were grown in the greenhouse in half-strength Hoagland's nutrient solution, prepared without P, and modified to include Na and C1 (Table 1). Phosphorus was added as KH_2PO_4 for the P treatments in the amounts of 0.00, 1.25, 2.50, 5.00, 10.00, 20.00, 40.00 and 80.00 mg P per 6 plants, i.e., per 20 liters of solution. Aeration of all solutions was started 5 days after transplanting. The pH values of the solutions were adjusted to 5.3-5.7 with either 1.0 N H_2SO_4 or 1.0 N NaOH. This was done initially after adding the salts, and thereafter daily as required. The tanks were painted on the outside with aluminum and on the inside with a non-toxic plastic (Amercoat No. 33). Masonite covers for the tanks were varnished with valspar on the underside and with aluminum on the upper side. Six holes, about equally spaced, served to hold six sugarbeet seedlings in each cover.

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[2] Numbers in parentheses refer to literature cited.

Table 1.—Chemical composition of nutrient solution when sugarbeet seedlings were transplanted*.

Salts added in mmol/l		Microelements in mg/l	
KNO_3	3.00	B, as H_3BO_3	0.250
K_2SO_4	0.50	Mn, as $MnSO_4 \cdot H_2O$	0.250
$Ca.(NO_3)_2 \cdot 4H_2O$	2.50	Zn, as $ZnSO_4 \cdot 7H_2O$	0.025
$MgSO_4 \cdot 7H_2O$	1.00	Cu, as $CuSO_4 \cdot 5H_2O$	0.010
$CaCl_2 \cdot 2H_2O$	0.25	Mo, as MoO_3	0.005
NaCl	0.50	Fe, as E•D•T•A(8)	2.500

* The eight P treatments are given in the text.

Sugarbeet seeds *(Beta vulgaris),* var. F58 554 H1, i.e., F_1 hybrid, were planted on May 1, 1967. They were treated with Phygon XL at the 1% rate and planted about 2 cm deep in vermiculite held in Amercoated metal germinating trays. The seeds and then the small seedlings were watered daily with one tenth strength modified Hoagland's nutrient solution, without phosphorus addition.

The seedlings appeared on May 5, 1967 and were transplanted May 15, 1967, when they were in the cotyledon stage. The individual plants were supported inside a cork ring by non-absorbent dacron fiber. The outside and inside diameters of the ring were approximately 6 and 4 cm, respectively, and 2 cm in thickness. Six of the seedlings were taken at random for each 20-liter capacity tank. The eight phosphorus treatments were replicated ten times and were arranged in the greenhouse in a randomized complete block design.

Harvesting

The plants were harvested on May 26, only 11 days after transplanting. The plants at harvest showed a gradation of deficiency symptoms from severe to none.

Twelve plants from two pots of the same treatment and adjacent blocks were combined, in order to have enough plant material for chemical analysis. The tops were cut from the fibrous roots, at lateral root initiation, weighed and separated into a) cotyledons, b) first pair of leaves (older), c) second pair of leaves (younger) and d) hypocotyl. The leaves were separated into blades and petioles. The blades of the younger leaves were designated as YB, and their petioles as YP; the blades of the older leaves as OB, and their petioles OP; the cotyledons as Cot, the fibrous roots as FR, and the hypocotyl as Hypocot, as used in the tables and concentration comparisons.

Each kind of plant material was weighed and dried separately. The fibrous roots were treated as outlined below.

Preparation of samples

As soon as the plants were separated and weighed, as described above, they were placed into paper bags and dried at 70° to 80° C in a forced-draft oven to constant weight; dry weights were then taken. The fibrous roots were washed with distilled water, centrifuged for 5 minutes at 39 X *g*, weighed fresh and after drying.

The other plant parts were not washed, because the air of the greenhouse was carbon filtered free of dust and the plants had not been sprayed or dusted with fungicides or insecticides.

All dried plant material was ground in a Wiley mill, equipped with a 40-mesh stainless steel sieve and steel cutting blades. The small amount of plant material from the low P treatments was ground in an oscillatory ball mill made of plastic (Wig-L-Bug amalgamator, Crescent Dental Mfg. Co., Chicago, Illinois). The ground plant samples were stored in plastic containers until analyzed.

Chemical analysis of plant material

The dry ground plant material was analyzed for: a) Soluble Phosphate in 2% acetic acid, and for total phosphorus by the ammonium-molybdate-stannous-chloride method (9); b) Nitrate-nitrogen by the phenoldisulfonic acid method, after removing chlorine (9); c) Potassium and sodium by the flame emission technique (9), using a Beckman model D.U. spectrophotometer with flame attachment in conjunction with a photovolt model 520 photomultiplier unit and d) Calcium and magnesium by the atomic absorption spectroscopy technique (2), using the Perkin-Elmer instrument, model 303. The digestate for the total P determination was also used for the K, Na, Ca and Mg analyses.

Results and Discussion

Visual symptoms of phosphorus deficiency

Plants deficient in phosphorus were smaller in size. The leaves and cotyledons were shorter in length and narower in width and had a characteristic dark green color. As the deficiency became more intense the leaves and cotyledons developed golden areas, that became necrotic. The fibrous roots of the healthy plants were almost white, whereas those of deficient plants changed in color from a brown to dark brown and then to a dark gray. As a rule, the stunting and reduced growth of phosphorus deficient plants was easily recognized by making direct comparisons to adjacent non-deficient plants.

Effect of phosphorus supply on seedling dry weight and dry matter percentage

The degree of deficiency, the mean dry weight of various plant parts, total tops, fibrous roots and the dry weight ratio of tops to roots of sugarbeet seedlings in relation to phosphorus treatment are given in Table 2. An increase in P supply affected the growth of the older blade tissue most and the cotyledons least. The tops were influenced more by phosphorus supply than the fibrous roots.

A statistical analysis of the dry weight of the tops for two adjacent treatments indicated that all P additions from 0 through 10 mg P/6 plants produced significant increases over the preceding treatment. An increase in P supply from 0 to 80 mg P/6 plants increased the dry weight of the older blades by as much as 15 times but the cotyledons only 2 times and the dry weight of the tops 11 times and the fibrous roots only 4 times.

The growth of the plant parts, younger blades, older blades, younger petioles and older petioles, increased significantly with increased P supply until 10 mg P/6 plants had been supplied. The growth of the cotyledons, hypocotl and fibrous roots stopped at the 5 mg P/6 plants supply.

Tops, relative to fibrous roots, increased from a ratio of 1.36 to 3.94 as the plants changed from a state of phosphorus deficiency to non-deficiency. These data indicate that with a limited P supply most of the phosphorus was retained by the fibrous roots resulting in the growth of the fibrous roots at the expense of the tops.

The percentage dry matter of sugarbeet seedlings decreased significantly as the phosphorus supply increased (Table 3) and the seedlings were no longer deficient in phosphorus. The values decreased up to 5 mg P supply/6 plants for the cotyledons and up to 10 mg P supply for the older blade, older petiole, hypocotyl and fibrous root tissues. Thereafter these percentages did not change significantly.

Effects of P supply on distribution and accumulation of P in sugarbeet seedlings

The soluble phosphorus in 2% acetic acid was the highest in the older blade and cotyledon tissues and least in the younger petioles, older petioles and hypocotyl tissues (Table 4). In terms of percentage change the soluble P concentrations of the OB^3, Cot^3, YB^3, FR^3, OP^3 and YP^3 tissues at the 20 mg. P supply increased by 229%, 219%, 108%, 65%, 31% and 13% over that of the $hypocot^3$ tissue, respectively.

[3] Symbols used: Y = young; O = Older; B = blade; P = petiole; Cot = cotyledon; Hypocot = hypocotyl; FR = Fibrous root.

Table 2.—Effects of phosphorus supply on deficiency symptoms of tops and fibrous roots and on dry weight of various parts of sugarbeet seedlings.

P supply mg/6 pls	Deficiency symptoms	Dry weight, mg/12 plants*									
		YB**	OB	YP	OP	Cot	Hypocot.	FR	Tops	Total	Top: FR
0.00	severe	..... +	85a	.... +	... +	112a	51a	183a	248a	431	1.36a
1.25	severe	. .. +	165b	.. +	.. +	140b	54a	238a	359b	597	1.51a
2.50	moderate	..... +	356c	... +	37a	160b	94b	373b	660c	1032	1.77a
5.00	slight	244a++	765d	20a	109b	208c	198c	713c	1542d	2505	2.16ab
10.00	none	486b	1044e	48b	158c	226c	240c	809c	2202e	3011	2.72b
20.00	none	583b	1171e	59b	173c	241c	258c	691c	2483e	3174	3.59c
40.00	none	646b	1261e	82b	184c	258c	253c	735c	2683e	3418	3.65c
80.00	none	919b	1310e	88b	190c	258c	274c	721c	2838e	3559	3.94c

* All values are means of five replications, except where shown otherwise in Tables 4-10.

** Symbols used: Y = young; O = older; B = blade; P = petiole; Cot = cotyledon; Hypocot = hypocotyl; FR = fibrous root.

\+ No plant material in these categories.

\+ + Means in a column followed by the same letter are not different at the 1% level of significance.

\+ + + The number of values in the mean is less than five, Tables 4-10.

Table 3.—Effect of phosphorus supply on percentage dry matter of sugarbeet seedling material.

P supply mg/6 pls	Percentage dry matter*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	. . _ +	15.5c	... +	..... +	10.4c	10.4c	14.0b
1.25	..... +	10.2b	. +	+	10.3c	9.6c	13.6b
2.50	... +	9.4b	. +	10.0c	8.3b	10.0c	11.5b
5.00	10.4b++	8.2b	8.7a	7.5b	7.3a	9.8c	9.3b
10.00	9.1ab	7.3ab	7.4a	5.9a	6.8a	9.0ab	7.9ab
20.00	9.0ab	7.1a	7.3a	5.7a	6.9a	8.8a	7.0a
40.00	9.9b	7.6ab	8.0a	5.9a	7.3a	9.4ab	7.2a
80.00	10.1b	7.6ab	8.2a	5.9a	7.2a	9.3ab	6.9a

* For footnotes see Table 2.

Table 4.—Effects of phosphorus supply on 2% acetic acid soluble phosphorus concentration of sugarbeet seedling material.

P supply mg/6 pls	Soluble P concentration in ppm (dry basis)*						
	YB**	OB	YP	OC	Cot	Hypocot	FR
0.00	.....+	590a	.....+	... ...+	240a	190+++	300a
1.25	..... +	1240a	..... +	... ... +	430a	400a	910a
2.50	. ... +	1470ab	..... +	610+++	560ab	500a	1430ab
5.00	2010a++	2110ab	1170+++	940a	1720ab	990a	1900ab
10.00	2960b	4230ab	1880+++	1930b	4970c	2130b	3070abc
20.00	7150c	11280c	3880+++	4490c	10950d	3430b	5670c
40.00	8480c	12660c	4430+++	4960c	11820d	3600bc	7130c
80.00	8060c	11990c	4170	4640c	11620d	3320bc	8240c

* For footnotes see Table 2.

The total phosphorus concentration of the OB and Cot tissues was higher than that of any other plant part, and the least in the OP and hypocot tissues, at the 20 mg P supply (Table 5). In terms of percentage change the total phosphorus concentration of OB, Cot, YB, FR and YP was 140%, 125%, 103%, 29% and 28% higher than that of the hypocot and OP tissues, respectively.

Table 5.—Effects of phosphorus supply on total phosphorus concentration of sugarbeet seedling material.

P supply mg/6 pls	Total P concentration in ppm (dry basis)*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	..... +	720a	..... +	..... +	320a	500+++	770a
1.25	..... +	1690a	..... +	. +	600a	800a	1770a
2.50	..... +	1810ab	... +	1320+++	840ab	1090a	2800ab
5.00	4250a++	2650ab	3000+++	1870a	2420ab	2180b	3340b
10.00	5660a	5380c	4420+++	2820b	5920c	3630c	4450b
20.00	10610b	12510d	6700+++	5090c	11750d	5220d	6710c
40.00	12400b	13380d	7680+++	6000c	12630d	5350d	8280c
80.00	12230b	13520d	7410	5660c	12600d	5410d	9600c

* For footnotes see Table 2.

Numerous experiments have demonstrated (1, 3, 4, 5, 12, 16) that the phosphate ion tends to accumulate in those portions of the plant undergoing the most rapid growth. On the basis of total phosphorus concentration the different plant parts can be classified as follows:

OB > Cot > YB > FR > YP > OP > Hypocot.

But since the Cot had already stopped growing at the time of harvest, this method of classifying tissues in relation to growth appears not to be a valid one.

Relation of tissue phosphorus to growth

The soluble P and the total P concentrations of the 1) OB, 2) OP, 3) Cot and 4) hypocot tissues were plotted against the corresponding fresh and dry weight of the plant part and fresh and dry weight of the tops.

These results in general show that the use of soluble P concentration of the various plant parts is preferable to total P to diagnose the P status of the sugarbeet seedlings, because it is easier to determine this form of P and it reflects the P status of the plant satisfactorily. But if it is necessary to determine cations at the same time, the total P concentration can be used, since this form of P can be determined easily from the digestate of the HNO_3-$HCLO_4$ digestion. The specific plant part to use for chemical analysis is more difficult to decide. If the selection is based on the sharpness of the transition zone, the hypocot and OP tissues would be preferable. But unfortunately the hypocot tissue has the disadvantage of a narrow phosphorus range. On the other hand the curves for the OB and Cot tissues do not have a sharp transition zone but they do have a wide range of P concentrations. Similar results were observed for the total P concentration of the plant parts. So the general conclusion may be drawn that for the determination of the critical P concentration of the sugarbeet seedling it is preferable to analyse the OP tissue. But when it is too difficult to sample it, the OB or the Cot tissues may be used as well.

Plottings of the dry weight of the tops against the soluble P concentration in the 1) OB, 2) OP, and 3) Cot tissues are given in Figures 1, 2 and 3. The figures illustrate how the soluble P concentration in these plant tissues change with P supply and plant growth. The nearly vertical portion of the curve, which includes the treatments from zero to 5 mg P/6 plants, shows a zone of P deficiency. This zone is characterized by increases in top weight, with an increase in P supply. From 10 mg up to 80 mg P/6 plants there is a zone of P adequacy.

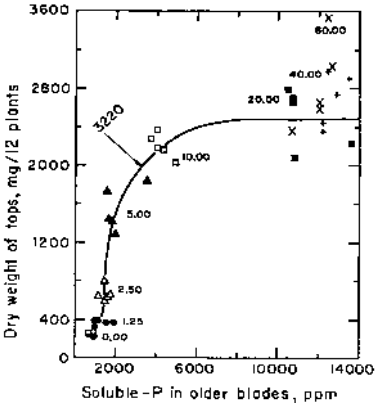

Figure 1.—Relation of dry weight of tops to soluble P concentration in older blade tissue of sugarbeet seedlings. The critical soluble P concentration at a 10% reduction from satisfactory growth, indicated by arrow, is approximately 3220 ppm. The numbers with the accompanying symbols show the mg of P per six plants added as KH_2PO_4.

In this case when the P supply increases the soluble P concentration in the plant tissues increases, but the top weights remain relatively constant. The portion of the curve between these two zones is the zone of transition (18). This zone for the OB, OP, and Cot tissues includes a range from 2110 to 4230, 940 to 1930 and 1720 to 4970 ppm of soluble P, respectively.

The soluble P concentration at a 10% reduction from the satisfactory top growth is approximately 3220 ppm, 1460 ppm and 3320 ppm for the OB, OP, and Cot tissues, respectively (Figures 1, 2, and 3). These values are referred to as the critical soluble P concentration, and can serve as a convenient point of reference in estimating the P status of sugarbeet seedlings. Sugarbeet seedlings containing much less P than their critical concentration at the time of sampling are deficient in P. These plants can be expected to respond favorably to P additions if all other factors are adequate for growth and the period of P deficiency is not too short. Plants with P values within the transition zone might increase slightly in growth, but plants with tissue values above the critical concentration cannot be expected to increase significantly in growth with increased P supply.

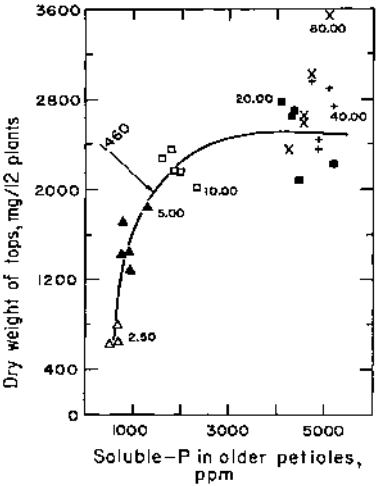

Figure 2.—Relation of dry weight of tops to soluble P concentration in older petiole tissue of sugarbeet seedlings. The critical soluble P concentration at a 10% reduction from satisfactory growth, indicated by arrow, is approximately 1460 ppm. The numbers with the accompanying symbols show the mg of P per six plants added as KH_2PO_4.

The estimated critical soluble P and total P concentration values, at 10% reduction from satisfactory growth, are given in Table 6. An inspection of this table shows that the estimated critical values for phosphorus are almost the same, for a particular plant part and form of phosphorus, whether the fresh or dry weight of the tops or of the plant part are used for plotting the data. The only exception is for the Cot tissue, where the critical values differed appreciably for the plottings of fresh or dry weight of tops or of Cot. This is perhaps due to the fact that at the time of harvest the Cot tissue had stopped growing.

Effects of phosphorus supply on tissue nitrate N concentration

The nitrate N values of the various parts of the sugarbeet seedlings are given in Table 7. As the P supply was increased the values for the OB, YB and YP tissues increased to a maximum. Then they decreased with P addition. In the Cot tissue the nitrate N concentration was relatively low for all P treatments.

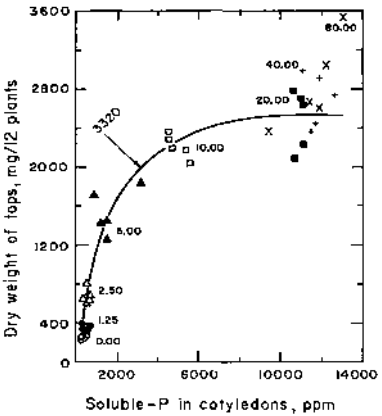

Figure 3.—Relation of dry weight of tops to soluble P concentration in cotyledon tissue of sugar beet seedlings. The critical soluble P concentration at a 10% reduction from satisfactory growth, indicated by arrow, is approximately 3320 ppm. The numbers with the accompanying symbols show the mg of P per six plants added as KH_2PO_4.

The nitrate N values decreased at first with increased P supply and then they followed the same pattern as in the OB tissue.

In the OP and FR tissues the nitrate N concentrations increased to a maximum with P treatment and then did not change significantly with a larger P supply. The values for the hypocot tissue decreased at first with P treatment, as in the Cot tissue, and they followed the pattern of the OP and FR tissues. Apparently phosphate enhances nitrate uptake preferentially. This is also suggested by the low top to FR ratio since the FR, because of small top size, should have the capacity to absorb nitrate to meet top needs.

Effects of phosphorus supply on K, Na, Ca and Mg concentration of seedling parts

The potassium concentration of various plant parts, as a rule, increased as the P supply was increased and after it reached a maximum it did not change significantly with P supply (Table 8). This general increase in K concentration with P supply may indicate that K uptake depends on the uptake of phosphate (7) or nitrate (15), directly or indirectly, or on the increase in K concentration as the P supply, from KH_2PO_4, in the culture

Table 6.—Critical phosphorus concentration values in ppm (dry basis), at 10% reduction from satisfactory growth.

Measurement and plant part used for growth comparison	Soluble P, ppm				Total P, ppm			
	OB*	OP	Cot	Hypocot	OB	OP	Cot	Hypocot
F.W. Tops	3,640	1,460	3,330	1,640	4,250	2,290	4,580	3,070
D.W. Tops	3,220	1,460	3,320	1,640	4,250	2,290	4,370	3,070
F. W. OB	3,220	.....	. .	.. ....	4,160	—..	.....	.....
D.W. OB	3,170	.....	... .	. .....	4,060	....	. .....	..... .
F.W. OP	.. ....	1,590	.....	. ..	....	2,340	.....	.....
D.W. OP	.....	1,460	.....	.....	.....	2,240	.....	. .....
F.W. Cot	....	....	2,180	.....	.....	.. ..	2,960	. ....
D.W. Cot	.....	—.. .	1,560	.....	... ...	.....	2,240	....
F.W. Hypocot	.. ..	.....	.....	1,510	.....	. .....	.. ....	2,860
D.W. Hypocot	. ...	..... .	.... .	1,300	.....	.....	.....	2,650

* Symbols used: O = older; B = blade; P = petiole; Cot — cotyledon; Hypocot = hypocotyl; F.W. = fresh weight; D.W. = dry weight.

Table 7.—Effects of phosphorus supply on nitrate nitrogen concentration of sugarbeet seedling material.

P supply mg/6 pls	Nitrate-nitrogen concentration in ppm (dry basis)*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	..+	340a	.....+	. ....+	2690b	11230+++	1610a
1.25	... +	1160a	.... +	. . +	1970b	9910+++	1910a
2.50	..... +	890a	. . +	5140+++	760a	7770+++	5340ab
5.00	5030b++	1880ab	9670+++	13620a	1370a	7130a	7140b
10.00	7520c	3950c	10340+++	19200b	2810b	9360a	9460b
20.00	4550b	2260ab	13620+++	21580b	2280b	9200a	9450b
40.00	2650b	1500ab	11810+++	20450b	1720ab	8950a	9100b
80.00	2060a	1190a	11600	20850b	1490ab	8840a	9720b

* For footnotes see Table 2.

Table 8.—Effects of phosphorus supply on potassium concentration of sugarbeet seedling material.

P supply mg/6 pls	% K concentration (dry basis)*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	.....+	4.34a	..... +	. ...+	7.9a	9.6+++	3.58a
1.25	...+	7.20b	..+	. ....+	9.0b	11.2d	3.32a
2.50	. ...+	7.17b	. ... +	9.1+++	9.4c	8.8c	3.40a
5.00	5.58a++	8.24b	8.2+++	11.4a	10.9c	7.5b	4.84b
10.00	6.55a	9.11b	9.4+++	13.3b	12.0d	8.0a	5.99b
20.00	7.90ab	8.82b	10.4+++	13.9b	12.1d	8.3a	5.85bc
40.00	7.83ab	7.53b	10.0+++	13.5b	12.3d	8.1a	6.07b
80.00	7.90ab	8.21b	10.1	13.8b	12.1d	8.2a	6.52bc

* For footnotes see Table 2.

solution was increased. It is also to be noted that all K values were relatively high, with values for the OP tissue reaching 13.9% K, dry basis.

All the sodium concentration values, except for the Cot and

Table 9.—Effects of phosphorus supply on sodium concentration of sugarbeet seedling material.

P supply mg/6 pls	% Na concentration (dry basis)*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	.....+	0.50b	... +	. +	1.35b	0.24+++	0.18b
1.25	..... +	0.87bc	. ....+	.... +	1.37b	0.21c	0.25b
2.50	. ....+	1.38c	.....+	0.64+++	1.64b	0.17c	0.36b
5.00	0.38c++	0.85bc	0.10+++	0.26b	1.35b	0.04b	0.09a
10.00	0.20b	0.51b	0.05+++	0.08a	1.15a	0.00	0.09a
20.00	0.12a	0.37a	0.04+++	0.06a	0.96a	0.00	0.08a
40.00	0.14a	0.38a	0.02+++	0.06a	1.04a	0.00	0.11a
80.00	0.11a	0.54a	0.01	0.05a	0.90a	0.00	0.09a

* For footnotes see Table 2.

OB tissues, were very low (Table 9), and these tended to increase and then decrease with P supply. The decreases in Na concentration with P supply may be due to a potassium-sodium competition (15) since, when P was increased, K supply was also increased by about 3.23%. It is also known (6, 10, 11, 14) that the Na concentration in plants increases in case of poor root aeration. But here the greatest Na uptake was not associated with the most root injury, i.e. lowest P supply.

The highest Ca and Mg concentrations were observed in the P deficient plants, with exceptions for the zero P treatment, where the Mg concentrations of the OB and Cot tissues were the smallest (Tables 10 and 11). With adequate P supply the Ca and Mg concentrations within a plant part remained relatively constant. Among the plant parts the photosynthetic tissues, including the Cot, were much higher in Ca and Mg than contiguous conducting tissues. The Ca and Mg values tended to parallel each other except in the FR tissue, where, unexpectedly, the Mg values differed by being very much higher than these of Ca and by decreasing rapidly with P treatment. The Cot tissue were relatively high in Ca and Mg and changed little with P treatment.

Summary and Conclusions

1) Sugarbeet seedlings at the cotyledon stage responded to phosphorus treatment within a few days after transplanting to culture solutions.

2) Deficiency symptoms of leaf blades and cotyledons increased with decreased phosphate concentration of all plant parts.

3) Phosphorus deficiency increased the percentage dry matter of all parts of the seedling.

4) The phosphorus status of sugarbeet seedling can be determined from the soluble or total phosphorus values for petioles

Table 10.—Effects of phosphorus supply on calcium concentration of sugarbeet seedling material.

P supply mg/6 pls	% Ca concentration (dry basis)*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	.... +	1.79b	... +	.....+	1.95b	0.53+++	0.69b
1.25	.....+	3.28c	...+	. . . .+	2.98c	0.57c	0.67b
2.50	.... +	2.26b	... +	2.27+++	2.72c	0.56c	0.56b
5.00	0.86a++	1.46a	0.75+++	1.40b	1.97b	0.34b	0.49ab
10.00	0.81a	1.36a	0.44+++	0.96a	1.90b	0.29b	0.49ab
20.00	0.81a	1.33a	0.46+++	1.01a	1.83b	0.27ab	0.46a
40.00	0.80a	1.35a	0.36+++	0.99a	1.77a	0.26ab	0.54ab
80.00	0.78a	1.30a	0.36	0.96a	1.79a	0.27ab	0.54ab

* For footnotes see Table 2.

Table 11.—Effects of phosphorus supply on magnesium concentration of sugarbeet seedling material.

P supply mg/6 pls	% Mg concentration (dry basis)*						
	YB**	OB	YP	OP	Cot	Hypocot	FR
0.00	.... +	1.03a	..... +	...+	1.27a	0.16+++	5.22b
1.25	... +	1.75c	.....+	.....+	1.78b	0.48c	4.64b
2.50	... +	1.32b	..... +	0.75+++	1.71b	0.37b	3.13b
5.00	0.74a++	1.29b	0.46+++	0.82c	1.60b	0.26a	2.56a
10.00	0.81a	1.35b	0.45+++	0.68b	1.59b	0.26a	2.81a
20.00	0.75a	1.29b	0.37+++	0.56a	1.49ab	0.24a	1.65a
40.00	0.71a	1.24ab	0.35+++	0.55a	1.42a	0.23a	1.80a
80.00	0.66a	1.19a	0.36	0.51a	1.40a	0.24a	1.48a

* For footnotes see Table 2.

of the first pair of leaves formed or for their blades or cotyledons. This cannot be done effectively by analyzing the entire seedling or its tops. The critical soluble phosphorus value is approximately 1460 ppm and 3220 ppm for the older petiole and blade tissues, respectively, and 3320 ppm for the cotyledons, dry basis.

5) Phosphorus deficiency decreased the nitrate nitrogen concentrations in the fibrous roots and older blade tissues. Smaller decreases in nitrate concentration took place in the conducting tissues and cotyledons. Similar decreases in potassium concentration took place in the fibrous roots, cotyledons, petioles and blades. All potassium values were relatively high with values for the older petioles reaching 13.9% potassium, dry basis.

6) Phosphorus deficiency increased the sodium and calcium concentrations in all plant tissues, and that of magnesium in the fibrous roots and hypocotyl.

7) Among the plant parts analyzed the photosynthetic tissues were much higher in calcium and magnesium than contiguous conducting tissues. The calcium and magnesium values tended to parallel each other except in the fibrous roots, where the magnesium values became very much higher than those of cal-

cium. The magnesium values decreased rapidly with phosphorus supply and after they reached a minimum did not change significantly with more phosphorus added.

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Development of Sugarbeet Breeding Lines and Varieties Resistant to Yellows

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Yellowing diseases caused by viruses reduce sugar yields in many sugarbeet growing areas of the world. In the United States losses have been severe in areas where sugarbeets, or other susceptible plants, are present during the entire year (1)[2]. Two yellowing diseases, beet yellows (BY) and beet western yellows (BWY), are common on beet (1,4). The diseases are caused by two distinct viruses, BYV and BWYV, both of which are spread by aphids. In controlled experiments Bennett and McFarlane (2) reported root-yield losses of 24.4%-41.7% for BY and 10.8%-18.2% for BWY. When both diseases were present the yield reductions were additive.

Breeding to find resistance has been underway in Europe since 1948 and in the United States since 1955. Rietberg and Hijner (8) developed selections in which yield reductions did not exceed 14-16%. Russell (9) reported a useful degree of tolerance to BYV and to beet mild yellowing virus (BMYV). McFarlane and Bennett (5) found the third and fourth successive selections from US 75 to be significantly more resistant to BYV than the parent variety.

Experimental Methods

Selections for yellows resistance were made from field plantings at Salinas, California. The rows were 28 inches apart and the beets were thinned to a spacing of 24-30 inches between plants. This wide spacing tended to equalize competition between plants and reduced the danger of selecting large, noncompetitive beets. Inoculations were made with a combination of BYV and BWYV when the plants were about seven weeks old. Virulent strains of the two viruses were used. The inoculation procedure was similar to that described by Bennett, Price and McFarlane (3). Green peach aphids, *Myzus persicae* (Sulz.), were produced in the greenhouse and acquired virus from infected source plants. Leaf pieces containing 5-10 aphids were

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[2] Numbers in parentheses refer to literature cited.

removed from the source plants and placed on plants to be inoculated. The aphids were killed by spraying with an aphicide in 24-48 hours to prevent the spread of virus to nearby beets.

Selections were based on freedom from disease symptoms and root size. Approximately six weeks after inoculation the plants were examined and rogued. Plants with severe symptoms and plants that had escaped infection were removed. Superior plants were staked. Roguing of plants with severe yellows symptoms was repeated once or twice more during the growing season. At harvest, large, well-shaped roots were selected from plants that showed the least number of dead leaves. The selected roots were placed in a cold room (40-45° F) for four months and then transplanted either to greenhouse pots or to greenhouse isolators *(6)* for seed production. Successive selections were made in a similar manner.

Studies by McFarlane and Bennett (5) have shown a low correlation between reduction in root yield and stunting, yellowing or necrosis of tops in plants affected by yellows. Resistance can best be determined from yield comparisons of inoculated and noninoculated plots. To obtain an accurate comparison, the noninoculated plots must be maintained free of infection. Aphid populations remain high in the coastal valleys of California during the entire growing season, and the spread of yellows cannot be prevented even though the plots are sprayed frequently with an aphicide. Summer temperatures are much higher in the Central Valley of California and aphid-vector populations are usually very low during the midsummer months. By delaying planting until the aphids have largely disappeared (usually in May), little difficulty has been experienced in maintaining infection at a low level in the noninoculated plots.

Resistance evaluation tests were made at Davis, California, in cooperation with the University of California. A split plot design was used. The treatments, consisting of a noninoculated check and a combination BYV and BWYV inoculation, were arranged in randomized strips across each of five replications. The variety subplots were two rows wide and 41 feet long. Stand counts were made following thinning and plant populations adjusted so that a similar number of plants remained in the inoculated and noninoculated plots of any given entry in each replication. Inoculations were made with a virulent strain of BYV and a virulent strain of BWYV approximately seven weeks after planting. The beets were harvested when five to six months old.

In 1966 and 1967, tests were also made at Salinas. The tests were planted in December in single-row plots, 50 feet long and, the plots were replicated 10 times. All plants in the test were'

inoculated in April with a combination of BYV and BWYV. The tests were harvested in September. The varieties and selections were also evaluated in adjacent noninoculated tests sprayed with an aphicide. Yellow infection was delayed, but nearly all plants became infected before the end of the growing season. In addition to sucrose percentage and root yield, the amino N, Na, and K content of the roots were determined for these tests.

Hybrids utilizing yellows-resistant selections were also included in U.S. Department of Agriculture and sugar company variety tests located in the major sugarbeet producing areas of California.

Results

Evaluation tests at Davis

Evaluation tests to determine the yellows resistance of selections made at Salinas were grown at Davis, California, between 1963 and 1966. Both self-sterile and self-fertile selections were tested. The tests also included the parental lines from which the selections had been made.

In the 1963 test, the combination of BY and BWY caused root-yield losses ranging from 21.0-49.5% (Table 1). Three selections from US 75 showed significantly less damage from yellows than US 75. The fifth successive selection 413 was more resistant than the fourth successive selection 011. The loss in

Table I.—Reduction in yield of yellows-resistant selections and of unselected sugarbeet lines when inoculated with a combination of BYV and BWYV at Davis, California.

No.	Description	Root yield (T/A) of inoculated beets			Percent yield loss from yellows		
		1963	1964	1966	1963	1964	1966
011	4th YRS US 75	16.7	19.6	....	37.9	26.1	....
413	5th YRS US 75	18.5	22.1	....	30.3	13.9	....
490	5th YRS US 75	17.4	21.7	....	35.9	21.6	....
513	7th YRS US 75	....	....	23.5	....	....	18.2
530	7th YRS US 75	....	....	22.8	....	....	30.1
368	US 75	13.8	17.7	16.6	49.5	37.2	40.0
221	2nd YRS 671	18.5	....	....	29.8	....	....
321	3rd YRS 671	....	18.6	....	....	30.7	....
521	5th YRS 671	....	....	19.7	....	....	26.8
671	Open-pollinated line	14.6	15.8	16.0	45.9	36.8	37.7
337	1st YRS 663	17.0	19.0	....	45.7	39.1	....
537	3rd YRS 663	....	....	20.6	....	....	35.8
663	Open-pollinated line	17.1	18.4	18.8	42.5	38.5	34.3
F62-63T	Tetraploid 663	20.0	23.1	....	32.5	30.3	....
338	1st YRS F57-85	....	15.9	....	....	31.9	....
538	3rd YRS F57-85	....	....	19.6	....	....	26.0
F57-85	Open-pollinated line	....	12.2	12.4	....	40.0	36.4
234	YRS from Netherlands	22.2	22.9	24.7	21.0	18.4	16.3
544	Inc. (430 × 234)	....	....	24.0	....	....	22.1
	LSD (5%)	2.1	1.9	1.9	5.6	6.4	7.1

221, the second successive selection from 671, was significantly lower, by approximately one-third, than the parental line. A selection from 663 failed to show any improvement, but the tetraploid 663 showed significantly less damage than diploid 663. The selection 234, developed by the Instituut voor Rationele Suikerproductie in The Netherlands, was outstanding in this test and showed a significantly lower yield loss than other selections tested.

Sucrose losses from yellows were more variable than root-yield losses and ranged from 0.5-1.9 percentage points (Table 2). The 234 selection showed the lowest loss followed by the US 75 selections.

A similar group of self-sterile selections and parental lines was tested in 1964. Root-yield losses ranged from 13.9-40.0% and sucrose losses ranged from 1.1-1.7 percentage points (Tables 1 and 2). Selection 413 showed about one-third the damage of US 75. However, the results of this test indicate that a reduction in both root yield and sucrose percentage occurred in this selection. A sister selection 430 showed about two-thirds the loss of US 75.

The selections 321 and 337 failed to show a significant improvement in yellows resistance over the parental lines from which they had been selected. The tetraploid of 663 again showed significantly less damage from yellows than diploid 663. Selection

Table 2.—Reduction in sucrose percentage of yellows-resistant selections and of unselected sugarbeet lines when inoculated with a combination of BYV and BWYV at Davis, California.

No.	Description	Percent sucrose of inoculated beets			Percentage points loss in sucrose		
		1963	1964	1966	1963	1964	1965
011	4th YRS US 75	12.3	12.1	...	1.2	1.4	..
413	5th YRS US 75	12.0	11.3	...	1.1	1.1	...
430	5th YRS US 75	12.6	11.6	...	1.2	1.2	...
513	7th YRS US 75	....	....	12.9	....	....	0.8
530	7th YRS US 75	....	....	12.4	....	....	1.1
368	US 75	11.7	11.6	12.5	1.9	1.7	1.6
221	2nd YRS 671	11.9	....	....	1.9	....	....
321	3rd YRS 671	....	11.4	....	....	1.2	....
521	5th YRS 671	...	....	12.3	....	..	1.6
671	Open-pollinated line	12.0	11.9	13.0	1.7	1.3	1.6
337	1st YRS 663	12.0	11.7	....	1.4	1.4	...
537	3rd YRS 663	...	....	11.8	..	....	1.6
663	Open-pollinated line	12.2	12.0	12.7	1.3	1.1	1.4
F82-651	Tetraploid 663	11.8	10.7	...	1.3	1.2	...
838	1st YRS F57-85	....	12.5	..		1.4	..
538	3rd YRS F57-85	....	....	13.0	..	.	1.2
F57-85	Open-pollinated line	.	12.0	13.6	..	1.7	1.0
234	YRS from Netherlands	13.0	12.4	13.8	0.5	1.3	0.9
544	Inc. (430 × 234)	....	..	12.9	....	.	1.1
	LSD (5%)	0.5	0.6	0.6	0.7	NS	NS

338 showed an improvement in both yellows resistance and root yield over the parental variety F57-85. The 234 selection from The Netherlands performed well from the standpoint of yield and sucrose percentage and was similar to the better US 75 selections in yellows resistance.

A group of self-fertile inbred lines was also tested at Davis in 1964. Included were nine inbreds which had been selected for yellows resistance. Nonselected inbreds commonly used as parents in hybrid varieties were also tested. Root-yield losses ranged from 7.6-41.1% among inbred lines selected for yellows resistance and from 32.6-38.4% among unselected inbreds (Table 3). The most resistant inbred, 742, showed only a 7.6% loss from yellows and also had a very satisfactory yield and sucrose percentage.

Table 3.—Reduction in yield and sucrose percentage of sugarbeet inbreds when inoculated with a combination of BYV and BWYV at Davis, California, in 1964.

No.	Description	Performance of inoculated beets		Loss from yellows	
		Root yield	Sucrose	Root yield	Sucrose
		Tons/acre	Percent	Percent	Pct. points
742	YRS (928-9 × NB1)	17.2	12.0	7.6	1.0
754[1]	YRS (671 × 9716-10)	13.2	10.6	13.3	1.0
740	YRS (928-3 × NB1)	15.8	11.5	15.1	1.0
757[1]	YRS (911 × 716-4)	15.3	10.1	17.6	0.9
743	YRS (928-20 × 561-3)	15.0	12.3	20.2	1.0
753[1]	YRS (671 × 716-4)	16.1	10.1	24.1	0.7
747	YRS (928-29 × 577-2)	15.9	11.7	26.2	1.1
768	YRS (926-56 × 716-8)	15.5	11.0	28.6	1.7
763	YRS 583 inbred	12.9	12.3	41.1	0.7
502HO	CMS of NB1	11.8	11.1	38.3	1.7
539	NB7 inbred	12.7	10.1	32.6	0.9
569	Monogerm inbred	10.8	....	38.4	....
	LSD (5%)	2.2	0.7	12.6	NS

[1] Replicated two times, not included in statistical analysis.

In 1966 the combination of BYV and BWYV caused root-yield losses ranging from 16.3-40.0% and sucrose losses ranging from 0.76-1.63 percentage points among open-pollinated varieties and selections (Tables 1 and 2). Line 513, the seventh successive selection from *US* 75, showed less than one-half the loss in both root yield and sucrose percentage as did US 75. Line 530, a selection from 430, yielded better than 513 in the noninoculated plots, but showed higher yield and sucrose losses from yellows. Selections from 671 and F57-85 showed significant improvements in yellows resistance. No improvement was demonstrated in the third successive selection from 663. Selection 234 again showed good performance and resistance equal to that of 513. Selection

544, from a cross between 430 and 234, performed similar to 513.

Root-yield losses among inbred lines tested in 1966 ranged from 15.0-48.7% and sucrose losses from 0.7-2.1 percentage points (Table 4). The 742 inbred which showed good resistance in 1964 again showed the smallest percentage yield loss but produced a low root yield. From the standpoint of both yellows resistance and other desirable characteristics, the most promising inbred was 760, a selection from a cross between a US 75 selection and a self-fertile line. This inbred remained green following inoculation and showed a yield loss of 18.8%. In addition, 760 possesses good curly top and bolting resistance. The susceptible 511 inbred showed the greatest loss from yellows.

Table 4.—Reduction in yield and sucrose percentage of sugarbeet inbreds and of F_1 hybrids when inoculated with a combination of BYV and BWYV at Davis, California, in 1966.

No.	Description	Performance of inoculated beets		Loss from yellows	
		Root yield	Sucrose	Root yield	Sucrose
		Tons/acre	Percent	Percent	Pct. points
Inbreds					
734	YRS (927 × 577)	23.8	12.9	23.5	0.7
760	YRS (911 × 717)	16.0	13.7	18.8	0.9
716	YRS (US 56 × NB1)	15.6	12.9	34.0	1.1
757	YRS (911 × 716)	15.1	11.9	32.2	1.3
768	YRS (926 × 716)	14.9	15.5	33.1	1.5
742	YRS (928-9 × NB1)	13.6	14.5	15.0	0.7
753	YRS (671 × 716-4)	12.1	11.7	30.7	1.8
754	YRS (671 × 716-10)	12.0	12.0	42.2	1.9
511	NB2	10.1	11.9	48.7	2.1
	LSD (5%) for inbreds	1.9	0.9	9.1	1.0
F_1 Hybrids					
716H3	562HO × 716	24.2	13.3	24.8	1.3
760H4	562HO × 760	23.7	13.9	20.5	1.2
753H4	562HO × 753	19.5	13.3	27.2	1.2
754H4	562HO × 754	18.2	12.9	29.4	1.5
569H3	562HO × 569	10.6	15.3	30.4	1.4
	LSD (5%) for hybrids	1.9	0.6	7.6	NS

Root-yield losses among four single-cross hybrids between monogerm male steriles and yellows-resistant inbred pollinators ranged from 20.5-29.4%. The F_1 hybrid 569H3, in which neither parent had been selected for yellows resistance, showed a yield loss of 30.4%. Losses in sucrose percentage ranged from 1.2-1.5 percentage points and the differences between hybrids were not significant. Hybrid 760H4 showed the best performance with a yield loss of 20.5%. The performance of this F_1 hybrid was similar to that of 513, the seventh successive selection from US 75 (Table 1) which was included in an adjacent test.

Performance of yellows resistant selection from US 75

Selection work performed between 1957 and 1961 (5) demonstrated that US 75 was heterozygous for yellows resistance and offered greater opportunities for improvement in resistance than did most other varieties that were tested. Successive selections were made from US 75. These selections were then evaluated for resistance and performance (Tables 1 and 2). Results with 513, the seventh successive selection from US 75 are summarized in Table 5. In four tests, under severe yellows, 513 produced an average 53% higher root yield and was 0.8 percentage points higher in sucrose than US 75. These tests were inoculated with BYV and BWYV. Under condition of moderate yellows (natural infection), 513 produced an average 47% higher root yield and was 0.2 percentage points higher in sucrose than US 75.

Table 5.—Performance of 513, the seventh successive yellows resistant selection from US 75, compared with the performance of US 75 in 1966 and 1967 California variety tests.

Location	513		US 75	
	Root yield	Sucrose	Root yield	Sucrose
	Tons	Percent	Tons	Percent
Severe yellows				
Salinas - 1966	26.0	16.2	16.3	14.3
Davis - 1966	23.5	12.9	16.6	12.5
Salinas - 1967	30.0	13.1	17.8	12.9
Davis - 1967	17.3	12.5	12.5	11.7
Average	24.2	13.7	15.8	12.9
Moderate yellows				
Brawley - 1966	20.4	15.7	13.9	15.8
Salinas - 1967	37.1	13.2	25.1	13.5
Brawley - 1967	32.2	14.1	20.7	13.2
Brawley - 1967	51.3	14.0	22.6	14.0
Average	30.3	14.3	20.6	14.1
Light yellows				
Davis - 1966	28.8	13.7	27.5	14.0
Davis - 1967	22.4	12.9	22.1	13.1
Average	25.6	13.3	24.8	13.6

Performance was also determined at Davis in 1966 and 1967 from noninoculated plots only lightly infected with yellows. In these two tests the performance of 513 and US 75 was similar both in root yield and sucrose percentage. Root-yield loss from yellows inoculated plots at Davis in 1966 was 18.2% for 513 and 40.0% for US 75. In 1967 the root yield loss for the selection was 22.5% compared with 42.7% for US 75.

In the two 1967 Salinas tests amino N, Na and K contents of the roots were determined. Amino N and Na contents of 513 were significantly lower than those of US 75. The K content of the selection was significantly greater than that of US 75.

Performance of hybrids with yellows resistant selections

Hybrids that utilized open-pollinated yellows-resistant selections as pollen parents were included in yield tests exposed to yellows in varying degrees of severity. Results (Table 6) showed that many of these hybrids performed better than US H7 when grown under conditions of moderate to severe yellows. US H7 is a monogerm hybrid variety that is extensively grown in California and is used as a standard check in variety tests. None of the parents of US H7 has been selected for yellows resistance. Comparisons of the relative yellows-resistance of the various hybrids should not be made because they were tested in different locations and the growing conditions varied from one test to another.

Hybrids that utilize the 413 selection from US 75 (Tables 1 and 2) as the pollen parent have been most widely tested. In tests at Davis, California, yield losses from yellows averaged 27% for 13H4, 28% for 13H8, and 40% for US H7. In 17 tests under conditions of moderate to severe yellows, 13H4 produced an average 22% more sugar per acre than US H7 (Table 6). In 11

Table 6.—Performance of hybrids with yellows resistant selections as pollen parents expressed in percent of the performance of US H7 in California variety tests.

Hybrid No.	Description	No. of tests	Gross sugar yield	Percent sucrose
Severe yellows infection				
13H4	569H3 × 413	5	124	101
13H8	546H3 × 413	5	129	104
13H11	550H4 × 413	1	124	100
30H4	569H3 × 430	3	111	101
37H4	569H3 × 337	3	103	100
37H8	546H3 × 337	2	110	100
44H4	569H3 × 544	3	127	104
44H11	550H4 × 544	2	131	100
34H11	550H4 × 234	2	143	104
Moderate yellows infection				
13H4	569H3 × 413	12	121	102
13H8	546H3 × 413	6	125	102
13H11	550H4 × 413	8	122	101
30H4	569H3 × 430	1	103	99
37H4	569H3 × 337	2	102	100
37H8	546H3 × 337	2	104	100
44H4	569H3 × 544	4	114	102
44H11	550H4 × 544	7	124	101
34H11	550H4 × 234	2	131	106
Light yellows infection				
13H4	569H3 × 413	10	115	102
13H11	550H4 × 413	6	117	100
30H4	569H3 × 430	5	103	100
37H4	569H3 × 337	2	101	100
44H4	569H3 × 544	1	105	100
44H11	550H4 × 544	2	106	97

tests, 13H8 produced a 27% higher sugar yield than US H7. Sucrose averaged 0.3 percentage points higher for the 413 hybrids than for US H7.

In 1967 Salinas tests (Table 7) under conditions of moderate to severe yellows the amino N, Na, and K content tended to be lower in the roots of 13H4, 13H8, and 13H11 than in US H7.

Tests under conditions of light yellows infection showed an average 15% higher sugar yield for 13H4 and 17% higher yield for 13H11 than for US H7. Sucrose averaged 0.2 percentage points higher for the 413 hybrids than for US H7.

Hybrid 30H4 that utilized selection 430 from US 75 (Tables 1 and 2) as the pollen parent produced 9% higher sugar yields under moderate to severe infection and 3% higher yields under light infection than US H7. In two Salinas tests the Na and K contents of the roots of 30H4 were similar to that of US H7, but the amino N content was higher in the roots of 30H4. The 37H4 hybrid produced only 3% higher sugar yields under moderate to severe yellows and 1% higher yield under light infection than US H7. The 437 pollinator line in 37H4 was selected for yellows resistance from 663, the pollinator in US H7. No improvement was observed in the yellows resistance of the selection (Tables 1 and 2). The small gain in sugar yield of 37H4 was significant in only two of five tests under moderate to severe infection.

In four tests 34H11 produced an average 37% higher sugar yields than US H7. The pollinator line 234 was the most resistant of a large group of European selections that were tested at Salinas. Additional tests are required to determine how the resistance of the 234 hybrid compares with that of the 413 hybrid. An increase of a cross between 234 and 430 was also used as a pollen parent. The resulting 44H11 hybrid produced an average 26% higher sugar yield than US H7 when tested under moderate to severe infection and an average 6% higher yield under light infection.

Discussion

Breeding studies over the past 13 years have demonstrated that a marked improvement can be made in resistance to the yellowing viruses of sugarbeet. Tests by McFarlane and Bennett (5) with more than 350 sugarbeet varieties and breeding lines showed that a wide range of resistance to BY exists within *Beta vulgaris* L. Results reported in this paper show that segregation for resistance occurs in many varieties and breeding lines.

The low correlation between reduction in root yield and stunting, yellowing, or necrosis of tops in plants affected by yellows (5) has made the selection process both difficult and

Table 7.—The amino nitrogen, sodium, and potassium content of the roots of sugarbeet in four tests at Salinas, California, in 1967.

Hybrid or Selection	Amino Nitrogen				Sodium				Potassium			
	Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3	Test 4
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
13H4	593	....	859	652	239	..	510	439	2199	..	2249	1581
18H8	....	607	737	....	..	382	534	..	....	2088	2194	....
18H11	588	....	744	636	265	..	544	570	2065	..	2155	1344
30H4	....	972	1291	....	....	306	536	....	....	2247	2308	....
44H4	....	873	1041	....	....	371	667	..	....	2222	2368	....
US H7	668	861	871	696	334	303	679	581	2308	2295	2351	1515
554	....	642	755	....	..	513	793	....	....	1890	2269	..
550	....	778	1337	....	..	325	586	..	....	2514	2812	....
513	..	587	684	....	....	382	619	....	..	2447	2726	....
US 75	....	745	1136	..	....	506	765	..	....	2307	2480	....
LSD (5%)	56	138	255	NS	91	110	118	NS	129	171	185	128

costly. Selections must be made on the basis of root performance as well as on freedom from top symptoms, and this limits the size of populations that can be examined for resistance.

Studies by McFarlane and Bennett (5) and by Russell (10) showed that differences exist within sugarbeet breeding material for resistance to yellows infection. In California these differences have been observed when the amount of infection was low to moderate. When aphid populations have been high and infection heavy, differences in resistance to infection have not been evident. Results, thus far, indicate that a useful level of resistance to infection will be difficult to achieve. Primary emphasis has, therefore, been placed on resistance to damage caused by the viruses.

Resistance is needed to both BYV and BWYV. Selections can be made from plants infected with the individual viruses or with both viruses. The selection work described in this paper was done at Salinas where both viruses occur in nature and the aphid vectors are present throughout the year. Even when aphicides are applied regularly, natural infection cannot be prevented and a portion of the plants become infected with both viruses. To eliminate this source of variation, the plants were inoculated with a combination of BYV and BWYV. Likewise, the selections were evaluated by comparing the performance of plots inoculated with the two viruses with that of noninoculated plots.

The results show that root-yield losses from yellows have been reduced by more than 50% in a selection from US 75. This improvement in yield has been accompanied by a higher sucrose percentage in yellows-infected beets, even though the selection was based primarily on root size. To insure that no reduction in quality occurs, methods have been modified to include selection on the basis of sucrose percentage as well as root size.

The testing program has failed to show how much of the reduction in yield losses is contributed by resistance to BYV and how much by resistance to BWYV. A portion of the hybrid tests were grown in areas where BWY is known to be the predominate virus, and the performance of hybrids involving yellows-resistant selections was markedly superior to that of US H7. These results suggest that a portion of the resistance was to BWYV. Results of tests by Bennett and McFarlane (2) indicate that selections made for resistance to BYV may also show resistance to BWYV. Additional work is needed to positively determine the relationship of resistance to the two viruses and the desirability of selecting for resistance to the individual virus or to the combination of viruses.

As might be expected the various varieties and breeding lines differ in their heterozygosity for yellows resistance. Greatest

progress has been made with selections from US 75. Root yields under conditions of severe yellows were 53% higher for the seventh successive selection than for the parent variety. Even with this marked increase in production, the selection is not suited for use as a commercial variety. The selection is multigerm and its performance does not equal that of our present hybrid varieties when grown under yellows-free conditions.

Practically all sugarbeet seed used in areas subject to yellows is monogerm and hybrid. The hybrids are produced by crossing a cytoplasmic male-sterile monogerm parent with a multigerm pollinator. Multigerm yellows-resistant selections with good combining ability can be used as pollen parents in hybrids. The 413 selection from US 75 has performed well as a pollen parent and was used to produce 13H4, 13H8, and 13H11 (Table 6). Two of these hybrids, 13H4 and 13H8, have been released as commercial varieties with the designations US H9A and US H9B (7). In addition to moderate yellows resistance these hybrids possess resistance to bolting and curly top.

Even though a wide range of yellows resistance exists within *Beta vulgaris* L. and marked progress has been made in the breeding program, immune or highly resistant lines have not been found. Successive selections within the more resistant self-sterile and self-fertile lines are yielding progressively smaller gains in resistance. The inheritance of resistance has not been determined but the results indicate that inheritance is complex and probably due to the action of multiple genes. If this is true, genes responsible for resistance in the various selections may differ. Crosses have been made between the more resistant selections with emphasis on crosses between selections from diverse sources. Selections will be made from F_2 and succeeding generations of these crosses.

Summary

Selections for resistance to beet yellows virus (BYV) and beet western yellows virus (BWYV) were made on the basis of freedom from disease symptoms and on root size. Improvements in resistance were obtained in both self-sterile and self-fertile sugarbeet lines. Root-yield losses from yellows averaged 20.4% for a seventh successive selection from US 75 compared with 41.4% for the parent. Under severe yellows, the selection produced an average 53% higher root yield and was 0.8 percentage points higher in sucrose than US 75. Under light yellows infection the performance of the selection was similar to that of the parent.

Hybrids between monogerm male steriles and yellows-resistant selections performed well under both severe and light yellows infection. Two hybrids that utilized a US 75 selection as the

pollen parent have been released as commercial varieties with the designations US H9A and US H9B. In 17 tests under moderate to severe yellows, US H9A produced 22% more sugar per acre than did the standard check variety. In 11 tests, US H9B produced a 27% higher sugar yield than the check. Sucrose percentage averaged 0.3 percentage points higher for US H9A and US H9B.

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Variety performance tests were made by Mr. K. D. Beatty, at the U.S. Southwestern Irrigation Field Station, Brawley, California. Varieties were also evaluated in each of the major sugarbeet growing areas of California by the Holly Sugar Corporation, Spreckels Sugar Company, and the Union Sugar Division, Consolidated Foods Corporation.

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Rapid Digestion Procedures for Determination of Metallic Ions and Total Nitrogen in Sugarbeet Samples[1]

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Introduction

Chemical digestion of sugarbeet material is always a problem because of the high sugar content involved. The sugar causes excessive foaming and charring and, consequently, the digestion is usually a long, tedious process. When there are many samples to be analyzed, digestion time can become an important factor.

This discussion is limited to digestion of sugarbeet samples for atomic absorption spectrophotometric analysis and for total nitrogen determinations. It should be noted, however, that the digested samples which result from the procedures can be used sometimes for other analyses. Atomic absorption spectrophotometry is an accurate and highly sensitive method for determination of many metallic ions. For this analysis, samples must be in a solution that can be easily aspirated through the sample tube of the burner-atomizer and that has the fewest interfering ions possible. Total nitrogen determinations are important in purity, quality and genetic studies; therefore, a reliable rapid digestion procedure for analysis of micro-samples is desirable.

Digestion for Atomic Absorption Analyses

Most atomic absorption spectrophotometric procedures suggest digestion of samples with nitric acid (10)[3] or a mixture of nitric and perchloric acids (1,6). Sometimes a mixture of nitric, perchloric, and sulfuric acids is used (5). We have found that nitric acid, if used alone, is unsatisfactory for sugarbeet samples because of excessive foaming and incomplete digestion. The nitric-perchloric acid mixture will eventually give complete digestion; but several additions of the acid mixture to the digesting sample must be made before complete digestion takes place. Both acids are quite volatile and decompose upon heating. This

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[3] Numbers in parentheses refer to literature cited.

procedure is also dangerous. If the mixture is allowed to boil down too far, a violent explosion can result through dehydration and decomposition of some of the components.

A mixture of nitric, perchloric and sulfuric acids is much safer to use. Since sulfuric acid is more stable at higher temperatures, its presence helps prevent dehydration which may cause the formation of the explosive compounds during the digestion. Again, this mixture requires at least 1 hour for complete digestion, and usually requires addition of more acid during the process.

Bolin and Stamberg (3) suggested the use of a digestion mixture of perchloric and sulfuric acids with some molybdenum added as a catalyst for determination of phosphorus. The presence of the molybdenum markedly increases the rate of oxidation of organic matter. This mixture is satisfactory for digestion of many samples, but in atomic absorption analyses, the high sulfate ion content that results sometimes causes interference. Bolin and Stamberg suggested the following proportions:

30 g sodium molybdate ($Na_2MoO, \cdot 2H_2O$) in 150 ml H_2O
150 ml concentrated sulfuric acid (H_2SO_4)
200 ml of 70 - 72% perchloric acid ($HC10_4$)

This procedure was modified for atomic absorption sample preparation to decrease the sulfate ion content (8) . We use the following combination:

350 ml 70 - 72% perchloric acid (HCIO4)
100 ml Conc. sulfuric acid (H_2SO_4)
1500 ml Conc. nitric acid (HNO_3)
2 g sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 100 ml H_2O

The amount of sodium molybdate suggested by Bolin and Stamberg was reduced to decrease the light scattering that results from an intense sodium flame. Excess scattering may decrease the sensitivity in atomic absorption analysis for other cations.

Sugarbeet thin juice samples, prepared according to Carruthers and Oldfield (4), may be read spectrophotometrically without digestion. Pressed juice and dried leaf and petiole samples must be digested. The ratio of the digestion mixture to the amount of sample may be adjusted as required. The procedure we use is as follows:

To 0.5 g of finely ground dried leaf or petiole sample in a pyrex digestion tube, add 5 ml of the acid digestion mixture. If this mixture is allowed to stand overnight, foaming is reduced considerably when first heated. Heat at medium temperature on a digestion rack. We use an electric rotary digestion rack. The sample will boil with some foaming and charring. Then a rapid stage of oxidation takes place, during which the solid material disappears and the sample becomes colorless. After this

reaction, continue heating at a high temperature until the sulfuric acid begins to reflux up the tube. This refluxing action digests any sample particles remaining on the sides of the tube. A total of about 25 minutes is required for the complete digestion. Cool. Dilute the sample to 25 ml. If necessary, further dilution of the sample is made if the concentration of the metallic ions is not within the sensitivity range of the atomic absorption spectrophotometer. In some determinations, additions of other reagents may be necessary as indicated in methods outlined in the procedure for atomic absorption analysis for a particular cation. For example, in calcium determinations (7), lanthanum chloride is added to mask interference by phosphorus and aluminum ions which may be present. Also, the sulfate ion concentration in the diluted sample and in the standard solution should be about 1%. All samples are read in comparison with standard solutions on an atomic absorption spectrophotometer.

This digestion procedure is satisfactory for atomic absorption analysis for various cations, except sodium and molybdenum. The presence of molybdenum causes no interference in any of the analyses we have made.

Digestion for Total Nitrogen

Total nitrogen determinations are time-consuming and difficult, especially when it is desirable to determine the nitrate nitrogen along with other nitrogen present.

Many variations of the Kjeldahl procedure have been used. The procedure which we have found to be the most satisfactory, and which will also determine nitrate nitrogen, is described in the Association of Official Agricultural Chemist's "Methods of Analysis" (2). This procedure uses salicylic acid in concentrated sulfuric acid along with sodium thiosulfate. Copper and potassium sulfate (Kel Pak) are added as catalysts and to raise the boiling point. The total digestion time required for this digestion of dried plant or juice samples is about 4 to 5 hours. The nitrate reacts with the salicylic acid in the presence of strong acids and, thus, is eventually converted to the ammonium ion in the digestion process.

The proposed digestion mixture given here still maintains the use of salicylic acid along with concentrated sulfuric acid. In addition, perchloric acid is added as a strong oxidant and sodium molybdate as a catalyst. The reagents are made up as follows (Reagent I should be made fresh just before use.):

Reagent I
150 ml cone. H_2SO_4
25 g salicylic acid
Mix well
Add 200 ml 70-72% $HC1O_4$

Reagent II

10 g $Na_2MoO_4 \cdot 2\ H_2O$ in 150 ml H_2O

In digesting thin juice samples, place 0.5 ml thin juice in a 50 ml-calibrated pyrex digestion tube or micro-Kjeldahl flask. Boiling stones are added to prevent bumping. Add 1.5 ml of Reagent I and allow to stand overnight to let the nitrate react with the salicylic acid. Just before digestion, add 1.0 ml of Reagent II (sodium molybdate solution). Place on a digestion rack and heat at medium temperature. After initial foaming and charring, the samples go through a rapid stage of oxidation during which the solid matter disappears and the mixture becomes colorless. Turn to high heat until the sulfuric acid refluxes up the sides of the tube. This will digest any remaining sample particles on the sides of the tube, and drive off excess hydrogen chloride and other volatile components which may interfere with the color formation if direct nesslerization is used later. After digestion, the samples may be read by direct nesslerization, by nesslerization of an aliquot of the digested sample, or by a steam distillation method. If nesslerization is used on aliquots of the digested sample, or if a steam distillation method is utilized in reading the sample, larger amounts of sample and digestion mixture can be used.

The direct nesslerization procedure involves, first, adding about 15 ml of distilled water to the cooled, digested sample in the calibrated digestion tube. Some of the excess acid is neutralized by the addition of 2 to 3 ml of 10 percent sodium hydroxide. Allow this mixture to cool. Add 1 ml of 2% gum ghatti solution to aid in stabilization of the colloidal solution when it is formed later. Using a pipet, blow in 12 to 14 ml Nessler's solution. Make up to 50 ml with distilled water. Mix well and read at 490 mμ on a spectrophotometer. The amount of sodium hydroxide and Nessler's solution should be adjusted to the samples being analyzed. Some types of samples may require more acid in digestion than others. The Nessler's solution must be sufficient for the colored colloidal compound of dimercuric ammonium iodide to form in ratio to the ammonium radical ion present. The pH of the resulting solution should be about 12. If the pH is too low, a red precipitate will form. If the pH is too high, the resulting solution may become cloudy. Cloudiness may also result if the Nessler's solution is not mixed thoroughly as it is added to the sample.

High concentration of contaminates, such as silicates or soluble salts, may also cause precipitation of the colloidal compound. For this reason, it is difficult to read digested leaf or petiole samples by direct nesslerization. However, these samples

may be read by the nesslerization method if an aliquot of the sample is used. To do this, dilute the digested sample to 50 ml with water. After thorough mixing, measure a 2 ml-aliquot into a second 50 ml-calibrated tube. Add about 15 ml of water. With a pipet, blow in 5 ml of Nessler's solution. Dilute to 50 ml, mix well, and read at 490 mμ as above. The dilution factor must be included in calculating the amount of nitrogen in the original sample. The colloidal solution resulting from nesslerization, if properly prepared, should be golden to reddish-brown in color and sparkling clear.

A steam distillation method may be used, if preferred, when a high concentration of interfering ions are present in the digested sample. It is then preferable to digest the samples in micro-Kjeldahl flasks which can be used later with the steam distillation apparatus. After digestion, the sides of the cooled sample flask are washed down with about 10 ml of distilled water. Connect the flask to the steam distillation apparatus and then add sufficient 40 percent sodium hydroxide to more than neutralize the acid present. The ammonia, which is driven off by steam distillation, can be collected in Nessler's solution and read as above. We prefer collecting the ammonia in a 2 percent boric acid solution which contains bromcresol green indicator, and titrating the resulting solution with 0.0143 N sulfuric acid (9). Each ml of the 0.0143 N sulfuric acid used is equivalent to 0.2 mg nitrogen in the sample.

Table 1 shows the average amount of total nitrogen, in mg per 100 ml thin juice, obtained on three thin juice samples. Each thin juice sample was analyzed three ways as follows: 1) Duplicate 0.5 ml portions of each sample were digested with 1.5 ml of the proposed digestion mixture (H_2SO_4, $HC1O_4$, salicylic acid and Na_2MoO_4) and read by direct nesslerization. The amount of sample and reagents added is kept to a minimum here because direct nesslerization is used for reading after digestion. 2) Duplicate 1.0 ml portions were digested with 5.0 ml of the proposed digestion mixture and read by the steam distillation-boric acid-sulfuric acid method. 3) Duplicate 1.0 ml portions were digested with 5.0 ml of the standard AOAC digestion mixture (H_2SO_4, salicylic acid, $Na_2S_2O_3$, and Kel Pak) and read by the steam distillation-boric acid-sulfuric acid method.

Thin juice samples were also run with known amounts of nitrate nitrogen added to the samples. The check results on these tests samples were satisfactory.

When analyzing pressed juice samples, best results are obtained when at least 5.0 ml of the proposed digestion mixture are used for each ml of pressed juice. Again, three pressed juice samples were analyzed using the proposed digestion mixture.

Table 1.—Mg of nitrogen per 100 ml thin juice obtained on three samples analyzed by three methods.

Sample	Method I	Method II	Method III
	Proposed digestion mixture		AOAC digestion mixture
	Direct nesslerization	Steam distillation	Steam distillation
1	18.38 mg N/100 ml	19.06 mg N/100 ml	18.88 mg N/100 ml
2	14.15	14.00	15.95
3	61.75	61.90	61.80

The results were checked against results obtained on the same three samples digested with the AOAC digestion mixture. The steam distillation-boric acid-sulfuric acid method was used in each case to read the sample after digestion. Table 2 shows results of these samples with the total nitrogen given in mg nitrogen per 100 ml pressed juice.

Table 2.—Mg of nitrogen per 100 ml pressed juice obtained on three samples digested by two methods.

Sample	Proposed digestion mixture	AOAC digestion mixture
1	82.25 mg N/100 ml	82.25 mg N/100 ml
2	153.00	153.25
3	61.00	60.60

Finely-ground dried leaf or petiole samples may also be digested with the sulfuric-perchloric-salicylic acid mixture with sodium molybdate added as a catalyst. Again, it is best to allow the samples to stand overnight after addition of the acid digestion mixture to allow the nitrate to react with the salicylic acid and to reduce foaming. Best results were obtained when 6 to 10 ml of the digestion mixture were used with 0.2 gram dried sample. Just before digestion 1.0 ml of the sodium molybdate solution is added. The digestion procedure is carried out the same as with thin and pressed juice samples.

Table 3 gives the mg nitrogen per 100 g sample for a single dried leaf sample. Four 0.2 g samples were digested using 10 ml of the proposed digestion mixture, and four 0.2 g samples were digested using 6 ml of the AOAC digestion mixture. All were read by the steam distillation method in which the ammonia was collected in weak boric acid solution and titrated with 0.0143 N sulfuric acid.

Table 3.—Mg nitrogen per 100 g dried leaf sample obtained in four duplicate analyses by two digestion methods on the same sample.

Sample	Proposed digestion mixture	AOAC digestion mixture
1	4650 mg N/100 g	4670 mg N/100 g
2	4680	4680
3	4620	4700
4	4670	4660

Summary

Good results have been obtained using new, faster digestion procedures for analysis of sugarbeet samples. The digestion mixture for sample digestion for atomic absorption spectrophotometry is made of concentrated nitric, perchloric, and sulfuric acids. A small amount of sodium molybdate is added as a catalyst. For total nitrogen determinations, a mixture of concentrated sulfuric and perchloric acids are used with the addition of some salicylic acid to aid in conversion of the nitrate nitrogen to the ammonium radical ion during digestion. Sodium molybdate again is added as a catalyst. The amount of digestion mixture is adjusted to the kind and amount of sample used.

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The Determination of Trace Metals in Process Juices and White Sugar

T. D. CARPENTER AND S. E. BICHSEL[1]

Received for publication April 18, 1968

Introduction

The analysis for trace metals is rapidly becoming routine in many industries. Metals as well as non-metals have been determined quite accurately for many years, but in most cases have required analytical procedures that are extremely time consuming. Many colorimetric procedures are very accurate when care and good technique are applied. However, there are usually a number of steps where small errors can be made that are multiplied in the final results.

The primary purpose of this paper is to determine "if" and "how" atomic absorption spectrophotometry can be applied to the determination of trace metals in process juices and white sugar. Of equal importance is the evaluation of atomic absorption and its application in providing rapid and accurate analysis for research and industrial problems.

The use of atomic absorption spectrophotometry as an analytical tool is now firmly established. (2, 3, 6)[2] This is apparent from the number of technical papers that have been published during the past few years. The field of analytical chemistry is developing so rapidly that much of the published information is out-dated before it reaches the reader. There has been an increasing interest in the presence of trace amounts of metals in certain products. An analytical report that simply states "trace" or "not detected" in many cases is no longer accepted. With this thought in mind, sugar technologists must explore new, faster, and more sensitive, direct means of determining trace metal content of white sugar and process juices.

Theory

In emission, which is the basis for flame photometry, atoms are excited and raised to a higher energy level. As these atoms return to their ground state they emit energy in the form of light of a specific wavelength characteristic of that element. Therefore, the intensity of this light is proportional to the concentration of the excited atoms.

However, in atomic absorption, as shown in Figure 1, light from a hollow cathode lamp made from the element of interest is passed through the flame and into the spectrophotometer where a resonant wavelength is isolated by the monochromator.

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[2] Numbers in parentheses refer to literature cited.

When a sample is atomized into the flame nearly all of the atoms are in the ground state and are capable of absorbing the resonant wavelength. This absorption is directly proportional to the number of atoms being atomized and can be related to concentration.

The limit of detection is dependent on the intensity of the primary light source, signal to noise ratio, ionization potential and frame temperature. Sensitivities vary with the elements to be determined. For example, magnesium produces a higher intensity light than iron and consequently can be detected at much lower concentrations. Magnesium is detectable down to 5 ppb whereas iron is only detectable to about 200 ppb.

As illustrated in the iron spectrum many lines are produced. It would appear that the line produced at 3720 Angstroms (A) would be the best choice. However, the most sensitive lines are 2483 A and 2488 A. The line at 3720 A is about 10 times less sensitive due to effects of nearby lines of emission. Copper produces a very strong line at 3247 A which allows detection limits as low as .05 ppm (Figure 2).

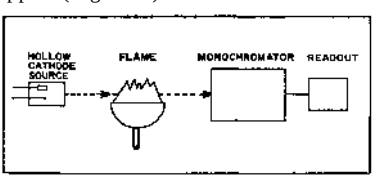

Figure 1.—Schematic diagram of atomic absorption single-beam instrument.

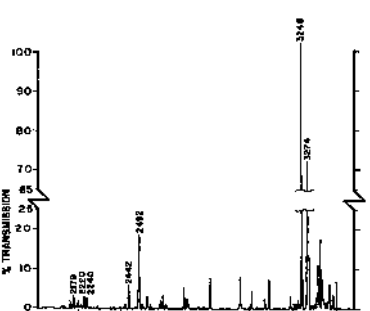

Figure 2.—Copper transmission spectrum.

It is now possible to determine virtually all metals by atomic absorption. If a hollow cathode lamp can be manufactured from the metal then this metal can be determined by atomic absorption. If a hollow cathode lamp cannot be manufactured from a particular metal such as phosphorus, then indirect methods may be employed. Non-metals may be determined in certain cases by quantitatively precipitating with a standard metal solution and determining the concentration of the unreacted metal. In these areas of analytical chemistry applications are virtually unlimited with atomic absorption.

Equipment and **Procedures**

A Beckman Model 979 atomic absorption unit coupled with a 10-inch Sargent Model SRLG linear-log recorder was used for all atomic absorption determinations. The Beckman unit was equipped with an Aztec Model AB-41 total consumption air-acetylene burner head. This burner was selected on the basis of its high dissolved solids handling capacity, ease of cleaning, and variable light path feature. Hollow cathode tubes of the single and multiple element type manufactured by Westinghouse and Aztec were used during this study.

In preparation of samples for analysis for atomic absorption, it was necessary to concentrate certain metals in white sugar. Since the sensitivity of this instrument is only 0.2 ppm on iron and 0.05 ppm on copper it was necessary to remove these two metals from the sugar and concentrate them to the range of the instrument. This was accomplished by passing 300 grams of white sugar in a 30 Brix solution through 15 ml of IRC-120 analytical grade cation resin in the hydrogen form. Water used to take the sugar in solution was decationized and blanks were run along with the samples. The cations removed from the sugar were then removed from the resin with two 25 ml aliquots of hot 3N analytical grade hydrochloric acid and rinsed with 50 ml of hot decationized water. The solution was evaporated to dryness and taken up in 10 ml of 5% HC1 solution. This solution was analyzed for iron, copper, magnesium, calcium, sodium and potassium.

Magnesium and calcium have much lower detection limits, so it was possible to determine these two elements on dilute solutions of white sugar. Dilutions necessary to avoid viscosity and interference problems were 15g of white sugar diluted to 100 ml with decationized water containing 0.1% lanthanum. To avoid interference problems in process juices it was necessary to dilute raw juice, thin juice, thick juice and molasses to approximately 5 Brix. Recovery studies were made on all samples by the method of standard additions. Analyses were also carried out on ashed samples for comparison.

Figure 3 gives the flame characteristics and instrument settings for each element. Once these settings have been established the standard curve can be reproduced very quickly. Fuel and support mixture is important as well as slit width and lamp current. Each of these settings is established for best operating parameters.

Figure 4 illustrates recovery of iron from white sugar by the ion exchange methods. This sample was duplicated and 100% recovery was obtained.

	IRON	COPPER	MAGNESIUM	MANGANESE	ALUMINUM	SILICON
WAVELENGTH	3483Å 3486Å 3720Å	3248Å 3274Å	4227Å 2300Å	2933Å 2939Å 2794Å	3900Å 3638Å 3961Å	2516Å
SLIT	0.2mm	0.05mm	0.10mm	0.10mm		
FUEL	ACETYLENE 9 psig	ACETYLENE 9 psig	ACETYLENE 9 psig	ACETYLENE 9 psig	ACETYLENE	ACETYLENE
SUPPORT	AIR 14 psig	AIR 14 psig	AIR 14 psig	AIR 14 psig	NITROUS OXIDE	NITROUS OXIDE
LAMP CURRENT	12 mA	12 mA	12 mA	12mA		
FLAME	OXIDIZING NON-LUMINOUS	OXIDIZING NON-LUMINOUS	REDUCING FUEL RICH LUMINOUS	REDUCING FUEL RICH LUMINOUS		

Figure 3.—Flame characteristics and instrument settings.

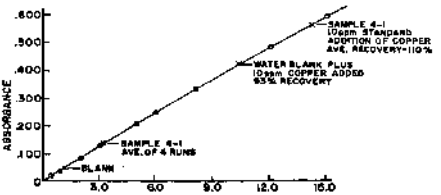

Figure 4.—Iron recovery from white sugar using IRC-120 analytical grade cation resin.

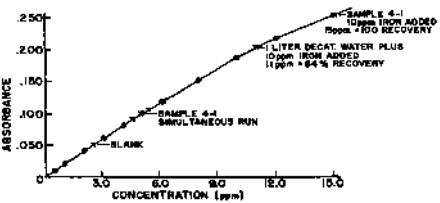

Figure 5.—Copper recovery from white sugar using IRC-120 analytical grade resin.

In Figure 5, on an average of four runs, copper recovery amounted to an average of 110%. Only one blank was run which gave 93% recovery. Differences in samples might possibly account for the high recovery figure.

Experimental Results

The first action was the determination of calcium, magnesium, iron, copper, sodium and potassium in process juices. Some interferences were encountered in the determination of magnesium and calcium but were eliminated with the addition of lanthanum.

Interferences in the determination of calcium and magnesium have been thoroughly explored (3, 6). Phosphates, sulfates, silica and aluminum are known interfering elements. The addition of lanthanum eliminates virtually all of the known interferences by tying up these compounds and allowing the calcium and magnesium to be completely atomized in the flame. No interferences have been reported in the determination of iron and copper (3). Figure 6 shows copper recovery in molasses. Dilutions of 1:20 were necessary to obtain 100% recovery.

Average metal concentrations in process juices are listed in Table 1. Since potassium is consistent throughout the processing cycle, results are calculated as percent on potassium. Results indicate possible elimination and corrosion patterns throughout the factory process. If these results are consistent, in future studies it may be possible to pinpoint corrosion problems or other trouble areas. Results show that these metals are present and can be traced throughout the factory. Analyses of ashed samples were consistent with analysis on straight process juices indicating that process juices can be analyzed directly with accurate results.

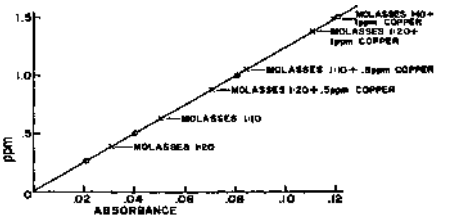

Figure 6.—Copper recovery in molasses.

Table 1.—Trace metals in process juices.

	Brix	Mg. sug. ml.	Conc. units	Na	K	Mg	Mg ashed	Ca	Ca ashed	Fe	Fe ashed	Cu
Diffusion Supply Water			P.P.M.	78	162	20.5	12.1	37.0	13.0	16.0	13.3	.019
			% on potassium			12.7		22.8		9.88		.17
Raw juice	15.3	134.6	P.P.M.	610	1740	355	306	79.0	80	22.6	40.5	0.11
			% on potassium			20.4		4.5		1.3		.0063
Thin juice	15.0	139.8	P.P.M.	580	1910	6.5	6.0	63.8	62.5	2.2	2.2	.015
			% on potassium			3.4		6.0		0.12		.0079
Thick juice	66.8	798.5	P.P.M.	2040	6400	38.8	40.0	328	272	19.5	50.0	0.6
			% on potassium			6.1		5.1		0.3		.022
Molasses	87.75	812.2	P.P.M.	13600	28500	214	204	1330	1264	100	212	4.1
			% on potassium			7.5		4.7		0.35		.027

Copper seemed to be one exception, but results from ashed samples were disregarded when it was established that the hydrochloric acid used to dissolve the ash contained copper impurities and the acid was possibly enhancing the copper absorption signal.

Figure 7 shows the calibration curve when determining magnesium in low concentration in diluted white sugar samples. It also shows the detection limits based on 1% (.005) absorption which is equivalent to .005 ppm (5). This is an extremely low concentration but quite reproducible when maintaining noise and interference at a minimum. Figure 8 illustrates the calibration curve for magnesium and its detection limits in single pass-normal burner position. This does not mean that magnesium and calcium can only be determined in these low concentrations. Actually, by turning the burner head for minimum light passage through the flame it is possible to read up to 20 ppm magnesium and 50 ppm calcium. This means that the range is at least 1000 fold on these two elements. However, the range is only approximately 20 fold in the case of iron.

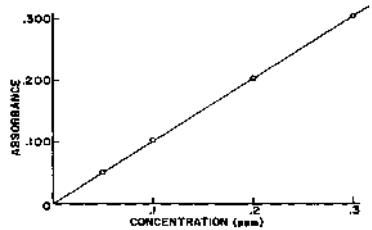

Figure 7.—Calibration curve for magnesium using triple pass.

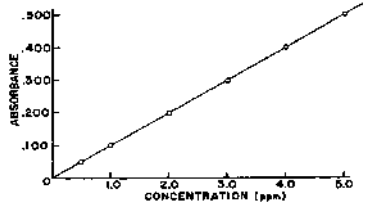

Figure 8.—Calibration curve for magnesium using single pass-normal burner position.

Table 2.—Magnesium and calcium in white sugar (15g diluted to 100 ml).

	Calcium						Magnesium				
	Absorbance		ppm				Absorbance		ppm		
Sample	1	2	1	2	Avg	Avg*	1	2	1	2	Avg
1-A	.02	.027	2.27	2.20	2.24	1.95	.013	.013	.07	.07	.07
1-B	.086	.086	7.07	7.07	7.07	8.45	.048	.044	.33	.31	.32
2-A	.017	.017	2.10	2.10	2.10	2.40	.015	.016	.09	.10	.10
2-B	.060	.060	4.93	4.93	4.93	5.55	.018	.017	.11	.11	.11
3-A	.023	.021	1.87	1.83	1.85	....	.025	.020	.16	.13	.15
3-B	.014	.014	1.20	1.20	1.20	...	.034	.030	.23	.20	.22
4	.126	.024	10.40	2.00	...	.... **	.037	.250	.25	1.67	. **
5	.057	.058	4.80	4.80	4.80		.009	.010	.05	.05	.05
6	.040	.039	3.33	3.33	3.33		.120	.119	.87	.87	.87
7	.026	.024	2.13	2.00	2.07		.015	.015	.09	.09	.09

* Average from ashed sample.

Table 2 lists results obtained by directly aspirating unfiltered samples of white sugar (15 grams diluted to 100 ml with decationed water). These white sugar samples represent high and low color and turbidity samples from four different sugar companies. Absorbance is quite low on high quality white sugar. Note that duplicate samples are quite reproducible and absorbance readings are well within the detection limits. Some readings are just above the lowest detection limit, but concentrations of this magnitude are of little concern. However, readings in the order of 0.126, as in the high oxalate sugar, may be quite significant. It should be pointed out that sampling is important

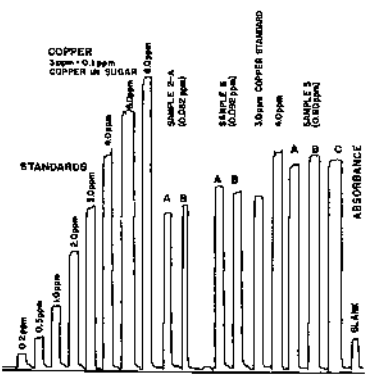

Figure 9.—Copper determinations on 10 milliliter samples concentrated from 300g of white sugar.

as is demonstrated by the range of results on sample 4 which is a high oxalate sugar. This indicates a non-homogeneous mixture and the presence of these elements in random particulate matter.

In Figure 9 the resulting data for copper are shown for several determinations on the 10 milliliters concentrated from 300g of white sugar through the ion exchange resin.

Table 3 gives complete analyses of all samples. Magnesium ranges from .05 ppm in high quality sugar to 1.67 ppm in low quality sugar. Calcium ranges from 1.20 ppm to 10.4 ppm. Copper ranges from .07 ppm to .230 ppm and iron from .058 ppm to .273 ppm.

Sodium and potassium also show a wide range. In sample series 1 through 3 (A) and (B) sugar samples are high and low quality sugars respectively.

Table 3.—Trace metals in white sugar—averages from Table 2 and 4.

Sample	Ca (ppm)	Mg (ppm)	Cu (ppm)	Fe (ppm)	Na (ppm)	K (ppm)
1-A	2.21	0.07	.077	.058	8.13	5.99
1-B	7.07	0.33	.126	.193	8.00	6.66
2-A	2.10	0.10	.082	.104	8.53	7.32
2-B	4.95	0.11	.230	.138	15.0	22.98
3-A	1.80	0.15	.023	.148	10.53	14.82
3-B	1.20	0.20	.062	.190	17.86	28.64
4	2.00 to 10.40	0.25 to 1.67	.119	.200	9.78 to 24.92	....
5	4.80	0.05	.110	.205	4.80	1.33
6	3.33	0.67	.092	.273	2.92	1.66
7	2.00	0.09	.138	.158	4.13	....

Table 4.—Copper and iron determination in white sugar.

Sample	Iron			Copper		
	Run A	Run B	Average	Run A	Run B	Average
1-A	.057		.058	.072	.083	.077
1-B	.193	.193	.193	.113	.145	.125
2-A	.113	.095	.104	.083	.081	.082
2-B	.138	.138	.138	.230	.230	.230
3-A	.158	.158	.148	.023	.023	.025
3-B	.213	.178	.190	.060	.064	.062
4	.180	.220	.200	.117	.121	.119
5	.221	.190	.205	.107	.113	.110
6	.283	.263	.273	.093	.090	.092
7	.158		.158	.138		.158

Summary

Experimental data were presented which indicates the utility of atomic absorption techniques in determining trace metal concentrations in white sugar, process juices and liquors.

With certain refinements the atomic absorption methods presented should attain an accuracy and precision equal to or surpassing classic trace metal extraction methods. The advantage of the direct method of analysis in time saved is apparent. Extension of direct trace metal determinations to zinc, aluminum and silica in white sugar, process juices and liquors should not pose any significant problem.

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Simultaneous Fumigating and Planting of Sugarbeets

RONALD C. JOHNSON[1]

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Soil fumigants have proven to be an effective means of controlling sugarbeet nematode, *(Heterodera schachtii).* They have not been used as extensively as they could be for two reasons: 1. the cost of the material; and 2. a delay in planting of from 10 days to 2 weeks was required. The object of the experiments reported in this paper was to evaluate the possibility of eliminating the waiting period that was required between when the fumigant was applied and the beets planted.

Methods

All experiments were conducted in commercial sugarbeet fields. Four tests were conducted in 1966 of which three were in Utah and one was in Washington. One test was conducted in 1967 to further evaluate some of the results obtained in the 1966 tests.

The tests were primarily conducted to determine if Telone[2] could be applied to soil and beets planted at the same time and the other fumigants were included as checks. Previously conducted tests had already proven that Telone was an effective fumigant and these tests were designed to determine the extent on stand of simultaneous fumigating and planting (2, 4)[3].

The fumigants, in all experiments, were applied with a chisel applicator at a depth of 6 to 8 inches. The beets were planted just as soon as the fumigation was completed or the fumigant was sidedressed shortly after the beets were planted. There was never a delay of more than half a day between the time the fumigant was applied and the beets planted.

1966 Utah Tests

There were three tests conducted in Utah in 1966. In all of these tests the fumigants were applied 6- to 9-inches deep. In the first field Telone was applied at 20 gallons per acre and Vidden D[4] at 25 gallons per acre. The fumigants were applied

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[2] Telone - the trademark of the Dow Chemical Company's 1-3 dichloropropene soil fumigant.
[3] Numbers in parentheses refer to literature cited.
[4] Vidden D - Another trademark of Dow Chemical Company's 1-3 dichloropropene soil fumigant.

Table 1.—The effect on stands of simultaneous applying 20 gallons of Telone per acre and planting sugarbeets. Utah 1966.

Location	Description	Beets per 100 feet of row	
		Untreated	Telone
Field No. 1	Same direction as beet row*	104	101
Field No. 2	Diagonal to planted row	103	107
Field No. 3	Sidedressed along side of row after beets were planted	97	94

•Beets planted as soon as fumigation completed.

in the same direction the beets weere planted and the chisels were 11 inches apart. The soil was a sandy loam.

In this test the Vidden D killed all of the beets either before or shortly after emergence. There were no visual adverse effects on stand from the Telone and there were only slight differences in stand between the Telone treatment and the untreated area as shown in Table 1.

The Vidden D treatment was not made in the other two fields because of the disasterous effect in this field and similar but less severe results in commercial fields where there was insufficient time between fumigating and planting.

The second field had 20 gallons of Telone applied diagonal to the direction the beets were planted and on the third field the fumigant was applied as a sidedressing after the beets were planted. These are shown as fields 2 and 3 respectively in Table 1. Field No. 2 was a sandy loam and number 3 was a clay loam. There was no apparent adverse effect from the Telone in either of these fields and the final thinned stands were as good in the treated as untreated areas.

The portions of beet row just above the chisel marks were examined carefully and no adverse effect could be determined. The concentration of fumigant should have been highest in these areas and still the beets showed no visual damage (1).

To further evaluate the value of the fumigation and the effect on yield and sucrose of the sugarbeets, harvest data were taken on the field that had been treated after the beets were planted. Table 2 indicates nearly a 3-ton increase in yield but a decrease in sucrose percentage from applying the fumigant. There was a net increase of 739 pounds of sugar per acre.

Table 2.—The effect on yield and sucrose of applying 20 gallons of Telone immediately after planting beets. West Jordan, Utah, 1966.

Treatment	Tons per acre	Percent sucrose	Lbs of sugar per acre
Untreated	18.29	16.2	5,927
Telone, 20 gal. per acre	21.09	**15.8**	6,663

1966 Washington Tests

In the 1966 test in Washington, a comparison was made between Telone and Vorlex[5]. The fumigants were applied in the same direction as the beet rows and were applied immediately before the beets were planted, or side-dressed shortly after planting. The chisels were spaced either 11 or 22 inches apart for the treatments applied before planting and 11 inches apart for the treatment applied after the beets were planted. The field was a sandy loam and the lightest soil in any of the tests reported.

Table 3 indicates that all of the Telone treatments gave good yield increase and that on this light soil, the 15 gallon rate was equal to the 25 gallon treatment. The Telone treatment made after planting yielded as high as the pre-plant treatments. There was some difference in stands but other factors may have caused some of this difference. Satisfactorily thinned stands resulted from all of the Telone treatments. The large decrease in sucrose percentage in the after planting treatment is unexplainable.

Table 3.—Soil Fumigation test conducted in Washington in 1966 to evaluate two fumigants and the possibility of simultaneous fumigation and planting.

Treatment	Tons per acre	Percent sucrose	Pounds of sugar per acre	Beet stands	Cyst count after harvest
Untreated	24.27	14.6	7,087	good	20
25 gal. Telone 11" shank space	27.87	14.5	8,082	fair	12
15 gal. Telone 11" shank space	31.96	15.0	9,588	fair	16
25 gal. Telone after planting	30.58	13.7	8,379	good	13
4 gal. Vorlex 22" shank space	22.55	14.8	6,675	fair	15
7 gal. Vorlex 22" shank space	25.05	14.6	7,315	fair	24
G gal. Vorlex 11" shank space	25.60	14.7	7,526	poor*	24

•Eighty percent of stand lost. Replanted April 20.

Vorlex was applied at 4 and 7 gallons per acre with the chisels 22 inches apart, and at 6 gallons per acre with an 11-inch spacing between the chisels. The 6 gallon treatment with 11-inch spacing between the chisels caused a reduction of approximately 80% of the original stand and had to be replanted. The other two did some injury to the stand but satisfactorily thinned stands were obtained. None of the Vorlex treatments gave sufficient increases to justify their use.

[5] Vorlex - The trademark of a soil fumigant of the Morton Chemical Company.

Table 3 shows that there were only small differences in the cyst count after harvest of any of the treatments. Fumigation does not kill all of the nematodes and those that survive seem to increase more rapidly on beets that grow well and produce high tonnages. The population of nematodes in a treated field generally increases so that a second year of beets without fumigation is not profitable (3).

This test would indicate that Telone can be applied just before or after the beets are planted and that large increases in yield and gross sugar can be obtained. It also shows that Vorlex did not show enough beneficial effects to be recommended as a treatment at or near the time the beets are planted.

1967 Tests

The 1966 tests had indicated that Telone could be applied simultaneously with planting without seriously damaging the beets. In 1967 a test was conducted to determine the effect on the beets if conditions were favorable for beet damage. In this test 26 gallons of Telone were applied per acre at a depth of 7 to 8 inches and with the chisels spaced 11 inches apart. A harrow followed immediately behind the fumigator and the seed drill behind the harrow. The beets were planted less than 5 minutes after the fumigant was applied. The field received 0.6 of an inch of moisture starting approximately 2 hours after the planting was completed.

These conditions should have made the Telone as toxic as possible to the beets. Table 4 indicates that there was an increase in tons of beets and gross sugar from the Telone application. It also indicates that the thinned stand was fairly consistent in the untreated check with a variation in counts of 92 to 103 per 100 feet of row. The Telone treated area varied from 58 to 104 with the low counts coming from areas where the chisel mark or application furrow was directly below the planted row. In some areas where one was exactly on top of the other, there was frequently 6 to 7 feet of row where there were no beets. The fact that the beet row was planted at a slight angle to the direction the fumigant was applied kept these areas from possibly being longer and more numerous.

Table 4.—The effect on yield, sucrose and stand of applying Telone the same time as planting beets and making conditions favorable for fumigation damage.

	Tons per acre	Percent sucrose	Lbs of sugar per acre	Thinned stand of beets per 100 feet
Untreated	27.13	16.0	8,682	92-103
Telone*	30.27	16.1	9,747	58-104

*26 gallons per acre.

Discussion

The 1966 tests indicated that Telone can be applied at the same time the beets are planted. They also indicated that Vidden D and Vorlex should not be used without a waiting period between application of the fumigant and planting the beets. The Washington test indicated that for sandy soils the 15 gallon rate was as effective as the 25 gallon application.

The 1967 test indicated that under some conditions the application of Telone could be detrimental to the stand of beets and that a grower should use caution in the practice of simultaneous fumigating and planting.

The direction of application as oriented with the direction of the planted row showed little effect in the 1966 tests. However, the 1967 tests indicated that under some conditions there are disadvantages in having the planted row exactly over the fumigated furrow. Observations of these plots would indicate an advantage of having the fumigant applied diagonal to the planted row.

Summary

From the results of these tests the following recommendations are made:

1. Telone can be applied simultaneous with the planting of beets.
2. Fifteen to 20 gallons of Telone is sufficient for light to medium soils.
3. It is probably best to apply the fumigant diagonal to the direction the beets will be planted.
4. A few hours delay can definitely be a safety factor, especially if moisture is expected.
5. Irrigation should not be applied immediately after simultaneous fumigating and planting.

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Control in Carbonation

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The primary aim in the control of first carbonation is to maintain the alkalinity of the carbonated juice uniformly at the optimum value. The customary control system consists of a pair of electrodes in the effluent, a pH recorder-controller and a control valve in the kiln gas line. When the pH of the effluent differs from a preset value, the controller transmits a signal to the kiln gas valve and adjusts the gas flow to return the effluent pH to the setpoint. This type of control is commonly referred to as feedback control because an error signal is fed back to the controlled variable. Disregarding the relatively low variations in the buffering capacity of the raw juice, the deviations of the pH from the control point are primarily due to changes in the flow rate of milk of lime or, in Steffen houses, of saccharate milk.

By its very nature, feedback control cannot prevent process upsets; it can only restore the process after an upset has occurred. The time required for restoration depends to some degree on the magnitude of the upset. When the saccharate flow rate changes frequently, the pH of the effluent may oscillate above and below the control point for extended periods, because with feedback control, corrective changes of the controlled variable are not instantaneous.

Obviously, if the flow rate of kiln gas could be changed instantaneously to the proper value, when a change in the flow rate of saccharate occurs, pH deviations from the control point would be eliminated. The instrumental requirements for such a control system become evident when we regard the carbonation process as a continuous acid-base titration. When the composition and flow rates of kiln gas and saccharate milk are held constant, the pH of the carbonated juice will remain essentially constant because the ratio of acid/base flow is constant. Conversely, to maintain a constant pH, it is necessary to maintain a constant ratio of kiln gas/saccharate milk flow rates.

Based on these considerations, a carbonation control system was developed and installed at the Woodland, California factory

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of Spreckels Sugar Company. The control principle involved here is primarily feedforward control, wherein the kiln gas flow rate is maintained proportionately to the saccharate flow rate. The heart of this system is a ratio relay which maintains the desired ratio of the two reagent streams. The ratio is continuously variable over the range of 0:1 to 2:1. The response of the relay to input changes is shown on the graph (Figure 1).

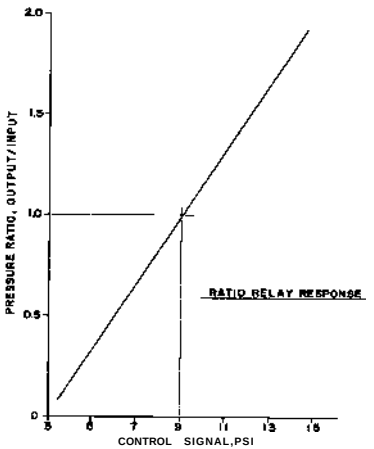

Figure 1.—The ratio of relay response to input changes.

Since our control system is pneumatic, relay functions are shown here in units of air pressure. The output pressure from the relay is a function of the input pressure and the control signal pressure. With a control signal of 9 psi, for example, the ratio of output/input pressures is unity. Thus, if the input pressure is 10 psi, the output pressure is also 10 psi. When the control signal pressure is increased to 12 psi, while the input pressure remains at 10 psi, the output pressure from the relay increases to 15 psi. Conversely, when the control pressure is reduced to 6 psi, the output pressure drops to 5 psi. Thus, the ratio of output/input pressure is set by the control signal pressure.

With a control pressure of 6 psi, for example, the ratio is 0.5. An input pressure of 8 psi will thus produce an output pressure of 4 psi; an input pressure of 10 psi produces an output pressure of 5 psi, etc.

When the relay is connected into the carbonation control system, in a manner where the input pressure represents the flow rate of saccharate milk to the carbonator, then the output pressure can be utilized to control the flow of kiln gas. The alkalinity of the carbonated juice can then be controlled by adjustment of the control signal pressure. The control pressure can be derived from the output of the pH meter. It is only necessary that the control pressure be increased, when the pH is above the desired value and vice versa. The basic control system used at our Woodland factory is shown in Figure 2.

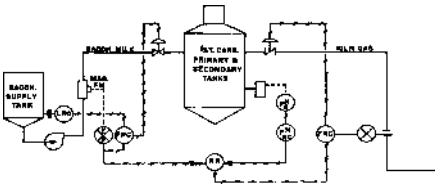

Figure 2.—The basic control system used at the Woodland factory of the Spreckles Sugar Company.

Woodland is a Steffen house and the production rate of saccharate milk is subject to cyclic variations which are the result of periodic filter changes. The saccharate milk supply tank—at the left of the drawing—acts as a buffer tank to smooth out the flow of saccharate to the carbonation system. Saccharate milk temperature and density are controlled automatically.

The saccharate supply tank has a level sensing device and a level recording controller (LRC). The output from the level controller is connected to the saccharate flow recorder-controller (FRC), in cascade. Thus, the saccharate flow rate follows the tank level; when the level increases, the flow rate also increases, and vice versa. The saccharate flow rate is sensed by a magnetic flowmeter; its electric output is converted into a pressure signal by the transducer (EP). This pressure is proportional to the flow rate.

The pressure signal is fed to the ratio relay (RR) as the relay input pressure. As shown earlier, the relay output pressure is always proportional to the input pressure. The output pressure

is cascaded into the kiln gas flow recorder controller (FRC) and thereby maintains a gas flow rate which is proportional to the flow rate of saccharate milk. A change in the flow rate of saccharate milk thus produces instantaneously a proportional change in the flow rate of kiln gas, and the alkalinity of the carbonated juice remains essentially constant.

The ratio of kiln gas/saccharate milk flow rates is controlled by the output pressure from the pH recorder-controller. The alkalinity of the carbonated juice can thus be controlled by adjustment of the setpoint of the pH controller.

The system described here is a combination of feedforward and feedback control. Process upsets due to changes in the flow rate of saccharate milk are eliminated by the feedforward control function. Feedback control from the pH meter stabilizes the process and maintains the selected pH value. Campaign tests have shown that control of carbonation juice alkalinity with the new control system is far superior to that achieved with the original feedback control. Installation of the new system in other Spreckels factories is nearly completed.

The system is by no means limited to Steffen houses. In straight houses, an additional ratio relay can be profitably employed to keep milk of lime flow proportional to raw juice flow, thus maintaining a constant percent CaO. The balance of the system then remains substantially as shown. When changes occur in raw juice flow, simultaneous changes in milk of lime and kiln gas flow assure constant percent CaO and constant carbonation juice alkalinity. The carbonation control at the Chandler factory of Spreckels Sugar Company, a straight house, employs a system of this type.

Observations on the Absorbancy of Sugar Solution

KARLHEINZ W. R. SCHOENROCK[1]

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Introduction

The evaluation of sugar quality is one of the prime factors in the manufacture of refined sugar. Among the quality tests for sugar, the determination of sugar color is one of the more important ones and, consequently, has received a great deal of attention in recent years.

In spite of the unreputed importance of so-called sugar color, the industry, and with it the consumer, are far from being in agreement on such questions as:

1. What does sugar color mean?
2. How is sugar color precisely measured?
3. What impurity is responsible for the color?
4. How can we relate color to impurity?
5. How can the coloring impurity be removed most economically in the refining process?

Carpenter & Deitz (l)[2] called attention to the disparity in the objective of measuring so-called sugar color.

The practice of blueing sugar is a well-known method of improving the visual color rating without enhancing real quality. On the other hand, a common European practice of grinding larger sugar crystals to a specific grain size causes a loss of sparkle and luster to visual appearance without changing basic quality.

ICUMSA has proposed several tentative methods for measuring sugar color in solution. However, the scientific evidence to promote specific guidelines such as cell length, sugar concentrations or wavelength respectively is very weak. Most generally accepted is a wavelength of 420 m/μ, a concentration of 50% sugar and a cell length of about 5-10 cm.

The objective in the past was primarily an attempt to assess the visual, yet superficial, discoloration rather than to measure the precise amount of color-causing impurities. By sticking too close to the former guidelines, we may forsake the opportunity to expose the answer to our real color problem.

[1] Research Chemist, The Amalgamated Sugar Co., Twin Falls, Idaho.

[2] Numbers in parentheses refer to literature cited.

Procedures and Discussions

All tests were carried out with a Beckman DU Spectrophotometer with power supply and photomultiplier. Matched sets of 10 mm silica cells accurate to within ± 1% T of each other at 100% transmittance filled with double-distilled, deionized water and tested at a wavelength of 220 m_μ were used. Careful cleaning and preparation of the cells were found to be essential to avoid erratic results. Larger cells for solution depth of 5 cm and 10 cm respectively seemed to give fair results above 400 m_μ but were obviously not suitable at the shorter wavelength.

The effect of optical abberation due to a change in the wavelength was investigated and found to be negligible within the scope of this investigation. The slit width was ranged between 0.15 - 0.3 mm for the tungsten lamp and between 0.2 - 0.6 mm for the hydrogen lamp. The pH variations between pH 4.8 - 10.0 had very little effect on absorbancy readings above 250 m_μ for granulated sugars. Below 250 m_μ the high pH sugar solutions gave substantially higher absorbancy readings.

Attenuation indices were similar to those found by Carpenter & Deitz (1) throughout the ultraviolet spectrum when using a hydrogen lamp as a source of energy. However, type 50 invert solution gave peak absorbancy around 280 m_μ while most granulated sugars peaked around 265 m/x.

With a tungsten lamp as a radiation source and a stray energy filter a sharp absorbancy peak was found at 295 m/x for all specimens tested. Peak absorbancy occurred at 300 m/x for the tungsten lamp without the use of the stray energy filter. This comparison is shown in Figure 1 for type 50 invert solution.

No explanation is offered at this time for the disparity in spectral response to the two energy sources with and without

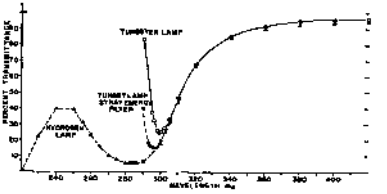

Figure 1.—Transmittance curves for Type 50 Invert. Variation due to different radiation sources with DU Spectrophotometer. 35% sugar solution.

the stray energy filter. The primary objective of this stduy was to take advantage of the maximum absorbancy with the tungsten lamp at 300 m_μ.

Figure 2 compares the transmittance curves over the upper U.V. spectrum for a beet sugar and a type 50 invert sugar produced from the beet sugar through cold inversion. Attention is called to the fact that both sugars had nearly identical attenuation indices at the popular wavelength of 420 m_μ and using the Bernhard-Phoenix Sphere Spectrophotometer.

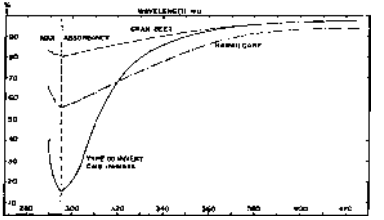

Figure 2.—Transmittance curves for 35% sugar solutions. Model DU, 10 mm cells, tungsten lamp with corex filter below 310 m_μ.

However, when viewed through large solution depth, e.g. 10 ft, the type 50 had a more intense yellow cast. It is not unreasonable to expect a certain amount of degradation even through cold inversion. A cane sugar has been added for comparison.

Further treatment of the type 50 syrup over the CI- form of a strong base anex resin elevated the attenuation index of the syrup over that for the original sugar.

The impurities thusly removed from type 50 were subsequently stripped from the anex with salt solution. A family of transmittance curves over the U.V. spectrum was prepared from the salt solution to explore maximum absorbancy. Figures 3 and 4 illustrate this comparison.

Note a pronounced peak absorbancy at 295 m_μ. Another shallow peak can be observed at 265 m_μ. Figure 5 verifies that the color impurities removed from the type 50 syrup obey the law of Beer at maximum absorbancy. Although observance to that law is exhibited at 420 m_μ the steeper slope at 295 m_μ allows for superior measurements.

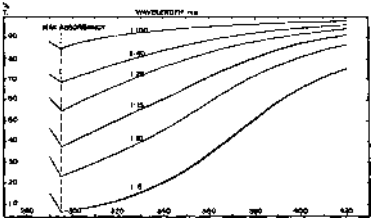

Figure 3.—Transmittance curves for coloring matter isolated from Type 50 invert (cold inversion). Model DU, 10 mm cells, tungsten lamp with corex filter.

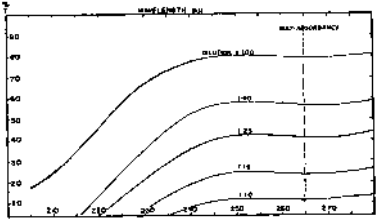

Figure 4.—Transmittance curves for coloring matter isolated **from** Type 50 invert (cold inversion). Model DU, 10 mm cells, hydrogen lamp.

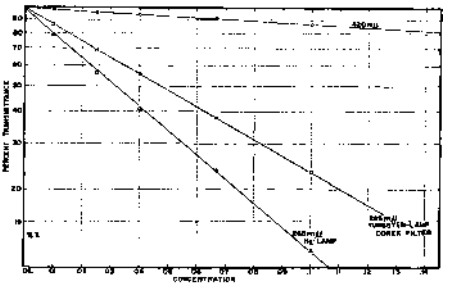

Figure 5.—Concentration vs transmittance for coloring matter isolated from Type 50 invert.

Obedience to that same law is illustrated for various sugars in Figure 6. The data for Figure 6 was obtained at 300 m_μ with the tungsten lamp and without the "corex" filter.

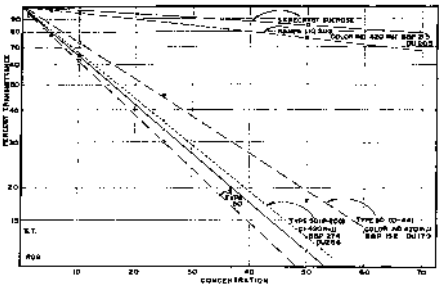

Figure 6.—Concentration vs transmittance for various sugars at 300 m_μ. Model DU, 10 mm cells, tungsten lamp.

Of significant importance is a comparison of the color index at 420m_μ for the Narapa liquid sugar and the 0-44 Type 50 respectively. It is obvious that absorbance at 420 m_μ did not reflect the normally expected inferior color quality for Type 50. The close agreement between the values obtained via Bernhard-Phoenix Sphere Spectrophotometer and model DU Spectrophotometer is also of interest suggesting that turbidity had negligible bearing on these results.

No attempt was made to compensate for the influence of scattering for any of the tests with the D.U. The selection of peak absorbancy at the short wavelength allows reduced sugar concentration and cell depth respectively thus minimizing the effect of scattering.

A variety of sugars, including local beet, Hawaiian cane, Japanese cane and beet and European beet from different areas were investigated for their peak absorbancy.

Figures 7 and 8 show some of the results.

Some highly refined sugars such as triple re-crystallized sucrose as well as sugar from liquors treated via ion exchange were included in these tests. All sugars exhibited maximum absorbancy around 300 m_μ with a tungsten lamp as a radiation source and 265 m_μ using a hydrogen lamp, respectively.

Grading of all sugars tested with regards to their refining qualities was an easy task on the basis of their absorbancy at 295-300 m_μ, while grading was somewhat obscured when based on absorbancy at 420 m_μ.

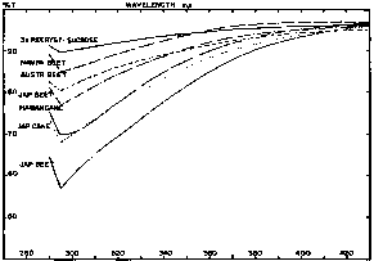

Figure 7.—Transmittance curves for various sugars 35% Model DU, 10 mm cells, tungsten lamp with corex filter.

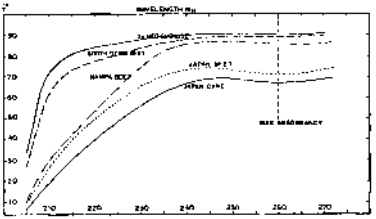

Figure 8.—Transmittance curves for various sugars 20% Model DU, 10 mm cells, hydrogen lamp.

As a rule, cane sugars did not fare as well as beet sugars on this basis. This perhaps can be traced to the relatively high invert load which most cane liquors carry. It is furthermore no secret that the cane industry has a more severe color problem than the beet industry. The two factors, namely invert and color, are undoubtedly connected. Absorbancy at 300 m_μ clearly reveals this problem while absorbancy at 420 m_μ does not contribute towards identification of impurities.

Standard liquor was also investigated for its transmittance spectrum in the U.V. spectrum. Trends similar to that for sugar were found. Figure 9 shows the results.

Prey and co-workers (2,3,4,5) have done extensive pioneering work on the absorbancy of specific model substances which are germain to the liquors in the sugar processing industry. These reaction products, usually degradates of invert, protein

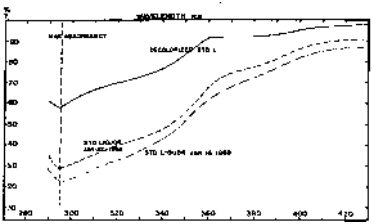

Figure 9.—Transmittance curves for sugarbeet processing liquors 1 RDS, Model DU, 10 mm cells, tungsten lamp with corex filter.

and pectin, exhibit maximum absorbancy in the U.V. spectrum. According to published data maximum absorbancy around 300 m_μ was usually observed for the primary reaction products of dihydroxyaceton, glycerinaldehyde, fructose, glycyl -1- tyrosin and others during hydrolysis.

Since peak absorbancy at 300 m_μ, seems to be specific for all sugars, it appears feasible to relate the absorbancy to a specific impurity thus expressing color as a more realistic quantitative entity.

Furthermore, it could be deducted from the studies of Prey and co-workers that the substances with peak absorbancy around 300 m_μ are merely intermediate forms giving rise to highly colored substances when exposed to further hydrolysis. This seems to be in agreement with the observation that the solution of an inferior sugar discolors faster when heated than a comparable test with a high quality refined sugar. Invert type sugar solutions deteriorate faster yet. The heating test of the Braunschweiger point system is apparently predicated upon this observation.

Conclusions

It appears that impurities with specific absorbancy bands around 300 m_μ and using a tungsten lamp as the energy source are common to all refined sugars.

Although the tungsten lamp is normally not recommended as an energy source for spectral measurements around 300 m_μ, its ability to resolve apparent differences in the concentration of colorant impurities suggests its application for sugar quality evaluation at the specified short wavelength.

The possibility of using relatively low sugar concentrations, e.g. < 35%, short cell depth, e.g. 10 mm, at maximum absorbancy

should avoid interference from normal turbidity. Identification of the coloring impurity may allow its expression as a quantitative entity.

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Waste Water Recirculation as a Means of River Pollution Abatement

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The Manitoba Sugar Company Limited operates a beet sugar factory in Fort Garry, Manitoba adjacent to the Red River in a suburban area upstream from Metropolitan Winnipeg. Water from the river is available for processing purposes but, because of the heavily populated area through which the river flows, the return of the factory effluent is undesirable though this was tolerated for the first 15 years of operation.

As the city grew the river could not absorb the increasing load of domestic and industrial wastes, and the establishment of a government sponsored commission to control this pollution was inevitable. The Commission was given considerable powers to force both industry and municipalities to conform to standards of quality for effluent discharge, and these powers were later augmented by the formation of a "Metro" system of government for Winnipeg in 1960.

Industry was required to contribute to the cost of sewage treatment in the metropolitan area on the basis of the volume of untreated wastes discharged to the river or public treatment facilities; effluents exceeding set levels of quality were subject to additional surcharges. The Company's sewage charges had exceeded $64,000 per year prior to the installation of the recirculation system, and, therefore, it was under considerable economic as well as public pressure to reduce or eliminate its pollutional load to the river. This posed a formidable problem for, although the factory wastes were similar to those from other beet sugar operations, the soil conditions and extreme climate contributed difficulties not commonly experienced elsewhere.

Climatically, extreme cold can be expected during much of the processing season, and freezing weather is usually continuous after November 1st. Thus, all harvesting must be completed before freeze-up which means that some two-thirds of the beet crop is piled. The cold weather further influences the handling methods because moving beets in rail cars from outside points in temperatures below 10°F is difficult, if not impossible. For that reason, beets grown in more distant areas are railed to the

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factory during harvest to be unloaded with clamshell buckets into piles. This procedure plus subsequent reloading and transport to the wet hopper causes a great deal of mechanical damage to the beets and provides broken surfaces from which sugar is leached by the flume water. Sugar pick-up by the flume water is also increased if there is any deterioration of the beets in the storage piles, which will occur should "hot spots" develop.

The soil in much of the beet growing area is classified as clay loam, commonly called "gumbo". In wet seasons, large amounts adhere to the beets so that the flume water picks up an enormous load during the transport of beets into the factory and during the washing process.

From the foregoing it can be seen that any treatment system mast accommodate dissolved organic matter and suspended solids in unusually large amounts.

Methods and Description

Before describing the recirculation system, it is sufficient to state that pulp press water and lime cake are not discharged; the former is returned to the diffuser and the latter is lagooned. Plans are presently underway to install a semi-dry lime mud handling system.

Formerly, flume water supply was passed through the evaporator and pan condensers prior to being used to transport beets. Since the quality and amounts of water required for the two functions are unrelated, it was obvious that separation of the two streams would simplify the design of a recirculation system.

First, it was established that water from the Red River for condenser injection could be returned to the river without penalty provided levels of contamination were sufficiently low. Since facilities existed for pumping water from the river, no attempt was made to establish a closed recirculating cooling water system.

Knowledge of recirculation systems used by the British Sugar Corporation provided a basis for study while the report presented by S. Force[2] of The Great Western Sugar Company provided further important data. From these, a scheme applicable to Manitoba conditions was worked out and put into operation in 1965.

For fluming, an estimated flow of 5,000,000 imp gal per day would have to be maintained in a closed system, requiring a volume of about 350,000 imp gal to fill a clarifier, surge tank,

[2] Force, S.L., 1965. The Findlay Flume ani condenser water svstem. J. Am. Soc. Sugarbeet Technol. 13(6): 478-491.

sludge beds, and pipelines and provide detention time for clarification.

Examination of the flume water effluent in the laboratory had indicated that excellent coagulation and sedimentation could be obtained by adding lime to an alkalinity of pH 11.5+. Further tests were made by a clarifier manufacturer in designing a settler that would handle the flume water flow, and an overflow acceptable for transporting and washing beets. This was incorporated into the system, illustrated in Figure 1, consisting of a screening station, clarifier, sludge handling and clarified water supply system.

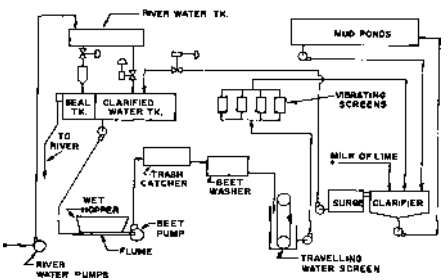

Figure 1.—Flow diagram of flume water system.

The flume water screening station consists of a travelling water screen which gives a primary removal of coarse organic material for the entire flow. In series with this are four 4 X 8 ft vibrating screens with decks of 6 X 6 mesh operating in parallel. The screened water flows to the intake launder of the clarifier where water-slaked milk of lime is added to raise the alkalinity to the desired level (pH 11.5 +). The clarifier overflow enters a surge tank and is pumped to the flume water supply tank as required.

Provision has been made to use clarified water in the evaporator condenser to maintain the temperature of the water at a suitable level during extremely cold weather. This has proven to be simple and effective.

The underflow from the clarifier is pumped to a disposal area which is graded to give good drainage to a collection ditch. Sedimentation of the sludge has been rapid and the clear water flows to a pump house to be returned to the clarifier. Under

most conditions, this water has a higher clarity than the clarifier overflow.

There has been a tendency to form lime scale in the pump and pipe lines of the return water system from the sludge beds. This has been easily controlled by flushing the pump with muriatic acid at infrequent intervals, and alternating the direction of flow between the outgoing mud line and the return water line every 3 weeks. The scouring action of the soil in the underflow has been sufficient to prevent a build up of scale in the lines.

The sludge collection beds (Figure 2) consist of two adjacent areas 1,360 ft long by 450 ft wide with an average depth of 2 ft. Each area is divided by cross dikes into cells to give one section 440 ft X 450 ft and four sections 230 ft X 450 ft. The two adjacent areas are used in alternate years in order that the collected sludge will have a year or more to stabilize before it is excavated. This cell structure provides better drainage than one large area where low spots allow water to accumulate and become

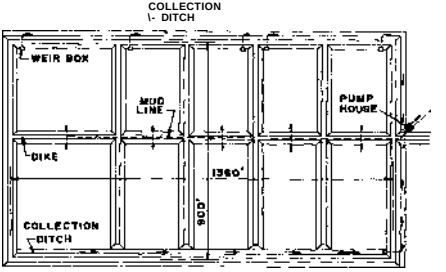

Figure 2.—Mud settling ponds.

septic. An odor problem does not occur where good drainage exists.

The clarified water is used for all fluming operations and in the beet washer. Provision was made for a final spray rinse with fresh water as the beets pass over the roller table between the washer and elevator, but recirculated flume water has been used here without any problems. Thus for all practical purposes, recirculated clarified flume water successfully replaces fresh river water in every way for fluming, washing and rinsing beets.

It may be observed here that recirculation of flume and condenser water has already had a considerable effect on water

economy of several factories, and selection of new factory sites no longer depends on the availability of large volumes of fresh water.

Some problems with foam were encountered particularly in the flume and beet washer. However, effective control has been achieved by the use of commercial defoaming agents. Tests carried out during the 1967 campaign show emulsified tallow to be the most effective and economical defoaming agent used to date.

A closed circuit operation requires careful control of any loss or gain in the volume of water contained in the system. Make-up to compensate for loss is relatively easy to regulate by automatic injection of fresh water on low level signal from the flume water supply tank. A gain in volume is a more difficult matter and originally was handled by using clarified water to prepare lime cake slurry for pumping to the lime lagoon. This gave a continuous bleed-out from the system and allowed a corresponding fresh water make-up.

During the two summers following the use of clarified water in the lime slurry, very strong and unpleasant odors developed in the lime pond. While poor drainage undoubtedly contributed to this, the highly contaminated flume water was considered to be a contributing factor. Hence in 1967, fresh water was used for lime cake disposal and any excess of flume water in the clarifying system was run out to some spare land. Due to unusually dry fall weather this was absorbed without difficulty and the effect of this practice in wetter years must wait for future developments.

It should be pointed out that the soil in the Fort Garry area allows little loss by percolation and that precipitation is about equal to the natural evaporation. Thus, there is little hope that any large quantity of the waste water can be disposed of in this manner.

The installed cost of the system including the clarifier, surge tank, two mud ponds and associate pumps, piping and controls, but excluding the travelling water screen and four vibrating water screens amounted to approximately $300,000.

Apart from the cost of providing the facilities and their maintenance, the only continuing item of expense is the lime required to promote flocculation and to raise the alkalinity to prevent fermentation. It has been found that the dosage of lime increases with time and appears to bear a relationship to the increasing concentration of dissolved solids. The lime addition has varied between 1.5 and 4.5 tons of CaO per day; it is obtained from the factory kiln and slaked with water to make milk of lime.

The concentration of dissolved solids in the recirculated water builds up steadily during the first 6 weeks of operation but levels out at about 10,000 ppm of total dissolved solids and 6,000 ppm BOD. The progress in this change in concentration is illustrated in Figure 3.

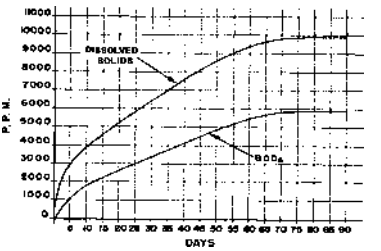

Figure 3.—Build up of dissolved solids and BOD_5 in the recirculated water.

The ultimate dissolved solids concentration in the recirculated flume water is presumed to correspond to an osmotic pressure equivalent to that inside the beet, so that no osmotic pressure differential exists to promote further extraction of the sugarbeet solids.

This situation can arise at dissolved solids concentrations in flume water appreciably below those in the sugarbeet because of the lower molal weights associated with the calcium and hydroxyl ions. The ions nevertheless have the same osmotic pressure as molal amounts of sugars and proteinaceous materials contained in the sugarbeet. As no fermentation occurs in the flume water, due to its high alkalinity, once this equilibrium has been established, the extraction of sugar may be inferred to approach zero.

The effect of this observation on loss of sugar in flume water is illustrated by estimating the quantity released in a non-recirculating system. Based on a usage of 1,700 imp gal of flume water per ton of beets which contains 200 ppm BOD_5 having a sugar equivalent of 1.4 times BOD_5, the quantity of sugar per 1,000 tons of beets is 4,760 lbs. Thus a significant saving of sugar results when the equilibrium point has been reached, which is an observation worthy of some attention.

As far as the sludge beds are concerned, there has been no difficulty with odor, except where water has lodged in small pools which did occur after the 1966 campaign. The collected soil apparently stabilizes within a year, for the first lagoons were

partially excavated about 7 months after being deposited, when soil was required for land-fill, and no obnoxious smell was released. It is expected that no problems will arise in disposing of the dry sludge.

Conclusions

The project described in this presentation has been successful in controlling a serious source of river pollution in a heavily populated area. By this action, the Company has been saved substantial disposal charges that would have been levied had the effluent been passed on to the Metro treatment plants. On this basis, the recirculation system has been financially successful. At the same time, one of the aims of the Metro government was to restore the aesthetic and recreational value of the Red River and it is, therefore, a matter of satisfaction to the Company that they have been able to contribute towards this objective.

Acknowledgments

Recognition is given to Mr. A. Penman, Director of the Waste Disposal Division of the Metropolitan Corporation of Greater Winnipeg, for his co-operation and technical advice.

This recirculation system was designed by the staffs of the Manitoba Sugar Co. Ltd. and the B. C. Sugar Refining Co. Ltd., and was based on the work of the B. C. Research Council which investigated the problems of sedimentation and stabilization of flume water.

The assistance of Dr. C. C. Walden of the B. C. Research Council who contributed to the development of this system and the preparation of this paper is gratefully acknowledged.

Virus-yellows Infected Sugarbeet Varieties: Effects of Harvest Dates and Nitrogen Fertilization

R. T. LEWELLEN, J. S. MCFARLANE, AND I. O. SKOYEN[1]

Received for publication May 1, 1968

Investigations in California have shown that the yellows disease of sugarbeet can cause up to 65% root yield loss, depending upon the yellows viruses involved, the virulence of the virus strains, time of infection and variety (1,2,3,6,9,10).[2] For a particular variety under similar virus conditions, root losses were consistent from test to test. However, the effect of yellows infection on sucrose percentage has remained inconclusive.

Yellows infection was also shown to influence purity. Bennett, Price, and McFarlane (3) found that under some conditions purity was slightly decreased with increases in sodium and potassium, but no change occurred for amino nitrogen. Goodban *et al.* (8) noted a slight decrease in processing quality and increased soluble nitrogen. Fife (7) demonstrated amino acid differences in infected and healthy plants.

Until recently, varieties with resistance to yellows were not available for comparison to determine the effect of yellows infection on root yield, sucrose percentage, or purity. From the breeding program at Salinas, California, several lines that show moderate resistance to yellows have been developed. Until lines with higher resistance are developed, these lines will be used as sources of resistance and as components in hybrid varieties. Currently, these lines with moderate resistance can provide the best control from losses by yellows. Because these lines will have to be grown under potential yellows infection, it is of interest to know how they perform in comparison to susceptible varieties.

The purposes of this experiment were to determine: (a) when sucrose losses occur in yellows infected lines; (b) how yellows affect a moderately resistant line in comparison with a more susceptible parental variety; (c) how yellows infection influences purity constituents; and (d) how the level of nitrogen fertility affects sucrose loss, resistance and purity factors for these populations. Nitrogen is known to influence sugar percentage, yield and quality characters of sugarbeet. Differences in nitrogen fertilization should help us to interpret the effects of yellows infection on sucrose loss and varietal resistance.

[1] Research Geneticists and Research Agronomist, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Salinas, California.

[2] Numbers in parentheses refer to literature cited.

Methods and Materials

The primary data for this experiment were obtained at Salinas, California in 1967. Additional data were obtained from the 1967 yellows-resistance evaluation trial at Davis, California. The varieties used were open-pollinated US 75 (11) and moderately resistant selections 413 and 613. Line 413, a fifth successive yellows-resistance mass selection from US 75, was used in the Salinas test. Selection for resistance was based upon root size and freedom from yellowing in infected populations. Line 613, used in the Davis test, was selected two additional times for yellows resistance, but *in* several tests it appeared to be identical to 413. These selections will be called line 13.

The Salinas test was planted February 23, 1967. The Davis planting was made May 23 after aphid flights had ceased and the likelihood of natural infection was low. A combination of a virulent strain (strain 7) of beet yellows virus (BYV) and beet western yellows virus (BWYV) was used to inoculate the beet plants in approximately the 10-leaf stage, as previously described by Bennett *et al.* (3). The Salinas and Davis inoculations were made on May 2 and July 20, respectively. Entire plots were inoculated at Salinas because natural spread cannot be prevented (9). At Davis comparisons were made between inoculated and noninoculated subplots.

A split-block design with eight replications was used at Salinas. Each replication was divided into two nitrogen fertility treatments. Treatment 1 received 110 pounds of nitrogen, applied as ammonium sulfate in equal applications, at planting and on May 16. This treatment represented slightly less than our normal application to varietal trials for this planting date. Treatment 2 received the same applications as treatment 1 but in addition had 60 pounds applied to it on July 6. Treatment 1 was designed to become nitrogen deficient as the season progressed, whereas treatment 2 was designed to provide sufficient nitrogen throughout the growing season.

Within each split block at Salinas, the two varieties and eight harvest dates were completely randomized. At 2-week intervals, from July 25 to October 31, plots were harvested. Individual plots were single rows 32 ft long. On each side of the plots buffer rows were planted to prevent border effects due to differential dates of harvest. Each harvested plot was placed in two bags and cleaned weights were taken. The roots were rasped and the **pulp** frozen until four harvest dates could be run at the same time. Data were obtained on root yield, sucrose percentage,

amino nitrogen, sodium and potassium. Amino nitrogen (NH_2-N), Na and K were determined from part of the lead-acetate filtrate used for the sucrose determination. Amino nitrogen (or noxious nitrogen) was determined in a spectrophotometer with Stanek-Pavlas copper reagent. A flame spectrophotometer was used for Na and K determinations.

To determine the nitrogen status, petioles were collected at each harvest date and petiole nitrates were determined by the method of Ulrich *et al.* (19).

The Salinas experiment was sprinkler-irrigated each week. Irrigation rates were governed to avoid as much movement of nitrogen between plots as possible.

The Davis data for US 75 and line 13 were derived from a variety trial, with blocks split for inoculated and noninoculated treatments. The trial was harvested on October 23. Data were obtained for yield, sucrose percentage, NH_2-N, Na and K. Natural spread of yellows from inoculated to noninoculated plots was minimal.

Results and Discussion

The results of the Salinas and Davis tests are presented in Tables 1 to 4 and Figures 1 and 2. Table 1 gives the levels of significance obtained for varieties, dates of harvest and nitrogen treatments at Salinas, and varieties and infection treatments at Davis, for the characters studied. Figures 1 and 2 give the mean values within each date of harvest for the seven characters at Salinas. Table 2 gives the means within each variety, nitrogen, or infection treatment. Tables 3 and 4 give the means for the significant, first-order interactions.

Table 1.—Levels of significance obtained for main effects and interactions for varieties, dates of harvest, and nitrogen levels at Salinas and varieties and infections at Davis for seven characters.

	Root yield	% sucrose	Gross sucrose	Petiole Root nitrate	NHs-N	Na	K
			Salinas				
Varieties	**	**	**	**	**	**	NS
Dates	**	**	**	**	**	**	**
Nitrogen	**	**	NS	**	**	**	NS
V × D	**	**	**	*	**	**	NS
V × N	NS	NS	NS	**	**	**	NS
D × N	NS	**	NS	**	NS	NS	NS
V × D × N	NS	NS	NS	**	NS	NS	NS
			Davis				
Varieties	**	NS	**	....	**	**	**
Infection	**	**	**	...	(*)	NS	NS
V × I	**	*	**	....	(*)	NS	NS

(*) = Signficance at the 10% level.
* = Significance at the 5% level.
** = Significance at the 1% level.

Dates of Harvest

Duncan's multiple range test (0.05 level of probability) showed that the mean yield of beets over all varieties and treatments significantly increased every harvest from the first (9.1 T/A) to the sixth date (19.9 T/A) with the rate of increase being nearly linear (Figure 1). The sixth and seventh dates were not significantly different, but the eighth date showed a significant yield reduction from the seventh date.

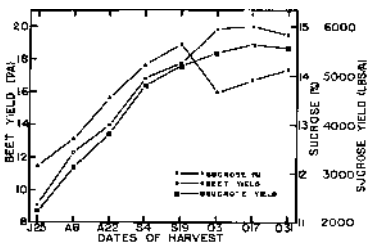

Figure 1.—Mean values for each date of harvest over all treatments for beet yield, sucrose percentage, and sucrose yield at Salinas.

Sucrose percentage also significantly increased for each date in a linear fashion through the fifth date from 12.2% to 14.6%. Between the fifth and sixth dates the decrease was significant to 13.6%. Increases for the remaining two harvests were slight. The highest sucrose percentage occurred in mid-September, with decreased percentages in late September and October.

The decrease between the fifth and sixth dates was probably not caused by increased NO_3 availability. Because sprinkler irrigation was used, a light rain (.18 inch) that occurred just before harvest could not have caused appreciable NO_3 leaching as suggested by Stout (16).

The growth curve for gross sucrose is the product of the curves for root weight and sucrose percentage. Therefore, the gross sucrose curve forms a more accurate indication of sucrose production than the curve of either of its components. Significant increases in gross sucrose occurred through the sixth date. However, after the sixth date, sucrose production leveled out. The eighth date showed a slight decrease from the seventh. The sixth, seventh, and eighth dates were not significantly different. Figure 1 shows that the mean gross sucrose curve does not have

the significant drop between the September 19 and October 3 dates as shown by the sucrose percentage curve. Instead, there appeared to be compensation by increased root yield.

It is of interest that reduced sucrose percentage and increased beet yield occurred during this part of the year, especially when one expects root growth to cease and sucrose percentage to increase. In the coastal valleys of California, summer temperatures may be lower than the temperatures of early fall. At the time sucrose percentage decreased, there was a warmer period which may have stimulated increased plant growth.

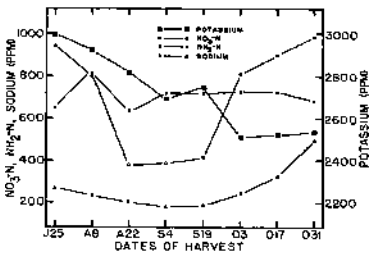

Figure 2.—Mean values for each date of harvest over all treatments for petiole nitrate, amino nitrogen, sodium, and potassium at Salinas.

As shown by Figure 2, petiole-NO_3 concentration started at a relatively high level, rapidly decreased and remained low through the part of the season in which rapid growth occurred, and then rapidly increased again when growth essentially ceased. The values for the third, fourth and fifth dates were significantly different from the values for the other five dates. This pattern was probably produced by the relatively high availability of NO_3 at the beginning of the experiment followed by decreasing availability and continued rapid growth and use of NO_3 through mid-September. However, during the last 6 weeks, growth and use of nitrogen were apparently reduced and NO, increased in the petioles. Other studies have not shown this increase in nitrate concentration during the fall months (4,13,17).

The concentration of root NH_2-N did not follow a curve similar to petiole-$N0_3$ over the 4 months that plots were harvested, but remained nearly constant at 680 to 730 ppm except

for a significantly higher level at the second date and significantly lower levels at the first and third dates.

Sodium generally showed a decreasing concentration in the roots while rapid growth was occurring, but the concentration increased in the fall as growth slowed and stopped. Na concentration significantly decreased from 270 ppm for the first harvest to 180 ppm for the fourth. After the fifth harvest Na increased significantly for each harvest and by the eighth date was twice as high as the first and nearly three times as high as the fourth.

There was a significant decrease in K concentration from the first date to the eighth date of harvest. Except for a slight increase for the fifth harvest, concentrations decreased to the sixth harvest. The seventh and eighth dates showed slight increases that corresponded with the period of decreased growth rates for beet roots.

Variety and Infection Effects and Variety X *Infection Interactions*

The US 75 variety and resistant selection 13 were significantly different for all characters except K at Salinas and sucrose percentage at Davis (Table 2). At Salinas, under uniform yellows infection, 13 was about a percentage point higher in sucrose and had 56% greater root weight to yield 68% more gross sucrose than US 75.

Table 2.—Mean values within variety or nitrogen treatments at Salinas and variety or infection treatments at Davis.

	Root yield T/A	Sucrose %	Gross sucrose lbs/A	Petiole nitrate ppm	Root NH_2-N ppm	Root Na ppm	Root K ppm
Salinas							
Varieties							
US 75	12.6**[1]	13.1**	3340**	905**	815**	342**	2654
Line 13	19.7	14.1	5620	499	601	189	2784
Nitrogen							
110	15.9**	13.9**	4480	510**	639**	235**	2675
170	16.4	13.4	4480	894	778	297	2763
Davis							
Varieties							
US 75	17.3**	12.4	4350**		978**	786**	3193**
Line 13	19.8	12.7	5050		655	432	3495
Infection							
Check	22.3**	13.0**	5780**		762(*)	566	3341
BYV-BWYV	14.9	12.1	3620		871	652	3347

[1] Indicates level of significance for each pair of means where (*), *, and ** are at the 10%, 5%, and 1% levels, respectively.

Yellows infection at Davis significantly reduced yield, sucrose percentage and gross sucrose as compared to noninoculated checks (Table 2). Significant variety X infection interactions occurred for yield, sucrose percentage and gross sucrose (Tables 1,3), whereas NH_2-N interacted significantly only at the 10% level and Na and K showed nonsignificant differences.

It appeared that the variety difference in sucrose yield was primarily due to differences in resistance to yellows. At Davis US 75 and 13 were not significantly different for root yield or sucrose percentage when grown under noninoculated conditions (Table 3); but under inoculated conditions selection 13 was 0.8 percentage point higher in sucrose and had 38% greater root weight to yield 48% more gross sucrose than US 75. These data suggest that in selecting line 13 from US 75 for resistance to yellows, the production factors for gross sucrose were not appreciably changed under virus-free conditions, but that the increased performance under yellows is due to resistance factors that allow existing yield genes to function more efficiently.

The mean petiole-NO_3 concentration of line 13 was significantly lower than for US 75 at Salinas. Petioles were not sampled at Davis, and the influence of yellows was, therefore, not determined.

Table 3.—Mean values within nitrogen or infection treatments for varieties at Salinas or Davis, respectively, for those characters that showed siginificant variety x infection or variety x nitrogen interactions.

Variety	Root yield T/A	Sucrose %	Gross sucrose lbs/A	Root NH_2-N PPm	Root Na PPm	Petiole nitrate ppm	Root NH_2N PPm	Root Na PPm
	BYV-BWYV Noninoculated (Davis)					110 lbs N (Salinas)		
US 75	22.1	13.1	5780	870	700	614	716	304
Line 13	22.4	12.9	5780	654	432	406	562	166
	BYV-BWYV Inoculated (Davis)					170 lbs N (Salinas)		
US 75	12.5	11.7	2920	1086	871	1196	915	381
Line 13	17.3	12.5	4320	656	433	592	641	213
LSD (.05)	2.1	.7	550	164	202	161	32	26

At Salinas, 13 showed a significantly lower NH_2-N concentration than US 75 (Table 2). At Davis, line 13 had nearly identical NH_2-N levels under yellows-inoculated and noninoculated conditions while US 75 showed significantly different levels under the two infection treatments (Table 3). Both US 75 treatments were significantly higher than 13's concentrations.

At Salinas and Davis, the 13 selection had a significantly lower Na concentration than US 75 (Table 2). As with NH_2-N at Davis, 13 had nearly identical Na levels under inoculated and

noninoculated conditions; but US 75 did not show a significant difference between infection treatments, even though the inoculated treatment showed a mean increase of 171 ppm (Table 3).

US 75 and selection 13 differed significantly in K concentration at Davis but not at Salinas. Both K and Na concentrations were higher at Davis than Salinas, perhaps indicating greater availability of these elements at Davis. At the higher Davis concentrations, any real differences between varieties would probably be greater and might account for the significant difference. Unlike Na, however, 13 showed the higher K concentration.

Contrary to the sucrose yield factors, it appears that our selection procedure for yellows resistance has decreased NH_2-N and Na and increased K in the resistant line. These two lines have genotypic differences for the levels of these constituents. How or why this occurred is not known, because selections were made without prior information on NH_2-N, Na or R levels.

Whether these variety differences are specific for just the 13 selection from US 75, or whether they occur for all lines that show greater yellows resistance than their parental line, is yet to be determined. If decreased NH_2-N and Na and increased K are linked with resistance to yellows, singly or together, they may serve as a selection criterion or at least as a supplement to our present scheme for obtaining yellows resistance. Russell (14) found that yellows tolerance in sugarbeet was associated with high K content and suggested a close genetic linkage between good tolerance and high K.

Nitrogen Effects

The two nitrogen levels caused highly significant differences in the measured characters except for gross sucrose and K (Table 2). As generally demonstrated with nitrogen treatments, the higher level of nitrogen caused the lower sucrose percentage and the higher root yield. However, in terms of gross sucrose produced, the two treatments produced identical amounts.

As would be expected, the high-nitrogen treatment caused significantly higher levels of petiole-$N0_3$ and NH_2-N than the low-nitrogen treatment. Sodium concentration was also significantly higher for the high-nitrogen treatment, but the K concentration was not significantly influenced by nitrogen. Similar results are reported in the literature for the influence of increasing nitrogen availability on purity characters (5,12,15,16).

Variety X Nitrogen and Date X Nitrogen Interactions

Because there was no significant variety X nitrogen interaction for sucrose percentage, yield, gross sucrose or K, US 75 and 13 were apparently performing in a similar manner for

these attributes at the two nitrogen levels tested (Tables 1,3). The nitrogen levels did not indicate that the resistance of 13 for gross sucrose yield was partially dependent upon the nitrogen fertility.

Significant variety X nitrogen interactions did occur for petiole-$N0_3$, NH_2-N and Na. While selection 13 showed only a mean increase of 186 ppm for petiole-$N0_3$ from the low to high nitrogen treatments, US 75 had a mean increase of 582 pp. Similarly both NH_2-N and Na increased more in US 75 than in 13 from the low to high nitrogen treatments.

Although the selected nitrogen treatments caused these significant responses for the two varieties, it appeared that greater differential rates of application would have given additional information on the influences of nitrogen. According to Ulrich (18), the critical level between deficient and sufficient levels of nitrogen is 1000 ppm $N0_3$-N in the petioles. The only treatment mean above the critical level for sufficient nitrogen is US 75 at 170 pounds nitrogen per acre whereas 13 at this level was in the deficiency range at 592 ppm.

The only significant interactions for date X nitrogen occurred for sucrose percentage and petiole-NO_3 (Table 1). This suggested that the nitrogen level does not cause differential responses for the other characters, or that the nitrogen treatments were not different enough to detect response differences.

Variety X Date Interactions

Significant variety X date interactions occurred for beet yield, sucrose percentage, gross sucrose, petiole-$N0_3$, NH_2-N and Na (Tables 1,4). These interactions indicated that the rate of change for the specific character being measured from date to date was not the same for both varieties. There was no interaction for K.

Under uniform yellows infection at Salinas, 13 and US 75 showed a differential rate of root growth with 13 growing proportionately faster than US 75. Increased growth occurred to the seventh harvest date for both varieties, then decreased for the eighth date. For this decrease in weight only US 75 showed a significant loss.

In the case of sucrose percentage, line 13 increased in sucrose concentration proportionately faster than US 75. At the first harvest there was less than 5% difference, but this difference consistently increased until there was greater than 11% difference at the end of the experiment.

The environmental influences that caused fluctuations in sucrose percentage from date to date appeared to be nearly equal

Table 4.—Mean values within variety treatments for dates of harvest for those characters that showed significant variety x date interactions.

Date of harvest	Root yield* T/A	Sucrose %	Gross sucrose lbs/A	Petiole nitrate ppm	Root NH_2-N ppm	Root Na ppm
			US 75			
1. July 25	7.34[a]	11.89[a]	1740[a]	1282[a]	781[abc]	853[d]
2. Aug. 8	9.71[b]	12.45[b]	2420[b]	1127[b]	959[c]	288[bc]
3. Aug. 22	10.96[c]	13.17[c]	2890[c]	493[a]	725[a]	248[ab]
4. Sept. 4	12.75[d]	13.81[d]	3520[d]	527[a]	825[cd]	225[a]
5. Sept. 19	14.20[e]	14.12[e]	4020[e]	410[a]	810[bcd]	228[a]
6. Oct. 3	15.40[fg]	13.07[c]	4020[e]	1150[b]	863[d]	316[cd]
7. Oct. 17	15.64[g]	13.82[e]	4170[e]	1090[b]	810[bcd]	433[e]
8. Oct. 31	14.86[ef]	13.30[c]	3960[e]	1180[b]	751[ab]	647[f]
			Line 13			
1. July 25	10.82[a]	12.41[a]	2680[a]	611[bcd]	514[a]	186[ab]
2. Aug. 8	14.82[b]	12.95[b]	3840[b]	479[abcd]	651[e]	177[ab]
3. Aug. 22	16.94[c]	13.94[c]	4730[c]	271[ab]	543[ab]	152[a]
4. Sept. 4	20.84[d]	14.58[d]	6080[d]	245[a]	616[c]	137[a]
5. Sept. 19	21.28[d]	15.11[e]	6430[e]	411[abc]	624[c]	137[a]
6. Oct. 3	24.35[e]	14.24[cd]	6930[f]	486[abcd]	602[bc]	166[ab]
7. Oct. 17	24.42[e]	14.54[d]	7090[f]	707[cd]	646[c]	216[b]
8. Oct. 31	24.07[e]	14.95[e]	7200[f]	786[d]	616[a]	343[c]

* Means within varieties followed by the same letter are not siginficantly different (0.05 level of probability) by Duncan's multiple range test.

for both varieties. For example, the reduction between the fifth and sixth dates affected both varieties. However, whereas 13 nearly regained the high sucrose level of the fifth date, US 75 remained stationary with nearly no resumption of increasing sucrose percentage.

Because both sucrose percentage and root weight showed significant interactions, gross sucrose would be expected to, and does. Again 13 increased faster in total sucrose than US 75. For 13 there was an increase in gross sucrose for every progressive date of harvest. US 75 essentially stopped increasing after the fifth harvest and showed a slight decline for the eighth harvest. For US 75 there was no significant difference for harvest dates five through eight. For 13 the sixth through eighth dates differed nonsignificantly but sucrose yield showed a continued increase.

These variety X date of harvest data indicated that the reduction of sucrose yield for the more susceptible US 75 occurred continuously following virus infection and that both sucrose percentage and beet yield were affected. Furthermore, as a consequence of its greater susceptibility, US 75 was unable to sustain continued sucrose increase as the environment became less conducive to sugarbeet growth after the middle of September, whereas moderately resistant 13 maintained some sucrose production.

For petiole-$N0_3$ US 75 and 13 showed a significant variety X date interaction at the 5% level (Tables 1,4). US 75 showed much higher concentrations for the first two and last three dates of harvest, but there was little difference for the third, fourth, and fifth dates.

US 75 and 13 showed a significant interaction for NH_2-N concentration through the course of the season. At every date of harvest US 75 had a higher concentration than 13, and although the curves for each variety were broken and changes from date to date were inconsistent, the trend was for US 75 to have decreasing and 13 to have increasing concentrations.

The interaction for Na resulted primarily from the first and last three dates of harvest. For the last three dates US 75 increased in Na concentration proportionately faster than 13. For the second through fifth dates there was proportionately little difference with US 75 continuing to show higher concentrations.

Under yellows conditions these data for 1967 indicated that harvesting after mid-September resulted in only slight yield increases. However, these increases were associated with rapidly deteriorating quality since several melassigenic components rapidly increased in concentration as the growth rate had decreased or stopped. This was particularly true with susceptible US 75.

Summary

Field tests at Salinas and Davis, California, in 1967 compared the effects of yellows infection on the moderately resistant selection 13 and its more susceptible parental sugarbeet variety, US 75. A combination of beet yellows virus and beet western yellows virus was used to inoculate the tests. At Davis inoculated and noninoculated plots were compared. At Salinas the uniformly infected test was grown under two nitrogen levels and harvested at eight dates with 2-week intervals starting July 25 and ending October 31. Root yield, sucrose percentage, and NH_2-N, Na and K concentrations were measured for all treatments. Petiole-NO_3 levels were measured at Salinas.

Under infected conditions at Salinas, root yield and sucrose percentage increased faster in the 13 line than in the US 75 variety. Line 13 increased in gross sucrose through October 31 whereas US 75 showed no increase after September 19. Decreases in the Na, K and petiole-$N0_3$ concentrations corresponded with the period of rapid growth. However, these constituents increased in concentration as the growth rate decreased in September and October. Amino nitrogen showed little change through the course of the season.

When free from yellows, US 75 and selection 13 were not different for root yield, sucrose percentage, or gross sucrose but were significantly different for Na, NH_2-N and K. The Na and NH,-N concentrations were higher and the K concentration lower for US 75.

When infected, US 75 and selection 13 were significantly different for root yield and sucrose percentage with selection 13 yielding 68% more gross sucrose. Infection with yellows caused no significant change in Na, NH_2-N or K levels in selection 13 but caused increased concentration of these impurities in US 75. Resistance in line 13 to yellows was not due to selection for increased vigor or yielding ability but to selection for resistance factors.

The high nitrogen treatment caused increased root yields, decreased sucrose percentages, and increased petiole-$N0_3$, Na and NH_2-N concentrations, but did not influence K significantly. The nitrogen treatments caused greater changes in US 75 than in moderately resistant 13. The nitrogen level did not appear to influence the resistance of line 13.

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Sugarbeet Yields Unaffected by Afternoon Wilting[1]

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Sugarbeet irrigation has been studied extensively in the western United States. The present "state of the art" has been reviewed by Loomis and Haddock (2)[3]. Most field experiments have been run at three or more levels of soil moisture—wet, medium and dry. Differences in root and sugar yields under these moisture regimes have not been strikingly different so long as the "dry" treatment did not cause prolonged wilting, and so long as the "wet" treatment did not cause leaching of nutrients.

One of the more interesting aspects associated with sugarbeet water relations is the tendency of the leaves to lose turgor on a hot afternoon, even though the soil moisture content is high. Plant moisture stress which causes this afternoon loss of turgor may interfere with growth. If the stomata close, photosynthesis may be reduced by a lack of CO_2. There is also evidence that moisture stress affects growth in other ways. Cell elongation may decrease at low turgor pressures. Moisture stress appears to decrease DNA and RNA contents in new leaves. These and other effects of water relations on the biochemistry of plant cells have been reviewed by Slatyer (5). Shah and Loomis (4), working specifically with sugarbeets, found that there was a direct effect of stress on the biochemistry associated with RNA and protein metabolism. This occurred even before wilting was visibly evident. While these observations were made under greenhouse conditions, they do raise questions about the detrimental effects of afternoon wilting on sugar production and the growth of beets in the field.

This question largely has been one of academic interest. If the plants lose turgor on a hot afternoon even when the soil surface appears moist in the shade of the leaves, what more could one do for them? However, with the development of solid-set sprinkler systems and automatic sequencing valves, some control of the microclimate may be conveniently incorporated

[1] Contribution from the Northwest Branch, Soil and Water Conservation Research Division, Agricultural Research Service, USDA; Idaho Agricultural Experiment Station cooperating.

[2] Research Soils Scientists, Snake River Conservation Research Center, Kimberly, Idaho.

[3] Numbers in parentheses refer to literature cited.

with the process of restoring transpired soil moisture. For example, would it. be better to sprinkle a crop continuously for one 12-hour period each week, or for three 30-minute periods during the heat of each afternoon?

As part of an experiment concerning the water relations of sugarbeets, Owen (3) found that sprinkling plants in pots twice a day did not totally control their wilting. Preliminary greenhouse experiments at this Center indicated, however, that loss of turgor could be controlled by frequent light sprinklings. Under high light intensity, a 10- or 15-minute sprinkling caused a drop in leaf water stress of 2 to 3 bars within 20 minutes or less, even when all the soil in the pot was moist. These results, coupled with the report of Shah and Loomis (4), provided the impetus to conduct a field trial using an automated sprinkler system.

Methods

The experiment was conducted as a randomized complete block design with three replications. The three irrigation treatments were: (1) Surface irrigation to fill the root zone when soil moisture stress at the 18-inch depth reached 0.65 bar; (2) intermittent sprinkling during the time of high evaporative demand to replace water use for the day to maintain soil moisture stress at the 18-inch depth between 0.5 and 0.65 bar; and (8) identical intermittent sprinkling at night to serve as a check on treatment No. 2. All treatments were to receive approximately the same amount of water. Treatment No. 2 received an additional inch of water in early September to equalize soil moisture stress.

The plot area of Portneuf silt loam was Fall fertilized with 66 lb of P and 50 lb of N per acre. Pelleted monogerm sugarbeet seed was planted on April 8. The area was irrigated and thinned, and on June 13 the field was sidedressed with 100 lb of N per acre and cultivated.

An automatic solid-set sprinkler irrigation system was installed on the plots to be sprinkled. Due to cool weather, cloudiness and precipitation, another irrigation was not needed until June 29, on which date the surface-irrigated plots were irrigated and the sprinkler irrigation system was turned on. Surface-irrigated plots were again irrigated on July 8, 14, 22, August 5, 17 and September 2 and 19. Sprinkled plots received between $7^{1/2}$ and 10 minutes of irrigation during each 40-minute period from 10:30 A.M. to 5:10 P.M., depending on evaporative demand. Average application rate was about 0.16 inch per hour.

Climatic conditions at Kimberly, Idaho produced higher evaporative demand conditions during 1967 than normal. August was the third hottest August, and September the second hottest September on record. There were 33 days during which evapotranspiration exceeded 0.25 inch.

During July the 18-inch tensiometer readings on the sprinkled plots averaged slightly below 0.5 bar. The soil moisture stress was allowed to increase to an average of 0.56 bar during August. By early September, the soil moisture stress was allowed to increase to an average of 0.68 bar, with the day-sprinkled plots averaging 0.06 bar higher than the night-sprinkled plots. At this time, the day-sprinkled plots received approximately 1 inch of additional water to reduce the soil moisture stress to that of the night-sprinkled plots.

During the growing season, stomatal resistance, plant moisture potential, soil moisture potential, leaf area index, and leaf temperature were measured. Beet root and sugar yields were determined at harvest on October 16. Stomatal resistance was measured with the unit developed by van Bavel (6). Plant moisture potential was determined in a vapor pressure psychrometer similar to the Peltier unit described by Zollinger et al. (7). Leaf temperature was measured with a Barnes Model IT-3[4].

Results and Discussion

The day-sprinkling treatment was very successful in controlling afternoon wilt. The leaves remained extremely turgid throughout the season. In contrast, the plants in the check plots showed typical afternoon drooping, particularly toward the end of each watering cycle.

The relative stomatal opening of the beet leaves was measured on several occasions in July and August. The meter was unable to detect a difference between any of the leaves on any treatment. All leaves supplied water vapor at the same rate as a wet piece of filter paper, indicating the stomata were well open. This included leaves on plants in the check plot on July 7 which obviously had low turgor and were beginning to droop.

The plant moisture stress measurements are summarized in Figure 1. Each point is a mean of two or three measurements taken about midday on the date indicated. Individual measurements occasionally varied as much as 3 bars from the mean.

[4] Trade names and company names are included for the benefit of the reader and do not infer any endorsement or preferential treatment of the product listed by the U. S. Department of Agriculture.

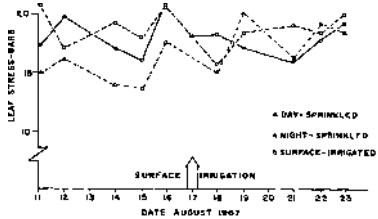

Figure 1.—Sugarbeet moisture stress measurements.

This resulted from random sampling of plants in the plots and reflects moisture stress variations from leaf to leaf. However, these data indicated that the day-sprinkled plants were maintained at a generally lower plant water stress. Between August 11 and 23, the mean midday stresses were 15.8, 17.2, and 18.2 bars for the day-sprinkled, night-sprinkled, and surface-irrigated plots respectively. The lowest field stress of 15.8 bars was about 5 bars higher than the stresses that can be maintained in potted plants growing in the greenhouse. This was evidently due to the difference in environment and may be significant when extrapolating greenhouse studies to field conditions.[5]

Leaf temperatures were measured by placing the sensing head of the infrared thermometer on a tripod 5 feet above soil level and aiming it downward at 40° below the horizontal. A majority of the leaves in view of the instrument were upper leaves, though several were shaded. The instrument recorded a mean integrated temperature for the leaves in its field of view.

When leaves in the sprinkled plots were dry, there was no measureable difference in leaf temperature between the irrigation treatments. Leaf temperatures apparently varied with air temperature, wet bulb temperature, and cloud cover. The temperature of the leaves in view of the thermometer tended to be midway between wet and dry bulb temperatures. On a partially cloudy day, leaf temperatures dropped from 2.5° to 4.5° C when a cloud passed in front of the sun.

Sprinkling reduced leaf temperatures from 2 to 3° C, depending on the initial leaf temperature and the wet bulb temperature; the higher the initial temperature or the lower the wet bulb temperature, the greater the cooling. Soon after sprinkling ceased,

[5] The authors are indebted to H. D. Fisher for making the plant moisture measurements.

the leaves began warming and usually returned to the initial temperature in about 10 minutes. Due to the reduction in leaf temperature during sprinkling the average seasonal temperature of the day-sprinkled leaves would be slightly lower than that of the other treatments. However, on any one day the differences in temperature were small compared to naturally occurring fluctuations.

Tabic I.—Mean leaf area indices, beet root yields, and sugar yields for surface— and sprinkler-irrigated plots.

	Leaf area index	Beet root yield	Sugar yield
		tons/acre	tons/acre
Surface-irrigated	7.98	**23.0**	3.13
Sprinkled daily	**9.07**	**24.5**	3.29
Sprinkled nightly	**7.75**	**25.2**	3.45

Table 1 shows the mean leaf area indices and yields of beet roots and sugar for the plots. There was no statistically significant difference at the 5% confidence level between any of the results. The day-sprinkled plots did tend to have the largest average leaf area index, although all plots had higher leaf area indices than is considered optimum for efficient sugar production. If there was any trend created by the lowering of the average leaf moisture stress, it was apparently reflected only in greater leaf growth. Since the leaves have priority on the use of nutrients and photosynthetic products, excessive top growth is not necessarily desirable for root crop production. Campbell and Viets (1), working in Montana, obtained their largest sugar production when the leaf area index did not exceed 3 during the growing season.

As there was no significant difference in the yield of either roots or sugar between the treatments, it appears that the afternoon loss of turgor observed in sugarbeets is not an important economic factor in southern Idaho.

Summary

A study was conducted to determine if daily intermittent sprinkling of sugarbeets would control afternoon wilt and if this, in turn, would affect the yield of beet roots and sugar. Daily and nightly intermittent sprinkling was compared with recommended practices of surface irrigation. Plant moisture stress, leaf area index, leaf temperature and yield were measured. Complete control of afternoon wilt was achieved on the sprinkled plots, but yield was not significantly increased.

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Sorting Sugarbeets by Specific Gravity to Obtain More Uniform Samples for Post-Harvest Studies[1]

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The large variability in sucrose content, clear juice purity, etc. in replicate samples from a single lot of beets grown under controlled field conditions has long been recognized. Where the purpose of an experiment is to compare the changes that occur or are induced by various post-harvest conditions or treatments; greater uniformity would result in more sensitivity with fewer or smaller samples. Since suitable storage space is frequently at a premium, smaller samples are desirable, and when time-consuming chemical analyses are involved, fewer samples are helpful.

The purpose of this paper is to describe a flotation method for removing a relatively homogeneous segment of beets from a harvested sample, and to demonstrate the efficiency in the reduction of variability.

Materials and Methods

All beets were uniformly topped, sufficient to open the cavity that is common in the crowns, and washed free of soil. All very large, small or ill-shapen beets were discarded. In 1966, a mixed lot of beets was used, composed of several varieties and treatments. In 1967, a lot of beets from one variety grown on uniform land in a perfect stand was used. A preliminary measurement of specific gravity (2)[3] was occasionally made to indicate the approximate mean of the lot to be sorted.

In 1966, the highest and lowest one-fourth (about 40 beets each) in specific gravity were separated and used as single samples. The one-fourth nearest the mean of the lot was removed from the residue and divided into ten 3-beet samples weighing nearly 6.0 pounds each. These were designated as the "sorted" group. Ten 3-beet samples of equal weight, but composed of randomly selected beets, was the "unsorted" group.

In 1967, a salt solution was quickly adjusted by adding saturated salt solution or water so that 10 of a 30-beet random sample sank and were discarded. With this solution in a 6-foot "horse-trough" (with a false bottom of woven wire) the third

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[3] Numbers in parentheses refer to literature cited.

highest in specific gravity was removed and discarded. The floating beets (two-thirds of the original lot) were removed to a 50 gallon drum containing a solution that was diluted so that half of them sank and half floated. The floating beets, constituting the third lowest in specific gravity, were then discarded. The beets that were saved were rinsed in a drum of water followed by rinsing with a spray nozzle. In general, a difference of about 0.006 in specific gravity in the two solutions was needed to isolate the center third, presumably near the mean in percent sucrose. Previous trials (1) (2) indicate that within this group there was a probable variation of about 1.5% sucrose in individual beets.

Results and Discussion

Table 1 shows the variance for the sorted and unsorted lots and the relative efficiency in the reduction of the variance by sorting. It is evident that the specific gravity sorting was effective in improving the uniformity of the analytical values. If the sorted and unsorted samples are the same size, then, obviously the number of beets harvested to obtain the sorted sample must be 3 or 4 times as large as if no sorting was done. The greater uniformity of the sorted samples makes a reduced number of analyses feasible.

Table 1.—Ten (1966) and 15 (1967) 3-beet (6 pound unsorted samples and 10 and 15 sorted samples were analyzed for percent apparent sucrose and clear juice purity. The variance and relative efficiency are shown in the table.

$$\text{Relative Efficiency} = \frac{\text{Variance unsorted}}{\text{Variance sorted}}$$

	% Sucrose		% Clear Juice Purity	
	1966	1967	1966	1967
Sorted	0.554	0.225	0.767	0.333
Unsorted	0.941	0.720	2.130	0.780
Efficiency %	170	320	278	234

Table 2 shows the percent sucrose and clear juice purity of the top and bottom fourths in 1966 in specific gravity separation. It is evident that sorting removed many beets either high or low in sucrose content.

Table 2.—Percent apparent sucrose and clear juice purity, and pounds extractable sugar per ton of the beets of the top and the bottom one-fourth in specific gravity of a mixed sample.

	% App. Sucrose	% App. Clear Juice Purity	ESPT, Lbs.
High one-fourth	18.77	96.12	345
Low one-fourth	13.39	94.91	238

The 40 beets in each lot in Table 2 were examined by sugarbeet experts before and after passing through the brei saw. The lot of beets had been selected, before sorting, to a rather uniform size, and the average weight per beet of the sorted and unsorted lots differed by about 0.01 pound per beet. No expert could distinguish between the 40 beets that were high and those that were low in specific gravity.

In 1967, all beets intended for storage experiments were sorted in this way. The improvement in uniformity was evident at a glance, even though the samples contained fewer beets than in previous years. Not only were the samples smaller and easier to store under optimum conditions but fewer beets were put through the brei saw. Analyses of the clear juice for sodium showed no increase as a result of sorting in sodium chloride solution. The improvement in the reliability of the samples seemed to more than repay the extra labor involved in sorting.

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Sugarbeet Responses to Selective Gametocide Sodium 2,3-Dichloroisobutyrate and Related Chemicals

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Inbred lines of sugarbeet are widely used by sugarbeet breeders in the United States to produce hybrid varieties. Many of these inbred lines are highly self-fertile (3)[2] and crosses between lines are difficult without the incorporation of some form of pollen sterility. Cytoplasmic male sterility (4) is used in the production of commercial hybrids by introducing it into one of the inbred parents. To cross individual plants of self-fertile inbreds the breeder usually must resort to mechanical emasculation of one of the plants. Sugarbeet flowers are small and stigmas easily damaged during emasculation so that crosses made by this means frequently result in few, if any, hybrid seeds. The report by Eaton (2) that cotton plants were emasculated by the selective gametocide, 2,3-dichloroisobutyrate (FW-450), led to preliminary testing of selective gametocide effects on sugarbeet by Dudley (1). Objectives of the studies at Salinas, California paralleled those of Dudley's testing at Fort Collins, Colorado (1). The evaluation of gametocidal properties included optimum concentration and timing of applications, effects on seed yield and germination, and chemical toxicity to treated plants. A further objective was the study of the effects of gametocide chemicals on sugarbeet plants with the longer flowering periods that occur in cool coastal climates.

Materials and Methods

The selective gametocide chemicals used in these tests were FW-450, sodium 2,3-dichloroisobutyrate and the salts of two other chlorinated organic acids, FW-676 and G-315 (as prepared by Rohm and Haas Company, Philadelphia, Pa.). The chemicals were applied as aqueous sprays to runoff giving plants equivalent amounts of chemical in relation to size and foliage area. Initial spray applications were made when the earliest flowers were in the early bud stage.

1960 Gametocide Tests

A field test was conducted in a planting made September 16, 1959 at Salinas, California. The test plant was a green hypocotyl

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sib-pollinated breeding line, SP579-01, obtained from U.S.D.A. sources at Beltsville, Maryland. The pollen source was a red beet derivative developed by Holly Sugar Corporation. The dominance of red hypocotyl (RR) to green hypocotyl (rr) permitted seedling identification of hybrids in the progeny of treated rr plants. Six of the 24 rows in the test were planted to SP579-01. Each row of test plants was bordered by two or three rows of the red beet. Test rows were divided into six 12-foot plots with 6-inch plant spacings within plots. Three concentrations of FW-450 applied at three intervals comprised the treatments with four replications.

An evaluation of seed yield on treated plants was originally intended; however, late bolting of the red beet pollen source necessitated cutting back the test plants for over 2 months. This made accurate yield evaluations impossible.

Three concentrations of FW-450, 0.15%, 0.22% and 0.3%, were applied in a series of three applications at intervals of 5, 10 and 15 days for each treatment. Applications were started June 10, 1960, when new flower branches were in early bud. Treated plots were harvested September 12-13, 1960. Seed was bulked from the plants in each plot.

As a check on natural cross pollination, roots of SP579-01 and the red beet derivative were transplanted to an isolation plot on March 28, 1960.

The greenhouse test in 1960 compared FW-450 with the related chemicals, FW-676 and G-315. Greenhouse-grown plants of SP579-01 and the red beet were used in the test. Plants of both varieties were given 3 months of thermal induction, starting when plants were 6 weeks old. The plants were transferred to the greenhouse July 6, 1960.

Gametocide applications were started July 27, 1960, when the SP579-01 test plants were in early bud. A total of 72 green hypocotyl plants were treated with the chemicals; 24 plants with each chemical and eight plants with each concentration. Untreated check plants and pollinator were moved to a separate greenhouse before flowering commenced. The 8-plant groups were alternated with rows of the red beet pollinator. Half of the plants were treated twice at 7-day intervals and the other half twice at 14-day intervals. Applications were made with 0.15%, 0.225% and 0.3% concentrations of each chemical. Seed was harvested individually from each test plant on October 25, 1960.

1961 Gametocide Test

Plants of the self-fertile multigerm inbred, NB1, were treated in the field with the FW-450 and FW-676 gametocides at con-

centrations of 0.15% and 0.3%. Stecklings were transplanted into a total of 24 plots of three plants each on March 24, 1961. Every other row was planted to red beet pollinator. Gametocide applications were started May 15, 1961, when test plants were in early bud. Each plot received a series of three applications at intervals of 6 or 10 days.

At harvest, on August 22, 1961, seed from two replications of the treated plots was separated into one lot of mature seedballs and one of maturing seedballs for each plot. Seed was bulked into one lot for each of the plots in the third replication. The separation of mature and maturing seed was made in order to estimate from counts of hybrid seedlings in each seed class the duration of maximum gametocide effects.

Seed yield on check plants was not obtained for the 1961 field test.

Results

Concentration, dosage, timing and number of applications

Tests with the 0.15% concentration showed that pollen sterility of the treated plants was comparable to that induced by higher concentrations (Tables 1, 2, and 3). Phytotoxicity was also reduced with the lower gametocide concentrations.

Treating plants to runoff resulted in high dosage rates. For the field tests, the calculated rate was 245 gallons per acre per application for the 1960 test and 320 gallons per acre per application for the 1961 test. A rate of 17 ml per plant was used in the greenhouse test. Observations of treated plants indicated that satisfactory sterility probably could be obtained by reducing carrier rates to 100 to 150 gallons per acre.

The timing and number of applications of gametocide chemicals are important considerations in a coastal climate such as

Table 1.—Effect of gametocide FW-450 treatments on cross-pollination, seed yield, and seed germination of the green-hypocotyl SP579-01 line in a 1960 field test.

Concentration	Treatment interval	Plants	Cross-pollination	Seed yield/plant	Germination
%	Days	No.	%	g	%
0.15	5	55	78	53.3	88
0.22	5	56	82	38.5	83
0.30	5	61	75	30.9	84
0.15	10	55	77	32.6	80
0.22	10	60	81	27.0	79
0.30	10	57	84	21.9	80
0.15	15	55	85	46.2	78
0.22	15	63	83	28.9	72
0.30	15	61	84	22.4	73
LSD 5%			N.S.	4.5	N.S.
Check		24	11	52.6	94

that at Salinas, California. Cool summer temperatures and frequent foggy periods promote prolonged flowering in sugarbeet plants, so that induced pollen sterility must be effective longer than in regions where higher temperatures shorten the flowering period. Two to three gametocide applications induced and maintained pollen sterility at the rates used in these tests. Two applications under greenhouse conditions sterilized treated plants for periods of 40 to 50 days. Treated plants were pollen sterile with most yellow anthers failing to dehisce in about 10 days of the initial applications. In the field, three applications prolonged pollen sterility more effectively than two applications. Pollen sterility reached a maximum 10 to 15 days after the first application in the field, and was effective for periods of 35 to 50 days after the final application. Lower dosages probably would require an additional application in the field.

Table 2.—Effect of gametocide treatments on cross-pollination, seed yield, and seed germination of the green-hypocotyl line, SP579-01, in a 1960 greenhouse test.

Chemical	Conc.	Treatment interval	Cross-pollination		Seed yield/plant	Germination
			Total of all seed	Mature seed[1]		
	%	Days	%	%	g	%
FW-450	0.15	7	100	100	5.0	33
		14	100	100	5.2	69
	0.225	7	97	100	3.7	77
		14	93	100	2.4	68
	0.3	7	86	100	5.4	93
		14	73	100	0.7	37
		Mean	92	100	3.7	63
FW-676	0.15	7	88	100	7.8	49
		14	89	100	6.1	62
	0.225	7	93	100	5.0	83
		14	81	100	4.1	80
	0.3	7	93	99	6.4	39
		14	69	89	3.4	59
		Mean	86	98	5.5	62
G-315	0.15	7	71	99	4.6	55
		14	69	95	5.3	81
	0.225	7	79	99	5.3	57
		14	62	94	3.5	72
	0.3	7	91	98	4.1	58
		14	72	84	1.3	49
		Mean	74	95	4.0	62
Check			51		16.3	42

[1] Refers to seed fully ripe at harvest.

Table 3.—Effect of gametocide treatments on cross-pollination, seed yield, and seed germination of the green-hypocotyl self-fertile NB1 inbred in a 1961 field test.

Chemical and cone.	Treatment interval	Cross-pollination			Seed yld. per plant	Germination
		Total all seed[1]	Mature seed[2]	Maturing seed[2]		
%	Days	%	%	%	g	%
FW-450						
0.15	6	**64**	65	41	44.7	48
0.30	6	**68**	78	53	**12.4**	22
0.15	10	**60**	80	37	31.2	24
0.30	10	**57**	65	26	**9.5**	**18**
FW-676						
0.15	6	**62**	81	27	25.0	26
0.30	6	**72**	89		12.5	29
0.15	10	**63**	74	54	26.3	**31**
0.30	10	**59**	65	41	**5.0**	26
Mean		**63**	75	40	20.8	28
Check		**0.5**				90

[1] Average percentages for all three replications.
[2] Average percentages for two replications with seed separated into maturity classes at harvest.

Tests showed that gametocide applications, started when bolting plants were in early bud stage, induced pollen sterility in the earliest open flowers. All gametocide treatments delayed flower opening and the highest concentrations caused the longest delays.

Results indicated that 5- to 7-day treatment intervals were slightly more effective than 10- to 14-day intervals (Tables 1, 2 and 3). In general, percent hybrids, seed yield, and germination were higher for the shorter intervals for all chemicals and concentrations.

Phytotoxicity

The three gametocide chemicals tested produced similar phytotoxic effects on treated plants (Figure 1). Leaf, stem, and flower tissues showed damage by contact burn within 2 to 3 days of application. Prolonged phytotoxic effects developed as chlorosis of branch tips, necrosis of severely chlorotic areas, and as thickened and distorted growth of leaves and flower structures. Contact burn occurred where drops of spray liquid collected. The chlorosis was more severe in the greenhouse than in the field and began developing 2 to 4 days after initial treatment. Chlorosis continued to develop for 20 to 30 days, often involving 3 to 4 inches of a branch tip. Chlorotic tissues eventually died back to normal green tissue. Seed set seldom occurred in chlorotic areas but mainly on the basal areas of affected branches and on new floral branch growth.

Distortion and thickening of plant parts was characteristic of new growth on treated plants (Figure 2). Severe thickening of sepals made flowers appear closed. Bracts were several times thicker than normal in cross section. New growth originating

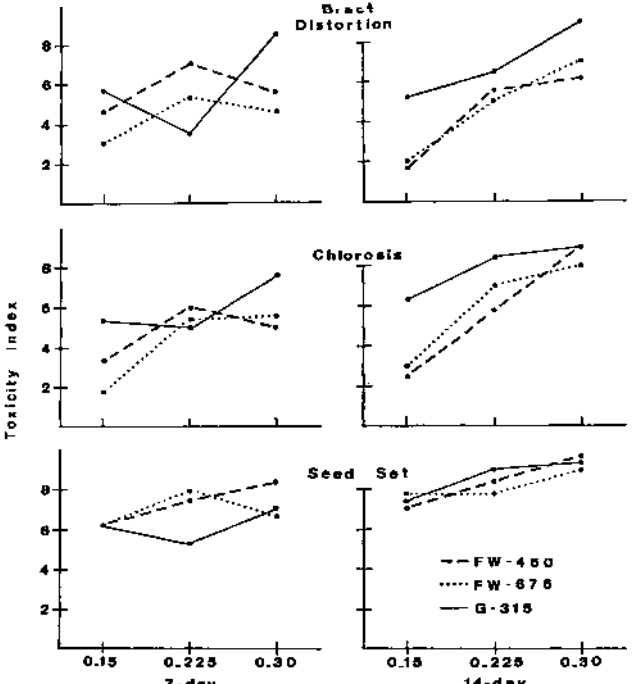

Figure 1.—Relative phototoxicity of gametocide chemicals to plants of SP579-01 treated at two intervals with three percentage concentrations in a greenhouse test in 1960. (1 = no damage, 10 = severe damage).

from axillary buds often showed extreme branch thickening and profuse bract development. Affected areas of new branches often produced few functional flowers. Frequently 1 to 3 inches of new branch growth had no flowers, and then flower formation would recur on the branch. Gametocide applications spaced 6 to 7 days apart generally produced less phytotoxic damage than those spaced 10 to 14 days apart.

Cross-pollination

Results of treating plants of the highly self-fertile NB1 inbred with gametocides in the 1961 field test are presented in Table

Figure 2.—Floral branch from sugarbeet plant treated with a gametocide chemical on the left, branch from untreated plant on the right. Note profuse bract growth and distorted, often flowerless branch development.

3. In this test, seed fully matured on treated plants at harvest averaged 75% hybrids for all treatments, but untreated plants averaged only 0.5% crosses with the red beet. Hybrid seed on plants treated at 6-day intervals averaged 7% higher for both mature seed and for the total of all seed than that on plants treated at 10-day intervals. Depending on the treatment, cross-pollination in matured seed at harvest was 20% to 54% higher than in maturing seed. Evidence that gradual recovery from gametocide effects, including pollen shedding, was occurring 5 to 6 weeks before harvest. This shows that maximum pollen sterility lasted for a period of 5 to 7 weeks after the final gametocide application. However, the percent hybridization also shows that plants were not completely emasculated at Salinas; scattered flowers usually produced some pollen throughout the period following treatment.

Cross-pollination percentages were high for both tests with SP579-01 (Tables 1 and 2). The mean was 81% hybrids over all concentrations and intervals for the 1960 field test. For the greenhouse test, the mean was 84% over all chemicals, concentrations, and intervals. However, the net increase in hybrid seed

on treated plants over that on untreated check plants was 70% and 33% for the field and greenhouse tests, respectively. The SP579-01 line was essentially self-sterile.

Effects on seed yield and germination

Seed yield comparisons for the greenhouse test showed yields per plant, for individual treatments, ranged from 4.3% to 48% of untreated check plants (Table 2). The lowest seed yield was on plants treated with 0.3% FW-450 at 14-day intervals and the highest was on plants treated with 0.15% FW-676 at 7-day intervals. Considering the chemicals together, yields for the 0.15% concentration and 7-day interval averaged nearly 36% of the check, slightly higher than other treatment combinations. Comparisons of treatment intervals show that seed yields for the 14-day interval were 5%, 29% and 66% lower than that of the 7-day interval for the 0.15%, 0.225% and 0.3% concentrations, respectively.

Although seed yield comparisons with untreated check plants of NB1 could not be made for the 1961 field test, plant damage from gametocides obviously resulted in severe yield reductions. Single-plant seed yields averaged 31% (22 grams) higher for the 0.15% treatments than for the 0.3% treatments with each chemical (Table 3). Likewise, the seed yields from 6-day treatments averaged 24% higher than those of 10-day treatments. FW-450 treated plants also yielded higher than FW-676 treated plants with the exception of the 0.3% treatment at 6-day interval which gave a yield of about 12.5 grams with both chemicals.

The seed yields for treated plants that had seed stalks cut back for over 2 months are shown in Table 1. In general, each increase in concentration and treatment interval lowered seed yield. Differences between concentrations and intervals were highly significant.

Germination of seed produced on treated plants was evaluated for all three tests reported. The germination tests were run for a three-week period. For the 1960 field test, germination percentages were generally high for all treatment intervals and concentrations of FW-450 (Table 1). The results showed a 10% reduction in germination as treatment intervals increased from 5 to 15 days, but the differences were not significant. Seed of the check germinated 94%, 6% to 22% higher than seed from treated plots.

Germination of seed from different treatments in the greenhouse ranged from 33% to 93% (Table 2). Treated plants of SP579-01 exhibited a range of sensitivity to gametocide chemicals; this is reflected in the range of average germination percentages.

This is also shown by the variation in the seed set, chlorosis, and distortion evaluations presented in Figure 1. The seed from check plants, located in a separate greenhouse, germinated only 42% which probably resulted from the greenhouse accidentally remaining closed during a period of high daytime temperature in late August. Seed from untreated plants of SP579-01 grown in the field germinated 94%.

Germination was reduced severely in the 1961 field test and averaged 28% for all treatments, compared to 90% for seed from untreated plants of NB1 (Table 3). The 6-day treatment with 0.15% FW-450 showed appreciably higher germination than other treatments. The low germination probably was partly due to the high dosage rates required to wet the plants to runoff and partly to sensitivity of the NB1 inbred to gametocide chemicals, particularly the higher concentrations used.

Deformed seedlings and polyploidy

In the course of germination testing of seed produced on treated plants in the greenhouse, deformed seedlings were frequently observed in the populations. Counts of affected seedlings were made in three separate tests. Results of these tests were averaged for each treatment and are shown in Figure 3. Out of the 69 treated plants producing viable seed, the progeny of 58 plants had deformed seedlings. Seed from plants treated with FW-450 at 7-day intervals showed 55% and 65% more deformed

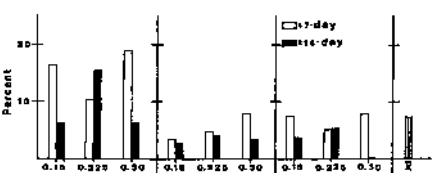

Figure 3.—Frequency of deformed seedlings in the progeny of plants of SP579-01 treated at two intervals with percentage concentrations of gametocide chemicals in a greenhouse test in 1960.

seedlings for all concentrations than plants treated with FW-676 and G-315, respectively.

Affected seedlings showed thickened hypocotyls, twisted thickened cotyledons, were slow growing, and often had poor root development (Figure 4). The number of deformed seedlings averaged 7.2% overall. The deformed seedlings resembled colchicine treated seedlings and suggested that possibly the deformities were due to polyploidy. A test for polyploidy was made by counting chromosomes in the tissue of young true leaves. Thirty-eight red (Rr) F_1 seedlings, progeny from 19 treated plants, were examined. The 38 plants included 22 seedlings labeled deformed by visual inspection and 16 which appeared normal. The normal plants were all 2n but 12 (55%) of the deformed plants had 4n tissue. The polyploid plants were mostly 4n, but one plant was found with both 4n and 8n tissue. Triploid plants were not found, a factor which indicates that in the surviving embryos fertilization of haploid gametes occurred followed by zygotic nuclear division without cell division.

Deformed seedlings were infrequent in germination tests of seed produced on treated plants for both field tests.

Utilization of gametocide chemicals in the breeding program at Salinas

Fifty-five crosses were made in 1963 in which the female parents had been treated with the gametocide FW-450. Treated plants included a self sterile variety, several self-fertile inbred

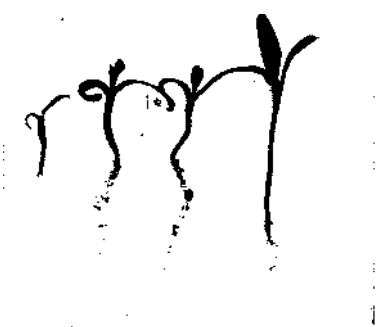

Figure 4.—Progeny from a gametocide treated plant of SP579-01 showing three deformed seedlings on the left and a normal seedling to the right. Note deformed hypocotyls, cotyledons, and relative root development.

lines and tetraploid lines. All treated plants were green hypocotyl (rr) and were crossed wherever possible with red hypocotyl (R-) pollinators.

Forty-eight crosses produced seed and the yields per cross ranged from eight seeds to seven grams. In all, 34 crosses were made between gametocide treated green-hypocotyl monogerm plants and red-hypocotyl pollinators. The progeny of these crosses produced 11 populations with all red-hypocotyl (Rr) seedlings, 21 with 58% red-hypocotyl seedlings and two populations with all green-hypocotyl (rr) seedlings. Germination of seed from the 34 crosses averaged 81%.

Discussion

The effects of gametocide chemicals on sugarbeet are similar to those produced on cotton (2). The results reported show that selective gametocide chemicals used at concentrations which cause pollen sterility also produce a degree of phytotoxicity and destruction of plant tissues. These effects may have been particularly severe in these tests because of the high dosages. Observation of treated plants suggested that lower dosages, and possibly lower concentrations, would reduce toxicity. The effect of lower toxicity on the percent cross-pollination in treated plants is unknown, although it is unlikely that hybridization levels would be greatly different than those obtained in these tests. Reduced toxicity would also cause less damage to flower ovules, increasing embryo survival through to mature seed. Observations of treated plants indicated that few flowers set seed during the period toxicity responses were developing.

Different sugarbeet varieties and inbred lines respond differently to applications of selective gametocide chemicals. Dudley (1) reported differences for seed yield and germination between two sugarbeet lines sprayed three times with FW-450. Similar varietal responses have been reported for cotton (2). Approximately 20% of the treated plants of SP579-01 showed some tolerance to gametocides in the 1960 greenhouse test. This is illustrated by the curve variations for chemicals, concentrations, and intervals in Figure 1. The low germination percentages and seed yields shown in Table 3 indicate that the NB1 inbred line is sensitive to gametocides.

The presence of deformed and polyploid seedlings in the progeny of treated plants of SP579-01, in the greenhouse test, revealed a particularly undesirable side effect of gametocide chemicals (Figure 3). When gametocides are used, progeny from treated plants should be observed critically, or examined cytologically, for the presence of polyploids. Deformed seedlings

were rare among the progeny of plants treated in the field test using the same variety. Origin of the polyploid seeds is unknown, but these may have developed by inhibition of one of the early cell divisions following fusion of gametes in the egg.

The level of cross-pollination obtained in the test with the NB1 inbred (Table 3), a mean of 63% for all treatments, shows that pollen formation *in* some self-fertile lines of sugarbeet can be quite effectively controlled with gametocides. Seed of check plants had only 0.5% cross-pollination. Plants treated at 6-day intervals with 0.15% FW-450 yielded an average 44.7 grams of seed each which averaged 48% germination. Dudley (1) showed percent hybrids increased from 3.51 to 40.27 when a self-fertile line was treated with three applications of FW-450. Seed yield and germination were reported to be only slightly reduced (1). The use of selective gametocides provides the breeder a means, in addition to crossing individual self-fertile plants, of producing quantities of hybrid seed for testing combining ability or disease reactions of self-fertile lines.

The data show that gametocide chemicals can be useful tools in sugarbeet breeding work, particularly when dealing with self-fertile breeding stocks. Some knowledge of varietal responses to selective gametocide chemicals appears essential to their utilization. The use of gametocides in commercial sugarbeet seed production is not feasible in view of the phytotoxicity associated with the concentrations required to induce pollen sterility.

Summary

Effects of selective gametocide chemicals on two sugarbeet lines were compared in a greenhouse test and two field tests. The chemicals used were FW-450, (sodium 2,3-dichloroisobutyrate) and two related chemicals, FW-676 and G-315. Chemical applications, two for the greenhouse and three for the field tests, were started when flowering plants were in early bud.

Concentrations of gametocides which produced pollen sterility in sugarbeet also caused a degree of phytotoxicity. High dosage rates probably intensified phytotoxic responses. Toxicity symptoms occurred as initial contact burn, gradual chlorosis of floral branch tips, necrosis of burned and chlorotic areas and distorted growth of leaf, stem and flower tissues. Symptoms developed over a period of a few days to about 4 weeks. The two sugarbeet lines tested responded differently to gametocide chemicals. Treated plants were seldom completely emasculated and occasional anthers in a single flower or all the anthers in a flower were observed shedding pollen.

Progeny of plants treated in the 1960 greenhouse test averaged 7.2% deformed seedlings, and a proportion of these proved to

be polyploids when examined cytologically. Deformed seedlings were rarely found in progeny of field treated plants. Seed from FW-450 treated plants showed the most deformed seedlings.

Cross-pollination of mature seed produced on treated plants of the self-fertile NB1 inbred ranged from 65-89% for the chemicals, concentrations, and intervals used. The lowest concentration of chemicals and the shortest treatment intervals tested generally depressed seed yields and germination the least.

Hybrids between plants of self-fertile lines were successfully produced with the use of the FW-450 gametocide in the breeding program at Salinas, California, in 1963. Out of 48 crosses producing seed, 34 were crosses between self-fertile lines. In 32 of these crosses, cross-pollination ranged from 58-100%.

Acknowledgment

The author is indebted to Dr. B. L. Hammond for making the necessary cytological examinations.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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Determination of Aflatoxins in Moldy Sugarbeet Pulp

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Numerous analytical procedures for aflatoxins in many agricultural commodities have been reported in the literature (1,2, 3, 5)[2]. However, none of the current methods can successfully serve for moldy sugarbeet pulp. They fail because their cleanup methods cannot effectively eliminate many interfering fluorescent compounds inherent in the pulp extract. The developing solvents generally used for separating aflatoxins on silica gel thin layer plate such as chloroform-methanol (1, 3), chloroform-acetone (2) and benzene-alcohol-water (1, 5) are not satisfactory when applied to sugarbeet pulp. This paper presents a method which quantitatively and rapidly determines aflatoxins in moldy sugarbeet pulp. The method consists of a modification of the extraction procedure of Eppley (2), a proposed thin layer chromatographic (TLC) clean-up and a new developing solvent (6).

Method

Reagents

(a) Solvents. - ACS grade or redistilled; chloroform, n-hexane, acetone, benzene and anhydrous ethyl ether.

(b) Aqueous acetone. - 70% acetone.

(c) Anhydrous ethyl ether-acetone (98:2, v./v.). - Prepare 100 ml for use.

(d) Chloroform-acetone-n-hexane (85:15:20, v./v.). Prepare 120 ml of this mixture. This mixture can be reused many times.

(e) Silica gel. - Silica-TLC-4GF. Mallinckrodt Chemical Works, St. Louis, Missouri.

(f) Cupric carbonate. - Reagent grade, powder.

(g) Quantitative and qualitative aflatoxin standards. Obtained from USDA-ARS, Southern Utilization Research and Development Division, New Orleans, Louisiana **70119.**

Apparatus

(a) Osterizer blender for grinding sample. - Model No. 10, 115 volts, 2.2 amps AC-DC, mounted with one quart standard pyrex container manufactured by John Oster Mfg. Co., Milwaukee, Wisconsin, or equivalent.

(b) Waring Laboratory Blendor for extracting sample. - Consists of three parts: 1. Explosion-proof motor, GE, Model No.

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[2] Numbers in parentheses refer to literature cited.

5BA60VL22, 1/5 HP, 115 volts, 4.2 amps and 60 cycles DC. 2. Stainless steel container, type 302, one quart; and 3. Explosion-proof switch. Obtainable from Waring Products Company, Winsted, Conn., or equivalent.

(c) Thin layer chromatography equipment. - Model B-200 Chromatofilm assembly, Research Specialities Co., 200 South Garrard Blvd., Richmond, California, or equivalent.

(d) Thin layer plate scraper. - Corning 39580, 30 M.

(e) Fluorescent Viewer. - Chromato-Vue cabinet equipped with one long wave 15 Watt lamp, Ultraviolet Products, Inc., San Gabriel, California.

(f) Desiccating storage cabinet. - Applied Science Laboratories, Inc., State College, Pennsylvania.

(g) Microbell jar with suction side arm. - A. H. Thomas, Cat. No. 2126.

Sample Preparation

Grind 200 g moldy beet pulp or pellets for 4 min in an Osterizer blender. Determine percent moisture on a portion of the ground sample. Weigh out a sample of 50 g into **a** Waring Laboratory Blendor. Add water to bring the sample to a moisture level of 50% (if percent moisture of a sample is over 50%, leave as it is.) Then add chloroform in an amount of 5 ml for each g of dry weight. Blend sample for 4 min. Filter through double layered cheese cloth on a Buchner funnel under vacuum. Measure 50 ml of the filtrate (equivalent to 10 g of sample) in a 100 ml cylinder. To the cylinder add 0.5 ml ethyl alcohol and a half teaspoon of cupric carbonate (7). Invert the contents several times, filter through a fluted Whatman No. 5 filter paper containing some filter aid (Celite (545). Rinse the cylinder with two 20 ml portions of chloroform. Pour them through the filter paper. Collect all the filtrates in a 100 ml beaker. Evaporate sample to 1-2 ml on a steam bath. Continue to dryness in a hood (without steam bath).

Transfer sample to a 5 ml beaker with 5 times of 1 ml 70% aqueous acetone. Aflatoxins will be dissolved in the 70% acetone and quantitatively transferred to the beaker, thus, leaving most of the colored material behind in the original extract. Evaporate off the aqueous acetone to dryness on a steam bath in a hood. Cover the sample with aluminum foil. Sample is then ready for TLC cleanup.

Thin Layer Plate Preparation

In a 500 ml glass stoppered flask mix 25 g SilicAR-TLC-4GF with 62.5 ml distilled water for 30 sec. Immediately pour the slurry into an applicator, spread a layer of 250 mm over 5 plates 20 X 20 cm. Let plates air dry for 30 min then dry at 80° C in an oven for 2 hours. The activated plates are stored in a desiccating storage cabinet.

TLC Sample Cleanup

On a TLC plate, two spots of 5 ml of the qualitative aflatoxin standard are spotted 2.0 cm from the side and 2.0 cm from the bottom. Connect these two spots by an imaginary line. A third 5 ml of qualitative standard is spotted at the mid-point of the line. Sample is then taken up in a 200 ml of benzene. Between the standard aflatoxin spots along the line evenly spot all the 200 ml of sample. (Cover the 5 ml beaker with aluminum foil to prevent the evaporation of benzene while spotting. Pipet the sample by inserting Hamilton syringe through the foil.) Develop the plate to full length in an ascending manner in an unlined TLC tank with 100 ml of the solvent mixture, anhydrous ethyl ether-acetone (98:2, v./v.).

Remove the developed plate. View the plate upon a fluorescent viewer. The standard aflatoxins show up in four well defined spots (Figure 1) and are free of most naturally occurring fluorescent materials. Mark out the bands opposite the aflatoxins with a sharp pencil. Carefully suck the marked bands into a scraper under vacuum. Place the scraper in a microbell jar. Elute the sample with 2 ml acetone into a 5 ml beaker under mild vacuum. Continue to elute with 3 X 1 ml acetone. Concentrate eluate on a hot water bath to dryness. The sample is then ready for quantitative TLC anatoxin analysis.

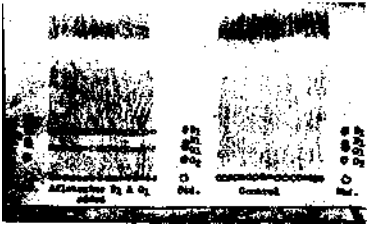

Figure 1.—Moldy sugarbeet pulp extract cleaned up by proposed **TLC method.**

Spot quantitative aflatoxin standard of 2, 3, 4 and 5 ml **and** one 5 ml of qualitative standard on TLC plate according to Pons et al. (3). Sample is taken up with 200 ml of benzene (4) **and** spotted as *2,* 6, 10 and 20 ml. A 120 ml volume of developer, chloroform-acetone-n-hexane (85:15:20, v.v.), is poured into **an** unlined TLC tank. The plate is then placed in the tank for ascending development. Time for developing to **a** height of 14 cm takes about 50 minutes. Withdraw the plate and view upon fluorescent viewer.

Calculation of Aflatoxin B_1 and G_1

Of the four aflatoxins, aflatoxin B_1 is the most toxic and usually of the greatest concentration, followed by G_1, whereas B_3 and G_2 are less toxic. Thus in reporting the quantity of aflatoxins present in samples usually B_1 and G_1 are considered. The spotted amount of sample (ml) is also a weight basis (the 2 ml is equivalent to 0.10 g of initial sample, 6 ml is 0.30 g, and so forth) on which aflatoxins B_1 and G_1 can be calculated, that is that if the 6ml sample spot has B_1 which matches 4ml of aflatoxin standard G_1

$$\text{then, } B_1 = \frac{4 \times 0.00057^{*}\ \mu g}{300{,}000\ \mu g} = \frac{22.8 \times 10^{-4}\ \mu g}{3 \times 10^{5}\ \mu g} = 7.6 \times 10^{-9} = 7.6 \text{ ppb}$$

By the same token, G_1 may be calculated as follows, if the 6 ml sample spot matches 4 ml standard aflatoxin G_1

$$\text{then, } G_1 = \frac{4 \times 0.00035^{*}\ \mu g}{300{,}000\ \mu g} = \frac{14 \times 10^{-4}\ \mu g}{3 \times 10^{5}\ \mu g} = 4.7 \times 10^{-9} = 4.7 \text{ ppb}$$

Results and Discussion

Four moldy sugarbeet pulp samples were fortified with quantitative anatoxin standard (B_1, 16 ppb; G_1 10 ppb). Three were extracted and cleaned up according to literature, respectively (2, 3, 5). One was prepared as the described method. All cleaned up extracts were then spotted on a TLC plate and developed as previously described. Figure 2 shows such a TLC chromatogram. The backgrounds of the chromatogram of the four differently prepared samples show the one by the proposed method to be the cleanest. This clean quality obtained was due to a three-step cleanup procedure: The addition of cupric carbonate to adsorb pigments from chloroform extraction (7); the use of 70% aqueous acetone to transfer anatoxins to a 5 ml beaker from the residual chloroform extract which has been evaporated to dryness; and the final cleanup of TLC.

* Quantitative standard aflatoxins B_1 and G_2, according to USDA-ARS Southern Utilization Research and Development Division.

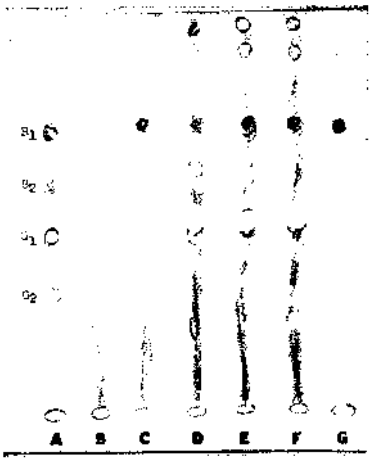

Figure 2.—Comparison of interferences on a thin layer chromatogram from a sugarbeet pulp sample extracted by different aflatoxin assay methods: A-quantitative standard, B-no aflatoxin added pulp cleaned up by proposed method, from C to F-aflatoxins B_1 and G_1, added pulps cleaned up by proposed method, Stoloff et al. method. Eppley method, and Pons et al. method, respectively, G - quantitative standard.

Table 1.—Recovery of alfatoxins added to moldy sugarbeet pulp.

Alfatoxin B_1			Aflatoxin G_1		
Added ppb	Found ppb	Recovery %	Added ppb	Found ppb	Recovery %
32	30.0	93.7	20	20.0	100.0
32	25.3	79.0	20	18.2	91.0
64	50.3	78.6	40	34.3	85.0
64	52.0	81.2	40	35.6	89.0

A set of aflatoxin-fortified samples were run for percent recovery by the proposed method. Results listed in Table 1 show recoveries of 79.0 to 93.7% for aflatoxin B_1 and 85.0 to 100.0% for aflatoxin G_1.

The fairly high percent recoveries permit a detection of low level of aflatoxins. A pulp sample fortified with 8 ppb aflatoxin B_1 has been positively identified in a sample spotted as 0.5 g dried weight and 4 ppb as 1.0 g dried weight.

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Transferring Cercospora Leaf Spot Resistance From Beta Maritima to Sugarbeet by Backcrossing[1]

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Introduction

Leaf spot, caused by *Cercospora beticola* Sacc. (I)[3], is one of the most widespread diseases of sugarbeet and thus is a serious problem in sugarbeet production throughout the world. The disease damages the leaves and consequently reduces yield of roots, percentage sucrose, and purity (4).

Control of leaf spot can be effected by dusting or spraying with commercial fungicides, but the development of resistant varieties or strains of sugarbeet now offers a more practical solution to the problem. A number of varieties with moderate levels of leaf spot resistance have been introduced by the U.S. Department of Agriculture and U.S. beet sugar companies in an attempt to meet the requirements in various districts (2). Such varieties have given only partial control of the disease, and the production of varieties with higher levels of resistance is needed.

The wild beet, *Beta maritima* L., seems to be an excellent source of high resistance to leaf spot. This study was conducted to appraise *B. maritima* as a source of leaf spot resistance and to evaluate the backcross method of plant breeding as a tool for transferring the high resistance of *B. maritima* to sugarbeet.

Materials and Methods

The crosses, selections, and reproductions involved in this breeding study were made under the direction of J. O. Gaskill at the U.S. Sugarbeet Research Station in Fort Collins, Colorado. The first cross, US 22/4 [multigerm (MM), curly top resistant (CTR), leaf spot susceptible (LSS)] X *B. maritima* [MM, leaf spot resistant (LSR)], was made in 1956. F_1 hybrid seed from this cross was planted the following year. Eighteen hybrid plants were chosen after selection for leaf spot resistance, root size and shape. These 18 selected plants were backcrossed to SL 539

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[3] Numbers in parentheses refer to literature cited.

[monogerm (mm), CTR]. From the first backcross generation, eight beets were selected for leaf spot resistance, root size, and shape. These beets were planted in the field and allowed to interpollinate. The resultant seed (B_1OP_1) was planted in the greenhouse; the young seedlings were photothermally induced (3); and 33 plants were selected for the monogerm character from the segregating generation. No selection was made in this generation for leaf spot resistance. The 33 selected plants were allowed to interpollinate, and the resultant seed was planted in the field the following year. After selection for leaf spot resistance, root size and shape, 14 roots were chosen. These again were backcrossed to a leaf spot susceptible variety, McF. 663 (MM. CTR). The resultant second-backcross seed was planted the following year and selections were made for leaf spot resistance, root size and shape. Thirty-five selected beets were planted in 1965 for the production of an open-pollinated generation (B_2OP_1). No selection was made for sucrose after either the original cross or the backcrosses.

Four populations were planted in the field in 1966 in a randomized complete block design with 22 replications:

1. A leaf spot susceptible sugarbeet variety—an increase of McF. 663.
2. *B. maritima.*
3. B_1OP_1—first open-pollinated generation following the B_1.
4. B_2OP_1—first open-pollinated generation following the B_2.

Single-row plots, 20 feet long, with 20 inches between rows, were alternated with rows of a susceptible sugarbeet line. Plants in the entire field were inoculated by means of a spore suspension prepared from diseased sugarbeet leaves collected in the preceding year. Supplemental sprinkling of the field, in addition to normal furrow irrigation, was used as an aid in developing an epidemic of leaf spot. Leaf spot readings were made on 16 individual plants in each plot at the peak of leaf spot development. Individual plant weight records and sucrose analyses also were made after harvest in all populations except in *B. maritima.* Ten plants were taken from each plot for this purpose. A composite sample of plants was taken from each plot of *B. maritima* for weight and sucrose analyses because of the small branched roots in this population. The scale used for leaf spot readings was "O" for no leaf spot and "10" for completely defoliated plants, a scale commonly employed by Sugarbeet Investigations, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture.

Results and Discussion

Some bolters (i.e., plants with seedstalks) occurred in populations having *B. maritima* parentage. To decide whether or not to use the leaf spot readings of bolters, *t* tests were applied to the means of readings of bolters and non-bolters. The means and their standard errors are given in Table 1. The differences between the leaf spot reading means of the bolters and non-bolters were significant at the 1% level. Consequently, all bolting plants were excluded in subsequent analyses of the data. Of the plants to which leaf spot readings were given in each plot, the first 10 non-bolters were used for these analyses.

Population means and standard errors for leaf spot readings, weight per root and percentage sucrose are given in Table 2. The open-pollinated backcross populations had acceptable weights and sucrose percentages, and their leaf spot readings were substantially lower than the readings for the sugarbeet variety (population no. 1).

Some fluctuations may be observed in total within-plot variances given in Table 3. These variances were obtained by calculating the variance within single plots and dividing the sum of the single-plot variances by 22 for a given population. This

Table 1.—Leaf spot reading means and standard errors for bolters and non-bolters.

Pop. No.	Non-bolters	Bolters
1	6.07 ±0.13	
2	2.35±0.12	4.75±0.42
3	4.93±0.10	7.05 ±0.23
4	4.83±0.14	5.75±0.48

Table 2.—Population means and standard errors for leaf spot, weight per root, and percentage sucrose.

Pop. No.	Leaf spot reading	Weight per root (kg)	Sucrose %
1	5.990±0.07	0.483±0.01	14.105±0.09
2	2.415±0.06	0.060±0.02	10.920±0.17
3	4.936±0.07	0.395±0.01	14.869±0.09
4	4.850±0.07	0.492±0.01	15.039±0.07

Table 3.—Total within-plot variances for leaf spot readings, weight per root, and percentage sucrose.

Pop. No.	Leaf spot readings	Weight per root	Percentage sucrose
1	0.7464	0.0349	1.3185
2	0.8614		
3	0.8828	0.0290	1.4940
4	0.8479	0.0416	1.0985

method excludes the variation due to replications and that due to the interaction of population X replications. It gives one an opportunity to look at random and genetic variability within the plots.

Population 1 had the lowest total within-plot variance for leaf spot and provided the best available estimate of the environmental variance for leaf spot. This population is assumed to be fairly homozygous for leaf spot susceptibility. However, some genetic variance must remain in this population. Therefore, heritability ratios based on this assumption are conservative broad-sense estimates or minimum estimates. Calculated broad-sense heritability ratios were 0.134, 0.155, and 0.120 for populations 2, 3, and 4, respectively.

Univariate frequency distributions for leaf spot readings are presented in Figure 1. It is clear from these results that the open-pollinated backcross populations had valuable individuals for leaf spot resistance. For example, there were five plants with a leaf spot reading of 2 in the B_2OP_1. This is a relatively high

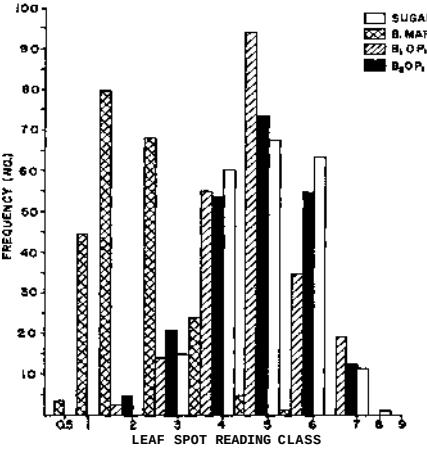

Figure 1.—Univariate frequency distributions for leaf spot readings in all populations.

proportion of individuals in a population of 220, and it is considered very promising to have 2.3% highly resistant individuals in a leaf spot breeding program as a basis for further selection. Root weights and sucrose percentages for the five leaf spot resistant plants are given in Table 4.

Table 4.—Root weights and sucrose percentages of the five highly resistant individuals in the B2OP1 population.

Leaf spot reading	Root weight	Sucrose
	(kg)	(%)
2	0.90	15.0
2	0.60	15.6
2	1.05	15.6
2	0.45	15.0
2	0.75	14.8

It is apparent that the open-pollinated backcross populations included plants with valuable leaf spot resistance and acceptable root weight and percentage sucrose. Thus, the results of this study demonstrate the feasibility of transferring the leaf spot resistance of *B. maritima* to sugarbeet by backcrossing. Assuming a large population on which to base selection, it is very likely that much progress could be made in a short time toward leaf spot resistance, without appreciable sacrifice of root yield and sucrose.

Summary

This study was made as an evaluation of: (a) *Beta maritima* as a source of *Cercospora* leaf spot resistance; and (b) the backcross method of plant breeding as a tool for transferring leaf spot resistance from *B. maritima* to sugarbeet.

A comparison of the leaf spot readings on bolting vs. non-bolting plants showed that the bolting phenomenon was positively associated with leaf spot susceptibility in this experiment; thus it was concluded that leaf spot readings should not be taken on bolting plants in a leaf spot breeding program.

Genetic variation and heritability ratios for leaf spot were relatively low in open-pollinated backcross populations due to an over estimation of the environmental variance. However, it was apparent from the study of leaf spot frequency distributions that there were highly resistant individuals with acceptable weight and sucrose percentage in the backcross progenies, especially in the B_2OP_1. Selection of such individuals in a large population should make possible substantial progress toward the development of a leaf spot resistant sugarbeet variety.

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Sugarbeet Virus Diseases in Arizona, 1965-1968

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Most virus diseases known to affect sugar beets in the United States were recognized within the past 20 years in the seed crop in Arizona. However, little, if any, information was available in regard to the relative incidences and epidemiology of the diseases. It was conceivable that the advent of a large commercial root-crop acreage could alter the disease situation. Thus, a study was undertaken to determine the relative incidences of sugarbeet virus diseases in Arizona and to follow their epidemiology over several seasons.

Methods

Monthly disease surveys were conducted in sugarbeet fields throughout the Salt River Valley in central Arizona from November through June of 1965-66, 1966-67 and 1967-68. Six experimental strip-fields were included the first year and 30 commercial fields were surveyed in each of the latter two seasons. In each experimental field, an area of approximately 4500 ft^2 was selected for plant-by-plant disease counts. Virus incidence in commercial fields was assessed by counting diseased plants in 20 samples of 50 plants each, situated at equal intervals along two random diagonals of the fields. New diagonals were traversed each month. Data included the monthly incidence of yellows (beet and western), curly top, beet mosaic, cucumber mosaic, yellow vein and rosette virus diseases. All fields were planted with Spreckels' Sugar Company's curly-top resistant variety S 301-H.

Results and Discussion

In 3 years the relative incidence of the virus diseases were similar. That is, yellows was the most prevalent, followed by curly top, the mosaics, yellow vein and rosette. Only trace incidences (mean == less than 0.8%) of beet and cucumber mosaic, yellow vein and rosette were observed each year. The yellow net and savoy diseases were not encountered.

Monthly mean incidences of yellows and curly top are presented graphically in Figure 1A & B. During the 1966-67 cam-

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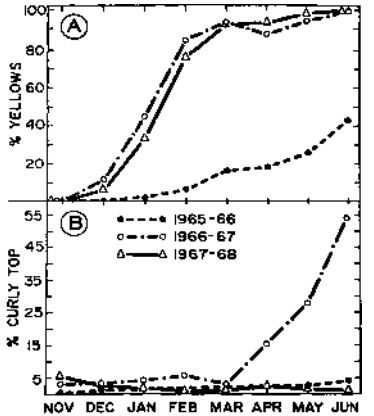

Figure 1.—Mean monthly incidence of sugarbeet yellows (beet and western) and curly top in central Arizona from November through June in 1965-66, 1966-67, and 1967-68; A, yellows incidence, and B, curly top incidence.

paign, when the first commercial root crop was grown, the incidence of yellows and curly top rose appreciably over that of the previous year. In December 1965, only a trace of yellows was encountered; in December 1966, the mean incidence of plants examined was 11%, with a range of 0 to 56% between fields. Final mean incidence of yellows in 1965-66 was only 42%; whereas in 1966-67, 100% of the plants exhibited symptoms of the disease. The mean incidence of curly top remained below 5% in 1965-66. Observations of curly top were discontinued from January to March because recovery (symptom masking) of infected plants made it almost impossible to detect the disease.

Curly top-infected plants were evident throughout the 1966-67 season, and by June the disease reached a mean incidence of 54%, with a range of 14 to 83% between fields. The more rapid build-up of yellows and the 10-fold increase in curly top in 1966-67, as compared to the previous season, can be attributed in part to environmental conditions that were more favorable to the insect vectors in the latter season. An unusually wet and cold winter in 1965-66 suppressed reproduction of aphids and leaf-

hoppers in Arizona. Increased acreage of sugarbeets also provided an abundance of host beets for the viruses and their vectors in 1966-67.

The mean monthly incidence of yellows during the 1967-68 season closely paralleled our results from the previous year. Although monthly incidence of the disease was somewhat lower until March, the final incidence of yellows reached 100% by June. Curly top incidence remained below 5% after our December survey.

Attempts were made to qualitatively correlate disease incidence and yields during the last two seasons. No consistent, general relationships were found; however, yields of certain individual fields apparently were adversely affected by early and high incidence of virus diseases, particularly curly top. For example, one field in the 1967-68 surveys had an initial incidence of 48% curly top and also an early high incidence of yellows. This field yielded about 16 tons of beets per acre with 15% sucrose, whereas the average yield in the Salt River Valley was about 22 tons with 15% sucrose.

The unusually high incidence of curly top in the 1966-67 season cannot easily be explained. The occurrence of more virulent strains of the virus late in the season could account for the increase in incidence and disease severity (2)[2]. However, isolates of curly top collected in March 1968 proved to be more virulent than Giddings' severe "Strain 11" (3) and almost as virulent as Bennett's (1) Los Banos strain (McFarlane, personal communication). Yet, recovery of the beets from early infection made it almost impossible to detect curly top infection late in the 1967-68 season. Apparently, factors other than the presence of highly virulent strains are necessary for the development of severe curly top symptoms in central Arizona.

The incidences of the sugarbeet virus diseases probably will vary from year to year depending on several factors. The effects of environment on the insect vectors and the reservoir hosts of the viruses are important considerations. Also, the maintenance of a beet-free period coupled with complete field cleanup operations of previous beet crops would reduce the potential of beets serving as reservoirs of virus for succeeding beet crops.

No consistent directional migration of green peach aphids *[Myzus persicae* (Sulz.)] or beet leafhoppers *[Circulifer tenellus* Baker)] into central Arizona has been observed. However, a hypothetical division of the Salt River Valley into four equal quadrats revealed that yellows incidence always was higher in the northwest quadrat early in the season. Qualitative estimates

[2] Numbers in parentheses refer to literature cited.

of green peach aphid populations early in the 1967-68 season nicely correlated the high incidence of disease with the greater aphid populations. Curly top, conversely, always has shown a higher early incidence in the southeast quadrat. Such information may be of help in searches for primary-inocula reservoir hosts of the viruses and the oversumraering habitats of the insect vectors.

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Mineral Composition of Sugarbeet Plants as Affected by Varieties and Genotypes[1]

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Haddock and Stuart (3)[3] demonstrated in nutrient culture studies that a specific monogerm sugarbeet variety, SLC 126, can be characterized for high yield and quality by a narrow seasonal range in chemical quality factors. If the wide variety of sugarbeet plants now being grown commercially, and those likely to be developed in the future, could be characterized nutritionally by reference to this same narrow range in chemical composition, rapid progress could be made in adjusting fertilizer practice to obtain high yield and quality of sugarbeets.

Ulrich et al. (8) concluded from a widely distributed geographic study in 1958 that low sugar yields may be more closely related to inadequacies in nutrition than to climatic limitations.

If a wide variation in chemical composition of plant tissue exists among varieties of commercial sugarbeets when grown under the same soil fertility conditions, it may not be possible to establish satisfactory chemical composition reference standards based on one variety which would apply to all other varieties.

Methods and Procedure

In 1966, we planted 48 sugarbeet genotypes on Millville silt loam in a variety testing program. We selected 21 of the widely varying genotypes for chemical study. The source of the genetic material for the 19 four-way sugarbeet hybrids used are shown in Table 1. In addition to the 19 hybrids, which included several with common male and female parents, two current commercial varieties were used as checks.

The Millville silt loam on which these varieties were grown is a deep, well-drained, calcareous soil derived from dolomitic limestone. The profile is relatively uniform in texture to a depth of more than 20 feet. The pH of the soil varies from 7.9 to 8.2 and contains 45 to 70% calcium carbonate equivalent. The average moisture at 1/3-atmosphere tension is 21%, and at 15 atmospheres it is 8.7%. The electrical conductivity (EC X 10^3

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[3] Numbers in parentheses refer to literature cited.

Table 1.—Lines selected in 1966 variety test I for leaf-petiole and leaf-blade chemical composition study.

Code No.	Description	Comparisons: Common d" parent: CT9	129	515	Common ? parent: 308	3611	OV. 3	Local sources of lines: 00.5	0%	25%	50%	75%
101	1114 Check											
104	(308 X 00.5) X (F.C. 503 X Rf)				X			X	X			
107	(129 X OV.l) X (CT9 X Rf)	X								X		
108	(308 x 00.5) X (CT9 X Rf)	X			X			X				
109	(308 x 129) X (CT9 X Rf)	X			X							X
111	(129 X 3611) x (CT9 X Rf)	X				X					X	
112	(308 x CT5B) X (CT9 X Rf)	X			X							X
113	(128 x OV. 3) X (CT9 X Rf)	X					X					X
114	(AM X OV.3) x (CT9 x Rf)	X					X				X	
115	(308 x 00.5) X (128 X Rf)				X			X		X		
117	(308 x 3611) x (128 x Rf)				X	X					X	
122	(308 x CT5B) x (129 X Rf)		X		X							X
123	(AM x OV.3) x (129 x Rf)		X				X				X	
127	(CT9 X OV.3) x (129 x Rf)		X				X					X
128	(308 x CT9A) X (129 X Rf)		X		X							X
134	(AM x OV.3) x (C515 x Rf)			X			X			X		
135	(AM x 503) x (C515 X Rf)			X					X			
141	(AM x 3611) x (C515 x Rf)			X		X			X			
144	(308 x 00.5) x (AM X Rf)				X			X				
148	UI Commercial check											
149	TASCO commercial check											

at 25 C) of the saturated extract varies from 0.35 to 0.52 millimhos per cm. The cation exchange capacity is 13.3, with calcium and magnesium constituting 12.4, sodium 0.4 and potassium 0.5 milli-equivalents per 100 g of soil. The irrigation water contains 1,10,85 and 240 lb of K, Na, Mg and Ca, respectively, per 24 acre-inches of water. The soil and water environmental conditions are therefore very favorable to the growing of sugarbeets.

The soil Avas fertilized with 40 lb of nitrogen and 50 lb of P_2O_5, per acre. Beets were planted May 2, 1966, and sprinkled for 6 hours immediately thereafter. The varieties were planted in two-row plots 36 feet long and 22 inches apart. Individual beets were spaced 12 inches apart. Successive sprinkler irrigations were applied May 30, June 8 and at weekly intervals thereafter until harvest. Beets were thinned on June 6 and harvested on October 10. It was cold and dry early, and remained dry throughout the season. No external nutrient deficiencies were observed during the growing season.

Sampling Procedure

Leaf and petiole samples were obtained on August 15 (the period of most rapid growth) in order to have the plants under highest possible stress for nutrient absorption. Eighteen recently mature blades and petioles were selected from each plot, washed in deionized water, dried in a forced draft oven at 70 C and ground in a stainless steel mill to pass a 40-mesh screen.

One gram of finely ground leaf petioles was extracted with 100 ml of 2% acetic acid solution. Sodium was determined on diluted aliquots by flame photometry. Soluble organic-nitrogen plus ammonia-nitrogen was obtained by the micro-kjeldahl method using 20 ml of solution. Nitrate-nitrogen was obtained from the extract by the spot-plate diphenylamine-color method. Total soluble nitrogen is the sum of soluble organic, ammonia and nitrate-nitrogen.

Leaf blades were wet-digested with HNO_3, followed by $HC10_4$ acid after the method of Gerritz (2). Phosphorus was determined by Barton's (1) procedure. Potassium was determined on a diluted aliquot of the above described digest by means of atomic absorption. Kjeldahl nitrogen (excluding nitrate-nitrogen) was obtained on 0.2 g of finely ground (< 40 mesh) plant material by means of micro-kjeldahl digestion distillation and titration procedure.

Sugarbeet pulp samples were dried in a forced-draft oven at 70° C, ground to pass a 40 mesh screen and analyzed for nitrogen,

phosphorus and potassium. Nitrogen was determined on a 0.5 g sample of dried pulp using the micro-kjeldahl procedure. Nitrate-nitrogen was not included in this determination. Phosphorus was determined by Barton's (1) procedure and potassium was determined by atomic absorption techniques from a diluted aliquot from a HNO_3 - $HClO_4$ digest of 2 g of dried pulp.

Experiment Results

Yield of gross sugar is shown (arranged in descending order) in Table 2 for the 21 varieties used in this study. This arrangement is maintained in Table 2 and Figures 1 to 3 which indicate the relation of other composition factors to yield of sugar. The mean value for the two commercial varieties used as checks is indicated by the broken horizontal line in Figures 1 to 3. It will be observed in Table 2 that there are three varieties significantly higher in sugar yield than the commercial check. It will also be noted in Table 2 that yield of gross sugar is closely related to yield of roots. Five of the varieties are significantly

Table 2.—Yield and quality of sugarbeet roots and chemical composition of petioles as influenced by genotypes, 1966.

Treatment No.	Gross sugar lbs/A	Yield roots T/A	Sucrose (percent)	Aug. sampling of petioles PPM	
				Soluble - N	Soluble~Na
101	7810	26.01	15.02	8850	6600
117	7764	25.10	15.50	9963	5313
128	7655	26.10	14.68	7088	6638
127	7435	24.93	14.89	6763	5513
115	7359	25.52	14.37	6975	7000
108	7152	26.11	13.66	7413	5888
113	6995	23.76	14.72	6813	5863
114	6978	22.24	15.02	8238	6225
109	6956	23.51	14.82	7738	6875
112	6951	23.13	14.99	8038	6588
123	6883	22.44	15.34	7075	6575
141	6839	21.75	15.74	7650	7675
107	6801	22.98	14.77	7900	6713
122	6800	23.49	14.41	6263	5988
104	6646	23.54	14.16	6338	7213
111	6602	20.59	15.87	5938	7825
148	6598	22.24	14.85	9088	8925
149	6539	21.51	15.16	6450	6550
135	6390	20.53	15.55	6838	6225
134	6382	20.89	15.23	7913	6463
144	6299	22.30	14.12	7000	6663
Mean	6932	23.27	14.91	7444	6534
S.E. of M	334	1.08	0.22	686	530
Sig. @ .05	933	3.00	0.60	1915	1482
C. V.	13.62	13.07	4.10	26.00	13.70
F - Value	1.71	2.74	6.96	2.16	4.48

higher in root yield than the commercial checks, but 14 are not different in yield.

It is evident from the tabular data in Table 2 that there is no positive relation between sucrose percentage and yield of sucrose or yield of roots. There are two varieties significantly different from the commercial checks, numbers 111 higher and 108 lower.

Data shown in Table 2 indicate that nitrogen concentration in petioles in August is not related to yield of gross sugar in October. Two varieties are significantly different from the checks; number 111 is lower in nitrogen and number 117 is higher.

Statistically, the sodium concentration in petioles shown in Table 2 is positively associated with gross sugar yield. (It is difficult to see this relationship from the tabular data in Table 2). Number 117 is the only variety significantly different from the commercial checks in sodium concentration in petioles.

The quantity factor proposed by Haddock and Stuart (3) as optimum for sugarbeet leaf blades is 450. None of the genotypes that we used reached this value. However, one variety, number 104, varied significantly from the commercial checks in this measurement (Figure 1).

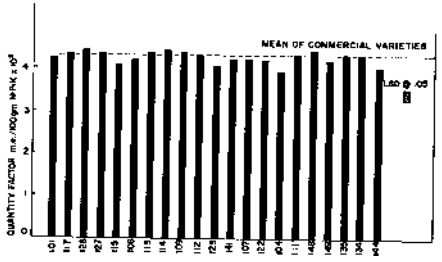

Figure 1.—Quantity factor of sugarbeet blades as influenced by genotypes, 1966.

The optimum nitrogen quality factor for sugarbeet leaf blades is 65. The mean value for the two commercial checks was 71.3. Nevertheless only number 122 was significantly below the checks (Figure 2).

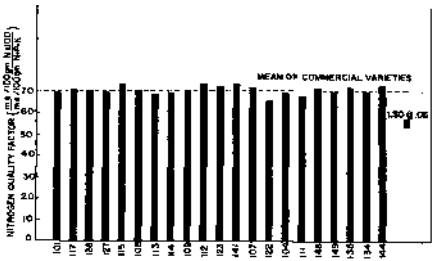

Figure 2.—Nitrogen quality factor of sugarbeet leaf blades as influenced by genetypes, 1966.

The nitrogen quality factor proposed as optimum for sugarbeet pulp is 63.2. This is considerably below the mean shown in Figure 3 for the commercial check sugarbeet roots. Note that three varieties are significantly below the mean for the commercial checks in Figure 3 (i.e., 108, 109 and 122).

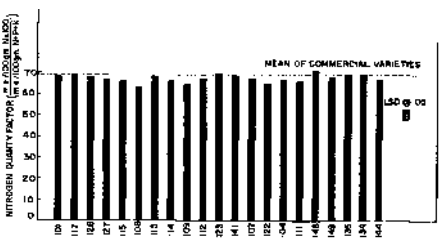

Figure 3.—Nitrogen quality factor for sugarbeet pulp as influenced by genotypes, 1966.

Discussion

We expected to find wide variation in chemical composition among the varieties studied. However, it is obvious (Table 2 and Figures 1 to 3) that variation is small. Roboz (6) stated that the quantity of harmful nitrogen in beet roots varies with the variety. She showed that 50% of a lot of 300 beets were from 50 to 100% higher in harmful nitrogen than the low nitrogen beets. Payne et al. (5) found no difficulty in showing a range of 40% in the sodium and potassium content of thin juice in their study of 20 varieties. Ryser et al. (7) found significant differences among nine genotypes for root composition of amino N, sodium, potassium and for petiole composition of nitrate-nitrogen.

There were significant differences among the 21 genotypes in almost every chemical studied, but these differences were very small for the diversity of genotypes examined. The coefficient of variation is modest for biological field material varying mostly from 5 to 15%.

The quantity-quality factors show less variation than shown for the specific concentration of a particular element; e.g., nitrogen, phosphorus and potassium.

There are, undoubtedly, sources of genetic material which would show a greater divergence of chemical composition than the material used in this study. The 18 four-way hybrids were each made up of diverse genetic material; however, they all had the same pollen restorer inbred parent. All but two entries had an Ovana parent (308, Ov 1 or Ov 3). In addition, several of the hybrids had one or two other parents in common (Table 1). Inbred lines could be produced which would consistently show higher or lower values in specific chemical constituents than the commercial beets used as check plants.

Although the 21 genotypes were quite diverse in genetic origin, they did not show great variability in yield, quality or chemical composition of leaf-blades, roots or petioles. The limited range of genetic material suggests that neither macro- nor micronutrients would be altered markedly by a breeding and selection program. However, other hybrids derived from inbreds with a broader genetic base for chemical constituents may have shown greater variation.

From the limited range of genetic material studied to date there is little basis for concern lest standards of nutritional adequacy used in 1968 will become outmoded by new releases of commercial varieties to be used in 1988. Of greater concern is the danger that sugarbeet growers will fail to use, to their advantage, standards of nutritional adequacy now available to them.

Summary

Twenty-one sugarbeet varieties were grown on the same soil and analyzed for various plant nutrients. Statistically significant differences were obtained among the 21 genotypes for each nutrient element studied. The range in chemical composition, while statistically significant, was relatively small. This suggests that it is feasible to use a standard chemical analysis for critical levels (and more particularly to use quantity-quality factors) in appraising the nutritional status of newly released commercial varieties, without danger of being misguided in the use of fertilizer or soil amendments.

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Emergence and Development of Sugarbeets as Influenced by Various Soil Mulches[1]

D. E. MILLER[2]

Received for publication January 7, 1968

In irrigated regions there are two important problems associated with the establishment of good stands of seedlings. In early spring when the surface soil is moist, soil temperatures often remain so low that seed germination and seedling development are inhibited. Also, there is danger of plant injury by freezing. Later in the season when soil temperatures are more favorable, the soil may be too dry for satisfactory establishment of seedlings.

The microclimate of the seed zone can be drastically changed through the use of mulches (l)[3]. Clear plastic strips over the soil increase soil temperatures and hasten seedling growth (9). However, plastic strips are not economical at present for sugarbeets and there is the problem of removing the plastic at the end of the growing season.

Recently, asphalt mulches have been used to obtain earlier and better stands of seedlings. Water emulsion asphalt possesses many characteristics of a desirable soil mulching material. Its application can be easily mechanized and combined with planting and chemical weed control operations. The material does not injure seedlings and, at the rates involved, does not induce undesirable effects *in* the soil. At the end of the growing season the asphalt residue is simply incorporated into the soil in the usual cultural operations. The cost is such that it can be economically used.

Takatori et al. (12) used asphalt mulches to obtain earlier germination and maturity of sweet corn. Soil temperatures were increased during the day by the mulch, and a band 6 inches wide was nearly as effective as 12- or 24-inch bands. They (7) also reported a decrease in loss of soil water by evaporation with mulching. The effect was greater as the asphalt band width was increased from 3 to 24 inches, although differences between the 12- and 24-inch widths were small. Soil water content 16 days after planting was about 4% by weight greater under asphalt than in bare soil. Bement et al. (2) reported increased soil temperatures and soil water contents with asphalt mulches.

[1] Contribution from the Soil and Water Conservation Research Division, Agricultural Research Service, USDA, in cooperation with the College of Agriculture, Washington State University, Pullman.

[2] Research Soil Scientist, USDA, Prosser, Washington.

[3] Numbers in parentheses refer to literature cited.

Fletcher (4 reported by 6) reported yields of sugarbeets were increased 2 tons per acre by 5-inch bands of asphalt mulch. The increase was attributed to higher soil temperatures, increased water and reduced crusting. Increases in soil temperatures as a result of use of asphalt mulches have been reported by Johnson et al. (5), Miller (8), Schales and Sheldrake (11), Sale (10) and many others. An excellent bibliography with abstracts on the use of asphalt and other mulches on agricultural crops has been prepared by Lippert et al. (6).

The primary objective of this study was to evaluate the influence of asphalt mulches on early spring soil temperatures and on emergence of sugarbeets. Two other mulches also were compared: (a) covering the soil between seeded rows with standard greenhouse glass; and (b) covering the soil between seeded rows with a layer of water held in clear polyethylene bags. Glass allows short-wave radiation from the sun to pass through but restricts the long-wave radiation emitted by the soil. Evaporation is reduced by the glass so that more of the incoming radiation is converted to sensible heat and used to warm the soil. Thus, a glass mulch should allow a soil to become warmer during the day and remain warmer at night than a bare soil. A water layer absorbs the short-wave radiation from the sun. It acts as a heat sink during the day and as a heat source at night. A glass or water mulch should increase the minimum nighttime soil temperatures. Danger of frost damage to seedlings should be reduced by conduction of heat to the air around the plant from the soil, in the case of the glass mulch, and from the soil and water if a water mulch is used. The beneficial effect of a water mulch was demonstrated by Bowers (3).

Materials and Methods

Mulch Treatments Three replications of four treatments were combined in a randomized block design. Each plot consisted of four rows, 25 feet long and 22 inches apart. Two guard rows were planted on each side of the experimental area.

The treatments were: (a) bare soil; (b) water emulsion asphalt, applied by spraying in a band about 12 inches wide over the seeded row; (c) standard greenhouse glass plates placed between rows with a space of about 1 inch left uncovered over the seeded row; and (d) polyethylene bags containing water placed between rows with a space of about 1 to 2 inches left between bags over the seeded row. The rate of asphalt application was about 450 gallons per acre of treated soil or 240 gallons per acre, total area. Prior to application of the emulsion, the rows were smoothed with a garden rake to allow good emulsion-soil contact. The

glass plates were placed at seeding time and left for about 6 weeks. In the polyethylene bags the depth of water varied from about 1 to 3 inches because of land side-slope. It was difficult to keep the water-filled bags off the seeded row because they tended to roll with the slope. Some seedlings lying under the bags were damaged. This trouble also made it necessary to remove the bags from the plots sooner than desirable. Some of the trouble was eliminated in the second seeding by welding a seam down the center of each bag and thus using two water-filled compartments in each bag. The water-filled bags were placed at seeding time and left for about 6 weeks.

Seeding Dates Two seeding times were used—February 17 and March 16. The two seeding areas were adjacent but statistically independent. The normal seeding time in the area is early- to mid-March.

A commercial seeder was used to plant at a rate of 12 seeds per foot at a depth of 1.5 inches. Seedling stands were thinned to 8- to 10-inch spacings.

Fertilization Nitrogen was broadcast and plowed under at the rate of 160 pounds nitrogen per acre as ammonium nitrate. Soil tests indicated no need for phosphorus and potassium.

Weed Control Weeds were controlled with ethyl N-ethyl-N-cyclohexylthiolcarbamate[+], applied at 4 pounds per acre and disked in twice to a depth of 3 inches.

Temperature Measurements A millivolt strip-chart recorder was adapted to record temperatures indicated by copper:constantan thermocouples. The thermocouples were installed at the end of one row in each plot at seed depth. Temperatures from the three replications of each treatment were averaged and plotted as a function of time of day. An integrated temperature for each day was obtained by measuring the area under each time:temperature curve with a planimeter. Maximum and minimum temperatures were obtained each 24-hour period from the same curves. Temperatures were measured for 3 weeks following each seeding.

Results

Soil Temperatures Soil temperature data at a depth of 1.5 inches are shown for February 20 (first seeding) in Figure 1 and for March 23 (second seeding) in Figure 2. Both of these days were bright and sunny. Integrated, minimum and maximum temperatures are presented in Table 1 for the period of about

[4] A product of StaufTcr Chemical Company, Code Number R-2063, presently sold under the name "Ro-neet." Trade names and company names are for the benefit of the reader and do not imply any endorsement or preferential treatment of the named product by the U. S. Department of Agriculture.

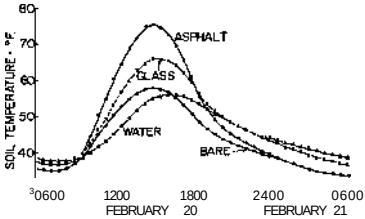

Figure 1.—Soil temperatures at a depth of 1.5 inches as measured February 20 and 21, 1966. First sugarbeet seeding.

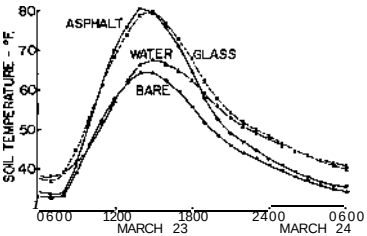

Figure 2.—Soil temperatures at a depth of 1.5 inches as measured March 23 and 24, 1966. Second sugarbeet seeding.

3 weeks following each seeding. The maximum temperatures were highest with asphalt and glass mulches, and the minimums were lowest with asphalt mulch or bare soil. The glass and water mulches decreased the night cooling rate compared with asphalt cover and bare soil. As the season progressed with longer days, greater solar radiation and shorter nights, the night soil temperatures under asphalt remained slightly above those of the bare soil.

Several times during the first few weeks of the first seeding the soil under the asphalt or without cover was frozen. The soil under the glass and water mulches did not freeze although ice formed in the water bags a number of times.

Emergence Emergence rates for the two seedings of sugarbeets are shown in Figures 3 and 4. As indicated in Figure 3 for the first seeding, emergence was most rapid with the glass mulch followed by the water and asphalt treatments. Very few seedlings emerged from the bare soil. A severe frost on March 17 killed

most of the seedlings on the asphalt plots and few on the glass treatments. None were killed on the water-mulch plots. This damage is indicated in Figure 3 by the decrease in emergence after March 18. Seedlings had not then emerged from the bare plots. On all plots about 30 to 40% of the plants that survived the early frost bolted later in the season.

In addition to the frost injury to seedling on the glass-covered plots, injury was observed from sand blowing. Apparently the

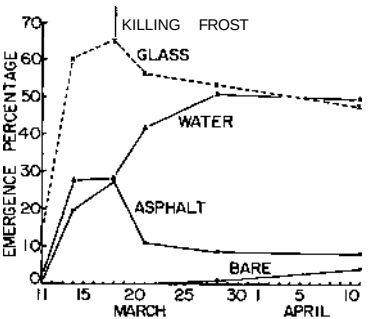

Figure 3.—Rate of emergence of sugarbeet seedlings as affected by various mulches. First seeding, 1966. Planted February 17.

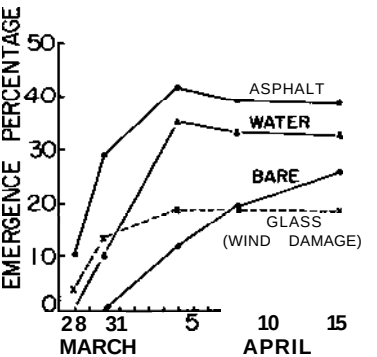

Figure 4.—Rate of emergence of sugarbeet seedlings as affected by various mulches. Second seeding, 1966. Planted March 16.

smooth glass surface encouraged injury from windblown sand. This was more serious in the second seeding than in the first.

Emergence from the second seeding was most rapid with the asphalt treatment followed by the water mulch (Figure 4). From the temperature data in Table 1, one would expect the greatest emergence from the glass treatment. The severe sandblast injury observed with the glass treatments in the second seeding may account for the lower apparent emergence. Seedlings killed by sandblasting would be missed in subsequent seedling counts.

Table 1.—Integrated, minimum and maximum soil temperatures at a depth of \-Vz inches as influenced by various mulches, 1966.

First seeding of sugarbeets—February 19 through March 13

	Integrated OF	Minimuin °F	Maximum oF
Bare	**40.8**	33.6	50.9
Water	43.7	37.4	51-5
Asphalt	**44.1**	33.7	62.6
Glass	45.3	36.2	58.1
L.S.D. .05	0.5	0.4	**1.2**
.01	0.7	0.5	**1.6**

Second seeding of sugarbeets—March 17 through April 5

	Integrated "F	Minimum oF	Maximum "F
Bare	52.2	39.7	67.2
Water	56.6	45.0	70.0
Asphalt	57.0	40.3	79.2
Glass	60.5	44.3	81.2
L.S.D. .05	0.6	0.6	**1.1**
.01	**0.8**	**0.7**	**1.4**

Emergence was low[r] for all treatments in the second seeding. The only apparent reason is that soil water was marginal. Bolting in the second seeding was negligible.

Plant Development and Yield- Plant development was much faster on the mulched plots than on bare soil, but there were no great differences in growth rates among the various mulches. The seedling stands were generally more uniform on the asphalt-treated plots than on the other treatments. Yields were not taken from the early seeding because of the frost damage. In the second seeding, yields from the mulched plots were not significantly (5% level) greater than those from the bare plots.

Summary

Early spring soil temperatures were increased and germination and seedling development of sugarbeets were hastened by application of asphalt mulch over the seeded row. Glass and water mulches were effective also although they are not practical for sugarbeets. When sugarbeets were planted a month earlier

than normal, good stands were obtained on all mulched plots but few seedlings emerged on the bare plots. Most of the seedlings that had emerged on the asphalt plots were killed by frost and about 30 to 40 percent of the surviving plants bolted later in the season. The glass and water mulches prevented seedling loss by freezing but did not prevent the bolting.

Yields were not taken from the early seeding because of the frost damage. With a normal seeding time yields were not significantly increased by the mulches.

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Trends in Nonsucrose Constituents of Central California Beets

II. Comparison of Molasses Produced at a Straight House and Steffen House.

P. H. MILLER[1]

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This is the second of two papers dealing with the trends of nonsucrose constituents in beets. The first paper concerned itself exclusively with impurities associated with Steffen-house operations^)[2].

In this paper the relative levels of nonsucrose constituents found in molasses produced at a straight house will be compared with those of Steffen molasses[3]. An attempt will be made to explain some of the differences noted.

Materials and Methods

Samples of daily molasses-produced composites from straight-house and Steffen-house operations were composited on a weekly basis. The samples were stored at ambient temperature.

The methods used to determine the various nonsucrose constituents in molasses have been given in a previous report(2).

Results and Discussion

Total Nitrogen

The nitrogen data presented are averages of the last four crop years. A plot of the total-N content of molasses produced at a straight house as well as a Steffen house is shown in Figure 1. The data are calculated as percentages on beets. The figure indicates that the same general trend of total-N is found in the two types of molasses produced. The major difference seems to be in the relative level.

The levels are higher in Steffen molasses. This is because the amount of molasses produced at a Steffen house, calculated as a percent on beets, is higher, thereby inflating the total-N level.

It should be noted that the trends in the nitrogen content of beets, as shown in Figure 1, are repeated each year at approximately the same time of year. Only the relative amount differs. At the present time we are not completely certain as to the cause of the trends.

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[2] Numbers in parentheses refer to literature cited.
[3] The straight-house factory is located in Manteca, California. The Steffen-house factory is located in Woodland, California.

There is a highly significant correlation between total-N, calculated as a percent on beets, and the following straight-house statistics: molasses produced [+0.65], cossette apparent purity coefficient (apc) [—0.80], and percent nonsugars in cossettes [+0.89]. A lower but significant correlation was also found between total-N and sugar extraction.

The same correlations of Steffen-house data were generally lower than found for straight-house data. However, they were all significant except for cossette apc.

A significant correlation does not exist between total-N, calculated on a beet basis, and cossette percent sugar. This was due primarily to spring campaign data. As can be seen in Figure 1, total-N content of beets declined quite rapidly in beets harvested after March. In order for a significant correlation to exist, cossette percent sugar would have had to show a similar increase. Representative factory data did not indicate that an appreciable increase occurred.

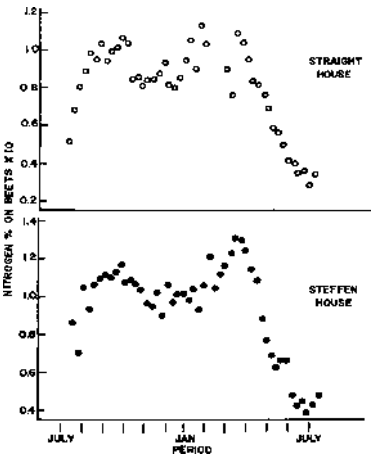

Figure 1.—Total nitrogen in molasses for crop years 1963-1966, straight house and Steffen house.

Amino Nitrogen

Figure 2 contains a plot of the amino-N and PCA-N content of straight-house and Steffen-house molasses produced during the last four crop years. Except for an increase during the February-

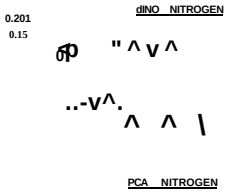

Figure 2.—Amino-N and PCA-N in straight-house and Steffen-house molasses, 1963-1966.

March period for straight house and the December-January period at the Steffen house, amino-N content of beets generally declines throughout the crop year.

Three samples of molasses produced at the Steffen house during the 1966 crop year were analyzed for individual amino acid content using the method of Gehrke (1). The samples represented molasses produced during early fall, late fall and late spring operations. The results are tabulated in Table 1 and are expressed on a nonsugar basis (mM/100 NS).

Of the twelve amino acids, alanine, aspartic acid, glutamic acid and the leucines account for over 70% of the amino acids

Table I.—Amino acids found in Steffen House molasses, 1966 crop year.

	Early fall		Late fall		Late spring	
Amino acid	mM/ 100NS	rel cone (%)	mM/ 100NS	rel cone (%)	mM/ 100NS	rel cone (%)
Alanine	12.6	24	10.7	21	1.4	31
Valine	4.0	8	2.8	6	0.5	11
Glycine	2-6	5	3.4	7	0.2	6
Isoleucine	4.0	8	3.2	6	0.3	7
Leucine-threonine	3.8	7	2.4	5	0.1	2
Proline	0.5	1	0.3	1	0.3	8
Serine	3.9	8	3.9	8	0.4	9
Aspartic acid	9.9	20	11.1	22	0.5	11
Glutamic acic	7.9	16	9.5	19	0.7	15
Tyrosine	1.7	3	2.3	5		
Total	50.9		49.6		4.4	

found in Steffen molasses. There do not seem to be any big differences in the concentration of amino acids found in early fall and late fall molasses produced. Whereas alanine, valine and proline decline, they are replaced almost quantitatively by an increase in glycine, aspartic acid, glutamic acid and tyrosine. Late spring molasses contain less than 10% of the amino acids which are found in late fall molasses. All of the amino acids decline except proline.

Alanine constitutes approximately $i/_3$ of the amino acids associated with late spring molasses as compared to $i/_4$ during the early fall.

The data in Table 1 show that not all of the glutamine or glutamic acid found in the beet was converted to PCA during the factory process. Calculations indicate the conversion to be approximately 85% during the fall campaign, increasing to 95% in the spring.

The amino acids shown in Table 1 account for approximately 70% of the amino-N found using the ninhydrin method of Harris. This is consistent with results reported on the Steffen factory liquors (2).

The average PCA-N content of straight and Steffen-house molasses produced during the last four crop years is plotted in Figure 2. The trends of PCA-N in straight-house molasses follow that of the total-N data. The trends, however, are not quite as prominent.

The PCA-N level in Steffen-house molasses remains quite constant during the crop year until the beginning of March. Here we see a rise through March followed by a decline of almost 80%.

Nitrate and Betaine Nitrogen

A plot of the nitrate-N and betaine-N contents of straight and Steflen-house molasses produced during the last four crop years will be found in Figure 3. The data are calculated as a percent on beets. The straight-house data indicate a general increase in nitrate-N throughout the fall campaign and into the spring. This is followed by a decline.

In contrast, the nitrate-N content of Steffen molasses remains somewhat constant during the fall campaign. Then in the middle of January it increases almost 100%. This is followed by a decline.

The trends and levels of betaine-N, calculated as a percent on beets, are quite similar in the two molasses types. The trends are also similar to those found for total-N.

A summary of the nitrogen data from molasses produced at straight and Steffen-house operations during the last four crop

years will be found in Table 2. The data have been calculated on a nonsucrose basis (mgN/100 NS) to show the relative concentration of the nitrogen components in each molasses.

During the last four crop years straight-house molasses contained an average of approximately 6% more nitrogen com-

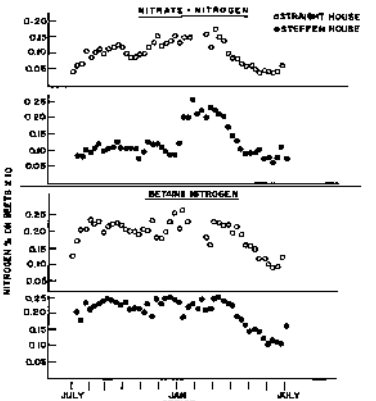

Figure 3.—Nitrate- and betaine-nitrogen in straight- and Steffen-house molasses, 1963-1966.

Table 2.—Summary of nitrogen, Data 1963-1966 crop years, mgN/100 NS.

Straight House						
Nitrogen	1963	1964	1965	1966	Average	Rel cone %
Total	6107	5794	5950	5099	5738	100
Amino	903	837	767	654	790	14
PCA	1140	1122	1182	886	1083	19
Nitrate	755	648	765	602	693	12
Betaine	1479	1354	1454	1202	1372	24
Other	1830	1832	1776	1757	1800	31
Steffen House						
Nitrogen	1963	1964	1965	1966	Average	Rel cone %
Total	5902	5238	5339	5196	5419	100
Amino	836	852	859	898	861	16
PCA	961	921	878	788	887	16
Nitrate	986	593	776	544	725	13
Betaine	1171	1124	1150	1141	1147	21
Other	1948	1747	1675	1829	1799	34

pounds, calculated on a nonsucrose basis, than was found in Steffen molasses. This difference is due mainly to the betaine-N and PCA-N fractions. Of the nitrogen fractions associated with straight-house molasses, the other-N seems to remain the most constant from year to year, fluctuating approximately 4% during the last 4 years. Nitrate-N fluctuates the most with no consistent trend being evident.

The only nitrogen fraction found in straight-house molasses which shows a consistent trend during the last four crop years is the amino-N. It has declined almost 30% during this period. Of the nitrogen fractions associated with Steffen-house molasses, betaine-N concentration seems to remain quite constant from year to year. Nitrate-N fluctuates the most.

The only consistent trends found in the various nitrogen fractions associated with Steffen molasses produced during the last 4 years are in amino-N and PCA-N. The former has shown a consistent increase from 836 to 898 mgN/100 NS. PCA-N, however, has more than offset this amino-N increase by declining from 961 to 788 mgN/100 NS. The data suggest that either the beets contain less glutamine relative to amino-N or that the conversion of glutamine to PCA in the factory is declining. The latter is probably true.

Approximately 51% of the total amino-N (amino-N plus PCA-N) found in Steffen-house molasses is accounted for as PCA-N. This is much lower than the 58% reported for molasses produced at a straight house. The data suggest that a lower percent of the glutamine and glutamic acid found in beets processed at a Steffen house is converted to PCA.

Inorganic Constituents

The average potassium and sodium content of straight and Steffen-house molasses produced during the 1965 and 1966 crop years have been plotted in Figure 4. The data have been calculated as a percent on beets. The same general trend is prevalent for both molasses types. Only the relative concentration is different.

During the last two crop years a highly significant correlation has been found between potassium, calculated as a percent on beets, and Steffen molasses apc [+0.61]. Correlation of straight-house data indicate a highly significant relationship exists between potassium and molasses apc for the 1965 crop year but not for 1966.

The relative concentrations of potassium and sodium remained quite constant during the last two crop years. The calcium data from straight-house and Steffen-house molasses produced during

the 1965 and 1966 crop years are plotted in Figure 5. The same general trends are evident in both types of molasses. It is interesting to note that the same trends have been found in Steffen-house diffusion juice (2).

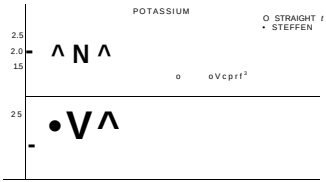

Figure 4.—Average potassium and sodium content of straight- and Steffen-house molasses, 1965-66 crop years.

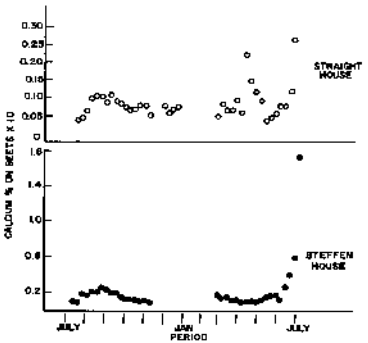

Figure 5.—Calcium data from molasses produced in straight house and Stef fen house, 1965-66 crop years.

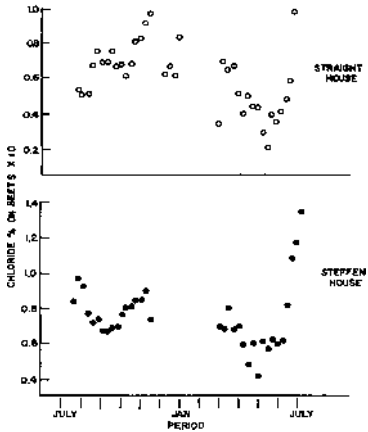

Figure 6.—Chloride data from straight- and Steffen-house molasses, 1965-66 crop years.

Chloride data for the last two crop years are plotted in Figure 6. Of the inorganic constituents studied thus far, chloride seems to fluctuate the most during the crop year and also from year to year. The chloride trends found in the two molasses types vary considerably during the fall campaign. The Steffen data are so widely scattered that it is impossible to find any trend.

A consistently significant correlation between chloride level, calculated as a percent on beets, and molasses apc or true purity data has not been found. This is contrary to data presented by Stark (3).

A summary of the inorganic fractions found in straight and Steffen-house molasses produced during the 1965 and 1966 crop years will be found in Table 3. The results have been calculated on a nonsugar basis (g/100 NS).

Straight-house molasses has approximately 5% more of the inorganic nonsugars studied than Steffen-house molasses. This is due primarily to a higher sodium level.

Calcium levels are much higher in Steffen molasses. This indicates that lime salt production is higher in a Steffen house than a straight house. This would be expected because of the reintroduction of these salts into the factory from the Steffen process.

Table 3.—Summary of inorganic data, 1965-1966 crop years, (g/100 NS).

Inorganic	Straight House	Steffen House
Sodium	**6.4**	**5.6**
Potassium	**10.8**	**10.4**
Chloride	**4.4**	**4.3**
Calcium	**0.9**	**1.1**
Magnesium		
Total	22.5	**21.4**

Table 4.—Nonsucrose content of molasses, 1965-1966 crop years (Relative Concentration)

	Straight House	Steffen House
N-compounds	**41**	**39**
Inorganic	**23**	**22**
Other NS	**36**	**39**

A summary of the nonsucrose constituents found in straight and Steffen-house molasses produced during the 1965 and 1966 crop years is given in Table 4. The data have been categorized under three major headings: nitrogen compounds; inorganic which includes only those covered in this report; and other nonsugars (other NS), presumably carbohydrates and other inorganic. The results are expressed as percentages of the nonsucrose constitutents found in molasses.

The data indicate that the nonsugar fraction of Steffen molasses contains relatively more other NS than straight-house produced molasses. However, it should be noted that at any particular time of the crop year the relative concentration of these fractions will change. This has been explained in an earlier paper on Steffen factory liquors (1).

Summary

The data show that the relative concentration of various nonsugars associated with straight-house and Steffen-house molasses vary considerably during the crop year. In some cases these variations are predictable from year to year. Possibly this information could be used as an aid to predicting optimum harvest time for beets.

The data indicate that straight-house molasses contains relatively more of the nonsugar fraction in the form of nitrogen and inorganic compounds than Steffen produced molasses. This is primarily due to increased amounts of betaine and sodium.

Acknowledgment

The author would like to thank Mr. F. G. Eis, General Chemist & Director of Chemical Research, for his assistance; and Mrs. A. Holland and Mr. T. Morrill, technicians, for analyses.

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Damage to Sugarbeet Roots from Various Degrees of Wilting at Various Temperatures[1]

S. T. DEXTER, M. G. FRAKES AND R. E. WYSE[2]

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When beet roots are removed from the ground, loss of fresh weight is prompt and evident from the loss of turgor. The percentage of sucrose in the wilted beet may rise rather markedly, particularly if the beet is wilted at a low temperature. The relatively new methods of determining pounds of extractable sugar per ton of beets, (3), (5), (9)[3], make it possible to study the loss of extractable sugar per ton of original beets during storage at different temperatures when the beets have wilted to various degrees. It is a well known fact that wilting is excessive in the beets on or near the surface of storage piles, particularly on the side exposed to the sun or prevailing winds. Damage may be considerable even in cases where no freezing or thawing or breakdown of tissue results, and covering with plastic, etc., may greatly reduce such wilting. It was the purpose of this investigation to determine the magnitude of the loss of extractable sugar per ton of harvested beets when wilted at different temperatures without freezing injury and to compare this with the injury from combined freezing, thawing and wilting in commercial piles.

Literature Review

No papers were found that dealt specifically with this problem. However, the results of the extensive research of Pack (7) regarding the loss of total sugar in beets as a result of wilting are common knowledge in the industry. Although the percentage of sucrose in a beet may increase as a result of wilting, the amount of sucrose in the beet decreases. Conversely, the ready uptake of water by beets immersed in water or very damp soil may amount to 5 to 7% of the weight of a "normal" beet (4) reducing the percent sucrose. When beets were immersed in NaCl solutions of various concentrations, equilibrium, with no loss or gain in weight over a period of several days, was found to occur at a concentration of from 2 to 3% salt, (Dexter, unpublished data) or an osmotic value in the vicinity of 20 atmospheres. This value

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station, East Lansing, as Journal Paper No. 4394. Parts of Mr. Wyse's research toward the Ph.D. degree are included in this paper.

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[3] Numbers in parentheses refer to literature cited.

agrees with Pack's figures. By conversion to vapor pressures it would appear that an ordinary beet would neither gain nor lose moisture in an atmosphere of 97-98% R.H. No work was seen, however, relating the ease of wilting to osmotic values in the beet. In Pack's work, large differences in wilting at a constant controlled vapor pressure deficit occurred in beets of essentially identical sucrose percentages, and he suggests that beets could be selected for the genetic character of the slow shrinkage. He found a good positive correlation between shrinkage and sucrose loss, but beets that were high in shrinkage were never low in loss of sucrose. Pack made no analyses for change in purity, invert sugar or raffinose, and in no case was apparent sucrose corrected for invert or raffinose, nor were beets stored under "storage pile" conditions of variable atmospheric conditions.

A survey of the world literature on sugarbeet storage (8) cites and emphasizes European papers relating to moisture loss under various conditions of temperature and relative humidity and the respiration and sugar losses associated with such dehydration. A translation of a part of "Sugarbeet Storage" by S. Vajna, together with literature citations is included in this survey.

Materials and Methods

Beets from the 1966 crop were washed and 10-beet samples weighed into mesh bags before storage. Analyses for percent sucrose (5) and clear juice purity (3) were made before storage and on each date of removal. Analyses for reducing sugars *(I)* and raffinose were made on occasion, in order to correct apparent sucrose, clear juice purity (CJP) and extractable sugar per ton (ESPT). Raffinose was determined by using a coupled enzyme system based on the reaction of galactose oxidase, with free and bound galactose. The enzyme preparation was purchased from Worthington Biochem., Freehold, New Jersey, and the procedure used was similar to that of McCready and Goodwin (6). Beets, Table 1, stored in walk-in U.S. Army refrigeratores, out-of-doors, were protected from dehydration to various degrees in order to produce severe wilting, moderate wilting and slight wilting at two temperatures. This was accomplished by enclosing the mesh-bagged 10-beet samples in single canvas bags, double canvas bags, and in polyethylene bags, in which a perforated bag of damp wood chips was enclosed. Samples of two other lots of beets, A and B, (Table 2) were stored in five other refrigerators with various degrees of ventilation and temperature control. In the company pile approximately 300 10-beet weighed samples (Table 3) were placed in excavations within the first, second and third foot from the outside on the top, shoulder, north and south sides, as well as in the body of

the pile. Samples were removed on three dates, reweighed to the nearest 0.01 pound, and the percent of loss of fresh weight determined. Sucrose analyses were corrected to the original weight of the sample. From this corrected value and the clear juice purity the extractable sucrose per ton of original beets was calculated. In many cases, sucrose was determined by both the Sachs Le Docte (SLD) and the Dexter, et al (DFS) method (5). With severely wilted beets, reduced weights of brei were made up to volume to improve the accuracy of the determination as commonly recommended. (2).

Results and Discussion

Table 1 shows the data for apparent and corrected sucrose, CJP and ESPT for beets stored and removed as shown, to give slight, moderate and severe wilting. The analytical results shown in Table 1 are the mean of those from three 10-beet samples on each date. From the table it may be seen that as wilting increased, ESPT was lowered and that this damage from equal wilting was greater at 7 than at 2C. When the "apparent" and "corrected" (for rafinose and invert) values for percent sucrose, CJP and ESPT are compared, it may be seen that at low temperature and little wilting, raffinose content was relatively high in comparison with invert sugar, and a substantial negative correction was necessary in order to compare the values. Thus, after 70 days of storage at 2C and slight wilting, the apparent ESPT was 16 pounds higher than corrected ESPT, while at 7C and severe wilting, apparent ESPT was 1 pound higher than corrected ESPT. Although the general recommendations of low temperature and no wilting for storage with low loss of ESPT remain valid, the degree of apparent damage was decreased in the wilted beets stored at higher temperature.

After 140 days of storage, it is clear (last column Table 1) that the increased pol due to raffinose was overbalanced by the lowered pol due to invert sugar in cases of severe wilt or storage at higher temperature. From a factory standpoint, the degree of difficulty in handling the problems due to raffinose versus those of invert sugar may be highly pertinent, as well as the loss of ESPT per se.

Sixteen replicates of lot A and of lot B were analyzed before storage (Table 2). The remaining samples were stored in mesh bags at the temperatures shown, reweighed on removal from storage and analyzed. In spite of well-stirred or circulated air, in most boxes, our experience has been that considerable differences in moisture loss occur due to differences in exposure of samples, even though the temperatures are essentially constant.

Table 1.—Loss of pounds extractable sugar per ton of original beets resulting from various degrees of wilting at different temperatures in refrigerated storage.

Storage treatment			Rrel		C.J.		In Beets		
Days and temp.	Degree of wilt	Final Wt. / Orig. Wt.	App. sucrose	Corr.* sucrose	App. CJP	Corr.* CJP	App. ESPT.	Corr.* ESPT.	To correct App. ESPT
		1.000	%	%	%	%	Lbs.	Lbs.	Lbs.
As harvested	None		18.10	18.00	94.23	93.71	320	314	—6
70 days									
2° C.	a**	.994	18.93	18.64	95.15	93.75	341	325	—16
2° C.	b	.952	18.70	18.50	95.69	94.68	340	330	—10
2° C.	c	.794	18.42	18.23	94.15	93.24	323	315	—8
7 C.	a	.986	18.41	18.35	93.34	93.12	319	317	—2
	b	.845	18.25	18.18	93.81	93.45	319	316	—3
	c	.594	17.35	17.35	92.84	92.84	297	296	—1
140 Days	a	.977	18.73	18.49	94.39	93.20	331	320	—11
2 C.	b	.854	17.70	17.54	93.03	92.20	304	296	—8
	c	.532	17.93	17.90	88.82	88.69	278	277	—1
7 C.	a	.964	17.37	17.35	90.64	90.53	283	282	—1
	b	.633	16.71	16.82	87.33	87.94	249	254	+5
	c	.393	16.69	16.78	86.28	86.73	240	246	+6
Mean	a	.980	18.36	18.21	93.38	92.65	319	310	—9
	b	.816	17.84	17.76	92.49	92.06	308	300	—3
	c	.578	17.59	17.56	90.64	90.43	286	285	—1

* Corr % S in C.J. = App. % S, C.J. $-\frac{1.59\ (\text{mg/ml Raffin.}) - 0.302\ (\text{mg/ml Invert})}{10 \times \text{Density}}$

** a. In polyethylene moisturized bags
b. In double canvas bags
c. In single canvas bags

Table 2—Values for apparent sucrose, clear juice purity (CJP) and extractable sugar per ton (ESPT) are shown for lots A and B before storage and after storage and wilting under varying conditions for 140 days.

Sample	Reps	Final wt. Orig. wt.	App. sucrose	App. CJP	ESPT
		LOTA	%	%	Lbs.
Orig. Mean, Lot A	16	1.000	19.06	94.78	341
A stored 2C	8	.883	18.12	93.33	314
A stored 2C	8	.839	17.74	92.68	303
A stored 4C	8	.869	17.98	92.70	307
A stored 4C	8	.840	16.90	91.99	292
A stored 7C	8	.992	17.75	92.55	302
A stored 7C	8	.921	16.70	90.60	271
		LOTB			
Orig. Mean, Lot B	16	1.000	19.39	94.53	346
B stored 0 to 7C	16	.977	17.61	93.00	304
B stored 0 to 7C	16	.807	15.41	86.20	219
B stored —1 to]C	6	.788	16.99	91.34	282
B stored —1 to 1C	6	.654	16.68	89.68	264
Mean A and B in storage	46	.917	17.71	92.72	303
	46	.815	16.53	89.95	264

Attempts to thoroughly humidify the boxes have resulted in severe icing (and incapacitation) of the refrigerator coil because of the unduly large temperature difference between the coils and the atmosphere.

The degree of wilting was computed for each sample. The replicates were divided into two equal parts, those highest and those lowest in wilting and the two wilting means computed. For each pair of values for wilting in Table 2, the mean analytical values for sucrose, CJP and ESPT are comparable. In each case these means may be compared with the means of the comparable lot, A or B, before storage.

From Table 2 it may be observed that in every case the replicates that wilted most at any temperature lost more ESPT than those with less wilting. In the storage of Lot B beets, with a temperature range from 0 to 7C, considerable condensation of water often occurred. This led to considerable molding on the surfaces of the beets that had been damaged by wilting. With lot B beets, at -1 to 1 C, excessive ventilation led to rather severe wilting even in the "low shrink" samples, and damage was considerable.

On three dates during storage *in* the company pile, three bags of beets were taken from the 1 foot level at three locations

on the top, the shoulder, the north side and the south side (36 bags from the 1 foot level on each date). Similarly, 36 bags were taken from the 2 foot level and 36 from the 3 foot level on each date, or 108 bags on each date. The beets in each bag were reweighed and shrinkage determined, as well as percent sucrose, CJP and ESPT. On January 22 bags were recovered from the body of the pile as the beets were removed for processing.

Table 3 shows the greatly condensed data for storage in this one year. The winter weather was exceptionally mild until a very heavy snowfall occurred that kept the piles well covered during the end of the storage period, and freezing of beets was relatively slight.

Table 3 shows the shrinkage and the loss of extractable sugar per ton of beets in the outside foot, the second foot, the third foot and in the body of the pile in 78 days of storage (October 27 to January 13), as judged by washed, weighed samples located at those depths on the south side, the north side, the top and the shoulders of the pile. In this season, the loss of ESPT in the third foot was about the same as that in the body of the pile. When the detailed analyses were examined, by far the greatest proportion of the loss of apparent sucrose occurred from October 27 to the first removal date, December 12. In these 46 days about 80% of the loss occurred; in the remaining 32 days about 20%. Pile temperatures taken over several years have indicated that a "safe" temperature of below 50F (IOC) is generally stable after the first week in December.

Table 3 calls attention to a fact frequently noted. Moisture in the warm air rising from the body of the pile generally condenses near the surface. The top outside foot of beets took up water substantially during the period under snow, and the beets on the shoulders with traditionally poor ventilation (due to trash) had wilted considerably less than the body of the pile.

Table 3—Shrinkage and loss of extractable sugar per ton of original beets stored in the outside foot, the second foot, the third foot and deep in the company pile. Stored October 27; removed December 12, 26 and January 13 for analysis.

Location	Wilting		Average of location	Loss ESPT lbs.	Removal date for analysis
	1/13/67	Av 3 dates			
S. side, outside foot	.622	.657			
S. side. 2 and 3 foot	.868	.860			
N. side, outside foot	.790	.767			
N. side, 2 and 3 foot	.934	.925			
Shoulder, outside foot	.751	.749	Outside foot	87.3	1/13
Shoulder, 2 and 3 foot	.968	.969	Second foot	59.6	1/13
Top, outside foot	.919	.808	Third foot	30.3	1/13
Top, 2 and 3 foot	.979	.961	Body of pile	30.0	1/22
Body of pile	.935 (1/22)				

Table 4—Mathematical procedures used in correcting values of apparent sucros ein brei and clear juice for content of raffinose and invert sugar, and the consequences in the calculation of clear juice purity and cxtractable sugar per ton.

Treatment	Brei			Clear juice									Beets	
	App. sucrose	Corr.*** sucrose %	Density* C.J.	App. Sucrose %	Raffin mg/ml	Suc. Eq.	Invert mg/ml	Suc. Eq.	Corr.** sucrose %	RDS	CJP app. %	Corr. CJP	App. ESPT	Corr. ESPT
Harvest	18.10	18.00	1.075	17.98	0.80	1.26	0.73	0.22	17.88	19.08	94.23	93.71	320	314
140 days, 2°C.														
No wilt	18.73	18.49	1.066	16.68	1.62	2.56	1.14	.34	16.47	17.67	94.39	93.20	331	320
Moderate wilt	17.70	17.55	1.065	15.63	1.19	1.88	1.48	.45	15.50	16.80	93.03	92.26	304	295
Severe wilt	17.93	17.90	1.056	13.04	0.17	0.27	0.27	.08	13.02	14.68	88.82	88.69	278	277

* From RDS concentration of clear juice, (table)

** Corr % S in C.J. = App. % S, C.J. — $\frac{1.59 \text{ (mg/ml Raffin.)} - 0.302 \text{ <'mg/ml Invert)}}{10 \times \text{Density}}$

*** Corr % S in Brei = Corr. % S in C.J. — $\frac{\text{/A}}{\text{App. \% S in C.J.} - \text{(ADD.}}$ % S in Brett

H

The beets on the north side consistently wilted less than those on the south.

For piles of the dimensions common in Michigan, about 20% of the beets are in the outside 3 feet and 80% in the body of the pile. Computing on this basis in 1966, about 36 pounds of ESPT were lost from an average ton of beets piled October 27 and removed January 13.

In this storage season of unusual snow cover, freezing was slight below the first 2 feet. Wilting was as high in the body of the pile as in the second and third feet and severe only in the outside foot. It seems likely that leaching from the frozen, periodically thawed and pulpy beets in the outside 2 feet was responsible for a considerable amount of the 90 and 60 pounds of ESPT lost there, although the wilting in the surface foot was certainly harmful.

In the case of piled beets that have been repeatedly frozen and that must receive rough treatment during washing, there is always excessive loss of soft pulpy tissue and sugar. In the comparisons in Table 3, all beets were sampled with the brei saw and no tissue was lost in determining shrinkage. Had the beets in the outer layer been subjected to washing, the losses of ESPT of orignal beets would certainly have been far higher than the 87 pounds shown since the sugar in many of the soft beets is lost completely. Washing the beets before they are piled and avoiding washing after freezing and thawing could eliminate this loss.

In a following paper the occurrence of raffinose and invert sugar in beets will be examined in detail, and the magnitude of the errors in apparent sucrose, clear juice purity and extractable sugar per ton under different conditions will be presented. Table 4 very briefly shows, by the use of examples with wilted beets, the mathematics involved in the corrections shown in Table 1.

The method of correcting the percent sucrose in the brei, by referring it to the degree of correction in percent sucrose in the clear juice, seemed legitimate in view of the fact that the relatively mild lime and heat treatment of the expressed juice appeared to destroy little or no invert sugar during the preparation of the clear juice. The main correction of the ESPT is the result of the generally conspicuous change in clear juice purity, when computed from the corrected, rather than from the apparent sucrose in the clear juice. The changes in percent sucrose in brei are, in most cases, little more than the unavoidable variances in percent sucrose in the beet samples. However, as shown in Table 1, the changes in percent **CJP** may be considerable and in either direction.

Summary and Conclusions

Wilting of beets resulted in a substantial loss of ESPT in comparison with beets stored without wilting. Damage from the

same degree of wilting was somewhat more as the temperature became higher, and as the temperature rose degree of wilting tended to be more. When corrections for raffinose and invert sugar content of the clear juice were made, percent clear juice purity often changed considerably, either increasing or decreasing depending upon the proportions of these sugars. Since ESPT is computed from the percent sucrose in the beet and the clear juice purity, differences between apparent and corrected ESPT were often sufficient to influence the interpretation of the experimental findings.

When beets near the surface of the pile wilted to a degree similar to those in refrigerated storage, losses of ESPT were greater than those stored without freezing and thawing. Losses of ESPT were far greater per day in the pile-stored beets in the early period of storage when freezing and thawing were negligible, but wilting was considerable. Protection from even relatively slight wilting seems important from the work of Pack and this investigation. It is suggested that the irrigation practice of "misting" the piles (8) might be highly desirable in both cooling the air and beets, and maintaining a higher relative humidity of the cooled ambient air.

From the data with several lots of beets stored at several temperatures, but with various degrees of wilting, the specific effects of temperature and of wilting can be distinguished, both in types of compositional changes and in ESPT.

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Effect of Water Extract of Sugarbeet on the Germination of Other Crop Seeds[1]

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The presence of germination inhibitors in seeds and fruits has been reviewed (2)[3]. The water extract of sugarbeet seed balls was found to retard the germination of sugarbeet seed and kill the radicles of emerged seedlings (5). Stout and Tolman (4) reported that the toxicity of the water-extract of sugarbeet was not specific to sugarbeet seed but also totally inhibited or retarded the germination of other seeds.

The present study was conducted to investigate further the effect of sugarbeet seed water-extracts of different dilutions on the germination of crop seeds and subsequent growth of seedlings.

Materials and Methods

Water extract of sugarbeet *(Beta vulgaris* L.) variety Maribo autopoly, lot number 7015, was prepared by soaking 10 g of the whole seed balls in 100 ml distilled water. The soaking was done in the dark at 10°C for a period of 24 hours. The leachate was then filtered through Whatman filter paper No. 4 and was referred to as the whole seed ball extract of 100% concentration. Sugarbeet seed balls were also crushed by means of a small hand mill and the true seed separated from the pericarp tissue. The water extract of each part (crushed seed balls, pericarp tissue and true seed) was prepared in the same manner as mentioned above and the effect of each was then tested on the speed of germination of true (excised) sugarbeet, alfalfa and sorghum seeds. The effect of the whole seed ball extract was tested on the initial germination and radicle length of sunflower *(Helianthus annus* L.), sugarbeet *(Beta vulgaris* L.), cabbage *(Brassica oleracea* L.), cotton *(Gossypiurn herbaceum* L.), alfalfa *(Medicago sativa* L.), vetch *(Vicia sativa* L.), rice *(Oryza sativa* L,.), barley *(Hordeum vulgare* L,.), perennial rye grass *(Loliurn perenne* L.), sorghum *(Sorghum vulagre* Pers.), wheat *(Triticum vulagre* L.), and oats *(Avena sativa* L.). Dilutions of 50% and 75% of the extract were prepared and their effect was compared with that of the undiluted extract on seed germination and subsequent seedling growth of alfalfa, sorghum and oats.

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For each test duplicates of 20 seeds were germinated on two filter papers in petri dishes. Three ml of solution was added to each dish. Germination was done in a Stults seed germinator at alternating temperature of 20-30°C. Germination in distilled water was used in each test to serve as the check. At an arbitrary time that was specific to each crop, seeds that produced radicles of one or more cm in length were considered germinable. Growth of ten seedlings was determined by measuring the fresh weight of radicles and hypocotyls or plumules after pressing the seedlings gently between two filter-papers to remove any surface water. Where the effect of the extract on subsequent growth of seedlings was studied, 200 seeds of the crop tested were germinated between blotters in distilled water and 2 samples of 20 germinated seeds with radicles one cm in length were selected for the test. All the data reported in this paper are the means of four replicated experiments.

Results and Discussion

Results (Table 1) indicate that the water extract depressed the germination percentage and the average radicle length of all the crop seeds tested. The extract affected germination of barley, alfalfa, sunflower, perennial ryegrass, cabbage, rice, and sugarbeets more than sorghum, oats, vetch, cotton, and wheat. The results are in agreement with Stout and Tolman (4) who reported that the toxicity of the water extract from sugarbeet seed balls was not specific to sugarbeet but also affected the germination of several other seeds.

The origin of the inhibitory material in the aqueous extract was found to be in the pericarp tissue (Table 2). The water extracts of the pericarp tissue and the crushed seed balls inhibited to the same magnitude the radicle development of sugar-

Table 1.—Effect of the water extract of sugarbeet seed on the germination percentage and radicle length of different crop seeds.

Crop seed	Germination period (hr)	Germination (% of check)	Radicle length (% of check)
Barley	84	1.7	18.2
Alfalfa	72	3.3	12.0
Sunflower	96	4.2	28.6
Perennial rye grass	144	4.2	11.4
Cabbage	84	10.3	12.9
Rice	204	19.8	14.3
Sugar beet (inhibitor-free)	96	35.4	36.1
Sorghum	72	53.2	17.1
Oats	132	66.7	6.7
Vetch	84	78.4	52.2
Cotton	96	79.5	42.5
Wheat	72	90.9	25.3

Table 2.—Effect of different extracts on the speed of germination of true sugarbeet, alfalfa and sorghum seeds.

Extract	Fresh weight 10 radicles after 72 hrs germ. (mg)		
	Sugar beet true seed	Alfalfa	Sorghum
Control (Dist. HaO)	20.71	55.69	**118.50**
Sugarbeet true seed	19.39	49.69	**118.19**
Pericarp tissue	2.35	13.75	38.13
Crushed seed balls	1.40	**10.69**	33.75

beet, alfalfa, and sorghum. The extract of the sugarbeet true seeds did not show any inhibitory properties when compared to distilled water.

The effect of different dilutions of the whole seed ball extract on seed germination of alfalfa, sorghum and oats is given in Table 3. The extract did not inhibit the germination at a dilution of 75%. The full strength of the extract (zero dilution), on the other hand, significantly decreased the germination percentage of all the seeds tested. The effect, however, was differential at 50% dilution since it significantly decreased the germination of alfalfa and oats but not of sorghum.

The reduction in germination percentage was due mainly to the inhibtory effect of the extract on radicles. The fresh weights of radicles of alfalfa and sorghum were not reduced by the 75% dilution but were significantly reduced by the 50% dilution. Reduction in the fresh weight of oat radicles was obtained only when the oat seeds were germinated in the undiluted extract. These results indicate that a minimum concentration of the extract is needed to cause radicle inhibition and that this minimum concentration is not the same for all seeds. Oat radicles were stimulated by 75% dilution of the extract (Table 3).

The undiluted extract significantly reduced the development of the sorghum plumule at 1% level, of the alfalfa hypocotyi at 5% level and had no significant effect on the oat plumule. No inhibition of the plumules was produced by the diluted extracts. Instead, at 75% dilution the plumules of oats and sorghum were significantly stimulated at 1% level. No significant stimulation of the hypocotyls of alfalfa was observed. This stimulation effect of the extract on the plumules of sorghum and oats might be due to the presence of growth-promoting substances in the extract that are stimulatory only when present at low concentrations.

The influence of the dilutions of the whole seed ball extract on the growth of seedlings of alfalfa, sorghum, and oats is shown in Table 4. The radicles of sorghum were significantly inhibited

Table 3.—Effect of various dilutions of the sugarbeet seed ball extract on seed germination and seed vigor[1] of alfalfa, sorghum and oat.

% dilution of extract	Alfalfa observed after 144 hrs			Sorghum observed after 120 hrs			Oats observed after 120 hrs		
	% germ.	Fresh weight 10 radicles	Fresh weight 10 hypocotyls	% germ.	Fresh weight 10 radicles	Fresh weight 10 plumules	% germ.	Fresh weight 10 radicles	Fresh weight 10 plumules
		Mg	Mg		Mg	Mg		Mg	Mg
Control[2]	90.2	67.1	144.5	95.0	122.8	73.6	63.9	62.5	60.1
75	89.4	63.0	173.5	97.5	137.8	100.9	55.0	81.6	96.1
50	75.6	26.1	163.6	90.0	87.4	57.7	46.0	61.5	71.1
0[3]	8.1	9.6	93.4	41.3	30.8	48.0	39.6	39.6	58.0
LSD 5%	12.0	22.7	43.8	13.9	34.7	17.7	11.1	11.0	16.0
LSD 1%	17.3	32.6	63.1	20.1	49.9	25.4	15.9	15.8	23.0

[1] Seed vigor was determined by measuring the fresh weight of radicles and plumules or hypocotyls.
[2] Distilled water.
[3] Whole seed ball extract of 100% concentration.

Table 4.—Effect of various dilutions of sugarbeet seed extract when applied on seedlings of alfalfa, sorghum and oats.

% dilution of extract	Alfalfa observed after 48 hrs		Sorghum observed after 72 hrs		Oats observed after 72 hrs	
	Fresh weight 10 radicles	Fresh weight 10 hypocotyls	Fresh weight 10 radicles	Fresh weight 10 plumules	Fresh weight 10 radicles	Fresh weight 10 plumules
	Mg	Mg	Mg	Mg	Mg	Mg
Control[1]	53.1	99.0	114.4	54.8	94.1	187.8
75	49.6	111.6	88.8	46.9	113.6	247.8
50	30.9	130.6	75.9	76.5	105.9	252.0
0[2]	13.5	98.5	48.8	93.2	68.3	241.3
LSD 5%	13.4	23.3	12.5	10.1	5.4	17.6
LSD 1%	19.3	33.4	18.9	15.2	7.8	25.4

[1] Distilled water.
[2] Whole seed ball extract of 100% concentration.

by the extract diluted to 75%. The magnitude of inhibition increased with the decrease in dilution. The growth of alfalfa radicles was reduced significantly (1% level) when the seedlings were allowed to grow in the 50% dilution of the extract. In contrast, the growth of radicles of oats was stimulated at the 75% and 50% dilutions. Radicle development of alfalfa, sorghum and oats was seriously inhibited by the original concentration of the extract.

The growth of plumules of sorghum and oats and hypocotyls of alfalfa was not inhibited by any concentration of the extract. Instead, the plumules of oats were significantly stimulated at 75%, 50% and zero dilutions, of sorghum at 50% and zero dilutions, and the hypocotyls of alfalfa at 50% dilution.

DeKock et al. (1) reported that the germination of cress seeds was inhibited by the water extract of the seed balls of sugarbeet. But, if the seeds treated with the inhibitor were washed and then put to germinate, the speed of germination was more rapid and the hypocotyls were much stronger than those of the controls. The authors concluded that some phase of the growth process is being retarded whereas other phases may proceed normally or be stimulated. Snyder (3) reported that the whole seed balls from two progenies of the sugarbeet variety US 401 germinated more rapidly than the naked seeds obtained from the same plants indicating a stimulating effect of the seed balls. Also, the growth of seedlings was found to be stimulated by the seed balls. In the present investigation the stimulating effect of the seed ball extract on other crop seeds appeared to be due to the presence of growth promoting substances in the extract that is stimulatory only when present at low concentrations.

Summary

The effect of the water extract of sugarbeet seed was studied on the speed of germination and seedling growth of different crops.

The water extract of the whole seed balls inhibited the germination and radicle development of many crop seeds. The degree of inhibition varied with seeds of different varieties.

The origin of the inhibitory material was found to be concentrated in the pericarp tissue and absent from the true sugarbeet seeds.

Extracts of the whole seed balls apparently contained substances that stimulated seedling growth of alfalfa, sorghum and oats. Stimulation was more prominent on plumules and hypocotyls than on radicles.

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Millipore-Antimony Pentachloride Colorimetric Method for the Rapid Determination of Saponin in Refined Beet Sugar

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In the past 16 years or so, methods have been devised for the characterization and determination of floc (3,7,8,10,13)[2] in refined beet sugar. Positive correlations were generally found to exist between saponin content and relative floc content (5,12,14). Walker (12) introduced the first reliable quantitative method for determination of saponin content in refined beet sugar. He utilized an antimony pentachloride reagent. Later, others (14,1) followed with adaptations of his method.

Gaddie and West (4) revealed the secret for the production of floc free sugar: Maintenance of a high pH in the white pan. Others (5,6) confirmed their findings. Thus the rule of thumb for production of floc free sugar was to maintain a pH in the white pan that was high enough to take care of the saponin level present in the massecuite.

Even with the general ability to produce floc-free sugar and to confirm that fact by relative floc tests or by quantitative saponin tests, problems still persist. Strikes of floc-free sugar lose their identity in the company of floc sugar in bulk sugar bins. The problem was lack of time to get a floc grading or saponin analysis of bulk or liquid sugar at time of shipment. Thus a method had to be developed which would be simple to perform and would determine if a sugar is floc-free, in less than 10 to 15 minutes.

Over the years we have filtered the saponin from a sugar solution acidified to pH 1.0 with HC1 onto a F-fritted glass Buchner funnel disk of 4-5.5 microns porosity, washed out the sugar with dilute acid, and then after drying, extracted the saponin with methanol followed by color development with concentrated sulfuric acid (1). It usually required about 4 hours for an acidified solution of 100 grams of sugar to filter through such a funnel. In tests with various filter media, the 1.2 micron Millipore filter proved to be very retentive and required less than 2 minutes for complete filtration of an acidified solution of 100 grams of beet sugar.

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[2] Numbers in parentheses refer to literature cited.

Stansbury and Hoffpauir (11) retained cane floc from an acidified cane sugar solution on a Millipore filter. They eventually extracted the floc from the filter for further analysis. Our thoughts were directed originally to the idea of extracting the saponin from the Millipore filter with methanol, and eventually developing the saponin color with concentrated sulfuric acid. This plan was readily abandoned when it became apparent that the Millipore filter was dissolved by methanol which in turn gave a color development with concentrated sulfuric acid.

The Millipore technical literature (9) indicated that qualitative spot tests could be performed on the Millipore filter surface. It was noted in the Millipore Chemical Resistance Table (9) that the Millipore filter was not affected by chloroform. We were able to demonstrate that a chloroform solution containing antimony pentachloride gave the characteristic pink color test for saponin on the Millipore filter surface without destroying the filter.

Grab samples of sugar from each of our mills were evaluated for floc and for relative saponin content as listed in Table 1. In the American Crystal Sugar Company adaptation of the Spreckels' floc method, an acidified 31 Brix sugar solution of pH 2.0 with HC1, is brought to boil and allowed to simmer for 5 minutes. A floc grade, by The Seven-Up grading system, not exceeding 2 at the end of 72 hours is considered to be Bottlers sugar.

Table 1 points out the excellent agreement of the floc method and of the saponin Millipore-$SbCl_5$ method.

The Saponin Millipore-$SbCl_5$ test was run on these same sugars several times by different individuals. Each time the results duplicated those listed in Table 1. By determining the relative saponin contents by the Millipore-$SbCl_5$ method (10-15 minutes), we were able to predict the floc performance (at the end of 72 hours) of those sugars.

Table 1.—Original evaluation of saponin by Millipore-$SbCl_5$ method.

Factory	Floc grade	Saponin by Millipore-$SbCl_5$	
		Rank	Color intensity
A	4	7	Intense Pink
B	4	7	Intense Pink
C	4	7	Intense Pink
D	2	6	Mildly Pink
E	1	1	Barely Pink
F	1	1	Barely Pink
G	1	1	Barely Pink
H	1	1	Barely Pink
I	1	1	Barely Pink

Since a sugar with a floc grade no greater than a plus 2 at the end of 72 hours will meet the Bottlers standard, such a sugar should then serve adequately as a control. Thus if the developed saponin color of the unknown strike of sugar is less than or equal to that of the control sugar then it is considered to be floc free and thus acceptable to the beverage industry. By running the control at the same time or just prior to the unknown, then the parameters of the test are the same for the control and the unknown. Great care should be exercised in selecting the control. It is possible that the control may allow some floc sugars to be graded as flce free sugar by the Millipore-$SbCl_B$ method. Historically we have found this rule of thumb, that if a sugar contains 1 ppm saponin or less it will produce a floc not greater than a plus 2. Thus if the control contains 1 ppm saponin or less it should reject all floc sugar. This rule of thumb may not exist for all companies, due to the purity of the saponin standard, to the kind of floc test and to the final time of observation of the acidified sugar solution for floc grading.

If the chemist is satisfied that an appropriate control has been obtained, then a sufficient quantity of that lot of sugar should be set aside.

Antimony Pentachloride Reagent

The following antimony pentachloride solution (2) after aging 3 to 4 days serves quite adequately for producing a pink color with saponin on a Millipore filter:

5 ml $SbCl_5$
95 ml CHC1,
1 g $SbCl_8$

Antimony pentachloride as it appears commercially contains free chlorine. The free chlorine inhibits the saponin-antimony pentachloride reaction. One gram of antimony trichloride added to 100 ml of 5% antimony pentachloride-chloroform solution will tie up the chlorine to make antimony pentachloride. An excess of antimony trichloride will impede the color reaction of saponin with antimony pentachloride.

Antimony pentachloride has great affinity for water, thus producing pyroantimonic acid and hydrochloric acid. High relative humidities can have a quenching effect on the color development of the saponin. Glassware used to prepare the antimony pentachloride reagent can become coated with a residue of pyroantimonic acid that is practically impossible to remove. Glass stoppered bottles containing the antimony pentachloride reagent freeze shut requiring special efforts to unstopper.

We have found the use of glass bottles with Teflon lined screw caps **to** be quite successful for the preparation and storage

of reagent. The glass bottles are calibrated for various volumes with chloroform.

These unopened bottles of 5% antimony pentachloride reagent for saponin should last indefinitely. An 8-year old bottle of 5% antimony pentachloride reagent for saponin containing .5 grams of antimony trichloride per 100 ml of solution was found to have great sensitivity for saponin.

A 10% antimony pentachloride reagent gives a much stronger pink color for saponin than the 5% reagent. In our opinion, however, if fats are present with saponin, a brown color may form with the 10% reagent while the normal pink color of the saponin is developed with the 5% antimony pentachloride reagent.

Reagents and equipment:

1. 5% $SbCl_5$ solution
2. ph 2.0 HC1 solution
3. Control sugar (plus 2 floc at end of 72 hours)
4. Millipore filter, Type RA White, 1.2 microns porosity, diameter 47 mm
5. Millipore filter holder
6. Glass pipette
7. Saponin from Beet Molasses (optional)
8. 1% NaOH solution (optional)
9. Schleicher & Schuell 507 filter paper (optional)

Glass pipette:

A suitable pipette can be made from 5-7 mm diameter glass tubing. The glass tubing opening at one end is drawn down to a diameter of about .5 mm or less. The pipette can dispense a drop of reagent that covers an area as small as 2 mm in diameter on the filter surface. By using a tall 2 oz screw cap glass bottle for the 5% antimony pentachloride reagent, it is possible to get enough reagent in the pipette without mouth pipetting to dispense at least 5-8 drops.

Another tall 2 oz bottle containing chloroform is used to rinse the reagent from the pipette. If the pipette is not cleaned after each use, it will become permanently plugged.

With practice, proper finger pipetting of the reagent may result in the dispensing of reagent at the rate of 1 drop per 1 to 2 seconds.

Saponin Millipore-$SbCl_5$ Control Method:

This method is used for rapid and routine analysis of refined beet sugar for saponin content and thus for floe forming potential. The following procedure is employed for the control sugar and the sugar of unknown saponin content.

Procedure:

1. Dissolve 100 grams of refined beet sugar in 200 ml of 2.0 HC1. (Time: 1 to 3 minutes)
2. Filter with vacuum through a 1.2 porosity Millipore filter. (Time: 1 to 2 minutes)
3. Rinse with 100 ml of pH 2.0 HC1. (Time: less than 1 minute)
4. Oven dry Millipore filter at about 105° C. (Time: 2 to 5 minutes)
5. Apply a drop of 5% $SbCl_5$ reagent to Millipore filter and to the control Millipore filter, compare at room temperature and/or at warmer temperatures.

Although high relative humidity may impede the saponin-antimony pentachloride pink color development at room temperature, this interference does not occur at temperatures as high as 105° C. If one is not satisfied with the color development, then he should apply reagent again to unused areas of the filters of the control and of the unknown and re-evaluate. These Millipore filters after evaluation have been retained enclosed as long as 4 months without any apparent decomposition and have still given a saponin test with antimony pentachloride reagent.

The Millipore $SbCl_5$ Control Method was tested at various factories this past campaign. The chief chemists provided their own control sugars. The results from four different factories are listed in Table 2.

The chief chemists from the four factories were impressed with the method. The chief chemist from D factory was not able to recheck the one sugar which failed the Millipore-SbCK test. He thought that a control sugar with a slightly higher saponin content would have allowed more actual floc-free sugars to be graded floc free by the Millipore-$SbCl_5$ method. His control sugar was found to contain .8 ppm saponin.

The chief chemist from F factory reported that the Millipore-$SbCl_5$ method, graded as floc-free, 85 to 90% of those strikes of sugar found to be floc-free at the end of 72 hours by the Spreckels' floe method. He also thought that on several occasions the

Table 2.—Floe test versus Millipore-SbCls control method at four factories.

Factory	No. of samples	Bottlers sugar	
		Floe test	Millipore-SbCIs
D	60	37	20i
E	30	16	16[2]
F	60		"85 to 90% of floe test"
G	16	14	12

[1] On one occasion floc-free by Millipore-SbCIs became floe sugar by Spreckels' floe method at the end of 72 hours.

[2] On two occasions floc-free by Millipore-SbCIs became floe sugar by Spreckels' floe method at the end of 72 hours.

Millipore-SbCls method graded as Hoc-free, sugars that became floc sugar at the end of 72 hours by the Spreckels' floc method.

It should be pointed out that in the original instructions to the chief chemists they were to use pH 1.0 HC1 for solution of the 100 grams of sugar for testing. At pH 1.0, partial charring and brittleness of the Millipore filter on oven drying, and depressed color development of the saponin-$SbCl_5$ reaction occurred. Subsequently it was found that at pH 2.0 these problems disappeared. The data from D, E and F factories are composed mostly of pH 1.0 results. The results of G factory represent pH 2.0.

It may be that less saponin is filtered out at pH 2.0 than at ph 1.0; however, this should have no bearing on the method as long as the control sugar or standards are also made up at pH 2.0.

Weekly composites of sugar from each of our mills were sent to the Research Laboratory for analysis. Table 3 compares the floc test and the Millipore-SbCl-, method.

The Millipore-SbCls method rejected all of the floc sugars. The control sugar at the Research Laboratory contained .8 ppm saponin.

Saponin Millipore-$SbCI_5$ Quantitative Method:

Originally it was not possible to quantitate directly the saponin content of beet sugar on the Millipore filter. The pink color of the saponin from beet sugar was evenly distributed over the area of the spot on the Millipore filter surface when antimony pentachloride reagent was applied. This is not the case for purified saponin obtained from limecake or from sugarbeet skin. Such saponin is washed out to the periphery of the spot on treatment with antimony pentachloride, thus making it impossible to quantitate. More recently it was found that saponin obtained from beet sugar molasses acts similarly to the saponin found in beet sugar, thus allowing for direct quantitation on the Millipore filter.

Table 3.—Floe test versus Millipore-SbCl^ control method at research laboratory.

Factory	No. of samples	Bottlers sugar	
		Floe test	Millipore-SbClo
A	3	1	1
B	13	13	13
C	15	7	5
D	16	0	0
E	16	16	13
F	17	16	13
G	16	0	0
H	15	13	11
Total	111	66	56
% Acceptable		59.5	50.5

Beet sugar free of saponin is fortified with various levels of saponin from molasses. Usually .1000 grams saponin are dissolved in 1 liter of methanol. Thus 1 ml of the methanol solution of saponin is added to 100 grams of saponin-free beet sugar to produce a 1 ppm saponin standard. It is very difficult, if not impossible, to find refined beet sugar free of any saponin. Originally it was thought that cane sugar could be used for preparation of the saponin standards. It was found that the saponin added to cane sugar was partially or completely masked on color development with antimony pentachloride.

We were able to locate a strike of sugar that had no more than, and possibly much less than, .1 ppm saponin. This lot of sugar is being used for preparation of saponin standards. Previous to finding this lot of sugar, we simply fortified with saponin the filtrate of an acidified solution of 100 grams of refined beet sugar of low saponin content which had been filtered through a 1.2 micron porosity Millipore filter.

Table 4 compares the actual floc grade with the amount of saponin present in the sugar.

The control sugar which we had been using to determine Bottlers sugar contained .8 ppm saponin.

Saponin Schleicher & Schuell - $SbCl_5$ Quantitative Method:

It was found that filter media other than the Millipore filters work well with the Millipore Filter Apparatus. Schleicher & Schuell Red Ribbon 589 Filter Paper is fairly retentive. Where it takes less than 2 minutes for a 1.2 micron porosity Millipore to filter an acidified solution of 100 grams of sugar, it takes less than 10 minutes for S 8c S RR 589 Schleicher & Schuell 507 filter paper is extremely retentive, requiring at least *20* minutes for comparable filtration. The saponin on Red Ribbon 589 paper is best visualized by dipping in antimony pentachloride reagent. The whole filtration area develops a uniform pink color. 507 paper may be visualized either by dipping or by spotting.

Table 4.—Quantitative analysis of saponin by Millipore-SbCls.

Sugar	Floe grade	Millipore-SbClo saponin, ppm
A	1	.3
B	2	1.3
C	2+	1.3
D	3+	1.5
E	2+	1.5
F	4	3.5
Control Sugar	2	.8

Standards: .00, .25, .5, 1.0, 1.5, 2.0, 3.0 and 4.0 ppm Saponin

Sugar	Floc grade	Millipore-SbCl$_3$ Saponin rank	SS 507-SbCl$_3$ Saponin, ppm
A	1	1	.2
B	1	2	.4
C	1+	3	.5
D	1+	4	.7
E	2+	7	1.3
F	2+	6	1.3
G	4	8	4.0
Control	2	4	.8

Standards: .25, .5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 ppm saponin

All of the sugars evaluated with the 507 paper, be they unknown saponin content or the standards, were first made up as basic solutions and filtered through a 1.2 micron porosity Millipore. This was done to remove fine sediment which would impede filtration through the very fine porosity 507 paper. The basic sugar filtrates and washings were then adjusted to pH 2.0 with concentrated HCl. The acidified sugar solutions were filtered through the 507 paper. The Millipore filters of the basic sugar solutions were tested for saponin content and found to contain none.

Conclusion

The authors recommend the use of the 1.2 micron Millipore filter for routine control work and especially where quantitation is desired. By this means the analyst should be able to complete a test easily in less than 15 minutes.

Where cost of the filter media is of consideration and the time element not critical, regular fine grade filter papers may be used.

In the event of background interference from the presence of fine sediment on the filter, it is recommended that this material be removed by filtration from an alkaline solution before proceeding with the test.

Acknowledgment

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Determination of Nitrate Nitrogen in Sugarbeet Petioles and Soils With 2-6-Dimethyl Phenol[1]

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Today farmers must produce high yields to survive in the face of the growing costs of production. High amounts of available nitrogen are essential for high yields although excessive nitrogen reduces the processing quality of the roots. Plant tissue analyses have proven valuable in determining whether proper amounts of nitrogen have been used. In addition, soil analysis for nitrate nitrogen is helpful in estimating the amounts of nitrogen fertilizer to apply. Nitrate nitrogen is not the only source of nitrogen available to plants, but it cannot be ignored when present in large amounts. For example, soils in the sugarbeet growing area of Eastern Oregon and Southwestern Idaho have been found to contain from 25 to 325 pounds of nitrate nitrogen per acre in the top 2 feet of soil prior to fertilization for a sugarbeet crop.

The analysis of large numbers of plant or soil samples is hindered by the lack of a method which will quantitatively measure nitrate nitrogen in a relatively fast and easy manner. Hartley and Asai (l)[3] describe a method for water analysis which is simple to use, direct and rapid. They state that "The precision of the method expresses as relative standard deviation averages less than 1% in the nitrate range of 5-29 P.P.M. nitrate nitrogen (original sample concentration). Because of the extreme rapidity in carrying out a determination and its conformity to Beer's law, the 2, 6-dimethyl phenol procedure should be very useful for determination of nitrate in samples containing nitrate nitrogen in the parts per million ranges." Their method as adapted for plant and soil analysis is given below.

Procedure for Plant Tissue

Weigh out 0.5 or 1.0 g. of dried ground plant material, depending on nitrate nitrogen content. If nitrate nitrogen is above 15,000 ppm, use 0.5 g. or change dilution of extract from that recommended below. Add 100 ml of 2 percent acetic (V/V) to each sample and shake for 10 minutes on a platform or wrist action shaker and filter. Two ml of these filtered extracts are diluted to 50 ml with distilled water. In addition to nitrate nitrogen the

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[3]Numbers in parentheses refer to literature cited.

solutions may now be used to determine phosphorus, potassium and sodium if desired.

Two ml of the diluted extracts are pipeted into test tubes. The test tubes are placed in a container of water at room temperature (18-28°C). Eight ml of 2:1 sulfuric acid-water (V/V) is added to each test tube. The tubes may be removed from the water bath while adding the acid but must be returned to the water bath as soon as possible. When the solutions have cooled, add 2 ml of 2, 6-dimethyl phenol in glacial acetic acid, (12.217 g per liter glacial acetic acid). Stopper the tubes, shake each sample separately to mix and place back in water immediately. The percent transmission may be read at 325 Mu 15 minutes after but no longer than 1 hour after mixing with the 2, 6-dimethyl phenol reagent. The solution must now be kept in the water bath until read or the reaction will proceed uncontrolled and results will be erratic and high. A reagent blank is carried through with each set of determinations

Procedure for Soils

Weigh 5.0 grams of soil, add 50 ml of one normal ammonium acetate (pH 7.0) and shake for 5 minutes. Filter the sample and develop the colors as in the procedure for plant material. Soil extracts usually do not need to be diluted. This extract also may be used to determine available potassium.

Comparative Accuracy and Factors Affecting the Analysis

To determine the comparative accuracy of this method it was compared with a method for plant analysis for nitrate nitrogen similar to that of Ulrich, et al (2) in which phenol disulfonic acid was used. Eight petiole samples ranging from 3000 to 12000 ppm nitrate nitrogen were analyzed by both methods. A simple correlation between the two values gave an r value of 0.99. The average value using phenol disulfonic acid was 7950 ppm and 7446 ppm using 2, 6-dimethyl phenol. A similar comparison between the two methods with 20 soils gave an r value of 0.97. The average value here was 50 ppm with both methods. The exceptionally high r values show that the two methods gave basically the same results.

Hartly and Asai (1) describe interferences from temperature, nitrite nitrogen, chlorides and organic matter. These interferences are discussed below as they affect the analysis of plants and soils.

Temperature affects the reaction by decreasing reaction time as the temperature is increased. This does not affect the determination providing the analytical procedure is followed as outlined. Results will be high and eractic when the temperature of the

solutions are allowed to rise above room temperature after the 2, 6-dimethyl phenol reagent has been added to the reaction. *Nitrite nitrogen* interferes directly with the reaction (Table 1). Table 1 shows that the extent of the interference depends directly on the

Table 1.—Effect of Nitrite nitrogen on nitrate nitrogen recovery.

Nitrite nitrogen (ppm)	Nitrate nitrogen (ppm)	% Recovery
0	1	100
1	1	190
2	1	240
4	1	320
6	1	380
8	1	435
10	1	490

amount of nitrite present. Actually nitrite nitrogen could be determined with the same procedure in the absence of nitrate nitrogen. The presence of nitrites can be easily detected because of a yellow color in a lake on the top of the solution that is formed when 2, 6-dimethyl phenol reagent is added to develop the color. The yellow color rapidly turns to orange when the sample is shaken indicating the presence of nitrite nitrogen. The addition of a small crystal of sulfamic acid to the pipeted sample before any other reagents are added will remove nitries without affecting the nitrate determination. *Chlorides* in excess of 200 ppm in the diluted sample will cause interference. To obtain this high level plant material must contain over 50% chloride and soil samples must contain over 4000 pounds chloride per acre to a depth of 6 inches of soil. This is true only when samples are diluted as the procedure indicates. *Organic matter* does not appear to be a problem in the method mainly because the high dilution of plant samples; and neutral normal ammonium acetate does not remove organic matter from the low organic matter soils where this method has been used.

The presence of sucrose was found to interfere with the determination of nitrate nitrogen. Samples from sugarbeet petioles produce a reddish color when analyzed which soil samples and standard solution do not. This red color is very pronounced when samples of fresh beet pulp are analyzed. The cause of the red color was found to be due to fructose which is formed when sucrose present is hydrolyzed in the hot acidic solution to glucose and fructose. This occurs when the sulfuric acid is added to the pipeted sample. The fructose, not glucose, then reacts with the solution to produce the red color. The amount of sucrose or fructose present determines the extent of the interference as seen in Table 2. The greater the concentration in solution the higher the percent recovery of nitrate nitrogen since the two are

additive. Fresh beet pulp is comparatively low in nitrate nitrogen (usually less than 500 ppm) and, therefore, cannot be diluted as much as petiole solutions. As a consequense, the sucrose pres-

Table 2.—Effect of sucrose and fructose on nitrate nitrogen recovery.

Nitrate nitrogen (ppm)	% Sucrose	% Fructose	% Recovery
2	0	0	100
2	0	.004	100
2	0	.008	101
2	0	.020	105
2	.004	0	100
2	.010	0	100
2	.016	0	101
2	.040	0	105
2	.080	0	110

ent gives high values in nitrate nitrogen determination of pulp samples. It is concluded that this method cannot be used for extracts of fresh beet pulp or for any solution which contains more than 0.01 per cent sucrose. Because of the great dilution in the procedure, no interference will occur in a plant sample containing less than 25 percent sucrose. This is higher than the concentration occurring in most plant material. This does not limit the method however, because this method, just as any colorimetric method, is not as accurate as the present transmission of the solution approach 100 percent. In other words, this method as outlined is less accurate when the nitrate nitrogen is less than 1000 ppm. This could easily be corrected by not diluting as much, but this may bring the concentration of sucrose up to a point where it will cause high nitrate values.

The standard curve for nitrate nitrogen using Baush and Lomb Spectronic 20 spectrophotometer at a wave length of 340 millimicrons is given below. Full scale deflection cannot be obtained on this spectrophotometer at 325 Mu as recommended in the original paper. The per cent transmission for 1, 2, 3, 4, 5, 6, 7, and 8 ppm nitrate nitrogen are 80.5, 65.0, 53.0, 41.0, 33.5, 27.0, 22.0 and 17.5 respectively.

Conclusions

Hartley and Asai (1) concluded that, "Use of 2, 6-Xylenol as a reagent for the spectrophotometric determination of nitrate compares favorable with three colorimetric methods most commonly used for nitrate determinations: the phenoldisulfonic acid, 2, 4,-Xylenol, and brucine procedures. This procedure compares favorable with the above mentioned colorimetric methods on the basis of the four requirements: rapidity, specificity, sensitivity, and reproducibility."

The main value of this procedure is the comparative ease of use as a routine method for large numbers of samples which must be analyzed in a short period of time with reasonable accuracy. In actual use the need for special precautions, as used in other methods such as silver sulfate for chlorides, sulfamic acid for nitrites and the use of chelating agents, have not proven necessary. These precautions can easily be incorporated into the procedure if needed.

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Effect of Row Width, Plant Spacing, Nitrogen Rate and Time of Harvest on Yield and Sucrose Content of Sugarbeets[1]

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Sugarbeets were first grown for sugar in Arizona with limited success in the early 1900's. The development of varieties with bolting and curly top resistance, and improvements in irrigation and fertilization practices have since made beets an economically feasible crop for the desert. In April of 1964, Arizona was alloted 20,000 acres for sugarbeet production starting with the 1966 crop year.

Commercial experience with row widths for sugarbeets in the Arizona desert has been confined to 40-inch, double-row beds used in the production of seed beets in the Salt River Valley. Under comparable climatic conditions, growers in the Imperial Valley of California make use of both double and single-row beds. Research information is not available on row widths and plant spacings for optimum sugarbeet production under Arizona conditions.

Row width and plant spacing studies conducted in other areas generally indicate that a row width of 20 inches and a plant spacing of 12 inches in the row are most desirable (1, 2, 3, 4, 5, 6, 8)[3]. Yield of roots and sucrose content were generally decreased by greater row and plant spacings. Several of these studies discussed the influence of plant distribution patterns in relation to plant populations and cultural systems. Nitrogen requirements of the sugarbeet have received much attention. Stout (7) has reviewed the influence of nitrogen fertilizer on sugarbeet quality.

The present experiments were designed to determine the effect of row width, plant spacing, nitrogen rate and time of harvest on root yield, sucrose content and gross sugar production of sugarbeets.

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[3]Numbers in parentheses refer to literature cited.

Materials and Methods

Sugarbeets were grown during the 1965-66 and 1966-67 seasons on Laveen clay loam soil at the University of Arizona Agricultural Experiment Station, Mesa, Arizona. Seed of the variety S-301H was planted on September 24, 1965, and September 29, 1966. Safflower was the preceding crop each year. Experimental design was a split-split-split plot in randomized blocks with four replications.

Plantings were made on 40-inch double and 24- and 30-inch single row beds. Row widths on the double-row bed averaged 20 inches, but were actually 12 inches on top and 28 inches between the beds. This treatment is hereafter referred to as the 12-28-inch width. Both the 12-28-inch and 30-inch widths are commonly used in the production of various other crops in the area. Beets were hand thinned to in-the-row spacings of 5, 10, and 15 inches.

Rates of fertilization were 70, 175 and 250 pounds of elemental nitrogen per acre. All plots received an application of 200 pounds per acre of 11-48-0 fertilizer prior to planting. Plots receiving 175 and 250 pound rates of nitrogen were sidedressed with ammonium nitrate at thinning time and again in January, while those receiving the 70-pound rate were sidedressed only at thinning.

Adequate soil moisture was provided by 12 furrow irrigations both years. The percentage of plants producing seed stalks was low, less than 15 percent both growing seasons. Many of these were bolters which remained vegetative. Virus diseases were present in the plantings each year, beet western yellows being most prevalent.

Harvests were made on April 20, June 7 and July 13 in both 1966 and 1967 corresponding to early-, mid- and late-season commercial harvest periods. On each date beets from 24 feet of row in each plot were harvested. Roots under 2 inches in diameter were considered commercially unharvestable and were weighed separately in April. At later harvests the proportion of small roots was negligible. One sample of 14 roots from each plot was analyzed for sucrose content at Spreckels Sugar Company laboratories.

Results and Discussion

Root yields were generally higher at each harvest date in 1966 than in 1967 (Tables 1 and 2). Yields obtained both years indicate that April 20 was early for harvest of a late September planting. Root growth was rapid (1.3 tons per week) between April 20 and June 7 and then declined.

Table 1.—The effect of row width and plant spacing on root yield, sucrose content and gross sugar production of beets harvested at different dates. 1966.

Row width	Plant spacing	Plant population	Yield of roots Harvest date				Sucrose content Harvest date				Gross sugar Harvest date			
			Apr. 20	June 7	July 13	Ave.	Apr. 20	June 7	July 13	Ave.	Apr. 20	June 7	July 13	Ave.
Inches	Inches	Per acre	Tons per acre				Percent				Pounds per acre			
12-28	5	62,730	17.0	25.8	30.5	24.4	11.6	14.4	14.7	13.6	3920	7430	8970	6770
12-28	10	31,360	17.7	28.9	32.3	26.3	12.1	14.4	14.2	13.6	4210	8290	9130	7210
12-28	15	20,910	19.6	30.4	33.0	27.7	11.6	14.1	13.5	13.1	4470	8480	8860	7270
Average						26.1				13.4				7080
24	5	52,270	16.0	25.6	29.2	23.6	11.7	14.1	14.6	13.5	3710	7200	8540	6480
24	10	26,140	18.1	26.8	30.5	25.1	11.9	14.4	14.1	13.5	4250	7680	8620	6850
24	15	17,420	17.7	28.0	30.9	25.5	11.3	13.7	13.2	12.7	3970	7660	8150	6590
Average						24.7				13.2				6640
30	5	41,820	16.4	23.8	28.6	22.9	11.5	14.5	14.1	13.4	3760	6920	8090	6260
30	10	20,910	20.0	26.9	31.7	26.2	10.8	13.5	12.1	12.1	4310	7240	7640	6400
30	15	13,940	18.2	25.4	28.6	24.1	11.4	13.9	12.9	12.7	4140	7070	7340	6180
Average						24.4				12.7				6280

Table 2.—The effect of row width and plant spacing on root yield, sucrose content and gross sugar production of beets harvested at different dates 1967.

Row width	Plant spacing	Plant population	Yield of roots Harvest date				Sucrose content Harvest date				Gross sugar Harvest date			
			Apr. 20	June 7	July 13	Ave.	Apr. 20	June 7	July 13	Ave.	Apr. 20	June 7	July 13	Ave.
Inches	Inches	Per acre	Tons per acre				Percent				Pounds per acre			
12-28	5	62,730	16.8	25.1	27.8	23.2	13.5	15.1	14.6	14.4	4530	7580	8160	6760
12-28	10	31,360	17.5	26.9	29.1	24.5	13.4	15.0	14.8	14.4	4660	8040	8570	7090
12-28	15	20,910	16.3	26.4	30.1	24.3	13.2	14.8	14.5	14.2	4260	7800	8720	6930
Average						24.0				14.3				6930
24	5	52,270	16.2	25.9	29.1	23.7	13.6	15.0	14.9	14.5	4370	7780	8680	6940
24	10	26,140	17.5	29.0	31.4	26.0	13.0	14.4	14.6	14.0	4530	8330	9100	7320
24	15	17,420	15.7	26.4	26.3	22.8	12.7	14.6	14.3	13.9	3990	7660	7490	6360
Average						24.2				14.1				6870
30	5	41,820	14.8	22.6	23.9	20.4	13.5	14.9	14.4	14.3	3960	6740	6840	5850
30	10	20,910	16.5	23.9	25.4	21.9	12.9	14.5	14.0	13.8	4220	6910	7090	6070
30	15	13,940	14.9	23.0	24.9	20.9	12.8	14.6	14.0	13.8	3800	6670	6970	5810
Average						21.1				14.0				5910

Sucrose content was lower in 1966 than in 1967, with the largest differences in April. Each year sucrose content tended to reach a maximum in June and then decrease by mid-July, when maximum and minimum temperatures are commonly above 100° F and 65° F, respectively. However, late season losses in sucrose content were more than offset by increases in root yields.

Row Width: An analysis of variance revealed significant differences in root yield between row width treatments (Table 3). The 12-28 inch width produced significantly higher root yields than the 30-inch width both years. There were significant differences between yields at the 12-28-inch and 24-inch widths in 1966, but not in 1967. The largest yield differences were obtained at the June and July harvests.

There were no significant differences in sucrose content as influenced by row widths until July. At that time the 12-28- and 24-inch widths contained the highest average sucrose concentration. The decrease in sucrose content which occurred each year during June and July was greatest at the 30-inch width.

Row width treatments resulted in differences in gross sugar production both years. In 1966 the 12-28-inch width produced the highest sugar yield, while in 1967 the 12-28-inch and 24-inch widths produced the highest yields. The 12-28-inch width yielded an average of 910 pounds more sugar per acre than the 30-inch width.

Plant Spacing: Plant spacing intervals of 10 inches resulted in significantly higher yields than 5-inch sacings both years.

Table 3.—Analysis of variance for root yield, percent sucrose and gross sugar production.

		Mean squares					
		Root yield		Percent sucrose		Gross sugar	
variation	d f	1966	1967	1966	1967	1966	1967
Row width	2	702*	2,483**	10.96*	3.84**	33.95**	67.97**
Error A	6	75	168	1.09	0.19	0.90	3.43
Plant spacing	2	1,292**	681**	11.08*	5.61**	5.12NS	11.52**
RW x PS	4	25 INS	163**	6.20NS	0.76NS	1.58NS	4.04**
Error B	18	212	22	2.24	0.49	2-28	0.65
Nitrogen rate	2	3,575**	4,026**	39.96**	39.09**	23.11**	39.18**
PS x NR	4	579**	199**	0.09NS	0.14NS	9.76**	3.46*
Error C	54	70	40	0.56	0.30	1.80	1.00
Harvest date	2	35,820**	30,244**	208.35**	77.28**	1079.86**	850.60**
HD x RS	4	198**	2,558**	2.02**	6.96**	6.66**	7.66**
Error D	162	37	57	0.38	0.27	0.91	1.29

*= F ratio exceeds the .05 probability level.
**= F ratio exceeds the .01 probability level.
NS= No significant differences.

Yields at 15-inch spacings were as high as those at 10-inch spacings in 1966 but were lower in 1967. Yield was generally less at 5- or 15-inch spacings when used with the 30-inch row width.

Large numbers of very small roots were produced by the 5-inch spacing at the April harvest (Table 4). In 1966 unharvestable roots represented over 3 tons per acre of the total yield. The greatest loss occurred on the 12-28-inch row width where the plant population was highest. Beet plantings intended for early harvest in the Salt River Valley should be thinned to a plant spacing of at least 10 inches to promote development of harvestable root sizes.

Table 4.—The effect of plant spacing on total and harvestable root yields of sugar beets for April harvest.

	Yield of roots			
	1966		1967	
Plant spacing	Total	Harvestable	Total	Harvestable
Inches	Tons per acre			
5	16.6	**13.4**	15.9	14.0
10	18.7	**18.2**	**17.1**	16.9
15	18.6	18.5	15.6	15.6
LSD .05	**1.6**	**1.7**	**1.2**	1.4

Note: Comparison of total and harvestable yields by *t* test indicates differences were significant **at** 5% level each year.

The 5-inch plant spacing gave the highest average sucrose content both years. As the interval was increased from 5 to 15 inches, sucrose concentration was decreased more than one percent. Late season losses in sucrose content were greatest where beets were spaced 15 inches apart.

Total sugar production was not significantly influenced by plant spacing treatments in 1966. However, in 1967 the 10-inch spacing resulted in the highest average sugar yield.

Results obtained in this study demonstrated the effects of both plant population and distribution pattern on root yield and sucrose content. Per acre plant populations greater than 31,360 or less than 17,420 generally were related to decreased yields. Sucrose content was highest where the plant population was highest.

The plant distribution pattern which more closely approached a square appeared most efficient. For example, with the population at 20,910 plants per acre, yields averaged 26.1 tons per acre at the 15-inch spacing on 12-28-inch rows compared to 24.2 tons per acre at the 10-inch spacing on 30-inch rows. In addition, sucrose concentration decreased as the space allotted became more rectangular.

The disadvantage of rectangular space allotments can also be seen at the 5-inch spacing. When this interval was used on 30-

inch rows, the average yield was 21.8 tons per acre compared to 24.0 tons per acre on 12-28-inch rows. Although the populations at these spacings were not the same, the lower population on 30-inch rows would be expected to be more favorable. At very high plant populations sucrose content was unaffected by distribution pattern.

Nitrogen Rate: The effect of nitrogen fertilizer on root yields and sucrose content was similar each year. As the nitrogen rate was increased from 70 to 175 pounds per acre, yields increased but sucrose concentration decreased (Table 5). The 175 pound rate, however, resulted in higher sugar yields than the 70 pound rate both years.

Table 5.—The effect of nitrogen rate and plant spacing on root yield, sucrose content and gross sugar production. Data from 1966 and 1967 were combined.

Nitrogen rate lbs/acre of N	Plant spacing (inches)[1]			Average		
	5	10	15			
	Roots tons/A	Roots tons/A	Roots tons/A	Roots tons/A	sucrose	Gross sugar lbs/A
70	**21.9**	22.4	20.9	**21.7**	**14.2**	6160
175	24.2	26.6	25.3	25.4	**13.7**	6960
250	23.4	26.3	26.7	25.5	**13.0**	6640
LSD .05				**0.4**	**0.3**	420

[1] Plant spacing by nitrogen rate interaction was highly significant both years in terms of root yield.

A significant plant spacing by nitrogen rate interaction occurred each year. The 250-pound rate resulted in an increase in yield at the 15-inch plant spacing but not at the 5-inch spacing, where a response might be expected. Evidently, nitrogen was not a limiting factor for yields at the very high plant populations. Since, in these experiments, the beets received adequate moisture for optimum yields, another factor, possibly light, was critical at the close plant spacings.

Summary

The influence of row width, plant spacing, nitrogen rate and time of harvest on root yield, sucrose content and gross sugar production of sugar beets was studied under Arizona conditions.

When row width was extended from 12-28 inches to 30 inches, root yield, sucrose content and gross sugar yield were decreased. An in-the-row plant spacing interval of 10 inches was most efficient in terms of root yield. Decreasing plant spacing to 5 inches resulted in a higher sucrose content but reduced root yields. Large numbers of small, unharvestable roots were produced in April at the 5-inch spacing. Populations greater than 31,360 or less than 17,420 plants per acre generally resulted in

reduced yields. Performance appeared most efficient when individual plants were allotted an area more closely approaching a square.

As nitrogen rate was increased from 70 to 175 pounds per acre, sucrose content decreased but root and gross sugar yields were increased. A plant spacing by nitrogen interaction indicated nitrogen was not the limiting factor for yield at the closest spacing.

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Improved Total Phosphorus Method for Determination of Disyston in Dried Sugarbeet Pulp

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Measurement of total phosphorus by phosphomolybdate-blue in isobutanolbenzene medium for the determination of residues of Disyston, 0,0-Diethyl S-[2-(ethylthio) ethyl] phosphordithioate and its oxygen analogs in plant material has been described by Anderson (1)[2]. The method was found to be inadequate when applied to dried sugarbeet pulp. The inadequacies were in the procedures of extraction and total phosphorus determination. This paper presents a method which has simplified the original procedure and yet improved its precision and accuracy for dried sugarbeet pulp.

Method

Apparatus and Materials

(a) Osterizer blender for grinding sample. - Model No. 10, 115 volts, 2.2 amps AC-DC, mounted with one quart, standard, pyrex container, manufactured by John Oster Mfg. Company, Milwaukee, Wis., or equivalent.

(b) Waring Laboratory Blendor for extracting sample. - Consists of three parts: (1) Explosion-proof motor, GE, Model No. 5BA60VL22, 1/5 HP, 115 volts, 4.2 amps and 60 cycles DC: (2) Stainless steel container, type 302, one quart; (3) Explosion-proof switch, obtainable from Waring Products Company, Winsted, Conn., or equivalent.

(c) Chromatographic columns. - K-42028, with 2 A Teflon plug, size D-2. Kontes Glass Company, Vineland, New Jersey.

(d) Kjeldahl digesting unit. - S-63200, Micro, Pregl, 6 place, gas heated, E. H. Sargent and Company.

(e) Spectrophotometer. - Model DU, Beckman Instruments, Inc.

(f) High vacuum evaporator. - VE-1000-B, Rinco Instrument Company, Inc., Greenville, **111.**

(g) Digestion tubes. - Folin-Wu, 50 ml.

(h) Separately funnels. - Kimax 29048-F, 30 ml, with Teflon plug, Kibzle Products, Toledo, Ohio. (For use, mark at the 15 ml level.)

(i) Activated carbon. - Darco 20 X 40, granular, Atlas Chemical Industries Inc., Wilmington, Del.

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[2] Numbers in parentheses refer to literature cited.

(j) Aluminum oxide. - Merck 71695, acid washed, chromatographic grade, A. Daigger and Company, Chicago, 111.

(k) Superbrite glass beads. - Size 130-5005, Serial No. P-4495, Minnesota Mining and Manufacturing Company, St. Paul, Minn.

Reagents

(a) Acetone, chloroform. - Reagent grade and redistilled, discard the very first and very last parts of the distillate, use only mid-part of distillate.

(b) Iso-butyl acetate. - Reagent grade.

(c) Molybdate solution. - Dissolve 10.69 g of analytical reagent grade ammonium molybdate tetrahydrate, $(NH_4)_6\ Mo_7 0_{24}\ *4H_2 0$, in double distilled water and dilute to 1 liter.

(d) Nitric acid. - Reagent grade, 8 N.

(e) Perchloric acid (70%).[1]

(f) Hydrochloric acid. - Reagent grade. Concentrated and 2 N.

(g) Sulfuric acid. - Reagent grade, 1 N.

(h) Sodium hydroxide. - Reagent grade, concentrated and 0.1 N.

(i) Thymol blue (0.1%). - Dissolved in ethanol.

(j) Phosphorus stock solution. - Dissolve 0.4393 g of reagent grade, oven dried, $KH_2PO.$, in double distilled water and dilute to 1 liter. This stock solution is 100 ppm in phosphorus. Working solution: dilute 2, 4, 6, 8, and 10 ml of stock solution to 100 ml, respectively. They are 2, 4, 6, 8, and 10 ppm phosphorus.

(k) Stannous chloride. - Dissolve 10 g of stannous chloride dihydrate in 25 ml of HC1 in a brown bottle. For use: dilute 1 ml to 20 ml with 1 N H_2SO_4.

Extraction

Grind 100 g dried beet pulp or pellets in an Osterizer blender for 5 minutes. Blend only 25 g of the ground sample with 100 ml double distilled water and 200 ml acetone in a Waring Laboratory Blendor for 5 minutes. Filter through Whatman No. 5 filter paper on a Buchner funnel. Record the recovered volume of filtrate. Shake the filtrate with 250 ml chloroform. Repeat the extraction with two 50 ml portions of chloroform. Combine all chloroform extracts and evaporate to dryness in a high vacuum evaporator at temperature of 40° C.

Chromatographic Procedure

Chromatographic column packing procedure is according to Anderson (1). Place a plug of glass wool in the bottom of column. Open stopcock slightly. Pour 15 g Superbrite beads into column. Wash column with 50 ml acetone. Drain off some acetone. Close the stopcock. Add 10 g aluminum oxide. Wash down with an-

other 50 ml acetone. Finally add 10 g Darco carbon into column. Tap the sides of column to make each layer as tight as possible. Rinse the packed column with 200 ml acetone at a flow rate of 3-4 ml per minute. During the column rinsing tap the sides several times.

Before the acetone rinse penetrates the carbon layer by not more than one-half inch rapidly transfer the vacuum dried sample from the evaporatory flask to the column by means of 10 ml of chloroform-acetone (30:70, v./v.). Rinse the evaporatory flask with 3 portions of 5 ml acetone and successively pour to column before sample starts to enter into column. Collect the eluate. Pour 300 ml acetone to the column. Evaporate the eluate to 1-2 ml in a 250 ml evaporatory flask under vacuum.

Digestion

Transfer the concentrated sample to a digestion tube. Use two portions of 2 ml acetone to remove the remaining sample. Evaporate it down to about 0.5 ml in a water bath (40° C) with a jet of air. Cool the content to room temperature. Add in order, 2 ml of 8 N HNO_3, chill in ice water, 0.5 ml of 70% perchloric acid, and a glass bead. Heat gently on a Kjeldahl digestion unit to boiling. Shut off the flame. Allow the billowy brown fume to subside. Continue to digest the sample until no more white fume remains. Time required for digestion is about an hour. Final volume of the digest is about 0.5 ml.

Colorimetric Determination of Phosphorus

Allow the digest to cool. Transfer it with 3 portions of 2 ml of double distilled water to a 30-ml separatory funnel containing 6 ml of molybdate solution. To the mixture add 2 drops of thymol blue, and swirl the mixture well. Neutralize the mixture first with concentrated NaOH to turn indicator from red to yellow. Continue neutralizing with 0.1 N NaOH until the yellow color remains after shaking (2). To the neutralized mixture add 1 ml concentrated HC1. Bring volume to 15 ml mark with double distilled water. Shake the mixture with exactly 10 ml iso-butyl acetate for 1 minute to extract phosphomolybdic acid from aqueous phase to organic phase (3). Discard the aqueous phase. Wash the organic phase with 5 ml of 2 N HC1 for 30 seconds. Again discard the aqueous phase. Remove any remaining droplets of water from stem of funnel. Drain the organic phase to a 25 ml graduate cylinder. Its volume should be still 10 ml. Add 6 drops of dilute stannous chloride solution. Read absorbance against a water blank at 730 mμ on a Beckman DU spectrophotometer. Reading is best taken after blue color has developed for 10 minutes but not over 25 minutes.

Preparation of Standard Curve

Pipette 1 ml of each working standard phosphorus solution to five 30 ml separatory funnels containing 6 ml molybdate solution, respectively. To the solution add 7 ml double distilled water and 1 ml concentrated HC1. Allow solution to stand for 5 minutes. Shake the solution for 1 minute with exactly 10 ml iso-butyl acetate. Then follow through rest of steps as previously described.

Calculation

$$\text{ppm Disyston} = \frac{(A)}{(B)} \frac{(274)}{(31)} \frac{(4)}{(C)}$$

A = Absorbance of sample.

B = Absorbance of a 4-microgram phosphorus standard.

$$C = 25 \text{ g} \times \frac{\text{ml of extract recovered}}{\text{300 ml of extraction solvent}}$$

Molecular weight of Disyston = 274.

Results and Discussion

The apparent residues of Disyston in controlled dried beet pulp varied with sample size and sample form (molasses pulp or plain pulp). In dried molasses pulp 50 g samples had apparent residues from 0.1 to 0.4 ppm, 25 g samples had 0.0 to 0.2 ppm; whereas dried plain pulp samples were from 0.5 to 1.0 ppm for 50 g samples and 0.2 to 0.5 ppm for 25 g samples. It appeared that a sample weight of 25 g assured the method a better sensitivity. This was further evidenced when percent recovery of added Disyston from 25 g dried molasses sample versus those of 50 g and 12.5 g were compared (Table 1).

Extraction solvent used here was 100 ml water and 200 ml acetone to a sample of 25 g. This aqueous acetone proved itself a most promising extractant over Anderson's solvents (1) as shown in Table 2.

Acids used for digestion of organic phosphate pesticide in this paper were similar to those of Stellar and Curry (4). The digest was brought to neutral with NaOH (2). Again the digest was acidified by 1 ml of concentrated HCl to 0.8 N. The purpose

Table 1.—Percent recovery of added Disyston from dried molasses sugarbeet pulp.

Sample grams	Disyston ppm, added	% Recovery
50	1.0	147
	0.5	140
25	1.0	100
	0.5	116
12.5	1.0	98
	0.5	50

Table 2.—Comparison of extraction solvents

Extraction by		Sample grams	Disyston ppm, added	% Recovery[1]
Anderson's Moist[2]		25	.5	96
Anderson's dry[3]		25	.5	87
Modified	moist[4]	25	.5	100

[1] Average of 2 runs.
[2] 140 ml water and 250 ml acetone, blended for 5 minutes.
[3] 400 ml chloroform reflux extraction for 24 hours.
[4] 100 ml water and 200 ml acetone, blended for 5 minutes.

of doing this is to insure the same acidity for each sample as well as for standards.

The phosphomolybdic acid was reduced to blue color in a medium of isobutyl acetate (3) instead of isobutanol-benzene (1). Because the selectivity of iso-butyl acetate for extraction of phosphomolybdic acid is as good as isobutanolbenzene, yet with no

Table 3.—Percent recovery of added oxygen analogs of Disyston from dried sugar-beet pulp.

Sample 25 g	Sulfone		Sulfoxide	
	ppm, added	% recovery	ppm, added	% recovery
Molasses pulp	0.2	120	0.2	100
	0.5	100	0.5	112
	1.0	102	1.0	100
Plain pulp	0.2	120	0.2	124
	0.5	89	0.5	114
	1.0	104	1.0	110

loss of the phosphate to the aqueous phase for iso-butylacetate is insoluble in water. Also phosphorus determined in iso-butyl acetate should be free of interference from most ions (3).

The percent recovery of oxygen analogs of Disyston obtained with the improved method showed excellent results (Table 3). Those had recovery slightly over 100%, possibly resulting from glassware or unusual samples of high apparent residues.

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The Effect of Nitrogen on Yield, Percent Sucrose, and Clear Juice Purity of Sugarbeets

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Introduction and Literature Review

The importance of nitrogen in sugarbeet production has received considerable research attention. As early as 1912 Headden (3)[2] showed that a relationship existed between beet yield and sucrose content. Since then many reports have been given to illustrate that excessive nitrogen tends to lower the sucrose content of the beet roots (1,4). Recently, increasing interest has been shown in the relationship between nitrogen and the clear juice purity of the beet roots. In 1967 Snyder (5) reported a decrease in purity with increasing rates of nitrogen.

The nutrition of nitrogen is difficult to control due to the biological nature of its various forms and the fact that it is markedly influenced by many cultural practices. This research was designed to study the effect of varying rates of nitrogen on the yield, percent sucrose and clear juice purity of sugarbeets. The data presented in this manuscript involves the years 1965, 1966 and 1967. Each year this research was conducted at three sites thereby giving a total of nine locations during the three years.

Materials and Methods

The soils on all locations were classified as clay or clay loam in texture, and were tile-drained at 40-foot intervals. Chemical analyses of the soils indicated a medium to high level of available phosphate and a high level of water soluble potash. On all sites the pH ranged from slightly acid to slightly alkaline in reaction.

The plots were laid out in a randomized plot design with six replications. Each plot contained four rows of beets and was 8 feet wide and 20 feet in length. Eight levels of nitrogen were studied (0, 30, 60, 90, 120, 150, 180, and 210 pounds of N per acre). The nitrogen was applied as a mid-June sidedressing with anhydrous ammonia. The soils at each location were fall plowed to a depth of approximately 8 inches. All plots received an application of 0-20-20 at 1000 pounds per acre. This was broadcast and disced in just prior to seeding. Monogerm seed was planted in 24-inch rows, and the beets were hand-thinned to one beet

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[2] Numbers in parentheses refer to literature cited.

per 12 inches of row. Thinning time each year occurred in mid-June. Planting of the beets took place in late April to mid-May.

The beets were harvested during the second week in October. Just prior to harvest a visual Cercospora blight *(Cercospora beticola)* rating was made on each plot according to the Kleinwanzlebener Rating Chart[3]. All beets were hand-harvested and hand-topped. Root and top weights were taken from 15 feet of each of the two center rows. From each plot 10 beets were selected at random and scrubbed free of soil for sucrose and purity determinations. The clear juice purity and extractable sugar determinations were made using the modified Carruthers method (2).

Results and Discussion

Effect of Rate of Nitrogen on Extractable Sugar Yield

Maximum extractable sugar was obtained when nitrogen was applied at the rate of 30 pounds per acre (Table 1). This corresponds very closely with a recent report in the literature (5). There appeared to be no reduction in sugar yield until nitrogen was applied at rates in excess of 90 pounds per acre. In other words, there were no sugar yield differences when nitrogen was applied at 30, 60, and 90 pounds per acre. On the average, extractable sugar yield was reduced by 651 pounds per acre by increasing the rate of nitrogen from 30 to the 210 pound rate.

Significant sugar yield differences were obtained on only three of the nine locations studied (Table 2).

Effect of Rate of Nitrogen on Percent Clear Juice Purity

Each year the highest percent clear juice purity was obtained

Table 1.—Effect of rate of nitrogen application on the yield of extractable sugar.

Nitrogen (lbs/A)	Year			Avg
	1965	1966	1967	
	lbs per acre			
0	5451	7019	5936	6135
30	5548	7534	6400	6494
60	5239	7185	6624	6549
90	5381	7214	6770	6455
120	5275	6883	6566	6241
150	5023	6740	6519	6094
180	4852	6760	6263	5958
210	4760	6620	6148	5843
L.S.D. (0.05)	742	902	796	

in beets that did not receive nitrogen (Table 3). On the average there was a gradual reduction in purity over the entire range of

[3] Kleinwanzlebener Cercospora-Tafel. Verlag Dr. Buhrbauk and Co. K. G. Berlin und Holzminder.

Table 2.—Summary of the statistical significance[1] of the various yield parameters at the nine locations.

Year	Location	Tops	% Sucrose	Roots	% C.J.P.	Ext. sugar
	1	*	*	N.S.	N.S.	N.S.
1965	2	*	N.S.	N.S.	*	*
	3	*	*	*	N.S.	N.S.
	4	N.S.	*	N.S.	*	N.S.
1966	5	*	N.S.	N.S.	*	N.S.
	6	*	N.S.	N.S.	N.S.	*
	7	*	N.S.	N.S.	N.S.	N.S.
1967	8	*	N.S.	*	*	*
	9	N.S.	N.S.	N.S.	*	N.S.

[1] Where an asterisk appears this implies that the data was statistically significant at the 0.05 probability level. N.S. denotes non-significance.

nitrogen applications. Over this range the purity decreased by 1.4%. The beets from the check plot had a 94.7% purity, whereas those from the plots that received 210 pounds of nitrogen had a purity of 93.3%.

The reduction in purity seemed to be most evident with rates of nitrogen that were in excess of 60 pounds per acre. On the average, the percent clear juice purity was lowered by 0.2 percent with every 30 pound increment of nitrogen.

Table 3.—Effect of rate of nitrogen application on the percent purity of sugarbeets.

Nitrogen (lbs /A)	Year 1965	1966	1967	Avg
	% purity			
0	94.2	95.1	94.9	94.7
30	93.8	95.0	94.7	94.5
60	93.9	94.9	94.8	94.5
90	93.5	94.2	94.7	94.1
120	93.5	94.4	93.9	93.9
150	93.2	93.9	94.1	93.7
180	92.1	93.9	93.0	93.0
210	92.6	94.0	93.4	93.3
L.S.D. (0.05)	1.50	0.75	1.19	

Significant differences in clear juice purity readings were obtained in five of the nine locations (Table 2).

Effect of Rate of Nitrogen on Root Yield

As illustrated in the data in Table 4, there were no significant root yield responses obtained in 1965 and 1966. In 1967 slight significant differences were obtained. Generally the root yield showed very little response to added nitrogen even though the nitrogen status of the soil was at a relatively low level prior to beet planting. This response closely corresponds to previous data

from this Station (1). In 1967 where some differences were observed, the only significant difference occurred between the beets taken from the check plot and those from the plot receiving 150 pounds of nitrogen. The maximum yield of roots was obtained at the 90 pound rate of nitrogen. This was only 1.5 tons greater than the yield from the plots that did not receive nitrogen.

Significant differences in root yields were observed in only two of the nine locations (Table 2).

Table 4.—Effect of rate of nitrogen application on the yield of sugarbeet roots.

Nitrogen (lbs /A)	Year			Avg
	1965	1966	1967	
	tons per acre			
0	20.6	22.8	18.8	20.7
30	21.5	24.0	19.6	21.7
60	20.5	23.8	20.6	21.6
90	21.3	24.6	20.6	22.2
120	21.2	23.8	20.1	21.7
150	20.8	23.5	21.6	21.9
180	21.3	24.5	21.1	22.2
210	20.3	23.9	20.7	21.6
L.S.D. (0.05)	N.S.	N.S.	2.65	

Effect of Rate of Nitrogen on Percent Sucrose

The average of the 3 years' data indicates that there was no reduction in percent sucrose until the 90 pound per acre level of nitrogen was reached (Table 5). A gradual reduction in percent sucrose occurred with rates of nitrogen in excess of 60 pounds per acre. In 1965 the sucrose percent was the highest in the beets from the check plots and a slight gradual reduction occurred thereafter. In 1966 the highest percent sucrose occurred at the 30 pound nitrogen rate and no reduction was recorded

Table 5.—Effect of rate of nitrogen application on the percent sucrose content of sugarbeets.

Nitrogen (lbs /A)	Year			Avg
	1965	1966	1967	
	% sucrose			
0	15.0	17.2	17.5	16.6
30	14.9	17.4	17.5	16.6
60	14.7	17.2	17.8	16.6
90	14.8	16.7	17.6	16.4
120	14.4	16.4	17.2	16.0
150	14.1	16.6	17.1	15.9
180	13.7	16.2	17.1	15.7
210	13.7	16.2	16.9	15.6
L.S.D. (0.05)	0.80	0.76	N.S.	

until the 90 pound nitrogen level. In 1967 the nitrogen rate did not significantly affect the percent sucrose of the beets. On the average, the sucrose percent dropped one percentage point over the entire range of nitrogen application. The beets from plots that did not receive nitrogen tested 16.6 in percent sucrose whereas the beets from the plots that received 210 pounds of nitrogen tested 15.6%.

From a statistical standpoint relatively few significant differences were observed (Table 2). Here we note that in only three of the nine locations were any of the differences significant.

Effect of Rate of Nitrogen on Beet Top Yield

Each year the maximum yield of sugarbeet tops was obtained with the highest level of applied nitrogen (Table 6). In general, there was a very consistent increase in stover yield with each additional increment of nitrogen. On the average there was an approximate one ton increase with each 30 pound increment of nitrogen. This implies an almost straight line relationship with no leveling off occurring. The check plot average yield was

Table 6.—Effect of rate of nitrogen application on the yield of sugarbeet tops.

Nitrogen (lbs /A)	Year 1965	1966	1967	Avg
	tons per acre			
0	10.1	18.4	10.2	12.9
30	11.4	19.7	12.1	14.4
60	13.2	21.3	11.9	15.5
90	14.2	22.7	13.3	16.7
120	15.4	23.0	14.2	17.5
150	14.8	22.6	16.0	17.8
180	15.7	23.5	16.0	18.4
210	17.1	24.6	16.6	19.4
L.S.D. (0.05)	3.17	2.74	2.61	

12.9 ton per acre compared to a yield of 19.4 tons per acre for the plots that received 210 pounds of nitrogen. The beet top yield gave the most significant response of all factors studied (Table 2). Statistical significance in top yield was obtained in seven of the nine locations under study.

Cercospora Leaf Blight Rating

Leaf blight was evident in only two of the nine locations. There was no incidence of blight in 1965 and 1967. Two of the locations in 1966 had blight to a very slight degree. On these sites the beets that received 0 or 30 pounds of nitrogen had slight infestations of blight. Beets in plots that received 60 pounds or more nitrogen did not show any evidence of this disease.

Summary

The effect of nitrogen on the yield, percent sucrose, and clear juice purity of sugarbeets was studied. Nitrogen was side-dressed in 30 pound increments ranging from 0 to 210 pounds per acre. This experiment involved nine locations during 1965, 1966, and 1967. Maximum extractable sugar was obtained when nitrogen was applied at 30 pounds per acre. The yield of sugar showed marked reductions when nitrogen was applied at rates in excess of 90 pounds per acre.

On the average there was a slight gradual reduction in clear juice purity over the entire range of nitrogen application. Beets harvested from the check plots had a 94.7% purity, and those harvested from plots receiving 210 pounds of nitrogen had a purity of 93.3%.

Rate of nitrogen had little significant effects on beet root yields. The maximum yield of roots was obtained when nitrogen was applied at 90 pounds per acre. However, this was only 1.5 tons greater than the yield from the plots that did not receive nitrogen.

There was no reduction in percent sucrose until the 90 pound level of nitrogen. From the 90 pound rate to the 210 pound rate a slight gradual reduction was obtained. The beets from the check plots contained 16.6% sucrose whereas beets that received 210 pounds of nitrogen tested 15.6%.

The highest yield of sugarbeet tops was obtained from plots that received the highest rate of nitrogen (210 pounds per acre). On the average, yield of beet stover increased approximately one ton per acre with every 30 pound increment of nitrogen added.

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Relationship of Date of Planting and Date of Harvest to Incidence of Disease, Stand Survival, Yield and Sugar Content of Sugarbeets at Yuma, Arizona[1]

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In the warm desert valleys of southern Arizona and California, sugarbeets are grown during the fall and winter for harvest in the spring. The campaign begins in late April and continues into July, but processors are seeking ways to extend factory operations over a longer period.

Since the normal harvest campaign in the Imperial Valley or the Salt River Valley is in the late spring when ambient temperatures are increasing daily, it is not feasible to store beet roots for later processing. Rates of catabolism within the root tissue are so high under these conditions that the root is rendered unfit for processing within less than 72 hours after it has been lifted from the soil. Processors manage the beet harvest so that each day growers deliver to a factory only that tonnage of roots which can be processed within 24 hours.

The possibility of leaving the beets in the soil for harvest during the summer has been considered. Price et al. (8)[3] reviewed the records of the Holly Sugar Corporation for the Imperial Valley and found that whenever the harvest period had extended beyond mid-July there was a decline in acre yields of sugar, due principally to reduced sugar percentage of the beets. However, from a date of planting study in the Imperial Valley, they concluded that the harvest period could be profitably prolonged by planting beets in July and August and harvesting them earlier than is the current practice.

The primary objective of this study was to determine the feasibility of planting beets at various times of the year at Yuma, Arizona in order to have them available for harvest over the longest period of time possible. If beets could be profitably harvested at Yuma during April or August they could be shipped to either Chandler, Arizona or Brawley, California, thereby extending the length of the processing campaign. Harvests often begin as early as April 20 in the Imperial Valley and May 1 in

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[3] Numbers in parentheses refer to literature cited.

the Salt River Valley, and are usually completed in both valleys by August **1.**

Materials and Methods

The 1962-1965 Experiment

US H2 sugarbeets were planted on approximately the fifteenth of every month during the three-year period, June 1962 through June 1965. Each planting consisted of four standard 40-inch beds 300 feet long with two rows of beets per bed spaced 12 inches apart. The plants were thinned to an 8-inch within-row spacing as soon as possible after stand establishment. The soil was Glendale silty clay loam underlain with fine sand at depths varying from 24 to 48 inches.

All plantings received 100 pounds per acre of N as ammonium sulphate by sidedressing after the first cultivation. Residual phosphorus in the soil was considered to be adequate from applications on previous crops. Supplies of potassium and other nutrients are normally adequate in this alluvial soil. Plots reserved for later plantings were kept free of weeds by cultivation.

Stand percentages were determined periodically on the basis of 1.5 plants per foot of row as a 100% stand. Plants in the plots were rated for diseased condition during the season. Disease symptoms, as expressed on the leaves, were evaluated visually with no attempt to isolate the specific disease causing organisms. A scale of 0 to 10 was used to indicate the range from no apparent disease to dead plants.

Periodically from December to September, three 5-foot double row segments were harvested from inside beds of each planting for yield and sugar content. Sugar percentage estimates were made by multiplying refractometer readings by the factor 0.8 which was derived by comparing refractometer readings with laboratory analyses of the same beet samples.

The 1965-1966 Experiment

The presence of old beets in the field constituted a source of disease infection for each new planting and probably resulted in higher levels of disease than would have occurred if beets had been used in rotation with other crops. In order to determine the disease incidence, rate of growth, and summer decline for beets planted following a beet-free period, all plots were plowed and free of beets by September 1, 1965. On November 12, a new field previously planted to alfalfa was used for a sugarbeet date of harvest study. There were eight replications of six harvest dates. All other procedures were similar to those of the preceding experiment. One plot from each replication was harvested every 2 weeks from May 23 to August 9.

Results and Discussion

The 1962-1965 Experiment

Disease The average disease index readings for the three-year period are shown in Table 1. Disease symptoms were evident early in the investigation. Curly top was observed in October 1962, mosaic in November, and "yellows" in March 1963. Disease symptoms were slower to appear in the fall and early winter plantings, but appeared quickly in the late winter and early spring plantings and increased rapidly in all plantings during the spring. Observations made in May 1963 indicated that the effects of disease were especially severe in plantings made during the previous summer months, while plantings made during the fall and winter were not severely affected.

Since beets were grown continuously in the experiment, they undoubtedly served as a source of infection for each new planting. Duffus (7) showed that new plantings were infected with disease much earlier as the distance from older diseased plants was decreased. The summer plantings of 1963 and 1964 started to show symptoms of disease earlier in the fall than did the 1962 summer plantings, while the fall, winter, and spring plantings in 1963-1964 and 1964-1965 followed a pattern similar to that of 1962-1963. Apparently the earliness of infection is dependent upon both the proximity of the disease source and the presence and numbers of efficient insect vectors.

Summer planted beets were always heavily diseased by the time growth was retarded by the cold weather of December and January. Therefore, they were in poor condition when growth resumed in February or March. Beets planted in October, or later, were not as severely infected with disease, withstood the cold weather better, and made better growth during the spring months. Apparently many of the common viruses which infect sugarbeets were present. In 1960, Bennett (1) reported that sugarbeet yellows disease was widespread in the Salt River Valley of Arizona and the Imperial Valley of California, and he named several weed hosts. Those common to the Yuma Valley include species of *Chenopodium*, *Amaranthus*, and *Atriplex*. Coudriet and Tuttle (4) reported on the movements of several efficient insect vectors of plant viruses in southern Arizona, the viruses transmitted, and the plants affected. According to them, curly top is spread in the cantaloupe fields of the Yuma Valley by the beet leaf hopper, *Circulifer tenellus* (Baker), which migrates in large numbers from the desert to the cultivated areas throughout the year, with peaks of abundance in both spring and **fall.**

Table 1.—Three year average disease index readings on monthly plantings of sugarbeets at Yuma, Arizona, 1962-1965.

Month of reading	Month of planting											
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May
July	.3[1]											
August	2	2										
September	2	2	1									
October	2.3	2.3	2.3	1								
November	3.3	3.3	3	2	0							
December	4	4.3	3.7	2.7	0	0						
January	5	5	4.7	3.3	.3	.7	0					
February	5.3	5.3	5.3	4	.3	.7	.3	.3				
March	6	6	5.7	4.7	1.3	1.7	1.3	1.3	2			
April	7	7	6.3	5	2	2.3	2	1.7	2.7	3.3		
May	7.3	8	7	6.3	2.3	3	3	3	3.7	5	3	
June	8	8.3	8	7.3	3.3	4	4	4.3	4.7	6.7	6.7	4.3
July		9	9	8.3	4	5	5	5	6	8.3	8.7	8
August					6	6	6.3	6.3	7.3	9.3	9.7	9.7
September								9	9	10	10	10

[1] Readings made on general diseased conditions using a scale of 0 to 10 to indicate the range from no disease symptoms to dead plants. The high readings of July, August and September include effects of high temperatures in combination with disease.

Beet yellows and beet western yellows both occur and are transmitted by the green peach aphid, *Myzus persicae* (Sulzer). Winged forms of this insect appear in September and gradually increase in population density during the winter. Maximum population density usually occurs between March 15 and April 15. Cucumber mosaic is common in cantaloupes at Yuma and readily transferred to sugarbeets by the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), which is prevalent in the Yuma area on johnsongrass, sorghum, and barley. This aphid has two distinct periods of winged form, September to December and February to May.

Reynolds et al. (9) reported that properly timed applications of insecticides during the normal growing season in the Imperial Valley (September - July) reduced the incidence of both curly top and yellows and increased both sugar percentage and tonnage of beets. However, he noted that in late spring tremendous numbers of alate aphids were flying and he doubted that any insecticide could protect the plants from infection with either beet yellows or beet western yellows under these conditions. Cook (3) reported that the beet leafhopper breeds continuously during the warmer months and that in California and Arizona nymphs are produced in every month except December and January. This fact and the migratory habits of the insect make control by ordinary methods very difficult.

Insects In addition to the vectors of virus diseases, there were other insects which damaged sugarbeets through the summer and fall. Flea beetles, principally *Systena blanda* Melsh., and the beet armyworm, *Spodoptera exigua* (Hiibner), were active from June through September. Insecticides used were 5% Malathion dust, *2%* Endrin dust, and Endrin spray. Applications made at intervals ranging from 14 to 30 days reduced populations but control was generally unsatisfactory.

From October 1963 to June 1965, all plantings were treated with Thimet. Twenty pounds per acre of ten-percent granules were applied in bands over the drill rows prior to each germination irrigation. No perceptable decline in either disease incidence or insect damage was noted as a result of these treatments.

In late October and early November, salt-marsh caterpillars, *Estigmene acrea* (Drury), moved into the beet plots from adjacent cotton fields and destroyed emerging seedlings by cutting them off at ground level. The most effective control was a 6-inch high aluminum-foil barrier placed around the field to keep the caterpillars out, a method commonly used to protect fields of emerging lettuce seedlings.

Stand persistence Early in the experiment it became apparent that high summer temperatures were a major factor in

determining when sugarbeets could be planted and grown for economical production at Yuma. On the average, afternoon temperatures reach 100 F from June 8 to September 13 and 105 F from June 26 to August 16 (10). Although all plantings resulted in fair stands (Figure 1), plantings which were beyond the seedling stage by the time they were subjected to the summer heat were dead or dying by August. However, seedlings from June, July and August plantings survived the first summer but quickly declined when, as mature plants, they were subjected to the heat of the following summer. Death appeared to result from the interaction of disease, insects and high temperatures.

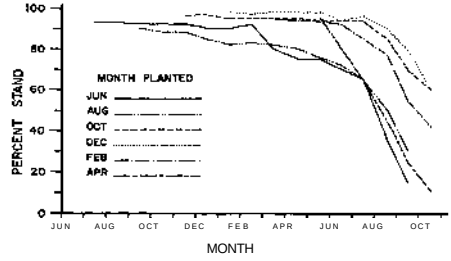

Figure 1.—Three-year average stand persistence. Each curve extends through the life-span of plantings made in the indicated month at Yuma, Arizona. 1962-1965.

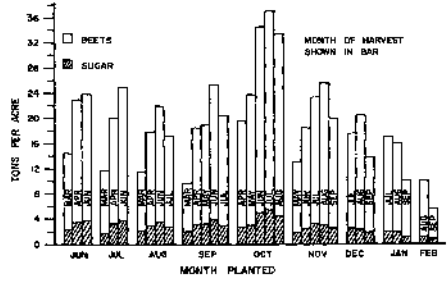

Figure 2.—Three-year average yields of roots and sugar from periodic harvests of beets planted monthly at Yuma, Arizona from **June 15, 1962 to June 15, 1965.**

Yield Highest average yields of both roots and sugar were obtained from beets planted in mid-October and harvested in late June or early July (Figure 2). Even with continuous beets, 41 tons of roots per acre were harvested on July 26, 1965 from the planting made October 15, 1964.

Examination of Figure 2 shows that the beets planted in October doubled in root size between mid-April and mid-July. The explanation of this occurrence is found in Table 1 which shows that beets planted in October were free of disease symptoms for at least 60 days after planting and then continued to show evidence of much less disease through the spring growing period than did the earlier plantings. Bennett et al. (2) reported that when infection by beet yellows was delayed 49 days, reduction in root weight was 22% less than when the beets were infected at the 12- to 16-leaf stage. Not only was the damage less as infection was delayed but the average daily reduction in root weight tended to decrease with the delay of infection.

If beets were to be harvested at Yuma as early as mid-March or early April, most of their development would have to be made during the previous summer and fall before growth was retarded by low temperatures in December and January. The highest average root yields obtained in March and April were from the Tune plantings which produced approximately 14 tons per acre by mid-March of the following year and 23 tons per acre in mid-April. The March and April yields from July, August and September plantings were progressively lower. When harvest of these summer plantings was delayed until Tune, their maximum yield potential was not realized because of the effects of disease (Table 1).

The possibility of extending the harvest campaign by planting beets in the winter or early spring for harvest in August does not seem promising. As shown in Figure 2, whenever the harvest was delayed until mid-August, regardless of the planting date, there was a decline in yield of sugar even though the tonnage of roots was slightly higher in some instances. In all cases the root sugar content decreased, plant growth rates were retarded and many plants died.

The 1965-1966 Experiment

Since production of maximum yields was not an objective of this experiment, planting was delayed until November 12 so that all beets and weeds could be completely removed from the plots of the 1962-1965 experiment. Apparently this precaution had little effect on the incidence of disease in this planting as is shown by comparison of the disease index readings in Tables 1 and 3. "Yellows" was observed early in March and was the most

prevalent disease. This was probably beet western yellows. Duffus (5, 6) found that beet western yellows occurs extensively in California and is caused by a virus which is persistent in the aphid. Once the insect acquires the virus, it can transmit beet western yellows the rest of its life. On the other hand, Bennett (1) showed that the beet yellows virus is semipersistent. After acquiring the virus most of the aphids in his experiments lost the ability to transmit it in 24 hours. In a few aphids the virus was able to persist 72 hours but not for 96 hours.

Curly top was observed as localized infections during March and April, but increased rapidly in July. Mosaic, probably cucumber mosaic (4), developed rapidly during May, June, and July. This disease complex was widespread by early August (Table 2).

Disease symptoms appeared to be strongly influenced by temperature since they increased rapidly with the advent of warm weather in the spring. Conversely, plants weakened by disease were apparently unable to survive the high temperatures of July and August (Table 2). The stand was materially reduced by August 5. Beets left in the field through August were nearly all dead by September.

Table 2.—Stand survival as related to disease index readings and maximum daily temperatures for sugarbeets planted November 12, 1965 at Yuma, Arizona.

	Jan. 8	Feb. 5	Mar. 5	Apr. 9	May 8	June 4	July 4	Aug. 5
Disease index[1]	0	0	2.1	3.6	4.3	4.6	5.0	7.0
Maximum temperature[2]	66	68	69	93	99	98	106	104
Percent stand[1]	100	100	98.5	92.6	91.8	91.4	88.8	69.9

[1] Data are averages of 8 replications.
[2] Ten-day average maximum daily temperature ending on date indicated.

Table 3.—Relationship of date of harvest to disease index, stand survival and production of sugarbeets planted in November following a beet free period at Yuma, Arizona in 1965.[1]

Harvest date	Disease index	Percent stand	Percent sucrose	Yields in tons per acre[2]	
				Roots	Sugar
May 23	4.3	91.8	14.4	14.72 b	2.12 b
June 6	4.6	91.4	13.8	15.94 b	2.19 b
June 21	4.8	90.1	12.6	18.87 a	2.36 b
July 9	5.0	88.8	13.3	20.18 a	2.67 a
July 20	6.0	79.5	11.9	20.03 a	2.38 ab
August 9	7.0	69.9	10.6	15.94 b	1.69 c

[1] Data are means of 8 replications.
[2] Group means followed by the same letter do not differ significantly at the .05 level.

Table 3 shows yield and sugar content of beets, along with disease index and stand when harvested at two-week intervals between May 23 and August 9. Although disease symptoms increased and stands declined slightly, yields of roots and sugar tended to increase steadily until approximately mid-July. Apparently, this was the end of the productive growing season. The two remaining harvests showed a quickening decline in stand and sugar content with corresponding loss in yield of beets and of sugar.

The incidence of disease in sugarbeets had a direct relationship to the date of planting. Whenever beets were planted in the summer or allowed to go into the summer from earlier plantings they became highly infected with disease. Beets planted between June 1 and September 15 were highly diseased by midwinter, while beets planted after October 1 were relatively free of disease symptoms until March or April.

Beets planted in June, July, or August persisted through the summer as seedlings and made some growth in the fall, but not enough to be harvested before growth was retarded by the low temperatures of December and January. These beets were relatively susceptible to frost injury and recovered very slowly in early spring. This was probably due to the stage of plant growth, the effects of disease, or a combination of these factors. Beet seedlings from the October plantings were relatively free of disease during December and January. They withstood the low temperatures well and quickly resumed growth in early spring. By mid-May, they were equal in root size to beets planted in the summer months and, for a mid-June harvest, beets planted in October produced 8 tons of roots per acre more than the plants seeded earlier.

Conclusions

Summer plantings had three distinct disadvantages: 1) They occupied the land and required cultivation and irrigation for a full year. 2) They required an intensive insect control program. 3) They became highly infected with disease which limited their yield potential, regardless of the date of harvest. However, summer plantings could be advantageous if a premium was placed on an April harvest. Experimental results indicated that root yields of about 20 tons per acre could be harvested in mid-April from beets planted the previous June if insects were controlled.

There is little probability of extending the harvest season beyond July, regardless of planting date. Insects, disease, and high temperatures appeared to be the principal factors responsible for the decline and death of mature beets left in the soil during the summer months.

Summary

US H2 sugarbeets were planted on approximately the fifteenth of each month during a three-year period. Periodically, plants were rated for disease and stand percentage, and harvested for root yield and sugar content.

In general, summer plantings were subjected to insects and disease over a long period of time and produced low yields whenever they were harvested the following spring or summer. Beets planted in the fall, preferably in October, endured the low temperatures of December and January well and made most of their growth before the disease infection increased in late spring. These plants produced good yields when harvested in June or July. Nearly all plants beyond the seedling stage at the beginning of summer died from the effect of disease, insects, and high temperatures during July, August, and September.

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Inheritance Studies with a Pollen Fertility Restorer Sugarbeet Inbred[1]

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Introduction

Genes which partially restore pollen fertility in sugarbeets, *Beta vulgaris* L. are common; however, strong pollen fertility restoration genes are scarce. In an extensive study with curly-top resistant material, Owen (6)[3] observed only one line that he considered of strong restorer genotype. He noted that normal hermaphrodite beets crossed to cytoplasmic male steriles (CMS) frequently segregated male sterile, partial fertile, and fertile offspring, but there were no lines having 100% fertile progeny. He attributed this to the fact that these pollinator lines carried sterile rather than normal cytoplasm.

In 1958 progeny of a subline of US 201-20 showed a high degree of pollen fertility when crossed with CMS. Inheritance studies involving this pollen restorer (R_f) line have been carried out and are reported in this paper.

Materials and Methods

S_2 plants of US 201 R_f were crossed with SLC 03 CMS, a BC_6 annual male-sterile tester at Salt Lake City, Utah in 1959. Subsequent Fi, F_2 and BC_X progenies were classified visually for pollen fertility.

Three S_3 lines of the same pollen restorer were again crossed to SLC 03 CMS in an effort to confirm the earlier findings. The fertility of the F_x, F_2, and Bd generations were checked visually and also microscopically by staining pollen with aceto-carmine. All generations were evaluated in a greenhouse maintained at approximately 21 C. x^2 statistics were utilized to evaluate goodness of fit to genetic ratios.

In 1964 the S_4 Rf pollinator was crossed to 19 biennial CMS inbreds from diverse sources. F_x progenies were overwintered at St. George, Utah and Salinas, California and were classified for fertility during the following summer. Because of the striking differences noted in the field, three hybrids, NB-1 CMS x R_f, CT9 CMS x R_f, and SLC 129 CMS x R_4 were grown as stecklings,

[1]Joint contribution of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, the Beet Sugar Development Foundation and the Utah State Agricultural Experiment Station. Approved as journal paper no. 856, Utah Agr. Exp. Station.

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[3]Numbers in parentheses refere to literature cited.

photothermally induced in a cold chamber at 5 C and classified for fertility in the greenhouse at 21 C during the winter of 1966. Four F_1, nine F_2 and five BC_1, R_f populations were evaluated for pollen fertility in the field at St. George in 1966.

Results

All F_1 plants of the first SLC 03 CMS x US 201 R_f, cross were vigorous fertile annuals (Table 1) and were remarkably uniform in growth habit and foliar characteristics in the greenhouse. Some plants produced great quantities of viable pollen, but there was striking variability from plant to plant and between different branches of the same plant. Careful observation showed that these plants were much more sensitive to environmental conditions than were normal hermaphrodite beets. When subjected to low temperatures or moisture stress, pollen production and dehiscence was poor.

Table 1.—Segregation of SLC 03 CMS x US 201 Rt S_2 pollinator in the greenhouse at Salt Lake City, Utah in 1959.

	No. of plants				Goodness of fit to 3:1 ratio
	Male sterile	Partial fertile	Fertile	Total	P*
F_1	0	0	113	113	
F_2 Observed	41	43	65	149	
Expected (3:1)	37.25	111.75			.50-.30
BC_1 Observed	116	117	1	234	
Expected (1:1)	117	117			.90-.80

*Probability for caluculated y" ratio.

Vigor was reduced in the F_2 generation but some individual plants appeared to be excellent pollen producers. Approximately 25% of the plants bore white empty anthers, which gave an excellent fit to a 3 fertile: 1 male sterile ratio (Table 1).

In the BC_1 generaion, growth was also vigorous and uniform, but pollen production was drastically suppressed as compared with the F_1. Only one plant was classified as completely fertile. Most of the partial fertiles bore badly shrunken yellow anthers with only a trace of viable pollen. Half of the offspring resembled the annual CMS parent. A x^2 test of pollen producers versus white anther male steriles, gave excellent agreement with an expected 1:1 ratio for monogenic inheritance.

Data from the crosses made in 1963 did not confirm the results of the earlier SLC 03 CMS x US 201 R_f cross. (Table 2). Three lines of family RB 3105 gave segregation ratios indicative of monogenic inheritance. Conversely, family RB 3104 lines showed a better fit to a 9 fertile: 7 male sterile F_2 ratio and a 1:3

Table 2.—Segregation of SLC 03 CMS X S_3 families derived from US 201 Rf, in the greenhouse at Logan, Utah in 1964.

					χ^2 Probabilities			
		No. plants[1]			F_2	Ratios	BC_1	Ratios
Family	Gen.	F	MS	Total	3:1	9:7	1:1	1:3
RB 3104-7	F_1	32	0	32				
	F_2	25	12	37	.30-.50	.10-.20		
	BC_1	12	31	43			<.01	.50-.70
-15	F_1	30	0	30				
	F_2	41	23	64	.02-.05	.20-.30		
	BC_1	68	160	228			<.001	.05-.10
-16	F_1	31	0	31				
	F_2	26	22	48	<.001	.95-.98		
	BC_1	7	25	32			<.001	.50-.70
-17	F_1	38	0	38				
	F_2	70	36	106	.02-.05	.02-.05		
	BC_1	11	34	45			<.001	.90-.95
Total	F_1	131	0	131				
	F_2	162	93	255	<.001	.01-.02		
	BC_1	98	250	348			<.001	.10-.20
RB 3105-1	F_1	12	0	12				
	F_2	41	15	56	.80-.90	.01-.02		
	BC_1	17	14	31			.50-.70	<.001
-11	F_1	38	0	38				
	F_2	21	4	25	.30-.50	<.01		
	BC_1	16	19	35			.50-.70	<.01
-13	F_1	46	0	46				
	F_2	9	1	10	.20-.30	.02-.05		
	BC_1	23	27	50			.50-.70	<.001
Total	F_1	96	0	96				
	F_2	41	20	91	.30-.50	<.001		
	BC_1	56	60	116			.70-.80	<.001

[1] F = stainable pollen with aceto-carmine and MS = white or brown anther flowers with no stainable pollen.

BC_1 ratio. Considerable variation in dehiscence and the percent stainable pollen was again noted in this material. Some plants produced abundant pollen while others bore shrunken yellow non-dehicing anthers, and had less than 50% stainable pollen.

In field plantings, F_1 restorer hybrids involving 19 biennial CMS lines produced fertile and partial-fertile offspring, but no white anther male steriles (Table 3). Performance of the individual hybrids was quite similar for the two locations. All plants were vigorous and a majority of them shed pollen profusely. At St. George the hybrids ranged from 24% to 100% fertile plants while at Salinas the fertility range was 39% to 100%. The NB-1 CMS x R_f hybrid had the lowest fertility percentage of the lines

Table 3.—Pollen fertility readings of F_1 restorer hybrids at St. George, Utah and Salinas, California in 1965.

	St. George, Utah				Salinas, Calif.			
	No. plants[1]			%	No. plants			%
Hybrid	MS	PF	F	F	MS	PF	F	F
SL 211H5 × Rf	0	5	30	86	0	24	20	45
SLC 127 × Rf	0	0	22	100	0	2	7	78
SLC 129 × Rf	0	0	18	100	0	0	5	100
SLC 128 × Rf	0	7	18	72	0	1	5	83
AI-1 × Rf	0	11	17	61	0	3	16	84
AI-10 × Rf	0	1	2	67	0	1	4	80
CT9 mm × Rf	0	1	25	96	0	7	10	59
NB-1 × Rf	0	16	5	24	0	11	7	39
C515 × Rf	0	0	2	100	0	0	17	100
F54-22-H-14 × Rf	0	1	16	94	0	1	55	98
S3317-5 × Rf	0	1	37	97	0	2	27	93
F.C. 502 Rf	0	0	2	100	0	1	5	83
S3317-14 × Rf	0	0	3	100	0	1	22	96
F.C. 503 × Rf	0	2	6	75	0	0	37	100
308HO 1 × Rf	0	1	30	97	0	1	31	97
2937 × Rf	0	0	30	100	0	0	41	100
2938 × Rf	0	5	26	84	0	0	35	100
SLC 126 × Rf	0	0	64	100	0	1	59	98
SL 0130 × Rf	0	0	55	100	0	1	41	98
Total	0	51	401	....	0	57	444	....
Average	0	2.7	21.1	88.7	0	3.0	23.4	88.6

[1]MS = white or brown shrunken anthers with no pollen, PF = partial fertile plants having yellow anthers with none or little dehiscent pollen, F = yellow anthers with abundant dehiscent pollen.

studied. At Logan 76% of the offspring for this cross were partial-fertile plants with relatively little pollen dehiscence. This same line also showed a greater tendency to resist pollen restoration than the other hybrids evaluated at Salinas.

Greenhouse readings on NB-1 CMS x R_f, CT 9 CMS X R_f and SLC 129 CMS x R_f hybrids confirmed the field readings that NB-1 CMS was a better emasculator than other CMS lines (Table 4). SLC 129 CMS and CT 9 CMS hybrids averaged over 80%

Table 4.—Fertility of F_1 restorer hybrids grown in the greenhouse at Logan, Utah in 1966.

	No. plants by upper class limits (% fertile)[1]											Total no. plants	Avg. % fertility
Description	MS	T[2]	10	20	30	40	50	60	70	80	90		
SLC 129 CMS × Rf	0	0	0	0	1	0	0	4	4	4	28	41	85
CT 9 CMS × Rf	0	0	0	3	1	1	1	0	2	7	37	52	81
NB-1 CMS × Rf	6	25	39	10	0	6	1	1	1	2	0	93	14

[1] Fertility determined by percent aceto-carmine-stained pollen at anthesis of flowers on main stem of seed stalk.

[2] T = trace, 1 or 2 stainable pollen grains per thousand.

fertility, whereas NB-1 CMS progeny tended to group around the 10% fertility class. Six plants of this cross had white shriveled anthers devoid of stainable pollen and appeared to be similar to their NB-1 CMS parent.

In the 1966 field planting, four F_1 hybrids produced 100% pollen-producing plants with varied degrees of fertility (Table 5). Consistently there were more pollen-producing plants than white anther steriles in the F_2 and vice versa for the BC generation. This suggests that a complementary type of gene action is governing the inheritance of pollen restoration. The SLC 128 CMS hybrid gave an excellent fit to a 9 fertile: 7 male-sterile ratio in the F_2 and a 1 fertile : 3 male-sterile ratio in the BC_1. Segregation of SLC 129 CMS x R_f was similar to that of the SLC 128 x R_f cross, but the F_2 fit was not as satisfactory. Segregation of CT 9 CMS x R_f and All CMS x R_f crosses gave a better fit to a 3:1 ratio than to a 9:7 ratio in the F_2. Flowever, the backcross data did not support the premise that there was only a single gene responsible for pollen restoration in these crosses.

Table 5.—**Pollen** fertility of F_1, F_2, and BC_1 generation restorer hybrids determined in St. George field planting in 1966.

Hybrid	Gen.	No. plants[1] F	MS	χ^2 Probabilities (3:1)	(9:7)
SLC 129 CMS × R_f	F_1	130	0		
CT 9 × CMS × R_f	F_1	102	0		
NB-1 CMS × R_f	F_1	131	0		
2938 CMS × R_f	F_1	191	0		
SLC 129 CMS × R_f	F_2	915	643	<.001	.02-.05
SLC 128 CMS × R_f	F_2	32	24	<.01	.80-.90
CT 9 CMS × R_f	F_2	46	19	.30-.50	.01-.02
AI-1 CMS × R_f	F_2	65	15	.10-.20	<.001
NB-1 CMS × R_f	F_2	428	163	.10-.20	<.001
308HO 1 × R_f	F_2	275	72	.05-.10	<.001
211H8 × R_f	F_2	62	40	<.001	.30-.50
S 3317-5 × R_f	F_2	351	61	<.001	<.001
S 3317-14 × R_f	F_2	54	25	.10-.20	.02-.05
2937 × R_f	F_2	565	70	<.001	<.001
2938 CMS × R_f	F_2	438	129	.20-.30	<.001
F. C. 503 CMS × R_f	F_2	102	35	.80-.90	<.001
				(1:1)	(1:3)
SLC 129 CMS × R_f	b_1	56	188	<.001	.50-.70
SLC 128 CMS × R_f	b_1	6	21	<.01	.70-.80
CT 9 CMS × R_f	b_1	49	105	<.001	.05-.10
AI-1 CMS × R_f	b_1	88	231	<.001	.20-.30

[1] Visual observation. MS = white-anther plants and F = yellow anther plants with **varied** degrees of pollen dehiscence.

Several of the other hybrids gave a better fit to a monogenic F_2 ratio than to the complementary two gene model. Three hybrids, 2938 CMS X R_f, 308HOI x R_f, and All CMS x R_f gave excellent fit to a 13:3 F_2 ratio. The observed segregation ratio in the backcross for AI- CMS x R_f, however, was <.001 probability of the 3 fertile : 1 male sterile expected ratio. The BC, generation of the other two hybrids was not evaluated.

Two crosses, S 3317-5 CMS x R_r and 2937 CMS x R_f failed to satisfactorily fit any of the expected ratios for 1, 2, or 3 factor pairs. In all cases failure to fit the complementary 9 fertile : 7 male-sterile ratio was due to an excess of plants in the fertile class.

Discussion

Several attempts (1, 2, 3, 5, 8, 9) have been made to improve upon the original model that Owen (G) proposed for the inheritance of CMS. This is because the breeding behavior of some CMS X hermaphrodite crosses could not be explained on the basis of the original premise. The isolation of a line of sugarbeets having strong pollen recovery genes made possible another approach to this problem.

In our study, the consistent segregation of more fertile plants in the F_2 and more CMS plants in the BC, tends to support Owen's (6) original hypothesis that two complementary chromosmal factors govern male sterility or fertility. However, only in the case of the annual population RB 3104, and 3 biennial crosses, SL 211H3 x R_f, SLC 129 CMS x R_f, and SLC 128 CMS x R_f, did we observe a good statistical fit to the expected 9:7 F, and 1:3 fertile : male sterile BC_1 ratios.

The reason for the plant-to-plant variation which was observed is not readily explainable. Some of it can be attributed to environmental influences such as moisture stress and low temperature. Environmental variation is demonstrated by the differences observed between the same hybrids grown in the greenhouse and in the field. Conversely, the similarity of readings made at Salinas and St. George on several hybrids shows that environment is not the complete explanation for the observed variation. Pfahler (7) noted assortive mating between four homozygous lines of corn in that pollen carrying the white endosperm allele (y) was 5-33% less effective than pollen with the yellow endosperm allele (Y). Inasmuch as an excess of fertile (pollen producing) plants was observed consistently for the F_2 and BC_1 generations of several hybrids (Table 5), there may be assortive mating in sugarbeets, wherein pollen carrying the fertile allele(s) is more effective than pollen with the male-sterile **allele(s).**

Our data supports that of other scientists (2, 4, 5, 8) that much of the inter-plant variability in pollen fertility of a segregating generation from a given cross is under chromosomal genetic control. Progeny of NB-1 CMS crossed with Type O pollinators such as SLC 129 or the reciprocal cross of SLC 129 CMS x NB-1 pollinator gives 100% male-sterile offspring. Only when both male steriles are crossed to the same restorer line, as was done in this study, did they show differences in the interaction of genetic factors. NB-1 tended to resist pollen restoration more than any of the other lines studied, which suggests that this line is a superior emasculator.

We have noted, as have others (4, 8), that non-type O lines show more variation due to environment and minor modifying genes than do the Type O pollinators. The same was true for pollen restorer crosses. The hybrids were considerably more variable in fertility than the selfed generations of the hermaphrodite pollen restorer parent.

It is apparent that extremely refined experiments will be necessary to further delineate the inheritance and interactions of cytoplasmic male sterility and pollen-restorer genes in sugarbeets.

Summary

1. An inbred carrying strong pollen restorer genes has been isolated from the variety US 201.
2. Studies of inheritance tend to confirm Owen's original premise that cytoplasmic male sterility is governed by complementary genetic factors. However, interaction between minor modifying genes and cytoplasms, and the influence of environment resulted in poor fits to classical genetic ratios.
3. NB-1 CMS is a superior emasculator tending to resist pollen fertility restoration when crossed with the 201 R_f inbred.
4. Extremely refined experiments will be necessary to further delineate the inheritance and interactions of cytoplasmic male sterility and pollen restorer genes in sugarbeets.

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Screening Sugarbeet for Resistance to Heterodera schachtii Schm.

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The cyst nematode, *Heterodera schachtii* Schm., has long been one of the most destructive pests of sugarbeets in many of the major sugarbeet growing areas of the world. Control has been largely by crop rotation. Resistance to other *Heterodera* species has been found and successfully incorporated into other crop plants, such as oats (4)[2], potato (5), and soybeans (1,2). Resistance in these crops has been largely qualitative and selection has been based on nematode cyst counts.

An apparent qualitative resistance has been found in the *Patellares* section of *Beta* (3,7). However, interspecific breeding has been extremely difficult and the incorporation of this resistance into the cultivated sugarbeet has not yet been achieved. Screening for a qualitative resistance in the cultivated sugarbeet has been carried on in the past without success. However, screening for different levels of quantitative resistance has achieved moderate success (6).

The purpose of this study was to determine if programs could be made within the *Beta vulgaris* species by selecting for different levels of quantitative resistance based on white female counts.

Methods and Materials

A great deal of plant-to-plant environmental variation for number of nematode cysts has been observed (8); therefore, the following technique was developed to reduce this variation. Instead of planting in nematode-infested soil, test plants were inoculated with surface-sterilized sugarbeet nematode larvae. The larvae were hatched and surface sterilized by a method developed by Whitney and Doney (9).

Figure 1 shows the type of containers used. These were 55 and 185 ml clear plastic vials with a hole in the bottom for drainage. The vials were filled with a dark, well aerated, sandy soil. In order to prevent growth of algae, masonite covers were placed on flats and the vials were placed in holes drilled through the tops as shown in Figure 2. Seedlings grown from surface sterilized seed were transplanted into these containers and allowed to grow from 1 to 2 weeks before inoculation. About 1,500 surface sterilized larvae were pipetted onto the soil surface of the 55

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[2]Numbers in paratheses refer to literature cited.

Figure 1.—Clear plastic vials used for nematode counting. White dots along roots are white females.

Figure 2.—Flats in which vials were placed.

ml vials, and 3,000 larvae onto the 185 ml vials, when good root growth was observed at the soil-vial interface. White females were counted about 4 weeks after inoculation.

Three separate studies were conducted in which the number of white females at the soil-vial interface was correlated with the toal number of cysts. In all three tests a correlation of greater than .90 was obtained. Thereafter, the counting consisted of white females at the soil-vial interface only.

Table I.—Mean number of white females at the soil-vial interface.

Variety or selection	Parent or source	Tests					
		615	607	608b1	608b2	608a1	608a2
590-1	S2	50.4[a]	123.9[a]	27.1[a]	138.1[ab]	30.1[a]	85.7[bc]
S2	Spreckels Sugar Co.	.....	150.5[a]	31.8[a]	178.8[ab]	9.2[b]	87.8[bc]
228-1	US 41	30.3[c]	169.8[a]	31.1[a]	141.3[ab]	15.3[ab]	94.2[abc]
US 41	USDA	42.0[abc]	187.8[a]	32.5[a]	143.2[ab]	11.0[b]	109.0[ab]
592-3	US 33	32.1[bc]	149.1[a]	28.8[a]	141.1[ab]	8.3[b]	112.8[a]
US 33	USDA	33.1[bc]	160.6[a]	33.0[a]	131.1[ab]	12.0[b]	97.2[abc]
594-2	US 22	29.1[c]	116.6[a]	29.1[a]	173.0[a]	15.8[ab]	82.7[c]
56-408	Amer. Crystal	37.8[abc]	154.4[a]	34.9[a]	129.8[ab]	12.6[b]	114.9[a]
Acc 107	Klein E mix[a]	45.8[ab]	189.8[a]	33.2[a]	108.9[b]	7.1[b]	107.2[ab]
62-9135, F1	R. Hecker	.....	189.4[a]	28.9[a]	107.3[b]	.....	.....
C5600	B. Hammond	12.7[d]	150.4[a]	29.8[a]	92.1[b]	15.4[ab]	106.4[ab]
US 15	USDA	41.8[abc]	.....	33.5[a]	157.7[ab]	10.2[b]	.....

Note: Any two means followed by the same letter are not significantly different at P = .05.

• A mixture of nematode tolerant lines from Klein E seed obtained from G. J. Curtis, Cambridge, England.

The plant materials selected for these trials were as follows: (1) several of the best sugarbeet nematode tolerant selections; (2) parents, if available, of the above mentioned selections; (3) other open-pollinated varieties of different origin having a broad genetic base; and (4) a uniform hybrid and a homozygous line for estimation of environmental error. Altogether, a total of 27 different lines were tested. Each line was tested in at least two tests, and some lines were tested in as many as six tests. Each test consisted of approximately 800 plants.

In order to evaluate quantitative resistance and compute the expected progress, an estimate of the genotypic variance is necessary. When evaluating a heterozygous population on a per plant basis, the total phenotypic variation is a combination of the environmental variation plus the genotypic variation as illustrated below.

$$\text{Phenotypic variance (Heterozygous)} = Var_e + Var_g$$

where: Var_e = the environmental variance

Var_g = the genotype variance

The total phenotypic variation of a homozygous population gives an estimate of the environmental variance as illustrated below.

$$\text{Phenotypic variance (Homozygous)} = Var_e$$

An estimate of the genotypic variance can be obtained from the difference between these two variances. An F test for the homogeneity of variance is the appropriate test for a significant genotypic variance.

Results

The mean number of white females per plant for each line in each test is shown in Tables 1, 2, and 3. In most of the tests there was little difference between the varieties for number of white female nematodes. However, there were a few tests in which significant differences were found between varieties. In no test did the selections have significantly fewer white females than their parents. On the other hand, there was a large variation among tests. Because of the nature of the tests statistical inferences could not be made" on the pooled means over tests; therefore, tests were not combined.

There was a large variety times test interaction; i.e., varieties differed in their relation to each other from test to test. However, two varieties were somewhat consistent from test to test. US 41 (Table 1) was relatively high in number of white females in all tests it appeared in. One of the check varieties (C5600) was consistently high in nematode counts in the series of tests shown in Table 2. However, it ranged from low to high in the other two series of tests (Tables 1 and 3). To measure the consistency

Table 2.—Mean number of white females at the soil-vial interface.

Variety or line	Source	Tests 708g1	708g2	708g3
F58-554H1	J. McFarlane	9.40[b]	13.64[b]	69.46[a-b]
C5600	B. Hammond	15.70[a]	18.50[a]	74.03[a]
GW 359	Great Western	11.10[b]	18.86[a]	45.95[d]
C877	Great Western	8.50[b]	14.48[b]	53.50[c-d]
C878	Great Western	11.30[b]	.....	.....
B888	Great Western	10.70[b]	13.65[b]	67.19[a-b]
B889	Great Western	9.70[b]	14.23[b]	57.87[c]
54-604-0	American Crystal	10.60[b]	11.60[b]	50.97[c-d]
60-604-0	American Crystal	10.40[b]	13.38[b]	52.70[c-d]

Note: Any two means followed by the same letter are not significantly different at P = .05.

Table 3.—Mean number of white females at the soil-vial interface.

Variety or line	Source	Tests 708d1	708d2
F58-554H1	J. McFarlane	19.31[c]	21.67[a-b]
C5600	B. Hammond	35.40[b]	27.17[a]
Tetra-Tri-Polanowice	G. Coe (European origin)	43.30[a]	25.22[a]
Budapest Poly Beta 4	G. Coe (European origin)	40.30[a-b]	25.94[a]
Poly Mono Poli-0	G. Coe (European origin)	19.20[c]	.....
Budapest Poly Beta 2	G. Coe (European origin)	25.00[c]	26.80[a]
A. J. Poli 2	G. Coe (European origin)	19.70[c]	24.59[a]
Buszcynski P Poly	G. Coe (European origin)	35.50[b]	26.01[a]
56-gH#3-M-1	American Crystal	29.50[b]	18.80[b]

Note: Any two means followed by the same letter are not significantly different at P = .05.

of varieties in relation to each other from test to test, varieties were ranked in each test and Spearman's rank correlation coefficient computed for each pair of tests. A significant ranked correlation was not found between any pair of tests.

Even though differences were observed between varieties in some tests, when the large variation among tests and the large variety times test interaction are considered, no variety consistently produced fewer females than another.

For each test, the total environmental variance and the total phenotypic variance for the heterozygous populations were computed and tested for a significant genotypic variance (Table 4). In no test was there a significant total genotypic variance. In addition to the total variances, the individual varieties in each test were tested for a genotypic variance. There were 7 varieties that exhibited a significant genotypic variance in one test. Even though each variety was tested several times, no variety exhibited a significant genotypic variance for the number of white females in more than one test.

To test the reliability of these results a selection scheme was set up in a population of 1,300 plans of several heterozygous varieties. From this population 215 plants that had less than 10

Table 4.—Estimated total phenotypic variances for white female counts.

Test	Homozygous Pop'n (Vare)	Heterozygous Pop'n (Vae + Va_g)
607	8,254	6,063
608a1	178	211
608a2	3,077	2,642
608b1	350	277
608b2	9,208	11,222
708g1	112	94
708g2	1,409	1,081
708g3	**124**	**104**
708d1	288	284
708d2	231	377

Var_e — environmental variance.
Var_g — genotypic variance.

white females at the soil-vial interface were selected. These were classed as resistant. Another group of 75 plants with high nematode counts was also selected from this populaion and classed as susceptible. These plants were tested in two more successive tests. At the conclusion of the third test the selection group classed as resistant had almost the same number of white females per plant as the selection group classed as susceptible (Table 5).

Table 5.—Selection for nematode resistance from an initial population of 1,300 plants.

Test	No.	X nematode count of Resistant selections	No.	X nematode count of Resistant selections
1	215	5.98	75	56.60
2	207	63.50	73	81.64
3	203	50.60	73	49.60

Discussion and Conclusions

These results indicate that there is either little or no resistance to the sugarbeet nematode in the cultivated sugarbeet; or, the environmental variation involved in this method of selection is too. great to detect small differences. This indicates that very little progress can be expected by selecting for a quantitative resistance based on this technique.

Many attempts have been made to reduce this large environmental variance. The technique developed in this study was one attempt. Different methods of inoculation have been studied by the authors. One rather important factor contributing to this variation is the number of available fibrous roots. When inoculation takes place the number of available fibrous roots may vary greatly from plant to plant as a result of genetic and environmental variation. This variation may be reflected later in the white female counts.

An estimate was made in one test of the number of observed roots at the time of inoculation with the later white female counts. A significant correlation of .34 was obtained.

If the more vigorous seedlings have more available fibrous roots for nematode invasion at the time of inoculation, greater numbers of white females may be observed on the more vigorous seedlings. Thus, by selecting plants that have fewer white females, selection may then be for the least vigorous seedlings. However, if the plant to plant variation in amount of fibrous roots is largely environmental, selection for fewer white females would not change the seedling vigor.

Summary

A technique was developed for counting white females of *Heterodera schachtii* Schm. on the soil-vial interface without disturbing the roots. Twenty-seven sugarbeet varieties and selections from different sources of origin were tested for a genotypic variance and number of white females.

Differences between varieties were found in some tests, but these differences were not consistent from test to test. Selections did not have significantly fewer white females than their parents. There was a large test times variety interaction and test variance. Little genotypic variance for number of white females was observed.

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Relative Incidences of Beet Yellows and Beet Western Yellows Viruses in the Salt River Valley of Arizona, 1965-1968

E. G. RUPPEL[1]

Received for publication October 3, 1968

Visual symptoms in the field usually are inadequate criteria for distinguishing between sugarbeets infected with the beet yellows virus (BYV) and beet western yellows virus (BWYV). Therefore, concurrent with monthly disease surveys (6)[2], samples collected from yellowed plants were indexed in the greenhouse to determine the identity, relative incidences, and epidemiology of the two viruses in central Arizona. This study was begun 1 year before the first commercial acreage of beets was grown for sugar and was extended through two commercial campaigns.

Methods

Leaves from sugarbeets with typical virus yellows symptoms were collected monthly from widely scattered fields throughout central Arizona. The samples were washed thoroughly and placed in flasks of water in separate insect-proof cages. Aviruliferous green peach aphids *[Myzns persicae* (Sulz.)] were allowed to feed on the diseased leaves for about 24 hours and then transferred (10 or more per plant) to healthy seedlings of *Capsella bursa-pastoris* (L.) Medic, *Chenopodium capitatum* (L.) Asch., *Beta vulgaris* L. 'S 301-H' (sugarbeet), and *Sonchus oleraceus* L. The technique was essentially the same as described by Duffus (4). BYV induces distinctive symptoms in C. *capitatum* but does not infect *C. barsa-pastoris,* whereas BWYV induces symptoms in C. *bursa-pastoris* but not in *C. capitatum* (1, 3). *S. oleraceus* was included to detect the beet yellow stunt virus. The latter virus induces symptoms in *C. capitatum* that are milder but similar to those induced by BYV (Duffus, personal communication). This virus was not encountered throughout this study. After aphids had fed for 48 hours, they were killed with an aphicide and the plants were placed on the greenhouse bench for observations. Periodic sprays with insecticide and frequent fumigations

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[2] Numbers in parentheses refer to literature cited.

of the greenhouse prevented contaminations from stray aphids. A total of 724 samples were indexed between December 1965 and June 1968.

Results

The results of the indexings (Figure 1) indicated that beet western yellows was the most prevalent yellows disease in central Arizona from 1965 to 1968, inclusive. In 3 years, BYV was not detected until April or May and only in mixed infections with BWYV. Although the overall recovery of BYV was low as a percentage of total samples, in 1966, 1967, and 1968 the virus was recovered from 42%, 58%, and 27% of the June samples, respectively. Thus, once the virus was introduced into the beet fields, secondary spread occurred quite rapidly.

Discussion

The prevalence of BWYV over BYV in the Salt River Valley agreed with an earlier study by Coudriet (2). However, he recovered BYV in November, whereas I first detected the virus in late spring. Nevertheless, Coudriet obtained the greatest percentage of BYV-infected plants in April. Apparently, BWYV is more prevalent than BYV in other sugarbeet areas in western United States (5).

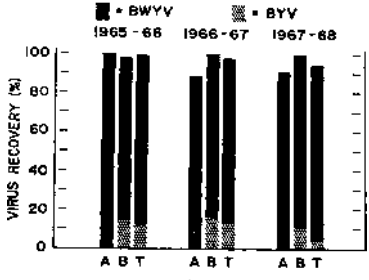

Figure 1.—Percentage recovery of beet yellows virus (BYV) and beet western yellows virus (BWYV) from field sugarbeets showing foliage yellowing in central Arizona from November through June 1965-66, 1966-67, and 1967-68; A, indexes made from November through February, B, indexes made from March through June, and T, total results.

The late detection of BYV might be explained in two ways. First, if a low percentage of incoming aphids were carrying the virus, the few plants that subsequently would have become infected might have escaped **the monthly samples. Then,** as second-

ary spread occurred there would have been a greater probability that BYV-infected plants were included in the indexes. Bennett (1) indicated that the perennial Australian saltbush *(Atriplex semibaccata* R. Br.) might be the source of BYV inoculum in Arizona. This species is a host of BYV, but only rarely does one find alate green peach aphids on these plants. Nevertheless, the relatively few aphids that begin to feed on diseased saltbush could acquire the virus and carry it to the beet fields. It also is conceivable that the primary inoculum (reservoir hosts) of BYV were located some distance from the commercial sugarbeet areas. BYV is non-persistent in the aphids. That is, most aphids lose their ability to transmit the virus within 24 to 48 hours after acquisition (1). Thus, the aphids would have to establish a succession of BYV-infected host plants until they were in close proximity to the beet fields. Such a succession of infected plants would take time and could account for the tardy occurrence of BYV in the spring (2).

BWYV and BYV are capable of inducing serious losses in sugarbeets; losses induced by simultaneous infection with both viruses are additive (5). The widespread distribution and prevalence of BWYV in Arizona, the large host range of the virus (3,5), and the virus-vector relationships of BWYV (3) make control exceedingly difficult. However, if measures can be taken to prevent widespread and early occurrence of BYV in commercial beet fields, disastrous additive losses could be avoided. Since the beet itself apparently is the chief reservoir host of BYV, the maintenance of a beet-free period between the harvest of one beet crop and the planting of a succeeding beet crop is strongly recommended (1). Destruction of escaped beets, beets that resprout after harvest operations, and perhaps, saltbush also should reduce reservoirs of primary inoculum.

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Effect of Phenolic Acids on Alpha-Amylase in Vitro, and Early Growth of Sugarbeet[1]

J. M. SEBESON[2], EARL MITCHELL[3], AND F. W. SNYDER[2]

Varner, et al. (4)[1] have reported an in vitro procedure for measuring a-amylase activity upon starch solutions. The reserve energy for the germinating sugarbeet embyro is the starchy endosperm or perisperm. Therefore, at least one enzyme would be required to hydrolyze the starch into a utilizable form. Juliano[5] has extracted from sugarbeet seeds (germinated four days) and identified by means of gel electrophoresis the enzyme a-amylase.

Since 1957, a number of potentially inhibitory phenolic acids have been isolated from sugarbeet fruits and identified. Five of those reported, caffeic (1,2), ferulic (1,2), gallic (3), p-hydroxybenzoic (1,2), and vanillic (2) acids were evaluated for their inhibitory effect on a-amylase activity in vitro. An attempt also was made to ascertain the degree of inhibition of these phenolic acids on germination of seeds excised from the fruits of sugarbeet.

Methods and Materials

The a-amylase solution from barley and the sodium acetate buffer, iodine reagent, and starch solution were prepared according to the methods of Varner, et al. (4).

Aqueous solutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}M caffeic, ferulic, gallic, p-hydroxybenzoic, and vanillic acids, and 10^{-2}M, gallic acid were used. The latter concentration was unattainable with the other acids because of their insolubility in water.

The incubation procedure was as follows: To 0.1 ml of a-amylase (containing 10 mg protein/ml), add 0.1 ml of phenolic acid, then add 0.8 ml of sodium acetate buffer (pH 4.82) and incubate at 25 C for 60 minutes. The phenolic acid was omitted from the control.

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For the assay, 0.1 ml of the test aliquot was removed from the incubation medium and diluted to 1 ml with sodium acetate buffer. The reaction was started by adding 1 ml of starch solution and allowed to run at room temperature for exactly 4 min. The reaction was stopped by adding 1 ml of iodine reagent. After adding water to make a final volume of 8 ml, the contents of the test tube were thoroughly mixed. The optical density was determined in a colorimeter at 620 mu.

The computations of ΔA_{starch}, units of activity, and the percent of activity were determined as follows:

$$\Delta A = A_b - A_s$$

where A_b = absorbance of the starch-iodine blank
and A_s = absorbance of the sample

$$U = \frac{\Delta A \times V}{a \times t}$$

where U = units of activity
V = volume (in this case 1 ml)
a = aliquot (0.1 ml)
t = time in minutes

$$P = \frac{Ui \times 100}{U_e}$$

where P = percent of activity
Ui = units of activity with enzyme plus inhibitor
U_e = units of activity with enzyme

The activity of the pure enzyme was 2-85 or 100%. The percent of inhibition of the phenolic acids was assumed to be the percent of activity subtracted from 100%.

The influence of calcium ions and dithiothreitol on the activity of a-amylase in the presence of 5×10^{-4} M gallic acid was determined. Crystalline a-amylase was incubated either with or without gallic acid. The assay was for 1 min. The assay medium consisted of 0.1 ml enzyme solution (25 mg of protein) and 0.9 ml of one of the following buffer solutions:

1. Control-O.OOIM acetate buffer with 0.01M $CaCl_2$ pH 4.8.
2. High Calcium-O.OOIM acetate buffer pH 4.8 with 1.0M CaCU.
3. Dithiothreitol—0.001 M acetate buffer pH 4.8 with 0.01M $CaCl_2$ and 0.2M dithiothreitol.

Sugarbeet seeds, excised from the fruits, were placed on a filter paper in a Petri dish and the solution of phenolic acid was added. Concentrations used were 10^{-3} and 10^{-4}M, except caffeic acid did not dissolve completely at the 10^{-3} concentration at room temperature. Thus, the caffeic acid at the higher concentration was a saturated solution and at the lower concentration it was

diluted ten-fold. The limited supply of excised seeds permitted only three replications of 10 seeds each for the higher concentration and two replications of eight seeds each for the 10^{-4} M treatments. Root lengths of the seedlings were measured after 5 days.

Results

The data (Table 1) indicate that a-amylase activity in vitro is inhibited most strongly by gallic acid. In the range of 10^{-3} to 10^{-5} M concentrations, caffeic and p-hydroxybenzoic acids were intermediate in inhibition, while ferulic and vanillic acids were least inhibitory.

Table 1.—Effect of five phenolic acids on a-amylase activity in vitro.

Conc. acid	$A_{620\ \mu}$	$\Delta A_{620\ \mu}$	Units	Percent act.	Percent inhibit.
10^{-3}M Gallic	1.35	0.13	0.32	11.2	88.8
10^{-3}M Gallic	1.35	0.13	0.32	11.2	88.8
Ferulic	0.85	0.63	1.57	55.0	45.0
Vanillic	0.89	0.59	1.47	51.6	48.4
Caffeic	0.98	0.50	1.25	43.8	56.2
p-Hydroxy benzoic	1.19	0.29	0.68	23.8	76.2
10^{-4}M Gallic	1.35	0.13	0.32	11.2	88.8
Ferulic	0.76	0.72	1.80	63.1	36.9
Vanillic	0.78	0.70	1.75	61.4	38.6
Caffeic	0.99	0.55	1.37	48.0	52.0
p-Hydroxy benzoic	0.93	0.55	1.37	48.0	52.0
10^{-5}M Gallic	0.85	0.63	1.57	55.0	45.0
Ferulic	0.72	0.76	1.90	61.7	38.3
Vanillic	0.77	0.71	1.77	62.0	38.0
Caffeic	0.80	0.68	1.70	59.6	40.4
p-Hydroxy benzoic	0.82	0.66	1.65	57.8	42.2
10^{-6}M Gallic	0.71	0.77	1.92	67.3	32.7
Ferulic	0.65	0.83	2.07	72.5	27.5
Vanillic	0.52	0.96	2.40	84.2	15.8
Caffeic	0.65	0.83	2.07	72.5	27.5
p-Hydroxy benzoic	0.71	0.77	1.92	67.3	32.7
Control	0.34	1.14	2.85	100.	
Blank	1.48				

The marked inhibition of a-amylase activity by gallic acid was not reversed by calcium ions (required for enzyme stability) or by dithiothreitol (protects the SH groups). The % inhibition versus incubation time curve is given in Figure 1.

The effect of the five phenolic acids on germination and growth of the intact sugarbeet seedlings was much less striking. Root lengths were variable but roots of some of the treated seedlings were long and appeared to be uninhibited by the phenolic acids.

We have estimated the concentration of gallic acid in sugarbeet fruits to be in the range of 10^{4} M. This concentration of gallic acids inhibited a-amylase activity in vitro by 89%. Concentrations of the other acids in the fruits have not been estimated.

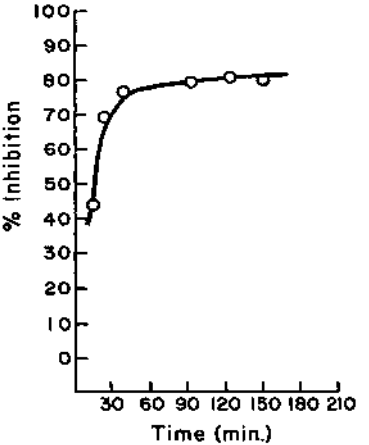

Figure 1.—Inhibition of alpha-amylase by gallic acid when incubated with 5×10^{-4}M gallic acid plus 0.01 M Ca^{++} and 0.01 M dithiothreitol.

Discussion

The presence of substances in sugarbeet fruits which delay or inhibit germination of the seed, the identification of a number of these potentially inhibitory substances, and the development of the test for a-amylase activity in vitro provide a system for attempting to evaluate the importance of some of these substances in affecting germination and early growth.

The kinetics of inhibition by the phenolic acids in the in vitro system have not been investigated. However, it is possible that these phenolic compounds bind irreversibly with the enzyme, perhaps at the site of activity. Adding calcium and dithiothreitol did not alter the inhibition.

The inherent variability in the length-growth of sugarbeet roots complicates any statistical analysis of data when the treatments do not cause large differences in growth. On the basis of average length of roots, the phenolic acids at concentrations of $1O^{3}$ M retarded root growth more than at 1CH M. Although the variable root growth precludes any statistical significance, the trend may be indicative of increasing toxicity with increasing concentration.

Rate of germination and subsequent root growth of sugarbeet were only slightly retarded, but a-amylase activity in vitro was markedly inhibited (89%) by 10^{-3} M gallic acid. This disparity is very interesting, particularly since repeated efforts have been made to demonstrate a significant inhibitory effect of gallic acid on germination and subsequent growth. How can the observations be reconciled? If the 89% inhibition of a-amylase activity occurs in the seed, it seems doubtful that sufficient hydrolyzed material would be available to essentially permit as rapid growth as in a water-control. If this did occur, it would suggest a very large excess of a-amylase activity for the normal situation. Perhaps the degree of inhibition imposed by gallic acid (as well as the other phenolic acids) on a-amylase in vitro does not occur in the intact seed. Possibly the cells contain a mechanism which would detoxify these phenolic acids and thereby drastically reduce the concentration of the inhibitory substance.

Should some such mechanism as the above account for the above noted disparity, the dangers inherent in extrapolating the effect of substances on isolated enzyme systems to intact organisms become very apparent. The effect on the intact organism, therefore, must be determined directly through experimentation on that organism.

Summary

The effect of caffeic, ferulic, gallic, p-hydroxy benzoic, and vanillic acids on a-amylase activity in vitro at molar concentrations of 10^{-3} to 10^{G} was determined. Gallic acid was most inhibitory. Caffeic and p-hydroxybenzoic were intermediate, while ferulic and vanillic acids were least inhibitory in the range of 10^{-3} to 10^{5} M. At 10^{-4} M, they ranged between 37 and 89%; at 10^{-5} M between 38 and 45%, and at 10° M between 16 and 33%. and at 10^{6} M between 16 and 33%.

Sugarbeet seeds, excised from the fruits, were placed in 10^{3} and 10^{4} M solutions of the five acids. Root growth after 5 days was measured. Root lengths tended to be shorter at 10^{3} than at 10^{4} M, but not statistically significant. The marked inhibition of the acids on a-amylase activity in vitro does not occur in the intact seed during germination and subsequent growth.

Acknowledgment

We wish to thank J. E. Varner, MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Mich., for supplying the a-amylase enzyme and valuable advice.

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Relation of Certain Amino Acids to Other Impurity and Quality Characteristics of Sugarbeet[1]

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Received for publicaiton June 3, 1969

In 1966 and 1967 we conducted studies to determine the levels of certain amino acids of sugarbeet with different genetic backgrounds that were exposed to different nitrogen fertility levels. We studied the relationships of these amino acids to several impurity compounds and correlated them with each other and with some yield factors to find information as to where efforts for quality improvement might be most effective.

Sugarbeet quality is a general term intended to describe the relative processing characteristics of beets or the ease and completeness of sucrose recovery from the raw product. Anything that interferes with recovery of white sugar is considered undesirable. The increased use of nitrogen fertilizers in the production of sugarbeet emphasizes the importance of well coordinated chemical-genetic and soils studies as they pertain to processing quality.

Recent emphasis in studying quality has been on thin juice purity and individual impurity components. Currently, thin juice purity in experimental materials is determined on laboratory thin juice by the method developed by Brown and Serro (l)[4] and modified by Carruthers and Oldfield (2). This phosphated thin juice does not differ from factory second carbonation juice with respect to major impurity components. Carruthers and Oldfield (2) report that 70% of thin juice nonsugars are potassium and sodium salts, amino acids and betaine, and that they are not present in equal quantities.

The free amino acids remaining in the thin juice are an important source of nonsugars. Carruthers and Oldfield (2) state that one-half of the nitrogen in purified juice originates as amino

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*Numbers in parentheses refer to literature cited.

acids, with 50 to 80% of this from glutamine which has both an amide and amino nitrogen group. Rorabaugh and Norman (11) rank the amino acids second to chloride and carbonate salts in deleterious effect on sucrose crystallization rate. The amino acids in peptides or proteins are almost entirely removed in the juice purification leaving the free amino acids which carry through to the thin juice. These free amino acids are estimated by Silin (12) to be about 0.2% of the total beet (fresh weight basis).

Amino acids have been measured in total and also individually (12) in various beet juices, and 14 have been reported to be in sugarbeet. These individual quantitative determinations have been made by reflectance density of amino acid spots on ninhydrin developed paper chromatograms. A quantitative study of the amino acids in sugarbeet has not previously been made on an amino acid analyzer. One of the purposes of the research leading to this work has been to analyze quantitatively for individual amino acids by using an amino acid analyzer, and to determine the effect of genotype and nitrogen fertilization on the quantity and proportion of the amino acids.

Glutamine accounts for 50 to 80% of all nitrogen originating from amino acids (2). Glutamine is readily changed to pyrrolidone carboxylic acid (PCA) and glutamic acid (to a lesser extent) during juice purification and sucrose crystallization, so that it represents 10 to 17% of nonsugars in the molasses. PCA is particularly important in the later stages of the factory process (2). Since the amino acids are such an important impurity component, it is necessary that their intra-and inter-relationships with other impurity components be established along with the effect of genotype and nitrogen fertility.

The case against glutamine, PCA, and glutamic acid has two facets, since they are not only major contributors to sugar loss into the molasses, but also contribute to processing difficulties caused by deamidation of glutamine. This lowers the buffering capacity of juice and its alkalinity. This is true for anything that increases the ratio of certain nitrogenous compounds to inorganic cations; because with such a ratio, the available alkalinity in the clarified juice is inadequate for achievement of low lime salts, and maintenance of pH in the sugar end unless large amounts of soda ash are added. The addition of soda ash increases molasses.

Plants are able to synthesize 18 amino acids and two amides and to form the constituents common to most proteins. About one hundred other amino and imino acids have limited distributions in high plants, but for the most part are not incorporated

into proteins. Undoubtedly, ammonia forms the major, and possibly the only, inorganic nitrogen compound utilized directly for amino acid biosynthesis. Recent studies, using N^{15} —labeled forms of ammonia, nitrate and elementary nitrogen, have confirmed that nitrogen rapidly enters glutamic acid and glutamine molecules. Aspartic acid, alanine, arginine and other amino acids are more slowly labeled irrespective of the type of inorganic nitrogen nutrient supplied. A kinetic treatment of N^{15}-ammonia incorporation into « -amino groups of free and protein-bound amino acids has established that glutamic acid, and apparently glutamine, form the only primary products of assimilation. Alanine and aspartic acid have been shown to be secondary products of nitrogen incorporation.

Materials and Methods

Our study consisted of laboratory and statistical analyses of 36 yield, quality, and leaf component characters from 12 genetic populations at three nitrogen fertility levels over a two-year period. The experiments were grown under irrigation at the Colorado State University Agronomy Research Center in 1966 and 1967. In both years planting was done April 10-15, and harvesting on October 10-15.

In 1966, three populations were grown in a split-plot design with 10 replications. Thin juice amino acid determinations were made only on the first five replications. In 1967, 10 replications of 11 different genetic populations were grown, two of which were the same ones grown in 1966. In 1967, we determined eight characters on all 11 populations and 28 characters on three populations (Table 1). In both years nitrogen fertility treatments were main plots and populations were subplots.

The experimental areas each year had a uniform application of 20 pounds of actual nitrogen and 100 pounds of phosphorus pentoxide (P_20_5) prior to fall plowing barley stubble. Nitrogen as ammonium nitrate (NH_4N0_3) was applied the following spring and harrowed into the treatment plots. Nitrogen treatments in 1966 were 0 pounds, 100 pounds (preplant); and 250 pounds (100 pounds, preplant, and 150 pounds, side dressed, after thinning but prior to July 14). The only difference in 1967 was that the middle treament was 125 instead of 100 pounds. The treatment without nitrogen was clearly nitrogen deficient all season; the 100 and 125 pound application of nitrogen in both years resulted in deficiency symptoms just prior to harvest; the 250 pound application showed no deficiency symptoms.

Three populations were grown in 1966, two F_t hybrids and GW 359-52R (an open pollinated multigerm former commercial variety). One of the Fi's and GW 359-52R were included in the

1967 experiment along with nine other F_1 inbred and special populations.

In both 1966 and 1967, we determined root yield and sucrose content on the roots from which phosphated thin juice was later recovered (at time of purity determination). This thin juice, according to Carruthers and Oldfield (2), is equivalent to the factory second carbonation juice; it receives no further purification and the processor must contend with all remaining juice impurities in the extraction and refining process. We determined impurity components on this thin juice and did a complete analysis

Table 1.—Characters determined and their units.

1966		1967	
Roots		Roots	
Plot weight	kgs/size plot	Plot weight	kgs/size plot
Sucrose	%	Sucrose	%
Recoverable sucrose	kgs	Recoverable sucrose	kgs/size plot
Pressed Juice		Thin Juice	
Conductivity	millimhos/cm[2]	Purity	%
		Potassium	mg/100ml
Thin Juice		Sodium	mg/100ml
Purity	%	Total nitrogen	mg/100ml
Potassium	mg/100ml	Betaine[2]	mg/100ml
Sodium	mg/100ml	NO3 nitrogen	mg/100ml
Total nitrogen	mg/100ml	Amino nitrogen[2]	mg/100ml
8 amino acids[1] (asp, glu, gly, ala, val, ileu, leu, lys).	μ moles/ml	9 amino acids[2] (asp, glu, gly, ala, val, ileu, leu, tyr, lys).	μ moles/ml
		Leaves (Dried)[3]	
		Copper	mg/100g
		Cobalt	mg/100g
		Calcium	mg/100g
		Magnesium	mg/100g
		Iron	mg/100g
		Nickel	mg/100g
		Leaves (Fresh)[2]	
		11 amino acids (asp, ser, glu, pro, ala, val, ileu, leu, tyr, phe, lys)	μ moles/g
		Fresh root (Pulp)[3]	μ moles/g
		12 amino acids (asp, glu, gly, ala, val, ileu, leu, tyr, phe, lys, his, arg)	

[1] Determinations made on reps 1 through 5.
[2] Determinations made only on populations 1, 2 and 6.
[3] Partial analysis made on 1 to 4 reps of populations 1, 2 and 6.

for the free amino acids using the Technicon Amino Acid Analyzers. In 1967, we prepared samples for amino acid analyses from fresh leaves and roots as soon after collection as possible. We used acid digested dried leaf samples for the determination of several metallic ions. We collected leaf samples just prior to root harvest. We selected the first mature, fully expanded leaves, when going from the inner part of the crown to the outer part. There are usually several leaves of this type on each plant.

In each plot we sampled and pooled one leaf from each of 20 plants which helped compensate for sampling errors which were inherent in the technique. The leaves were carefully stacked, tip to tip and petiole to petiole, and placed in a plastic bag with an identification tag. A transverse center section was removed from each sample for the amino acid analysis. The remaining portions of each sample were dried for metal analyses. A complete list of the determinations made on the different tissues is outlined in Table 1. Table 2 lists the populations used in 1966 and 1967, along with some of the characteristics of the populations.

Table 2.—Populations used in 1966 and 1967 and some of their characteristics.

	Year and population	Characteristics
1966		
1.	GW 359-52R	Open pollinated commercial multigerm variety adapted in the Colorado plains; high yield, relatively high sucrose and thin juice purity.
2.	52-305CMS × 54-458,F1	Single cross hybrid; relatively low sucrose and thin juice purity; high thin juice nitrogen.
3.	52-430 × 52-307,F1	Single cross hybrid; relatively low thin juice nitrogen.
1967[1]		
1.	52-430 × 52-307,F1	Same as population 3 in 1966.
2.	52-305CMS × 52-407,F1	Single cross hybrid; low sucrose and purity; high thin juice nitrogen.
3.	52-430 × 54-346,F1	Single cross hybrid; high sucrose and purity.
5.	A59-2	High glutamic acid selection from American Crystal Sugar Co.
6.	GW 359-52R	Same as population 1 in 1966.
7.	52-305CMS	Inbred used in F1's.
8.	52-430	Inbred used in F1's.
9.	52-407	Inbred used in F1's.
10.	54-346	Inbred used in F1's.
11.	34	Inbred; curly top resistant. bolting resistant.
12.	Ovana	Open pollinated white fodder beet.

[1] Population 4 in original planting plan was deleted from study because of poor emergence and stand.

[6]Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

The sucrose content was determined using the standard Sachs Le Docte cold digestion method. Recoverable sucrose was calculated using an equation developed by the Great Western Sugar Company (7). Plot weight was determined in 1967 on a single row 19-foot plot bordered on each side by a uniform competitor, in 1966 on second row of 19-foot four row plots. Conductivity was determined on pressed juice by using a solubridge apparatus that measured conductance in millimhos/cm. Purity was determined on phosphated thin juice (2). The total nitrogen determination was based on the method reported by Payne *et al.* (8). Quantitative determinations of betaine were made by a procedure described by Focht *et al.* (3), modified by Payne *et al.* (9).

We determined sodium and potassium on a Baird-Atomic flame photometer. An atomic absorption spectrophotometer (Perkin-Elmer Model 290) was used to determine the other metallic ions in the samples. These metal analyses, with the exception of calcium and magnesium, were carried out according to the procedures outlined in Perkin-Elmer's Analytical Methods for Atomic Absorption Spectrophotometry (1966), as modified by Harrison and Andre (6).

Leaves for amino acid analyses were harvested on a plot basis and the samples were prepared immediately after harvest. A 25 g transverse section cut from the center of the stacked leaves was placed in 100 ml of 10% sulfosalicylic acid solution (w/v) and ground for 5 minutes in a Waring blender. The sulfosalicyclic acid denatured the proteins present in the sample. After grinding, the samples were allowed to stand until the liquid layer separated from the foam.

A 10 ml sample was withdrawn from the liquid layer with a pipette and centrifuged for 10 minutes. This sample was adjusted to a pH of 2.0 with 40% NaOH. The sample could have been analyzed at once or frozen (at -20°C) for later analysis. Frozen samples were warmed to room temperature before being placed on the amino acid analyzer. Any sediment in the sample was removed by centrifugation before being placed on the columns. Depending on the sample, 0.5 to 1.5 ml was used for the analysis. Norleucine was originally used as the internal standard for each chromatogram but our samples contained dihydroxyphenyl alanine (DOPA) which affected the area of the norleucine peak. For an internal standard we used L-a-amino-/3-quanidinopropionic acid. This internal standard was made by taking a 2.5 micromole/ml solution of L-a-amino-^-quanidinopropionic acid in 0.0IN HCI and adjusting the pH to 2.0. Ten ml of this solution plus 5 ml of 62.5% sucrose were placed in a 25 ml volumetric flask and diluted to volume with 0.0IN HCI.

The samples were chromatographed on a two-column Technicon Amino Acid Autoanalyzer. The 140 cm. X 6 mm. columns were filled with Technicon's type B, 8% cross-linked sulfonated resin beads. The colutnn temperature was maintained at 56.6° C. Gradient buffers, ranging in pH from 2.875 to 5.00, were pumped through the columns, along with the sample, under a pressure of 250-300 psi for 21 hours. Before analysis of a sample, three or four duplicate runs were made using 0.2 ml of Technicon's 18 amino acid standard solution plus 0.2 ml of the internal standard on each column. The 18 amino acid standard was diluted from 2.5 micromoles per ml to 1.0 micromole per ml by using 2.0 ml of the standard, 2.0 ml of 0.01 N HC1 and 1.0 ml of 62.5% sucrose solution. The areas were calculated on the standard curves, and the average area for each amino acid was used to compute the amount of the individual amino acids found in the samples by comparison to the standard areas (13). The internal standard (0.2 ml) was also used on each column with each sample analyzed, so that adjustments could be made for any variation betw7een analyses due to possible changes in the sensitivity of the color producing reagents. Some changes (4, 10) were made in the procedure along with the arranging of the modules so that two 21-hour analysis could be run simultaneously (5).

One of our objectives was to study the individual amino acids in beets of different genetic backgrounds while exposed to different fertility levels. In order to get a measurable amount of some of the other amino acids and keep the glutamic acid curve on the paper, the analyzer worked best under the conditions of concentration and pH described above for a single run. By this procedure we were able to study a primary amino acid product and some secondary amino acid products of nitrogen assimilation.

The autoanalyzer system was modified so that the ninhydrin and hydrazine sulfate were kept separate until they had passed through the proportioning pump. They were completely dissolved in each other in the first mixing coil in discrete units separated by nitrogen. In like manner, the sample was mixed in this solution in a second mixing coil before going through the reaction bath where it was heated to 90°C for approximately 24 minutes for color reaction to take place.

This procedure determined the following amino acids in micromoles per gram of fresh leaf: aspartic acid (asp), threonine (thr), serine (ser), glutamic acid (glu), proline (pro), glycine (gly), alanine (ala), valine (val), isovaline (ival), cystine (cys-S-S-cys), methionine (met), isoleucine (ileu), leucine (leu), tyrosine

(tyr), phenylalanine (phe), ornithine (orn), lysine (lys), histidine (his), tryptophan (try) and arginine (arg). Glutamine and asparagine were also present but not measurable. Unknown amino acids with peaks appearing between alanine and glycine and between ornithine and lysine are being studied. Ammonia was not measured.

A composite root pulp sample was prepared on a plot basis from the individual beets with a rasp. A sample was taken from the crown to the tail of each individual root in the plot, and the composite sample was mixed before taking out a 25 g sample of the pulp which was immediately placed in 100 ml of 10% sulfosalicylic acid solution and ground for 5 minutes. The ground root sample was then treated in the same way as the leaf samples. The stored samples were brought to room temperature and centrifuged before a 1 ml sample was withdrawn and analyzed on the Technicon amino acid analyzer. Micromoles of amino acids per gram of root sample were calculated against standards as before.

The thin juice was prepared according to Carruthers and Oldfield (2). The thin juice was adjusted to a pH of 2 and the samples were frozen until they were put on the amino acid analyzer. A thin juice sample of 0.5 ml was chromatographed along with the internal standard. The results were compared to amino acid standards and reported as micromoles per milliliter.

Results

The experimental results will be presented for each year, and relationships between years will be injected wherever pertinent. Also, discussion necessary to maintenance of continuity will be included with the results.

Year 1966

Means for plot characters and thin juice characters in 1966 along with multiple range tests for differences among all means are shown in Table 3. The hybrid 52-430 x 52-307, F, (pop. 3) had relatively low root yield and was apparently unable to benefit from the additional nitrogen in the 250 pound per acre treatment. The high rate was about 150 pounds in excess of the commercially recommended rate. This same hybrid declined in sucrose with increasing nitrogen but attained its highest purity at the 100 pound nitrogen rate. Hence, this genotype had a considerably different nitrogen response from the commercial variety (pop. 1) and the other hybrid (pop. 2). Populations 1 and 2 were rather typical in their nitrogen response. Recoverable sucrose was maximum at 100 pounds of nitrogen. In populations 1 and 2 the additional root yield at 250 pounds of nitrogen was more **than** offset by lower sucrose and purity.

Among the thin juice impurity components in hybrid population 3, we found that potassium and sodium were not much different from populations 1 and 2. However, the total thin juice nitrogen at 250 pounds of nitrogen per acre was lower than in populations 1 and 2. In fact, total nitrogen did not increase with the additional 150 pounds of nitrogen. This F, genotype was not capable of utilizing the additional nitrogen which functioned more as a repressor or inhibitor than growth stimulator. This peculiarity in total thin juice nitrogen of population 3 was partly a reflection of the amino acids in the thin juice. In the case of population 3 there was no significant change in any of the amino acids in going from 100 to 250 pounds of nitrogen. The absence of change may mean that the nitrogen incorporation into the secondary products was deterred or that the process was slowed down. The amount of glutamic acid reported in thin juice is not the amount of glutamic acid in the root, because at the pH of the sample, and with time, some glutamine changes to pyrolidone carboxylic acid (PCA) and, to a lesser extent, to glutamic acid. Also, the change of asparagine to aspartic acid goes on under these conditions to a minor extent. In populations 1 and 2 there was a marked increase in all eight amino acids from both nitrogen increases. This in turn was reflected in the population means where population 3 never had a higher content of any amino acid than the other two populations. Amino acid quantities increased significantly with the increase in nitrogen fertilization. Thus, the individual amino acids left in the thin juice increased with an additional supply of nitrogen but maximized under abundant nitrogen at some point that was genotype dependent.

The eight amino acids listed in Table 3 were those present in the thin juice in measurable quantity and were not occluded on the chromatogram.

In all samples we had difficulty separating the amino acid peaks in the threonine-serine area of the chromatograms when we used the buffer system and temperature necessary to obtain separation of the other amino acids in a single run. The medium sized threonine peak was usually occluded to the larger peak in the serine position. Therefore we could not accurately calculate the amount of threonine present in the sample. The large peak in the serine position often showed two, sometimes three, closely occluded peaks of different sizes. We attempted to identify these occluded peaks and their respective positions by use of known amino acids. As a result we believe them to be serine, asparagine,

Table 3.—Means and tests of differences for plot and thin juice characteristics in 1966.

Population &/or nitrogen treatment	Root weight (kg/plot)	Sucrose %	Conduct, (millimhos)	Thin juice purity%	Recov. sue. (kg/plot)	Thin juice		
						K	Na (mg/lOOml)	N
1. GW 359-52R								
0 lbs N	7.070a[1]	18.16a	0.88b	96.880a	1.1925a	64.834b	11.745b	26.709c
100 lbs N	7.735a	17.31b	0.96b	96.086a	1.2257ab	63.706b	14.848b	33.733b
250 lbs N	8.005a	15.56c	1.42a	92.616b	1.0580b	86.895a	32.192a	54.562a
2. 52-305CMS X 54-458,Fi								
0 lbs N	6.585b	17.40a	0.75c	96.755a	1.0679a	63.277b	8.841b	25.017c
100 lbs N	7.240ab	16.75a	0.97 b	95.55 la	1.1045ab	63.531b	13.150b	35.123b
250 lbs N	7.575a	14.96b	1.50a	91.579b	0.9423b	95.318a	27.842a	62.952a
3. **52-430** x 52-307,Fi								
0 lbs N	5.135a	17.14a	0.73b	94.429a	0.7767a	56.011b	11.307b	24.299b
100 lbs N	5.645a	16.62a	0.83b	95.522a	0.8502a	61.079b	15.642b	36.064a
250 lbs N	4.770a	14.14b	1.32a	94.084a	0.5964b	73.129a	41.137a	41.792a
Population means								
1	7.603a	17.01a	1.09a	95.194a	1.1587a	71.812a	19.595 b	38.335a
2	7.133a	16.37b	1.07a	94.628a	1.0382b	74.042a	16.611c	41.031a
3	5.183b	15.97c	0.96b	94.678a	0.7411c	63.406b	22.695a	34.052b
N Treatment means								
0 lbs	6.263b	17.57a	0.79c	96.021a	1.0124a	61.374b	10.631c	25.342c
100 lbs	6.873a	16.89b	0.92b	95.720a	1.0601a	62.772b	14.547b	34.973b
250 lbs	6.783ab	14.89c	1.41a	92.760b	0.8656b	85.114a	33.724a	53.102a

[1] Means followed by the same letter do not differ significantly (P<0.05)

Table 3—(Continued)

Population &/or nitrogen treatment	Amino acids in thin juice (μ moles/ml)							
	asp	glu	gly	ala	val	ileu	leu	lys
1. GW 359-52R								
0 lbs N	0.342c	0.760c	0.034c	0.136b	0.078c	0.122b	0.116b	0.022b
100 lbs N	0.512b	1.074b	0.062b	0.246b	0.150b	0.198b	0.204b	0.032b
250 lbs N	0.866a	1.608a	0.112a	0.662a	0.290a	0.430a	0.508a	0.064a
2. 52-505CMS × 54-458,F1								
0 lbs N	0.422b	0.954b	0.048b	0.154a	0.086b	0.136b	0.144b	0.030b
100 lbs N	0.460b	1.088b	0.048b	0.168a	0.114b	0.206b	0.208b	0.034b
250 lbs N	1.082a	2.066a	0.090a	0.280a	0.198a	0.390a	0.538a	0.070a
3. 52-430 × 52-307,F1								
0 lbs N	0.268b	0.894b	0.034a	0.088a	0.056a	0.058b	0.070a	0.022a
100 lbs N	0.468a	1.292a	0.044a	0.154a	0.094a	0.148ab	0.156a	0.032a
250 lbs N	0.554a	1.374a	0.038a	0.150a	0.084a	0.126a	0.142a	0.030a
Population								
1	0.573a	1.147b	0.069a	0.348a	0.166a	0.250a	0.276a	0.039a
2	0.655a	1.369a	0.062a	0.201b	0.133b	0.244a	0.297a	0.045a
3	0.430b	1.187b	0.039b	0.131b	0.078c	0.111b	0.123b	0.028b
N Treatment								
0 lbs	0.344c	0.869c	0.039c	0.126b	0.073c	0.105c	0.110c	0.025b
100 lbs	0.480b	1.151b	0.051b	0.189b	0.115b	0.184b	0.189b	0.033b
250 lbs	0.854a	1.683a	0.080a	0.364a	0.191a	0.515a	0.396a	0.055a

and glutamine. In thin juice and root samples the largest occluded peak seems to be glutamine. We hope with further work on this problem that we can find a method to separate these peaks so that each can be measured accurately. Other amino acids detected in trace quantities were proline, cystine, methionine, tyrosine, phenylalanine, arginine, histidine and tryptophan. Any quantity with an optical density reading on the chromatogram charts of less than 0.020 was considered to be a trace.

Among the eight prevalent measurable amino acids, aspartic and glutamic acids were most abundant and there was roughly twice as much glutamic as aspartic acid. This was as expected because glutamic acid and glutamine are primary products of nitrogen assimilation and aspartic acid and alanine are secondary products of nitrogen assimilation. In populations 1 and 2 at 250 pounds of nitrogen, alanine, valine, isoleucine and leucine were present in quantities which we consider significant in relation to quality; however, this was not true in population 3. In population 3 only glutamic and aspartic acids were present in significant quantities.

It should be remembered that these determinations were made on thin juice which had been purified and the amino acids present would have been primarily free of soluble amino acids. The amino acid content of thin juice is not necessarily a reflection of amino acids in the raw juice or root. Also, free amino acids in an organism tend to be very labile and represent a metabolic pool which may vary considerably, being a function of both environment and genotype. The amino acids in thin juice would be expected to be less labile than in leaf tissue.

Thin juice samples came from roots harvested at different hours of the day, since it usually takes several hours to harvest an experiment. They were sampled at slightly different lengths of time after harvest, since sampling in an experiment also takes several hours. However, the relationships among amino acids and with other characters, as shown by correlations, were quite constant and did not show a lot of random or unaccountable variation. This constancy was also reflected in the analyses of variance by the total absence of population x nitrogen x replication interaction for any of the amino acids.

The difference in amino acids was generally greater due to nitrogen treatment than to population effect, at least for combinations selected. There were increasing amounts of amino acids with increasing nitrogen except in population 3 as pointed out

previously. This increase appeared to be generally nonlinear, depending on both the particular amino acid and the population. There was a significant population x nitrogen interaction for all 8 amino acids, usually due to population 3 at 250 pounds of nitrogen. This represents a differential genetic response to increased nitrogen. The direction of the response in population 3 was desirable, but this appeared to have resulted from the inability of this genotype to utilize higher nitrogen rather than utilizing it in a more efficient and desirable manner.

Simple correlations among individual thin juice amino acids in 1966 were high and significant within populations (52 of 54 r values were significant, the nonsignificant r s were all in pop. 3) particularly within populations 1 and 2. This was probably due to the rather direct response of all amino acids to nitrogen fertilization. The same type correlations within nitrogen treatments were not as high, particularly with glutamic acid. This latter fact was interesting since glutamic acid was the most abundant. This general reduction in correlation was a reflection of the secondary importance of the genotype compared to nitrogen fertility level in influencing the quantity of amino acids.

The simple correlations for all characters within populations and within nitrogen treatments for 1966 were not tabulated, therefore only the information derived from these correlations will be discussed. Correlations within nitrogen treatments within populations were generally not significant, due to only three degrees of freedom for testing r. An examination of relations was made on the 1967 amino acid data where complete data were available on 10 replications instead of only five.

Year 1967

Data from the 1967 experiment were more extensive than those in 1966. Eleven populations were included and eight characters (noted in Table 1) determined on each. On populations 1, 2 and 6 a total of 36 determinations were made, all on 10 replications within the three nitrogen fertility levels. Means for the eight characters are shown in Table 4. Population 1 was the same Fi hybrid that appeared as population 3 in 1966 and population 6 (GW 359-52R) was the same as population 1 in 1966. Populations 7 through 11 were inbred lines most of which were parents of the F_x populations 1, 2 and 3. Population 5 (A59-2) was an increase of selections for high glutamic acid from *Beta vulgaris* x *Beta macrocarpa,* F_4. Population 12 (Ovana was a fodder beet developed in Europe from a sugarbeet x fodder beet cross. It would be expected to be of poor quality by sugarbeet standards.

Table 4.--Means and tests of differences for plot and thin juice characters on all 11 populations in 1967.

Pop. &/or N treatment	Sue.	Root weight	Pur.	Rec. sue.	Thin juice K	Na	N	NO3-N
1. **0 # N**	15.66a[1]	8.05a	92.48a	1.059a	94.5b	31.2a	49.4b	7.22b
125# **N**	15.47a	8.48a	92.28a	1.109a	97.8b	33.8a	60.5b	13.01ab
250# **N**	14.24b	8.74a	90.27a	0.998a	126.7a	43.0a	74.4a	17.79a
2. **0 # N**	15.74a	12.55b	92.92a	1.68 1ab	121.2b	30.5b	52.3c	12.91b
125# **N**	14.96a	15.11a	90.10b	1.795a	133.7b	42.8a	69.6b	17.21ab
250# **N**	13.64b	14.68a	89.56b	1.576b	155.4a	48.8a	83.4a	24.47a
3. **0# N**	15.78a	10.79ab	93.30a	1.446a	77.3b	28.0b	29.7b	6.46b
125# **N**	15.21a	10.00b	93.29a	1.299a	89.7ab	39.3ab	44.6a	14.36a
250# **N**	14.16b	12.07a	92.01a	1.434a	102.0a	43.7a	52.2a	16.44a
5. **0 # N**	14.66a	12.54b	91.66a	1.529a	98.9b	41.2b	54.1c	12.69b
125# **N**	14.20a	14.36a	90.20ab	1.615a	111.4b	44.3ab	70.5b	15.57 b
250# **N**	12.96b	15.66a	88.32b	1.538a	134.8a	55.0a	89.2a	27.12a
6. **0# N**	15.45a	13.42a	93.15a .	1.782a	97.4c	32.3b	43.5c	9.76c
125# **N**	14.33b	14.50a	90.22b	1.666ab	114.1b	52.9a	67.7b	17.19b
250# **N**	13.50c	14.24a	89.05b	1.488b	135.5a	61.2a	83.8a	27.60a
7. **0# N**	16.35a	4.93a	93.36a	0.690a	98.1c	14.7a	50.5c	5.09b
125# **N**	15.62a	5.69a	91.15a	0.740a	117.3b	21.4a	66.3b	11.17ab
250# **N**	14.22b	5.56a	88.67b	0.611a	144.7a	19.9a	90.5a	15.65a
8. **0 # N**	14.89a	6.19a	92.17a	0.758a	98.9c	34.0b	45.8b	7.13b
125# **N**	13.54b	5.24a	89.35b	0.559ab	118.3b	47.6a	55.3b	11.84b
250# **N**	13.12b	4.64a	87.81b	0.461b	146.3a	45.1 ab	71.6a	19.40a
9. **0# N**	14.77a	5.81a	92.51a	0.721a	101.4b	30.8b	35.6b	7.52c
125# **N**	13.50b	6.86a	89.61b	0.727a	115.0b	56.2a	60.2a	19.91b
250# **N**	12.67c	6.10a	88.08b	0.587a	135.6a	57.1a	67.8a	27.89a

[1] Means followed by the same letter, within populations, do not differ significantly ($P<0.05$).

Table 4—(Continued)

Pop. &/or Nf treatment		Sue.	Root weight	Pur.	Rec. sue.	Thin juice			
						K	Na	N	NOs-N
10.	0 # N	15.67a	4.35a	95.74a	0.609a	73.3b	20.4 b	30.1b	3.81b
	125# N	14.92a	4.55a	91.87b	0.564a	80.6ab	32.2ab	36.6ab	6.92ab
	250# **N**	14.03b	5.04a	93.14b	0.607a	91.5a	43.5a	48.2a	13.75a
11.	0# N	15.88a	7.40b	92.90a	0.984a	85.6b	24.6a	40.7c	5.47b
	125# N	14.89b	9.66a	91.1 Sab	1.186a	97.5 b	33.0a	66.4b	8.95ab
	250# N	14.19b	10.08a	89.36b	1.122a	126.8a	30.4a	86.3a	13.60a
12.	0 # N	8.86a	18.66b	83.80a	1.045a	156.9c	84.4b	76.7b	35.83c
	125# N	8.25 a	21.60a	81.00b	1.038a	217.6b	115.3a	106.5 a	59.18b
	250# N	7.31b	21.84a	77.59c	0.803b	249.8a	121.2a	105.1a	76.26a
Population	1	15.12ab	8.42d	91.68bc	1.055cd	106.3de	36.0cd	61.4cde	12.68c
	2	14.78bc	14.11b	90.8ficd	1.684a	136.8b	40.7bc	68.4bc	I8.20b
	3	15.05ab	10.95c	92.87ab	1.393b	89.7f	37.0cd	42.2f	12.42c
	5	13.94de	14.19b	90.06d	1.561a	115.led	46.9b	71.2b	18.46b
	6	14.43cd	14.05b	90.8 led	1.645 a	115.7cd	24.4e	65.0bcd	18.19b
	7	15.40a	5.39ef	91.06cd	0.68 le	120.0c	9.3f	69.1 be	10.64c
	8	13.85e	5.36ef	89.77d	0.593c	121.2c	42.2bc	57.6de	12.79c
	9	13.65e	6.26e	90.07d	0.679e	117.3c	48.0b	54.6e	18.44b
	10	14.87bc	4.64f	93.58a	0.593c	81.8f	32.0d	38.3f	8.16c
	11	14.99ab	9.04d	9I.14cd	1.097c	103.3c	29.3de	64.5bcd	9.34c
	12	8.14f	20.70a	80.80e	0.962d	208.1a	107.0a	96.1a	57.09a
N Trmt.	0# N	14.88a	9.52b	92.18a	1.119a	100.3c	33.8c	46.2c	10.36c
	125# N	14.08b	10.55a	90.02b	1.118a	117.5b	47.2b	64.0b	17.76b
	250# N	13.09c	10.78a	88.53c	1.020b	140.8a	51.7 a	77.5a	25.45a

There were significant differences in sucrose content among populations, nitrogen treatments and nitrogen treatments within every population. Hence, without exception sucrose declined with increased nitrogen fertilization. There was no population by nitrogen treatment interaction; sucrose declined at the same rate in all populations.

In the case of root weight there were considerable differences among populations; the fodder beet had high yield while the inbreds had typically low yield. The mean for all populations within the 125 and 250 pound nitrogen treatment was not different, but both were greater than the 0 treatment. So on the average there was little or no yield response beyond 125 pounds of nitrogen. There was a significant population *x* treatment interaction; not all populations responded in the same manner to increasing nitrogen. This was particularly true of populations 2, 6, 7 and 8. This interaction was of interest because it indicated that there were genotypes which were more capable of utilizing the available nitrogen to increase root yield.

Thin juice apparent purity was significantly different from one population to another; this was of most interest in the hybrid and commercial populations. With increasing nitrogen, purity declined, in the same nonlinear fashion as sucrose content. There was no interaction of population x treatment indicating the dominating effect of nitrogen environment over genotype.

Recoverable sucrose was a function of weight, sucrose and purity, but weight was the dominant factor. Recoverable sucrose is more useful than gross sucrose since extractable sucrose is the economically valuable product. There were significant differences among populations with 2 and 6 producing the highest quantity. Because of the rapid decline in sucrose and purity and less rapid increase in root weight, recoverable sucrose was not different at 0 and 125 pounds of nitrogen, and both were higher than at 250 pounds. Within populations recoverable sucrose at 250 pounds of nitrogen was either the same or less than at 125 pounds. Therefore, 250 pounds of N would represent an economic loss at the levels of available N in the check treatment for this study. The amount of available N initially in the soil is an important factor to consider in determining the rate of fertilizer N to recommend in order to prevent a negative effect on recoverable sucrose. Among the 11 populations there was no interaction of populations and N treatments for recoverable sucrose.

The thin juice is probably the most important plant material for analysis of nonsugars, since this is considered to be the equivalent of second carbonation juice in the factory (2). All nonsu-

crose compounds in this juice persist through the sucrose extraction process and terminate in the molasses. Seven characters, in addition to nine amino acids, were determined on thin juice in 1967. Purity of this juice was mentioned above. Purity is an index or ratio indicating the portion of total soluble solids in the juice which is sucrose.

Potassium is a rather important cation in the thin juice. With respect to potassium content, there were significant differences among nitrogen treatments and among populations. There was also a significant population by nitrogen treatment interaction. Although potassium increased in every population with increased nitrogen fertilization, it did not increase at the same rate. Sodium in the thin juice behaved in the same manner as potassium.

Total nitrogen in the thin juice was significantly different for nitrogen treatments and for populations but their interaction was not significant. Hence, total nitrogen increased the same in all populations with increasing nitrogen fertilization. However, there was considerable difference among populations which represents the potential for developing populations with low total nitrogen along with other desirable characters.

Nitrate (NO_a) nitrogen in the thin juice was the only other character measured on all 11 populations. Nitrate (NO_3) nitrogen represents a form in which nitrogen is commonly translocated. There were significant differences in NO_3 among populations, among nitrogen treatments and among nitrogen treatments within populations (interaction). Averaged across the entire experiment about 6% of the total juice nitrogen was contained in NO_s. This compares with about 4% of the total thin juice nitrogen contained in glutamic acid. The total nitrogen contribution of a compound is not a good measure of its melassigenic power. Rather the effect of the compound on the sucrose crystallization rate and the effect on the pH or buffering capacity of the juice are the factors that determine how an impurity affects the recovery of sugar. It is generally considered that the nitrite-nitrate-nitrogen content of various factory juices changes little and goes through the factory unchanged with only slight elimination.

Means of 28 characters measured on three populations are listed in Table 5. The thin juice and leaf characters were determined on all 10 replications, but the root amino acids were measured on 1 to 4 replications and should be considered less reliable. Multiple range tests have been calculated across N treatments, across populations and across N treatments within populations. Within any group means followed by the same letter do not differ significantly.

Table 5.—Means and tests of differences for thin juice, leaf, and root characters measured on 3 populations in 1967.

Pop. &/or N treatment	Amino N	beta-ine	Thin juice								
			asp	glu	gty	ala	val	ileu	leu	tyr	lys
1. 0 # N	14.84b*	169.3b	0.995a	1.886b	0.084a	0.175 a	0.077a	0.147a	0.163a	0.056a	0.032a
125# N	18.74ab	180.9b	1.147a	2.159b	0.086a	0.167a	0.089a	0.0202a	0.206a	0.110a	0.026a
250# N	22.97a	205.2a	1.226a	2.484a	0.085a	0.229a	0.091a	0.202a	0.223a	0.154a	0.031a
2. 0# N	13.08c	168.1b	1.018b	1.551b	0.063b	0.112b	0.059b	0.143b	0.148b	0.086b	0.022b
125# N	19.45b	180.4ab	1.305ab	1.911a	0.085ab	0.175ab	0.119a	0.246a	0.280a	0.164b	0.039a
250# N	24.56a	190.4a	1.367a	2.208a	0.106a	0.216a	0.125a	0.301a	0.337a	0.277a	0.041a
6. 0 # N	14.52c	122.8b	0.777b	1.312b	0.086b	0.243b	0.121b	0.200c	0.217c	0.115c	0.042b
125# N	23.72b	133.7ab	1.146a	1.906a	0.145a	0.487a	0.182a	0.315b	0.346b	0.333b	0.052ab
250# N	29.75a	142.6a	1.423a	2.056a	0.166a	0.572a	0.216a	0.404a	0.450a	0.467a	0.057a
Population											
1	18.85b	185.1a	1.110a	2.176a	0.085 b	0.190b	0.086b	0.184c	0.197b	0.107c	0.030b
2	19.03b	179.6a	1.230a	1.890b	0.085b	0.168b	0.101b	0.230b	0.255b	0.176b	0.034b
6	22.66a	133.0b	1.115a	1.758b	0.132a	0.434a	0.173a	0.306a	0.338a	0.305 a	0.050a
N Treatment											
0 # N	14.14c	153.4c	0.917c	1.583c	0.078c	0.177c	0.086c	0.163c	0.176c	0.086c	0.032b
125# N	20.64b	165.0b	1.199b	1.992b	0.105b	0.276b	0.130b	0.254b	0.277b	0.202b	0.039a
250# N	25.76a	179.4a	1.339a	2.249a	0.119a	0.339a	0.144a	0.302a	0.337a	0.299a	0.043a

[1] Means followed by the same letter do not differ significantly ($P<0.05$).

Table 5—(Continued)

treatment		Dried leaf Cu	Co	Ca	Mg	Fe	Ni
1.	0 # N	**1.897a**	0.732a	383.95a	347.08a	23.559a	1.275ab
	125# N	**2.150a**	0.708a	415.57a	436.12a	26.811a	1.155b
	250#N	**2.212a**	0.756a	490.29a	498.97a	23.571a	1.305a
2.	0 # N	**4.260a**	0.804a	759.78a	584.59a	25.684a	1.230a
	125#N	**1.811b**	0.720a	757.46a	688.02a	24.710a	1.245a
	250# N	**1.756b**	0.768a	710.72a	733.73a	20.921a	1.200a
6.	0 # N	2.033a	0.804a	600.63a	464.70a	25.233a	1.200a
	125#N	**1.896a**	0.732a	527.96a	450.61a	25.013a	1.290a
	250#N	**1.711a**	0.708a	565.43a	554.59a	29.448a	1.215a
Population							
	1	2.086a	0.732a	429.94c	427.39b	24.647a	1.245a
	2	2.609a	0.764a	742.65a	668.78a	23.772a	1.225a
	6	1.880a	0.748a	564.67b	489.97b	26.565a	1.235a
N Treatment							
	0 # N	2.730a	0.780a	581.45a	465.46c	24.825a	1.235a
	125#N	1.952b	0.720b	566.99a	524.92b	25.511a	1.230a
	250#N	**1.893b**	0.744b	588.81a	595.76a	24.647a	1.240a

Table 5—(Continued)

Pop. &/or N treatment		Fresh leaf										
		asp	ser*	glu	pro	ala	val	ileu	leu	tyr	phe	lys
1.	0# N	0.344c	0.569b	0.328c	0.208c	0.792b	0.129b	0.072c	0.076b	0.072c	0.071b	0.040b
	125# N	0.428b	0.560b	0.376b	0.276b	0.784b	0.144a	0.084b	0.088a	0.080b	0.084a	0.044a
	250# N	0.532a	0.740a	0.412a	0.352a	0.872a	0.140a	0.132a	0.088a	0.108a	0.084a	0.040b
2.	0# N	0.496c	0.468c	0.320b	0.300c	0.852b	0.120c	0.048c	0.072b	0.056b	0.044b	0.044c
	125# N	0.560b	0.548b	0.352a	0.396b	0.872b	0.144b	0.084a	0.088a	0.068a	0.048a	0.058a
	250# N	0.628a	0.600a	0.344a	0.668a	0.932a	0.156a	0.068b	0.088a	0.064a	0.052a	0.048b
6.	0# N	0.356c	0.538c	0.340c	0.164c	0.804c	0.128c	0.064c	0.084b	0.064b	0.051c	0.040b
	125# N	0.476b	0.580b	0.380b	0.236b	0.852b	0.140b	0.072b	0.088a	0.068b	0.062a	0.044a
	250# N	0.612a	0.712a	0.436a	0.296a	0.904a	0.148a	0.112a	0.088a	0.100a	0.056b	0.044a
Population												
	1	0.435c	0.625a	0.372b	0.279b	0.8I6c	0.138a	0.096a	0.084b	0.087a	0.080a	0.041c
	2	0.561a	0.539b	0.339c	0.455a	0.885a	0.141a	0.067c	0.083b	0.063c	0.048c	0.048a
	6	0.481b	0.612a	0.385a	0.232c	0.853b	0.139a	0.083b	0.087a	0.077b	0.057b	0.043b
N Treatment												
	0 # N	0.399c	0.523c	0.329c	0.224c	0.816c	0.125c	0.061c	0.077b	0.064c	0.056b	0.041c
	125# N	0.488b	0.563b	0.369b	0.303b	0.836b	0.143b	0.080b	0.088a	0.072b	0.065a	0.047a
	250# N	0.591a	0.684a	0.397a	0.439a	0.903a	0.148a	0.104a	0.088a	0.091a	0.063a	0.044b

* Probably includes asparagine and glutamine.

Table 5—(Continued)

Pop. &/or N treatment	Fresh Root											
	asp	glu	gly	ala	val	ileu	leu	tyr	phe	lys	his	arg
1. 0# N	1.760	2.480	.040	.280	.160	.240	.280	.040	.040	.120	.080	.160
125# N	1.440	2.120	.040	.200	.160	.200	.240	.040	.040	.080	.040	.120
250# N	2.560	3.520	.080	.360	.200	.320	.404	.240	.080	.160	.080	.320
2. 0# N												
125# N	2.480	2.680	.040	.240	.200	.400	.520	.320		.120	.040	.080
250# N	2.640	3.240	.080	.360	.240	.480	.560	.480	.080	.160	.080	.160
6. 0# N	1.900	2.232	.080	.480	.260	.480	.460	.288	.068	.112	.060	.100
125# N	1.760	2.028	.100	.592	.248	.392	.440	.340	.060	.120	.040	.100
250# N	2.272	2.848	.140	.860	.352	.620	.688	.780	.080	.152	.080	.160
Population												
1	1.920	2.708	.052	.280	.172	.252	.308	.108	.052	.120	.068	.200
2	2.560	2.960	.060	.300	.220	.440	.540	.400	.080	.140	.060	.120
6	1.976	2.368	.108	.644	.288	.472	.528	.468	.068	.128	.060	.120
N Treatment												
0# N	1.872	2.280	.072	.440	.240	.376	.424	.240	.060	.112	.064	.112
125# N	1.828	2.152	.080	.468	.228	.360	.420	.288	.056	.112	.040	.100
250# N	2.380	3.028	.120	.692	.308	.548	.620	.640	.080	.152	.080	.188

Looking at thin juice amino N, there were significant differences due to N treatments both within and across populations and also differences due to population. Differences due to N treatments were greater than those due to populations. The proportion of amino N in the total N went up slightly from 0 to 250 pounds of N, 30.6 to 33.2%. This proportion varies more for populations, 27.8% for population 2 to 34.9% for population 6. Since amino N is one of the most deleterious type of nitrogenous compounds, the proportion of amino N in the total N could be an important consideration. Population 2 at 27.8% would be a more desirable genetic population than 6 at 34.9%.

Betaine was present in quite large quantity in the thin juice. It has low melassigenic power but was present in such large quantity that it became important. There were significant differences in betaine quantity among populations, N treatments and N treatments within populations. Betaine always increased with increasing nitrogen, but the differences due to genotype (populations) were greater than that induced by N treatment. The nitrogen in betaine in this experiment constituted about 39% of the total N at 0 pounds nitrogen down to 30% and 27% for 125 and 250 pounds, respectively. Hence, as available nitrogen increased, proportionately less was found in the form of betaine. This was unfortunate since the other more melassigenic compounds increased proportionately. The proportion that betaine N was of total N was 35%, 31% and 24% for populations 1, 2 and 6, respectively. The proportions were not greatly different from N treatments so one can say that genotype and nitrogen environment had about equal effect on the proportion of nitrogen tied up in betaine. It may be fortunate that such a large part of the thin juice nitrogen was present in betaine since betaine is one of the least noxious, with respect to sucrose recovery, of the nitrogen containing compounds, but unfortunate that the proportion of betaine declined with increased nitrogen fertilization.

The free amino acids present in measurable quantity in the thin juice are listed in Table 5. Glutamic and aspartic acids were the quantitatively important ones. Serine, threonine, asparagine and glutamine (not measurable because of occlusion) were less than glutamic but probably higher than aspartic acid. The other seven amino acids appeared in small quantity but jointly they became important. Seven other amino acids (methionine, phenylalanine, histidine, tryptophan, arginine, proline, ornithine) were present in trace quantities as well as three unknowns, one of which appeared in appreciable quantity.

Almost without exception the amino acids increased quantitatively with increased nitrogen fertilization. The amount of increase was dependent on genotype which led to the significant population by N treatment interaction in six of the nine measured amino acids. Nitrogen treatments caused a significant change in every case while aspartic acid was the only case in which populations showed no significant difference. Hence, the free amino acid content of thin juice was a function of available nitrogen while the rate of change was a function of genotype.

For example, aspartic acid increased by 83% in population 6 in going from 0 to 250 pounds N while in population 1 and 2 it increased only 28% and 34% respectively. Tyrosine was most responsive to increased N; it increased 175%, 222% and 306% in going from 0 to 250 pounds N in populations 1, 2 and 6 respectively. Tyrosine was rather minor in quantity, however. In general, populations 2 and 6 increased more rapidly in amino acids than did population 1, but the latter population was one which responded least to increased N in 1967 and hardly at all in 1966. It was a genotype which lacked the capacity to utilize nitrogen.

Even though one expects the quantity of free amino acids in a plant to be quite labile, those in the thin juice were very consistent and reflected quite distinctly the N treatment and population effect. Apparently the free amino acid content in the root at harvest time was quite stable.

Only limited data are reported for amino acids in fresh root. These amino acid analyses were made from samples of pulp or brei. Unfortunately, very few replications were sampled so that there were no errors or tests of differences for these means in Table 5. Twelve amino acids were present in measurable quantity including phenylalanine, histidine and arginine which were not present in thin juice. Those nine amino acids present in both thin juice and root rank quantitatively in exactly the same order in both sample sources, the only difference being that the amino acids in the root were only 30% to 40% as much per gram of pulp as per milliliter of thin juice. This could be expected since all proteins and insoluble solids that were present in the pulp were removed in the thin juice purification process; the thin juice can be considered more concentrated with respect to free amino acids.

The quantities of the three additional amino acids (phenylalanine, histidine and arginine) were insignificant. All the amino acids in the root responded to N treatment and population in virtually the same way as in thin juice. Again, tyrosine showed

the greatest response to nitrogen. It appeared that either root tissue or thin juice could be used equally well to determine the relative quantity of free amino acids in beet roots.

Leaves were also harvested just prior to root harvest. Samples for amino acid analyses were prepared from fresh leaves immediately after harvest. Dried leaf samples were used for analyses for metallic ions (or metals).

There were 11 free amino acids present in measurable quantity in fresh leaves. In comparison with thin juice, glycine was not present in measurable quantity, but serine, proline and phenylalanine were present and measurable. The quantitative rank of amino acids in fresh leaves was quite different from thin juice or root. Alanine was most abundant followed by serine (possibly with asparagine and glutamine), aspartic acid, glutamic acid, proline and valine in that order. With minor exceptions, the quantity of leaf amino acids increased with increased nitrogen fertilizer. The response was, on the average, slightly less than in thin juice.

There appeared to be little relationship between population rank for leaf and thin juice amino acids. It appeared that the relative quantities of leaf amino acids would not serve well as indicators of root or thin juice amino acids. One would expect free amino acids in the leaf to be a rather dynamic metabolic pool, changing over the season, from light to darkness, and even from hour to hour. No direct measure of this quality was made; but relatively large error mean squares for all the leaf amino acids indicated that there was more plot to plot variability than for thin juice amino acids.

The dried portions of the leaf samples were analyzed for copper, cobalt, calcium, iron, nickel and magnesium. There were no meaningful or consistent differences in quantity of these elements due either to N treatments or populations. These elements in the leaves did not appear to provide any useful information relative to quality and genotype performance.

In order to establish the relationships of 36 characters for which an orthogonal set was available, simple correlations of all 36 were calculated. Also, correlations of the 8 characters measured on 11 populations were calculated but not tabulated. Among the 8 characters across all 11 populations it was interesting to note that nitrate-nitrogen (NO_s-N) in the thin juice was more highly correlated with sucrose, root weight, purity, potassium and sodium than was total nitrogen in thin juice. These same correlations within populations across N treatments were about the same for NO_s-N and total N, except for population 12 which was the fodder beet. In this population NO_s-N was much more

closely related to the other six characters than was total N. This was particularly true for sucrose, root yield, purity and recoverable sugar. This must indicate that many times some of the nitrogen included in total N must be in a form which is not so detrimental to sucrose accumulation and purity.

Simple correlation coefficients gave some idea of the effect of nitrogen fertilizer on relationships of various characters across a fairly large sample of genotypes. Root weight was negatively correlated with sucrose to about the same degree in all N treatments (-0.61**, -0.56**, and -0.60**). The same was true for sucrose with purity (0.76**, 0.82**, and 0.83**) and weight with purity (-0.58**, -0.54**, and -0.50**). Recoverable sucrose was related to weight at 0, 125, and 250 pounds N (0.69**, 0.66**, and 0.62**). It was related to purity only at 250 pounds N (0.24*) and was related to sucrose only at 125 and 250 pounds of N (0.20* and 0.22*).

Thin juice K maintains the same correlations with other characters across all N treatments. It was not correlated with recoverable sucrose, nor were sodium, total N and NO_R-N correlated with recoverable sucrose at any of the N levels. Correlations of Na with the other characters were not greatly changed by N treatment. Correlations of total thin juice nitrogen with weight, sucrose, and K were highest at 125 pounds N, but correlations with purity and Na declined with increased nitrogen fertilizer. Nitrate-nitrogen in the thin juice was, with minor exception, most highly correlated with all other characters at all N treatments.

The correlations of NO_j-N with the other seven characters did not change with change in N treatment, except $N0_3$-N with total N where the correlation was greatly reduced at 250 pounds of N, from 0.82** and 0.83** at 0 and 125 pounds of N to 0.44** at 250 pounds. At this level of excess available nitrogen, NO_s-N was no longer a strong indicator of the nitrogen balance and relationship of nitrogenous compounds in the plant. It was of some concern that recoverable sugar was so poorly correlated with thin juice potassium, sodium, total N and NO_a-N, since net sugar was the character of ultimate commercial interest.

A few of these correlations were significant within populations, particularly populations 6 and 12 (data not shown). Correlations within N treatments within populations were calculated but had only 8 degrees of freedom for testing. Within the populations of greatest interest there was little difference in correlations from within population and within N treatment. Hence, the correlations were little affected by population x N treatment interaction.

Correlations of all 36 characters which were determined only on populations 1, 2 and 6 were calculated, but not tabulated within the entire experiment, within populations within N treatments, and within N treatments within populations.

Since correlations among the first eight characters already have been discussed over all 11 populations, only their relationship with the 28 additional characters will now be discussed. Sucrose was negatively correlated with all amino acids in both the thin juice and fresh leaves Among these correlations, those with thin juice amino acids were generally higher than those with leaf amino acids, except for aspartic and glutamic acids. This was of interest since aspartic and glutamic were the most abundant amino acids in the thin juice. Sucrose content was not significantly correlated with betaine, leaf metals and leaf phenylalanine and lysine.

Root yield correlations across all three populations and the three N treatments were positive and significant with all thin juice impurities except betaine, glutamic acid and lysine. Root yield correlations with leaf metals did not appear to be important; with leaf amino acids they were generally weaker than with thin juice amino acids. Correlations of thin juice purity with thin juice amino acids, other nonsugars and leaf amino acids were consistently significant and negative except for leaf valine, phenylalanine and lysine. Only magnesium among the leaf metals was correlated with purity (-.30**).

Recoverable sucrose was noticeable by its lack of correlation with most of the major juice impurities as well as sucrose and purity. Recoverable sucrose was largely determined by root yield, hence, tends to be related to other characters much like root yield was, only weaker. Thin juice potassium was positively correlated with most characters except the leaf metals, sucrose, and purity. Sodium followed the pattern of potassium quite closely even though it was correlated only $0.53^{\#\#}$. Amino N in the thin juice was most highly correlated with thin juice nitrogen, sodium, isoleucine, leucine and NO_a-N in that order. It was correlated with all leaf amino acids except lysine, but not as highly as with the thin juice amino acids. Nitrate-nitrogen was more highly related with weight, as well as thin juice potassium, sodium, alanine, valine, tyrosine and lysine than was total N, althought it was less highly correlated than amino N in most cases.

It appeared that thin juice amino N was generally a better determinant of root yield, sucrose, purity and recoverable sucrose than was thin juice total N and NO_a-N. However, it was still only weakly related to recoverable sucrose. Betaine, although generally present in large quantity in thin juice, was not very

highly related to any of the characters in the study. The most important measurable thin juice amino acids quantitatively were aspartic and glutamic acids. Those of minor importance were alanine, isoleucine, leucine and tyrosine, while glycine, valine and lysine were present in very small quantity, even with 250 pounds of N. Glutamine, serine and threonine are likely important, but were not measurable under conditions of this experiment.

Comparing aspartic and glutamic acids, glutamic acid had the highest correlation with recoverable sucrose and betaine while aspartic acid was most correlated with root weight, purity, potassium, sodium, total N, glycine, alanine, valine, isoleucine, leucine, tyrosine and lysine. Thin juice aspartic and glutamic acids were not generally as highly correlated with the leaf amino acids; of the two, glutamic generally had the highest correlation.

When these same correlations were calculated within populations some differences were noted. Namely, the correlations of aspartic and glutamic acids were generally weakest for population 1 and strongest for 6. Also, glutamic acid had in general higher correlations with all variables up through 18 than did aspartic acid. Hence, use of one amino acid as an indicator for other characters may be applicable only within specific populations. These same type correlations within N treatments showed that aspartic and glutamic acids were equally related with weight, sucrose, purity, recoverable sucrose, potassium, sodium, total N, amino N and betaine, but the relationships became weaker with increasing nitrogen. With the other thin juice amino acids, aspartic was more highly correlated than glutamic. These correlations were also reduced with increasing nitrogen.

In dried leaf samples the metals copper, cobalt, calcium, magnesium, iron and nickel were present in greatly different quantities. Calcium and magnesium were abundant relative to copper, cobalt, iron and nickel. There were very few significant correlations of these metals with any of the other characters.

The amino acids in fresh leaves at harvest had quite frequent correlations with other characters. The two most conspicuous observations about their correlations were that the frequency of significant correlations was sharply reduced with increasing N fertilization, and the quantity of a particular amino acid in the leaf was rarely correlated with the quantity of the same amino acid in the thin juice.

Discussion

The response of population 3 (an F_1 hybrid) in 1966 to increasing nitrogen fertilizer was somewhat unusual. It represent-

ed a marked genotype difference from populations 1 and 2 which seemed to respond rather typically. With increasing fertilizer N from adequate (100 lbs) to excess (250 lbs) the purified thin juice extracted from the roots of population 3 did not increase significantly in total N or any of the amino acids. This was a desirable genotype response with respect to quality, but it was accompanied by an unacceptably low root yield. Apparently, the individual amino acids in the thin juice quantitatively maximize at some point with increasing fertilizer nitrogen. If a high performing genotype had a low maximizing point this would be a very desirable characteristic. However, it would seem physiologically unlikely that this maximization point would be sufficiently low in high yielding genotypes. There may also be a year or location interaction with genotype in this case. An interaction with years was indicated in 1967 when this same F_1 hybrid did not maximize as soon or as decisively with increasing N fertilizer as in 1966.

From the sample of four genotypes analyzed for thin juice amino acids (1966 and 1967) it would appear that all amino acids present in measurable quantity increased with increasing available soil nitrogen to some maximum or plateau which was genotype dependent. The three populations with acceptable yield characteristics did not reach this point even with 250 pounds of fertilizer N. The increase in quantities of amino acids was not linear, however. They appeared to increase more between 0 and 100 or 125 pounds than between 100 or 125 and 250 pounds of nitrogen.

The rate of increase was also different for the different amino acids. For instance, in population 6, 1967, aspartic acid increased proportionately more than glutamic acid but the opposite was true for population 1. For population 2 the increase rates of aspartic and glutamic acids were about the same.

There was considerable difference in thin juice amino acids due to year. Part of this difference might have been due to differences in residual nitrogen in the fields in which the 1966 and 1967 experiments were grown, but it was very doubtful that most of the year difference can be attributed to this source.

The most abundant measurable amino acid in the thin juice was glutamic acid followed by aspartic. Threonine, serine, and probably asparagine and glutamine were present, but were occluded, and therefore not measurable under conditions of this experiment. All of the others were present in minor quantity, but glutamic acid, alanine, valine, isoleucine, leucine, tyrosine and lysine did contribute 2.83% of the total thin juice N in 1967 compared to 2.58% for aspartic acid and 4.34% for glutamic

acid; that was the average for all three populations over all N treatments.

Although the study was not designed to quantitatively analyze the thin juice for all nitrogenous compounds, some comparisons can be made. As fertilizer N was increased, the proportion of total thin juice N (accounted for by the amino acids, amino N and betaine) declined, while that of NO_3-N increased. From 65 to 71% of total N was accounted for within populations and 68 to 75% within N treatments. There remained unmeasured specific nitrogenous compounds which accounted for 25% or more of the total N. This seems quite high unless there was considerable unmeasured glutamine, asparagine, threonine, serine, pyrolidine carboxylic acid (PCA), and ammonia in the thin juice. Purines, pyrimidines and nucleosides probably make minor contributions to total N. No complete nitrogen analysis of this type laboratory thin juice has ever been published. Probably no analysis has ever been undertaken; but one should be, since it appears that this phosphated thin juice is being used increasingly in beet quality evaluation.

The differences in thin juice amino acid content due to N treatment were generally greater than that due to genotype. From the limited sample of genotypes one would conclude that amino acid content can be shifted and regulated much more by nitrogen culture than by genotype. The dominance of nitrogen environment over genotype was also evident in the sucrose content and thin juice purity in 1966 and 1967. In this study it appeared that genotype could do relatively little to overcome the effects of nitrogen on sucrose content and the various nitrogenous impurities. However, in previous work, two hybrids were found capable of producing high sucrose with low concentrations of total nitrogen in the thin juice the low total nitrogen appears not to be caused by amino acids.

There were no populations among the four analyzed for amino acids which demonstrated the possibility of finding or developing a high performing genotype with low thin juice amino acids. A poor performing genotype (population 3 in 1966 and 1 in 1967) did have lower thin juice amino acids and small response to increasing N fertilizer, but this was probably part of the reason for its poor performance.

Other thin juice impurities were quite deleterious but none so much as amino nitrogen compounds. Carruthers and Oldfield (2) rate potassium as 0.25 as deleterious. Potassium increased with increased nitrogen fertilization but at different rates in difference populations. Also, the increase of potassium was not linear in relation to N fertilizer.

Sodium, which is considered by Carruthers and Oldfield (2) to be more detrimental than potassium, was rated by them as 0.35 as melassigenic as amino N. The anions with which potassium and sodium are associated are perhaps quite important. The chloride determinations in this study have not been analyzed at this time. Carbonates were very labile and difficult to measure quantitatively. It would seem that these anions plus the sulfates may all be quite melassigenic and warrant further study. On the other hand they may necessarily be associated with potassium and sodium, so that a measure of potassium and sodium would provide as much quality information as a measure of both cations and anions. Potassium and sodium are easy to measure quantitatively relative to chlorides and particularly carbonates. The individual cation and anion relationships need to be studied before the anion content can be ignored as it has been in the past.

Betaine was the one nitrogenous compound measured whose quantity was affected more by genotype than by N fertilization. This was encouraging from the standpoint of breeding, since relatively low betaine genotypes in all nitrogen environments should be synthesizable. It was not apparent from this study that a betaine reduction was compensated for by any other melassigenic compound. It is a trimethyl product of glycine. It would be a valuable by-product of beet sugar refining if a commercial use for it were found.

Comparisons of free amino acids in the root tissue and thin juice indicated that either all free amino acids were retained in the thin juice process or the individual amino acids were eliminated in equal proportions. The amino acids ranked the same quantitatively in root and thin juice. This was not true of free amino acids in the leaves. Alanine and serine were most abundant in fresh leaf but were minor in both the root and thin juice. From this study it appeared that relative quantities of leaf amino acids would be of little value as indicators of root and thin juice amino acids, root quality or sucrose yield.

Since several of the metals measured in the leaves are present in some of the essential enzymes, we hoped that they might be related in some way to quality or yield characters. However, no useful relationships were found. As a matter of fact, very few significant correlations of any type were found in relation to these metals. From this study it appeared that quantities of copper, cobalt, calcium, magnesium, iron and nickel in the leaves at harvest were of little value in determining beet quality or sucrose yield.

Nitrate-nitrogen in the thin juice seemed to be of more importance than has been reported previously. Nitrate-nitrogen

was most highly correlated with the yield characters, potassium and sodium. This occurred in spite of the fact that it accounts for only 3.5 to 7.6% of total N in sugarbeet. This compared with much higher values for amino N and betaine. In the fodder beet NO_3-N accounted for more than twice as much of the total N, 13.4.%. Without exception the proportion of total N contributed by NO_3-N increased with increased N fertilization. This was the opposite of betaine and most of the amino acids.

We saw that the nitrogenous compounds make varying contributions to total N in the thin juice dependent on the nitrogen status of the soil and the genotype. Averaged over the three populations studied, amino N and NO_3-N increased in proportion to the total thin juice N while betaine, aspartic and glutamic acids decreased. A sizable portion of the total N and amino N was unaccounted for by the nitrogen-containing compounds analyzed.

In the thin juice it appeared that aspartic and glutamic are the only measurable amino acids worthy of much consideration although glutamine, asparagine, serine and threonine should also be measured. The other seven present in the thin juice would be important if considered as a group, but they were affected somewhat differently by genotype and N fertilizer. Hence, they did not respond as a group and would have to be treated individually with respect to fertilization and selection.

Summary

The quantity of some individual amino acids was determined in sugarbeet of different genetic backgrounds and at different nitrogen fertility levels. The relationships of impurity components with each other and with yield factors was determined to obtain new information about where efforts for quality improvement might be most effective.

The study consisted of laboratory and statistical analyses of 36 yield, quality, and leaf component characters from 12 genetic populations, at three nitrogen fertility levels, over 2 years. Only two populations were common to both years, and not all populations were analyzed for all characters.

The data of both years and of four genotypes showed that those measurable amino acids in the thin juice (equivalent to factory second carbonation juice) increased with increasing available soil nitrogen. There was an indication that they may reach some maximum or plateau which was genotype dependent. Every amino acid was present in larger quantity in 1967, which might be explained by a year interaction or by residual soil nitrogen. The most abundant amino acids in thin juice were glutamic acid and then aspartic acid, although asparagine, glutamine, PCA, serine and threonine may have been abundant but were not

measurable under conditions of this experiment. Glutamic and aspartic acids were the primary products of nitrogen assimilation.

The study was not designed to analyze the thin juice quantitatively for all nitrogenous compounds. Some comparisons can, however, be made:

1. The differences in thin juice amino acid content due to nitrogen treatment were greater than that due to genotype, indicating that amino acid content can be shifted more by nitrogen culture than by genotypes.
2. It appeared from limited evidence that genotype could do little to overcome the detrimental effects of excess nitrogen fertilization.
3. As nitrogen treatment was increased, the proportion of total thin juice nitrogen accounted for by most of the amino acids, amino nitrogen and betaine declined while that of nitrate-nitrogen increased.
4. From 65 to 71% of nitrogen was accounted for within populations and 68 to 75% within nitrogen treatments.
5. Unmeasured specific nitrogenous compounds accounted for 25% or more of the total nitrogen. This seems high unless there was decomposition of glutamine and asparagine.

There was no high performing genotype (high weight sucrose) with low thin juice amino acids. In previous work we found two genotypes that had low total nitrogen in the thin juice at harvest. This low total nitrogen appeared not to be caused by amino acids.

The amino acids ranked the same quantitatively in roots and thin juice. This was not true of amino acids in the leaves. There appeared to be little useful relationship of leaf amino acids and quality or yield characters.

Nitrate-nitrogen in thin juice seemed to be of more importance than has been reported previously. It was highly correlated with yield characters, K and Na. It was apparent that the nitrogenous compounds measured make varying contributions to total nitrogen in the thin juice, dependent on the nitrogen status of the soil and genotype. There was a portion of the total nitrogen and amino nitrogen which was unaccounted for.

Sodium and potassium increased in every population with increased nitrogen fertilization, but at different rates in different populations. Betaine was the one nitrogenous compound whose quantity was affected more by genotype than by nitrogen fertilization. Genotypes with low betaine should be synthesizable. Quantitites of copper, cobalt, calcium, magnesium, iron and nickel in the leaves at harvest were of little value in determining beet quality or sucrose yield.

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Trends in Nonsucrose Constituents of Central California Beets

I. Analysis of Steffen Factory Liquors

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Introduction

During the last few years diffusion juice, thin juice, carbon-treated thick juice, and molasses produced at a Steffen house were analyzed for nitrogen and inorganic content. It was noticed that the concentration of the nonsucrose constituents varied considerably during the crop year and that the changes occurred at about the same time each year.

This report will deal with the nonsucrose constituents found in various Steffen house liquors throughout the crop year. It will also indicate where in the factory process some of these compounds are eliminated. Significant correlations between certain nonsucrose constituents and pertinent factory data will be given.

Materials and Methods

Samples of diffusion juice, thin juice, and carbon-treated thick juice were taken at two-hour intervals, twice a week, during the day shift. These samples were frozen and stored immediately at -20°C.

Each day a sample of the daily molasses-produced composite from Steffen house operations was obtained and composited on a weekly basis. These samples were stored at room temperature.

Kjeldahl-N. The colorimetric method of Williams (10)[2] was used.

Amino and PCA[3]*-N.* The method developed by Harris (7) was used.

Betaine-N. The ion exchange procedure developed by Carruthers (4) was used to clean up all samples. Betaine-N was then determined colorimetrically by the method of Sano and Matsuno (9).

Nitrate-N. The samples were acidified with phosphoric acid and then passed through a 5 cm bed of Duolite A-7 anion exchange resin in the [OH]- form. The column was washed with 25 ml of deionized water. The nitrate-N was eluted from the column with 20 ml of 2N NaOH. The effluent was neutralized

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[2] Numbers in parentheses refer to literature cited.
[3] Pyrrolidone Carboxylic Acid.

with acid, and the nitrate-N determined colorimetrically using 2,6 xylenol (8).

Sodium and Potassium. Samples were ashed and determinations made using the Beckman DU flame photometer.

Calcium and Magnesium. An EDTA titrimetric method was used.

Chloride. "Quantabs," a product of the Ames Company, were used to determine chloride content of the samples.

Results and Discussion

A plot of the Kjeldahl-N content of diffusion juice, thin juice, carbon-treated thick juice (1) (CAP effluent), and molasses produced is shown in Figure 1. The data are averages of the last three years and are expressed on a nonsugar basis (mgN/100 NS). The figure indicates that during the crop year approximately the same concentration trend of Kjeldahl-N is prevalent in all of the factory liquors investigated. There is, however, one difference which is not readily seen in the figure; that is, the relative concentration of Kjeldahl-N found in diffusion juice during the spring campaign, as compared to that for thin juice. This point will be discussed later.

We have found a highly significant negative correlation between diffusion juice Kjeldahl-N and cossette ape [-0.86].

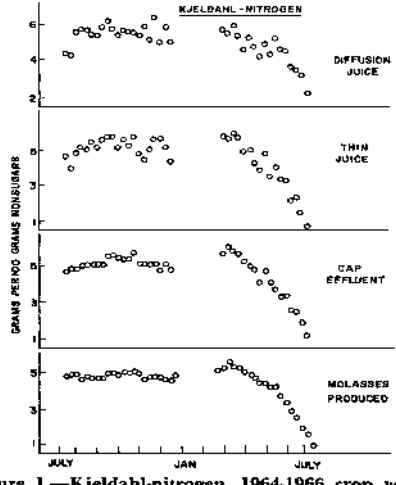

Figure 1.—Kjeldahl-nitrogen, 1964-1966 crop years.

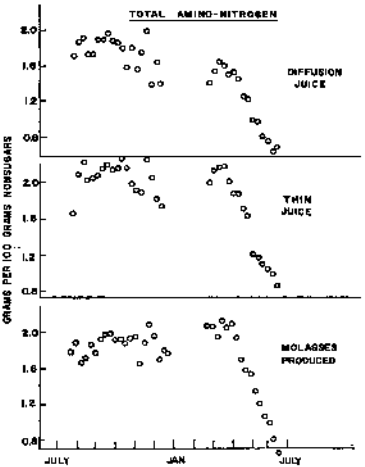

Figure 2.—Total amino-nitrogen, 1964-1965 crop years.

During the 1964 and 1965 crop years the Steffen factory liquors were analyzed for total amino-N. The data, calculated on a nonsugar basis, are plotted in Figure 2.

The CAP effluent data have not been plotted. However, the trends found were similar to those of molasses produced. The same general trend of amino-N is prevalent throughout the factory process. It should be noted that, in general, the concentration trends of amino-N found in the factory liquors are similar to those for Kjeldahl-N. The only difference occurs during the fall campaign. The amino-N curve is slightly more convex than the Kjeldahl-N curve.

A limited amount of work has been done on identifying the individual amino acids associated with Steffen factory liquors. The N-trifluoroacetyl *n* butyl ester derivatives (5) of the amino acids were separated using gas chromatography. The results are shown in Table 1 and are calculated on a solids basis (mM/100 rds) and also as a percent of total amino acids identified.

Twelve amino acids were identified. Alanine, serine, asparagine, and glutamine account for approximately 75% of the total. The table indicates that as the juice proceeds through the factory, the concentrations of glutamine and asparagine de-

Table 1.—Amino acids found in factory liquors, August 1967.

Amino acid	Diffusion juice		Thin juice		CAP Effluent	
	mM/100rds	%	mM/100rds	%	mM/100rds	%
alanine	2.9	20	2.5	23	2.9	31
valine	0.5	3	0.6	5	0.6	6
glycine	0.3	2	0.5	5	0.5	5
isoleucine	0.5	3	0.6	5	0.5	5
leucine	0.3	2	0.3	3	0.2	2
threanine	0.4	3	0.4	4	0.4	4
proline	0.3	2	0.2	2	0.3	3
serine	1.0	7	0.9	8	0.8	9
(asparagine aspartic acid)	2.1	15	1.6	15	1.4	15
(glutamine glutamic acid)	5.6	39	3.2	29	1.5	16
cytosine	0.2	1	0.1	1	0.1	1
lysine	0.2	1	0.1	1	0.1	1

cline. It is well known that glutamine is converted to PCA during the factory process. However, the fate of asparagine is unknown at this time. The relative concentration of alanine increases as the juice passes through the factory. This is due to the decline in the previously mentioned amines.

A total amino-N determination was made on the samples of juice. The results indicate that approximately 70% of the ninhydrin positive compounds are naturally occurring amino acids.

The PCA-N found in factory liquors is formed primarily from glutamine (2,6). As the juice moves through the factory, more of the glutamine is converted to PCA. In Table 2 we have listed the relative concentration of PCA-N as a percent of the total amino-N found in various factory liquors during the 1964 and 1965 crop years.

Table 2.—Relative concentration of PCA-N to total amino-N, 1965 crop year.

	% of Total Amino-N
Diffusion juice	8-10
Thin juice	16-24
Cap effluent	37-48
Molasses produced	49-56

In general, the maximum values shown in Table 2 occur during the spring campaign and minimum values in the fall. This could be caused by an increasing amount of giutamine being present in the beet during the spring campaign relative to the other amino acids.

The nitrate-N contents of diffusion juice, thin juice, CAP effluent, and molasses produced during the 1965 crop year are plotted in Figure 3. The same general trends are evident in all of the juices. Nitrate-N data for diffusion juice correlate

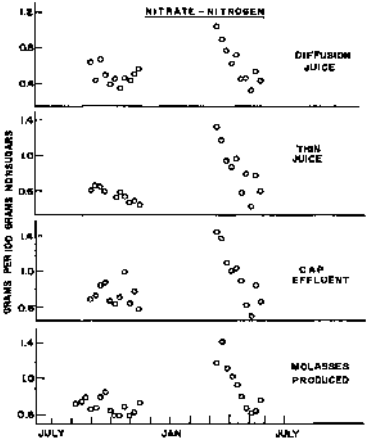

Figure 3.—Nitrate-nitrogen, 1965 crop year.

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Figure 3.—Nitrate-nitrogen, 1965 crop year.

quite well with cossette percent sugar during the fall campaign [—0.75]. The correlation is not significant during the spring campaign.

Figure 4 is a plot of the betaine-N content of the various juices for the 1965 crop year. The data are quite scattered. The only trend which is clearly shown is the decline occurring during the spring campaign. Our data suggest that there is a build up of betaine-N in the sugar end of the factory. This can be seen in Figure 4 and will be discussed later.

A summary of the nitrogen data for the various factory liquors produced during the 1965 crop year is given in Table 3. For comparison, the results have been expressed on a sugar basis (mgN/100 S).

Carbonation feed is denned as diffusion juice plus saccharate cake. The difference in nitrogen content found between carbonation feed and thin juice is the amount removed during the carbonation process. The data indicate that approximately 20% of the total-N in carbonation feed is removed during the carbonation process. This is primarily due to a reduction in the other-N [75%] and betaine-N [12%] fractions.

It has been found that as the spring campaign continues more of the nitrogen associated with carbonation feed is removed during carbonation. A prime example of this occurred during

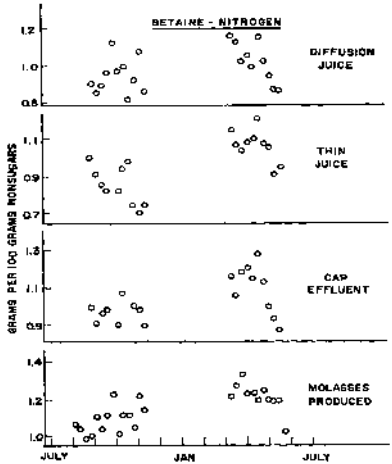

Figure 4.—Betaine-nitrogen, 1965 crop year.

Figure 4.—Betaine-nitrogen, 1965 crop year.

the 1966 crop year. Calculations indicated that the carbonation process consistently removed approximately 15% of the total-N found in carbonation feed throughout the fall campaign.

At the beginning of the spring campaign, nitrogen removal by the carbonation process was essentially the same as noted for fall operations. However, as the campaign progressed, nitrogen removal was accelerated, until during the May-June period approximately 40% of the nitrogen associated with carbonation feed was being removed.

As Table 3 indicates, amino-N and nitrate-N content of carbonation feed are not affected by the carbonation process. It is interesting to note that we have found a reduction of betaine-N during the carbonation process. This is contrary to published data (4).

Table 3.—Summary of nitrogen data, 1965 crop year, mgN/100 S.

Nitrogen	Diffusion juice	Carbonation feed	Thin juice	Cap effluent	Molasses produced	Ratio mol prod/cap
Amino	321	257	253	253	987	**3.9**
Nitrate	**111**	**89**	**90**	**99**	453	**4.5**
Betaine	182	146	122	123	672	5.5
Other	561	450	280	213	964	4.5
Total	**1175**	942	745	688	3076	**4.5**

During the evaporation process, the data in Table 3 indicate a 7% reduction of nitrogen in the juice. This is primarily in the form of ammonia from the conversion of glutamine to PCA. In fact, it accounts for approximately 70% of the nitrogen reduction.

The reduction of nitrogen in juices passing through the evaporation process remains quite constant throughout the crop year. Listed in the last column of Table 3 are ratios of each nitrogen fraction found in molasses produced to that for CAP effluent. If the ratios are different for a particular nitrogen fraction, it is because sugar end operations had a disproportional effect on the particular fraction.

Table 3 indicates that the amount of total-N, nitrate-N, and other-N calculated on a sugar basis increased approximately 4.5 times in molasses produced. However, betaine-N shows a higher increase and amino-N a lower increase. This indicates that the relative concentration of these two nitrogen fractions compared to total, nitrate, and other-N has changed in the sugar end of the factory.

Calculated on a nonsucrose basis, we have found that betaine-N in diffusion, thin, and CAP effluent declines approximately 30% during the spring campaign. However, its decline in molasses produced is only 10%. We feel that a similar situation might exist during the fall campaign. However, it is impossible to calculate because a definite trend does not exist in the diffusion juice, thin juice, and CAP effluent data.

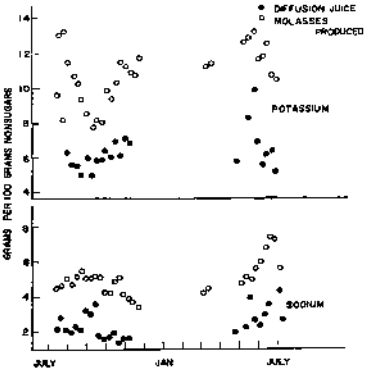

Figure 5.—Potassium and sodium, 1966 crop year.

Our data suggest that betaine-N is being formed in the sugar end of the factory, possibly at the expense of amino-N. However, further work will need to be done to substantiate this hypothesis.

Figure 5 is a plot of the levels of potassium and sodium found in diffusion juice and molasses produced during the 1966 crop year. These data have been calculated on a nonsucrose basis (mg/100 NS). The levels of these elements have also been determined in thin juice and CAP effluent. Because the trends were the same, the data were not plotted.

Probably the most noticeable difference between these data and the previously reported nitrogen data occurs during the spring campaign. While the trend in the level of potassium is downward, similar to that of the nitrogen fractions, sodium shows a marked increase.

A highly significant positive correlation has been found between thin juice sugar and potassium content. The relationship is so good that analysis of the potassium content of waters used for boiler feed may be warranted. This analysis could replace the continuous sugar detector now being used.

The levels of chloride ion found in diffusion juice and molasses produced during the 1966 crop year have been plotted in Figure 6. The trend during the fall campaign is similar to that of potassium.

During the spring campaign the diffusion juice data are so scattered that a trend is not seen. However, in molasses produced the chloride trend follows that of sodium.

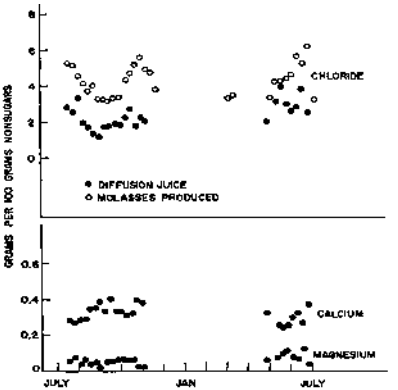

Figure 6.—Chloride, calcium and magnesium, 1966 crop year.

Levels of calcium and magnesium found in diffusion juice during the 1966 crop year are also plotted in Figure 6. The data for the other juices have not been plotted because they would only indicate lime salt levels and the efficiency of the carbonation process in removing magnesium. Magnesium is removed almost quantitatively by the carbonation process.

Although not shown, it should be noted that the trends in the level of calcium found in diffusion juice parallel lime salt concentration found in molasses produced. This would indicate that these salts are in the beet or are produced during the diffusion process. Carruthers has found that approximately 10% of the calcium content of the beet is extracted during the diffusion process (3). Due to the similarity of trends noted previously between calcium in diffusion juice and lime salts in molasses produced, it is possible that the only calcium extracted during the diffusion process is that which is attached to an acid molecule. More work will need to be done on this aspect.

A summary of the inorganic fraction associated with diffusion juice, thin juice, CAP effluent, and molasses produced during the 1966 crop year will be found in Table 4. For comparison reasons the results are expressed on a sugar basis (mg/100 S).

Table 4.—Summary of inorganic data, 1966 crop year, mg/100 S.

Inorganic ions	Diffusion juice	Carbonation feed	Thin juice	Cap Effluent	Molasses produced	Ratio mol prod/cap
Sodium	535	463	524	526	3072	**5.8**
Potassium	1381	1180	1216	1216	6362	**5.2**
Chloride	461	409	465	474	2657	5.6
Calcium	391	332	197	182	846	4.6
Magnesium	77	62			19	
Total	2845	2446	2402	2398	12956	5.4

The ratio of sodium:potassium:chloride in diffusion juice is 1.2:3.0:1. However, this ratio changes in molasses produced to 1.2:2.4:1, indicating a proportional increase in the sodium and chloride level as the juice passes through the factory. This is seen in the last column of Table 5. The sodium level is increased by addition of soda ash for lime salt control. Also, centrifugal wash water is softened by passing it through an ion exchange column where calcium and magnesium ions are exchanged with sodium. This sodium rich water is spun off and the solids eventually end up in molasses. Insufficient washing of the regenerated ion exchange resin used for softening centrifugal wash water could be another source of sodium as well as chloride content in molasses.

The effect of the carbonation process on nonsucrose constituents found in carbonation feed during the fall campaign is

much different from spring operations. To show this effect, the nonsucrose constituents have been categorized as either: nitrogen compounds; inorganic, which includes only the inorganic fraction discussed in this report; and other nonsugars (other NS), which is primarily carbohydrate material and some inorganic. Data used to calculate the amount of nonsugars found in carbonation feed are given in Table 5.

Table 5.—Nonsucrose content of carbonation feed, 1966 crop year.

Nonsugar fractions	Diffusion juice g/100S	Diffusion juice Rel. %	Saccharate cake g/100S	Saccharate cake Rel. %	Carbonation feed[1] Diffusion juice 80% g/100S	Carbonation feed[1] Saccharate cake 20% g/100S
			FALL CAMPAIGN			
Nitrogen Cmpds	8.60*	41	2.71	33	6.88	0.54
Inorganic	3.15	15	0.97	12	2.52	0.19
Other NS	9.31	44	4.55	55	7.45	0.82
Total NS	21.06	100	8.23	100	16.85	1.65
			SPRING CAMPAIGN			
Nitrogen Cmpds	7.77*	49	2.64	27	6.22	0.53
Inorganic	2.44	15	1.21	12	1.95	0.24
Other NS	5.80	36	6.04	61	4.64	1.21
Total NS	16.01	100	9.89	100	12.81	1.98

[1] Carbonation feed is a mixture of diffusion juice and saccharate cake on a sugar ratio of 4:1 in carbonation.

* mg total-N/100S × 7.4.

[1] Carbonation feed is a mixture of diffusion juice and saccharate cake on a sugar ratio of 4:1 in carbonation.

- mg total-N/lOOS X 7.4.

Saccharate cake addition to diffusion juice in the carbonation process is approximately 20% on a sugar basis. Because of the relatively low concentration of nitrogen in saccharate cake due to discharge of glutamic acid to Steffen waste, the concentration of N-compounds in carbonation feed is lower than that found in diffusion juice. Conversely, saccharate cake has a relatively high concentration of nonsucrose constituents in the other-NS fraction. This causes an increase in the proportion of this fraction found in carbonation feed compared to diffusion juice.

Carbonation elimination during the fall and spring campaigns of the 1966 crop year have been calculated for each of the three fractions of nonsucrose constituents associated with carbonation feed (Table 6). The data indicate that approximately 30% of the nonsugars associated with carbonation feed during the 1966 fall campaign are removed by the carbonation process. This was primarily in the form of compounds which are categorized as other-NS [57%].

During the spring campaign approximately 35% of the non-sugars in carbonation feed are removed by the carbonation process. This compares quite well with fall operations. The

Table 8.—Reduction of nonsucrose constituents by the carbonation process, 1966 crop year.

Nonsucrose fractions	Carbonation[1] feed g/100S	Thin[2] juice g/100S	Carbonation elimination g/100S	%
		FALL CAMPAIGN		
Nitrogen Cmpds	6.88	5.36	1.52	22
Inorganic	2.52	1.96	0.56	22
Other NS	7.45	4.51	2.94	39
Total NS	16.85	11.73	5.12	30
		SPRING CAMPAIGN		
Nitrogen Cmpds	6.22	2.34	3.88	62
Inorganic	1.93	2.08	(0.13)[3]	(6)[3]
Other NS	4.64	3.85	0.79	17
Total NS	12.81	8.27	4.54	35

[1] Includes only nonsugars from diffusion juice (Table 5).

[2] Includes nonsugars found in thin juice minus the amount from saccharate cake (last column Table 5).

[3] Increase.

difference between the two campaigns is in the kind of nonsugar removed.

The data indicate that 86% of the nonsugars removed by carbonation during the spring campaign are in the form of nitrogen compounds. The amount (g/100 S) is over 2.5 times that which was removed during the fall campaign even though the amount available in carbonation feed was lower. This indicates that the individual components which make up the nitrogen compounds fraction changed considerably during the crop year.

The data in Table 6 indicate that the inorganic fraction increases 6% during spring campaign operations. This is due primarily to addition of soda ash to thin juice.

This report indicates that the relative concentration of individual nonsucrose constituents found in beets changes considerably during the crop year. These changes probably account for certain processing problems such as color, pH drop, etc. being prevalent during one part of the year and relatively nonexistent during another time.

Summary

During the last few years qualitative and quantitative data have been compiled on approximately 65% of the nonsucrose constituents associated with various Steffen house factory liquors. Levels and trends in the concentration of individual nonsugars throughout the crop year have been shown.

Acknowledgment

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Germination Potential of Monogerm Sugarbeet Seed As Determined by Field Emergence And Laboratory Germination[1]

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Introduction

With the discovery of monogerm sugarbeets (9) in 1948, growers and fieldmen anticipated precision planting throughout the industry. When the monogerm varieties were introduced into commercial production a decade later, there was immediate concern because of low germination in many varieties. When precision planted, these varieties germinated poorly, causing irregular stands. This forced growers to use thicker field plantings which again required thinning as with the old multigerm varieties.

A constant problem in laboratory germination tests of sugarbeet seed has been to obtain full germination potential. This problem has existed for years, but caused less concern in multigerm varieties as usually one or more of the three to four seeds per seedball would germinate. Likewise, since thinning of multigerm varieties was mandatory, excess seedballs were planted to be assured of a stand. When monogerm varieties which consist of a 1:1 seed to fruit ratio were planted, poor germination was readily detected.

There have been many laboratory germination studies on multigerm varieties however, little information has been reported on non-germinability of monogerm seed. Conflicting results have evolved from the multigerm seed studies as to possible causes of poor germination. As a result, the most common factors reported to cause low germination are: chemical inhibitors (4,7, 12,13), physical restrictions of the seedball (10,11), and underdeveloped seeds (14).

When the low germination percentages of monogerm seed were initially reported, the validity of laboratory results was often questioned. Common concerns about these results were: 1) Are laboratories getting the full germination potential from a seed lot? 2) Are laboratory results as high as field emergence results?

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[3] Number in parentheses refer to literature cited.

The objective of this study was to investigate low germination of Oregon-grown monogerm sugarbeet seed using laboratory and field methods to determine germination potential. This potential is defined as the number of fruits having seeds with sufficient embryo and perisperm development to produce a normal seedling under ideal conditions. The methods used were: 1) Standard laboratory germination; 2) Hydrogen peroxide laboratory germination; and 3) Field emergence.

Materials and Methods

Twelve hybrid monogerm sugarbeet varieties were selected to represent the principal commercial seed lines currently grown in Western Oregon. Six of these varieties were composed of natural fruits as harvested and the other six were composed of partially decorticated fruits. Decortication is a process that removes the corky maternal tissue of the fruit.

Field Emergence Study

Plots were arranged in a randomized block design with four replications. Each replication contained one row with 50 seeds for each variety.

The soil was a fine, sandy loam which was approximately the same aggregate size as Hammerton (5) found to give maximum rate of emergence and number of seedlings. The fertility level was determined by soil tests to be adequate prior to seed bed preparation. All seed was treated with Ceresan "M" and planted on August 5, 1966.

Fifty seeds for each row were hand-planted 3 inches apart at a depth of ¾ inch on moist soil. Individual seeds were marked with small stakes at planting for positive identification at emergence. Seedling emergence counts began 7 days after planting and continued for 5 weeks. During maximum emergence (7 to 14 days), daily counts were made to ascertain emergence prior to damage by insects or pathogens. Only emergence of healthy seedlings was recorded and not the survival and eventual field stand.

Laboratory Germination Studies

Laboratory germination tests using the hydrogen peroxide and standard methods were initiated concurrently with field emergence studies. Eight 50-seed replicates of each variety were germinated by each method. Germination tests, using both methods, were conducted at the same time in the same germinator for each variety. Special precautions were taken to avoid contamination during germination tests. This involved cleaning all tools with water before planting and counting, covering blotters and beets while soaking, and covering beets while drying.

Standard Method

Each 50-seed replicate was soaked in 200 ml of water at 25°C for 2 hours. Following a five-second rinse in warm water, seeds were spread on paper towels to dry for 4 hours at laboratory temperatures. Seeds were then hand planted in blotter boxes prepared from 6 X 10 inch blue-gray blotting material (120 pound weight). The blotters were soaked in water for 1 hour and drained for 1 hour prior to folding into boxes as excess moisture can form beads on the fruits. The boxes were formed by folding the longest section across the center, leaving one half for a lid and folding up the three edges of the other half to form a support for this lid.

Planted seeds were placed in a germinator which was maintained at a temperature of 20C for 16 hours and 30C for 8 hours. No watering of medium (blotter boxes) was necessary during the test period. Germination counts were started at 3 days and continued for 14 days after planting. Normal and abnormal seedling evaluation was in accordance with the Association of Official Seed Analyst Rules (1). Diseased abnormal seedlings were removed at the interim counts to prevent contamination of healthy seedlings, but other abnormal seedlings were evaluated at the completion of the test.

After the final count (14 days) ungerminated fruits were hand-cut with razor blades and evaluated for firm ungerminated or underdeveloped seeds. Firm ungerminated seeds filled more than half of the fruit cavity and had white, chalky perisperm and firm white embryo. Underdeveloped fruits had either completely empty cavities or partially developed seeds. The seeds classified as underdeveloped (shrunken) were either discolored and watery or filled less than half of the fruit cavity (14).

Hydrogen Peroxide Method

Fifty seeds were soaked in 200 ml of a 0.1 % hydrogen peroxide solution for 16 hours. After the soak period, the solution was drained off and the seeds were rinsed in warm running water for 5 seconds. They were then placed on paper towels and allowed to dry for 2 hours. The seeds were carefully rinsed and dried to prevent any injury to protruding radicles which may be present at the end of the soak period. The remainder of the test, including planting, counting, evaluation of seedlings, and cutting was conducted as described for the standard method.

Results

Mean germination results of all methods are summarized in Table 1. This table includes germination percentages of each variety as determined in laboratory (columns a and b) and in

Table 1.—Germination results of two laboratory methods and field emergence for 12 varieties of mongerm sugarbeet seed.

Variety	Laboratory results		Difference between laboratory results	Field emergence	Difference between laboratory and field	
	H_2O_2 method	Standard method	(a - b)		(d - a)	(d - b)
	(a)	(b)	(c)	(d)	(e)	(f)
1	77.75	64.50	13.25**	79.50	1.75	15.00**
2-D[1]	72.75	71.00	1.75	79.00	6.25	8.00
3	89.50	81.75	7.75	82.00	(7.50)[3]	0.25
4-D	85.50	81.75	3.75	88.00	2.50	6.25
5-D	85.50	81.00	4.50	84.00	(1.50)	3.00
6	81.50	81.25	0.25	87.00	5.50	5.75
7	69.50	52.50	17.00**	77.50	8.00	25.00**
8-D	81.50	72.25	9.25*	82.00	0.50	9.75*
9	87.00	74.50	12.50**	94.50	7.50	20.00**
10	84.00	76.50	7.50	88.50	4.50	12.00**
11-D	89.00	90.00	(1.25)*	88.50	(0.50)	(1.50)
12-D	75.50	72.50	1.50	73.50	(2.50)	1.00
Mean	81.58	74.87	6.71*	83.67	2.09 ns	8.80*

* Significant difference at the 5% level as determined by Duncans Multiple Range Test.
** Significant difference at the 1% level as determined by Duncans Multiple Range Test.
[1] Partially decorticated varieties are indicated by (D).
[2] Brackets indicate standard results are higher than H_2O_2 results.
[3] Parentheses indicate laboratory results are higher than field results.

field studies (d). Differences between laboratory methods (c) and between the laboratory and field (e and f) are also shown.

The hydrogen peroxide method produced consistently higher results than the standard method for all varieties examined except 11-D. This difference (column c) was significantly higher at the 1% level for three varieties and at the 5% level for one variety. The laboratory methods differed less for those varieties with partially decorticated seed except variety 8-D. When comparing the mean of all varieties for each method, the hydrogen peroxide results were significantly higher than the standard method results at the 5% level. This difference was 6.71%.

The hydrogen peroxide results were not significantly different from field emergence results (column e) for the 12 varieties examined. The standard germination results were consistently lower than the results of field emergence for all varieties except 11-D and significantly lower for five of 12 varieties tested (column f). Four of the five varieties that were significantly lower had natural (not decorticated) fruits. When comparing the mean results of all varieties, the difference between the standard method and field emergence was 8.8% (significant at the 5% level); whereas, the difference between the hydrogen peroxide method and field emergence results was 2.09% (not significant).

Correlation coefficients comparing associations between the three methods are presented in Table 2. The standard and hydrogen peroxide methods were significantly associated (r = 0.862**). The hydrogen peroxide method was significantly associated with field emergence (r = 0.735**); but, the standard method and field emergence were not significantly associated (r = 0.546).

Table 2.—Simple correlation coefficients (r) for laboratory germinations and field emergence of 12 monogerm sugarbeet seed varieties[1].

	Field emergence	Standard method
Standard methods	.546	
Hydrogen peroxide method	.735**	.862**

[1] Values of r necessary for significance: .576 at 5% level, .708 at 1% level.

Germination percentage at the first count (7 days) in the field and at an early count (4 days) for both laboratory methods is shown in Figure 1. These results indicate that the hydrogen peroxide method closely followed the early field emergence for all varieties; whereas, the standard method was generally lower than the other two methods.

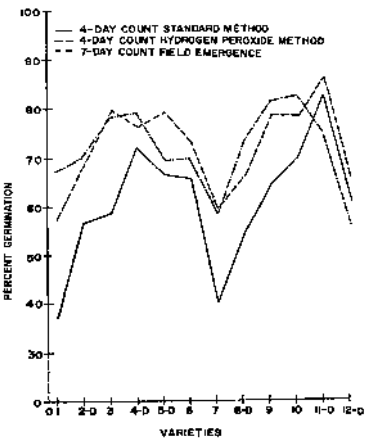

Figure 1.—Initial emergence of seedlings comparing the first count in the field and early laboratory counts of two methods.

Primary factors causing low laboratory germinations are shown on Table 3. Underdeveloped seeds ranged from 4.7 to 20.2% with a mean of 9.5%. The percentage of abnormal seedlings was consistently lower when using the hydrogen peroxide method than the standard method. Six varieties having natural (not decorticated) fruits had significantly higher abnormal seedlings at the 1% level when using the standard method. The mean of abnormal seedlings for all varieties using the hydrogen peroxide method was 4.9%. This was significantly lower at the 1% level than the mean of abnormal seedlings for the standard method which was 11.0%. The laboratory methods did not differ significantly in the number of firm ungerminated seeds at the final count except for variety 8-D. There was also no significant difference between the means of all varieties for firm ungerminated seeds when comparing the two laboratory methods.

Table 3.—Primary factors lowering the laboratory germination results for 12 varieties of sugarbeet seed.

		Abnormal seedlings		Firm ungerminated[1] seeds	
Variety	Underdeveloped seeds[1]	H_2O_2	Standard	H_2O_2	Standard
1	13.0	3.2	16.0**	6.5	4.5
2-D_2	20.2	4.7	6.0	0.5	3.5
3	6.9	1.7	9.2**	2.0	1.0
4-D	8.5	2.7	6.0	3.0	3.7
5-D	6.9	3.0	5.0	4.0	5.5
6	7.4	4.0	10.5**	3.5	2.2
7	11.7	13.5	28.7**	5.5	6.7
8-D	9.0	6.0	9.7	2.7	8.0*
9	4.7	5.2	16.7**	2.0	4.5
10	5.7	7.0	12.5**	4.0	4.2
11-D	7.0	1.7	3.0	0.2	1.5
12-D	12.9	5.5	8.0	5.2	5.2
Mean	9.5	4.9	11.0**	3.2	4.2

[1] As determined by cuitting test.
[2] D = indicates partial decortication of fruits.
* Significantly higher than H_2O_2 method at the 5% level.
** Significantly higher than H_2O_2 method at the 1% level.

Discussion

Contrary to previous speculation, field emergence results compared favorably to the laboratory results when using the hydrogen peroxide procedure. This hydrogen peroxide method was significantly associated with field emergence at the 1% level (Table 2) and did not differ significantly from field emergence results for any varieties examined. Early laboratory counts using the hydrogen peroxide method compare very favorably to the initial seedling emergence in the field (Figure 1). Also, those varieties which germinated poorly in the laboratory (1,7 and 12-D) follow the same trend under field conditions. Therefore, the hydrogen

peroxide method should provide an accurate indication of germination potential for field planting purposes. Every effort was made in the field emergence study to have ideal soil and moisture conditions and positive identification of emerged seedlings. Due to these precautions, the field emergence results were higher than previously expected. Under other soil types and environmental conditions, field emergence of these varieties may be lower than reported in this study.

Laboratory results re-emphasized the complex problems involved when attempting to gain accurate estimates of germination potential for sugarbeet seed. Underdeveloped seeds, abnormal seedlings, firm ungerminated seeds and decortication of fruits are among the factors found to influence or lower the germination results (Table 3).

The Association of the Official Seed Analysts Rules for Seed Testing (1) recommend the standard method (a water soak or wash period) for germination of sugarbeet seed. In this study, the hydrogen peroxide method gave consistently higher laboratory germination results than the standard method (Table 1). Differences between the two laboratory methods were greatest at the initial counts (Figure 1). These differences decreased with time although four varieties were significantly lower at the final count when using the standard method (Table 1). The close correlation between the two methods (Table 2) indicates that the same germination response was measured although the hydrogen peroxide method was usually higher than the standard method.

The chemical action of the hydrogen peroxide on germination of sugarbeet seed was not determined in this study. Stimulation of germination by hydrogen peroxide in other kinds of seed was attributed by Ching (3) to increased respiratory rate. In this study, a stimulus was indicated by the faster germination at the early counts (Figure 1) and some radicle emergence from fruits during the 16-hour hydrogen peroxide soak period. This early emergence of the radicle can be detrimental, however, as additional caution must be taken to prevent radicle damage while planting.

Other researchers (2, 6) have reported the effects of seedborne pathogens on sugarbeet germination. These pathogens usually attack the young seedlings just at emergence from the seedball and cause abnormalities in laboratory tests. The primary reason for lower germinations with the standard method in this study were abnormal (diseased) seedlings. The principal pathogens causing the abnormalities were *Phoma betae* Frank and *Peni-*

cillium spp. The number of abnormal seedlings was consistently higher for the standard method (Table 3) than for the hydrogen peroxide method. This difference was significant for six of the 12 varieties examined. The hydrogen peroxide apparently surface-sterilized the fruits, thereby reducing abnormalities due to surface pathogens.

A decortication process is often used by sugarbeet companies to aid in sizing monogerm seed for precision planting. This mechanical process has also been used to increase germination (2). In these studies there was less variation between the laboratory methods for those varieties having partially decorticated fruits. One reason for this was that the number of abnormal seedlings occurring in decorticated varieties (Table 3) was much lower than varieties having natural fruits. There were no significant differences in number of abnormal seedlings between the laboratory methods for those varieties having decorticated seed. This confirms other results (2, 6), that the seedball maternal tissue is a primary carrier of seed-borne pathogens, and that if portions of this tissue are removed, the effect of pathogens on germination is reduced.

Underdeveloped seeds have been reported as a primary cause for low germination of Oregon-grown monogerm sugarbeet seed (14). Underdeveloped seeds found in these varieties ranged up to 20% (Table 3). These underdeveloped seeds have a direct effect on the germination of a seed lot whether planted in the field or laboratory. As a result germination potential for lots having underdeveloped seeds will always be something less than 100%.

Specific chemical analyses were not conducted to determine the amount and kind of chemical inhibitors present. A good indication of the presense of inhibitors is the number of firm ungerminated seeds at the final count (Table 3). Except for variety 8-D there was little difference in number of firm ungerminated seeds remaining between the hydrogen peroxide and standard methods. Mean percentages of firm ungerminated seeds for all varieties was 3.2% for the hydrogen peroxide method and 4.2% for the standard method. Hence, the role of inhibitors was of less concern than that of abnormal seedlings or underdeveloped seeds.

Summary and Conclusion

Field and laboratory investigations were conducted on the low germination of Oregon-grown monogerm sugarbeet seed. Two laboratory methods (hydrogen peroxide and standard) and field emergence were compared for 12 varieties.

The standard laboratory method, using a water soak, had a slower speed of germination and lower total germination than the hydrogen peroxide method. Differences were less pronounced between the two methods for those varieties having partially decorticated seed.

Field emergence results compared favorably with laboratory germination results when using the hydrogen peroxide method. Standard germination results followed the same trend as the field emergence but were consistently lower. The germination potential measured by the hydrogen peroxide method gave an accurate indication of field emergence.

Primary factors contributing to low laboratory germination were: 1) underdeveloped seed, 2) abnormal seedlings, and 3) firm ungerminated seeds. Underdeveloped seeds, including empty fruits or fruits having shrunken seeds, directly affected the germination potential. Abnormal seedlings were caused primarily by seed-borne pathogens and were highest in those varieties having natural seedballs. Abnormalities were higher when using the standard method than with the hydrogen peroxide method. The number of firm ungerminated seeds was small and the role of inhibitors was considered minor.

No single laboratory method gave an accurate measure of the total germination potential. The hydrogen peroxide method, however, provided an accurate estimate of germination potential, provided a cutting test was conducted after the final count of the germination test. The number of underdeveloped seeds and firm ungerminated seeds determined by the cutting test aided in determining the total germination potential of a seed lot.

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Gallic Acid in Sugarbeet Fruits[1]

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Approximately ten organic compounds isolated from the fruits of sugarbeets, which may be potentially inhibitory to rate of germination, have been reported in various publications (10)[3]. Massart (6) identified by paper chromatography, vanillic, ferulic, p-hydroxybenzoic, and cinnamic acids in aqueous extracts of sugarbeet fruits and showed that they delay or inhibit germination. Koves and Varga (4), also using paper chromatography, identified caffeic, ferulic, p-hydroxybenzoic, p-coumaric and salicylic acids in an ether extract obtained from the water extract of sugarbeet fruits. They were unable to demonstrate the presence of vanillic acid, but they did observe an unknown inhibitor. Recently, Mitchell (7) identified cis-4-cyclohexene-l,2-dicarboximide in sugarbeet fruits and determined that it is inhibitory to lettuce and sugarbeet seeds. Makino and Miyamoto (5) and Miyamoto (8) identified oxalate in sugarbeet fruits and established that oxalate was inhibitory to germination. A statistically significant relationship ($r = -0.6^{**}$) was found between the quantity of water soluble oxalate in the fruit and speed of germination of sugarbeet (10). Evidence for an inhibitory substance(s), other than oxalate, also was obtained in this study (10). Furthermore, the phenolic carboxylic acids appear to play a role in the resistance of the sugarbeet to *Cercospora* leaf spot disease (2).

This paper reports the identification of gallic acid (3,4,5-trihydroxybenzoic acid) in various extracts of sugarbeet fruits by means of thin-layer chromatography (TLC), color reactions, and ultra-violet (UV) light interaction. The amount of gallic acid in the fruit extract was estimated and the influence of gallic acid on germination was determined.

Methods and Materials

Isolation

Extracts of air-dried fruits of a commercial monogerm sugarbeet variety were prepared by eight different procedures. The details of three of the procedures follow.

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station. Approved for publication as Journal article 4579, Michigan Agricultural Experiment Station.

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[3] Numbers in parentheses refer to literature cited.

Extract No. 1

Ten grams of fruits were soaked in 50 ml of water for 18 hours in a refrigerator. The pH of this extract was adjusted to 5 to 6. To 25 ml of extract, 15 ml of 15% $CaCl_2$ was added and the mixture was heated at 80 C for 1½ hours to promote precipitation. The precipitate was filtered and dissolved in 25 ml of 5% HCl. This solution was extracted three times with 15 ml of ether. The ether extracts were evaporated to a volume of 5 ml to concentrate the solutes.

Extract No. 2

Ten grams of fruits were digested with 30 ml of 5% HCl for 4 hours. After filtration, the solution was made alkaline with NaOH and evaporated to dryness under reduced pressure with the temperature held under 50 C. The residue was dissolved in 10% HCl and extracted twice with 15 ml of ether, and then the ether volume was reduced to 5 ml.

Extract No. 3

Ten grams of fruits were placed in 50 ml of water and were heated at 80 C for 12 hours. The solution was filtered, made alkaline with 0.1N NaOH, and evaporated to dryness under partial vacuum at a temperature below 50 C. The residue was dissolved in 10% HCl and extracted three times with 20 ml of ether. The ether extract was neutralized with 0.1N NaOH and evaporated to the aqueous phase (1 ml). The aqueous portion was acidified with H_2S0_4 and passed through a column packed with chromatographic grade Silica (50-200 mesh, G. F. Smith)[4]. The column was eluted with four fractions (a, b, c, d) of 5, 10, 20 and 30% 1-butanol in chloroform. Gallic acid was found in the third fraction (c). This method was adapted from one used by Goncharova (1).

The other procedures included various combinations of those given above, and in addition, initial soaking in 95% ethanol and final extraction in acetone.

The TLC stationary phase (0.25 mm in thickness) was made of Silica Gel G (Merck, Germany), using 30 g of silica gel in 60 ml of water, according to directions in the Operating Manual 103-B of Desaga/Brinkmann[4]. Because the presence of water in the thin-layer materially affects the R_f values, the plates were dried in an oven at 105 C for 2 hours. The dried plates were stored in a desiccator with potassium hydroxide pellets as the dehydrating agent. Before use, the plates were again heated in an oven at 105 C for 1 hour.

[4] The use of specific brand names and procedures does not indicate endorsement of product to the exclusion of others, but indicates procedural methods used.

For the majority of the determinations dried plates were used. In others, the internal water phase of the plates was increased by steaming the layers after spotting and before irrigation, according to the methods employed by Van Sumere, *et al.* (12). The plates were held over a beaker of boiling water until they were uniformly wet. (Care must be used here because oversteaming can destroy the thin-layers). After steaming, the plates were dried at room temperature for about 5 minutes before irrigation was started. (A double front will result if the steamed layer is used immediately.) The R_f values of the phenolic acids are dependent upon the water content of the thin-layers; hence, to get reproducible values, the steaming and the drying of the plates must be carried out under the same conditions each time.

Identification

The four most definitive (i.e. - they showed the greatest amount of separation among the spots) solvent systems used in the TLC procedure were as follows: 1) ethanol, ammonia, water (100:12:16 v/v); 2) benzene, methanol, glacial acetic acid (90:16:8); 3) benzene, dioxane, glacial acetic acid (90:25:4); and 4) methanol, 5N ammonium hydroxide (80:20).

The irrigation of the thin-layer plates was done in a standard Desaga glass chamber fitted with a ground glass cover. The time for development (10-15 min.) is short and is one of the advantages of TLC.

The developed spots were sprayed with bromcresol green (40 mg per 100 ml H_2O adjusted to a blue color with 0.1N NaOH), 1% ferric chloride, or diazotized p-nitroaniline. This latter spray was prepared by mixing 5 ml p-nitroaniline (0.5% in 2N HC1) and 0.5 ml $NaN0_2$ (5%, v/v), and then adding 15 ml sodium acetate (20%, v/v). Acidic compounds react with bromcresol green to produce yellow to green colors depending upon the degree of acidity. Ferric chloride produces shades of gray to brown. Best results were obtained with bromcresol green and ferric chloride sprays.

Poly-hydroxy aromatic compounds will fluoresce or absorb under UV light in the wavelength range 230-290 mμ, depending upon the substituent group or groups attached to the benzene ring, in addition to the OH groups. Some spots develop color after being sprayed with a 2N sodium hydroxide solution. Gallic acid is a pink-violet under UV light; the color is intensified after spraying with sodium hydroxide.

Effect on germination

Three experiments were conducted to ascertain any inhibitory effect of gallic acid on germination and early seedling growth

Table 1.—Chromatographic data for extracts of sugarbeet fruits developed in four solvent systems using TLC.

	Solvent system*							
	1		2		3		4	
Spot	R_f	Color**	R_f	Color**	R_f	Color**	R_f	Color***
1	0.11	Green	0.094a	Yellow	0.90a	Green	0.11	Decolorized
2	0.17a	Yellow	0.195	Yellow	0.20b	Yellow	0.17	Decolorized
3	0.27	Yellow	0.24b	Yellow	0.38a	Yellow	0.27	Grey
4	0.35a	Green	0.35a	Green	0.63ba	Yellow	0.35a	Brown
5	0.52	Yellow	0.39a	Green	0.73a	Yellow	0.52a	Brown
6	0.59b	Yellow	0.56	Green	....	....	0.68b	Brown
7	0.73a	Green	0.83a	Green	....	....	0.75a	Light Brown

* Composition given under Methods and Materials.
** Bromocresol green spray.
*** 1% ferric chloride spray.
a Spot fluoresced under UV light.
b Spot pink-violet under UV light and fulfills criteria for gallic acid.

of sugarbeets. Excised true seeds were placed on filter paper in Petri dishes to which was added 2 ml of either gallic acid solution or of distilled water for the control. Concentrations of gallic acid used were 10^{-1} and 10^{-3}M. Higher concentrations were not used since they would be unrealistically high in terms of the possible concentration in the fruit. Evaporation of liquid was avoided during the 4-day period of growth. Length of root was recorded for each seedling.

Results

Isolation and identification

A total of 180 chromatograms were made in this study. Usually six spots were placed on one plate. The volumes of extract applied to each spot varied from 10 to 200 /il.

After a number of exploratory chromatograms were made, we noted that one spot appeared with regularity on all chromatograms. The literature R_f values, color reactions, and the response to UV light indicated that the spot was gallic acid. Table 1 summarizes the results obtained. After preliminary indications that gallic acid separated on the chromatograms, pure gallic acid was spotted along with the extract spots. The R_f values of the pure gallic acid corresponded closely with the extract spots. Color and UV light reactions gave further positive proof of identification. The spot in each solvent system which corresponds to the position for gallic acid is indicated in Table 2, (11). Gallic acid absorbs UV light resulting in a pink-violet color and the test spots conformed closely. Also, due to the electron releasing substituents (the OH groups on the benzene ring) gallic acid should be a strong acid. The test spots reacted with bromcresol green to produce a yellow color, thus indicating strong acidity.

Since in the literature, ferulic, caffeic, oxalic, vanillic, and p-hydroxybenzoic acids have been reported as being present in

Table 2.—Rf values reported in the literature (11) for a number of acids which have been identified in extracts of sugarbeet fruits.

		Rf Value					
	Solvent system	Gallic acid	Ferulic acid	Caffeic acid	Oxalic acid	Vanillic acid	p-Hydroxy-benzoic acid
1	Ethanol, ammonia, water	0.59	0.52	0.36	0.098	0.55	0.44
2	Benzene, methanol, acetic acid	0.24	0.517	0.38	0.05	0.513	0.52
3	Benzene, dioxane, acetic acid	0.20	0.33	0.17	0.11	0.42	0.48
4	Methanol, ammonia	0.68	0.62	0.56	0.15	0.74	0.50

sugarbeet fruits, solutions of these acids and the extracts were spotted. In a number of the chromatograms, these acids appeared to be present in the extracts.

By using the same volumes and comparing the size of the spots with the sizes of the spots of pure gallic acid of known concentrations, it was possible to estimate the concentration of gallic acid present in the fruit. The concentration of free gallic acid in the extracts of the two seedlots analyzed was estimated to be 10^{-4}M.

A longer period of hydrolysis produced a higher concentration of gallic acid. Extract No. 2 had a high concentration, while samples soaked in 95% ethanol for 12 hours, which isolated only free gallic acid, produced the least. As would be expected, Extract No. 3 produced chromatograms with the best separations.

Up to six other acidic compounds of the phenolic type separated out on the chromatograms. As was pointed out above, some of these corresponded to ferulic, caffeic, vanillic, and p-hydroxybenzoic acids; some were not identified. This is an area for additional fruitful investigation.

Inhibitory action

Root length of 316 sugarbeet seedlings on the two concentrations of gallic acid and the water control did not differ significantly. A trend toward less root growth on 10^{-3}M gallic acid was noted, however. The root length of sugarbeet seedlings 4 days after the start of germination is usually inconsistent under any growth conditions. This variability actually prevents the attainment of statistically significant data with any manageable-sized experiment. In an attempt to reduce the variability, we used true seeds collected from a single plant which had loose seedcaps. The variability still was large and the differences were not significant.

Wheat grown, as a bioassay test (9), at the same concentrations of gallic acid as the sugarbeets also did not differ significantly from wheat grown in the water control.

The source of energy for the germinating sugarbeet embryo is starch. Thus, at least one enzyme is required to hydrolyze the starch into a substance which can be utilized by the embryo. To identify the hydrolyzing enzyme(s) in sugarbeet, Juliano[5] used endosperm of seeds 4 days after start of germination. Using the gel electrophoresis technique, he established that the hydrolytic enzyme is alpha-amylase.

[5] Dr. Bienvenido Juliano, Research Associate, MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan.

Gallic acid at 10^{-1}M concentration markedly inhibited (nearly 90%) and at 10^{6}M inhibited by one-third alpha-amylase activity in vitro[6]. Thus, the highly inhibitory effect of gallic acid on alpha-amylase activity in vitro is in sharp contrast with the statistically non-significant effect on germination and seedling growth in vivo.

Discussion

The literature abounds with evidence that gallic acid is present in numerous plants. Gallic acid readily forms 3-glucogallic, 4-glucogallic, and 5-glucogallic acids. Some gallic acid is found free in plants, but most of it is present as a glucoside.

Phenolic acids have been shown to inhibit germination significantly. The concentrations of the acids and phenols listed below cause a 50% inhibition of the germination of lettuce seeds (3).

Catechol	10^{-2}M	Ferulic acid	$5x10^{-3}$M
Resorcinol	$5x10^{-3}$M	Caffeic acid	$>10^{-2}$M
Salicylic acid	$1.5xl0^{-3}$M	Coumaric acid	$5xl0^{-3}$M
Gallic acid	$5xl0^{-3}$M	Pyrogallol	10^{-2}M

Although in the above data (3), gallic acid of $5xl0^{-3}$M concentration inhibited 50% of the lettuce seeds from germinating, we were unable to demonstrate a statistically significant reduction in germination or seedling growth of sugarbeet in 4 days by placing seeds in 10^{-3}M gallic acid.

Gallic acid strongly inhibited the enzyme, alpha-amylase in vitro, but only slightly inhibited germination and seedling growth of sugarbeet. Although the enzyme is required to hydrolyze the reserve starch to provide energy for germination and growth, these data indicate the danger of extrapolating inhibitory effects on enzyme systems in vitro to the possible effect on germination of the seed and subsequent seedling growth.

Summary

Air-dried fruits of sugarbeet were extracted with water, ethanol, or 5% HC1. The solution was then extracted with ether. These extracts contained gallic acid, based on the R_f values obtained with four solvent systems in thin-layer chromatography, a pink-violet color and absorption under UV light, and color reactions for a strong acid.

The concentration of gallic acid in the samples analyzed was estimated to be approximately 10^{-4}. A concentration of 10^{-3}M slightly inhibited germination and early root growth of sugarbeet. However, in vitro as little as 10^{-6}M gallic acid significantly inhibited alpha-amylase - the enzyme which hydrolyzes starch, source of reserve energy, for the germinating embryo of sugarbeet.

[6] Data of J. M. Sebeson, Earl Mitchell, and F. W. Snyder. See page 556

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The Problem of Underdeveloped Seeds Occurring in Monogerm Sugarbeets[1]

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Introduction

Underdeveloped[3] sugarbeet seeds may be defined as: "Those fruits having either completely empty (seedless) cavities or having partially developed shrunken seeds." Fruits with underdeveloped seeds have a direct effect upon the germination potential of a seed lot, although they cannot be visibly detected from completely filled fruits. In 1967, underdeveloped seeds were reported to be the primary factor contributing to low germination in Oregon-grown sugarbeet seed (14)[4].

Even though these underdeveloped seeds are presently considered to be a serious problem, little information has been reported on their occurrence in either multigerm or monogerm sugarbeet lots. Seedlessness has been reported in other crops, however, and has been noted by some researchers in sugarbeet seed. Grimm (4) found that the number of filled and empty seedballs can be determined by X-ray examination. Hogaboam (9) confirmed Grimm's techniques and found that in 19 monogerm plants examined, all contained some seedless or empty fruits which ranged from 2 to 35%. Hogaboam (9) also reported variation in embryo and perisperm development. Some seeds had a fully developed perisperm but an underdeveloped embryo, while in other seeds the opposite development was observed.

Other cases of empty seedballs or seedless ovarian cavities have been noted by researchers when conducting studies on problems of sugarbeet seed germination. Hogaboam and Snyder (10) found 10% seedless ovarian cavities of seedballs examined in sizing experiments. They also noted that the fruit size, whether it be diameter or thickness, was a poor indicator of the contents of the ovarian cavity. Processors of sugarbeet seed have been aware of empty or hollow seedballs for many years and have attempted to determine the number present in a lot by the "crack" test. This test involves cracking the seedball with a hammer on a heavy steel plate after which the contents are examined for internal seed development (16).

[1] Technical Paper No. 2558, Oregon Agricultural Experiment Station. This work was done under a project supported by the Beet Sugar Development Foundation.

[2] Assistant in Farm Crops and Assistant Professor, Seed Laboratory, Oregon State University, Corvallis, Oregon, respectively.

[3] Previously referred to as undeveloped seeds by author (14, 15).

[4] Number in parentheses refer to literature cited.

This study was conducted to evaluate the over-all problem of underdeveloped seeds. The research objectives were:

1. Evaluation of methods for determining underdeveloped seeds.
2. Determination of the location of underdeveloped seeds on a plant.
3. Investigation of the effects of final processing on the removal of underdeveloped seeds.
4. Survey of the magnitude of underdeveloped seeds in the major sugarbeet seed production areas.

Materials and Methods

To accomplish the objectives outlined, four separate studies were conducted bearing on each objective.

Study I. Evaluation of Methods

Methods used to determine underdeveloped seeds were: 1) Excision method; 2) X-ray technique; and 3) Germination-cutting method. All three methods were conducted on 10 lots of monogerm sugarbeet seed which had routinely germinated less than 85%.

The excision method was conducted on four 50-seed replicates of each lot. The true (naked) seed was removed from the sugarbeet fruit and germinated under standard conditions. This was accomplished by cutting the edge of the pericarp with a razor blade or scalpel after which it was spread apart and the seed removed. Only one replication (50 seeds) was excised at a time due to the slow, tedious procedure of this method.

After excision, the naked seeds were placed in two classes: good and underdeveloped. The good class consisted of seeds which were firm, filled and convex in shape. The underdeveloped class consisted of either no seed development (fruit cavities were completely empty) or shrunken seeds. The shrunken class included seeds that were flattened or concave in shape and filled less than half of the fruit cavity. After removal, both the good and underdeveloped classes were germinated following standard procedures as prescribed by the Association of Official Seed Analyst Rules (2). Daily germination counts were made on the excised seeds for 14 days after which the number of normal seedlings of both classes and the number of non-germinated underdeveloped seeds were recorded.

The X-ray technique involved the radiographing of sugarbeet fruits and evaluating the radiographs for internal seed development. The method used in this study was similar to methods previously proposed by Grimm (4) and Hogaboam (9). A Victor Self-Rectifying Mobile X-Ray Unit having an oil-immersed tube with a Beryllium window was used to take the radiographs. The

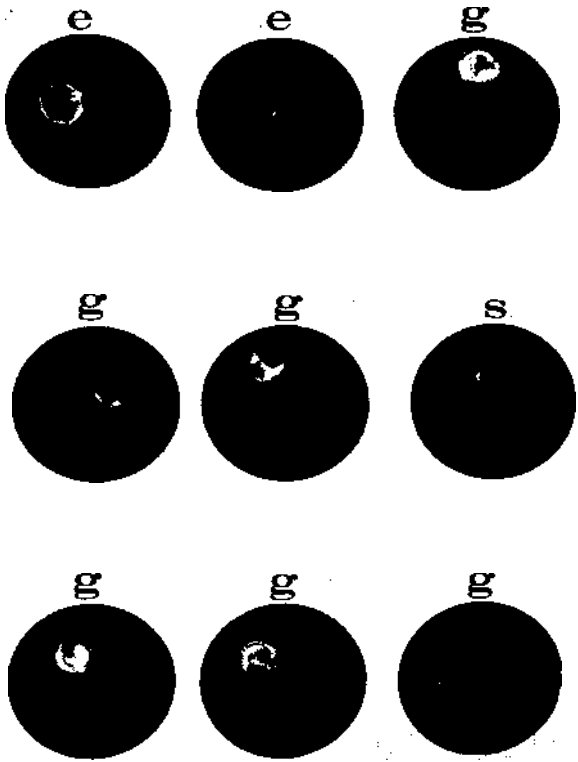

Figure 1.—Radiograph of monogerm sugarbeet seeds showing empty (e), shrunken (s) and good (g) seeds as determined by X-ray technique.

unit was operated at 45 KVP and 3-4MA. The procedure followed was to place the fruits on Industrial Type M film with the seedcap facing down (against the film). The focal distance was 18 inches and the exposure time was 10 seconds. Four 50-seed replicates were radiographed for each lot.

Developed radiographs were examined and estimates of seed development in each fruit were recorded into two classes, good and underdeveloped. Seeds classed as good appeared lighter in color on the radiograph and filled more than half of the fruit cavity (Figure 1). Structural outline of the perisperm and embryo can usually be seen in these good seeds. The underdeveloped class included fruit cavities that were completely empty and appeared dark colored on the radiograph. It also included the partially developed shrunken seeds which filled less than half of the fruit cavity or lacked perisperm or embryo development (Figure 1).

The germination-cutting method involved hand cutting and evaluation of fruits remaining at the final count of a 10-day germination test. Eight 50-seed replicates of the 10 lots were germinated following the hydrogen peroxide procedure (14). At the final count, the fruits with ungerminated seeds were placed

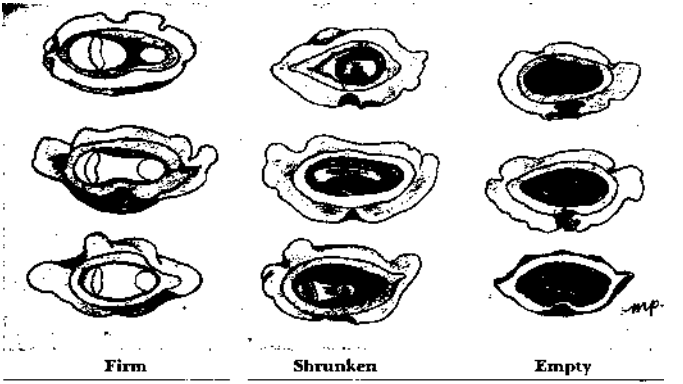

Figure 2.—Cross sections of internal structures of ungerminated seedballs examined at the final count of a laboratory germination test using the germination-cutting method. Firm ungerminated seeds (on left) have firm white embryo and perisperm. Underdeveloped seeds are either shrunken (middle) or completely empty (on right).

with the seedcap down and cut in half with a razor blade. The halves were examined internally for seed development and separated into two seed classes, firm ungerminated and underdeveloped. The firm ungerminated class included seeds which fill more than half the fruit cavity and have white, chalky perisperm and a firm white embryo (Figure 2). The underdeveloped class included fruits having completely empty cavities or those having partially developed shrunken seeds (Figure 2). The seeds classified as underdeveloped (shrunken) were either discolored and watery or filled less than half of the fruit cavity.

The percentages of underdeveloped seeds found by the three methods were compared statistically and significant differences between methods were determined using a Duncans Multiple Range Test.

Study II. Plant Location of Underdeveloped Seeds

To determine the location of underdeveloped seeds on a plant, investigations were conducted on 1) an entire plant, and 2) lateral branches only. Two monogerm varieties (variety 1, SL 126 X 128 and variety 2, F62-569H_3) were utilized for both the plant and branch studies.

Five plants which had the same date of anthesis were selected from each variety for the plant evaluation studies. At maturity, branches were harvested from eight locations on each of the 10 plants. The locations (Figure 3) were as follows: 1) The main stem from the apex down to the first lateral branch (A): 2-6) One primary lateral including side branches from each of five vertical locations on the main stem; Upper (U), mid-upper (MU), middle (M), mid-lower (ML) and lower (L); 7) Several secondary lateral brnaches taken from primary laterals at random locations on plant (S); and 8) Several tertiary laterals taken at random from secondary laterals on the plant (T). After removal of similarly located branches from each of the five plants, they were grouped into the eight groups for each variety and placed on wire mesh screens to dry. When completely dry, the branches of each group were threshed and stored. A standard germination test was conducted using eight 50-seed replicates for each location, and the underdeveloped seeds were determined by the germination-cutting method.

Two-hundred lateral branches from 12 plants in each of two varieties were used to determine the number of underdeveloped seeds at different positions on an individual branch. All of these branches had approximately the same peak flowering date and number of fruits per branch (60-80). At maturity, the branches were removed from the plant and the length of each branch

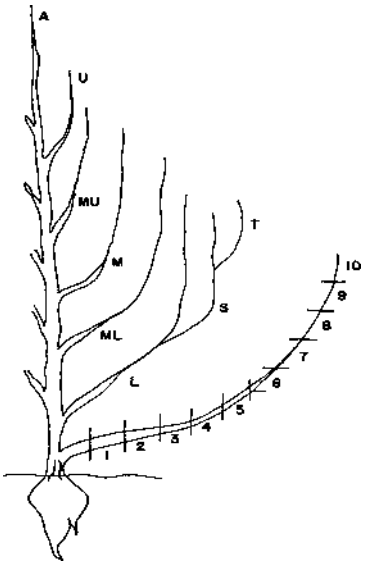

Figure 3.—Diagram of sugarbeet plant indicating the location of branches examined in Study II: Main axil (A); Primary laterals (U, MU, M, ML, L); Secondary laterals (S); and Tertiary laterals (T). Lowest branch indicates the 10 adjoining groups examined on individual lateral branches.

was measured. After measuring, each individual branch was cut into 10 equal sections (Figure 3 - lowest branch) with a razor blade leaving the fruits intact on the stem of each section. Eventually the similar portions from all 200 branches were grouped accordingly into 10 groups. Group number one is that portion of the lateral branch closest to the main axil, whereas group number 10 is the extreme tip of the lateral branch. The groups were dried on wire mesh trays after which the seeds were removed and counted into eight 50-seed replicates for germination following standard germination procedures. Underdeveloped seeds were determined using the germination-cutting method.

Study III. Effects of Final Processing

This study was conducted to determine if the final processing procedures were removing all of the underdeveloped seeds from

a lot. This final processing is completed by the sugar companies and includes cleaning with air blast machines, decortication and sizing procedures. Eight lots of seed having samples of both unprocessed (natural) and completely processed fruits were received from three sugar companies for this study. The processed fruits were in a size class which ranged from 8-10/64ths inch. The underdeveloped seeds occurring in these 16 samples were determined by the germination-cutting method.

Study IV. Survey of Production Areas

A survey was initiated to evaluate the occurrence of underdeveloped seeds in Oregon-produced seed lots in 1965. Forty seed lots which had been tested routinely for germination at the Oregon State University Seed Testing Laboratory also were examined for underdeveloped seeds by cutting at the final count. In 1966 and 1967, all sugarbeet seed lots routinely tested were examined for underdeveloped seeds using the germination-cutting method. This included 138 seed lots in 1966, 41 of which were seed for major commercial usage and the remainder from small stock fields. In 1967, a total of 108 seed lots were examined of which 22 were considered commercially important lots.

This survey was expanded to the Arizona sugarbeet seed production area for the 1966 and 1967 seed crops. In 1966, 10 seed lots were examined for underdeveloped seeds using the X-ray technique and in 1967, 20 seed lots were examined using the same technique.

For both seed production areas data were kept on all samples, and the yearly means were determined for the percent of underdeveloped seeds. For the Oregon production area, yearly means also were determined for percent germination and percent firm ungerminated seed.

Results and Discussion

Study I. Evaluation of Methods

Percentage of underdeveloped seeds as determined by the excision method, X-ray technique and germination-cutting method for 10 lots are shown on Table 1. There was no significant difference between the three methods when the means of all lots were compared. The percentages of underdeveloped seeds, as determined by the excision method, were consistently lower (9 of 10 lots) than those obtained by the other two methods. However, this difference was significantly lower (5% level) for only lot number 1. X-ray results were not significantly different from germination-cutting results for any of the 10 lots examined.

The principal factor causing the lower values with the excision method was the germination of shrunken seeds occurring in the underdeveloped class. It was observed that some shrunken

seeds germinated after excision, whereas seeds of similar development were found ungerminated by the germinating-cutting method. It has been shown that the maternal tissue surrounding the true seed mechanically restricts germination (13). It appears that a similar restriction prevented germination of shrunken seeds in intact fruits when using the germination-cutting method. These seeds did germinate, however, when excised from the fruit. The X-ray results of the underdeveloped seeds compare closely to the results of the germination-cutting method. This indicates that structural seed development gives a good measure of underdeveloped seeds present in a seed lot.

The excision method is a slow and tedious precedure which requires nearly 3 hours for one replication of 50 seeds. Since the method is time consuming and may give misleading results, cannot be considered as efficient or accurate as the X-ray or germination-cutting methods for determining underdeveloped seeds.

A cutting test can be conducted at the final count of a routine germination test (germination-cutting method) or on dry seeds prior to germination or processing. If conducted on dry seeds, it would be similar to the "crack" test that has been used by processors attempting to locate empty fruits while cleaning seed lots. The primary difference between the cutting test and the "crack" test is that each seed is examined for seed development by the cutting test, and those seeds which are only partially developed (shrunken) are classified as underdeveloped. The "crack" test involves forcibly breaking the fruits with a hammer and examining the contents for white perisperm and embryo (16). The seed is usually squashed in this procedure and the results can be misleading. Partially developed shrunken seeds have some white embryo and perisperm present and may be mistaken for good fully developed seed. Therefore, care must be taken when using this test to be certain of adequate seed development before classifying the seed as to germination potential.

The X-ray technique is a fast method for estimating the underdeveloped seeds in a sugarbeet seed lot and is only limited by the availability of an X-ray unit. Several X-ray units are presently marketed which provide the "soft" X-rays that are required for this type of examination. An X-ray units could be incorporated into a processing or cleaning operation to give immediate information on seed development. The recent addition of polaroid film for this X-ray technique could speed up this examination by eliminating the darkroom procedures previously required.

By comparing the germination of the 10 lots (Table 1), it can be concluded that the underdeveloped seeds play a major role in lowering the germination percentage. If these underdeveloped seeds could have been removed, the germination potential would have exceeded 90% in all cases (underdeveloped plus germination).

Table I.—Percentage of underdeveloped seeds a determined by three methods and the germination percent for ten lots of sugarbeet seed.

Lot	Method			Germination[1]
	Excision	Germination-cutting	X-ray	
1	28.0*	35.5	36.0	67.7
2	17.5	22.5	20.0	69.2
3	11.0	12.0	12.0	82.5
4	14.5	17.0	21.0	76.0
5	7.5	10.0	8.0	84.0
6	8.0	11.0	13.0	82.0
7	12.5	13.0	15.0	76.5
8	8.0	13.0	13.0	79.5
9	19.5	19.0	24.0	71.8
10	12.5	12.0	10.0	79.5
Mean	13.9	16.5	17.2	76.9

[1] Hydrogen Peroxide Method 8 x 5 0 seed replicates.

* Underdeveloped seeds determined by excision method siginificantly lower than other two methods at 5% level.

Study II. Plant Location of Underdeveloped Seeds

The percentage of underdeveloped seeds occurring at different locations on a sugarbeet plant (Figure 3) for two varieties are shown on Table 2. These results indicate that underdeveloped seeds occur at all locations on a plant with at least 5% occurring at each location. There was little variation in underdeveloped seeds from location to location except for an increase on the

Table 2.—Percent underdeveloped seeds occurring at different vertical locations on plants of two sugarbeet varieties.

Plant location[1]	Underdeveloped seeds		
	Variety 1 (SL 126 x 128)	Variety 2 ($F62\text{-}569H_3$)	Mean
Main stem (A)	6.0	15.5	10.7
Primary laterals			
Upper (U)	5.5	5.0	5.2
Mid-upper (MU)	7.0	6.5	6.7
Middle (M)	6.5	8.5	7.5
Mid-lower (ML)	7.5	9.5	8.5
Lower (L)	6.0	8.0	7.0
Secondary lateral (S)	6.0	8.0	7.0
Tertiary lateral (T)	9.5	22.5	16.0

[1] See Figure 3 for complete description of locations.

tertiary (T) branches. This increase would be expected due to the late development of these branches and the indeterminate flowering habit of this species. The increase in underdeveloped seeds on the main axil of variety 2 cannot be explained but may be an exception rather than the common result at this location. When additional plants were examined at this location, a similar increase was not noted.

Tabic 3.—Percent underdeveloped seeds occurring at adjoining locations branches for two varieties of monogerm sugarbeet seed.

Location on branches[1]	Underdeveloped seeds		
	Variety 1 (SL 126 x 128)	Variety 2 (F62-569Ha)	Mean
Lower 1	5.0	3.0	4.0
2	6.0	2.0	4.0
3	9.0	8.5	8.7
4	7.5	2.5	5.0
5	9.0	9.0	9.0
Middle 6	9.0	8.0	8.5
7	12.0	13.5	12.7
8	12.0	15.0	13.5
9	12.0	16.0	14.0
Tip 10	19.0	32.5	25.5

[1] For a diagram of the location of each adjoining group on a lateral branch, see Figure 3.

Underdeveloped seeds occurred at all adjoining locations on a lateral branch (Table 3) for both varieties. The percentage of underdeveloped seeds gradually increased toward the tip of a branch. Lower percentages of underdeveloped seeds were found at the locations closest to the main axil, yet the mean of underdeveloped seeds for the two varieties examined was not less than 4% for any one location. The percentage of underdeveloped seeds increased rapidly at the extreme tip of the branch especially for variety 2. As previously mentioned, this was expected due to the indeterminate flowering habit of sugarbeets which does not allow sufficient time for all seed to develop. The tip of a branch (group 10) represented extremely immature seeds or flowers which would be eliminated during normal harvesting operations.

When underdeveloped seeds at various locations on a plant or branch were compared, fruit size had little influence on the internal seed development. Underdeveloped seeds were found in fruits which were as large in size and development as fruits occurring at similar locations which contained completely developed seeds. This would correspond to results by Hogaboam and Snyder (10) that fruit size, whether it be diameter or thickness, is a poor indicator of the content of the ovarian cavity.

Study III. Effects of Final Processing

The final processing of fruits was only partially effective in the removal of underdeveloped seeds for the lots examined (Table 4). In lots 3, 7 and 8, the underdeveloped and empty seeds have readily been removed; whereas, in lots 1, 2 and 5 there is little or no change in the number of underdeveloped seeds from the percentage found in natural fruits. This variation was similar for the three companies that furnished seed for this study.

Table 4.—Effect of final processing on the removal of fruits containing underdeveloped seeds from eight lots of monogerm sugarbeet seed.

Lot number	Percent underdeveloped		Percent after processing
	Natural fruits	Decorticated fruits	
1	15.7	15.0	0.7
2	17.5	11.7	5.8
3	18.5	3.5	15.0
4	8.2	3.0	5.2
5	24.5	24.5	0.0
6	4.2	1.5	2.7
7	44.5	3.5	41.0
8	34.7	5.2	**29.5**

It has been suggested that the underdeveloped seeds occurring in a seed lot can be mechanically removed from that seed lot during the cleaning process. Hammerton (7) reports a higher germination for seeds cleaned with a gravity table than non-cleaned seeds of the same lot. It seems possible that a machine such as a gravity table or a pneumatic air separator may effectively remove underdeveloped seeds since one would expect the unfilled fruits to be lighter in weight than the good, filled fruits. These results substantiate this assumption for certain lots (7 and 8) where very few underdeveloped seeds remained in the lot after processing. In other lots, however, final processing appears to have no effect upon removal of underdeveloped seeds. If the underdeveloped seeds are not removed at final processing, it has an immediate effect on any precision planting of sugarbeet fields. Therefore, it is important to know the number of underdeveloped seeds in a seed lot up to and after final processing. These underdeveloped seeds can be determined very rapidly using either the cutting method or X-ray technique outlined above.

In attempting to correlate fruit size and weight with underdeveloped seed, a simple experiment using a laboratory model pneumatic seed blower was conducted. Each of several lots of seed, known to have underdeveloped seeds, was blown into four weight classes. The first fruits to be removed by the air separation contained primarily underdeveloped (empty) seeds. A com-

plete separation of fruits containing underdeveloped seeds from those fruits having good filled seeds could not be obtained at any setting. As the air was increased on the seed blower, a gradual increase in the percentage of good fruits was found in the blown portion, whereas, underdeveloped fruits were still present in the heavy portion. A percentage of underdeveloped seeds remained in the heavy portion, even at the final blower setting when many filled seeds had been blown out. This offers a partial explanation of why these underdeveloped fruits will not all be removed by final processing.

Study IV. Survey of Production Areas

In Oregon, the significance of underdeveloped seeds on germination was first recognized in 1964 when six lots which had low laboratory germinations were also found to have equally low field emergence results. When fruits from these six lots were excised, various stages of seed development were encountered. This development ranged from completely filled fruits to fruits containing no seed or only a partially developed seed. This latter category was termed as underdeveloped seeds and ranged from 9 to 28% in the six lots examined. Since this percentage was surprisingly high, the determination was continued on all seed lots examined at the Oregon State University Seed Laboratory starting in 1965.

The mean percentage of underdeveloped seeds occurring in all Oregon-produced lots for 3 years (1965, 1966, 1967) was maintained at nearly 20% (Table 5). The mean of firm ungerminated seeds, which are an indication of inhibitors present in a sugarbeet lot, ranged from 2 to 6% for the same period. The mean of all germination results for the three-year period remained at approximately 72%. When comparing underde-

Table 5.—Mean percentages of underdeveloped seed, firm ungerminated seed and germinated seed and germination in Oregon-produced lots for a three-year period. Mean percentage of underdeveloped seeds in Arizona-produced seed for 1966 and 1967.

Year	Lots examined	Underdeveloped seeds	Firm ungerminated seeds	Germination
		OREGON		
1965	40	19.0	3.0	72.0
1966	138	18.2	6.0	72.4
Comm.	41	12.7	6.5	78.2
Stock	97	20.4	5.7	70.0
1967	108	21.3	2.1	72.9
Comm.	22	13.6	2.0	79.9
Stock	86	23.0	2.1	72.9
		ARIZONA		
1966	10	**24.2**		
1967	20	**41.0**		

veloped seeds, firm ungerminated seeds and germination, it can readily be determined that the underdeveloped seeds were the primary cause for low germination for all 3 years in the Oregon production area. If these underdeveloped seeds are added to the germination percentage shown, the germination potential would exceed 90 percent in all cases.

The mean of underdeveloped seeds occurring only in the commercial lots was lower both years (about 13%) than the mean of all lots examined. These commercial lots, even though smaller in number, represent the major acreage of sugarbeet seed produced in Oregon annually. The lots indicated as "stock" lots are only small acreages, which are primarily being increased for future plant breeding use.

Little information was available on the significance of underdeveloped seeds in seed production areas other than Oregon. After examination of the 1966 and 1967 seed crop in Arizona (Table 5), however, it was found that the magnitude of underdeveloped seeds in this production area equaled that of Oregon in 1966 and nearly doubled it in 1967 (41%). Since these two areas produce a major portion of monogerm sugarbeet seed in the United States, it can be concluded that underdeveloped seeds are the primary cause for lowering the germination potential of commercial sugarbeet seed.

Even though underdeveloped seeds are found in nearly all lots of sugarbeet seed, their cause is uncertain. Hills (8) has shown that lygus bugs which feed on the soft developing fruits can cause the embryos to collapse and the resulting fruit cavity to be empty or hollow. He reported that the lygus nymphs do the most damage and that maximum damage is caused during the late bloom to early seed stages of development. He has shown that lygus bugs can be controlled by insecticides but states that rarely can satisfactory results be obtained in one application.

Parthenocarpy has also been suggested as a possible cause for empty sugarbeet fruits. This phenomenon, broadly defined by Gustafson (6) to include any seedless fruit, unless it is definitely known that fertilization and abortion have taken place, has been reported in many crop species including sugarbeets. Causes of parthenocarpy include: 1) Stimulus from foreign pollen of other species (18); 2) Artificial induction by application of synthetic auxins and hormones (5); 3) Stimulation due to extreme temperature conditions (1, 17); 4) Effects due to nutritional level of the plant (1,6,17); and 5) Effects due to insects feeding on the plant (6). It seems possible to theorize that one or more of these may under natural conditions be influencing the sugarbeet plant to cause a parthenocarpic seedless fruit. If

parthenocarpy were the primary cause for underdeveloped seeds in sugar beets, however, only the empty or seedless fruits would result. Since the partially developed seeds are also found in this classification, it is doubtful that parthenocarpy is the only cause.

Nutritional deficiencies of sugarbeet plants in seed production areas have also been theorized as a possible cause for underdeveloped seeds. In other crops, a close relationship of pollen germination and pollen tube growth to some nutrient elements may add support to this hypothesis (11). It has been shown that adequate supplies of boron (11), calcium (3) manganese (12) and other minor elements must be available for adequate pollen germination and growth. If these minor elements are not available, it may eventually affect fertilization and seed set. It seems probable that similar minor elements may affect seed development in sugarbeet plants if they are deficient in the seed production areas.

Summary and Conclusion

The percentage of underdeveloped seeds in a seed lot has a direct affect on its germination potential. When this percentage exceeds 20% as was shown in this study, the germination potential of that lot would never be higher than 80. If the germination percentage is in this range, it will seriously affect the field planting rates throughout the sugarbeet industry. This will force growers to use thicker planting rates instead of precision-spaced plantings.

Underdeveloped seeds have been found in all seed lots produced in Oregon over the last 3 years and are an important problem in Arizona as well. Since little information is presently known as to the cause of these underdeveloped seeds, adequate methods should be available for detecting them prior to germination testing, processing and planting. The X-ray technique and the germination-cutting method can both be used to accurately determine the number of underdeveloped seeds. Since the germination-cutting method was effective, it should be utilized in conjunction with all germination tests conducted on sugarbeet seed to determine the full potential of the seed lot. The X-ray technique provides a fast and simple method for determining underdeveloped seeds which could easily be implemented into a processing plant or a company research station.

Underdeveloped seeds occur at all locations on a sugarbeet plant. They are not directly associated with fruit size. Final processing and sizing may or may not be effective in removing these underdeveloped seeds from a lot.

Underdeveloped seeds are the single most important factor presently contributing to low germination of monogerm sugar-

beet seed lots in the Oregon and Arizona seed production areas. Therefore, to increase the germination potential of monogerm varieties, it is essential that these underdeveloped seeds be eliminated either by mechanical or other means.

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A Sugarbeet X Beta Procumbens Hybrid and Its Backcross Derivatives

R. K. OLDEMEYER[1]

Received for publication July 18, 1969

The species in the *Beta patellares* section of the genus *Beta* remain in the only known source of immunity or high resistance to sugar beet nematode, *Heterodera schactii.* Efforts to find an economical level of resistance to nematode within sugar beet varieties and other races of *Beta vulgaris* have not been successful. Only partial success has been achieved through recurrent mass selection within sugarbeet varieties by which a number of genes each with a minor effect have been concentrated (7)[2].

A number of research stations are apparently continuing a study of the Patellares species in attempt to transfer immunity to sugar beet (1,2,4,5). Most notable of these is the work of Helen Savitsky at Salinas, California (9) in which the major effort is on a polyploid level because it is easier to maintain tetraploid and triploid plants having Patellares germ plasm than diploid ones.

Plants having nematode resistance will be sought in the progeny of the hybrid plants. Resistance might be expected to be transferred as the result of crossing over or the development of trisomies. Irradiation or some other manipulation of trisomies could be used to make a permanent transfer of resistance germ plasm to the sugarbeet genome.

The purpose of this paper is to record the breeding behavior and describe the progeny of a *Beta vulgaris* X *B. procumbens* hybrid referred to as the Turkish wild hybrid because the *B. vulgaris* parent was an annual type commonly found in Turkey. The breeding behavior of sugarbeet hybrids involving this Turkish wild accession did not indicate that cytogenetic differences existed between it and sugarbeet. However, the Turkish wild naturally had cytoplasmic male sterility. The hybrid was first reported by Oldemeyer (6) and was analyzed cytogenetically by Helen Savitsky (8). A description of plants in three generations, F_1, backcross one and backcross two follows:

F_1 *hybrid:*

This F_1 hybrid was diploid (2n = 18), had indeterminant growth and was more or less continually reproductive; morphologically, it was typical of that reported for other Patellares

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[2] Numbers in parentheses refer to literature cited.

hybrids except that it lived on its own roots; it was completely pollen sterile. The F_1 hybrid plant was different from others reported in that it set a relatively large number of fruit when the flowering plant was exposed to sugarbeet pollen (estimated at 10% of the flowers). Examination of the seeds within the fruit indicated seed aborted at all stages of development, but a fraction of them were fully formed and viable. Savitsky's cytogentic investigation indicated, and the breeding behavior confirmed, that this fertility was apparently caused by a process allowing eggs with a non-reduced number of chromosomes to be formed resulting in viable triploid embryos.

Backcross-one plants:

About 150 viable backcross-one plants were produced from a total population of about 300. About one-third of the backcross-one plants died because they produced no secondary roots, behaving as has been reported typically for F_1 hybrids. A smaller group was unthrifty and lived for varying lengths of time, and very few of them grew to produce seed.

The thrifty plants were remarkably uniform morphologically; all were annual, being a little more vegetative than the F_1 hybrid; the new growth was less chlorotic than the F_1 hybrid. No nematode cysts were found on several backcross-one plants after they were grown in nematode infested soil. All the plants were completely male sterile and relatively female sterile although the population was quite variable as to number of fruit set when the plants were exposed to sugarbeet pollen. All plants produced a few seeds; several produced seed enough for a 20 to 80-plant backcross-two population.

Chromosome numbers of 10 of the thrifty plants, counted by Helen Savitsky, indicated they were triploid ($3n = 27$).

Backcross-two plants:

About 700 backcross-two plants were produced of which a few were inviable. The viable plants were of three distinct types. The predominant class was composed of annual or biennial plants which looked like sugarbeets and were fully female fertile. Pollen viability varied from apparently normal to sterile. Neither annualism nor male-sterility could be used as indicating an exchange of germ plasm, because the parental female *B. vulgaris* plant was annual and cytoplasmic male-sterile. Helen Savitsky's counts of the chromosomes of 50 of this class of plants revealed them to be normal diploids ($2n = 18$).

The next most frequent class was one with plants which were sugarbeet-like but were morphologically different from the predominant class. Several types of plants were represented in this class. These plants were generally less vigorous and less

fertile than the predominant class. Some had gigas characteristics, i.e. thick, stubby leaves, thick stems and thick expanded flower bracts. The growth of others was quite fine and leafy. The nine extraordinary plants were found to be trisomic (2n = 19) by Helen Savitsky.

The other class, composed of only four plants, was morphologically similar to the F_1 hybrid and backcross-one plants except they had gigas characteristics and were completely female sterile as well as male sterile. Chromosome counts indicated these plants were tetraploid and aneuploid. Two had 36, one had 37, and one had 38 chromosomes.

Cytogenetic conclusions:

The relatively high female fertility of the F_1 hybrid apparently was conditioned by a genetic complex which allowed nonreduced eggs to be formed. This was also manifested in the formation of triploid eggs for the production of tetraploid backcross-two progeny. It can be presumed the triploid backcross-one plants had two genomes of *Beta vulgaris* and one genome of *B. procumhens* while the tetraploid backcross-two plants had three genomes of *B. vulgaris* and one of *B. procumhens.* Where the extra chromosomes came from in the 37 and 38 chromosome plants of the backcross-two generation, is open to conjecture. Apparently, the only female gametes of the backcross-one plants which were viable, and which produced viable zygotes, embryos and plants were ones with a full complement of *B. vulgaris* chromosomes.

With the isolation and identification of eight or nine of the nine possible trisomics by Butterfass (3), it might be possible, by comparison to known trisomics, to determine if the extra chromosomes in these new trisomies are of *B. vulgaris* or Patellares origin.

Transfer of germ plasm for *B. procumhens* conditioning nematode resistance might occur as the result of crossing over. Helen Savitsky (9) has indicated three or four chromosomes of *B vulgaris* X *B. procumhens* or *B. webbiana* hybrids are associated during meiosis. Whether true pairing, with consequential crossing over and resultant germ plasm transfer occurs has never been proved.

It is presumed that nematode resistance is genetically dominant based on our observations of the resistance of plants known to contain Patellares germ plasm and from the similar observations of others (10,11).

Nematode resistance reaction of derivatives:

All pollen fertile backcross-two plants were allowed to interpollinate while the male-sterile ones were pollinated by sugarbeets.

Seed was harvested separately from individual plants. Some 8000 plants in about 400 of these maternal families were transplanted into nematode infested soil in flats in the greenhouse. A maximum of 25 plants were used per family. After 3 months, the soil was gently washed away from the roots of seedlings and the nematode-free plants selected. The intertwining of roots made selection very uncertain so that it was necessary to transplant 2300 plants individually into aluminum foil pots[3] which were filled with soil uniformly infested with nematodes. After about 6 weeks, the aluminum foil was rolled back and the roots examined. Wide degrees of resistance or tolerance were observed. At this time, several plants appeared to be immune of which about 100 plants from this screening were transplanted to 6-inch clay pots filled with nematode infested soil for further screening. After the plants had grown in clay pots for a time, 25 were selected as having no cysts and were therefore presumed to be immune. No one maternal family was represented by more than two plants in this group. Eventually, however, every plant of this group had at least some cysts.

Remnant seed of backcross-two maternal families was planted in a field known to be uniformly heavily infested with nematodes. Considerable inter-family variation as to vigor was observed. All plants of some families died or were very weak while some families were comprised of nearly normal plants, Figure 1.

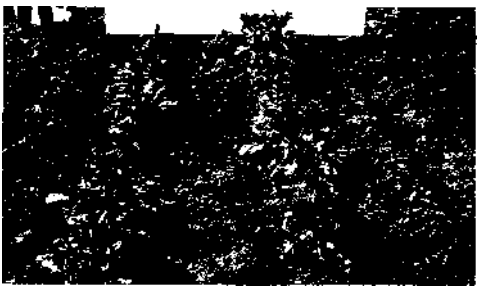

Figure 1.—Five maternal lines derived from a Turkish wild X *B. procumbens* hybrid grown in soil which had a high population of sugarbeet nematodes *(Heterodera schachtii).* Two lines appear to be tolerant while the alternate rows beginning on the left are highly susceptible. Initial stands were about equal.

[3] McFarlane, J. S. 1953. Mimeographed report to the Beet Sugar Development Foundation.

The roots of all the vigorous plants contained many cysts, indicating the vigor was not due to nematode resistance. No cysts were found on the roots of some of the weak plants, but the necrosis of the fine roots indicated nematode damage. There was no way to ascertain whether this variation was caused by germ plasm from *B. procumbens* or whether it existed in the *B. vulgaris* germ plasm.

With the development of a relatively large population of backcross-one plants, the nematode reaction of backcross-two plants was tested directly rather than growing seed from them. No immunity was found among 550 backcross-two plants.

Following the eventual infection of all plants in the greenhouse and the inconclusive observations in the field, the sugarbeet breeders of Great Western discontinued this project and placed remnant seed of all generations except the F_1 in storage.

It was concluded that the full-time services of a skilled cytologist and nematologist would be required before basic or practical results can be realized from a program such as this. It is hoped that eventually this research can be pursued to a successful end.

Monogerm seed from Procumbens hybrids:

The *Patellares* species cannot be considered a source of a new monogerm seed character. Although the seed clusters on hybrid derivatives, in some cases, were not as tightly fused, it is obvious that several fruits occur at one point and that in the case of Patellares species, their being "monogerm" is dependent upon abscission layers between fruits (10). Such abscission layers could be a definite disadvantage in commercial seed production because the seed would drop after ripening. Premature falling of seed was observed on some plants in this material and in other sugarbeet lines. Although the individual fruit of the multigerm clusters of backcross-one plants were loosely bound, the clusters in backcross-two plants were like those occurring on sugarbeet plants.

Summary

Viable backcross-one progeny of a *Beta vulgaris* X *B. procumbens* plant were uniformly sterile, although a few seeds were set and chromosome numbers of those examined were triploid (2n = 27). Chromosome counts of backcross-two plants revealed diploid (2n = 18), trisomic (2n = 19) or tetraploid (2n = 36,37, 38) plants. No nematode immunity was observed to be transferred to backcross-two from *Beta procumbens* as determined by extensive screening of hybrid derivatives.

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Factors Affecting Chloride Uptake and Implications of the Chloride-Nitrate Antagonism in Sugarbeet Mineral Nutrition[1]

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Received for publication May 29, 1969

Chloride has been identified as an essential plant nutrient element for sugarbeets and other plants (7,11).[3] Ulrich and Ohki (11) reported that CI concentrations in sugarbeet petioles of 0.2 milliequivalents per gram (meq/g) were associated with healthy plants but that CI concentrations approximating 0.005 meq/g were indicative of extreme CI deficiency and growth retardation.

When CI is readily available the amounts absorbed by plants greatly exceed the growth-limiting levels. Kretchmer *et al.* (9) state that increasing levels of CI in the substrate resulted in linear increases in CI content of 10 different plant species. This effect was independent of type of substrate or plant species. They reported dry-weight CI concentrations up to about 1.2 meq/g of plant tissue.

Another aspect of CI nutrition is that increasing levels of CI in plants are associated with decreasing levels of NO_3-N and vice versa (1,2,6,9,10,12,13). Thus, there is an antagonism or negative interaction between these two ions in nutrient absorption.

These kinds of observations prompted an investigation into the role of CI in central Washington-grown sugarbeets. Field experiments have been under way for several years which were not initially designed with the study of CI in mind. CI analyses proved to be very useful, however, in explaining some apparently anomalous results. In an earlier report (5), for example, it was shown that increasing rates of K fertilizer (as KC1) resulted in increasing levels of sucrose in the beet roots. Also, NO_3 in the petioles decreased with the K rates. The analytical results for petiole CI (not reported earlier) are presented here in Figure 1. The results for K uptake are repeated for comparison. Figure 1 indicates that CI concentration increased from below 0.2 meq/g to more than 1.2 meq/g. There is no indication in these results of an upper limit of CI absorption. It is apparent also that K and CI uptake are independent of each other.

[1] Scientific paper No. 3284. College of Agriculture, Washington State University, Pullman, Project No. 1607. Portions of this paper were given at the Fifteenth General Meeting of the American Society of Sugar Beet Technologists, Phoenix, February 1968.

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[3] Numbers in parentheses refer to literature cited.

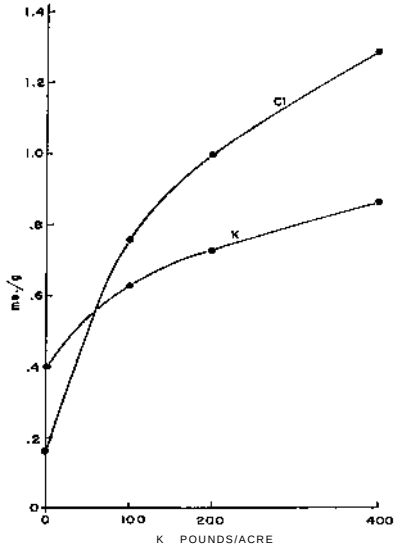

Figure 1.—Effect of applied K (as KC1) on K and CI content of sugarbeet petioles. Each point is the mean of 12 replications. For further details see (5).

The basic objective of a series of experiments conducted in 1967 and 1968 was to evaluate sugarbeet responses to N, P and K fertilizers under central Washington conditions. A preliminary report on the N aspects of this research has been made (4). The purpose of this report is to present the results of CI analyses from these experiments. Factors affecting CI availability and the implications of the $C1\text{-}N0_3$ interaction are discussed.

Procedure

Sugarbeets were studied at 40 different locations in 2 years. Data obtained included soil and petiole analyses and yield results. Experiments at 26 locations were carried to completion. Some information was recovered from several locations which were not carried to completion. Each experimental site consisted of approximately 1 acre in a larger commercial field of sugarbeets. Fields were selected which had not received any fertilizer

since the beginning of the previous growing season. Before crop establishment, the soil was sampled according to a standard procedure which takes into account the wide horizontal and vertical variations in soluble soil constituents which commonly occur in irrigated soils (3). Each experimental area was separated into 0.034 acre size plots and fertilizer treatments were applied according to the schedule given in Table 1. Phosphate fertilizer was used where necessary so that P was not a limiting factor in the growth of beets. In most instances the fertilizer was applied broadcast-plowdown. In a few cases the fertilizer was applied as sidedress after thinning. The fertilizer treatments were organized in a randomized complete block design in four replications. Planting and all cultural practices were done by the grower-cooperator.

Table 1.—Treatment combinations of nitrogen, and potassium fertilizer.

Treatment No.	Element rate—pounds per acre[1]	
	N	K[2]
1	0	0
2	100	0
3	200	0
4	200	200

[1] The element forms were: N, ammonium nitrate, and K, muriate of potash.

[2] When the preliminary soil test indicated a probable K deficiency, treatments 1, 2 and 3 received K, and it was then omitted in treatment 4. Thus, treatment 4 was either a positive or negative check treatment, depending on the location.

The sampling and NO_3 analytical procedures for petioles have been described (5).

CI was measured in distilled water extracts of soil and plant samples by an adaptation of the method given by Johnson and Ulrich (8). Petiole NO_3 and CI are reported in terms of meq/g dry weight. Soil NO_3 and CI are reported in terms of soil test indexes. Each index is the sum of the element ppm in all one-foot increments of soil through the zone sampled.

Results and Discussion

Petiole CI *versus Soil Test* CI

Petiole CI concentrations from the zero-K treatment at the respective experiments are presented in Figure 2 as a function of soil test CI. The results follow the same pattern for both years. In general, as soil test CI goes up the CI content of petiole goes up. The (o) data points are from an area that is irrigated by return-flow water. This water contains 18 ppm of CI. Seasonal fluctuations in this CI load are quite small. All other data points are from experiments that are irrigated with water directly from the Yakima and Columbia rivers. These waters have CI concentrations of 2.0 and 0.6 ppm, respectively.

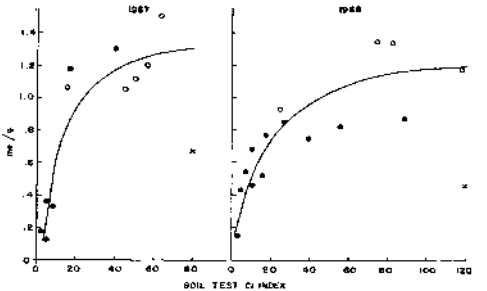

Figure 2.—The relationship between CI concentration of sugarbeet petioles and the soil test CI index is 2 years. Each point is the mean of four replications of treatment No. 1 from different experiments. See text for explanation of different symbols.

Some of the fields receiving low-Cl water have a history of KC1 fertilization. A few fields have undergone some degree of salinization.

The (x) data points are from fields that had a high amount of residual fertilizer N. Estimations from soil analyses indicate that the 1967 high-N field contained 310 pounds of NO_3-N per acre in the rooting zone, and the 1968 high-N field contained 460 pounds per acre. (These conversions are based on an average soil bulk density value of 1.45.) The results from these two sites indicate the depression of CI uptake by NO_3.

It is apparent from Figure 2 that water-soluble CI in the soil is a good indicator of CI availability. It is apparent also that CI availability at a given site can be predicted only after considering irrigation water input and the background of residual NO_3 The latter point will become more evident with subsequent discussions.

A very pertinent question arises from Figures 1 and 2. This has to do with the likelihood of having CI-deficient sugarbeets where both soil and water are very low in CI content. This question is being pursued.

The C1-N0$_3$ *Negative Interaction*

It has been shown that the soil test N index is a fair indicator of N availability for sugarbeets in central Washington (4). In light of this and the foregoing data on CI uptake, the background on which the fertilizer treatments were superimposed should be considered in interpreting the treatment effects. Sugarbeet re-

sponses to the fertilizer treatments, in terms of petiole Cl and NO_3 concentrations, are given in Figures 3, 4 and 5 for seven selected sites. The soil test indexes for these sites are given in Table 2. The selections were made to show the different ways in which the Cl-NO_3 uptake interaction manifested itself.

Table 2.—Soil test indexes for NO_3-N and Cl[1] (background data for Figures 3, 4 and 5).

Soil test index	Figure						
	3a	3b	4a	4b	4c	5a	5b
N	36	69	8	26	13	13	78
Cl	62	17	6	29	2	50	80

[1] Soil test index is the sum of the element ppm in all one-foot increments sampled. Sampling depths varied from 14 to 72 inches, depending on the depth of the rooting zone. The limiting soil layer was caliche.

Figure 3 shows two sets of results where 200 pounds of K per acre were applied with treatments 1, 2 and 3 (Table 1). The K rate was omitted with treatment 4. In Figure 3, as the N rate goes up the petiole NO_3 goes up and petiole CI goes down. With treatment 4, CI content drops and NO_3 increases. Note that without added KC1, there is more petiole CI than NO_3 in Figure 3a and less CI than NO, in Figure 3*b*. This shift is in agreement with the soil test indexes, Table 2.

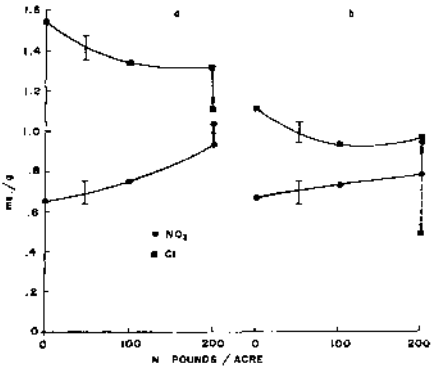

Figure 3.—The effect of NH_4NO_3 and KC1 fertilizer on NO_3 and CI concentrations of sugarbeet petioles. Each point is the mean of four replications. The vertical line segment is LSD (.05). Treatments 1, 2 and 3 included KC1 at 200 pounds per acre of K. The effects of omitting KC1 at treatment 4 are indicated by the arrows. Petioles were sampled (a) 6/22/67 and (b) 6/28/67.

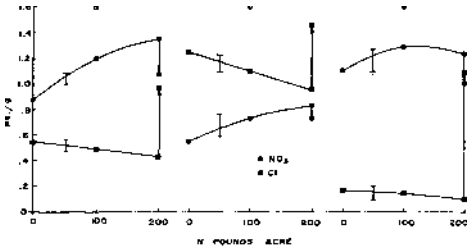

Figure 4.—Effect of NH_4NO_3, and KCl fertilizer on NO_3 and Cl concentrations in sugarbeet petioles. KCl fertilizer was introduced only with treatment 4. Petioles were sampled (a) 6/18/68, (b) 6/21/68, and (c) 6/4/68.

In Figure 4 results are shown for three sites where KCl was introduced only with treatment 4. At each site as the N fertilizer rate increased petiole NO_3 increased and with treatment 4 petiole NO_3 decreased. Petiole Cl decreased regularly with N rates in Figures *4a* and *b* and increased upon addition of KCl. The level of soil Cl at the site represented by Figure *4c* was apparently too low for the N rate to have much effect on Cl uptake. When KCl was added, however, there was a very large effect on petiole Cl. Again, the variation in soil test Cl is reflected in the results of Figure 4 by the relative position of the three Cl response curves.

At the site represented by Figure *5a* there was a large petiole NO_3 response to N rates. In *5b* there was no response to the N fertilizer. The effects of residual N (soil test 18 versus 78) are especially notable in these two sets of results. Petiole NO_3 was not affected by fertilizer KCl in Figure *5a;* this kind of null response occurred infrequently. The petiole NO_3 response to KCl in Figure *5b* followed the more common pattern. In both Figure *5a* and *b* petiole Cl decreased with fertilizer N and increased with KCl. Only one possible exception to this pattern has been noted.

Another kind of contrast in results may be seen by comparing Figures *4c* and *5a.* In both of these cases soil test N was identical and low. The two experiments were in different years, but the shift in positions of petiole NO_3 and Cl is evidence of the different between Cl soil tests: Figure *4c* had very low soil Cl background (coupled with low water CI content); and Figure *5a* had a relatively high soil Cl (as a result of water-borne Cl).

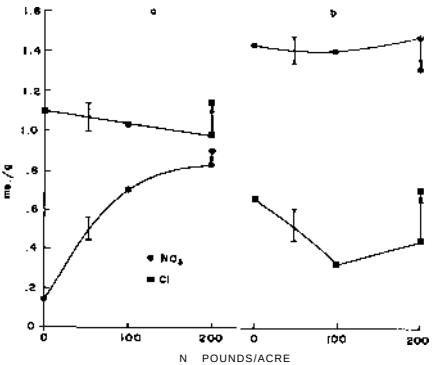

Figure 5.—Effects of NH_4NO_3 and KC1 fertilizer on NO., and Cl concentrations of sugarbeet petioles. KC1 was introduced only with treatment 5. Petioles were sampled (a) 6/22/67 and (b) 6/20 67.

The C1-$N0_3$ negative interaction in plant nutrition has been investigated under solution or sand culture conditions (1,2,9, 10,12,18). From the results presented here it appears that the antagonism clearly manifests itself under field conditions too. It is apparent also that this phenomenon has important implications in assessing either soil test N as an index of N availability or petiole NO, as an indicator of the N nutritional status of the plant. From all the results taken from these experiments, for example, as a first approximation, optimum N fertilizer rates will be 50 pounds per acre higher in the areas irrigated with 18 ppm Cl waters compared to the areas receiving low-Cl waters.

Root Yield and Sucrose Responses

It is not the main purpose of this report to elaborate on the overall crop performance in this series of experiments. It is well to point out in passing, however, that there was one site where there was a possibility of a direct response to K *per se* as judged by petiole K and Na levels and root yields. In addition there were six sites that had a root yield response to 100 pounds of N per acre. (Among the sites represented by the figures there were significant responses to N in *4a, 4c* and *5a.)* In all cases there was a reduction in sucrose percent with added N fertilizer. The negative response of sucrose percent to increased N availability was quite sensitive.

Of direct concern here is the effect of the C1-$N0_3$ interaction on crop performance. In this respect, there was no detectable effect on root growth. There was, however, some effect on sucrose. It was pointed out previously (5) that positive sucrose responses to KC1 fertilization have been noted quite frequently in this area. The increases ranged up to about 0.8%, generally averaging around 0.4%. Similar kinds of responses were observed in the 1967-68 experiments. These are summarized in Table 3. The criteria tor judging the KC1 effect was a difference of 0.2% sucrose between treatments 3 and 5 at each site. (The relative experimental error for sucrose percent is typically lower than for other plant nutrition and growth parameters. Differences of 0.2% sucrose are usually significant at the 1% level.) On this basis 54% of all experiments had a significant increase in sucrose, with 38% showing no effect. Eight percent showed a signficantly lower level of sucrose.

Table 3.—Changes in sucrose percent associated with additions of KCl fertilizer: A frequency distribution.

Type of response	No. of sites	Percent in category
Null	10	38
0.2% or greater increase	14	54
0.2% decrease	2	8
Total	26	100

The nature of the sucrose response to KCl fertilizer was not readily predicted by soil test or by tissue analyses. Nevertheless, when there was a positive sucrose response to KCl fertilization, the most logical explanation is that the sugarbeet is responding to reduced NO_3 uptake. In other words, the change in sucrose is an indirect response to the Cl-containing fertilizer. Many of the other factors that affect sucrose accumulation can be related to N. For example, sugarbeets under furrow irrigation typically show a reduction in sucrose after a rainstorm which was preceded by a prolonged period of no precipitation. In this instance, NO_3-N has been washed from its position of isolation in the dry surface soil into the active root zone. On the other hand, the crop responds with increased content of sucrose to excessive irrigation as a result of N leaching. Thus, several unrelated variables could cancel the apparent effect of KCl on sucrose accumulation.

The foregoing interpretation of the plant Cl-sucrose relationships assumes that there are no direct connections between K uptake and sucrose accumulation beyond the point where K content is non-limiting to normal metabolism. In the examples

given, the experimental design does not allow for a clear-cut separation of the effects of K and Cl. But increases in percent sucrose between treatments 3 and 4 (N_{200} versus N_{200}> K_{200}) occurred when K in the petiole ranged from .55 and 2.14 meg/g. These numbers are well above the tissue-K levels associated with K deficiency (5).

Summary and Conclusions

Experiments involving N and K fertilizers were conducted in commercial sugarbeet fields in 1967 and 1968. These experiments were not designed initially with Cl nutrition in mind but analyses of soils, irrigation waters, and plant materials were made in an attempt to explain some apparently anomalous results.

The investigations on Cl indicate that Cl is readily absorbed by sugarbeets. Uptake of Cl is essentially a exponential function of the amount of water-soluble Cl in the soil. Cl in the plant was also related to the load of Cl in irrigation water. The data indicate that certain areas of central Washington have very low Cl concentrations in both soil and water. This raises a question on the possibility of Cl-deficient sugarbeets in the area.

Results from fertilization with NH_4NO_3 and KC1 confirm the well-documented antagonism in plant uptake of NO_3 and Cl. The way in which the antagonism expresses itself can be related to the background concentrations of $N0_3$ and Cl in the soil. The evidence points up the necessity of analyzing soils and plants for both Cl and NO_3 in order to properly evaluate soil N availability and the N nutritional status of the plant.

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The Effect of Bolting and Seed Stalk Removal On Yield and Sucrose Content of Sugarbeets

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The occurrence of seed stalks in commercial sugarbeet fields is generally considered undesirable by both the grower and company personnel. A small percentage of bolters will make a sugarbeet field look extremely ragged. Growers frequently instruct their labor to remove the seed stalks at the same time they are weeding the beets. Only limited information has been published concerning the effect of bolters or the removal of these seed stalks on yield and sucrose content of sugarbeets.

The weather in February and the first part of March of 1967 was favorable for the planting of sugarbeets in much of the area in which the Utah-Idaho Sugar Company grows beets. However, in the later part of March, April and part of May it was adverse to planting or growing sugarbeets. This adverse weather caused excessive bolting in many of the early planted fields, which afforded an opportunity to evaluate the effect of bolting on the yield and sucrose content of sugarbeets.

Experimental Procedure

To fully evaluate the effect of bolting three separate tests or three parts of the same test were conducted. The first was to compare beets that had bolted with beets that had not bolted. The second was to compare beets from the same field but with different planting dates where the earlier planting dates had bolting beets and the later date did not. The third was to remove the seed stalks from alternate six-row strips and compare the strips that had the bolters removed with strips where the bolters were not removed. All tests were conducted with Utah-Idaho monogerm hybrid #2.

Paired Sample Comparisons

Samples were gathered intermittently from five fields by selecting two non-bolting beets with a bolting beet in between, or two bolting beets with a non-bolting beet in between. All samples were taken from areas that had full stands, which meant that the beets adjacent to the sampled beets and the beets in adjacent rows were not more than 15 inches apart with a minimum of 30 paired samples from each field. Four of the selected fields were in the Garland, Utah area and one was in the Toppenish, Washington area.

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All of the bolting beets would be classed as early bolters as there was well developed seed on all of the seed stalks. (Early bolting beets generally produce seed whereas late bolting beets produce a leafy stalk but no seed).

Four of the five fields yielded sizeable differences in tonnage in favor of the non-bolting beets. In the fifth field (Okada Bros.) the bolting beets gave the highest yield. In the four fields in which the non-bolting beets gave the highest yield, the bolting beets seemed a little tougher with a more coarse, woody fiber than did the non-bolting beets. This was not true in the Okada field where the bolting beets gave the highest yield. In this field there was no discernable difference in the fiber whether the beets had bolted or not. As the results of this field were so different from that obtained in the other fields, additional samples were taken which confirmed that the bolting beets were slightly heavier than the non-bolting beets.

The differences in sucrose were not nearly as pronounced as were the differences in yield. The non-bolting beets had the highest sucrose in three of the fields and the bolting beets in the other two. The average of the five tests, as recorded in Table 1, shows that the non-bolting beets yielded 3.10 tons more per acre, had 0.4% higher sucrose and produced 1,124 more pounds of sugar per acre than did the bolting beets. This confirms the work done in 1952 by Nelson and Deming **(l)**[2].

Table 1.—Comparison of bolting and non-belting beets from paired samples.

	Tons/acre		Sucrose %		Lbs of Sugar/acre		
Grower	Bolters	Non-bolters	Bolters	Non-bolters	Bolters	Non-bolters	Planting date
D. Bingham	15.36	20.19	14.1	14.9	4,332	6,017	March 25
Okada Bros.	26.13	24.39	13.4	13.3	7,003	6,488	March 27
L. Anderson	22.81	27.64	13.7	15.0	6.256	8.292	March 7
A. Jeppson	14.10	21.23	15.6	15.1	4,399	6,411	March 10
Company Farm*	18.53	19.01	13.5	14.2	5,003	5,399	February 5
Average	19.39	22.49	14.1	14.5	5,397	6,521	

* Washington

Difference Caused by Planting Dates

A field of sugarbeets was planted on a company farm in Washington on February 5. Part of the same field was held for weed control experiments and was not planted until March 10. By the first week in July the early-planted part of the field averaged approximately 80% bolters. The part of the field that was planted on March 10 had only an occasional bolter.

Sixteen samples were taken from each of the planting dates

[2] Numbers in parentheses refer to literature cited.

for yield and sucrose determination. Each sample was 15 feet long and contained not less than 12 beets. Yield does not seem to vary much with stands of beets from 75 to 125 beets per 100 feet of row (2).

The results in Table 2 show a large increase in yield from the later planting (March 10) as compared to the early planting (February 5). There was also a small increase in sucrose percentage in favor of the later planted beets that had only a few bolters. The net result was an increase of 1,290 pounds of sugar per acre or a 30% increase over the early planted part of the field.

Table 2.—Comparison of yield and sucrose of a February 5 planting that had 80% bolting and a March 10 planting that had only an occasional bolter.

Tons/acre		Sucrose %		Lbs of sugar/acre	
Bolters	Non-bolters	Bolters	Non-bolters	Bolters	Non-bolters
15.98	20.49	13.53	13.70	4,324	5,614

Removal of Seed Stalks

In the same field that was planted on February 5 and had 80% bolters, the seed stalks were removed from three six-row strips to determine the effect on yield and sucrose. The bolters were cut off on July 12 and good cultural practices continued until the beets were harvested on October 4. Eight 15-foot samples were taken from each strip or a total of twenty-four 15-foot samples were taken from areas having the seed stalks removed and adjacent areas where the seed stalks were not removed.

Table 3 shows a 1.1 ton increase from removing the seed stalks but there was no effect on the sucrose percentage. This resulted in an increase of 292 pounds of sugar per acre or a 7% increase from removing the seed stalks.

Table 3.—Comparison of bolting beets with beets that had seed stalks removed. Eighty percent of beets had bolted.

Tons/acre		Sucrose %		Lbs of sugar/acre	
Bolters	Cut off bolters	Bolters	Cut off bolters	Bolters	Cut off bolters
16.33	17.42	12.3	12.3	3,993	4,285

The test indicated that the tonnage increase would compensate for the expense and effort of removing the seed stalk and there were additional benefits in harvesting and processing these beets.

Discussion

These tests would indicate that non-bolting beets produced more than do adjacent bolting beets. This confirms the work done by Nelson and Deming (1). All of the beets in these tests were from fields that were planted early and bolted early. All of the bolters developed seed and would therefore be classed as early bolters though the bolter beets in one of the fields had most of the characteristics of medium or late bolters.

The second part of the test indicated that the part of the field planted March 10 that had only an occasional bolter produced more than the part of the field planted February 5 that had 80% of the beets bolt. Though much data have emphasized the advantage of early planting, it should be clarified that both dates of February 5 and March 10 would be considered early planting dates for this area. The February 5 date is earlier than the company has ever recommended for the area and this test indicates that a compromise early date of the last of February or first part of March is probably advantageous.

The third part of the test indicates there are some advantages to be gained from removing the seed stalk. The yield was affected more than was the sucrose and the 7% increase in pounds of sugar per acre should more than compensate for the effort and expense to remove the seed stalk. It should also be recognized that some additional benefits are generally gained in harvesting and processing the beet crop.

Summary

The comparisons made in these tests would indicate:

1. Non-bolting beets produce more than adjacent bolting beets.
2. Beets planted early enough that 80% of the beets bolted produced less than beets planted later that had no bolters. Both plantings were actually "early".
3. There are benefits to be gained from the removal of the seed stalks.

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Fertilizer Results on Sugarbeet in the Hereford, Texas Area

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In order to establish some guide lines for fertilizer recommendations to sugarbeet growers in the Hereford, Texas, area, it was necessary to design and carry out a testing program. Beet quality is easily adversely affected by excess nitrogen rates, especially in a climate which does not cool off in the fall as rapidly or markedly as it does in the northern states. Therefore, the program was designed to indicate those rates of nitrogen which would be most likely to give the highest yield while sacrificing the least sugar and purity. The objective of this paper is to report the results of these tests.

During 1966, the treatments were as follows: Three methods of application, *viz.:* 1. All material broadcast before listing up the beds; 2. All material chiselled into the center of the beds; 3. All materials chiselled in the bed 4" off center. Three rates of nitrogen, *i.e.,* 100, 175 and 250 lbs per acre, were applied.

Table 1.—Results of nitrogen and phosphorus application using several methods and rates on three farms near Hereford, Texas. 1966.

Treatment	Acre yield: Gross sugar Lbs/A	Acre yield: Net roots Tons/A	Percent sugar	Percent* purity	No. roots 100' row
Farm H	3980	14.25	14.0	93.2	94.6
Farm J	4845	20.23	12.0		153.8
Farm B	7571	29.32	12.9	90.9	136.5
Nitrogen 100	5407	20.38	13.35	92.58	134.0
Nitrogen 175	5530	21.43	13.02	92.20	128.0
Nitrogen 250	5458	21.95	12.55	91.51	123.0
Method I	5490	21.61	12.86	92.0	115.4
Method II	5514	21.34	12.99	92.3	136.9
Method III	5392	20.82	13.07	92.0	132.6
LSD .05 nitrogen, methods & farms	219	.83	0.28	0.79	7.2
Phosphorus 50	5410	21.00	13.00	92.0	129.2
Phosphorus 100	5520	21.52	12.94	92.2	127.5
General mean	5466	21.26	12.97	92.1	128.3
	Significance of calculated variance ratios				
Among farms	●●	**	*	**	*
Among N rates	NS	**	**	*	NS
Among methods	NS	NS	NS	NS	
Between phosphorus rates	NS	NS	NS	NS	NS

* Thin juice purity Method of Brown & Serro, as reported in Proc. Am. Soc. Sugar Beet Technol. 8: 274.

[1] Agronomists, Holly Sugar Corporation, Sheridan, Wyoming and Hereford, Texas, respectively.

The N source was ammonium nitrate. Two rates of phosphate, 50 and 100 lbs per acre of P_2O_5, were derived from triple superphosphate. There were four replications at three locations in randomized block design. The results of the test are shown in Table 1. Planting date was March 10, 1966 and harvest dates were October 24 and 26, 1966.

The treatments for the 1967 test were as follows: Four nitrogen rates, 75, 150, 225 and 200 lbs per acre, derived from ammonium nitrate. Three phosphorus rates, 0, 50 and 100 lbs per acre of P_2O_3 with triple superphosphate as the source. There were four replications arranged in random blocks at three locations.

The data which were collected are shown in Table 2. The beets were planted about March 20, 1967 and harvested November 2-4, 1967.

Table 2.—Results of nitrogen and phosphorus application using rates of application on three farms near Hereford, Texas. 1967.

Treatment	Acre yield: Gross[1] sugar Lbs/A	Acre yield: Net-[2] roots Tons/A	Percent[1] sugar	Percent[1] purity	No. roots[1] 100' row
Farm I	7492	28.260	13.26	89.06	159.0
Farm II	4492	21.758	13.76	90.86	165.6
Farm III	8189	32.202	12.73	88.05	84.5
Nitrogen 75	6556	24.394	13.57	89.98	137.7
Nitrogen 150	6795	25.504	13.57	89.88	139.8
Nitrogen 225	6774	25.918	13.16	89.00	133.1
Nitrogen 300	6752	26.560	12.76	88.44	135.0
Phosphorus 0	6664	25.482	13.25	89.50	137.4
Phosphorus 50	6817	25.918	13.27	88.88	137.8
Phosphorus 100	6664	25.395	13.26	89.58	133.9
General mean	6725	25.592		89.32	136.4
LSD .05 farm	332	0.66	0.24	0.700	8.43
.01 farm	440	0.87	0.32	0.926	11.14
LSD .05 N rates	NS	0.76	0.28	0.807	NS
.01 rates	NS	1.009	0.36	1.067	NS
Calc. "F" farms	**	**	**	**	**
N rates	NS	**	**	••	NS
Calc. P2O5 rates	NS	NS	NS	NS	NS

Notes: [1] There were no significant interactions among treatments.

[2] On Farm I, 225 lbs N yielded the greatest. On Farm II there were no differences due to N applied. On Farm III 150 lbs N yielded more than the other treatments. There were no other significant interactions.

Before fertilization, soil samples were taken at the three locations, and the results of the tests are shown in Table 3[2].

[2] We wish to express our appreciation to the Texas Agricultural Extension Service and Texas A. & M. University for the soil analyses.

Table 3.—Soil analyses. Fertilizer trials. Hereford, Texas. 1967.

Field	% OM	Pounds per acre		
		P	K	N as NO_3
I - 1	1.4	>132	>1000	51
I - 2	1.25	86	>1000	18
II - 1	1.25	25	>1000	2
II - 2	1.10	15	>1000	2
III - 1	1.22	7	>1000	40
III - 2	1.20	7	>1000	136

pH - 7-8; Ca - 4-5 tons

The data from both years show wide differences in all categories among farms. The lower yield and sugar of 1960 are a reflection of the heavier disease incidence during that season, curly top and *Cercospora* leaf spot.

The very low yield of Farm II, 1967 data, was due to two severe hail storms.

Differences among nitrogen rates followed the usual pattern of decreasing quality factors with increasing rates of nitrogen, and increasing tonnage with increasing nitrogen rates. It is practically and statistically significant that gross sugar did not increase with increasing nitrogen rates. This lack of effect of the increasing N rates was probably due to the high fertility status of many of the farms in this area, where large amounts of commercial fertilizer have been used for years in the production of high market value crops such as onions, lettuce, potatoes and other vegetable crops.

In the 1966 test, the only difference which could be assigned to the method of application was that broadcasting all of the fertilizer materials before listing up the beds caused a marked reduction in the stand of beets. This may have been due to a salinity factor; although it was not a sufficient reduction to cause a yield effect, it could become important in the case of planting to a stand.

In the 1966 test, there was a significant interaction among the nitrogen rates within the three farms, when measuring both gross sugar and tonnage. Farm J had significant decreases in gross sugar due to increasing nitrogen rate. This decrease amounted to about 600 lbs gross sugar per acre.

Farm H showed a significant increase in gross sugar and tonnage with each increment of added nitrogen. Farm B showed the highest yield with the 175-lbs rate and a decrease with the 250-lbs rate of nitrogen.

The planting and harvest dates at all locations were within one week of each other, both in 1966 and 1967.

There were no other significant interactions in the 1966 test.

In the 1967 test, there was only one significant interaction. This was among nitrogen rates within farms when measuring tons of roots. Farm I showed the most tonnage with the 225-lbs rate of N. Farm **II** showed no differences due to applied N. Farm **III** showed the highest yield with the 150-lbs rate.

There seemed to be a trend for a beneficial effect from 50 lbs of P_2O_5 per acre, and on Farm B (1966) a significant increase in tonnage was shown by the 50 lb rate over the zero rate.

Summary and Conclusions

Fertilizer tests were conducted in the Hereford, Texas area during the years 1966 and 1967. These tests were concerned with rates of nitrogen and phosphorus application.

Large variations in production were apparent among farms.

The highest yield of sugar per acre was attained with a rate of nitrogen of between 150 and 200 lbs of N per acre, although there were variations within the farms.

Rates of phosphorus tested did not have any effects on the variable measured, although there seemed to be an indication that at least 50 lbs per acre would be beneficial in some cases.

Acknowledgment

The writers wish to express their gratitude to the Texas Agricultural Extension Service and Texas A. & M. University and particularly, Dr. Art Onken, Assistant Agronomist and Mr. Harvey Walker, Assistant Agronomist, for their very considerable effort in designing the tests, applying the materials and consulting on the results. Without this service, the work would not have been done.

Thin Juice Deliming With Gel Type Cation Exchange Resins

C. D. Fox, L. LANGLOIS, A. PIETROLUCCI[1]

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In 1964, with the objective of getting the best possible use from an evaporator installation which was inadequate in capacity, a thin juice deliming plant was purchased from the Dutch firm Imacti Industrieele Maatschappij Activit N.V. and installed.

In anticipation of a future increase in plant capacity, the service conditions for which the plant was designed were set at 22,000 Imperial gallons of thin juice per hour, with an average lime content of .075 CaO/100 Brix or approximately 15 grains $CaCO_3$ per Imperial gallon. Since the resin chosen, Imac C-12, had an estimated exchange capacity of 20,000 grains $CaC0_3$ per cubic foot/hour, it was estimated that a column containing 176 cu ft would remain on stream 11.4 hours, thereby requiring 2.1/10th regenerations per 24 hours, under average conditions. Therefore, the primary part of the installation consisted of two columns, each containing 176 cu ft of Imac C-12 resin operating in the sodium cycle and using common salt for regeneration.

Imac C-12 is a strongly acidic cation exchange resin obtained by sulphonating the gel type copolymer divinylbenzene; it can be used at temperatures as high as 120°C.

In addition to the two resin columns, the remainder of the installation consisted of a sweetening-off tank, having a capacity of two bed volumes; a buffer-tank for regeneration water storage of approximately four bed volumes; a decantation tank for resin recovery, having a capacity of one bed volume; a concrete wet salt storage tank, having an effective capacity of approximately 40 tons of salt. Also included were the necessary valves, piping and flowraters. A brine filter which was not part of the original installation was added later. All internal surfaces of the entire installation were epoxy-coated.

The material put through the columns was filtered second carbonation juice without sulphitation. Sulphitation of thin juice was discontinued to avoid sulphating of the resin and to put the juice into the evaporators at a pH high enough to avoid inversion. Recently, considerable attention has been given to the effect of temperature on dissociation constants. From Figure 1 (from data provided by S. Stachenko, Canada and Dominion

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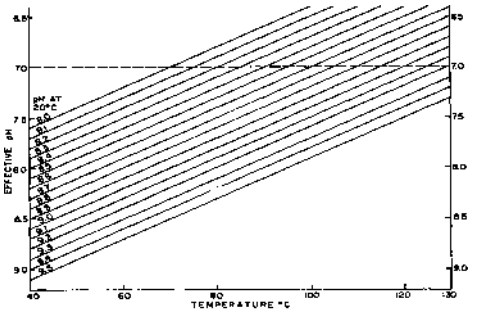

Figure 1.—Temperature effect on pH of thin juice.

Sugar Co. Ltd.), it will be seen that a pH of 9.2 at 20°C is actually an effective pH of 7.0 at 130°C. There was no decrease in pH due to lime removal.

The juice was put into the column at approximately 45 psi. pressure, estimated pressure drop across the column with a flow rate of 90 to 120 gallons per cu ft of resin per hour being 20 to 30 psi. It was planned to put the entire juice production through the resin until the effluent from the bed reached a lime content of 0.03 CaO/100 Brix after which the saturated bed would be sweetened-off and regenerated. It was estimated that a volume of sweetening-off water amounting to approximately twice the bed volume would be required and that the sweetening-off operation could be accomplished in approximately 20 minutes. Actually, the resin is sweetened-off to a solid content of 1° Brix as determined by small hand refractometer.

The sweet water is introduced into the evaporator supply tank through a control valve, operated on the split-range principle by the evaporator rate controller, in such a manner that no water can be added to the evaporator supply tank as long as sweet water is available. The pressure of the sweetening-off water is approximately 50 psi.

After sweetening-off, it is necessary to put the resin in suspension for efficient backwashing and reclassification of the bed. This is accomplished by an air purge requiring approximately 300 cu ft of air at a pressure of 7 psig supplied during a three-minute interval.

Following the air purge, the bed is vigorously backwashed counter-current to the juice flow until the effluent water is clean

and free from suspended matter, including resin fines. This water is sent to discard through the decantation tank which retains the suspended resin and holds it for eventual recovery. After backwashing, the bed is regenerated with brine made from common crushed rock salt which is dissolved in the wet salt storage tank from which it is removed at 24° Be, filtered and diluted to 12° Be before being put through the resin in a counter-current manner.

The theoretical amount of sodium chloride required is 1,984 pounds per regeneration, which in practical operation amounts to approximately 2,000 pounds of common rock salt, or about 800 gallons of saturated brine. The resin is normally in contact with the brine for approximately 1 hour per regeneration.

In the 1967 campaign, a polyphosphate material known as Watcon 116 was introduced with the brine during every fourth or fifth regeneration, at the rate of 1 pint per 100 gallons of 24° Be brine to assist in keeping the beds clean. After the brine treatment, the excess of salt is removed by counter-current backwashing. When the resin is free of salt, the column is again ready for another cycle. However, when the original installation was put into operation, certain unanticipated troubles developed.

The first difficulty concerned the method of brine preparation and withdrawal from the salt storage tank. As originally constructed, the solution water was introduced about midway in the salt bed, vertically, and the brine withdrawn from perforated transite pipes at the bottom of the tank. This resulted in plugging of the brine pump and lines. A revision was made, on advice from the Imacti Engineers, which involved constructing a barrier near one end of the tank, extending about halfway up the vertical height. This created a brine well to which the pump suction was connected through a filter. The solution water was then introduced at the bottom of the tank through the original withdrawal pipes. The brine overflowed the newly constructed barrier into the brine well from which it was pumped. No further difficulty from this source was experienced.

Secondly, it soon became apparent that the filtration of the second carbonation juice was completely inadequate. After a short time on stream, an excessive pressure drop across the column began to develop, reducing the column capacity and requiring the bypassing of more and more juice. This indicated the need for a trap filtration which was set up; at times, the results of the two nitrations were not always perfect.

Another difficulty was the occlusion of air in the juice, due to the fact that the juice flowed directly from the filter receiving boxes to the pump. This difficulty was eliminated by the in-

stallation of a surge tank between the filters and the resin columns, equipped with automatic level control.

A serious difficulty was traced to the fact that the brine was not being filtered. The salt purchased for regeneration has an insoluble content of .25% which is principally shale. When the difficulty became extreme, the columns were unloaded down to the supporting mat upon which was found a hard layer of shale, as much as 3" thick, the only juice flow occurring through an annular ring about 4" wide around the outer periphery of the column. This difficulty was eliminated by the installation of an Immedium brine filter.

After a considerable period of time, it was discovered, during a visit of one of the Imacti Engineers, that both the air for purging the columns and the water for backwashing them, was being supplied in insufficient volume. Inadequate air purge and backwash of the columns results in channelling, giving capricious and completely unsatisfactory results during both the regeneration and service cycles. On each regeneration, the resin must be put in suspension by the air purge and kept there by the backwash during the entire operation which results in some carry-over of resin into the decantation tank. There was a tendency on the part of the operators to limit the amount of carry-over by insufficient air purge and backwash because the resin from the decantation tank had to be recovered by a manual screening operation which turned out to be laborious and time consuming. To solve this problem, a small installation was set up for mechanically screening the resin and returning it to the columns, using a jet pump, pumping through a rubber hose to reduce damage to the resin to a minimum. This installation is shown in Figure 2.

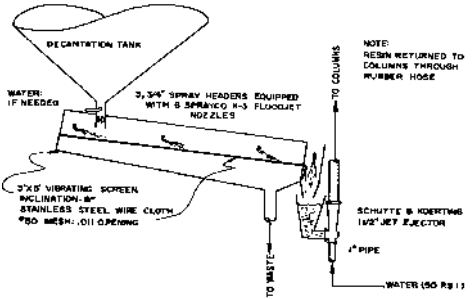

Figure 2.—Arrangement for screening resin.

After solving, or partially solving, the various difficulties, the deliming plant proved to be a valuable addition to the process equipment.

Although some of the various ramifications pertaining to the operation of the deliming plant, during the 4 years it has been in service, might be of interest, the scope of this paper does not permit their discussion. However, one important circumstance should be mentioned. It has never been possible to pass all of the thin juice through the resin columns.

During the 1967 campaign, the effluent from the columns had an average lime salt content of .019 while the lime salt content of the thin juice entering the evaporators was .035. Figure 3 indicates graphically that only 63% of the total juice was passed through the columns. This can be accounted for by one or more of the three following factors:

a) Filtration of the second carbonation juice is still inadequate.
b) The low raw melt is returned to the beet end causing a considerable increase in viscosity.
c) The estimated flow rate through the resin was too high and has not been realized.

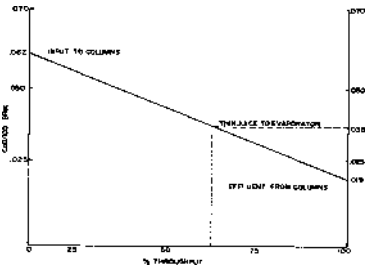

Figure 3.—Performance of Imacti deliming station, 1967 campaign.

Tables 1 and 2 show a very condensed comparison between certain results obtained in the 1963 campaign, which was the last operation without the deliming plant, and the 1967 campaign. As between the two campaigns, there was a 14% increase in slicing capacity. Lime salts decreased by 50%. The brix of the evaporator thick juice increased by 8%. Evaporator boil-outs decreased by 73%. Steam requirements, percent on beets, decreased by 12.2%. Much of the improvement indicated above can be credited to the deliming station.

Table 1.—Comparative data.

	Without deliming 1963"	With deliming 1967
Average daily slice	1341	1528
Lime salts, thin juice	.073/100 Brix	.035
Brix, evaporator thick juice	56.6	61.1
Evaporator boil-outs, Effects/100,000 tons[b]	8.0	2.18
Steam, percent on beets	97.75	85.89
Soda ash, lbs/ton beets	.93	Nil

[a] Last year of operation without deliming.
[b] Individual effects in five-effect system.

Table 2.—Cost of process materials for deliming.

	1967
Cost of salt/ton beets	$ .0360
Cost of polyphosphates/ton beets	.0057
Cost of replacement resin/ton beets[a]	.0046
Total cost per ton beets	$ .0463

[a] Replacement amounted to *1%* of resin in service.

Since the deliming plant has been placed in operation, soda ash has been introduced into process in significant amounts only in 1965 when, due to the working of a large tonnage of extremely badly deteriorated beets, the effective alkalinity dropped as low as —.1311. Prior to the installation of the deliming plant, the introduction of soda ash began when the effective alkalinity reached .005 or lower. This procedure was discontinued and soda was added only when the pH of the low raw massecuite dropped below 7.5.

After the deliming plant was placed in operation, there was a noticeable decrease in the viscosity of the sugar end products. This was particularly noticeable in the fluidity of the massecuites, and had a beneficial effect on centrifugal and crystallizers operations. At the centrifugals, even high purity massecuites discharge from the loading gates without chunking. At the crystallizers, higher densities can be maintained without danger of damage to the stirrer mechanisms.

Conclusion

A deliming station has been described, the operation of which has resulted in reduced evaporator boil-outs, increased hydrogen ion stability and reduced viscosity of sugar end products. Solutions of some difficulties encountered were also described.

Respiration of Sugarbeets Following Harvest in Relation to Temperature, Mechanical Injury and Selected Chemical Treatment[1]

DAVID R. DILLEY, RALPH R. WOOD AND PHILLIP BRIMHALL[2]

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The loss of sugar from sugarbeets during storage is a significant economic factor. Some factors which contribute to this post-harvest loss include dehydration, decay, mechanical injury, sprouting, freezing, respiration and sugar conversions. Although quite variable, sugar losses alone have been estimated (6)[3] at approximately 1 pound of sugar per ton of beets per day over the normal processing period, which may be as long as 4 months following harvest. Another estimate (2) places the average loss at more than 40 pounds of sugar per ton of beets piled, excluding processing loss. There is, therefore, great interest within the beet sugar industry to minimize this loss by improving the handling and storage techniques. Accurate assessment of the various factors contributing to the overall loss is of paramount importance in order to determine where improvement should be made, if proven economically feasible.

Knowledge of sugarbeet respiration and factors which influence its magnitude may be of value in developing improved handling and storage techniques. This study was initiated to determine the influence of temperature, mechanical injury and selected chemicals on the respiration rate of sugarbeets.

Materials and Methods

The sugarbeets (variety GW H-1) employed in these studies were grown near Fremont, Ohio. They were harvested on October 16 and November 6, 1967. Those from the first harvest were subjected to post-harvest chemical treatments, mechanical injury and ethylene treatment. Beets from the second harvest were employed for storage temperature and mechanical injury studies. The beets for both studies upon harvest were topped manually (cut just below the crown), and cleaned by a high pressure water spray to remove the adhering soil. The chemical treatment study consisted of 4 lots of approximately 30 beets each, fairly uniform in size, which received a 15-second immersion in one of the following treating solutions: 1) potassium azide - a res-

[1] Michigan Agricultural Experiment Station Journal Article No. 4406.

[2] Department of Horticulture, Michigan State University, East Lansing, Michigan, Northern Ohio Sugar Company, Fremont, Ohio and The Great Western Sugar Company, Longmont, Colorado, respectively.

[3] Numbers in parentheses refer to literature cited.

piratory inhibtor (3 lbs/100 gal water), 2) Merck HZ 3456 - a morphactin[4] (1 lb/100 gal water), 3) Botran - a fungicide[5] (1.5 lbs/100 gal water), or 4) water only, which served as the control. Within 6 hours of harvest, six samples of three beets from each treatment lot (sample weight of approximately 3 kg) were weighed and placed in the APRIL[6] system (4) to monitor the respiration rate in air at 20°C. Each sample was analyzed twice daily over a period of 10 days. In addition to the above treatments, two samples of four non-treated beets were cut in half lengthwise to simulate mechanical injury and opposite halves placed into two respirometer chambers. Two additional samples of three beets each were gassed with 1000 ppm of ethylene in a $C0_2$ free atmosphere for 12 hours at 20°C prior to commencing respiratory gas analysis.

For the temperature study, 5 replicates of three beets each were placed in respiration chambers at 0, 10, or 20°C, and respiratory analysis was commenced within 6 hours of harvest. In addition, five samples of three beets each were maintained at 0° C for 5 days prior to placement at 20° for respiratory measurements. This was to simulate a change of temperature effect on beet respiration. After $8^1/_2$ days beets maintained continuously at 0, 10 or 20° C, were momentarily disconnected from the gas analyzing equipment and subjected to mechanical injury. Beets in four of the five chambers at each temperature were sliced into equal halves. All samples were returned to the respiratory chambers and reconnected for gas analysis for an additional 5-day period. Beets in the 5th chamber were not cut. Manipulation of the beets in this manner increased the surface area for gas diffusion and was designed to simulate severe mechanical injury that may occur during the harvesting and handling operation. The respiration data was processed by appropriate programs on the CDC 3600 computer. Best fit equations were obtained by the least squares procedure. Cumulative respiration data was obtained for various durations by integration of these equations. In some cases, the cumulative $C0_2$ evolution was obtained by approximate integration employing Simpson's modification of the prismoidal formula (8). Carbon dioxide production rates were converted to sugar loss and expressed as sucrose from the fact that 1 ml of $C0_2$ is derived from 1.274 mg of sucrose, assuming all the $C0_2$ evolved arose from complete oxidation of sucrose.

[4] Methyl-2-chloro-9-hydroxy fiuorene-9-carboxylate.
[5] 2,6-Dichloro-4-nitroaniline.
[6] APRIL - Automated Photosynthesis and Respiration Integrating Laboratory.

Results and Discussion

The influence of various chemicals upon the respiratory rate of harvested sugar beets at 20° C is shown in Figure 1. Little difference was noted in the respiration rate of beets treated with the Botran fungicide or the morphactin compound in comparison to the non-treated beets. Similarity of respiration of control and fungicide treated beets indicates that microbial activity did not

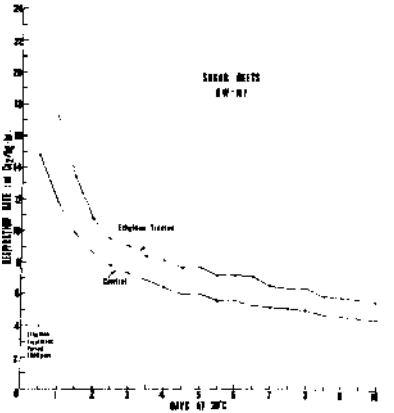

Figure 1.—Influence of post-harvest application of several chemicals on respiration rate of sugarbeets.

contribute significantly to the respiration measurements during the test period. The time-course change in respiration of non-treated beets is typical of results obtained by other investigators (7,10). The respiration rate declined sharply during the first 2 or 3 days following harvest after which a more or less steady respiration rate was observed through the test period of 10 days. The high rate observed for beets treated with potassium azide, a potent respiratory inhibitor, is difficult to interpret. It was presumed that the respiratory rate of potassium azide treated beets would be much lower than the control. However, potassium azide stimulated the respiration rate with the peak rate at approximately 1 $^{1}/_{2}$ days following treatment after which the rate declined, reaching a steady state value after 5 or 6 days. By the 10th day following treatment the potassium azide treated beets were respiring approximately 60% higher than control beets. Similar data (not shown) were obtained for oxygen consumption

for all samples, thereby showing that the various chemical treatments were of no effect upon respiratory quotient. Had potassium azide poisoned mitochondrial oxygen uptake, the respiratory quotients would have been greater than 1. The pronounced effect of potassium azide on the respiration rate is particularly interesting since the beets had only received a 15-second immersion in the treating solution. Further, it is unlikely that the chemical penetrated much beyond the surface in contact with treating solution. Microbial activity would most certainly be destroyed by potassium azide at the concentration employed and, therefore, cannot be considered as contributing to the measured respiration rates.

Fitted lines of the time-course change of respiration rate measured as O_2 consumption are shown in Figure 2 for the control and the potassium azide treated beets. A 4th degree polynominal describes the data for the potassium azide induced respiratory behavior (see footnote to Table 2). A 3rd degree polynominal adequately described respiration of control beets and those receiving Botran or the morphactin.

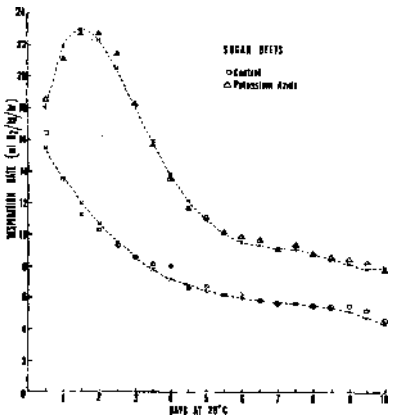

Figure 2.—Influence of potassium azide on respiration of sugarbeets.

The influence of these chemicals on the respiration rate expressed as cumulative respiration is shown in Tables 1 and 2. In Table 1, the respiration rates have been calculated for the 8th

Table I.—Influence of selected post-harvest treatments on respiratory activity of sugarbeets.

Treatment	Respiration rate (static)*20°C		Respiratory quotient
	CO_2 produced	O_2 consumed	
	(ml/Kg/day)		
Control	111	114	0.97
Potassium azide 3 lbs/100 gal	175	193	0.91
Merck HZ 3456 1 lb /100 gal	149	156	0.96
Botran 1.5 lbs/100 gal	127	133	0.95
Sawed (lengthwise)	441	495	0.89
Ethylene (1000 ppm-12 hr)	140	163	0.86

* Based on average respiration rate for the 8th through 10th day following harvest. Beets harvested 10/16/67 at Fremont, Ohio. Variety GW **H1.**

Table 2.—Influence of selected post-harvest treatments on respiration of sugarbeets*.

Treatment	Cumulative respiration		Respiratory quotient
	CO_2 produced	O_2 consumed	
	(ml per Kg per 7 days at 20°C)		
Control	1227	1408	0.87
Potassium azide 3 lbs/100 gal	2150	2526	0.85
Merck HZ 3456 1 lb /100 gal	1362	1554	0.88
Botran 1.5 lbs/100 gal	1390	1594	0.87
Sawed (lengthwise)	6942	7638	0.91
Ethylene (1000 ppm-12 hr)	1578	1815	0.87

•Beets harvested 10/16/67 at Fremont, Ohio. Variety GW H1.

The following equations describe the respiration of sugarbeets in response to the various treatments as a function of time following harvest and treatment. The time variable (^) is expressed as the number of 12 hour cycles. Respiration rate (y) is ml of CO2 or O2 per hour. These equations apply to Figures 1 through 4.

Control:

$$CO_2 = 0.00434\chi^4 + 0.1751\chi^3 - 2.374\chi + 16.15$$
$$O_2 = -0.00561\chi^3 + 0.1544\chi^2 - 2.312\chi + 17.72$$

Potassium azide:

$$CO_2 = 0.000144\chi^5 - 0.00892\chi^4 + 0.2044\chi^3 - 2.0435\chi^2 + 7.2813\chi + 11.28$$
$$O_2 = 0.000184\chi^5 - 0.01154\chi^4 + 0.26818\chi^3 - 2.7505\chi^2 + 10.468\chi + 10.03$$

Botran:

$$CO_2 = -0.003822\chi^3 + 0.1605\chi^2 - 2.306\chi + 17.26$$
$$O_2 = -0.003693\chi^3 + 0.1634\chi^2 - 2.525\chi + 19.87$$

Morphactin HZ-5456:

$$CO_2 = 0.003655\chi^3 + 0.1481\chi^2 - 1.982\chi + 15.56$$
$$O_2 = -0.005244\chi^3 + 0.1985\chi^2 - 2.02\chi + 17.20$$

Control + Ethylene:

$$CO_2 = 0.000815\chi^4 - 0.0123\chi^3 + 0.7861\chi^2 - 6.372\chi + 26.78$$
$$O_2 = 0.001186\chi^4 - 0.06143\chi^3 + 1.1408\chi^2 - 9.1549\chi + 35.30$$

Mechanical injury:

$$CO_2 = 0.00121\chi^5 - 0.06823\chi^4 + 1.5938\chi^3 - 12.098\chi^2 + 35.40\chi + 46.03$$
$$O_2 = 0.00129\chi^5 - 0.07263\chi^4 + 1.4783\chi^3 - 12.675\chi^2 + 35.16\chi + 58.99$$

through the 10th day following harvest, a period of relative stability of the respiratory activity. In Table 2, the data is cumulative respiration during the first week following harvest.

The beets receiving a 12-hour exposure to ethylene at a concentration of 1000 ppm respired at a consistently higher rate throughout the 10-day period following harvest (Figure 3). The time-course change of the ethylene treated beets exactly paralleled that of the control, indicating that no marked qualitative change

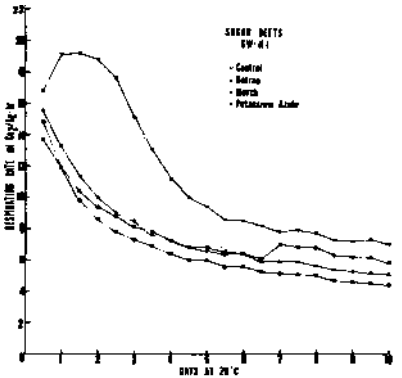

Figure 3.—Effect of ethylene treatment on respiration of sugarbeets as measured by CO_2 evolution.

in metabolism had occurred, but rather the general level of metabolic activity was increased. The effect of ethylene was maintained throughout the course of the experiment although the beets had only received a 12-hour exposure to ethylene. In fruit tissues, ethylene has been observed to induce a increased rate of respiration which is sustained if ripening processes are induced (1). This ethylene treatment was included since ethylene is naturally produced by plant tissues. Furthermore, ethylene may be a product of decay organisms developing on beets during storage. In addition, it has generally been observed that ethylene evolution is stimulated following wounding of a tissue (3). Ethylene did not alter the type of respiration, as the respiratory quotients are the same for ethylene treated as compared to control beets. Cumulative respiration data for ethylene treatment is also given for steady-state respiration, and respiration during the first week following harvest in Tables 1 and 2, respectively.

Mechanical injury caused by cutting the beets lengthwise induced a drastic increase in the respiratory rate of sugarbeets compared to uncut beets as measured by $C0_2$ production and O_2 consumption (Figure 4). There was no effect or injury on respiratory quotient throughout the time course of the experiment, indicating that respiratory metabolism was not quali-

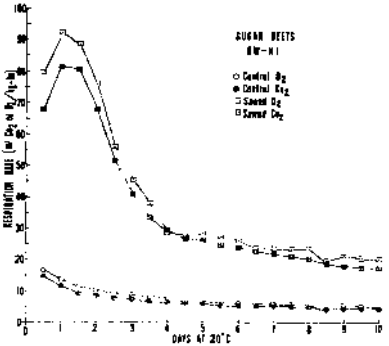

Figure 4.—Influence of mechanical injury on respiration rate of sugarbeets. Beets were cut longitudinally.

tatively altered from that of the control beets. Some of the increase in metabolic activity may be the result of a wound response at the cellular level, but only a small fraction of the total respiring cells were actually injured. The equation that best fits data for the mechanically injured beets is a 4th degree polynominal for both O_2 and CO_2 (see footnote to Table 2). A peak in metabolic activity was reached at approximately 1 day following mechanical injury, after which the respiration declined sharply reaching a steady rate of respiration at approximately 4 days. The steady-state respiration of mechanically injured beets remained from the 4th through the 10th day at a rate approximately 4 times that of the control beets. This respiratory behavior in response to mechanical injury may be explained in terms of a gas diffusion barrier. Cutting the beets exposes more surface area for gas diffusion and, in addition, the newly cut surface allows O_2 to gain entrance into the tissue very readily. Microbial development cannot be ruled out as a factor contributing to the high respiration rate of cut beets since no provision was taken to disinfect the beets. However, as noted earlier, beets dipped in Botran after topping and washing respired at the same

rate as control beets. Furthermore, potassium azide treatment would essentially surface sterilize the cut surface of the beets ruling out microbial activity as contributing to the respiration rate. In fact, potassium azide stimulated respiratory gas exchange as noted earlier. The rapid decline between the 1st and the 4th day following cutting may result from dehydration of the cut surface and creation of a less permeable diffusion barrier. It is not known if wound healing due to periderm formation was responsible for the decrease in respiration rates during this period. From the 4th through the 10th day following cutting, respiration declined slowly, but paralleled that of control beets. The fact that the respiratory rate of cut beets leveled out at a much higher value than control beets is probably explained in that the diffusion barrier at the cut surface, continued to be more permeable than the uncut surface of the control. In addition, the surface area of the cut beets was greater. The magnitude of respiratory activity of cut vs control beets is clearly seen in the data of Tables 1 and 2. The cumulative respiration calculated for the 8th through the 10th day following harvest for the cut beets is nearly four times that of control beets. When calculated for the first 7 days following harvest the difference is even greater in view of the markedly stimulated respiration during the first 4 days following the mechanical injury.

The influence of temperature on respiratory rate of sugarbeets is shown in Figure 5. The time-course change of respiration at 20°C for beets in this experiment paralleled, but was slightly higher than that obtained in the earlier experiment. At 10°C, however, the respiration declined more quickly following harvest and by the 5th day had reached steady-state values. At 0°C the respiration remained low and quite stable for a period of approximately 5 days, after which the rate declined and reached a steady-state value by the 8th day. An interesting aspect of this temperature data is seen in comparing 0 with 10°C respiration. Note that between the 4th and the 6th day following harvest the 10°C respiration is only slightly higher than that at 0°C. At the 5th day the temperature coefficient (Q_{10}) is approximately 1.1 between 0 and 10°C and 2.35 between 10 and 20°C. At $1^1/_2$ days following harvest the temperature coefficient between 0 and 10°C was 2.64 and between 10 and 20°C was 1.3. By the 8th day steady respiration rate was apparent for beets at 0, 10, or 20°C and the temperature coefficient between 0 and 10°C and between 10 and 20 °C was 1.41 and 1.46, respectively. One would expect that the temperature coefficient would remain approximately constant through this 8-day period providing metabolic activity was qualitatively the same. Since it did not, some inter-

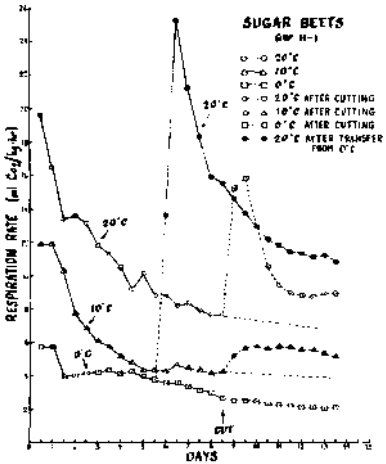

Figure 5.—Influence of temperature, change in temperature, and mechanical injury on respiration rate of sugarbeets as measured by CO_2 evolution.

mediary metabolite may have accumulated at 10°C that acted as a restraint on metabolism. Alternatively, conversion of oligisaccharides to readily metabolizable monosaccharides may account for the relatively high respiration rate at 0°C, since sugars accumulate in beets as in other storage organs at low temperatures (5). Further evidence is the respiratory response upon transfer from 0 to 20°C. Beets maintained for $5\frac{1}{2}$ days at 0°C and then transferred to 20°C exhibit markedly stimulated respiratory activity in comparison to those beets not exposed to the low temperature. Accumulation of readily metabolizable carbohydrates such as reducing sugars at the low temperature may be responsible for this increased respiration rate at the higher temperature. The decline in respiration at 20°C parallels, but remains at a higher level for beets receiving a low temperature treatment in comparison to those not.

Respiration data for mechanically injured beets at 0, 10 and 20 °C are also given in Figure 5. The respiratory response to mechanical injury at these three temperatures is further evidence that gas diffusion may be a limiting factor to the metabolic activity of sugarbeets. The metabolic demands for oxygen at

low temperature is sufficiently low, however, that gas diffusion is not a limiting factor in respiration. Furthermore, at the lower temperature, a greater quantity of oxygen would be dissolved in the tissue fluids. Therefore, as observed, beets cut at 0°C should have a respiration rate similar to intact beets. At 10°C, however, the cut beets exhibited a higher respiration rate than uncut beets, and at 20°C the effect was still greater. This response is what one would expect if the surface of the beet were acting as a barrier to entrance of O_2. The intercellular air space volume of beets and other root crops is very low, being of the order of 0.5 to 2% of the total volume (9). In tissues with a low intercellular air space volume, diffusion of gases into or out of these tissues is limited. The bulk of the gas diffusion must take place in solution and the rate is much slower than in a gas phase. Microbial activity may contribute to the respiration rates obtained in the temperature study since no special precautions were taken to retard their development. No visual signs of fungal or bacterial contamination were evident.

It is interesting to note that the effect of mechanical injury on respiration at 20°C was much less pronounced for beets harvested November 6 than for beets harvested October 16. For the early harvest of October 16, the respiration rate of mechanically injured beets reached a value of approximately 90 ml of COo/kg/hr on the 1st day; whereas control beets at the same time had a respiration rate of approximately 12 ml/kg/hr. Mechanically injured beets for the second harvest (November 6, reached peak values of approximately 16 ml/CO_2/kg/hr vs control values of approximately 8 ml within 1 day following mechanical injury, then declined rapidly. Steady-state respiration values were apparent in 3 days while those of beets harvested October 16 required 6 days. One marked difference between these two experiments was, however, that the beets of the second harvest were held for $8^1/_2$ days prior to mechanical injury. The respiration rates of control beets at 20°C for both the first and second harvest were similar (compare 20°C respiration rate in Figures 2 and 5) during the first $8^1/_2$ days. In fact, the effect of change in temperature from 0 to 20°C for beets of the second harvest induced a more marked respiratory response than cutting.

The marked difference in respiratory response to mechanical injury of beets at these two harvests may indicate that gas diffusion becomes less of a limiting factor on respiration rate as the harvest season progresses. This may be a result of an increase with growth in the amount of intercellular air space which markedly increases gas diffusion in bulky storage organs. Greater availability of readily metabolizable substrates in beets at a less

mature state in development may be responsible for the marked stimulation of respiration that is observed upon cutting. Further studies are needed to establish the factors responsible for the respiratory response to mechanical injury.

The similarity in the time-course change of respiration of mechanically injured beets to beets receiving a post-harvest treatment with potassium azide suggests an indirect effect of potassium azide, perhaps upon the permeability characteristics of the beets. The respiratory curves in both instances are described by similar 4th degree polynominals. Furthermore, the time required to reach the respiratory peak and to establish steady-state respiration are similar, differing in magnitude only. If microbial activity was responsible for the high respiration rate of mechanically injured beets, it is highly unlikely that the respiratory kinetics would so closely parallel that of the azide treated beets which should be quite free of microbial activity. As mentioned previously, it is doubtful that the potassium azide would affect the bulk of the cells beyond the surface.

The effect of temperature on respiration rate of sugarbeets at the various temperatures employed is summarized as cumulative respiration in Tables 3 and 4 for steady-state respiration and respiration during the first 7 days, respectively. In Table 3 it is evident that temperatures much below 10°C are not greatly beneficial in reducing the respiration rate; whereas, a temperature change between 10 and 20°C is of considerable influence. This

Table 3.—Influence of temperature on respiration rate of sugarbeets following harvest.

Temperature (°C)	Respiration rate (static)* CO_2 produced	O_2 consumed	Respiratory quotient
	(ml per Kgm per day)		
0	74	76	0.97
10	103	117	0.88
20	178	188	0.95

* Based on cumulative respiration for the 6th through the 8th day at the various temperatures following harvest. Harvest made on 11/6/67 at Fremont, Ohio. Variety GW **HI.**

Table 4.—Influence of temperature on respiration rate of sugarbeets during first week following harvest*.

Temperature (°C)	Cumulative respiration CO_2 produced	O_2 consumed	Respiratory quotient
	(ml per Kgm per 7 days)		
0	684	683	1.00
10	1054	1099	0.96
20	1896	1980	0.96

* Beets harvested 10/16/67 at Fremont, Ohio.

may have bearing on the practicality or design of low temperature storage facilities for sugarbeets since it would appear that provisions to maintain temperatures much below 10°C are not necessary. Maintenance of temperatures near 0°C, which is difficult since beets are stored out-of-doors and are, therefore, exposed to widely varying temperatures, does not appear to be warranted from this short-term storage temperature and respiration data.

The influence of various treatments calculated as sugar loss is given in Tables 5 and 6 for the steady-state respiration period and respiration during the first week following harvest, respectively. The magnitude of sugar loss, particularly with mechanically injured beets is rather striking with respect to the initial sugar content. An industry estimate of 1 lb of sugar lost per ton of beets per day from all sources during the storage season may be conservative. At 20°C (Table 5) beets may lose 14 lb. of sucrose per ton per day from respiration alone. Beets, however, are not stored at this high a temperature during the bulk of the storage period. Rather, temperatures are generally below 10°C

Table 5.—Influence of selected post-harvest treatments on loss of sugar from sugarbeets.

Treatment	Daily sugar loss at 20°C* gms of sucrose kg$^{m\ OI}$ beets per day	pounds of sucrose tons of beets per day
Control	0.141	0.282
Potassium azide 3.0 lbs/100 gal	0.223	0.446
Merck HZ 3456 1.0 lbs/100 gal	0.190	0.380
Botran 1.5 lbs/100 gal	0.162	0.324
Sawed (lengthwise)	0.562	1.124
Ethylene (1000 ppm-12 hr)	0.178	0.356

* Based on average daily CO_2 production from the 8th through the 10th day following harvest.

Table 6.—Influence of selected post-harvest treatments on loss of sugar from sugarbeets.

Treatment	Sugar loss (first week)* gms of sucrose kgm of beets	pounds of sucrose ton of beets
Control	1.563	3.126
Potassium azide 3 lbs/100 gal	2.739	5.478
Merck HZ 3456 1 lb /100 gal	1.735	3.470
Botran 1.5 lbs/100 gal	1.770	3.540
Sawed (lengthwise)	8.844	17.688
Ethylene (1000 ppm-12 hr)	2.010	4.020

* Based on cumulative CO_2 production during first week following harvest at 20°C.

through much of the storage season. However, warm harvest period temperatures of 20°C or higher are encountered. Furthermore, temperatures greater than 20°C are possible within the beet piles due to the heat of respiration.

Mechanical injury undoubtedly is a significant factor contributing to respiratory loss of sugar from sugarbeets. The damage to beets in the harvesting and handling operations may be extensive. The increase in surface area of beets as a result of mechanical injury (breakage, cracking, topping or chipping) markedly increases the respiration rate as seen in Figures 3 and 5, and in the sugar loss data of Tables 5 and 6. Possibly, suitable artificial diffusion barriers such as waxes or synthetic materials could be utilized to reduce gas diffusion, and thereby partial restriction of the respiration rate without causing fermentation, and, therefore, be of value in reducing respiratory losses.

Acknowledgment

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Nutritional Conditions in Sugarbeet Fields of Western United States and Chemical Composition of Leaf and Petiole Tissue, Including Minor Elements[1]

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Ulrich *et al.* (12)[3] concluded from a national study on the effects of climate on sugarbeet yield, that plants receiving ample supplies of nutrients and water, and kept free of plant diseases, produce as much in Michigan as in the best beet growing areas of the United States. It appears appropriate to inquire if high producing sugarbeet fields in various sugarbeet areas have acquired a common nutritional status for the growing of sugarbeets. Furthermore, Ulrich (11) and Haddock and Stuart (4) have proposed techniques for diagnosing well nourished sugarbeet plants. A number of diagnostic soil fertility tests have been proposed (7, 8, 10) for crop plants in general, without specific reference to sugar beets.

Research on sugarbeet nutritional requirements could be directed more intelligently if the present nutritional status of the more productive sugarbeet fields were known. During the past 20 years the percentage of sugar in sugarbeets grown in the United States has shown a persistent decline while yields have increased significantly (3). Even with this increase in yield, production of high quality sugarbeets is far below desirable and attainable goals. The production of high yields of high quality beets results from a combination of many cultural practices. Adequate plant nutrition has been one of the most influential in bringing about past advances. It promises great hope for future improvement in both yield and quality of sugarbeets. In each sugarbeet growing district there are farmers who consistently produce good yields of high quality beets. It was hoped that an examination of the soils used and plants produced by farmers may demonstrate the nutritional conditions of the soils which are conducive to high yields. This study was undertaken to indicate weaknesses or strengths inherent in natural soils and farmer practices of the Western United States.

[1] Contribution from the Southwest Branch, Soil and Water Conservation Research Division, Agricultural Research Service, USDA, in cooperation with the Utah Agricultural Experiment Station, Logan, Utah.

[2] Research Soil Scientist, USDA, Logan, Utah; and Soil Scientist, USDA, Reno, Nevada.

[3] Numbers in parentheses refer to literature cited.

Methods and Procedure

The five sugarbeet companies which cooperated in this study selected three highly productive farms in one or more of their respective districts. A 5-acre area was selected on each farm for soil uniformity, accessibility and high yielding ability.

Soil samples were collected by the authors or their colleagues before fertilization and before seeding. They were obtained from two depths, 0 to 6 and 6 to 12 inches, and from 20 borings. Each depth was composited separately, air dried promptly near the sampling site and mailed to Logan, Utah, for chemical and physical study.

Leaf-blade and petiole samples (30 leaves per sample) were obtained at three seasonal sampling dates. These were carefully washed in deionized water and dried at 70° C near the sampling area. All samples were packaged dry and sent to Logan for chemical analysis. Yield of roots and tops and sugar analysis were obtained by each cooperating company. Standard laboratory chemical procedures were used for both soil and plant tissues.

The authors wish to present a broad general understanding of the nutritional conditions of only the most productive sugarbeet fields in Western United States. Since the observations were restricted to a small segment of the sugarbeet producing area, conclusions which seem to apply to this limited segment of sugarbeet production must be applied with extreme caution to the broader area and with some caution to all high producing farms because of the sampling limitations.

The authors desire to present this broad appraisal of nutritional conditions in productive sugarbeet fields from four points of view, viz. (A) soil analysis, (B) petiole analysis, (C) leaf-blade nitrogen-potassium ratios, and (D) minor element status.

Experimental Results

Soil Characteristics of Farms Studied

Location—Cooperating sugar companies selected three presumably high producing fields in each district. Figure 1 and Table 1 show the distribution of farms brought under observation in this study. It will be noticed that there are 13 in Idaho, 2 in Oregon, 6 in Washington, 4 in Colorado, 1 in Montana, 1 in Nebraska, 15 in Utah and 6 in California.

Incubated Available Nitrogen—Few data are published on the desirable level of available soil nitrogen needed for the production of a good sugarbeet crop. Stanford and Han way (10) proposed the use of a soil incubation technique for appraising nitrogen availability. The senior author modified this technique using 25 grams of soil and found after several years of study

Table I.—Distribution of farms used in sugarbeet field study 1961.

State	Factory District	Farm
Idaho	Burley	Mai
		Bowen
		Stanger
	Twin Falls	Glenn
		Parrish
		Newberry
		Harrison
		Denney
	Nampa	Carlson
		Watanabe
		Christensen
	Idaho Falls	DeKay
Oregon		Itania
		Hart
		Walkayawa
Utah		Goodwin
		Amalgamated
	Lewiston	Jensen
		Maw
		East
	Ogden	W'ayment
	Garland	Wiedman
		Fukue
		Firth
	Gunnison	Sorensen
		Hansen
		Hawley
	West Jordan	Marcusen
		Gammon
		Hamilton
Washington	Moses Lake	Bingham
	Moses Lake	Lybbert
	Othello	Hirasawa
	Toppenish	Bauchey
	Toppenish	Benz
	Prosser	Willard
Nebraska	J Mitchell	S. C. D.
Montana	Huntley	Gabel
Colorado	Grand Junction	Long
	Grand Junction	Matchett
	Fort Collins	Johnson
	Fruita	Schlauger
California	Fresno	Cardwell
	Mendota	V. D. L.
	Firebaugh	Schimer & Frazier
	Gonzales	Oreggia
	Spreckles	Spreckles
	New Watsonville	Crossetti

that 60 ppm of incubated nitrate-nitrogen is ample under Utah conditions for at least 20 tons of sugarbeets. This may or may not be an adequate level for soils used in the wide range of

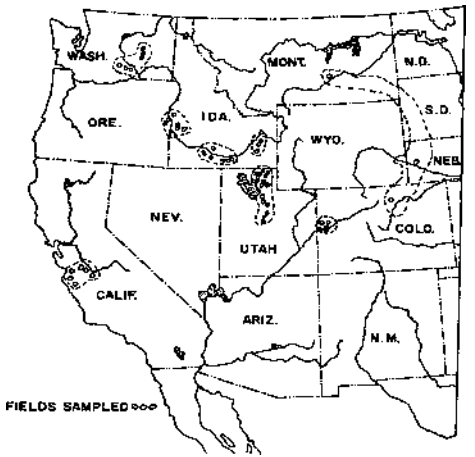


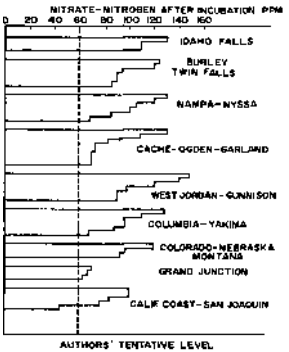
Figure 1.—Location of sample areas in the Western United States.

climatic conditions found in this study. Nevertheless, the authors have placed this value as a tentative reference point in Figure 2[4] This reference value is shown as a broken vertical line.

Two important conclusions appear obvious from the data shown in Figure 2. First, the range of available nitrogen from farm to farm is very wide; and second, based on the tentative adequate level, there are excessive quantities of available nitrogen in most of the sugarbeet fields under study.

Sodium Bicarbonate Soluble Phosphorus—There is no standard soil test value for $NaHCO_3$-soluble phosphorus for the sugarbeet crop which is generally accepted. Olsen *et al.* (6) have suggested 22 pounds per acre or 11 ppm as a phosphorus level above which a response is unlikely for wheat, oats, alfalfa and similar crops. They recognize that values may be higher for potatoes and other crops. This value is plotted at 15 ppm to accommodate for higher requirement of sugarbeets for phosphorus. The Utah Agricultural Experiment Station has recently revised soil test recommendations for sugarbeets. This value is placed at 22 ppm above which sugarbeets are unlikely to benefit

* Fields in each district are arranged graphically in descending order. Each field is allotted the same vertical space. Variation in soil composition within each district and between districts can be readily appraised.



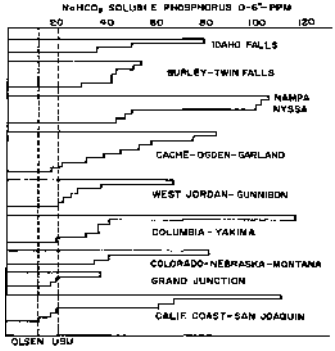


Figure 2.—(left) Incubated nitrate-nitrogen in 48 soils of Western United States. 1961.

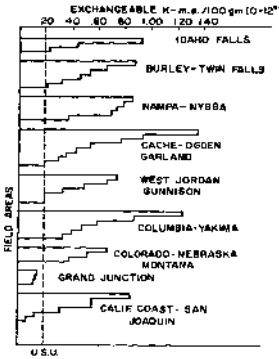
Figure 3.—(right) Sodium bicarbonate soluble phosphorus in 48 sugar-beet fields of Western United States. 1961.

from additional phosphorus. These values are shown by vertical dotted lines in Figure 3 for reference.

Two significant conclusions are justified from the graphical data in Figure 3. First, the soils under observation exhibit a wide variation in the quantity of available phosphorus; and second, the soils are heavily weighted on the side of excess phosphorus.

Exchangeable Potassium—There are few available data to support a soil test value in terms of available potassium. The authors have selected the recent recommendation of the Utah Agricultural Experiment Station of 0.2 of a me. exchangeable potassium per 100 grams of soil, as the value above which a soil is unlikely to show visible signs of potassium deficiency in sugarbeets. Only five of the fields studied in this survey showed less than 0.2 me. of exchangeable potassium or 156 pounds potassium per acre 6 inches. As with phosphorus and nitrogen, the soils showed a wide range of exchangeable potassium and generally an adequate supply relative to the standard reference (see Figure 4).

Exchangeable Sodium—The sugarbeet plant is thought to be tolerant and in many cases benefited by sodium in the nutrient solution. There was only one farm in California which was obviously too high in exchangeable sodium for sugarbeets. It is not known with any degree of certainty what concentration of



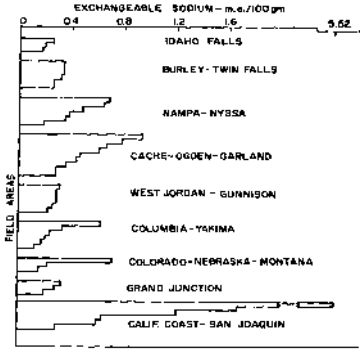


Figure 4.—(left) Exchangeable potassium in soils from 48 sugarbeet fields in Western United States. 1961.

Figure 5.—(right) Exchangeable sodium content of 48 sugarbeet field soils in Western United States. 1961.

sodium in the soil solution or in the exchange complex is optimum for the growth of sugarbeets. At least sodium is present in all western sugarbeet soils in rather high concentrations and exhibits considerable variation from field to field in all areas (see Figure 5).

Conclusions Based on Soil Analysis

Soil analysis methods now available for western soils are in need of refinement. Nevertheless, they show positively that, as a group, the 48 sugarbeet soils sampled in this study are well supplied with available phosphorus, nitrogen, and potassium. The very interesting feature shown in Figures 2, *3* and 4 is the wide range of available nutrients shown in most of the sugarbeet-growing districts. Few of the soils show any indication of insufficient major nutrients. The question might well be raised: Is it beneficial or harmful to have a soil highly charged with one particular plant nutrient and at the same time poorly provided with another nutrient?

Large quantities of sodium are present in all soils studied. Heimann and Ratner (5) lay great stress on the need of a high K:Na ratio in the sugarbeet plant for high yield, sucrose percentage, and sugar extraction percentage. The average exchangeable sodium and potassium in the 48 sugar beet fields studied were nearly identical, but the average soluble sodium concentration in the saturated extract was 3.47 times the potassium concentration (data not shown).

Petiole Analysis

Data from petiole analysis are given in Figures 6 to 17. These are given by individual farms and show a seasonal trend for available nitrogen, phosphorus and potassium. Ulrich's (11) "critical level" value is shown as a solid horizontal line with each figure for comparison of nutritional status.

Nitrogen—It is apparent from Figure 6 that only in the case of the Parrish and Dennie farms did the nitrogen level approach the critical level during the growing season. None of the farms

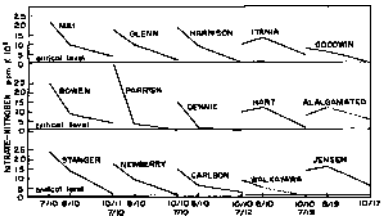


Figure 6.—Seasonal nitrate-nitrogen concentration in sugarbeet petioles from commercial fields in Idaho and Utah. 1961.

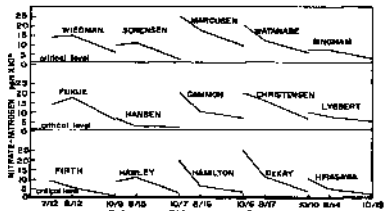


Figure 7.—Seasonal nitrate-nitrogen concentration in sugarbeet petioles from commercial fields in Utah, Idaho and Washington. 1961.

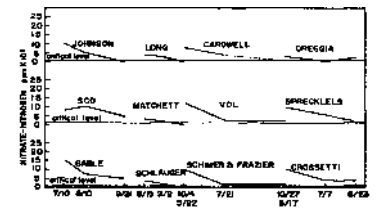


Figure 8.—Seasonal nitrate-nitrogen concentration in sugarbeet petioles from commercial fields in Colorado and California. 1961.

shown in Figure 7 indicated a nitrogen deficiency. In Figure 8 the three farms in Grand Junction showed a weakness in supplying available nitrogen as did the Oreggia farm in California. Figure 2 should be studied in this connection since it shows an agreement with data in Figures 6, 7 and 8 with respect to nitrogen availability. Three farms in the Ogden area, viz. Maw, East and Wayment show a low nitrogen content in petioles after mid season (Figure 9). The six line drawings on the right half of Figure 9 were obtained from pot culture studies and local fields with varying known levels of available nitrogen. These are presented for comparison with high producing farms. Check-N

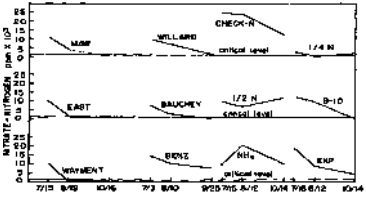


Figure 9.—Seasonal nitrate-nitrogen concentration in sugarbeet petioles from commercial fields in Utah and Washington and nutrient cultures. 1961.

represents a sugarbeet plant with well-balanced nutrition for optimum growth. $^1/_2$ N represents a plant slightly deficient in nitrogen. NH_4 represents a plant with ample nitrogen in the ammonia form but with high phosphorus. 14 N represents a sugarbeet plant deficient in nitrogen all season. B-10 represents a sugarbeet plant grown in a field with ample phosphorus and potassium but deficient in nitrogen. Exp. represents a plant grown on a soil with ample nitrogen but low in phosphorus and potassium.

Phosphorus—Data shown graphically in Figures 10, 11, 12 and 13 for seasonal phosphorus content in sugarbeet petioles indicate that there were no fields deficient in phosphorus. Ulrich's (11) "critical level" of 750 ppm of soluble phosphorus is shown for reference. These petiole values are in good agreement with Olsen's (6) soil test values. The broken vertical line is placed at 15 ppm available soil phosphorus (Figure 3). The six line drawings on the right half of Figure 13 are given for comparison with plant tissue composition from commercial fields. OK represents plant tissue sufficiently low in potassium to result in depressed yields. Nitrogen and phosphorus composition are adequate.

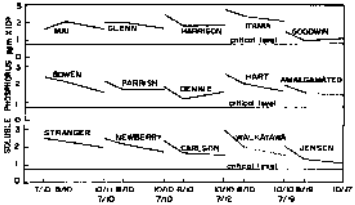


Figure 10.—Seasonal soluble phosphorus concentration in sugarbeet petioles from commercial fields in Idaho and Utah. 1961.

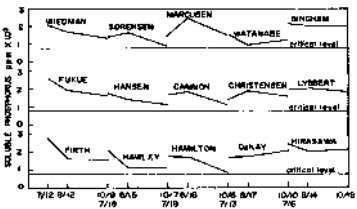


Figure 11.—Seasonal soluble phosphorus concentration in sugarbeet petioles from commercial fields in Utah, Idaho and Washington. 1961.

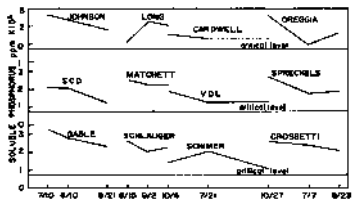


Figure 12.—Seasonal soluble phosphorus concentration in sugarbeet petioles from commercial fields in Colorado, Nebraska, Montana and California. 1961.

Potassium—Figures 14 to 17 show all petiole samples from commercial farms to be well above the critical level set for these tissues. Data in Figures 4 and 16 disagree with respect to the ability of the three soils at Grand Junction (Long, Matchett, Schlauger) and two in the San Joaquin area (Cardwell, Schimer) to supply potassium. Otherwise, the soil tests and petiole tests

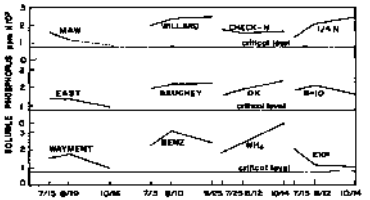


Figure 13.—Seasonal soluble phosphorus concentration in sugarbeet petioles from commercial fields in Utah and Washington and nutrient cultures. 1961.

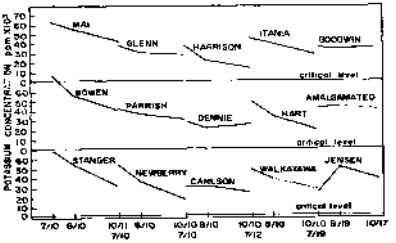


Figure 14.—Seasonal soluble potassium concentration in sugarbeet petioles from commercial fields in Idaho and Utah. 1961.

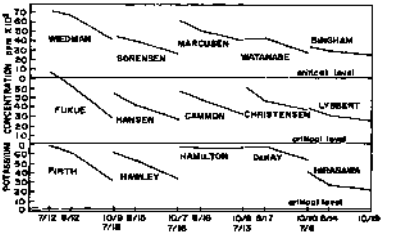


Figure 15.—Seasonal soluble potassium concentration in sugarbeet petioles from commercial fields in Utah, Idaho and Washington. 1961.

are in good agreement, indicating an ample supply of available potassium for sugarbeets. The six line drawings on the right half of Figure 17 with the possible exception of Exp. show petioles with adequate supplies of potassium.

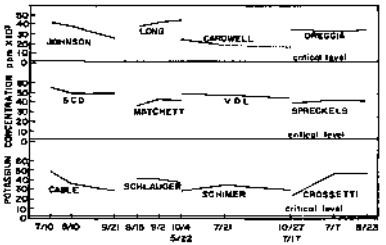


Figure 16.—Seasonal soluble potassium concentration in sugarbeet petioles from commercial fields in Colorado, Nebraska, Montana and California. 1961.

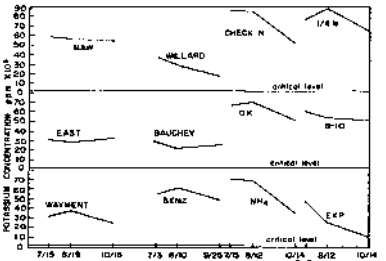


Figure 17.—Seasonal soluble potassium concentration in sugarbeet petioles from commercial fields in Utah and Washington and nutrient cultures. 1961.

Conclusions Based on Petiole Analysis

The seasonal petiole data indicate that the 48 selected farms are well supplied with the primary plant nutrients with few exceptions. One might well raise the question: Is there likelihood of injury from over-fertilization with either nitrogen or phosphorus? The use of "critical level" as used by Ulrich (1) makes weak provision for appraising excessive fertilization with nitrogen. On this basis beet petioles should contain less than 1,000 ppm NO_3-N several weeks before harvest. Also, on this basis two-thirds of the high producing sugarbeet farms are applying excessive quantities of nitrogen. Only one-third of the farms approached the critical level of 1,000 ppm NO_3-N on or before harvest time. It is well known that excessive nitrogen fertilizers are associated with low sugar content of sugarbeet roots. This may well explain the low sugar percentage shown in Figure 30.

Leaf-Blade Composition

Nitrogen-Potassium Ratio—It has been shown by the authors (4) that the N:K ratio in the leaf-blade is a good, sensitive indication of nutritional status of the sugarbeet plant, particularly if the phosphorus nutrition is adequate. Muller *et al.* (6) have laid great stress upon the necessity of having a wide $N:K_2O$ ratio (1:3) in fertilizer used for sugarbeets, especially when available soil nitrogen is high. It is obvious from the data in Figures 18, 19 and 20 that all of the high producing fields studied in Western United States produce plants with a high nitrogen-potassium ratio.

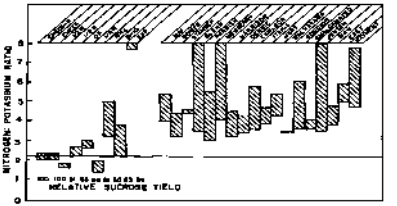


Figure 18.—Seasonal nitrogen-potassium ratio in sugarbeet leaf-blades grown in nutrient solutions and commercial fields. 1961.

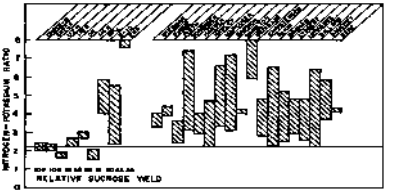


Figure 19.—Seasonal nitrogen-potassium ratio in sugarbeet leaf-blades grown in nutrient solutions and commercial fields. 1961.

We (4) have found in sugarbeet nutrient culture studies conducted over several years strong support for Muller *et al.* (6) claim that available potassium must be high when available nitrogen is high. The relative sucrose yield obtained from sugarbeets with varying N:K ratios are shown graphically in Figure 19 for reference with N:K ratios found in sugar beets from commercial fields under study. The seasonal range in N:K ratios

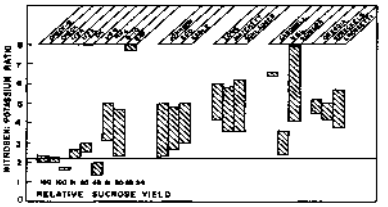


Figure 20.—Seasonal nitrogen-potassium ratio in sugarbeet leaf-blades grown in nutrient solutions and commercial fields. 1961.

is indicated by the length of each diagonally lined column. Check-N and Check exhibit an optimum N:K ratio relative to yield of sucrose. When nitrogen was deficient (1/2 N and 1/4 N) yield of sucrose was depressed to 91 and 61% respectively. When nitrogen was in excess (NH_1) yield of sucrose was likewise depressed (50%). When potassium was in deficient supply (1/2 K and 0 K) yield of sucrose was relatively low (85 and 68%). Sugarbeets grown on a local field (Exp.) were judged to be deficient in potassium and adequate in nitrogen and phosphorus while beets grown on field B-10 were thought to be adequate in nitrogen up to midseason and deficient in nitrogen the last of the season.

Conclusions Based on Nitrogen-Potassium Ratios

It appears to the authors that while the several farm soils under study were generally well supplied with adequate quantities of nitrogen, phosphorus and potassuim, relative excesses of available nitrogen predominated.

The evidence suggests that either nitrogen fertilization needs to be reduced or that potassium fertilization should be increased on the high producing sugarbeet farms of Western United States.

The practical problem of optimum plant nutrient concentration and balance in commercial sugarbeet fields needs immediate attention.

Minor Nutrient Composition

Iron—Data on iron composition of sugarbeet leaves showed them to range from 50 to 175 ppm (Figure 21).

Zinc—Zinc deficiency in sugarbeets with a critical level of 10 ppm has been reported by Boawn and Viets (2). Data in Figure 22 show all fields studied were well above the deficiency level.

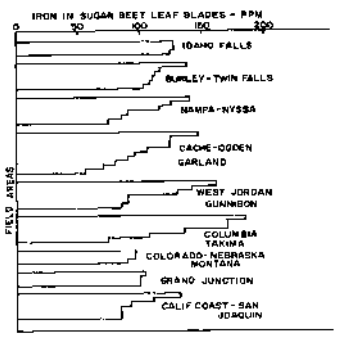


Figure 21.—(left) Iron concentration in sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

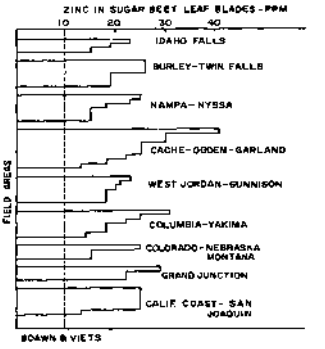


Figure 22.—(right) Zinc concentration in sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

Manganese—Bear, *et al.* (1) have proposed a deficiency level of 30 ppm in beet leaves. On this basis beets in the Grand Junction area were very close to the deficiency level. Otherwise, there appeared to be ample manganese in beet leaves on western high-producing farms as shown in Figure 23.

Copper—The authors could not find a report of copper deficiency in sugarbeets. Bear, *et al.* (1) report 3 ppm for clover leaves and 6 ppm for alfalfa tops as deficiency levels. It will be noted in Figure 24 that the lowest average value found in the 48 high producing farms was 7 ppm. It appears unlikely that copper deficiency is an immediate threat to soil productivity among sugarbeet growers.

Sulfur—Sulfur deficiency has been reported with increasing frequency in recent years. As shown in Figure 25 this element does not appear to be an immediate threat to high producing sugarbeet fields. On the other hand, some students of this problem believe the N:S ratio to be a more sensitive indication of sulfur need. The graphical data given in Figure 26 indicate that the Grand Junction area may become deficient in sulfur.

Chlorides—It is well known that western soils contain high amounts of chlorides. While sugarbeets are not thought to be

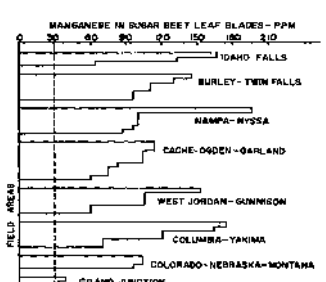


Figure 23.—(left) Manganese concentration in sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

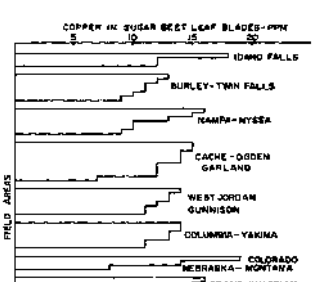


Figure 24.—(right) Copper concentration in sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

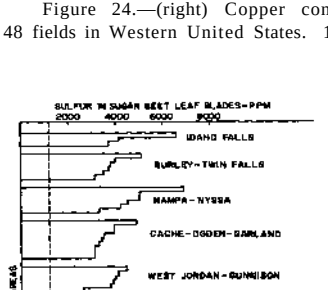


Figure 25.—(left) Sulfur content of sugarbeet leaf-blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

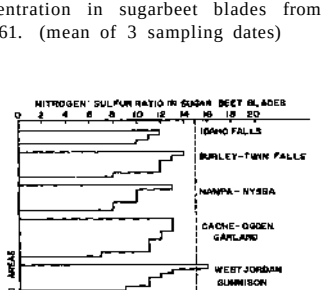
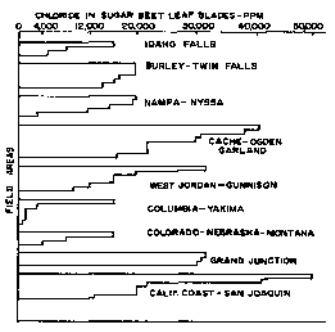


Figure 26.—(right) Nitrogen-sulfur ratio of sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

sensitive to these salts, there is little evidence that more than traces are useful. The wide range of chloride concentration found in sugarbeet plants in this study should throw suspicion on these salts. Particularly in the fact of the following statement by Rorabaugh and Norman (9): "Carbonate and chloride account for three-fourths of the total melassigenic power in beet sirups and should warrant most concentrated efforts." The range of chlorides in sugarbeet leaves shown in Figure 27 is 500 times greater in some plants from Utah and California than from those in the Columbia Basin. It would be surprising indeed if high concentrations of chloride do not adversely influence yield and quality of sugarbeets.

Boron—Data presented in Figure 28 show that sugarbeet plants, on the 48 high producing farms used in this study, were adequately supplied with boron on the basis of reports by Bear, et al. (1). The total range of boron concentration in leaves of beets sampled in this study was only from 32 to 95 ppm. The range between deficiency and toxicity is influenced by many factors other than boron concentration in the soil.



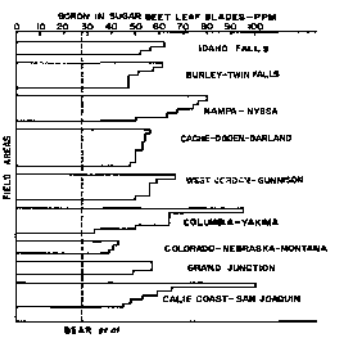


Figure 27.—(left) Chloride concentration in sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

Figure 28.—(right) Boron concentration in sugarbeet blades from 48 fields in Western United States. 1961. (mean of 3 sampling dates)

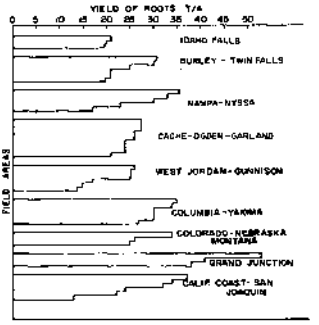
Conclusions on the Need for Minor Elements

Since the sugarbeet fields used in this study were selected because they were in a high state of fertility, one should not expect to find minor nutrient element deficiencies of any kind. At least in the present condition there is little or no evidence that any of the minor elements are bordering on serious deficiencies. However, immediate studies should be undertaken to establish toxic limits for all the minor elements in sugarbeets. An even more desirable goal would be to establish a range of optimum concentrations of all minor elements in sugarbeet tissue.

Yield and Quality of Sugarbeets

Yield—It should be expected that fields selected on the basis of anticipated high yields would produce well over 20 tons per acre. Data in Figure 29 show this assumption to be correct. The range of yield may be considered wide when only the high producing farms are included in this study. However, when one considers a wide range of soil conditions encountered, the range in yield does not appear great. The range in each district is frequently as wide as it is between districts, and is such as to suggest that something besides climate is exerting a powerful influence on yield.

Quality—Data shown in Figure 30 should be quite disturbing



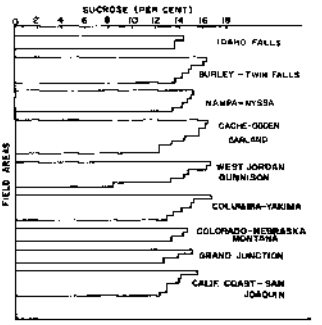


Figure 29.—(left) Yield of roots from 48 sugarbeet fields in Western United States. 1961.

Figure 30.—(right) Sucrose composition of sugarbeet roots grown on 48 farms in Western United States. 1961.

in view of the past 30 years' trend in sugarbeet quality. Nearly half of the best producing farms are delivering sugarbeets to the processor with a sugar percentage of 14 or less. There were only 4 of the 48 farms producing beets with 16% sugar, and none of them reached 17%.

Conclusions

It is evident that the most productive sugarbeet fields in Western United States are far from ideally fertilized. The processor of sugarbeets has been painfully aware of a serious and increasing unbalance in commercial fertilization of sugarbeets since the days of barnyard manure and superphosphate. The immediate problem appears less related to minor element fertilization, than to achieving proper balance of the primary nutrient elements in the soil. A secondary problem appears to be a proper appraisal of the influence of excessive salts, e. g. sodium, chloride, magnesium, calcium and sulfate, and finding ways of managing sugarbeet soils so as to take full advantage of the presence of these salts. Because this study was limited to high yielding fields of sugarbeets, it is not possible to formulate conclusions on nutrient deficiencies in low yielding fields. The wide range of soluble salts found in high yielding fields and in plant tissue grown in these fields suggest the possibility that excesses of various salts may be a greater barrier to proper nutrition of sugarbeets in Western United States than deficiency of nutrient elements.

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Relation of Sugarbeet Seedling Emergence to Fruit Size[1]

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In the Great Lakes area, industry personnel have observed that a higher percentage of sugarbeet seedlings emerge from plantings of large monogerm fruits than from small ones. The implications of these observations suggested the studies reported.

Previous research (1,2,3,4)[4] has shown that fruit- and seed-size are correlated. Fruit diameter of a monogerm variety was correlated (0.68**) with seed diameter (1). For a given monogerm seedlot, usually a greater percentage of the seeds in the larger-sized fruits germinate than those in smaller-sized fruits. Thus, under field conditions, when the same number of "seeds" are planted per unit length of row, fewer seedlings emerge from the smaller-sized fruits solely on this basis. However, this alone could not account for the large differences in field emergence usually observed.

The positive correlation between fruit and seed size suggests that seedlings developed from seeds in the larger fruits may be larger than those from the smaller fruits. Also, the quantity of reserve starch may be greater in larger fruits. Seedlings from the larger fruits may exert a greater force during the emergence process. Since the hypocotyl is involved in seedling emergence, hypocotyl size may influence emergence.

When seedlings from large and small fruits of sugarbeet were compared, those from the larger fruits had larger diameters of hypocotyls and a greater percentage of emergence from sand and soil, particularly as the seeds were planted deeper. Depth of planting in sand also affected hypocotyl size.

Methods and Materials

Monogerm fruits (single cavity with one viable seed), of a given variety were sized into two or three categories. When whole fruits were used, they were sized and then hand processed. For many of the experiments, commercially processed and sized fruits were used. Size categories were in inches/64. A 6 1/2-7 1/2 size-class indicated that all the fruits fell through a 7 1/2 round-hole screen

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station. Approved for publication as Journal article #4686, Michigan Agricultural Experiment Station.

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[4] Numbers in parentheses refer to literature cited.

but remained on a 6 1/2 round-hole screen. For the designation "On 12", all the fruits remained on a 12 round-hole screen.

Hypocotyl size

In the initial experiments, two sizes of fruits were placed on blotters in the germinator. Approximately 10-day old seedlings which had hypocotyl lengths over l1/2 cm, were selected and a 1-cm segment of the hypocotyl was removed by means of a guillotine. The segments were kept on moist paper until they were weighed and counted. The average weight per segment was calculated for each fruit-size class.

In later experiments, whole fruits of a single size-class (12-13) were planted in fine quartz sand (<1 to 0.1 mm) at 1/2- and 2-inch depths in covered plastic boxes. To determine the effect of depth of planting on hypocotyl size, we kept the seedlings in the dark for the 10-day experiment. The average weight of 50 of the 1-cm segments excised immediately above the transition zone was determined. The average weights of 50 of the intact hypocotyls of groups of seedlings grown in the dark and in the light in the greenhouse also were obtained.

Laboratory germination and emergence

In some experiments, the percentage germination at approximately 70 F in a germinator on two layers of blotter was compared with emergence from quartz sand at 72 to 74 F. Quartz sand, having 7 1/2% moisture, was placed in covered plastic boxes (5x7x4 in). Approximately 3/4 in of sand was placed in the box; the fruits were placed, and then covered with the moist sand to specified depths. Twenty-seven seeds of each of two size-classes of fruits were planted in each box. A minimum of three replications (boxes) per experiment was used for each depth. Other experiments involved soaking of the fruits and using sand with different moisture contents.

Since the fine quartz sand with 7 1/2% moisture will compact, care must be taken to apply the same amount of pressure to all containers. Where close comparisons are desired for certain fruit size-classes, the experiment should be designed to place the size-classes in the same container. Reproducibility is similar to blotter germination.

Field emergence

In order to correlate laboratory germination and emergence from sand with emergence under field conditions, we used two fruit sizes, small (6 1/2-7 1/2) and large (9 1/2-10 1/2), of commercially processed "seed" of Lot 7334 of variety US H20. The "seed" was treated with a fungicide.

Field plantings were made by two industry cooperators. Precautions were taken to avoid any bias of individual planting units of the commercial planters.

Results

Hypocotyl diameter was positively correlated with fruit size for each set of data listed in Table 1. In experiments involving emergence from sand, the hypocotyls of seedlings from commercially-processed, large fruits averaged larger than those of seedlings from small fruits.

Table 1.—Relation of seedling hypocotyl diameter to fruit diameter in monogerm sugarbeet.

Variety	Fruit size		Hypocotyl segments*	
	Whole	Pruress.	Avg weight	
	In./64	In./64	No.	Mg
Hybrid	On 12		30	4.6
	8½-9½		50	4.1
US H20	On 13		35	4.5
(Lot 6369)	8-9		22	3.1
US H20	On 12		159	4.3
(Lot 6432)	8 9		222	3.6
(SL126 × 128)ms ×	On 12	On 12	127	4.1
SP5822-0	10-11	8½-10½	172	3.7
(Lot 4504)	7½-8½	6½	205	3.0

* 1-cm segments.

For seedlings emerging in light, the hypocotyls from "seeds" planted 2 in deep in sand weighed more than those from "seeds" planted 1/2 in deep (37.4 versus 21.4 rag per hypocotyl). This weight differential occurred even though the seedlings from the shallow planting emerged earlier and photosynthesized more. For seedlings emerging in the dark, the hypocotyls from "seeds" planted 2 in deep also weighed more (59.4 versus 52.9 mg per hypocotyl). The 1-cm hypocotyl segments of seedlings grown in the dark from "seeds" planted 2 in deep in sand also weighed more than those from "seeds" planted 1/2 in deep (8.2 versus 6.9 mg per segment; t-test significance at 2% level). The length of hypocotyls was more variable for the deep planting than for the shallow.

Repeated experiments revealed that germination percentages on blotters were nearly identical to percentages of emergence from quartz sand when the fruits were covered with 1/2 in of sand, thus the percentage values can be used interchangeably.

Emergence of sugarbeet seedlings from quartz sand (seven experiments, total of 24 replications involving different varieties and seedlots) showed that: 1) Fewer seedlings emerged (18 to 65% fewer) when fruits were planted 2 in deep than when planted 1/2 in deep; and 2) Proportionately fewer seedlings emerged from the smaller fruits than from the large, when planted 2 in deep.

When small and large fruits of US H20 were placed in sand at 1/2-, 1-, 1 1/2-, and 2-in depths, progressively fewer seedlings emerged, but the percentage emergence declined more sharply between the 1 1/2- and 2-in depths. When large, processed fruits (either soaked in water for 20-30 min before planting or planted in the air-dried condition) were planted 1 in deep in sand having moisture contents of 3, 5, or 7 1/2%, seedlings emerged most rapidly from sand at 7 1/2% moisture. Soaking the fruits hastened emergence from sand at 3% moisture. The final percentages of emergence did not indicate any distinct trends or effects. However, when whole and processed fruits were planted (1 in deep in sand at 7 1/2% moisture) either in the air-dried condition or after soaking in water for 20-30 min, the percentage of seedlings emerging from the soaked fruits averaged 10 to 15% below that of the dried fruits.

Emergence of seedlings has been compared in firmly packed and relatively loosely packed sand. The fruits (9 1/2-10 1/2 size-class) were placed at a depth of 2 in. Firmly packed sand reduced seedling emergence to 46% of that from the loosely packed sand.

Field emergence study

Both size-classes of fruits contained at least 98% fully developed seeds. Percentages for blotter germination and emergence through 1/2 in of sand averaged 95% for both size-classes. Seventy-six percent of the seedlings from large fruits emerged through 2 in of sand, but only 53% of those from small fruits.

Field emergence data (Table 2) reveal the same trends that were obtained in the laboratory. With the exception of 1 1/4-in depth on the Adelsperger Farm, for any given planting depth, fewer seedlings emerged from the small than from the large fruits. Also, fewer seedlings emerged as the fruits were planted deeper and proportionately fewer seedlings emerged from smaller fruits planted deeper. J. L. Brown, Farmers and Manufacturers Beet Sugar Association, Saginaw, Michigan planted the two size-classes of fruits 1 in deep[5]. Seedlings emerged from 58% of the large fruits and from 37% of the small fruits.

Table 2.—Field emergence of sugarbeet variety US H20 as affected by planting depth in northern Ohio.*

Fruit size (inches/64)	6½ - 7½		9½ - 10½	
Farm — Depth planted (inches)	1¼	2	1¼	2
Adelsperger	46	28	47	42
Havens	18	13	36	34
Damschroder	47	....	70	....

* Data of P. Brimhall, Northern Ohio Sugar Co., Fremont, Ohio.

[5] Helmerich Farm, Bay City, Michigan.

Discussion

The technique of planting sugarbeet fruits at different depths in moist sand offers a simple procedure for differentiating the emergence potential of seedlots and particularly for various size-classes of fruits. It should be possible to standardize the emergence from sand so that the various size-classes could be given an emergence potential rating which then could be used by growers to minimize emergence problems.

The data from Ohio (Table 2) indicate the difficulty of trying to predict possible emergence for a given seedlot. However, where emergence tends to be a problem, seed with high emergence potential should be made available to the grower. If the grower employs space-planting to get the desired stand without thinning, the following guide lines should be useful: 1. When smaller "seeds" are space-planted, at least one-third more should be planted than when larger "seeds" are planted; 2. Since depth of planting affects the percentage of emergence much more for small "seeds" than for the large, smaller "seeds" should be planted shallow (probably always less than 1 in deep); and 3. Where lack of moisture may limit germination, particularly for later plantings, "seeds" probably should be planted somewhat deeper. In such cases, larger "seeds" should be planted because they have greater emergence potential.

In the future, sugarbeet varieties may have larger fruits and greater emergence potential, however, each seedlot will still have a range in emergence potential which is related to fruit-size. Also, the micro-environment in which the seed develops and matures may effect the emergence potential of the seed.

The erratic germination and emergence performance of soaked "seeds" seems to be related to the differential needs of individual seeds for oxygen and sensitivity to limited diffusion of oxygen through the moist fruit. Small increases in the moisture content of the fruit may impede the rate of diffusion of oxygen into the seed below that required for germination. Thus, in repetitive experiments, the inherent small differences in moisture content may lead to relatively large differences in percentages of germination.

The effect of depth of planting on the hypocotyl was unexpected. Since energy is expended during the emergence process, the hypocotyls from a deep planting logically might be expected to be smaller than those from a shallow planting. The data suggested that the expenditure of energy was not sufficient to reduce the size of the hypocotyl. Perhaps the increased diameter of the hypocotyls from a deep planting is related to the increased impedance during emergence. The restriction on elonga-

tion might exert greater turgor pressure on the lateral walls than would occur with unrestricted cell elongation, thus enlarging cell-diameters and concurrently the hypocotyl diameter. The depth of planting affected the length of time the seedlings grew in the dark and could affect the size of the hypocotyls, since light retards elongation.

Summary

Sugarbeet fruits were separated into large and small size-classes and placed on blotters for germination. Approximately 10 days after germination, 1-cm segments of hypocotyl were excised. The segments of hypocotyls from the large fruits weighed more than those from small fruits. The size differential could also be observed macroscopically.

The percentage emergence of seedlings from moist quartz sand was determined when fruits were placed at 1/2- and 2-in depths. As the fruits were planted deeper, fewer seedlings emerged. Significantly, at the 2-in depth, proportionately fewer seedlings emerged from the small fruits than from the large when compared to the l/2-in depth.

The trends in emergence potential obtained in sand in the laboratory were confirmed by emergence from soil under field conditions. The results indicate that seeds in large sugarbeet fruits have a greater emergence force and emergence potential than seeds in small fruits.

Acknowledgment

C. M. Harrison, Crop and Soil Sciences Department, Michigan State University, suggested the study of the effect of depth of planting on hypocotyl size.

Fred Russell, Buckeye Sugar Company, Ottawa, Ohio directed our attention to some of the field observations which suggested this study.

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Quantitative Growth Studies with Sugarbeets, Beta vulgaris[1]

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Total yield of dry matter from a crop depends upon the size, mode of display, and duration of leaf area or photosynthetic system in relation to seasonal income of solar radiation. Total dry matter production by sugarbeets is maximal where an optimum canopy is produced as early as possible, and where this canopy endures as long as possible. There is, however, a limit beyond which increased leaf area will not increase dry matter production, or a point at which mutual shading becomes a factor. In addition, sugar yield is a variable proportion of the total dry matter yield, depending on the balance of internal competition for assimilate. The proportion of assimilate transferred to sugar storage may be reduced by cultural treatments which increase top growth, such as nitrogenous fertilizers. Quantitative growth characteristics of sugarbeets in relation to quality and sucrose production are not well defined at this time, and some question arises as to what might be considered optimum leaf area. This experiment was conducted to intensify and expand present research in this field. Nitrogen fertilizer treatments and genetic populations were used as variables to fluctuate leaf area growth curves so that the effects of these variations in leaf area on such factors as net assimilation rate, root growth rate, dry matter formation and sucrose accumulation could be determined.

Materials and Methods

Sugarbeets were grown on an irrigated Nunn clay loam at the Colorado State University Research Center near Fort Collins. The soil was calcareous nonsaline and contained about 2% organic matter.

The field received 40 pounds of P_2O_5, per acre to insure an adequate level of this nutrient. Five nitrogen treatments were imposed by adding ammonium nitrate as follows: (1) Check (no nitrogen fertilizer); (2) 125 lb nitrogen per acre applied preplant in March; (3) 250 lb nitrogen per acre applied preplant in March; (4) 125 lb nitrogen per acre applied July 12; (5) 250

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lb nitrogen per acre applied July 12. The preplant application of fertilizer was broadcast March 26 and harrowed into the surface soil, the delayed application in July was placed about 2 inches deep below the furrow and irrigated.

Two genetic populations were planted March 31, 1966: (1) A56-3, a variety similar to those now distributed in northeastern Colorado, and (2) an F_1 hybrid (52-305 X 52-307) developed by the Sugar Crops Section of the Crops Research Division, U.S.D.A., Fort Collins, Colorado.

The experiment was a factorial with the ten treatments replicated four times. Plots were 16 rows wide and 60 feet long with rows 22 inches apart. The beets were hand thinned in mid-May to about a 10-inch plant spacing. Beets were harvested beginning June 6, 1966, and approximately every 2 weeks thereafter until the 12th harvest on November 8. Fifteen feet of row containing 17 to 19 beets were harvested each time to insure approximately equal competition within the row. Alternate rows were harvested during the season to maintain normal competition between rows throughout the season. The beets were divided into blades, petioles plus crowns and roots. The fresh and dry weights of each plant part were determined, as well as leaf area and leaf number. From these measurements root yield, leaf area index, leaf area duration, net assimilation rate and growth rate were calculated. Beginning July 5, sucrose content and apparent thin juice purity were determined for each harvest.

Leaf Area Measurement. At each harvest a representative beet was chosen from each plot. The leaves of this plant were removed, placed on blueprint paper and exposed to sunlight for a few seconds. Later development of this paper in ammonium hydroxide gave the outline of the leaf. The leaf pattern was cut from the paper and weighed to measure leaf area. Leaves for area measurements were dried and weighed to relate area to dry weight. The total dry weight of leaves was determined for each plot, then the leaf area per plot was calculated from the dry weight-leaf area relationship previously determined.

Leaf Area Index (LAI). Leaf area index is the leaf area per unit ground area. This value was obtained from the ratio of leaf area to ground area; both measurements were in the same units for 15 feet of row.

Leaf Area Duration (LAD). Leaf area duration is the integral of the leaf area curve over a given growth period. These values may be obtained by the use of a planimeter on leaf area index curves. For this experiment, however, the leaf area duration was approximated by summing the leaf area index for each 2-week sampling period. To express the LAD on a weekly basis the values for this experiment were doubled.

Net Assimilation Rate (NAR). Net assimilation rate is the rate of increase in dry weight per unit of leaf area (2,18)[3]. The equation normally used for calculating NAR assumes a linear relationship between changes in dry weight and leaf area between the two sampling dates in question, i.e.

$$NAR = \frac{(W_2 - W_1)\ (\ln A_2 - \ln A_1)}{(T_2 - T_1)\ (A_2 - A_1)}$$

W_2 and W_1 are dry weight estimates of the plant material in grams per square meter of ground area at times T_2 and T_1; A_2. and A_1 are leaf area index values at T_2 and T_1; $T_2 — T_1$ is the time interval in days (11,18).

Sucrose Percentage and Purity. Sugar percentages on the beet pulp were determined by a method standard with commercial sugarbeet companies and similar to the method outlined in A.O.A.C. (1). Thin juice purity was determined in the clarified extract of brei as outlined by Carruthers and Oldfield (3).

Percent Recoverable Sugar. The quantity of refined sugar from a crop of beets is a better value for expressing sugar production economically than is gross sucrose. A method has been proposed to estimate the amount of recoverable white sugar after processing (5). The recoverable sugar percentage is calculated from the percent sucrose in the beet, the purity of the second carbonated juice and a standard factory loss and molasses purity. Values for percentage recoverable sugar were obtained from tables generated using the Great Western Sugar Co. formula for calculating recoverable sugar, assuming a 62.5 molasses purity and 0.3% factory loss.[4]

Results and Discussion

Final Harvest Yields

The last three harvests did not differ significantly; therefore, they were analyzed together to give a better estimate of final yields. The results are shown in Table 1.

The main effects for root yield were significant for variety and nitrogen fertilizer. Root yield for the A56-3 and F_1 varieties were 22.7 and 20.6 tons per acre, respectively, for means of harvests and nitrogen levels. Husseini[5] compared the same two varieties in a greenhouse experiment and found that the F_1 gave superior yields. Variety-by-environment interactions are not uncommon, and this coupled with a more severe infestation of leaf spot caused by *Cercospera beticola* on the F_1 are possible explanations for this difference.

[3] Numbers in parentheses refer to literature cited.
[4] Wood, R. R., personal communication.
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Table 1.—Average main effects of harvest date, nitrogen fertilizer and variety on final root yield and quality, 1966.

Treatment	Root yield T/A	Sucrose %	Juice purity %	Recoverable sucrose T/A
Harvest date				
Oct. 8	21.3	17.2	93.2	3.13
Oct. 22	21.5	17.3	94.1	3.24
Nov. 8	21.5	17.5	94.3	3.35
Nitrogen#				
Check	15.9c	18.3a	95.1a	2.78b
125, March	22.7b	18.2a	95.0a	3.69a
250, March	24.9a	17.5ab	93.5c	3.90a
125, July	21.2b	16.9b	94.0b	3.09b
250, July	22.5b	15.8d	91.6d	2.76b
Variety				
A56-3	22.7	17.3	94.8	3.41
F_1	20.6	17.4	94.6	3.07
Significance (F-test)				
Harvest date	----	----	----	----
Nitrogen	**	**	**	**
Variety	**	----	----	*

* Significant at 5% level.

** Significant at 1% level.

Duncan's Multiple Range Test; values followed by the same letter are not significantly different at the 5% level.

Duncan's multiple range test (9) comparing nitrogen effects on final root yield gave the following results: (1) the yield of no-nitrogen check plant was significantly lower than yields for all other treatment at the 5% level; (2) there was no significant yield difference at the 5% level between applications of 125 lb nitrogen early, 125 lb late, and 250 lb late; (3) the 250 lb application in March caused significantly higher yields than all other nitrogen treatments at the 5% level.

The effect of variety was not significant for sucrose content; the A56-3 and F_1 genotypes averaged 17.4 and 17.3 percent, respectively, for all nitrogen fertilizer treatments. Nitrogen fertilizer, however, did show a significant effect on sucrose content. Roots from the check and 125 lb preplant treatments had higher sucrose contents than all other treatments. Application of nitrogen at planting had a definite advantage over side-dress applications in July, as plants from both the 125 lb and 250 lb nitrogen treatments applied in March had significantly greater sucrose contents at the 5% level than did plants receiving the same rates July 12. An interaction of variety by nitrogen was observed in the analysis of variance for sucrose. At lower rates of nitrogen

the A56-3 had the same or greater sucrose than the F_1, but at the 250 lb rates the F_1 was higher in sucrose for both early and late nitrogen applications.

Nitrate-nitrogen contents of the petioles for the growing season, as determined by the phenoldisulfonic acid method (8), explain the low sucrose percentages for plants receiving late nitrogen. Petioles were sampled three times during 1966 and analyzed for NO_3-N. These results are given in Figure 1. Varieties were averaged since there was no significant difference between them for NO_3-N. Ulrich (14) suggested that petiole nitrate should be less than 1000 ppm 4 to 6 weeks before harvest to promote sucrose accumulation prior to harvest. Concentrations above 1000 ppm late in the season decrease sucrose content and purity while concentrations below 1000 ppm early in the season may reduce root yield. It is evident from Figure 1 that NO_3-N content was high late in the season for beets receiving the 250 lb side-dress application in July and caused reduced sucrose percentages at harvest. It is apparent, also, that plants in the check treatment were too low in petiole nitrate early in the season, which accounts for the low root yield.

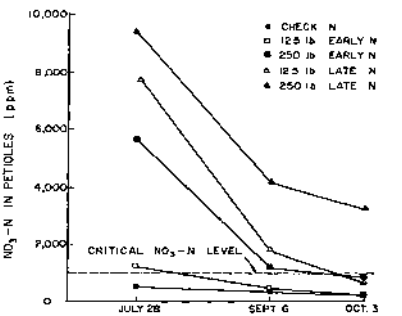

Figure 1.—Effect of nitrogen fertilizer on petiole nitrate content; varieties combined.

Thin juice purity for 1966 was relatively high for all treatments (Table 1). Varieties did not differ significantly for purity, but nitrogen fertilizer did have a significant negative effect on purity, caused primarily by the treatments receiving late nitrogen.

Recoverable sugar in tons per acre, which takes into account both percent sucrose and purity, was significant for the main effects of variety and nitrogen, but there was no significant inter-

action. The A56-3 and the F_1 varieties gave yields of 3.41 and 3.07 tons per acre, respectively. The difference between the varieties was caused principally by the difference in root yield between the two, since neither sucrose content nor purity differed appreciably.

The application of nitrogen had a highly significant effect on yield of recoverable sugar. The advantage of preplant over July applications of nitrogen was caused by the combined effects of root yield, sugar content and purity. When nitrogen was applied in March root yields increased, but there was no real reduction in sugar content. When nitrogen was applied in July root yields increased also, but less than with preplant applications. More significant, however, sucrose percentage and purity decreased markedly. The yield of recoverable sugar for the 250 lb nitrogen treatment in July was no greater than the check, although there was a large response in root yield (Table 1).

Seasonal Growth Analysis

Growth studies with a sugarbeet crop require records of dry matter production of whole or specific parts of the plants, sucrose production and leaf area. These measurements are usually made on repeated samplings at 1 or 2 week intervals. From these records growth rates and sucrose accumulation rates can be calculated, and an analysis can be obtained for yield in relation to leaf area as well as a comparison of the efficiencies of growth and sucrose accumulation. Published results (7, 15,16) indicate that yield of dry matter of sugarbeets is more closely related to leaf area than to net assimilation rate or to efficiency of the leaves. Goodman (7) has noted that this relationship may be of particular importance since the leanness is partially within the experimenter's control. Efficiency of leaves, however, responds more to factors such as temperature and hours of sunlight which are not directly controlled by the experimenter.

Leaf Area—The main effects of variety and nitrogen fertilizer on the maximum leaf area index for the season are given in Table 2. The application of 125 lb of nitrogen more than doubled the maximum LAI over the check treatment and the 250 lb application further increased the LAI. The A56-3 variety had the larger maximum values for all nitrogen treatments.

The effect of time and rate of application of nitrogen on LAI during the growing season is illustrated in Figure 2. Varieties were averaged because there was no interaction between variety and nitrogen treatments. Maximum leaf areas were reached by the first of August for all treatments receiving preplant applications of nitrogen, but when the nitrogen was delayed until mid-July, maximum leaf areas were not attained until the end of

Table 2.—Effect of variety and nitrogen fertilizer on growth of leaves and net assimilation rate.

Treatment	LAI Maximum	LAD Weeks	Leaf number Avg for season	NAR, avg[1] g/m²/day
Nitrogen				
Check	1.4	18.6	20.1	8.0
125, March	3.2	37.8	22.3	7.3
250, March	4.0	51.3	22.9	6.8
125, July	5.6	45.9	22.6	7.6
250, July	4.2	55.0	25 6	7.7
Variety				
A56-S	3.7	46.0	22.6	6.8
F_1	3.1	36.0	21.9	7.3

[1] Average NAR values calculated through the September 24th sampling.

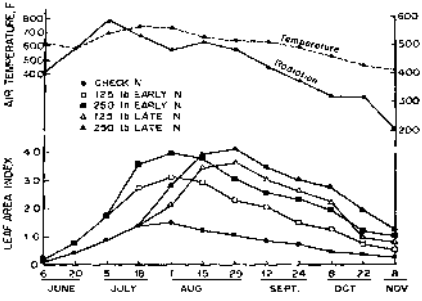

Figure 2.—Effect of nitrogen fertilizer *on* leaf area index (varieties combined), and average air temperature and total solar radiation for the same periods.

August. In all cases leaf areas decreased after a midseason peak. The temperature dropped to 22°F on October 15 and 16, which explains the marked decline in leaf area for the last two samplings.

Figures 3a and 3b show some leaf growth characteristics for the effect of early nitrogen (nitrogen rates and varieties combined). Figure 3a indicates that the decline in LAI throughout the later part of the growing season was attributed more to a decrease in size of leaf than to leaf number, since the number of leaves was actually increasing part of the time while LAI was decreasing. Figure 3b shows the change in the dry weight per unit leaf area with season. The ratio of dry weight to leaf area increased rather steadily throughout the growing season, indicating a thicker leaf. This effect was even more pronounced for the late nitrogen treatments.

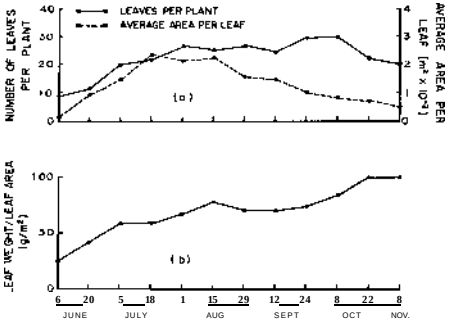

Figure 3.—Effect of preplant nitrogen (nitrogen rates and varieties combined) on (a) number of leaves per plant and average leaf area, and (b) leaf weight per unit leaf area.

Leaf area duration (LAD) has been proposed as a more significant measure of the plant's ability to conduct photosynthesis because it considers both the magnitude and persistence of leaf area (17). LAD is usually expressed in terms of weeks. Harvests for this experiment were at 2-week intervals, so that the LAI for each harvest was doubled and summed to give LAD for the whole season in terms of weeks (Table 2). The differences in LAD caused by nitrogen treatment can be explained in part by the small changes in average number of leaves per plant (Table 2). The application of nitrogen also increased the average leaf area. Thus, both the size of the leaf and number of leaves per plant were responsible for the increase in LAD when nitrogen was applied. Morton and Watson (10) found that both increased cell division and cell enlargement were responsible for larger leaves in sugarbeets when nitrogen fertilizer was added.

The late application of nitrogen produced as great a LAD for the season as did the early nitrogen, but sucrose yields for the early nitrogen treatments were higher, as noted in Table 1. Two reasons can be postulated for the lack of close relation between LAD and yield. First, the preplant nitrogen provided larger leaf areas early in the season when solar radiation and air temperatures also were high (Figure 2), thus increasing the potential to produce photosynthate. Second, nitrogen applied in July decreased sugar yield by inducing late vegetative growth which delayed the accumulation of sugar in the root.

Net Assimilation Rate—Air temperature and total solar radiation were measured daily at Fort Collins during the 1966 growing season. The average values for 2-week periods corresponding to harvest dates are plotted in Figure 2. Radiation was relatively high from mid-June to mid-September, but highest readings were present during early July. The leaf area curves show that the preplant nitrogen treatments produced greater leaf area for periods of highest radiation than did the July applications of nitrogen. The preplant application of nitrogen would be expected, therefore, to produce more photosynthetic material provided the leaf area was not so large as to cause excessive mutual shading. Stout (12) pointed out that a surplus of leaves may be a liability rather than an asset. As the leaf area increases and mutual shading increases, light intensity on the lower leaves may be so low that respiration exceeds photosynthesis, thus reducing total carbohydrate accumulation by the plant.

The rate at which dry matter was produced per unit leaf area (NAR) was computed for each harvest throughout the season and average rates were calculated through September (Table 2). Considerable variation was found in NAR for harvests after September 24. By the end of September, roots had attained most of their weight, leaf area was declining rapidly, and the total dry weight for the above-ground portion of the plant was decreasing. These factors are, no doubt, the principal contributors to the late season extreme variation in NAR.

No significant difference was found between average NAR values for the season for treatments, although the check treatment might appear to be most efficient (Table 2). The F_1 variety appeared also to be slightly more efficient than the A56-3 variety. This might be expected since the hybrid was known to develop greater root-top ratios[6]. The time at which nitrogen was applied also had different effects on NAR values during the season, even though final results were about the same.

The effect of time of application of nitrogen on NAR during the season is shown in Figure 4; the 125 and 250 lb rates of nitrogen and varieties were combined. After a maximum in early July, the NAR decreased with time throughout the rest of the season and approached zero in September. This decrease with time is in agreement with data presented by Watson (17) and Campbell and Viets (4). The NAR values declined more rapidly during July and August for plants fertilized with nitrogen in March than for those in the check treatment or the treatment that received late nitrogen in July (Figure 4). This may have been caused by the larger leaf area for the preplant treatment

[6] Husseini, op. cit.

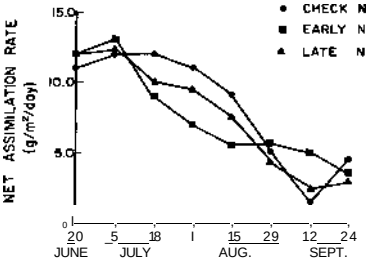

Figure 4.—Effect of time of application of nitrogen on net assimilation rate; nitrogen rates and varieties combined.

and less efficient exposure to solar radiation. Correlations between NAR and LAI were negative throughout the season, and the coefficients were more highly negative for periods with greatest leaf area. The correlation coefficient between LAD and average NAR for the season for all treatments was —0.60. This correlation is less than the —0.85 correlation obtained by Campbell and Viets (4) who worked with plants having much larger leaf areas. No significant correlation could be found between NAR and total dry weight for any of the harvests and values varied considerably from one harvest to the next. LAI, however, was significantly correlated with total dry weight for harvests throughout the season. The correlation coefficients for the various harvests ranged from 0.90 to 0.60. Net photosynthesis for the range of leaf area obtained in this experiment thus was dependent more upon the total leaf area than upon the efficiency of the leaf area. This is consistent with the findings of other workers (7, 15, 16).

Root Growth—'The accumulative root yield during the season is given in Figure 5. The two nitrogen rates for each time of applying nitrogen were averaged because there was no interaction. The varietal effect, significant at the end of the season, did not appear until September, and then only for those treatments receiving nitrogen. The lower yield of the F_3 may have been the result of a *Cercospora* leaf spot infection which was considerably greater on this variety during the latter part of the season. Differences in leaf area between varieties that appeared early in the season had little effect on early season root yield. Any advantage of the lower top-root ratio of the F_1 may possibly be realized only when grown at a higher stand.

The advantage of the preplant application of nitrogen on root yield appeared as early as June 20, and by July 12, at the time of the late application of nitrogen, the response over the check was 2 to 3 tons per acre. The difference in yield between plants receiving early and late applications of nitrogen remained about 2 tons for the rest of the season (Figure 5).

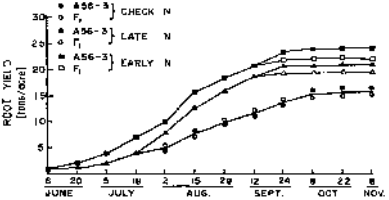

Figure 5.—Effect of variety and time of application of nitrogen fertilizer on fresh root yields; nitrogen rates combined.

From these data it would seem that timely leaf area is important for maximizing root yield. It was noted in Table 1 that the early application of nitrogen was more effective for increasing root yields than was the late application. The rate of root growth, for plants receiving early and late applications of nitrogen, is given in Figure 6 with corresponding LAI values; fertilizer rates and varieties were averaged for convenience in presenting the data. Early in the season, the rate of root growth, in tons per acre per 2 weeks, was greater for plants fertilized with nitrogen before planting; this was coincident with a greater leaf area for

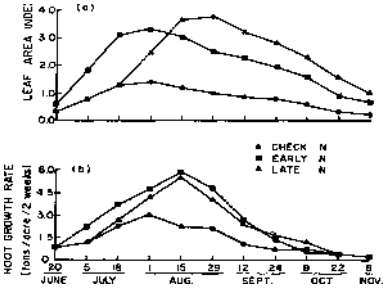

Figure 6.—Effect of time of application of nitrogen on (a) leaf area index, and (b) rate of root growth; nitrogen rates and varieties combined.

plants from the same treatment. Solar radiation and air temperature (Figure 2) also were higher early in the season. Ideally, leaf area probably should have reached a maximum even earlier than that attained with the preplant application of nitrogen.

Even though leaf area of the beets was greater after mid-August for the July nitrogen treatment, the rate of root growth was greater through mid-September for the preplant treatment. The slower rate of root growth for the July treatment during this period was due, apparently, to the high nitrogen status of the plant. With the early application of nitrogen there was a lower level of nitrogen in the plant during August and September (Figure 1) and more assimilate accumulated in the root. On the other hand, plants receiving nitrogen in July had a higher nitrogen status; therefore, these plants had a greater tendency for the assimilate to be used in top growth. After mid-September the leaf area of the beets for the preplant treatment was greatly reduced, but by this time the solar energy and air temperature were so low that the extra leaf area for the July application had little advantage (Figure 6).

The LAI for each harvest throughout the season was correlated with final root yield. Correlations were highest for the July 18 harvest, where a coefficient of 0.60 was obtained; thereafter, correlation of LAI values from later harvests with final root yield decreased as the season advanced. It would appear that leaf area early in the season had more bearing on the final root yield than did a comparable leaf area at a later date. The net effect is probably related to interactions among solar radiation, temperature, and nitrogen status of the plant. A correlation coefficient, significant at the 1% level ($r = 0.71$, 38 d.f.) was obtained for LAD and final root yield for all treatments. The data indicate that the correlation coefficient would be considerably higher if correlations were calculated for the early application only, and even larger if only one variety were considered.

Recoverable Sucrose—Variety differences in leaf area had no significant effect on percentage recoverable sucrose throughout the season, so varieties were combined for the following discussion. Plants that received the higher rates of nitrogen were lower in recoverable sucrose throughout the season, but because rates within an application date exhibited the same trend, they were averaged to indicate the effect of time of application of nitrogen on percentage recoverable sucrose (Figure 7). Beets that received an early application of nitrogen were low in recoverable sucrose early in the year but increased steadily throughout the season. Although plants receiving preplant nitrogen were lower in sucrose than those of the check for the season,

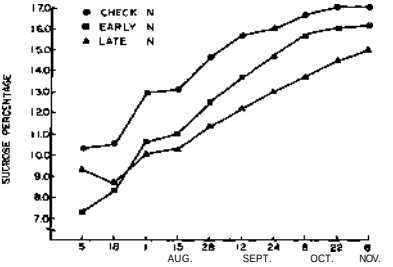

Figure 7.—Effect of time of application of nitrogen on the percentage of recoverable sucrose; nitrogen rates and varieties combined.

they still managed to give recoverable sucrose percentages over 16% by October. Nitrogen applied July 12 caused the recoverable sucrose to remain low until mid-August before it began to increase. Leaf area curves (Figure 2) show that it was mid-August before maximum leaf areas were achieved in plants that received late nitrogen. Thus, most photosynthate to that time was used for producing top growth.

LAD and final recoverable sucrose percentage for all treatments were negatively correlated ($r = -0.55$, 38 d.f.). Although significant at the 1% level, only about 30% of the variability in sucrose percentage could be accounted for by LAD. LAD was positively correlated with the yield of recoverable sucrose, but only about 22% of the variability in yield of sucrose ($r = 0.47$, 39 d.f.) could be attributed to LAD. This was a direct consequence of the higher positive correlation ($r = 0.71$, 38 d.f.) between LAD and final root yield.

Higher correlation coefficients were found for the relationship between LAD and percentage recoverable sucrose or yield of sucrose when each date of application of nitrogen was considered separately. This was because the relationship between leaf area and yield of roots or sucrose depended upon the time when nitrogen was applied (Tables 1 and 2). For example, LAD accounted for 85% of the variability in the yield of recoverable sucrose for the season ($r = 0.92$, 16 d.f.) when only preplant applications of nitrogen were considered.

Rates of accumulation of recoverable sucrose during the season were calculated to explain the influence of treatment on final yield of sucrose. Sucrose accumulation rates for the early and late applications of nitrogen with rates and varieties com-

bined (Figure 8) show the definite advantage of the preplant applications through most of the growing season. It is interesting to note that although the late applications of nitrogen developed leaf areas by mid-August as great as those given where nitrogen was applied preplant (Figure 2), the roots did not accumulate sucrose at a comparable rate until after mid-September; by this time accumulation rates were very low because of a less favorable climatic environment. Lower recoverable sucrose plus a slower rate of root growth for most of the season caused the lower yield of sucrose where nitrogen was not applied until July 12. It is also possible that the capacity of the root to absorb sucrose (sink capacity) may have an influence on sugar accumulation (13), although the magnitude of this effect is not known. If sink size is significant, then an early application of nitrogen would have had a definite advantage over the check or delayed applications of nitrogen by providing a larger sink earlier in the season.

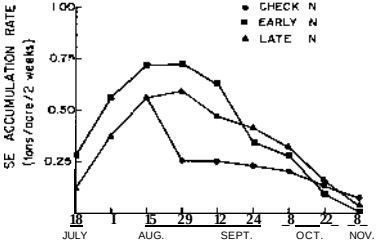

Figure 8.—Effect of time of application of nitrogen on the rate of accumulation of recoverable sucrose; nitrogen rates and varieties combined.

Results for the preplant nitrogen treatments (Figure 9) show that the rate of sucrose accumulation in the root was greater for the 125 lb rate of nitrogen than for the 250 lb rate until about September 1, although leaf area was greater for the 250 lb rate. The lower rate of accumulation of sucrose for the 250 lb treatment prior to September 1 was apparently the result of a higher nitrogen content of the plant (Figure 1). This caused more vegetative growth and less sugar accumulation. It should be noted here, however, that the sacrifice in early accumulation of sucrose is not necessarily detrimental. The results indicate that a large leaf area should be established early in the season when radiation and temperature are optimum. This promotes early root growth and greater sucrose accumulation later in the season. This was evidenced by the greater final yield of sugar

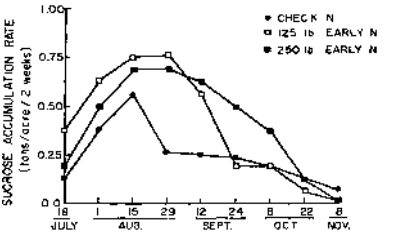

Figure 9.—Effect of preplant applications of nitrogen on the rate of accumulation of recoverable sucrose; varieties combined.

for the 250 lb rate over the 125 lb rate of preplant nitrogen. Roots from the check treatment, although highest in sucrose percentage, were not high-producing because plant growth was severely restricted much of the season.

Some question exists then, as to what should be considered optimum leaf area. Watson (16) suggested that a LAI between 6 and 9 may be near the upper limit of the agricultural range for maximum growth rate of sugar beets. Although values of this magnitude were not obtained in this experiment, the largest leaf areas for the season did produce greatest amount of dry matter. Since dry matter production was still increasing at the highest rates of nitrogen fertilization, it can be assumed that optimum leaf areas for dry matter production were above those encountered in this experiment. Although high leaf area often gives greater root growth and total dry matter production, a higher sucrose accumulation does not necessarily follow. Campbell and Viets (4) and Goodman (6) suggested that the optimum LAI may be closer to 3 for maximum sugar production. Results of the present study indicate that the optimum LAI may be 3 to 4 when attained early in the season. It is doubtful that a LAI greater than 3 or 4 would have been beneficial in this experiment unless the maximum was reached earlier in the season. The optimum leaf area would be expected to vary during the season because of fluctuations in temperature and radiation. A high LAI late in the season, caused by abundant supplies of nitrogen, was inferior not only because it delayed sucrose accumulation, but also because an excessive leaf area was presented at a time when climatic conditions were less favorable for photosynthesis. Differences in optimum leaf areas for sugar production may exist also between varieties. The F_1 hybrid of this experi-

ment was able to produce more sucrose per unit leaf area than the commercial variety. Since the hybrid had a lower leaf area and higher root-top ratio, sucrose yield for the F_1 might have been raised by increasing the stand to increase the LAI.

Summary

A factorial experiment that included two sugarbeet varieties and five nitrogen treatments was conducted on a calcareous Nunn clay loam to study the effect of variations in leaf canopies on the yield of roots and sucrose. Harvests were made at 2-week intervals throughout the growing season for growth analysis.

The genetically heterogeneous commercial variety grown in northeastern Colorado gave greater leaf areas, root yields and sucrose yields than did the F_1 hybrid. The hybrid, however, did have higher root-top ratios and higher net assimilation rates throughout the growing season. Varietal differences in final yields of sucrose were caused by varietal effects on root yields late in the season. No varietal differences were noticed for sucrose content or thin juice purity.

Nitrogen effects on leaf area were highly associated with yields of roots and sucrose. When nitrogen was applied in March before planting, the higher rates of nitrogen produced greater leaf canopies early in the season and caused greater yields of sucrose in the fall harvests. The greater sucrose yields were a consequence principally of larger root yields with only small reductions in sugar content.

When nitrogen was applied as a side-dress application in July, a comparable leaf area index was obtained about a month later than when nitrogen was applied in March. Root yields were lower, however. Sucrose content and sucrose production were reduced also when compared with the preplant treatment.

Optimum leaf area index values for sugar production for this experiment appeared to be 3 to 4. No single nitrogen treatment maintained highest sucrose accumulation rates throughout the season; highest accumulation rates during the season shifted from treatment to treatment and depended upon both leaf area and nitrogen status of the plant.

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Comparison of Sugarbeet Single-Cross Hybrids with Double-Cross Hybrids for Seed and Root Characteristics

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The use of F_1 crosses between inbred lines to produce superior corn hybrids was outlined by Shull in 1909 (4)[2]. However, hybrid corn on a commercial basis did not become a reality until after Jones (3) suggested the use of the double-cross system to overcome the economic problems involved in producing commercial single-cross hybrids. Hallauer (1) states that with the development of improved inbreds, improved cultural practices and a better understanding of gene action involved in heterosis, the trend is now toward single-cross and three-way hybrids. In the Iowa corn yield tests for potential commercial varieties, 1.6% of the entries were single crosses in 1957 while 30.8% of the entries were single crosses in 1965 (1).

Primary disadvantages of commercial single-cross varieties are:

1—Seed production costs are increased since inbreds produce the seed in the case of single-cross hybrids while F_1s are the seed bearing parents for double-cross hybrids.

2—Single-cross hybrids are less likely to be adapted to as wide an environmental range as three-way or double-cross hybrids.

Advantages from the use of single-cross hybrids are:

1—Higher yielding ability than three-way or double-cross hybrids (2).

2—Greater uniformity than more complex hybrids.

3—Restorer lines or lines with Mendelian male sterility are not required to produce seed for the commercial hybrid.

4—Testing of hybrids which combine three or more inbreds is not necessary.

Data presented in this paper were gathered over several years to weigh the advantages and disadvantages connected with the use of single-cross sugarbeet hybrids.

Materials and Methods

Single-cross sugarbeet hybrids have been tested by the Utah-Idaho Sugar Company for 5 years as potential commercial varieties. In 1967, a double-cross hybrid and four single-cross

[1] Geneticist, Seed Production Manager and Fieldman (now Senior Fieldman Idaho district); Utah-Idaho Sugar Company, respectively.

[2] Numbers in parentheses refer to literature cited.

hybrids, made up of inbreds contained in the double cross, were compared. Tests were conducted at Garland, Utah; Idaho Falls, Idaho; Toppenish, Washington and Moses Lake, Washington. Tests were planted in a randomized block design. Hybrids were replicated ten times at each location.

In 1968, the same double cross tested in 1967 was compared with two selected single crosses at West Jordan, Utah, as well as at the locations where the 1967 tests were grown. Hybrids were planted in a randomized block design replicated 10 times at each location. Gross sugar per acre, tons of beets per acre, percent sucrose and impurity index data were obtained for all hybrids at all locations for both 1967 and 1968.

In each of 3 years (1965, 1966 and 1967), cytoplasmic male-sterile inbred lines were compared with CMS F_1 crosses for yield of seed per acre, clean off percentage and percent germination. In 1968, six CMS inbred lines were evaluated for the above characters but were not compared with a CMS F_1.

CMS lines and crosses were planted in strips with a pollinator in an adjacent strip. In 1965, one non-replicated planting was made comparing four inbreds with one F_1. In 1966 and 1967, tests were conducted in each of three fields. Only two fields were used in 1968. All seed tests were conducted at St. George, Utah.

Results

The double cross hybrid in 1967 was significantly higher in production of sugar per acre, beets per acre and impurity index than either single cross having 11863 CMS as the female parent (Table 1). Single crosses with the 511866 pollinator were relatively low in percent sucrose (Table 1).

Table 1.—Comparison of a double-cross hybrid with single-cross hybrids.

Year	Hybrid	Pounds of sugar per acre	Tons of beets per acre	Percent sucrose	Impurity index
1967	(11863CMS × 12163) CMS × (511866 × 512066)	9153	29.63	15.56	7064
	11863CMS × 511866	8522[a]	27.55[a]	15.54	6192[a]
	11863CMS × 512066	8354[a]	26.18[a]	16.06[b]	6282[a]
	12163CMS × 511866	9131	30.66	15.02[a]	7324
	12163CMS × 512066	8952	28.43	15.87[b]	7060
1968	(11863CMS × 12163)CMS × (511866 × 512066)	7066	22.17	15.95	6160
	12163CMS × 511866	7536[b]	24.13[b]	15.63[a]	6405
	12163CMS × 512066	7632[b]	23.24[b]	16.39[b]	5828[a]

[a] Less than the double cross at the .05 level of significance.

[b] Greater than the double cross at the .05 level of significance.

The locations X hybrids interaction was significant for pounds of sugar per acre, tons of beets per acre and impurity index. Differences among hybrids were significant at the .05 level.

In 1968, 12163CMS X 511866 and 12163CMS X 512066 were significantly higher than (11863CMS X 12163) CMS X (511866 X 512066) for pounds of sugar per acre and tons of beets per acre (Table 1). As shown in Table 1, one single cross was higher and the other lower than the double cross for percent sucrose. Hybrids were significantly different for all characteristics studied but none of the locations X hybrids interactions were significant.

Table 2 lists the CMS inbreds grown for seed in 1965. In this non-replicated planting, seed yield of inbreds compared favorably with the CMS F_1. In 1966, the F_1 was superior to the inbreds for production of seed per acre (Table 2) but there was no significant difference for percent germination. The F_1 had a similar clean-off percentage to one inbred but was better than the other.

Table 2.—Seed characteristics of inbred CMS lines and F_1 CMS crosses grown at St. George, Utah.

Year	CMS		Yield of clean seed lbs/acre	Clean-off percent	Germination percent
1965	11563	(Strip)	4623	26.0	76
	11863	(Strip)	5413	25.0	66
	12165	(Strip)	4657	24.5	80
	13063	(Strip)	4470	19.2	75
	11863	(Blended)	4330	9.6	60
	11863CMS × 12163	(Blended)	5916	14.1	81
1966	11863CMS × 12163		4546a*	30.0a	81a
	12163		3922b	33.3a	83a
	1761		2151c	45.2b	82a
1967	11863CMS × 12163		4779a	19.3a	90a
	100363 × 12163		4735a	21.3a	92a
	100363		3262b	26.2a	91a
	12163		3139bc	24.5a	84a
	106666		2793c	28.3a	88a

* Duncan's multiple range test. Means having the same suffix letter are not significantly different at the .05 level.

Data in 1967 indicated no difference among F_1s and inbreds tested for clean-off percentage or percent germination (Table 2). Both F_1s had significantly higher seed yields than the inbreds. Inbred 12163 produced 86% and 66% as much seed as did 11863 CMS X 12163 in 1966 and 1967, respectively.

Using a monogerm CMS F_1 cross, production of commercial seed at St. George, Utah, averaged 4005 pounds of clean seed per acre for 1965 and 1966. Using a monogerm CMS inbred

for commercial sugar beet seed production, yield of clean seed per acre decreased to 3637 pounds in 1967 and to 2500 pounds in 1968. Yield of the best CMS F_1 crosses was about 10% above the yield of the CMS inbred in 1967 and about 35% above in 1968. Year to year differences as well as material grown would, of course, affect seed production.

Discussion

Inbred 11863 appeared to be the inferior component of the double cross, (11863CMS X 12163) CMS X 512066). Inbred 12163 had good combining ability for yield of beets but was not as good as 11863 for combining ability for percent sucrose. Using 12163 as the CMS and either 511866 or 512066 as pollinators resulted in single crosses superior to the double cross for all characters studied in 1968. Line 511866 had good combining ability for yield of beets while 512066 had good combining ability for yield of beets and excellent combining ability for percent sucrose.

Significant locations X hybrids interactions in 1967 indicated that hybrids responded differently at a given location. However, in 1968 when only the two best single crosses were tested there were no significant interactions. The single crosses were consistently superior to the double cross at all locations where tests were conducted.

Seed yields of inbreds decreased considerably as compared to F_1S. However, the production obtained from inbreds was high enough to make production of single crosses economically feasible. Selection of inbreds for greater seed production should be possible. Clean-off percentage and percent germination of the better inbreds was equal to that of the F_1s tested.

Summary

A high yielding sugarbeet double-cross hybrid was compared with four single-cross hybrids in 1967 and two selected single-cross hybrids in 1968. Single-cross hybrids tested were composed of two of the component inbreds from the double cross. Selected single-cross hybrids were superior to the double-cross hybrid for percent sucrose in 1967 and for sugar per acre, tons of beets per acre and percent sucrose in 1968. Location by hybrid interactions were significant in 1967 but not in 1968. The best single-cross hybrid was equal or superior to the double-cross hybrid at each location in both years. In 1968, the best single cross produced 8% more sugar per acre than the double cross over all locations.

Seed yield dropped from about 4000 pounds per acre to 3600 pounds per acre in 1967 and to 2500 pounds per acre in 1968 when CMS inbred lines were used instead of CMS F_1s. Clean-off percentage and percent germination were not affected.

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