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The objective of this study was to investigate the low seed germination of 12 varieties of Oregon grown monogerm sugar beet, Beta vulgaris L., using several methods to determine the germination potential. The methods were: (1) X-ray radiograph examination, (2) standard laboratory germination following official rules, (3) laboratory germination by hydrogen peroxide method, and (4) field emergence.

The X-ray technique was effective and accurate for determining the number of undeveloped seeds in the varieties examined. The use of this technique for determining germination potential is questionable at this time, since the abnormalities present in the seedballs could not be detected from the radiograph.

The speed of germination, as well as total germination, was higher for the hydrogen peroxide method than the standard method.

There was less difference between the two methods for those varieties having decorticated seed. The results of the hydrogen peroxide method compared more favorably with the field emergence results than did the standard method.

The primary factors contributing to low laboratory germination were: (1) undeveloped seeds, (2) abnormal seedlings, and (3) firm ungerminated seeds. The undeveloped seed class included the completely empty seedball cavities and those seedballs having shrunken seeds. The abnormal seedlings were caused primarily by seed-borne pathogens and were most frequent in those varieties having natural seedballs. Abnormalities were higher when using the standard method than with the hydrogen peroxide method. The role of inhibitors, as determined by the number of firm ungerminated seeds, was minor for all the varieties except one.

It was determined that of the three laboratory methods investigated, no single method would give an accurate estimate of the total germination potential of a variety. Therefore, either the X-ray technique or cutting should supplement one of the regular laboratory germination methods to gain additional information on the seedlot's potential.

GERMINATION POTENTIAL IN MONOGERM SEED OF BETA VULGARIS L.

by

Dennis Merlin TeKrony

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GERMINATION POTENTIAL IN MONOGERM SEED OF BETA VULGARIS L.

I. INTRODUCTION

Sugar beets, Beta vulgaris L., are cross pollinated plants with indeterminate flowering that produce either single (monogerm) or multiple (multigerm) sessile flowers at each bract (Figure 1). The flowers form a hard, dry fruit called the "seedball" which has a thick, corky maternal tissue (pericarp) surrounding the true seed (Figure 1).

Sugar beets are biennial plants, but by using the "winter annual method" (34), seed can be produced on a seasonal basis. To employ this method, the seed production area must have mild winters, yet cool temperatures of 45-55°F. for 100 days to satisfy the photothermal induction requirement. Areas of western Oregon, especially the Willamette River Valley, have proven to be well adapted for sugar beet seed production.

A constant problem with sugar beet seed has been the low germination obtained in seed laboratory tests. This problem existed in multigerm varieties, but often went unnoticed as normally one of the three to four seeds per seedball would germinate. With the discovery of monogerm sugar beet seed in 1948 (38), the sugar beet industry anticipated precision plantings which would reduce the hand labor previously needed for the multigerm seed. Yet, when the

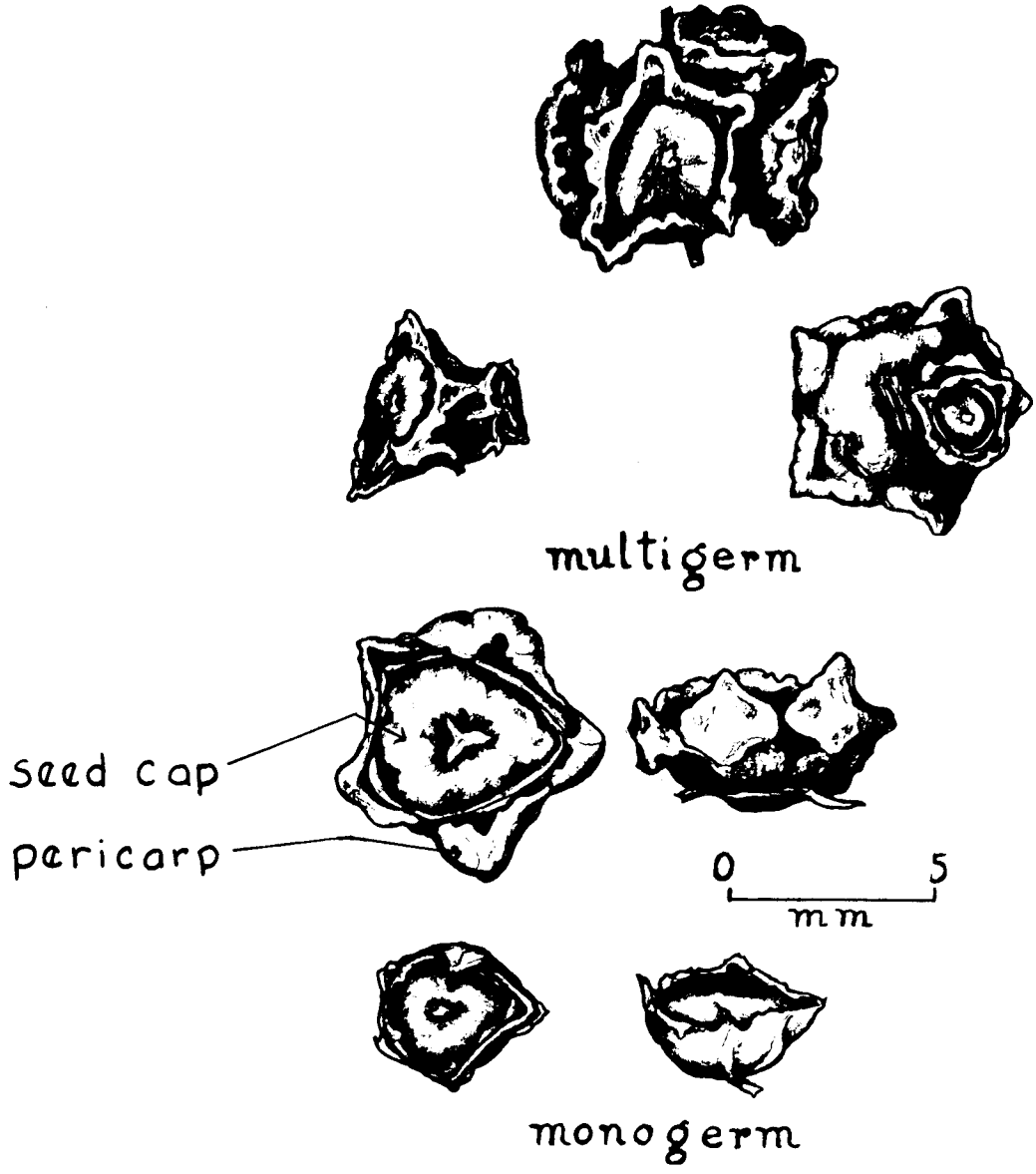

Figure 1. Morphological characteristics of multigerm seedballs (top) and monogerm seedballs (bottom).

monogerm seed varieties were introduced for commercial production, some ten years later, there was immediate concern because of low germination of many of these varieties. When the monogerm seedballs were precision planted at a direct one to one, seed to seedball ratio, poor germinations could be readily detected in field plantings, as well as laboratory tests.

There have been laboratory germination studies through the years on multigerm varieties; however, little information is available on the non-germinability of monogerm seed. Conflicting results have evolved from these studies of multigerm seed as to the possible cause of the poor laboratory germinations. As a result, chemical inhibitors, physical restrictions of the seedball, and environmental restrictions of the growing area have been cited as the most common reasons thought to cause the low germination.

The objective of this study was to investigate the low germination of Oregon grown monogerm sugar beet seed by using several methods to determine the germination potential. Potential germination is defined as "the number of seedball cavities having sufficient embryo and perisperm development to produce a normal seedling." The methods used were: (1) X-ray radiograph examination, (2) field emergence, (3) standard laboratory germination, and (4) hydrogen peroxide laboratory germination.

II. LITERATURE REVIEW

Monogerm sugar beet seed was discovered by V. F. Savitsky (38) in 1948. It was determined that this single-seed trait could be transferred by backcrossing into existent multigerm varieties (5). Therefore, the first monogerm varieties were ready for commercial production in the late 1950's. It was immediately determined that many of these new varieties had low germination. This problem, with reference to monogerm seed, is relatively new and little information has been published on it. Therefore, the information in this review pertains primarily to investigations of multigerm varieties that have been reported throughout the years.

Chemical Inhibitors

Germination tests using sugar beet seeds in the presence of other kinds of seed, have shown that the beet seeds have inhibiting action on germination and growth (12, 16, 51). In 1941, Froschel and Funk (16) suspected the inhibiting ability of sugar beet when they noticed the absence of the weed, Agrostema githago L., in beet fields. They later demonstrated that when the weed and beet seed were sown together, the Agrostema seed failed to germinate. In 1940, Tollman and Stout (51) found that water soluble substances in the seedball of sugar beet produced a toxic effect on germination by retarding

germination and killing the radicles. A year later, they identified this toxic substance to be largely due to the action of ammonia (49) which was liberated from the nitrogenous substances present in the seedcoat during germination.

Following these early investigators, many compounds which are potentially inhibitory to germination have been isolated from the seedballs of sugar beets. DeKock, Hunter and MacDonald (12) obtained from the steeped water extract of sugar beet seedballs an unsaturated yellow oil which acts as a powerful inhibitor when tested on cress seed, Lepidium sativum L., and other seeds. They showed that this inhibitor oil prevented respiration and salt uptake, but was removed by prolonged washing. They also concluded that the inhibitor was more potent in freshly harvested seed. Miyamoto (33) has isolated oxalic acid from multigerm seedballs and has shown that if present in sufficient concentrations, it will inhibit germination. Other researchers (48) have verified these water soluble oxalates to be present in monogerm sugar beet seed and have obtained a positive correlation between speed of germination and the amount of oxalates in water extract for most samples examined. They also determined that the oxalates were concentrated in the corky material of the seedball. In studies by Massert (32) and Koves and Varga (27), many additional compounds have been isolated from aqueous extracts of beet seed which may be potentially inhibitory to germination.

Among the identified compounds are the following: vanillic acid, ferulic acid, p-hydro-oxybenzoic acid, salicylic acid, p-coumaric acid, and p-hydroxycinnamic acid.

Even though many inhibitors have been isolated, the exact effect of these inhibitors on germination and the relationship between concentration of inhibitors in the seedball and the speed of germination is uncertain. In some instances, stimulatory effects have resulted from small concentrations of the same substances that had been isolated as inhibitors. DeKock, et al. (12) noted this stimulatory effect on cress seeds that had been inhibited by the yellow oil extract. When these seeds were washed and set out for germination, the rate of germination was rapid and hypocotyls were stronger. It was concluded that although some processes are retarded, others may proceed normally or be stimulated. Snyder (43) also found that a stimulator may be present in some sugar beet varieties but its presence was usually secondary to inhibitory substances in the seed. It was observed that betaine, which occurs in sugar beet, may also have a stimulatory effect on hypocotyl growth when applied in small amounts (53).

Morphological Variation

There is considerable evidence showing extreme variation of sugar beet seeds within a variety or lot in relation to their speed of germination (43, 48, 51). Ustimenko (52) observed that seeds of

monogerm fruits with a high weight per 100 fruits germinated slower and less completely than those fruits having lower weight. He ascribed this lower germination to a greater amount of pericarp on the larger fruits. Hogaboam and Snyder (23) confirmed this observation and showed a significant positive relationship between fruit size and speed of germination. The effect of temperature during anthesis and seed maturation has been found to affect fruit size (47). Fruits matured at lower temperatures with an average of 66°F. were larger and heavier and greater in number than those matured at higher temperatures of 76°F.

The physical restrictions of the maternal tissue of the seedball have been shown to influence germination. Snyder (43) concluded that the maternal tissues of the seedball usually hinder germination since seedballs notched to expose a portion of the true seed germinated more rapidly than natural seedballs[1] of the same variety. He (45) later concluded that speed of germination in sugar beet seeds was largely controlled by the physiochemical characteristics of the fruit tissue. Sedlmayr (39) confirmed that the speed of germination is controlled mainly by the maternal part of the seedball and that this is a heritable trait. The maternal tissue of the seedball, that lifts from the fruit during germination, is called the seedcap (Figure 1).

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[1] Natural seedballs are as harvested from the plant with no physical alterations by processing or decortication.

It is difficult to measure the exact restriction of this seedcap, although Gemma (17) attributed non-germination of Beta patellaris Moq. to the inability of the radicle to push up the seedcap, even when it absorbs enough water to germinate. Snyder and Hogaboam (47) found that seedcaps of sugar beet seed maturing at higher temperatures (76°F.) are looser and may be shed more readily than seedcaps from seed produced at lower temperatures (66°F.). Peto (36) felt that sugar beet seed produced in the lower temperature range of Vancouver, British Columbia, possesses a thicker, tighter seedcap than multigerm seed of the same sample.

Some evidence is available which indicates that the low germination of some sugar beet varieties may be heritable (39, 40, 54). Yet in an attempt to select for speed of germination, Snyder (44) could not get a consistent result from selected groups within the same variety and concluded that the speed of germination is still controlled by maternal tissues.

Even though there is evidence of varietal variation and some evidence that speed of germination is controlled by either physical or chemical restrictions, little information is available on seedlessness or empty seedballs of either multigerm or monogerm seed. Grimm (18) has shown that the number of filled and empty seedballs can be determined by X-ray examination. Hogaboam (22) confirmed Grimm's technique and found that in 19 monogerm plants examined, all

contained some seedless or empty seedballs which ranged from 10 percent to 35 percent. He also noted variation in the development of perisperm and embryos within the seedballs. He found that the X-ray technique had no deleterious effects on germination performance of seedballs. Empty seedballs or seedless ovarian cavities have also been noted by other experimenters when conducting studies on inhibitors or the effects of the seedball on germination (23, 47). Hogaboam and Snyder (23) found ten percent seedless ovarian cavities of fruits 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. The percentage of developed seed in multigerm seedballs was found to be much higher when plants were matured at lower temperatures (66°F.) than at higher temperatures (76°F.), although the seed matured at the higher temperature germinated more rapidly (47).

Processors have been aware of these empty seedballs and have attempted to determine the number present in a lot by the "crack" test. This test involves forcibly breaking the seedball with a sharp tool and examining the contents for white perisperm or embryo. If located, an attempt is made to remove the empty seedballs from a lot during the cleaning process with machines such as a gravity table. Hammerton (19) indicates that he had a higher germination for seeds cleaned with a gravity table than non-cleaned seeds of the same lot.

Even though the empty seedballs are found in most seed production areas, their cause is most often uncertain. Hills (21) has shown that Lygus bugs, Lygus elisus Van Duzee, which feed on the soft developing seedballs, can cause the embryos to collapse and the resulting seedball cavity to be empty. He has shown that satisfactory results can be obtained with applications of insecticides for the control of Lygus bugs.

Laboratory Germination

Throughout the years, seed laboratories have attempted to counter the various chemical and physical inhibitors that may be present in sugar beet seed and measure the true germination of a seed lot. As a result, various mechanical and chemical techniques have been used to remove these inhibitors from the seedball so that normal germination can take place. As early as 1926, Jackson (26) compared soaking sugar beet seeds to dry planting and concluded the best procedure would be to soak seeds at least two hours before germination. This procedure has been verified by many experimenters and has been followed in the official rules for seed testing of the Association of Official Seed Analysts (2). Several investigators (12, 33, 51) who have isolated various compounds as inhibitory, were usually able to increase germination and counteract this inhibitory action by washing in water. MacKay (30) found germination was higher

when seedballs were pre-washed and planted after drying than when planted wet. It has been determined that combinations of washing and drying for as many as four times are not detrimental to seed viability and usually increase germination (36).

Chemicals and growth stimulators have also been tried for increased laboratory germination. In 1930, Hanley and Woodman (20) found that a treatment of dilute sulfuric acid would increase total germination, due to increased permeability of the seedcoat. Anderson (1), Lackey (28), and Peto (36) have verified that sulfuric acid does increase germination, although Lackey and Peto felt the acid tends to dissolve the maternal tissue. Peto has also found increased germinations by using hydrochloric acid and the enzyme hemicellulase. Peterson's (35) experiments with spray applications of gibberellic acid did not increase the percentage or vigor of germination of sugar beet seed. Snyder (42) found similar results with soaking seedballs in giberrellin solution, although higher concentrations stimulated stem elongation. Dexter and Miyamoto (13) have shown that seedballs treated with hydrophilic colloids germinated faster because of increased water uptake by the seedballs.

The stimulating effect of hydrogen peroxide on seed germination and subsequent seedling growth has been noted in a number of species (8, 9, 10). Ching (9) attributed this stimulation in seed of Douglas fir, Pseudotsuga menziesii Franco, to increased water absorption

and a marked increased respiratory rate.

It has been shown that sugar beet seeds require a medium wetter than most other seeds to germinate (14, 24, 25). Hunter and Erickson (25) found that sugar beets had to contain a minimum moisture content of 31 percent before germination would take place. Cuddy (11) found that the best laboratory germination temperatures for natural seedballs were 20°C. constant or a 20-30°C. alternating, and for decorticated seedballs 20°C. constant.

Another factor that has been shown to affect laboratory germination results is seed-borne pathogens present in the seedball maternal tissue. These pathogens may either attack the seeds prior to emergence from the seedball or cause abnormalities and browning of the seedling after initiation of germination. Anderson (1) observed browning of sugar beet seedlings and partially attributed this to the fungus Rhizopus nigricans Ehr. Other experimenters (3, 7, 31) have found that abnormal seedlings in natural seed were due largely to seed-borne fungi, since they did not occur after seed treatments with fungicides. It was found that seed produced under humid conditions had a greater amount of fungus flora than seed produced in dry regions (3). Brandt (4) feels that this pathogen problem is so important that all seeds tested in his "Germat" procedure are treated with fungicides prior to germination tests.

A mechanical process of seedcoat abrasion, which is commonly

called decortication or processing, is another method that has been used to give increased germination of sugar beet seed. Anderson (1) found that the germination obtained from decorticated seedballs was similar to that of seedballs treated with sulfuric acid. Many experimenters (3, 19, 31) have verified this nearly equal response for germination of decorticated seed compared to chemical treatments or washing in water. In experiments with pre-soaking of whole and decorticated seed, Cuddy (11) found that whole seed responded much more to soaking than decorticated seed. During long soak periods, the decorticated embryos would begin to swell, and often a radicle would emerge from the seedball. Ustimenko (52) also observed that water absorption of decorticated fruits was completed after 40 to 50 hours, whereas whole fruits took 80 to 90 hours and only one-third to one-half as much water was imbibed as in the decorticated fruits.

Smith and Walters (41), in field tests using a precision planter and decorticated seed, felt that the removal of the corky material reduced the effect of inhibitors. Other experimenters (3) have reached the same conclusion when comparing decorticated to whole seeds in germination tests. Snyder et al. (48) found that decorticated seed had a smaller percentage of total water soluble oxalate compared to whole seed. Therefore, the inhibitor, oxalic acid, which they had determined to be concentrated in the corky material of the seedball, could be removed by decortication. Decortication is also

an effective method of removing seed-borne pathogens that may be in the corky maternal layer of the seedball (3, 31).

Field Emergence

Despite extensive precautions to insure an accurate measure of seed lot potential in laboratory tests, there has been much concern due to the poor correlations between the laboratory germination results and field emergence. Porter and Rice (37) showed that in multigerm seed varieties, field emergence was much lower than laboratory results but concluded that the field results were doubtful due to poor climatic conditions after planting. This emphasizes the problems that have been encountered in trying to correlate laboratory and field results. The field conditions are directly influenced by environment and will vary considerably from year to year and field to field. Hammerton (19), and Stout and Tollman (50), in experiments with washed beet seed, found there was little difference between washed and unwashed seed when germinated in soil. Stout and Tolman also observed that when seedballs were washed in laboratory tests, the germination was increased to a point comparable to field results, whereas unwashed seedballs were far below field results. They found that laboratory results with washed seedballs closely compared to filled seeds as determined by the "crack" test, although field results for all varieties were lower than laboratory results and

approximately 10 percent below the "crack" test. Bush (6) also found that field germination was consistently lower than blotter tests in the laboratory, regardless of whether the seed was whole or decorticated. The field results ranged from 10 to 50 percent below the laboratory results.

Both seed-borne and soil-borne diseases play an important part in associations between laboratory and field results. Byford (7) found that field emergence was inversely related to the number of disease infested seedballs and when seeds were planted untreated, the field showed a lower germination than did the laboratory. MacKay and Tonkin (31) found a positive association between field emergence and laboratory, if all the seeds were treated to control disease, although field emergence results were still much lower than laboratory results.

Limited information is available as to optimum soil, moisture and fertility conditions for maximum field emergence, although several researchers (14, 24, 25) have shown that sugar beet seed requires a soil wetter than most other seeds to germinate. It has been shown that with soil temperatures at 50°F., the emergence periods for all seed lots were more than twice as long as at 70°F. (29). Snyder and Dexter (46) found a better correlation between blotter germination at 70°F. and soil emergence at 70°F. than between blotter germinations at 70°F. and soil emergence at 50°F. In

extensive studies on the effects of soil aggregate size on field emergence, Hammerton (19) has determined that a fine soil aggregate gives the highest and most rapid seedling emergence. He felt this was due to a better moisture supply and lower mechanical impedence in fine soil compared to coarser soils. He determined that deep sowing at one and one-half inches had a delaying effect on emergence and reduced the ultimate emergence compared to shallower sowing at a depth of three-fourths inch. He attributed this effect to less mechanical impedence and a shorter period over which the seedlings were susceptible to pathogens. He also reported that seedling emergence was retarded by high levels of nitrogen (150 pounds per acre) in field studies, especially at low moisture levels. This confirmed previous results by Dubetz (15) in greenhouse tests, where he observed lower germination when ammonium nitrate was placed in close contact with the seedball.

III. MATERIALS AND METHODS

Studies were conducted at the Seed Laboratory and East Farm of the Farm Crops Department, Oregon State University. Twelve hybrid monogerm sugar beet seed varieties were selected to represent the principle commercial seed lines currently grown in western Oregon. Of these varieties, six contained natural seed as harvested and six contained decorticated or processed seed which had the corky maternal tissue of the seedball partially removed. (The decorticated varieties are so indicated by a (D) on all tables used in this study).

Four methods for determining germination potential of sugar beet seed were used for each variety. They were: (1) X-ray technique, (2) field emergence, (3) standard laboratory germination following Association of Official Seed Analysts (AOSA) rules (2), and (4) laboratory germination, hydrogen peroxide (H_2O_2) method. When using the X-ray technique, the germination potential is defined as "those seedballs having a filled cavity with perisperm and embryo development". For the laboratory and field germination methods, the germination potential is defined as "the emergence and development of those essential structures which indicate the ability to produce a normal plant."

X-Ray Technique

The X-ray technique used in these studies involved the radiographing of the sugar beet seedballs, after which the same seeds were germinated. The objective of this method was to follow and identify an individual seed from the time of radiographing throughout the entire washing, drying, planting, and counting processes necessary for germination.

A Victor self rectifying mobile X-ray unit having an oil-immersed tube with a beryllium window was used to take the radiographs. The unit was operated at 45 KVP and 3-4 MA. The seedballs were placed at a focal distance of 18 inches from the tube and exposed for 10 seconds on industrial type M film.

Four 50-seed replicates were radiographed and germinated for each variety. Two 50-seed replicates were radiographed at each exposure. The seedballs were placed on an X-ray template (Figure 2) with the seedcap facing the film. After the seedballs were radiographed and the film removed, they were dropped directly from the X-ray template into a washing template. The washing template (Figure 3) was made of 1/2-inch plastic and had 100 compartments in the same alignment as the X-ray template, so that a direct comparison could be made between the radiograph and germination for each seedball. Each compartment was 5/16 inch in diameter and

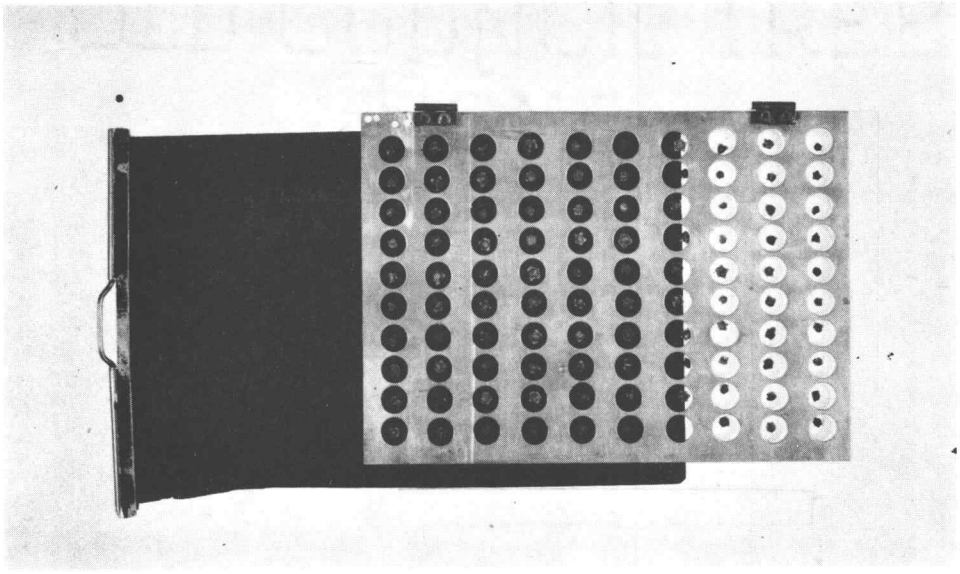

Figure 2. X-ray template with film holder (in black) being removed to allow radiographed seeds to drop directly into cavities of washing template (Figure 3).

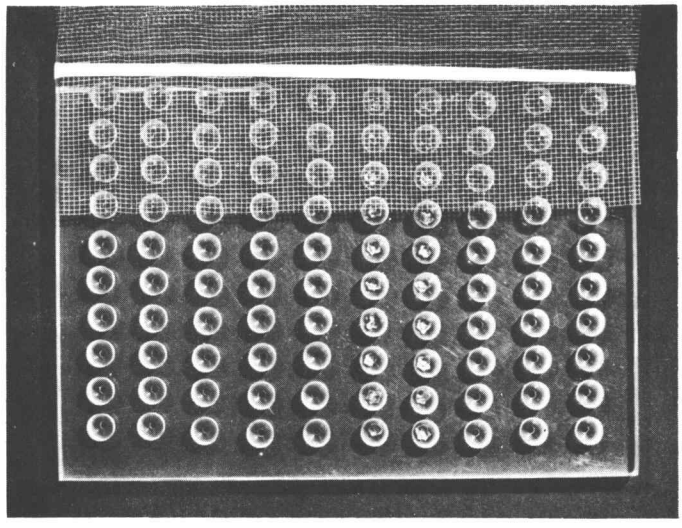

Figure 3. Washing template. The screen completely covers template during washing process to allow for movement of solution through each cavity.

had a small hole in the lower end of the cavity. The open (upper) end of each compartment was covered with a screen during the washing process, which allowed for movement of the solution around each individual seedball.

Prior to germination, the radiographs were examined and estimates of potential germination for each seedball were recorded into two classes: good and undeveloped. Seeds classed as good appeared lighter in color on the radiograph and filled more than half of the seedball cavity (Figure 4). The structural outline of the perisperm and embryo could usually be seen in these good seeds. The undeveloped class included those seedball 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 seedball cavity or lacked perisperm or embryo development (Figure 4).

After recording the radiograph estimates of germination potential of all seedballs, these same seedballs were washed for 16 hours in a 0.1 percent hydrogen peroxide solution in the washing template. They were then rinsed for five seconds in running water and dried for two hours on paper towels. At the completion of the drying, the seedballs were hand planted in blotter boxes in the same alignment in which they were originally radiographed. The planted

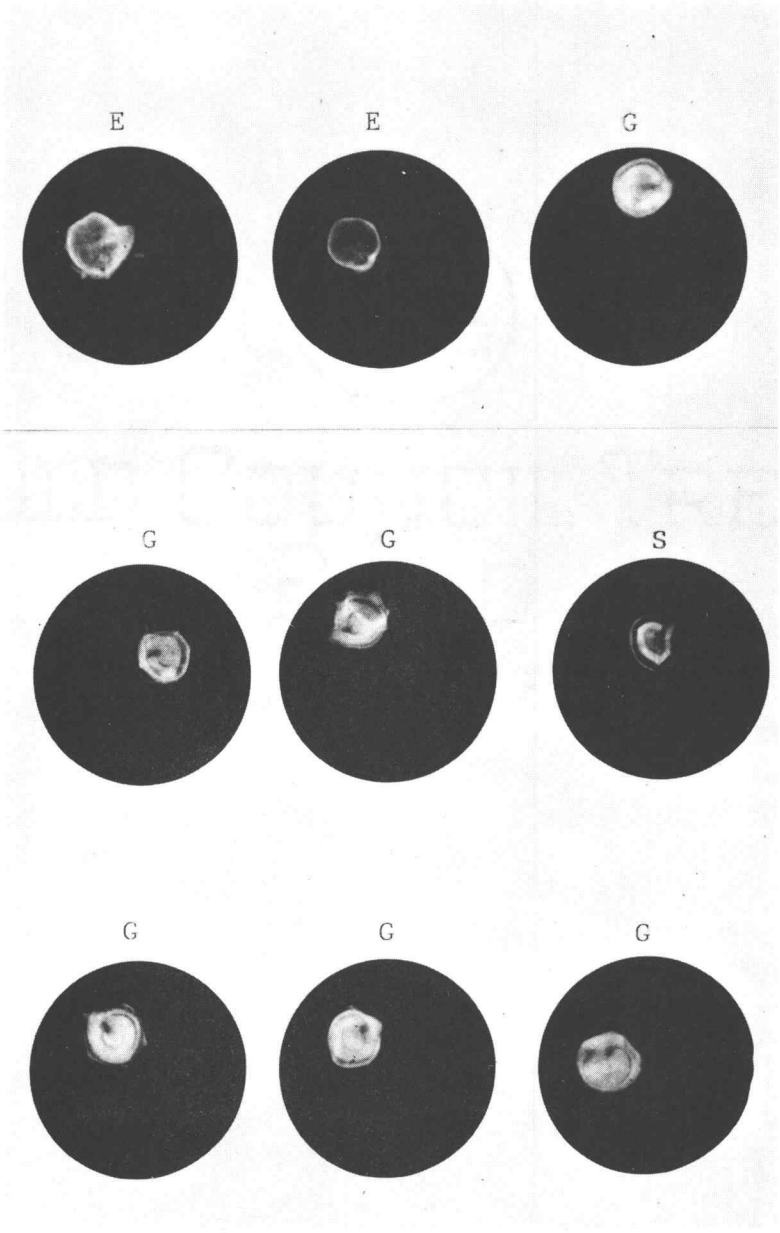

Figure 4. Radiograph of monogerm sugar beet seeds showing empty (E), shrunken (S), and good (G) seeds.

seeds were then placed in a Minnesota type germinator[2] which maintained a temperature of 20°C. for 16 hours and 30°C. for eight hours, and a relative humidity of 95 ± 2.5 percent. Normal seedlings were counted starting at two days and continuing daily for 14 days after planting. Abnormal (diseased) seedlings were removed at the interim counts, but other abnormal seedlings were evaluated at the completion of the test. Normal and abnormal seedling evaluation was in accordance with AOSA rules (2). Seedballs remaining ungerminated at the final count were hand cut and evaluated.

The procedure followed for cutting was to place the ungerminated seedballs with the seedcap down and cut each one in half with a razor blade. The halves were then examined internally for seed development and separated into two seed classes, (1) firm ungerminated and (2) undeveloped. The firm ungerminated class included seed which filled more than half of the seedball cavity and had white, chalky perisperm and a firm white embryo (Figure 5). The undeveloped class included seedballs having completely empty cavities or those having partially developed, shrunken seeds (Figure 5). The seeds classified as undeveloped (shrunken) were either discolored and watery or filled less than half of the seedball cavity. All undeveloped seeds determined by cutting were compared directly to

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[2] Minnesota style germinator No. 2000. Seedburo Equipment Company, Chicago, Illinois.

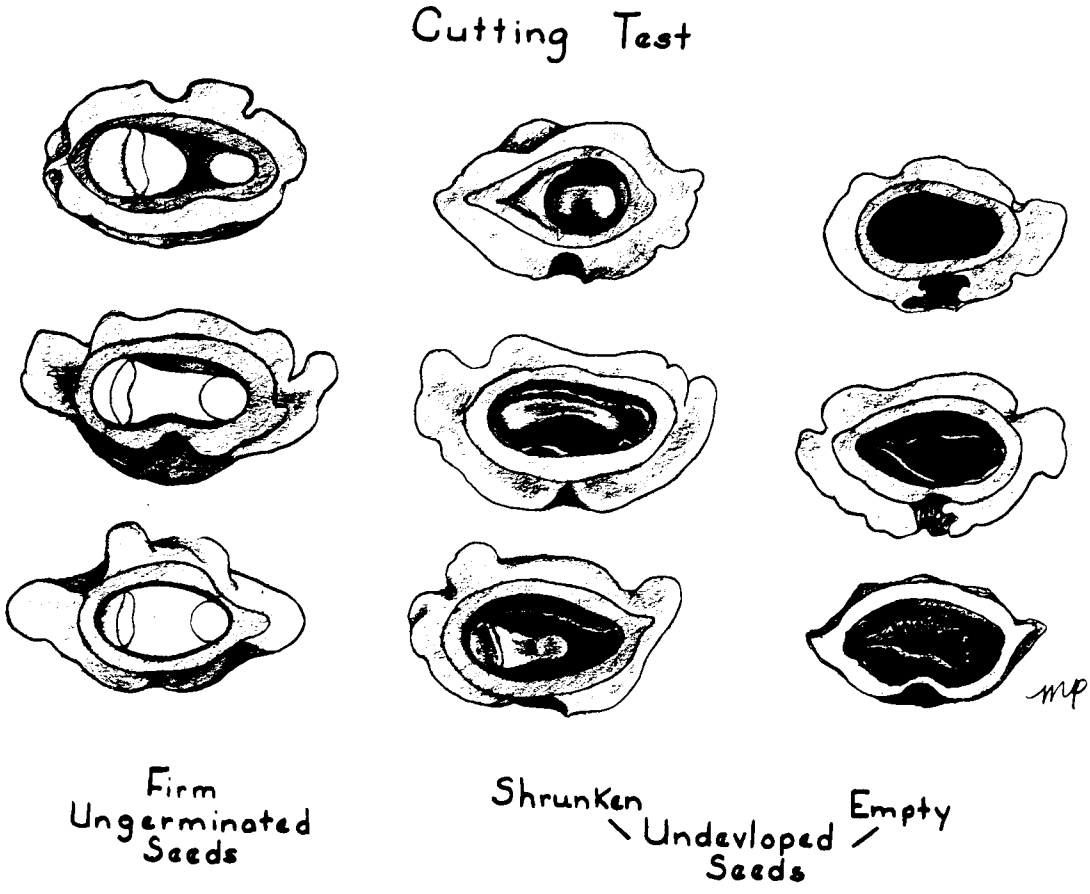

Figure 5. Internal structures of ungerminated seedballs cut in half at final count of a laboratory germination test. Firm ungerminated seeds (on left) have firm white embryo and perisperm. Undeveloped seeds are either shrunken (middle) or completely empty (on right).

the estimates of undeveloped seeds determined previously from the X-ray radiograph.

Field Emergence

The field emergence experiments were arranged in a randomized block design with four 50-seed replications for each variety. All seed was treated with a commercial fungicide prior to planting. The soil type was a fine sandy loam texture which was approximately the same aggregate size as Hammerton (19) found to give a maximum rate of emergence and number of seedlings. The fertility level of the field was determined by soils tests to be adequate and a good seedbed was prepared. All plots were planted in one day during the first week of August.

The 50 seeds for each replication were hand planted to a depth of 3/4 inch on moist soil and spaced three inches apart. All replications and individual seeds were marked with small stakes at planting for positive identification at emergence (Figure 6). Seedling counts of emerged seedlings were started seven days after planting and were continued until six weeks after planting. During the period of maximum emergence (7 to 14 days), daily counts were made to be certain of recording all emerged seedlings prior to damage by insects or pathogens.

Figure 6. Field emergence plot. Small white stakes mark planted seedballs to insure positive identification at emergence.

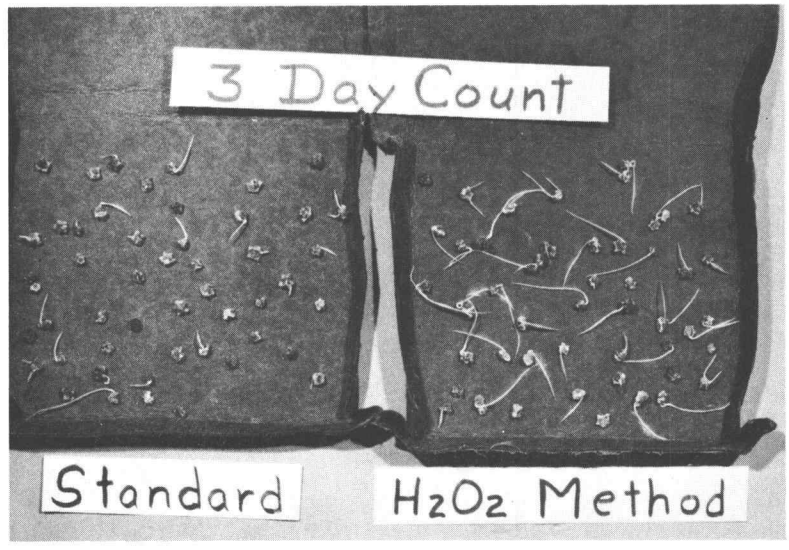

Figure 7. Stimulation of germination at the three-day count by the hydrogen peroxide method (right) compared to the standard method (left). The medium for both methods is blotter-boxes.

Laboratory Germination

Laboratory germination studies, using two methods, hydrogen peroxide and standard, were initiated at approximately the same time as the field emergence studies were conducted. Eight 50-seed replicates were germinated for each method and for each of the varieties examined. Germination tests using both methods were conducted at the same time in the same germinator for each variety. Sanitary precautions were taken to avoid outside contamination during the germination test. This involved cleaning all tools used for planting and counting, covering blotters while soaking and covering beets while drying.

Standard Method

Each 50-seed replicate was soaked in 200 ml of water at 25°C. for two hours. They were then rinsed in warm water for five seconds and placed on top of paper towels to dry for four hours at laboratory temperatures. After drying, seeds were hand planted in blotter boxes (Figure 7) prepared from standard germination blotter medium.[3] The blotters were soaked in water for one hour and drained for one hour to remove excess moisture prior to folding into blotter boxes. The planted seeds were placed in a Minnesota type germinator which maintained a temperature of 20°C. for 16 hours and 30°C. for

[3] Blue-gray seed germinating blotting 120-pound weights acquired from Rochester Paper Company, P. O. Box 185, Rochester, Michigan.

eight hours, and a relative humidity of 95 ± 2.5 percent. No watering of the medium was necessary during the test period. Normal seedlings were counted starting at three days and continuing daily for 14 days. Abnormal diseased seedlings were removed at the interim counts to prevent contamination of other seedlings. Other abnormal seedlings were evaluated at the completion of the test. Normal and abnormal seedling evaluation was in accordance with AOSA rules (2).

Hydrogen Peroxide Method[4]

Fifty seeds were soaked in 200 ml of a 0.1 percent hydrogen peroxide solution for 16 hours. After the soak period, the solution was drained off and the seedballs were rinsed in warm running water for five seconds. They were then placed on paper towels and allowed to dry for two hours. The seedballs were carefully rinsed and dried to prevent any injury to protruding radicles which are often present at the end of the soak period. The remainder of the test, including planting, counting, and evaluation of seedlings, was conducted as described for the standard method.

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[4] Developed at Oregon State University Seed Laboratory by Merle Pierpoint and L. A. Jensen.

Statistical Comparison of Methods

The associations between (1) the radiograph estimate of potential germination to blotter germination and (2) radiograph estimates of undeveloped seeds to undeveloped seeds by cutting, were determined by a correlation coefficient. The least significant differences were determined from an analysis of variance and comparisons were made between the mean of the X-ray estimate and the mean of the normal germination for each variety.

The means of germination of the two laboratory methods (H_2O_2 and standard) were compared to the mean of field emergence for each variety with a Duncans multiple range test. Correlation coefficients were determined to compare the results from the X-ray technique, standard method, hydrogen peroxide method and field emergence for determining germination potential.

IV. RESULTS

The results of germination potential as determined by the four methods used in this study are summarized in Table 1. Mean values for the methods involving all varieties are: X-ray technique, 92.16 percent; hydrogen peroxide method, 81.58 percent; standard method, 74.87 percent; and field emergence, 83.67 percent.

The estimated germination potential determined from the X-ray radiograph is compared to the germination test results of the radiographed seeds in Table 2. The normal germination was consistently lower than the radiograph estimate for all twelve varieties examined. This difference was significantly lower at the one percent level for five varieties and at the five percent level for two varieties. The factors contributing to the significant differences of the seven varieties were abnormal seedlings (five varieties) and firm ungerminated seeds remaining (two varieties). The mean for all varieties for the radiograph estimate was 92.16 percent, while the mean of normal germination was 85.12 percent. The correlation coefficient between the radiograph estimate and the normal germination was $r = 0.618$, which shows a significant association at the five percent level of significance.

The undeveloped seeds as determined by the X-ray technique and cutting are shown on Table 3. This number ranged from 4.5

Table 1. Results[1] of two laboratory germination methods and field emergence compared to X-ray technique for 12 varieties of monogerm sugar beet seed.

Variety	X-ray Technique	Laboratory Germination H_2O_2 Method	Laboratory Germination Standard Method	Field Emergence
1	88.50	77.75	64.50	79.50
2-D	82.50	72.75	71.00	79.00
3	94.50	89.50	81.75	82.00
4-D	94.50	85.50	81.75	88.00
5-D	92.50	85.50	81.00	84.00
6	90.00	81.50	81.25	87.00
7	91.00	69.50	52.50	77.50
8-D	94.50	81.50	72.25	82.00
9	95.00	87.00	74.50	94.50
10	95.00	84.00	76.50	88.50
11-D	94.50	89.00	90.00	88.50
12-D	93.50	75.50	72.50	73.50
Mean	92.16	81.58	74.87	83.67

[1] Results are expressed as a percentage.

Table 2. Percent of estimated germination potential as determined by X-ray compared to the percent germination of the same seeds for 12 varieties.

Variety	Radiograph[1] Estimate	Germination[2] Normal	Germination[2] Abnormal	Germination[2] Firm Ungerminated[3]
1	88.50	78.00**	2.00	9.00
2-D	82.50	77.00	2.50	3.50
3	94.50	88.00*	5.00	2.50
4-D	94.50	87.50*	2.50	4.50
5-D	92.50	91.50	2.00	0.50
6	90.00	85.00	2.00	3.50
7	91.00	77.50**	11.00	3.50
8-D	94.50	85.00**	5.50	3.50
9	95.00	93.50	1.00	1.00
10	95.00	84.00**	8.00	3.00
11-D	94.50	91.50	2.00	0.50
12-D	93.50	83.00**	8.00	3.00
Mean	92.16	85.12	4.29	3.16

1 r = 0.618* for radiograph estimate to normal germination

2 Hydrogen peroxide method (4x50)

3 Determined by cutting method at 14-day count

* Significantly lower at the 5% level

** Significantly lower at the 1% level

Table 3. The estimated percent of undeveloped seeds by X-ray compared to the actual percent found by germination and cutting for 12 varieties.

Variety	Radiograph Estimate[1]	Cutting Ungerminables
1	11.50	11.00
2-D	17.50	17.00
3	5.50	4.50
4-D	5.50	5.50
5-D	7.50	6.00
6	10.00	9.50
7	9.00	8.00
8-D	5.50	6.00
9	5.00	4.50
10	5.00	5.00
11-D	5.50	6.00
12-D	6.00	6.00
Mean	7.79	7.42

[1] $r = 0.987^{**}$ for radiograph estimate to cutting.

percent (variety 9) to 17.5 percent (variety 2-D) for all varieties with an overall mean of 7.79 percent with the X-ray technique and 7.42 percent with cutting. There were no significant differences between the number of undeveloped seeds estimated by X-ray and the number found by cutting. A correlation coefficient showed a highly significant association between the two methods at the one percent level of significance ($r = 0.987$).

A comparison of the germination differences at the 4, 7, and 14 day counts between the standard and hydrogen peroxide methods can be found on Table 4. The hydrogen peroxide method gave a higher germination than the standard method at the final count (14-day) for all varieties except one (11-D). This difference was significantly higher than the standard method at the one percent level for three varieties and at the five percent level for one variety. The hydrogen peroxide method consistently gave a faster germination than the standard method as indicated at the 4-day count. This response was significantly higher at the one percent level for seven of the twelve varieties at four days. There were smaller differences between the two methods for those varieties having decorticated seed except variety 8-D.

The number of abnormal seedlings found when comparing the two laboratory germination methods are shown in Table 5. The standard method consistently recorded a higher number of abnormal

Table 4. Differences in germination results between the hydrogen peroxide and standard methods at the 4, 7, and 14 day count for 12 varieties.

Variety	Percentage Difference H_2O_2 results minus standard results		
	4 days	7 days	14 days
1	25.50**	13.75**	13.25**
2-D	13.00**	3.25	1.75
3	21.50**	13.50**	7.75
4-D	5.00	1.75	3.75
5-D	12.50**	2.50	4.50
6	7.00	(2.00)[1]	0.25
7	16.00**	15.00**	17.00**
8-D	14.25**	6.25	9.25*
9	15.00**	12.50**	12.50**
10	8.75	7.00	7.50
11-D	4.00	(1.25)	(1.00)
12-D	4.00	1.50	4.00

[1] Brackets indicate standard results higher than H_2O_2 results

* Significantly lower at the 5% level

** Significantly lower at the 1% level

Table 5. The percentage of abnormal seedlings found by two laboratory germination methods for 12 varieties.

Variety	Abnormal Seedlings	
	Hydrogen peroxide method	Standard method
1	3.25	16.00**
2-D	4.75	6.00
3	1.75	9.25**
4-D	2.75	6.00
5-D	3.00	5.00
6	4.00	10.50**
7	13.50	28.75**
8-D	6.00	9.75
9	5.25	16.75**
10	7.00	12.50**
11-D	1.75	3.00
12-D	5.50	8.00
Mean	4.87	10.96

** Significantly higher at the 1% level

seedlings than did the hydrogen peroxide method. This difference was significantly higher at the one percent level for six of the twelve varieties. There were no significant differences between the two methods for those varieties that had decorticated seed.

The differences between the two laboratory germination methods and field emergence are shown in Table 6. The standard method gave results that were consistently lower than the field emergence for all varieties except one and significantly lower on five of the twelve tested. Four of the five varieties that were significantly lower had natural (not decorticated) seed. The hydrogen peroxide results more closely compared to the field emergence results with no significant differences recorded between the two methods. Figure 8 shows the germination percentage at the four-day count for the two laboratory methods compared to the seven-day count of the field emergence. The hydrogen peroxide method closely follows the field emergence results for all varieties, whereas the standard method is generally lower than the other two methods.

The correlation coefficients comparing associations between the X-ray technique, hydrogen peroxide method, standard method, and field emergence are shown on Table 7. The standard method and hydrogen peroxide method are significantly associated at the one percent level ($r = 0.862$). The hydrogen peroxide method showed a higher correlation to field emergence than did the standard method

Table 6. Germination differences between field emergence results and laboratory results using two laboratory germination methods for 12 varieties.

Variety	Percentage Difference (Field Emergence Minus Laboratory)[1]	
	Standard Method	H_2O_2 Method
1	-15.00**	-1.75
2-D	- 8.00	-6.25
3	- 0.25	7.50
4-D	- 6.25	-2.50
5-D	- 3.00	1.50
6	- 5.75	-5.50
7	-25.00**	-8.00
8-D	- 9.75*	-0.50
9	-20.00**	-7.50
10	-12.00**	-4.50
11-D	1.50	0.50
12-D	- 1.00	2.50

[1] Laboratory and field emergence results shown on Table 1

* Significantly lower at 5% level

** Significantly lower at 1% level

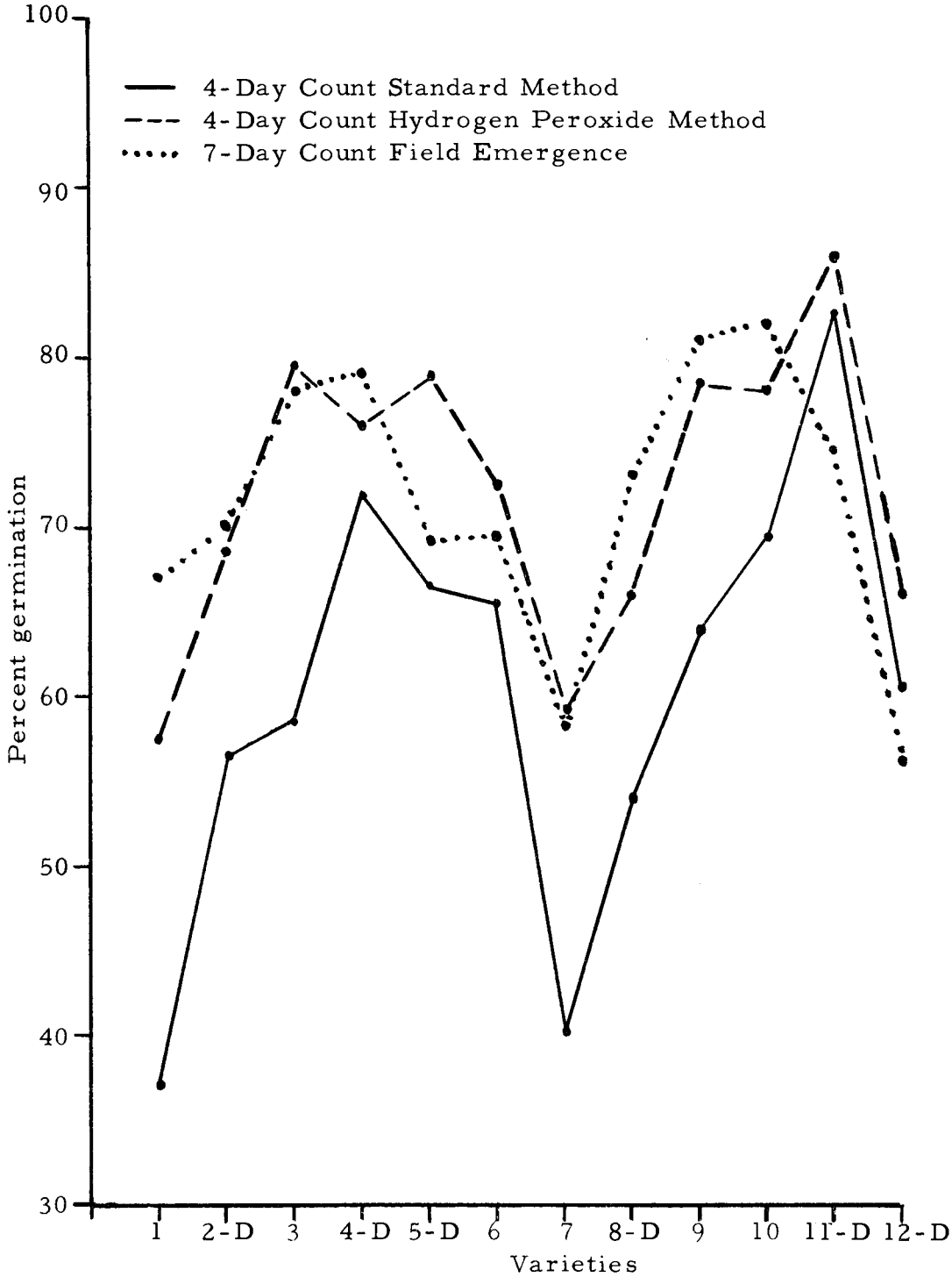

Figure 8. Initial emergence of seedlings. The first count in the field compared to an early laboratory count of two laboratory methods.

(r = 0.735). The hydrogen peroxide method also showed a higher correlation (r = 0.639) to X-ray potential than did the standard method or field emergence.

Table 7. Simple correlation coefficients (r) for laboratory germinations, field emergence and X-ray technique of 12 monogerm sugar beet seed varieties. [1]

	X-ray Technique	Field Emergence	Standard Method
Field emergence	0.435		
Standard method	0.164	0.546	
Hydrogen peroxide method	0.639*	0.735**	0.862**

[1] Values of r necessary for significance: 0.576 at 5% level, 0.708 at 1% level.

V. DISCUSSION

The results of these studies re-emphasize the complex problems involved when attempting to gain an accurate estimate of germination potential in monogerm sugar beet seed. Undeveloped seed, ungerminated seedballs, abnormal seedlings, and decortication of seedballs are among the factors found in these studies to influence or lower the germination results. It is difficult to obtain a single laboratory method that will overcome all of these obstacles and give an accurate estimate of germination potential that can be used as a guide to field performance.

A technique such as X-ray was known to be much faster than a regular germination test, but little information was available on its use or accuracy for routine measuring of germination potential. These results have shown that the estimates of potential germination by the X-ray technique were consistently higher than the normal germination of the radiographed seeds (Table 2). The differences ranged from 1.0 percent (variety 5-D) to 13.5 percent (variety 7), and were significant for seven of the twelve varieties examined. When significant differences did occur, they were clearly attributed to: (1) abnormal seedlings, (2) firm ungerminated seeds, or (3) a combination of the two. These abnormal or firm ungerminated seeds did not appear different on the radiograph from seeds that

produced normal seedlings. Therefore, when estimating the potential germination from the radiograph, they were classed as good seeds, which explains why the estimate was consistently higher than normal germination. Abnormal seedlings are commonly found in sugar beet seed, and they should be reported as abnormal rather than being recorded as a part of the normal (good) seedlings when conducting a germination test. It should be pointed out that the "soft" X-ray used in this study had no deletorious effect on normal germination.

Even though the use of X-ray for estimating germination is questionable at this time, it should be emphasized that the method still has advantages and that with improved technique it could be used in the future. The present official germination methods for all seeds are a slow and laborious procedure. These methods involve many personnel, much equipment, and a testing period of at least ten days. A single X-ray unit requiring few personnel could furnish germination results in one hour. This could revolutionize germination testing for seed laboratories and greatly hasten the movement of seed in marketing channels.

The X-ray technique did prove to be an effective and accurate method for determining the number of undeveloped seeds in a variety. The estimated number of undeveloped seed by X-ray compared very closely to the actual number found by cutting for all varieties (Table 3).

The results of X-ray and cutting were not significantly different for any variety and showed a highly associated correlation coefficient ($r = 0.987$). The X-ray technique could be used routinely to give the percentage of undeveloped seeds in a lot. These data would supplement a standard germination test and aid in explaining problem lots. The results could also be used by processors as a guide in cleaning operations or by farmers as a guide for field planting.

The number of undeveloped seeds found in these varieties ranged from 4.5 percent to 17.5 percent with an overall mean of 7.5 percent (Table 3). This revealed an important problem previously not recognized in Oregon produced seed lots. These undeveloped (empty, hollow) seedballs were known by seed processors to occur, but their role was considered to be minor. In routine seed laboratory tests, the internal portion of the seedball is not examined and the undeveloped seeds are not determined. Therefore, due to past research, nearly all non-germinating seedballs were assumed to be sound, and their lack of response was attributed to physical or chemical restrictions of the seedball.

The percentage of undeveloped seeds in a seed lot has a direct effect on the germination potential of that lot. This is especially true when 10 percent of a lot is composed of undeveloped seed, as was shown on varieties 1, 2-D, and 6 of this study. If these undeveloped seeds could be removed from the seed lot, the germination

potential of the lot would be substantially increased. This is indicated in Table 8, where the percentage of undeveloped seed were added to the normal germination in the hydrogen peroxide method. This increased the germination potential of nearly all varieties (except 7 and 12-D) to 90 percent or more.

The cutting method has been used to determine firm ungerminated seeds as a supplement to the standard germination test for tree seeds. This procedure also proved to be helpful in these studies, when used at the final count of a germination test. Although cutting is much slower than X-ray, it gave an accurate count of undeveloped seeds in the varieties examined. It also gave the number of firm ungerminated seeds remaining at the end of a germination test, which is a good indication of inhibitors present in a lot. For these two reasons (undeveloped seed and firm ungerminated seed), the cutting method could supplement a standard sugar beet germination test. This would provide much needed additional information on the non-germinating seedballs in a lot which is not obtained by the present official testing methods.

The percentage of completely empty cavities in the undeveloped class varied somewhat but generally accounted for 75 percent of all undeveloped seeds found. The remaining 25 percent were classed as shrunken or partially developed. By observation, it appears that size of seedball has no direct relationship to the undeveloped seeds

Table 8. An estimation of the germination of each variety if the percentage of undeveloped seeds is added to the normal germination percentage.

Variety	Normal Germination[1] (plus)	Undeveloped Seeds[2]	Possible Germination
1	77.75	11.50	89.25
2-D	72.75	17.50	90.25
3	89.50	5.50	95.00
4-D	85.50	5.50	91.00
5-D	85.50	7.50	93.00
6	81.50	10.00	91.50
7	69.50	9.00	78.50
8-D	81.50	5.50	87.00
9	87.00	5.00	92.00
10	84.00	5.00	89.00
11-D	89.00	5.50	94.5
12-D	75.50	6.00	81.5

[1] Hydrogen peroxide method (Table 1)

[2] X-ray technique (Table 3)

found. Hogaboam and Snyder (23) had previously concluded this while studying the influence of the size of fruit and seed on germination.

Even though the undeveloped seeds are found in most tests, their cause is uncertain. Hills (21) has shown that lygus bugs which feed on the soft developing seedballs can cause the embryos to collapse and the resulting seedball cavity to be empty or hollow. Parthenocarpy has been suggested as a possible cause for the empty seedballs. Other theories are failures during pollination, nutritional deficiencies of the plant, or environmental effects of the growing area on seed development.

Specific chemical analyses were not conducted in this study to determine the amount of chemical inhibitors present in the varieties examined. As previously mentioned, a good indication of the presence of inhibitors is the number of firm ungerminated seeds remaining at the final count (Table 2). Except for variety 1, this percentage was less than 5 percent for all varieties examined and had a mean of 3.16 percent. This indicates that the role of inhibitors seems to be of minor concern in these studies. The fact that variety 1 had 9 percent firm ungerminated seeds suggests that an inhibitor may be present in this variety.

The current AOSA rules recommend the standard method with water for germination of sugar beet seed. In these studies, the

hydrogen peroxide method gave consistently higher laboratory germination results than the standard method (Tables 1 and 4). This higher germination response was similar to that obtained by other chemical treatments such as dilute acids (28, 36).

The differences (Table 4) between the hydrogen peroxide and standard methods decreased with the duration of the test, although four varieties were still significantly lower with the standard method at the final count. The close correlation (Table 7) between the two methods indicates that they were both measuring the same germination response, although the hydrogen peroxide method was usually higher than the standard method.

The exact chemical action of the hydrogen peroxide on the germination of sugar beet seed was not determined in this study. Stimulation of germination by hydrogen peroxide has been shown in other kinds of seed, and attributed by Ching (9) to increased respiratory rate. This stimulation is indicated in these studies by the faster germination at the early counts (Table 4, Figure 7). The stimulus at the four-day count compared very favorably to the early count (seven-day) of field emergence (Figure 8). The radicles sometimes emerge from some seedballs during the 16-hour H_2O_2 soak period prior to planting, which seems to substantiate the suggestion that a stimulus is provided by the hydrogen peroxide. This emergence of the radicle can be a detriment in the H_2O_2 test, as additional caution

must be taken to prevent damage while planting.

Several researchers (3, 7, 31) have reported the effects of seedborne pathogens on sugar beet germination. These pathogens usually attack the young seedling just at emergence from the seedball and cause abnormalities in laboratory tests. It appears that the primary reason for the lower germinations with the standard method in these studies was the abnormal seedlings. The principle pathogens causing the abnormalities were identified as Phoma betae Frank, and Penicillium spp. The number of abnormal seedlings was consistently higher for the standard method (Table 5) than the hydrogen peroxide method. This was significant on six of the twelve varieties examined. For this reason, it was felt that the hydrogen peroxide tends to sterilize the surface of the seed, thereby reducing abnormalities due to surface pathogens.

The decortication process is often used by companies to aid in sizing monogerm seed for precision planting. This mechanical process has also been used to increase germination (3). It can be observed in these studies that there are less differences between the two laboratory methods with respect to decorticated varieties than natural varieties (Table 4). One reason for this is that the number of abnormal seedlings from decorticated varieties (Table 5) is much lower for both methods. With decorticated seed there were no significant differences in number of abnormal seedlings between

the two methods. This confirms results of other researchers (3, 31) that the seedball maternal tissue is the primary carrier of seedborne pathogens present in sugar beet and that, if a portion of this tissue is rubbed off, the effect of the pathogens on germination is reduced.

Snyder et al. (48) has demonstrated that a large percentage of the chemical inhibitors are concentrated in the seedball and can be removed by decortication.

The field emergence results for these studies were higher than would be expected, considering the potential of the varieties used. This was due in part to the fact that every effort was made to have the most ideal soil and moisture conditions for field emergence as well as positive identification and counting of emerged seedlings. It would be expected that under other soil types and environmental conditions, the field emergence of the same varieties could be considerably lower than those reported in this study.

The field emergence results compared very favorably to the laboratory results when using the hydrogen peroxide method, as shown by Table 6. This method was significantly associated with field emergence at the one percent level (Table 7). The standard method gave laboratory results that were below the field emergence and significantly lower for five varieties (Table 6). It can be observed from Figure 8 that generally those varieties which germinate poorly in the laboratory follow the same trend of poor germination

in the field.

In comparing the overall results of the two laboratory methods and field emergence, it appears that the hydrogen peroxide method would give a better indication of the germination potential for field planting. This method was consistently higher than the standard method, had fewer abnormal seedlings and compared much closer to field emergence. For these reasons, this method should be considered by the AOSA as an alternate or replacement of the standard method for sugar beet germination testing.

VI. SUMMARY AND CONCLUSIONS

Investigations were conducted on the low germination of Oregon grown monogerm sugar beet seed using several methods. The germination potential of twelve varieties was determined by each method and comparisons were made between methods. The methods used were: (1) X-ray technique, (2) standard method, (3) hydrogen peroxide method, and (4) field emergence.

The X-ray technique was effective and accurate for determining the number of undeveloped seeds present in a variety. Seeds which were abnormal or firm ungerminated in a laboratory germination test could not be distinquished from normal seedlings on the X-ray radiograph. Therefore, the X-ray estimate of germination was consistently higher than the normal germination of the radiographed seeds. Since this technique did not detect abnormalities present in the seedballs, it did not give a true estimate of the germination potential of the varieties examined.

The standard method, which is the "official" laboratory germination method following AOSA rules, had a slower speed of germination and a lower total germination than the hydrogen peroxide method in these studies. The difference was less pronounced between the two methods for those varieties having decorticated seed.

The field emergence results compared very favorably to the laboratory germination results of the hydrogen peroxide method. The results of the standard method followed the same trend as the field emergence results but were consistently lower.

The primary factors contributing to low laboratory germination of some of the varieties examined were: (1) undeveloped seed, (2) abnormal seedlings, and (3) firm ungerminated seeds. The undeveloped seed class included the completely empty seedballs or those having badly shrunken seeds and had a direct effect on the germination of a variety. The abnormal seedlings were caused primarily by seed-borne pathogens and were highest in those varieties for which natural seedballs were used. Abnormalities were higher when using the standard method than with the hydrogen peroxide method. Except for one variety, the number of firm ungerminated seeds remaining was small and the role of inhibitors minor.

From these investigations, it was concluded that no single method studied would give an accurate measure of the total germination potential for a variety. Therefore, either the X-ray technique or cutting should supplement one of the routine laboratory germination methods as part of a germination test. This combination would furnish the number of undeveloped seeds which would aid in determining the total germination potential of the seed lot.

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