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Experiments on the Origin and Nature of a Zinc Deficiency of Field Beans on a Portneuf Soil as Determined by the Application of Zinc 65

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EXPERIMENTS ON THE ORIGIN AND NATURE OF A ZINC DEFICIENCY OF FIELD BEANS ON A PORTNEUF SOIL AS DETERMINED BY THE APPLICATION OF $ZINC^{65}$

by

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INTRODUCTION

In a few instances an agricultural problem lends itself well to research into some of the more basic aspects of a problem, as well as to research to determine the exact nature of the problem and to prescribe a cure. Such a problem was found by the author to exist on a soil of the Twin Falls, Idaho area. Besides making an attempt to ascertain the exact nature of the problem, an experiment was set up in an attempt to understand more about the mechanism responsible for the problem.

The Portneuf silt loam soil comprises a large amount of the cropland in the Twin Falls area. Where the soil is irrigated, an unusual condition has occurred. Three years ago in some fields, field beans were grown following a sugar beet crop in which the sugar beet tops were plowed back into the soil. Under these conditions, the beans grew poorly and were thought by this author to be suffering from zinc deficiency.

The following year, two rows of beans were sprayed with a zinc sulfate spray in order to verify that the disease was zinc deficiency. They responded very well to the treatment, which helped confirm that this was a zinc deficiency. After this, a large sample of the soil was transported to Logan for further experimentation.

The Portneuf silt loam is a volcanically originated, highly calcareous soil of pH 7.6. This soil has a lime layer at 17 to 18 inches and is estimated to be of only moderate fertility.

This study was initiated in an attempt to determine if the zinc

deficiency in the field could be duplicated in a laboratory growth chamber by growing field beans on the same soil, which had been treated with dried sugar beet tops. Furthermore, this study was an attempt to determine what effect added sugar beet tops have on the plant's ability to acquire zinc and also if and where in the soil the inactivation of zinc occurs.

REVIEW OF LITERATURE

History

Zinc is one of the sixteen chemical elements generally considered to be necessary for the normal growth and reproduction of plants. It is also necessary in animal nutrition.

Zinc deficiencies in the field were first observed in Florida in 1927 on crops growing on peat soils. In the 1930's zinc was used to cure zinc deficiency of citrus in Florida and California (Seatz and Jurinak, 1957). Deficiencies have also been found to occur commonly in plants grown on calcareous and non-calcareous soils throughout the West, particularly in the case of deciduous and citrus orchards. Regions affected by zinc deficiencies include the Columbia Basin in Washington and the Sacramento Valley in California. Scott and Boshett (1949) described zinc deficiencies as occurring in plants grown on old corral areas in California. Viets (1951) indicated very definite deficiencies on soils of newly irrigated lands of the Yakima Valley and the Columbia Basin Project in Washington. Seatz and Jurinak (1957) indicate zinc deficiencies have occurred widely on the highly phosphatic soils of central Tennessee and Kentucky, especially where the soils are heavily limed. They describe two conditions which might cause the appearance of zinc deficiencies. Increased fertilization has produced larger yields which remove large stores of available zinc, and new cultivation of low zinc soils tends to develop deficiencies.

Factors affecting the availability of soil zinc

As in most types of plant nutrition, two factors govern the ability of a soil to provide sufficient zinc to the growing plant, (1) the total supply of zinc in the soil and (2) the availability of soil zinc. According to Seatz and Jurinak (1957), most soils in the United States have more than enough zinc to meet the requirements of normal plant growth. In spite of this, crops may develop zinc deficiency. Thus the major problem is one of availability. The following discussion covers the known factors affecting soil zinc availability.

Cation exchange capacity. According to the theory of Epstein and Stout (1951), a sandy soil must have about 1.5 parts per million (ppm) zinc, and a clayey soil, 15 ppm zinc. Concentrations below this represent percentages below 0.1 to 0.2 percent of the cation exchange capacity of the soil. They have found that the availability of metal ions to plants decreases sharply below these values. In other words, these values represent the critical range, assuming no interactions of pH, precipitation, chelation, and perhaps other factors. This apparently is not a valid assumption for many soils but serves as a good first approximation.

Brown (1950) indicated that when a soil was shaken with 0.01 M $ZnSO_4$ solution, there was a fast reaction which reached equilibrium with 4 to 20 milliequivalents (meq) zinc retained per 100 grams of soil, and that this equilibrium amount depended on soil pH, organic matter content, and cation exchange capacity.

Chandler et al. (1952), when they mixed soil with a known amount of $ZnSO_4$ and subsequently filtered and analyzed the filtrate, found that the fixing power of the soil ranged from 170 to greater than 1,000

ppm zinc.

Jamison (1943) reported that larger particles of zinc materials released more leachable zinc than smaller particles. Since the small, evenly distributed particles apparently allowed more intimate contact with the soil than did the larger particles, strong absorption in the upper layers of the soil was indicated.

Soil pH. One of the first factors to be correlated with zinc deficiency was soil pH. At first it was thought that as pH increased, zinc solubility decreased. Wear (1956) showed, with field tests in Alabama, that there was a reduction in corn yield of ten or more bushels per acre produced by adding one ton per acre of lime to a soil low in available zinc. Also, he showed that addition of lime cured zinc toxicity in plants grown in zinc dipped containers. Epstien and Stout (1951) showed that uptake of zinc by plants increased with increased hydrogen ion concentration and decreased with higher calcium ion concentration. Waltz et al. (1953) indicated a more pronounced fixation of zinc in the soil with the addition of limestone than with applications of phosphate. Peach (1941) concluded that as the pH of the soil is increased by addition of $CaCO_3$, the amount of extractable zinc diminishes and virtually all of the zinc is fixed at pH 9.0.

McGeorge (1948) found that adding sulfur and a sulfur-manure mixture to the soil increased the zinc content of plants. Gall (1936) showed that soils which absorbed the greatest amount of zinc were those high in organic matter, calcium, and colloid content. Mahoric (1936) suggested that this was a simple reaction of $ZnSO_4 + CaCO_3 \rightarrow ZnCO_3\downarrow + CaSO_4$. Wear (1956) indicated this was a lime-pH effect rather than a calcium-pH effect, since he showed reduced zinc uptake with pH

increased by Na_2CO_3. Jurinak and Thorne (1955) disputed this, however, as in an experiment they performed, zinc solubility reached a minimum at pH 5.5 to 6.7 in the Na and K systems and increased greatly with pH increases, suggesting the formation of soluble alkali zincates. In the Ca system, zinc solubility reached a minimum at pH 7.6 with no increase in solubility at higher pH.

Lott (1938) showed that applications of a few hundred ppm zinc to acid soils was detrimental to oat seedlings. However, addition of $CaCO_3$ alleviated the toxicity. This indicated that zinc is more available at low pH, and addition of $ZnSO_4$ causes the pH to go even lower in poorly buffered soils.

Thorne et al. (1942), in studies of zinc relationships in Utah soils, found no significant difference in concentration of zinc in calcareous and non-calcareous parent materials, but indicated that zinc is more readily removed by weathering from non-calcareous parent materials. Complete analysis of these Utah soils showed good correlation between organic matter and zinc and between pH and zinc (both total and available zinc).

Leyden and Toth (1960) showed that the zinc content of tomato tops decreased as the pH increased from 5.5 to 7.5. Also as the pH increased from 5.5 to 7.5, less fertilizer zinc was absorbed, and more came from the soil.

Leeper (1952) attributed the unavailability of zinc in calcareous soils to the $CaCO_3$ and showed that as the age of the precipitate increased, the zinc became less available due to the rearrangement of the crystal ions into a more ordered, compact arrangement (aluminum oxide, for example, dissolves easily in dilute acid when freshly

formed but not after standing for a few hours). Hibbard (1940) suggested that a portion of the acid extractable zinc may be held in the mineral lattice and only hydrogen ions would be small enough to enter and replace it.

Peech (1941) also found that zinc could be virtually all extracted from sandy soils with a normal NaCl solution of pH 3.0. As the pH of the soil was increased, the amount of extractable zinc diminished. Nearly all of the zinc was fixed at pH 8.0 even up to 300 pounds per acre. Nelson and Melstead (1955) did the same experiment with ammonium acetate extraction and found that with acid soils nearly all of the zinc could be removed, but in a calcareous soil a portion of the zinc could not be removed, except by extraction with 0.1 N HCl. Jurinak and Thorne (1955) postulated that at high pH available zinc precipitates in the form of insoluble calcium zincate. This acid soluble portion of the zinc increased in quantity with an increase in time that the zinc was exposed to the calcareous soil. Apparently, the acid soluble zinc did not occupy the exchange sites in calcareous soils since there was no reduction of base exchange capacity on formation of the acid soluble zinc form.

Fixation by organic matter. As mentioned above by Thorne et al. (1942), Gall (1936), and Scott and Boshett (1949), organic matter, as well as calcium and pH, affects availability of soil zinc. Apparently, increases in organic matter decrease the availability of zinc by some means such as chelation. Himes and Barker (1957) indicated that soil organic matter has a high affinity for zinc. The humic fraction of the soil absorbed about four times as much zinc as the fulvic fraction. This particular soil was 9.5 percent organic matter with a cation

exchange capacity of 12.8 meq per 100 grams. The soil absorbed 27.5 meq zinc per 100 grams of soil. The humic fraction composed 11 percent of the soil organic matter, yet it absorbed 59 percent of the added zinc. The fulvic fraction comprised 5 percent of the soil organic matter and absorbed 12 percent of the added zinc. The zinc held by the organic fraction could be removed to some extent by EDTA but was not removed to any appreciable extent by analine.

Fixation by soil minerals. Seatz and Jurinak (1957) suggested that zinc may be absorbed in a non-exchangeable form on commonly occurring lime minerals.

Wear and Sommer (1947) attempted to relate the amount of acid extractable soil zinc to observed deficiency symptoms. Using the acetic acid extraction method, they found that on one soil no deficiencies occurred at a zinc level of 0.6 to 3.5 ppm but that deficiencies appeared at levels of 0.2 to 0.5 ppm. Using the 0.1 N HCl extraction, levels were 1.2 to 4.7 ppm zinc showing healthy plants and 0.5 to 0.9 ppm zinc giving symptoms of deficiency.

Elgabaly and Jenny (1943) in their discussion of zinc associated with the soil mineral fraction suggested three forms of zinc were present in the soil: (a) replaceable zinc, (b) difficultly replaceable zinc (inner layer of the double layer), and (c) non-replaceable zinc (mineral lattice zinc). In another study, Elgabaly (1950) showed that 10 to 70 percent of the applied zinc became fixed (not replaceable by neutral ammonium acetate). He believed the fixed zinc had entered the mineral crystal lattice in one of three ways. First, it might fill cavities which are of comparable size in the aluminum octahedral layer. Theoretically this can occur easier in a 1:1 lattice (entrance

is more easily accomplished through holes produced by the distorted aluminum octahedra on the a - b planes). It seems improbable, according to him, that the zinc ion could enter the Al-Si-Al structure through the SiO planes. Second, the zinc might be adsorbed as a complex monovalent ion, thus becoming a part of the inner layers of the electrical double layer. Third, the zinc might replace magnesium in the octahedral coordination of the mineral lattice (Mg^{++} and Zn^{++} are of similar size). Jurinak and Bauer (1956) indicated that the change of zinc in the soil from ammonium acetate extractable to acid extractable (more difficultly removed) may be due to dehydration of the zinc ion after adsorbtion.

Zinc functions in the plant

Seatz and Jurinak (1957) also indicated that zinc is a necessary component of several enzyme systems which regulate the metabolic activities of the plant. Zinc is a part of the enzyme carbonic anhydrase which regulates the equilibrium between CO_2, water, and carbonic acid. It also functions as a part of two other enzymes, dehydropeptidase and glycylglycinedipeptidase, which have a part in specific aspects of protein metabolism.

Skoog (1940) showed that plants deficient in zinc were low in auxins, and auxin content of the plant increased when zinc was added. Leopold (1955) suggested that zinc was necessary for the normal production of tryptophan, a precursor of the auxin, indoleacetic acid. Other evidence (Elliot, 1952) indicates that zinc deficiency may limit tryptophan production by limiting serine formation (serine is considered to be condensed with indole to form tryptophan).

Zinc deficiency symptoms

Plants differ in their ability to extract zinc from the soil.

Viets et al. (1954) studied the ability of 26 different crops to remove zinc from the soil. They classified the plants in three categories. The plants which had poor ability to acquire zinc were beans, soybeans, corn, hops, grapes, lima beans, flax, and castor beans. The mildly sensitive group included potatoes, tomatoes, onions, alfalfa, sorghum, sudan grass, sugar beets, and red clover. Insensitive plants are peppermint, the cereals, asparagus, mustard, carrots, safflower, and grasses.

Zinc deficiencies in plants cause several abnormalities in structure. According to Seatz and Jurinak (1957), the palisade cells of leaves from most affected plants are larger and transversely divided, rather than columnar, as in normal leaves. Therefore, zinc deficiency may lead to cell enlargement rather than cell differentiation. Other abnormalities may occur as a reduction in the number of chloroplasts, absence of starch grains, presence of oil droplets in the chloroplasts, presence of calcium oxalate crystals, and the accumulation of phenolic materials in the leaves. These chemical changes in the plant indicate that zinc may be related to metabolism of carbon in plants.

Seatz and Jurinak (1957) indicated that the roots of zinc deficient plants may also be abnormal. Tomato roots may have a series of swellings with whorls of root hairs near the root tip. Cell structure is also unusual. Cells of the meristematic region may be enlarged and of an irregular arrangement with many air spaces between cells. Older tissue becomes necrotic, with flaky masses of exfoliated cells. Root metabolic products are also unusual. Tannins, fats, and calcium oxalate are present in abnormally large amounts while starch is absent.

Zinc deficiency in trees is known as little leaf, rosette, mottle

leaf, and yellows. Citrus and deciduous fruits are very susceptible to this disease. Little leaf describes one of the visual symptoms of many crops--small, sometimes distorted and chlorotic leaves. Zinc deficiency causes a decrease in growth of stem internodes, thus giving a rosetting effect. The leaf chlorotic color pattern is distinctive but may at times be mistaken for other minor element visual symptoms. The color pattern when used with the other mentioned physical deformities, however, gives reliable indication of zinc deficiency.

Sommers (1928) showed that, with a mild zinc deficiency, no visual symptoms of zinc deficiency were apparent on red kidney beans until flowering, at which time there was rapid absicission of leaves and flower buds.

Correction of zinc deficiency

Application of zinc compounds to the soil for relief of deficiencies has met with unreliable success. Leyden and Toth (1960) indicated very poor results with $ZnSO_4$ applied to the soil. They also showed that, as soil pH rose from 5.5 to 7.5, less of the fertilizer zinc was absorbed and more came from the soil. Peech (1941) called the results of $ZnSO_4$ applications to Norfolk soils to correct zinc deficiency of citrus, "disappointing." Shaw et al. (1954) indicated that the percent utilization of zinc fertilizer was very low and decreased with larger applications of zinc. In corn, zinc applied to the seed furnished most of the plant's zinc. The plants showed very little difference in utilization of zinc from $ZnSO_4$ and $ZnCO_3$. Boawn et al. (1957) tested zinc utilization from $ZnSO_4$, Zn chelate, stripping acid residue, blast furnace slag, ZnO, $Zn_3(PO_4)_2$, $ZnCO_3$, zinc frits, and commercial zinc granules. Results showed that the zinc chelate gave good improvement,

while there was no significant difference between $ZnSO_4$ and any of the other zinc sources--they all produced very little improvement of the deficiency problem. Speer et al. (1952) tested three zinc sources--$Zn(NO_3)_2$, $Zn(NH_3)_4(OH)_2$, and Na_2ZnO_2--and found no significant difference in availability of the different sources despite their chemical dissimilarity (Zn^{++}, $Zn(NH_3)_4^{++}$, and $ZnO_2^{=}$). Boawn et al. (1957) indicated that $ZnSO_4$ might otherwise show good results, but its high solubility allows the zinc to transfer rapidly to insoluble or otherwise unavailable forms. They concluded that zinc chelate is the only reliable form for soil application.

Viets (1951) indicated very good results with a foliar spray of 0.5 percent $ZnSO_4$ and 0.25 percent $Ca(OH)_2$. Seatz and Jurinak (1957) indicated that a spray of five pounds of $ZnSO_4$ per 100 gallons of water for application on citrus corrected zinc deficiency for one to three years. He indicated that foliage injury is reduced by adding two to three pounds of hydrated lime, soda lime, or lime sulfur to the spray. Wetting or adhesive agents sometimes give beneficial results. Sprays applied just before flush growth give the longest effect. He also indicated that concentrated sprays of 25 pounds $ZnSO_4$ per 100 gallons of water are effective on apples and pears when applied as a dormant spray just before buds open. Zinc sulfate sprays have also been satisfactory on field crops when the spraying is done early in the season before serious deficiencies arise. Dormant sprays of zinc sulfate have also been effective on deciduous fruits.

METHODS

General methods

The Portneuf soil was prepared by sifting to 2 mm size, after which the sugar beet top (organic matter) treatments were added. The sugar beet tops, including petioles, were harvested from a field near Tremonton, Utah, on October 15, 1960. The sugar beet tops were dried at 80 F in a forced air dryer, then ground in a Wiley mill. The ground sugar beet tops were added to the soils at the rates of 0, 15, 30, and 45 tons per acre equivalent green weight. For calculations, sugar beet tops were assumed to be 80 percent water before drying, and a soil weight of 2×10^6 pounds per acre was used.

The sugar beet tops were mixed with the soil, which was then placed in half-gallon plastic containers at the rate of 1.5 kg per pot. The soil was brought to approximately field capacity with tap water and allowed to incubate at 80 F before seeds were planted. Incubation periods were three weeks, five weeks, and six weeks for experiments one, two, and three, respectively.

This experiment was set up with four organic matter (sugar beet top) levels and eight replications, using a completely random design.

Great Northern bean seeds were planted at the rate of seven seeds per pot. Seven days after planting (about 3 to 3½ days after emergence), the plants in each pot were thinned to the three plants of most uniform size, using the whole stand as the standard.

This experiment and all future experiments were carried on in the growth chambers described in the appendix of this dissertation. A

phototemperature of 88 F and a nyctotemperature of 74 F were used in combination with a photoperiod of 13 hours and a nyctoperiod of 11 hours. These values were found to be near optimum for field beans, according to Went (1957).

The pots had no provisions for drainage, so the soil was kept moist by twice daily applications of tap water. Care was taken to prevent over watering.

On March 30, 1961, the plants were harvested. This constituted a growing period of about six weeks. The plants were beginning to flower at this time. After harvesting, the plants were dried at 80 F in a forced air dryer and then weighed.

Nitrogen addition

The equivalent of 200 pounds of N as NH_4NO_3 per acre furrow slice (2×10^6 pounds) was added to the soil of the second and third experiments in order to alleviate the apparent nitrogen deficiency noted in the first experiment. This was mixed with the soil at the time the organic matter treatments were introduced.

Zinc foliar spray

Seven days after thinning, half of the plants of the second and third experiments were sprayed with a solution containing 200 ppm of zinc as $ZnSO_4$ and a small amount of wetting agent. Care was taken to prevent any overspray or drip from touching or entering the soil. The plants were thoroughly wetted once with the spray.

Incubation and growth periods

For the third experiment, 32 pots were prepared with soil. However, only 16 pots received the four organic matter treatments and

the addition of radiozinc six weeks before planting. The other 16 pots were treated just prior to planting.

The initial 16 pots, after treatment with nitrogen, beet tops, and radiozinc, were brought to field capacity and placed in the growth chamber and maintained at 80 F for six weeks. This constituted the incubation period. Just prior to planting, the other 16 pots were treated in the same manner as were the first 16 pots.

Radiozinc methods

Upon arrival of the 10 microcuries (mc) of Zn^{65} as $Zn^{65}Cl_2$, the shipment was diluted, and approximately 0.3 mc was pipetted into twelve of the 16 pots constituting the incubated treatment. The radiozinc was placed on the surface of the soil, near the center of the pot. The water added to the pot to bring the soil to field capacity and future waterings were relied upon to distribute the Zn^{65} throughout the soil. Three samples of the Zn^{65} shipment were pipetted and set aside for making into counting standards, which was subsequently done.

After harvesting, plants were separated at ground level into aerial parts and roots. Because of their radioactivity, the plants were dried on waxed paper right in the growth chamber at 88 F.

Plant tops were pelleted, weighed, and then counted. A crystal well scintillation counter was used for counting all samples.

Soil fractionation methods

After harvesting of plants, the pots containing roots and soil were left in the growth chamber for two weeks to allow the soils to become air dry. The pots containing radiozinc were then transferred to the laboratory for processing.

The soil in the pot was saturated with 80 percent ethanol. Then the soil was dumped out into a large porcelain pan, where the roots were carefully washed from the soil with 80 percent ethanol. The roots from each pot were dried and pelleted, then weighed and counted.

The slurry consisting of soil and ethanol-water solution was filtered to separate the ethanol-water portion containing the water soluble soil Zn^{65}. The soil was washed three times to remove all water soluble soil Zn^{65}. Eighty percent ethanol was used in order to keep the H_2O concentration as low as possible to avoid removing Zn^{65} on the soil exchange complex by means of a high water concentration.

Then the soil was air-dried and thoroughly mixed, and two samples were taken, a 0.1-gram sample for direct counting, and a 10-gram sample for further soil fractionation. The small sample was counted and this total count used for comparison with the sum of each of the soil fractions.

The 10-gram sample was dried in a vacuum dessicator over sulfuric acid for five days, weighed, and then again saturated with 80 percent ethanol. The soil sample was filtered and washed three more times, and an aliquot of the filtrate was counted. The water soluble Zn^{65} which appeared in the filtrate was added to that which was counted from the first washes to give the total water soluble Zn^{65}.

Next, the soil sample was treated with a pH 5.0 buffer solution of sodium acetate-acetic acid. The soil was washed and filtered with this solution five times after the last effervescence reaction took place on addition of solution. This solution was used in place of a strong acid as it was thought that this buffered solution might affect the Zn^{65} associated with the exchange complex and the organic fraction

less than a strong acid. The Zn^{65} which came out in the pH 5 buffer treatment is termed the acid soluble Zn^{65} and is considered to be mostly the radiozinc which was held physically or chemically in the soil lime or lime minerals.

After the soil lime was destroyed, the sample was treated with 30 percent hydrogen peroxide by the method of Jackson (1958). After destruction of organic matter, the soil and solution were filtered, and an aliquot of the filtrate was counted. In this way, the Zn^{65} associated with the soil organic fraction was determined.

At this point, the soil sample was shaken with a one normal $CaCl_2$ solution, then filtered with three more $CaCl_2$ washes. An aliquot of this filtrate was counted to determine the Zn^{65} associated with the soil exchange complex.

After the calcium chloride treatment was completed, the soil was treated with 10 ml of one normal sodium hexametaphosphate to 100 ml of suspension to act as a dispersing agent. The soil clay fraction (to about five microns) was separated from the sand and silt fraction by stirring the suspension and allowing it to settle, using a rough figure of about one centimeter per second or faster for the settling time for particles larger than five microns. After the appropriate settling time, the liquid and materials in suspension were removed by pipette. This procedure was repeated three times. The total clay materials of each sample were dried and weighed, then mixed thoroughly, and a small sample taken for direct counting. The sand and silt fractions were also dried, weighed, mixed, and sampled for counting.

See Figure 1 for the flow diagram of the analysis of this experiment.

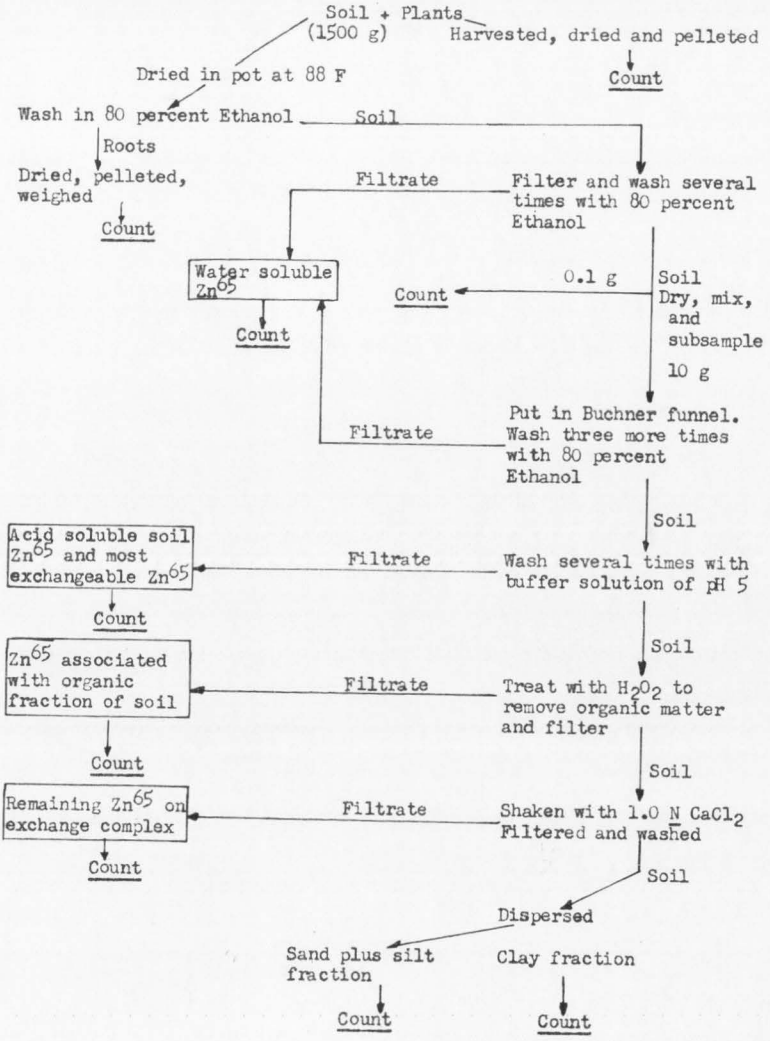

Figure 1. Flow diagram showing the steps in separating the various fractions of the Zn^{65} in the soil.

RESULTS

Preliminary experiment

The first experiment was designed primarily to determine whether the zinc deficiency of field beans noted in the field and attributed to an increased amount of sugar beet tops in the soil could be duplicated in the laboratory growth chamber.

In this test, 32 pots were used, with four organic matter (sugar beet tops) levels and eight replications. Plant top yields were used to give a measure of the effect of sugar beet tops added to the soil on plant yield (table 1).

Table 1. Dry weight yield of tops of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, preliminary experiment

Sugar beet tops	Replications								Avg.
	1	2	3	4	5	6	7	8	
tons/acre	grams per pot								
0	7.14	5.86	6.31	6.18	6.68	7.01	5.92	6.12	6.40
15	5.74	5.62	5.51	5.94	6.14	5.56	5.49	6.03	5.75
30	6.22	5.13	4.97	5.33	5.01	5.14	4.82	5.21	5.23
45	3.49	4.35	3.25	3.96	3.68	3.52	4.11	3.66	3.75

No statistical analysis was run on these data, as it seemed quite obvious that increased amounts of sugar beet tops decreased yields.

After four weeks of growth, some plants (especially those of the higher organic matter treatments) showed visual symptoms of patterned

chlorosis which matched well the zinc deficiency symptoms for beans described in the literature (Viets, 1954) and which were like those symptoms seen in the field the season before. However, the patterns were overshadowed by a general chlorosis which appeared to be due to insufficient nitrogen.

This test was successful in that it showed a suppression of yield due to increased amounts of sugar beet tops in the soil. It also showed limited visual symptoms of zinc deficiency. The experiment, however, failed to rule out the possibility of yield decrease due to nitrogen deficiency, since added organic matter can decrease soil nitrogen available to the plants.[1]

In the next experiment, nitrogen fertilizer was added to alleviate the nitrogen deficiency. Also, a zinc spray was included to prove more conclusively whether or not the plant was able to acquire sufficient soil zinc under these experimental conditions.

Intermediate experiment

This experiment was designed as a randomized block with four organic matter levels (0, 15, 30, and 45 tons per acre sugar beet tops). Two zinc spray levels were used, with three replications. This constituted a 4 x 2 x 3 experiment.

The dry weight yields of the plant tops are shown in table 2 and the statistical analysis in table 3.

The symptoms of nitrogen deficiency which were noticed in the first experiment were lacking in this experiment. The plants were healthy, from all indications, except in the higher level organic matter

[1]In order for soil microorganisms to decompose the added soil organic matter, nitrogen is required. If soil nitrogen is low, the microorganisms compete successfully with plants for the soil nitrogen.

Table 2. Dry weight yield of tops of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, intermediate experiment

Sugar beet tops	Spray	Replications 1	2	3	Total	
Tons/acre		grams/pot			grams	
0	+	3.68	3.74	3.72	11.14	3.71
15	+	3.20	3.31	3.26	9.77	3.26
30	+	4.16	4.22	4.19	12.57	4.19
45	+	4.37	4.10	4.24	12.71	4.24
0	-	3.77	3.65	3.71	11.13	3.71
15	-	3.14	3.06	3.09	9.29	3.10
30	-	2.19	2.30	2.24	6.73	2.24
45	-	1.60	1.83	1.69	5.12	1.71
		L.S.D. 0.05			0.014	.005

Table 3. Analysis of variance, dry weight of plant tops, intermediate experiment (data of table 2)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	6.60×10^{-4}	3.30×10^{-4}	n.s.
Sprays	1	8.074	8.074	0.001
Organic Matter	3	1.774	0.591	0.001
Sprays x Organic Matter	3	7.250	2.416	0.001
Error	14	8.89×10^{-2}	6.35×10^{-3}	

Coefficient of variation--2.44%

[a]Significance not measured beyond 0.001.

treatments of the unsprayed plants. In all cases, plant growth was quite uniform when observed in the growing state. All plants in one treatment initiated flowers within 36 hours of each other. Flower initiation on the plants showing zinc deficiency symptoms occurred later than that of healthy plants, and the number of flowers on the affected plants were decreased.

The 30 and 45 tons per acre equivalent organic matter treatments caused definite symptoms of zinc deficiency on the unsprayed plants, while the sprayed plants of the same treatments were healthy and growing vigorously.

Inspection of the table of analysis of variance on the results of this experiment reveals highly significant differences between sprays, organic matter levels, and interaction between sprays and organic matter levels.

Spraying the plant foliage with the zinc sulfate spray apparently allowed the plant sufficient zinc for normal or nearly normal growth. In all cases, the sprayed plants produced more dry matter than did the unsprayed plants. With the sprayed plants, there was no case of visual deficiency symptoms. Actually, the sprayed plants produced better yields on the higher organic matter levels, while the unsprayed plants produced the poorest yields on the higher organic matter levels. The significance of this interaction is shown in the analysis of variance table. Apparently, the spray furnished sufficient zinc to allow the plant to take advantage of better fertility or better soil conditions brought about by the addition of the sugar beet tops.

In the case of the unsprayed plants, addition of sugar beet tops markedly decreased yields. At this point it seems logical to assume

that this is due to decreased availability of zinc, brought about by the increase in organic matter. It was hoped that a further experiment would show this more conclusively and help shed some light on the changes taking place in the zinc status of plants and soil due to incorporation of sugar beet tops in the soil.

The analysis of variance table indicates no significant difference in replications in this experiment. This indicates the value of the uniform environment provided by the growth chambers. Actually, a coefficient of variation of 2.44 percent is extremely good for a biological experiment of this kind, indicating highly uniform conditions. In the greenhouse, this value might be expected to fall in the range of 20 to 25 percent.

Radiozinc experiment

In this experiment radiozinc (Zn^{65}) was utilized by direct application to the soil before incubation and planting. It was hoped that zinc concentrations within the plant and various fractions of the soil would give some clue as to how and where soil zinc is inactivated following incorporation of sugar beet tops into the soil.

The experiment was a randomized block design consisting of four organic matter levels (0, 15, 30, and 45 tons per acre equivalent of green sugar beet tops), two incubation periods (six weeks and no incubation period before planting), and two spray levels (no spray and sprayed as in the intermediate experiment) with two replications. After the uniform results of the intermediate experiment were observed, it was felt that two replications would give sufficient accuracy. This, of course, allowed another variable to be added and still allowed the experiment to be carried out within the space limitations of the growth

chamber. This gave a 4 x 2 x 2 x 2 experimental arrangement.

Actually, the experiment can be thought of as having four organic matter levels x two incubations x four replications as far as the soil analysis is concerned. It was felt that sprays of $ZnSO_4$ applied only to the plant foliage would have no measurable effect on concentrations of soil radiozinc. Therefore, all measurements of Zn^{65} made on soil fractions ignore the spray treatment and would appear as four replications, except that the fourth replication (second spray replication) was not treated with Zn^{65}. This provided a radiation check. If measurable tissue destruction due to radiation had occurred, it would have appeared as a significant difference in replications in the analysis of variance of dry weight yields. Therefore, when soil fraction Zn^{65} measurements appear in the data, they are treated as three replications.

Plant analysis. As the organic matter level of the soil was increased, the plants sprayed with $ZnSO_4$ increased in dry weight (table 4). This was true for both incubated and non-incubated treatments. The trend was exactly opposite for the unsprayed plants in the incubated treatments. Where the soil and organic matter were allowed to incubate for six weeks, and the plants were not given additional zinc through the spray method, increase in soil organic matter in the form of sugar beet tops decreased the dry weight of the plants.

Where the soil was not incubated but planted immediately after addition of sugar beet tops, plant dry weight increased in the 30 and 45 tons per acre treatments.

The plants sprayed with $ZnSO_4$ yielded better than the unsprayed plants.

The analysis of variance of this data appears in table 5. As in

Table 4. Dry weight yield of tops of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Spray	Incubation	Replications 1	Replications 2	Total
tons/acre			grams/pot		grams
0	+	+	2.344	2.033	4.377
15	+	+	2.292	2.668	4.960
30	+	+	2.688	2.581	5.269
45	+	+	2.745	2.850	5.595
0	-	+	2.313	1.989	4.302
15	-	+	2.006	1.964	3.970
30	-	+	1.657	1.891	3.548
45	-	+	1.585	1.544	3.129
0	+	-	2.268	2.252	4.520
15	+	-	2.305	2.508	4.813
30	+	-	2.675	2.872	5.686
45	+	-	2.975	2.911	5.886
0	-	-	2.162	2.407	4.569
15	-	-	2.187	2.178	4.365
30	-	-	2.888	2.798	5.547
45	-	-	2.923	2.679	5.602
			L.S.D. 0.05		0.007

Table 5. Analysis of variance on the data of dry weight of plant tops (data of table 4)

Source	df.	ss.	ms.	Sig.[a]
Replications	1	3.9×10^{-4}	3.9×10^{-4}	n.s.
Sprays	1	1.065	1.065	0.001
Organic Matter	3	0.6100	0.2033	0.005
Incubation	1	1.153	1.153	0.001
Organic Matter x Spray	3	0.648	0.216	0.001
Incubation x Spray	1	0.613	0.613	0.001
Incubation x Organic Matter	3	0.4834	0.1611	0.005
3 way	3	0.333	0.111	0.005
Error	15	0.3175	2.116×10^{-2}	

Coefficient of variation—6.11%

[a]Significance not measured beyond 0.001.

the previous experiment, there was no significant difference between replications. This again points out the benefits of good control of the growth environment. The coefficient of variation was 6.11 percent which is very good for a biological experiment of this type.

The dry weight yield of plant roots appears in table 6, and the analysis of variance of these data is set forth in table 7. The only apparent difference in the roots was due to organic matter levels and was significant only at the 0.20 level. Table 8 summarizes the data of six observations, where it is apparent that the overall effect of added sugar beet tops was to decrease the amount of root growth.

Looking at radiozinc-plant relationships, it is found in table 9 that the total Zn^{65} found in the plant tops was reduced by higher levels of sugar beet tops in the soil, in the unsprayed plants grown on the incubated soil. However, when the plants were sprayed, the trend was reversed. Analysis of variance of these data is presented in table 10.

Where the soils weren't incubated, there were slight increases in total Zn^{65} in the plants grown on soil with more incorporated sugar beet tops.

The total Zn^{65} uptake was greater on the soils not given an incubation period.

The data on the Zn^{65} concentration in plant tops appear in table 11. The most obvious difference here is between incubations. The incubated soil produced plants with much lower levels of Zn^{65} than did the non-incubated soil.

For the unsprayed plants, Zn^{65} concentrations were generally lower in the higher sugar beet soil application levels. This trend was reversed for the non-incubated soils.

The analysis of variance is given in table 12. As in several other

Table 6. Dry weight yield of roots of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	No spray		Spray
		1	2	1
tons/acre		grams/pot		
0	+	2.775	1.529	1.790
15	+	2.537	1.588	4.230
30	+	1.058	1.174	0.825
45	+	1.277	1.366	1.011
0	-	1.224	2.762	2.059
15	-	2.632	1.031	1.976
30	-	2.370	2.078	1.153
45	-	1.785	0.641	2.647

Table 7. Analysis of variance on the data of dry weight yield of plant roots (data of table 6)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	1.024	0.5121	n.s.
Incubation	1	5.98×10^{-2}	5.98×10^{-2}	n.s.
Organic Matter	3	3.476	1.1586	0.20
Organic Matter x Incubation	3	2.584	0.8614	n.s.
Error	14	8.867	0.6333	

Coefficient of variation—43.9%

[a]Significance not measured beyond 0.001.

Table 8. Effect of added sugar beet tops on root weights of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency, preliminary experiment

Sugar beet tops	Sum of all replications of each sugar beet top level
tons/acre	grams/6 pots
0	12.139
15	13.994
30	8.658
45	8.727

Table 9. Total zinc^{65} uptake by plant tops of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	No spray		Spray
		1	2	1
tons/acre		microcuries		
0	+	1.299	1.377	0.814
15	+	0.962	0.998	1.010
30	+	0.747	0.845	1.113
45	+	0.737	0.652	1.205
0	-	2.439	3.098	2.441
15	-	2.559	2.884	2.542
30	-	4.165	4.055	3.539
45	-	3.815	3.273	3.111

Table 10. Analysis of variance on the data of zinc^{65} uptake by plant tops (data of table 9)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	0.1287	6.435×10^{-2}	n.s.
Incubations	1	28.518	28.5187	0.001
Organic Matter	3	1.228	0.4094	0.01
Organic Matter x Incubation	3	2.331	0.7773	0.005
Error	14	1.166	8.328×10^{-2}	
Replications (1+2 vs 3) x Incubations	1	0.283	0.283	0.03
Replications (1+2 vs 3) x Organic Matter	3	0.157	0.0523	n.s.
Error difference	10	0.726	0.0726	

Coefficient of variation--13.96%

[a]Significance not measured beyond 0.001.

Table 11. Zinc^{65} concentration of plant tops of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	No spray		Spray
		1	2	1
tons/acre		microcuries/gram dry tissue		
0	+	0.562	0.692	0.401
15	+	0.480	0.508	0.441
30	+	0.451	0.447	0.431
45	+	0.465	0.422	0.423
0	-	1.128	1.287	1.084
15	-	1.170	1.324	1.103
30	-	1.557	1.412	1.226
45	-	1.305	1.222	1.046

Table 12. Analysis of variance on the data on zinc^{65} in the plant tops (data of table 11)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	9.621×10^{-2}	4.810×10^{-2}	0.01
Incubation	1	3.481	3.481	0.001
Organic Matter	3	3.775×10^{-2}	1.258×10^{-2}	0.10
Incubation x Organic Matter	3	9.104×10^{-2}	3.034×10^{-2}	0.01
Error	14	8.852×10^{-2}	6.322×10^{-3}	
Replications (1 + 2 vs 3) x Incubation	1	1.51×10^{-2}	1.51×10^{-2}	0.15
Replications (1 + 2 vs 3) x Organic Matter	3	0.42×10^{-2}	0.42×10^{-2}	n.s.
Error Difference	10	6.922×10^{-2}	6.922×10^{-3}	

Coefficient of variation--9.27%

[a]Significance not measured beyond 0.001.

experiments where the radioisotope was the determining entity, there was a significant difference between replications.

The total Zn^{65} uptake by the roots of the bean plants is set forth in table 13. The apparent result in this set of data is the fact that there was more total Zn^{65} in the plants grown on the non-incubated soil than in the plants from the incubated soil. There were no other relevant significant differences. The analysis of variance of these data appears in table 14.

As was the case with total Zn^{65} uptake of roots, the concentration of Zn^{65} in the roots showed a significant difference only between incubations. The Zn^{65} concentration of the roots was greater in plants grown on the non-incubated soil. This set of data appears in table 15 with analysis of variance in table 16.

Soil analysis. The water soluble Zn^{65} extracted from the soil appears in table 17. From the analysis of variance, table 18, it is clear that the only significant difference in these data is the difference between incubations. The soil which was not incubated yielded more water soluble Zn^{65} than did the incubated soil. The difference was significant at the 70 percent level.

The acid soluble zinc data are set forth in table 19, with analysis of variance in table 20. The non-incubated soil contained somewhat more acid soluble Zn^{65} than the incubated soil did. The amount of sugar beet tops in the soil somewhat affected the amounts of acid soluble Zn^{65} in the soil. While the acid soluble Zn^{65} in the soil decreased somewhat with increase in sugar beet top applications in the incubated soil, the amount of acid soluble Zn^{65} increased quite markedly with increased sugar beet tops in the non-incubated soil. This portion of the experiment also showed a significant difference between replications.

Table 13. Total zinc^{65} uptake by plant roots of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	No spray		Spray
		1	2	1
tons/acre		microcuries		
0	+	3.994	2.800	2.708
15	+	1.287	1.592	3.202
30	+	1.164	1.295	1.331
45	+	1.295	1.947	1.496
0	-	2.622	4.321	3.478
15	-	4.373	2.212	3.423
30	-	4.251	2.758	3.591
45	-	1.724	2.621	2.830

Table 14. Analysis of variance on the data of the total zinc^{65} uptake by plant roots (data of table 13)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	0.3954	0.1977	n.s.
Incubations	1	8.275	8.275	0.005
Organic Matter	3	5.664	1.888	0.03
Incubation x Organic Matter	3	3.155	1.052	0.20
Error	14	8.589	0.6135	
Replications (1 + 2 vs 3) x Incubations	1	0.0014	0.0014	n.s.
Replications (1 + 2 vs 3) x Organic Matter	3	1.145	0.3816	n.s.
Error difference	10	7.443	0.7443	

Coefficient of variation--30.20%

[a]Significance not measured beyond 0.001.

Table 15. Concentration of zinc^{65} in plant roots of Great Northern beans grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	No spray		Spray	Sum of sub-treatments in incubations
		1	2	1	
tons/acre		microcuries Zn^{65}/gram			
0	+	1.439	1.831	1.513	
15	+	0.507	1.003	0.757	
30	+	1.100	1.103	1.613	
45	+	1.014	1.425	1.480	14.785
0	-	2.142	1.564	1.689	
15	-	1.861	2.145	1.732	
30	-	1.394	1.327	3.114	
45	-	0.996	4.089	1.069	23.322

Table 16. Analysis of variance on the data on the concentration of zinc^{65} in plant roots (data of table 15)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	0.9318	0.4659	n.s.
Incubation	1	3.037	3.037	0.05
Organic Matter	3	0.6603	0.2201	n.s.
Organic Matter x Incubation	3	0.6167	0.2056	n.s.
Error	14	7.852	0.5609	

Coefficient of variation--47.16%

[a]Significance not measured beyond 0.001.

Table 17. Water soluble zinc^{65} in the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Replications 1	2	3	Sum of sub-treatments in incubations
tons/acre		microcuries/pot			
0	+	2.194	3.709	3.843	
15	+	3.590	3.182	2.318	
30	+	2.416	2.407	1.917	
45	+	1.994	1.762	2.896	32.228
0	-	3.149	2.556	3.847	
15	-	2.639	3.254	2.771	
30	-	2.601	1.811	4.330	
45	-	3.700	2.324	3.282	36.264

Table 18. Analysis of variance on the data on the water soluble zinc^{65} in the soil (data of table 17)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	1.158	0.579	n.s.
Incubation	1	0.679	0.679	0.30
Organic Matter	3	1.529	0.510	n.s.
Organic Matter x Incubation	3	1.199	0.399	n.s.
Error	14	7.603	0.543	

Coefficient of variation--25.78%

[a]Significance not measured beyond 0.001.

Table 19. Acid soluble zinc^{65} in the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Replications		
		1	2	3
tons/acre		microcuries/pot		
0	+	22.6	29.0	38.2
15	+	14.4	20.8	25.5
30	+	12.9	27.4	30.8
45	+	15.3	25.7	26.5
0	-	19.7	33.1	29.6
15	-	28.5	48.8	68.5
30	-	27.4	44.5	83.2
45	-	40.1	45.3	74.6

Table 20. Analysis of variance on the data on the acid soluble zinc^{65} in the soil (data of table 19)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	2402.5	1201.2	0.005
Incubation	1	2692.4	2692.4	0.001
Organic Matter	3	332.5	110.3	0.30
Organic Matter x Incubation	3	1125.7	375.3	0.01
Error	14	1264.5	90.32	

Coefficient of variation--27.39%

[a]Significance not measured beyond 0.001.

The Zn^{65} associated with the soil organic fraction occurs in table 21. Analysis of variance of these data occurs in table 22. There was more soil organic Zn^{65} in the incubated soil than in the non-incubated soil. In the incubated soil, there was more organic Zn^{65} in the soils which were treated with greater amounts of sugar beet tops. In the non-incubated soil, there was no definite trend in differences in organic Zn^{65} due to different levels of sugar beet tops.

Exchangeable Zn^{65} appears in table 23 with the associated analysis of variance in table 24. More exchangeable Zn^{65} occurred in the soil which was incubated than in the non-incubated soil. For the incubated soil, the amount of exchangeable Zn^{65} decreased with increases in amounts of sugar beet tops added to the soil.

There was quite a considerable amount of Zn^{65} associated with the mineral fraction of the soil. The Zn^{65} associated with the sand plus silt fraction is shown in table 25, with accompanying analysis of variance appearing in table 26. There was more Zn^{65} attached to the sand and silt in the soil which was incubated.

In the non-incubated soil, the sand and silt Zn^{65} decreased with increases in amounts of sugar beet tops added to the soil.

Although there was a large amount of Zn^{65} associated with the clay fraction, there was no significant difference in the amount of Zn^{65} on the clay fraction due to any of the treatments. These data appear in table 27 with analysis of variance as table 28.

A table has been prepared which totals all Zn^{65} in each fraction of the experiment, and compares it with the totals from every other treatment, which, theoretically, should be the same. Also included is the total count of the intact soil sample. This sample had only the

Table 21. Zinc^{65} associated with the organic fraction of the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Replications 1	2	3	Total	
tons/acre		microcuries				
0	+	6.02	4.25	5.22	15.49	
15	+	7.14	7.46	6.58	21.18	
30	+	6.84	8.06	10.55	25.45	
45	+	9.99	13.94	11.63	35.56	
						97.68
0	-	5.27	4.53	5.07	14.87	
15	-	5.72	7.16	5.24	18.12	
30	-	5.04	8.47	4.56	18.07	
45	-	4.98	4.38	7.66	17.02	
						68.08

Table 22. Analysis of variance on the data on the zinc^{65} associated with the soil organic fraction (data of table 21)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	3.5813	1.7906	n.s.
Incubation	1	36.5066	36.5066	0.001
Organic Matter	3	42.6287	14.2095	0.02
Organic Matter x Incubation	3	31.4841	10.4947	0.05
Error	14	30.8983	2.2070	

Coefficient of variation—20.4%

[a]Significance not measured beyond 0.001.

Table 23. Amount of zinc^{65} on the exchange complex of the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Replications			Total
		1	2	3	
tons/acre		microcuries			
0	+	2.856	4.324	4.446	11.626
15	+	3.042	2.680	4.443	10.165
30	+	2.948	2.457	2.597	8.002
45	+	2.688	2.722	3.183	8.593
0	-	2.960	2.128	3.428	8.516
15	-	2.861	2.523	2.873	8.257
30	-	2.382	2.556	2.743	7.681
45	-	2.992	3.495	2.534	9.021

Table 24. Analysis of variance on the data on amount of zinc^{65} on the exchange complex of the soil (data of table 23)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	0.9876	0.4938	0.10
Incubation	1	1.0049	1.0049	0.05
Organic Matter	3	1.7131	0.5710	0.10
Organic Matter x Incubation	3	1.2615	0.4205	n.s.
Error	14	4.0664	0.2904	

Coefficient of variation--17.99%

Table 25. $Zinc^{65}$ associated with the sand and silt fraction of the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Replications 1	2	3	Total
tons/acre		microcuries			
0	+	89.9	115.2	105.1	310.2
15	+	141.2	139.5	125.8	369.5
30	+	65.3	89.8	141.9	297.0
45	+	155.3	88.3	103.1	346.7
0	-	92.3	113.7	144.0	350.0
15	-	83.4	86.4	65.4	235.2
30	-	74.0	86.8	52.5	213.3
45	-	54.7	54.1	78.9	187.8

Table 26. Analysis of variance on the data on the $zinc^{65}$ associated with the sand and silt fraction (data of table 25)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	542.80	271.40	n.s.
Incubation	1	4876.30	4876.30	0.01
Organic Matter	3	2398.60	799.53	0.35
Incubation x Organic Matter	3	4038.50	1346.16	0.15
Error	14	8410.00	601.28	

Coefficient of variation--10.38%

[a]Significance not measured beyond 0.001.

Table 27. Zinc^{65} associated with the clay fraction of the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Replications		
		1	2	3
tons/acre		microcuries		
0	+	171.8	143.2	143.0
15	+	143.5	127.9	135.0
30	+	177.7	147.9	127.7
45	+	116.7	140.0	157.1
0	-	194.0	120.5	90.8
15	-	164.9	163.8	145.2
30	-	136.7	150.1	175.3
45	-	134.3	184.4	174.3

Table 28. Analysis of variance on the data on the zinc^{65} associated with the soil clay fraction (data of table 27)

Source	df.	ss.	ms.	Sig.[a]
Replications	2	541.6	270.8	n.s.
Incubation	1	440.3	440.3	n.s.
Organic Matter	3	287.7	95.9	n.s.
Organic Matter x Incubation	3	1840.2	613.4	n.s.
Error	14	10279.2	734.2	n.s.

Coefficient of variation--10.5%

[a]Significance not measured beyond 0.001.

water soluble Zn^{65} removed, and appears in the balance sheet which occurs as table 29.

Table 29. Balance sheet comparing total microcuries of Zn^{65} in each pot with the total obtained by summing the amount in the fractions of the soil on which Great Northern beans were grown in a growth chamber under conditions of apparent zinc deficiency induced by added sugar beet tops, radiozinc experiment

Sugar beet tops	Incubation	Spray	Plant top Zn^{65}	Plant root Zn^{65}	Water soluble soil Zn^{65}	Acid soluble soil Zn^{65}
tons/acre				microcuries per pot		
0	+	-	1.30	3.99	2.19	22.6
	+	-	1.38	2.80	3.71	29.0
	+	+	0.85	2.71	3.84	38.2
15	+	-	0.96	1.29	3.59	14.4
	+	-	1.00	1.59	3.18	20.8
	+	+	1.02	3.20	2.32	25.5
30	+	-	0.75	1.16	2.42	12.9
	+	-	0.85	1.29	2.41	27.4
	+	+	1.11	1.33	1.92	30.8
45	+	-	0.74	1.29	1.99	15.3
	+	-	0.85	1.95	1.76	25.7
	+	+	1.20	1.50	2.90	26.5
0	-	-	2.44	2.62	3.15	19.7
	-	-	3.10	4.32	2.56	33.1
	-	+	2.45	3.48	3.85	29.6
15	-	-	2.56	4.37	2.64	28.5
	-	-	2.88	2.21	3.25	48.8
	-	+	2.52	3.42	2.77	68.5
30	-	-	4.17	4.25	2.60	27.4
	-	-	4.06	2.76	1.81	44.5
	-	+	3.53	3.59	4.33	83.2
45	-	-	3.82	1.72	3.70	40.1
	-	-	3.27	2.62	2.32	45.3
	-	+	2.96	2.83	3.28	74.6

Table 29. Continued

Exchangeable soil Zn^{65}	Zn^{65} with soil clay	Zn^{65} in organic matter	Zn^{65} with soil sand and silt	Total Zn^{65}	Mean	Intact soil sample less water soluble Zn^{65} counted
			microcuries per pot			
n.s.	171.8	6.02	89.9	297.8		303.7
	143.2	4.25	115.2	299.5		298.6
	143.0	5.22	105.1	298.9		311.3
	143.5	7.14	104.2	275.1		291.4
	127.9	7.46	139.5	301.4		305.7
	135.0	6.58	125.8	299.4		306.2
	177.7	6.84	65.3	267.1		282.4
	147.9	8.06	89.3	277.7		289.9
	127.7	10.55	141.9	310.3		306.6
	116.7	9.99	155.3	301.3		311.0
	140.0	13.94	88.3	272.3		286.1
	157.1	11.03	103.1	303.9	292.1	300.6
	194.0	5.27	92.3	319.5		314.8
	120.5	4.53	113.7	281.8		291.6
	90.8	5.07	144.0	279.3		286.2
	1[illegible]4.9	5.72	83.4	292.1		299.8
	163.8	7.16	86.4	314.5		313.2
	145.2	5.24	65.4	293.1		301.0
	175.3	5.04	74.0	292.8		298.6
	150.1	8.47	86.8	298.5		302.5
	136.7	4.56	52.5	288.4		294.9
	174.3	4.98	54.7	283.3		297.0
	134.4	4.33	54.1	296.4		302.3
	134.3	7.66	74.0	299.7	294.9	309.2

DISCUSSION

The dry weight yield of plant tops of the radiozinc experiment followed closely the results of the first two experiments, i.e., where no additional zinc was supplied to the plants during the growth period, increased amounts of sugar beet tops decreased plant growth, apparently by somehow decreasing the availability of the soil zinc, which was already nearly insufficient in amount. Adding zinc in the form of a spray allowed plants to take advantage of increased soil fertility and/or better soil physical conditions brought about by the addition of the sugar beet tops.

Where no incubation period was allowed after addition of sugar beet tops to the soil, there apparently was insufficient time for zinc tie-up to take place before the plants were able to acquire sufficient zinc to allow nearly normal growth.

The bean roots showed a difference in only the overall effect of organic matter, with no difference in the replications (which would indicate no difference in spray treatments, as this experiment is set up). Generally, increased sugar beet tops decreased root weight. It is obvious from the high coefficient of variation (43.9 percent) for these data, that either root weights cannot be relied upon to indicate the growth status of the plant, or all of the root tissue was not recovered from the soil. Although great effort was made to recover all root tissue from the soil, it is difficult to pick out all of the very small root parts from a highly radioactive soil as one must work at least semi-remotely.

Approximately the same results were obtained for total Zn^{65} in the plant tops as were found with dry weight yield. The plants grown on the incubated soil without benefit of added zinc from the spray were not able to acquire sufficient soil zinc when sugar beet tops were added to the soil. In this case, the higher the sugar beet top concentration, the lower the amount of Zn^{65} in the plant.

Where the plants growing on the incubated soil were sprayed, apparently the added zinc increased the strength of the plant to the point where it was better able to feed on the semi-available soil zinc.

Where the soil was not incubated, apparently there was insufficient time for the soil Zn^{65} to become fixed, or "tied-up." Therefore, the beans grown on the non-incubated soil were able to acquire more of the soil zinc^{65}.

The Zn^{65} concentration of the plant tops was not as conclusive as the total Zn^{65} but gave the same indication. The plants grown on the non-incubated soil were able to acquire a higher concentration of Zn^{65}, again indicating that time is an important factor in the tie-up of added soil zinc. The unsprayed plants grown in the incubated soil had a lower concentration of Zn^{65} compared to the sprayed plants. These two criteria of measurement support each other well.

Root total Zn^{65} and Zn^{65} concentration were in agreement also. The roots growing in the incubated soil had less total Zn^{65} and a lower concentration of Zn^{65} than did the roots in the non-incubated soil. This again points out that time is required for the tie-up of zinc to take place in the soil.

Going to the soil analysis, it is seen that the water soluble Zn^{65} is decreased in the incubated soil, indicating that additions of sugar

beet tops decrease the water soluble soil zinc if sufficient time is allowed for the inactivation to take place.

The acid soluble soil Zn^{65} also decreases with additions of sugar beet tops, when properly incubated. The acid soluble soil Zn^{65} fraction contains the Zn^{65} which was physically and chemically associated with the soil lime and lime minerals. An acid-buffer wash of pH 5.0 was utilized to remove the acid soluble fraction. The buffer was composed of a normal sodium acetate-acetic acid buffer. This means that the soil zinc was subjected to dilute sodium, calcium (from the dissolved lime), and hydrogen ions. Therefore, it is reasonable to assume that some, and probably a considerable amount, of exchangeable zinc was removed by this treatment. Actually, this fraction called the acid soluble Zn^{65} is in reality the sodium, calcium, and hydrogen ion extractable Zn^{65}.

From the above discussion it seems evident that both exchangeable zinc and acid soluble zinc decrease with increases in sugar beet tops, when an incubation period occurs. Although the exact amounts of Zn^{65} associated with the acid extractable and exchangeable fractions are not available due to this confounding effect, it is apparent that the Zn^{65} in both of these soil fractions decreases when there is an increase in sugar beet tops added to the soil.

Although some of the exchangeable Zn^{65} was probably removed by the acid wash, it seems reasonable to assume that the Zn^{65} remaining on the soil exchange complex, and removed by the $CaCl_2$ washes, is proportional to that Zn^{65} which was present on the exchange sites before washing began. This is deduced from the fact that when cations are extracted from the exchange sites by consecutive washes of other

replacing cations, the amount removed is proportional to the amount present at the beginning of the wash, or to the amount of cations present before washing began.

Statistical analysis of the exchangeable zinc removed by the $CaCl_2$ wash indicates significant decreases of exchangeable Zn^{65} with increased sugar beet tops added to the soil.

With this measurement, we are dealing with amounts on the order of hundredths of a microcurie. The amounts were so low as to make counting of the sample very difficult with the counting method used. Counts were only a few above background, definitely in the range where a worker must question the accuracy of results somewhat. However, the coefficient of variation was quite acceptable (less than 18 percent), indicating that if errors were present in counting, at least they were consistent. Since exchangeable soil Zn^{65} was so low, it is not included in the balance sheet (table 29) where all Zn^{65} of the experiment is accounted for.

It has been shown that soil water soluble Zn^{65} and soil acid soluble Zn^{65} and all Zn^{65} removed by plants decrease with addition of sugar beet tops to the soil. Where does this zinc go?

The Zn^{65} associated with the organic phase also increased with addition of sugar beet tops, on the incubated soils. The fact that the soil organic Zn^{65} decreased with added sugar beet tops on the non-incubated soil indicates that soil Zn^{65} was continually moving into the organic phase as time progressed.

The other place where soil Zn^{65} appeared with increased time was that Zn^{65} which was attached to the sand and silt mineral fraction of the soil. An explanation for this last phenomena is not apparent.

About 83 percent of the 1500 grams of soil in each pot was sand plus silt, which gave an activity of about 0.08 microcuries per gram. Although this is low compared to the clay (about 0.7 microcuries per gram), it still accounts for about one-third of the Zn^{65} applied to the soil.

SUMMARY

Additions of organic matter in the form of sugar beet tops to a soil apparently already somewhat low in zinc tended to make the zinc present in the soil even more difficult for the plant to acquire. In the field this deficiency was overcome by a foliar spray of $ZnSO_4$.

The deficiency symptoms were duplicated with plants grown on this soil in the laboratory growth chamber. Additions of dried sugar beet tops to the soil caused and aggravated the deficiency. $ZnSO_4$ sprays alleviated the deficiency condition.

In the last experiment, time was shown to be an important factor in the tie-up of zinc in the soil. If the soil and added zinc were allowed to incubate for three to six weeks or more before planting, the added zinc became less available for plant use.

If the incubation period was utilized, additions of sugar beet tops decreased the uptake and concentration of Zn^{65} within the plant, as well as decreasing plant dry weight. If a $ZnSO_4$ spray was applied to the plant, the added sugar beet tops increased weight and zinc concentration in the plant, indicating that the added spray zinc strengthened the plant to a point where it could compete better for soil zinc and take advantage of some apparent benefit (such as better fertility or better soil physical condition) caused by the added sugar beet tops.

When incubated, the soil Zn^{65} was affected by the added sugar beet tops. Water soluble Zn^{65}, acid soluble Zn^{65}, and exchangeable Zn^{65}, apparently the three most available forms of soil zinc, were decreased

by addition of sugar beet tops to the soil. At the same time, Zn^{65} associated with the soil organic fraction and Zn^{65} associated with the soil sand and silt mineral fraction increased.

It appears that when a soil is on the borderline of sufficiency or deficiency of zinc, very little changes in management practices are required to upset the balance of the soil to the point where plants are unable to acquire sufficient soil zinc. Fortunately, soil zinc problems are easily rectified when recognized.

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A P P E N D I X

THE DESIGN AND CONSTRUCTION OF A PLANT GROWTH CHAMBER

Introduction

The growth of a plant is the result of the genetic makeup of the plant and of the environmental conditions to which the plant is subjected. Just as differences in the genetic constitution of plants produce differences in growth, so does environment produce variations. Variability is perhaps the most general and fundamental problem with which workers must contend in studying the growth and development of plants.

Environment in its broadest sense includes both soil and climatic factors. Although it must be kept in mind that climatic factors influence soil factors, this discussion deals primarily with the climatic factors.

The study of environmental effects on the plant covers a wide range of subjects primarily because each of the few environmental factors has a wide range of values. Each of these values may have a distinct effect on the plant. Also, many of these factors, each with a wide range of values, interact with each other in producing their effect on the plant. In other words, changing one factor may modify the influence of many of the other factors. This makes it extremely difficult to separate the effect on the plant of any one single factor, unless very careful control of the whole environment, with variation only in the one factor to be studied, is carried out to a fine degree.

Went (1957) attributes the existence of the large number of conflicting theories in the botanical sciences to conflicting experimental

evidence, attributable to the influence of unknown or unappreciated variables in the conditions of the experiment, principally environment control. In other words, inadequate experimental techniques prevent the development of a "Theoretical Botany", comparable to a generally accepted "Theoretical Physics."

According to Went, air conditioned greenhouses, temperature controlled artificial light rooms, and other controlled environment facilities are at present the most powerful tools at our disposal to improve experimental conditions and to minimize the experimental error.

After the reader acquaints himself with the general discussion of these factors in the literature review, it will be clearer why it is necessary to have close control of day and night temperature, light quantity and quality, day length, and relative humidity.

Literature review

Volumes have been written which contain the results of experiments of those who have looked into the effects of environment on plants. However, only a few studies are pointed out here in order to show that each of these variations in environment have an effect on the plant.

Temperature. Work with flower bulbs was instigated many years ago. This early work was facilitated by the fact that the environmental effects could be studied in easily-developed, dark temperature-controlled chambers. This is because bulbs of flowering plants initiate their flowers while in the resting period. This work was further facilitated by the fact that flower bulbs are genetically homogeneous and relatively small, taking up little space in the control chamber.

The work of Blaauw, which was extended by Van Slogteren, is

discussed by Hudson (1957). In the tulip, flower initiation is directly dependent on temperature, with the optimum temperature occurring at 17 to 20 C. At 9 C or lower and 28 C and higher, there is practically no development. Also, it is very important that, after development of the flower primordia, the temperature be decreased to 8 or 9 C. This is apparently necessary to allow elongation of the stem. It should be pointed out that this is only one case, and the optima for other species may differ greatly.

Diurnal thermoperiodicity. In 1943, another step in the control of environment was made by Went (1957), who constructed air conditioned glass houses in which light could be controlled to a certain extent. The first extensive studies by Went, made on the tomato, resulted in the concept of diurnal thermoperiodicity.

In the tomato, a constant temperature during day and night, hereafter referred to as phototemperature and nyctotemperature, respectively, results in considerably less growth than if the nyctotemperature was lower than the phototemperature. The optimum temperatures for the tomato have been found to be 26.5 C phototemperature and 13 to 18 C nyctotemperature, except that the very young plants have a nyctotemperature requirement of about 30 C for a short period.

As an explanation of this phenomenon, Went suggested that sugar translocation is reduced at relatively high temperatures, and gradually increased as temperature dropped. Verbeck, according to an interpretation by Hudson (1957), believes that respiration is relatively too high at high nyctotemperatures. Whether or not these are proper explanations, the fact remains that thermoperiodicity occurs in a large number of plants. In most plants, optimum conditions dictate a lower

nyctotemperature than phototemperature. As with most of the environmental conditions the optimum day and night temperatures vary with species. For example, the African violet (Saintpaulina ionantha) has its optimum as a higher nyctotemperature (23 to 26 C) than phototemperature (14 C). In some plants, there is evidence to show that there is no effect of thermoperiodicity. Fortanier, according to an interpretation by Hudson (1957), demonstrated that in the peanut diurnal thermoperiodicity does not occur.

Light and interactions of light and temperature. Photoperiodism and photosynthesis are the two processes in which light plays a predominating part. In both cases, light quantity, quality, and duration must be considered.

If rate of photosynthesis is plotted against light intensity, a saturation curve results. In other words, the photosynthetic rate goes up in proportion to amount of light, until a maximum is reached, and a lower maximum rate of photosynthesis is achieved at lower environmental temperatures. Went (1957) showed that, for the tomato, relatively weak light has the same morphological effect as relatively high temperature, giving thinner stems, lighter leaf color, smaller trusses with fewer flower buds and smaller fruits containing less starch and sugar.

The day lengths of a large number of plants have been studied. Workers have found that plants could be classified into groups on the basis of the relationship between day length and flowering: (a) short day plants, where flowering occurs only when day length is less than the critical day length (that day length which is just capable of inducing flowering); (b) long day plants, where flowering occurs only if day length is longer than critical day length; and (c) day length neutral plants, where flowering is independent of day length.

According to Van der Veen and Meijer (1959), day length also effects dormancy, leaf development, stem elongation, anthocyanin production, and vegetative reproduction in different plants.

Chlorophyll absorbs light of the blue and red regions most strongly. Such light triggered mechanisms as flowering and the other actions mentioned above are thought by most workers to be affected by light of the red-infrared regions. Infrared light is the antagonist of red light (in many cases, these two wavelengths have reversible, opposite effects on the mechanism).

It is not possible to regulate light quality over a wide range. However, balance of infrared-blue light can be varied somewhat by the use of more or less of the fluorescent and incandescent lamps. Incandescent lamps do not furnish much intensity of light in the blue range, but they furnish the infrared light not supplied by the fluorescent lamps (National Appliance Co., 1959).

At the present time it is not possible to duplicate bright sunlight intensities in the growth chamber by artificial lighting methods. However, recent technological advance allows approximation of one-third the value of full sunlight. VanderVeen and Meijer (1959) indicate that some plants can be grown at intensities of 5 percent of that of full sunlight under proper conditions of temperature. Generally, values of one-third full sunlight give satisfactory plant growth and actually produce as much or more light than the sun on a cloudy day.

Relative humidity. The influence on plants of water in the soil is generally recognized, but very little attention has been given to control of relative humidity, principally because it has not been easy to control.

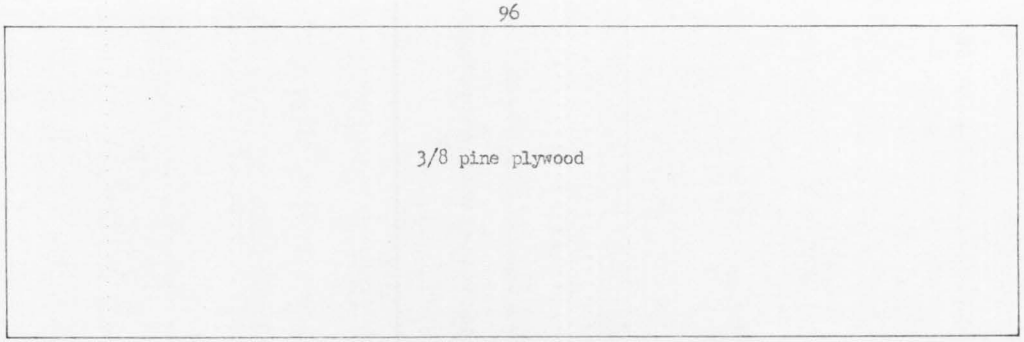
Although very little work has been done on the effects of relative humidity effects on plants, this may be a very important factor. Fortanier, according to Hudson (1957), demonstrated the effect of relative humidity of the air on the peanut plant. He showed a strong influence of the relative humidity on the number of flowers. In testing values from 50 to 90 percent, he found that for the photoperiod, 90 percent relative humidity gave the greatest number of flowers, and during the nyctoperiod, 90 percent relative humidity gave twice as many flowers as 50 percent relative humidity.

Description of the growth chambers

Construction of the chambers. The growth chambers consist of walls each of which is built from two pieces of three-eighths-inch plywood separated by spacer with 1½-inch rock wool insulation between (Figure 2). Each section is prefabricated and held together by wood screws which facilitates disassembly for moving. There are two chambers, each 8 feet long, 3 feet deep, and 3 feet high. This gives a floor space of 24 square feet per chamber. A complete set of construction drawings are given as Figures 3 to 8 and table 30.

Each chamber has two doors, 24 inches by 40 inches, which allow complete access to all parts of the chamber. The doors are offset and fitted to insure a proper seal (Figure 4, detail 1).

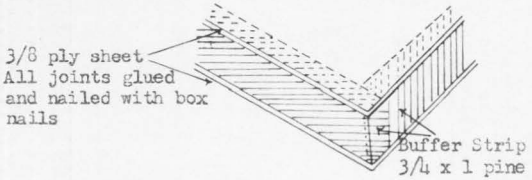
Air circulation is achieved by drawing air out of the chamber at the top at one end with a fan (320 cubic feet per minute capacity--see table 31). The air is forced through the conditioning unit (see Figure 2) and back into the growth chamber at the bottom, underneath a removable false floor. The false floor consists of four sheets of three-fourths-inch plywood which have three-fourths inch holes drilled



Bottom

Material

1. Insulation--20 ft.^{2} of 1 1/2 rockwool
2. Buffer stripping--21 ft.
3. Plywood--pine 3/8 x 29 x 96--two pieces
4. Box nails; glue



Construction Detail

Insulated with 1 1/2 inch rockwool

Figure 3. Construction detail of bottom of phytotron (all dimensions are inches unless noted).

Detail I--Adjustable vent

Cut hole 6 x 12. Line with buffer strip. Add 1/2 x 3/4 strips inside edge. Build door 4 7/8 x 10 7/8. Add 1/2 x 3/4 strips outside edge. Fasten with 2 hinges, 1 window latch. Cover opening with screen inside.

Detail II--Utility box

Cut hole 4 x 6. Line with buffer strip. Build door 2 7/8 x 4 7/8. Construct as per Detail I. Fasten with 2 window latches outside (one each side). Build utilities into this box.

Material

1. Insulation--9 ft.^{2}
2. Buffer stripping--23 ft.
3. Plywood--pine 3/8 x 32 x 36--two pieces
4. 1/2 x 3/4 pine--10 ft.
5. Hardware
   - 2 hinges--1
   - 3 window latches
   - 1 screen--7 x 13

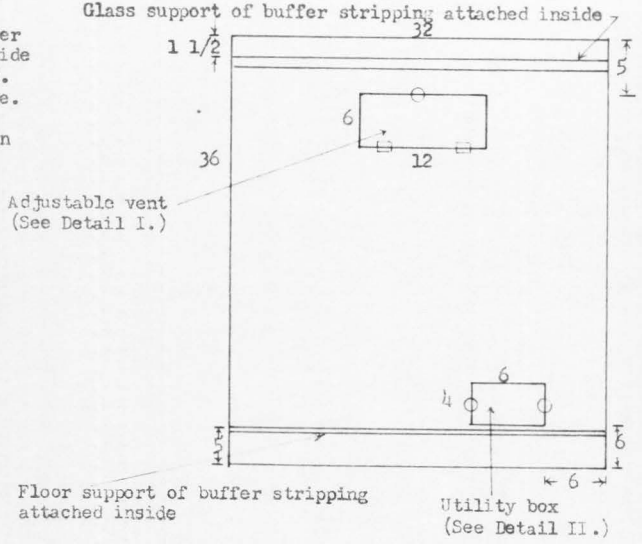
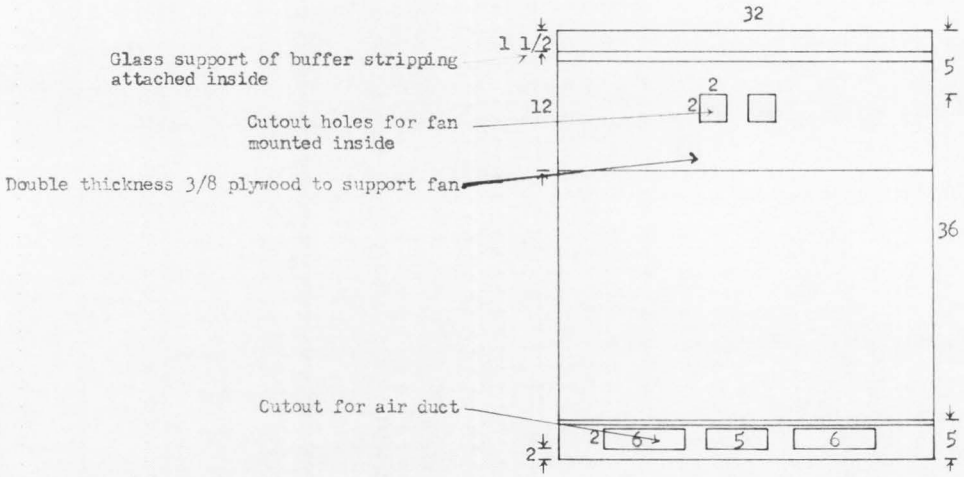


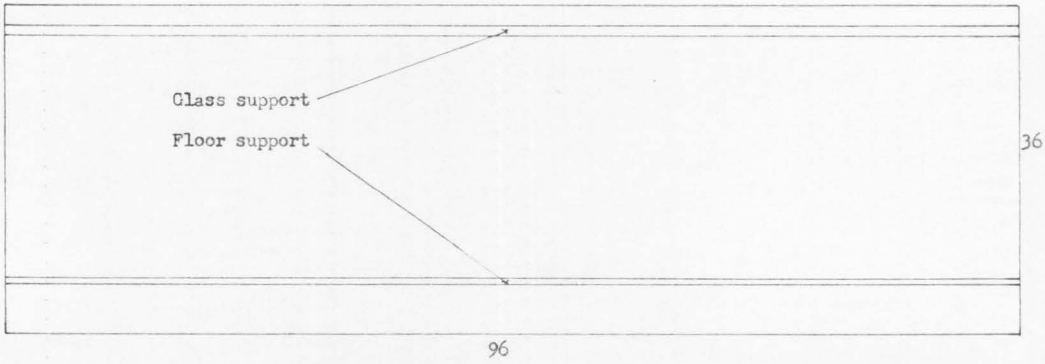
Figure 4. Construction detail of end of phytotron, insulated as in construction detail of Figure 3 (all dimensions are inches unless noted).



Material

1. Plywood--pine 3/8 x 32 x 36--one piece;
   3/8 x 32 x 12--one piece
2. Buffer stripping--6 ft.

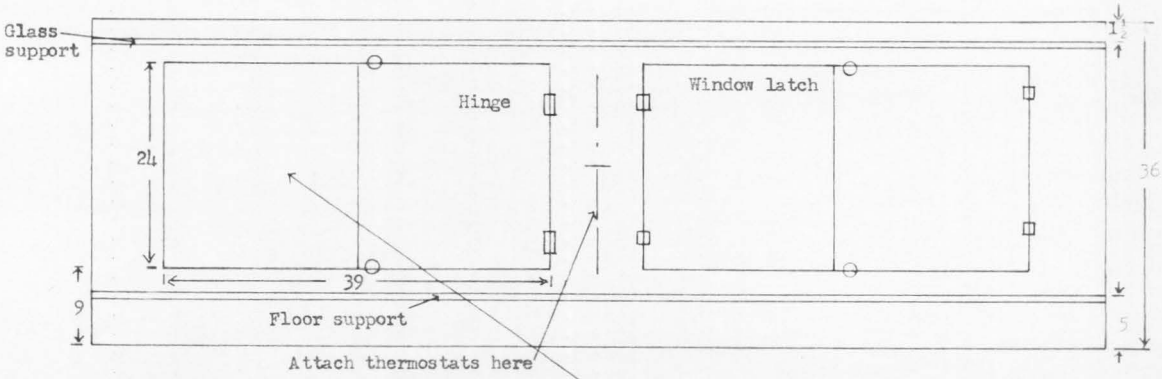
Figure 5. Construction detail of uninsulated end of phytotron (all dimensions are in inches unless noted).



Material

1. Insulation—24 ft.^{2}
2. Buffer stripping—38 ft.
3. Plywood—pine 3/8 x 36 x 96—two pieces

Figure 6. Construction detail of back of phytotron, insulated as in construction detail of Figure 3 (all dimensions are in inches unless noted).



Material

1. Insulation--24 ft.^{2}
2. Buffer stripping--92 ft.
3. Plywood--pine 3/8 x 36 x 96--two pieces
4. Clear pine stripping--1/2 x 3/4--45 ft.
5. Hardware
   8 hinges--2
   4 window latches

Doors
Cut out four--22 7/8 x 18 5/8.
Assemble and insulate as per detail Figure 3. Fit together as per detail I, Figure 4.

Figure 7. Construction detail of front of phytotron, insulated as in construction detail of Figure 3 (all dimensions are in inches unless noted).

Control system parts are mounted on outer side of Part C.

Material

1. Insulation--22 ft.^{2}
2. Buffer stripping--50 ft.
3. Plywood--3/8 pine:
   - 2 pieces 4 x 29
   - 4 pieces 15 x 29
   - 2 pieces 11 x 29
   - 2 pieces 21 x 29
   - 4 pieces 16 1/2 x 36
4. 1/2 x 3/4 pine--6 ft.
5. Hardware
   - 2 hinges--1
   - 1 window latch
   - 1 screen--7 x 13

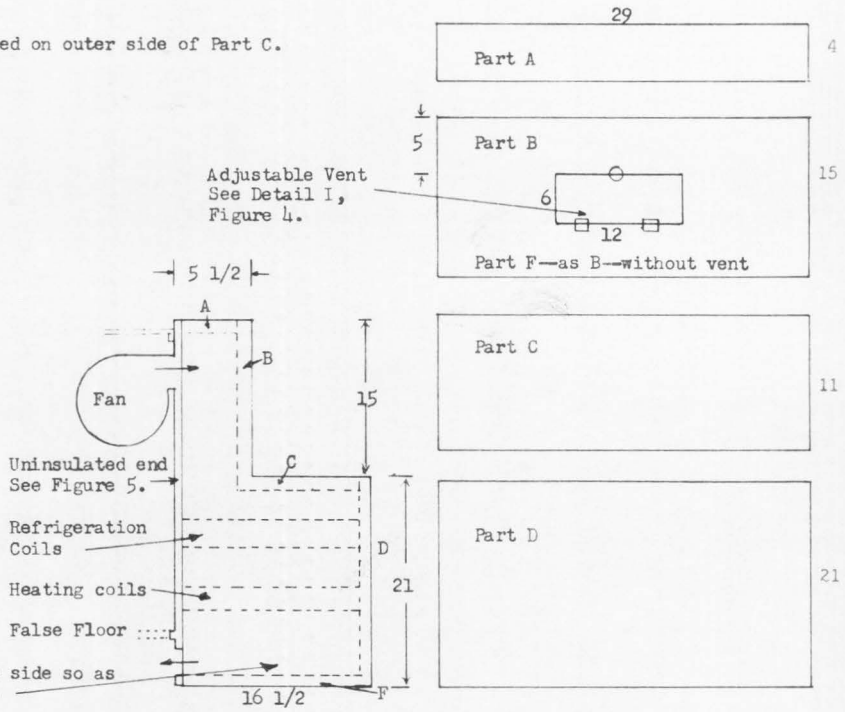


Figure 8. Construction detail of air conditioning unit of phytotron (all dimensions are in inches unless noted).

Table 30. Construction notes

1. Glass--4 pieces 28 3/4 x 23 7/8"--single strength. The glass, as divided, permits easy removal for frequent cleaning, yet fits tightly in place without appreciable air leaks.

2. False floor--4 pieces 28 3/4 x 23 7/8"--use 1/2" plywood. Perforate by drilling 99 3/4" holes (11 holes across the long side by 9 holes across the short side of each piece).

3. Parts are prefabricated as per drawings pages 61 to 67, are painted at least 3 coats with white enamel paint, and assembled with wood screws (3" x 10) as per drawing page 61. The air conditioning duct is attached with metal strips and wood screws to the main body of the unit. Any small cracks which are apparent are then filled with calking compound.

4. Lighting system--the fluorescent light fixtures are suspended above the glass top, allowing at least 5" clearance between the fluorescent bulbs and the glass. The incandescent fixtures are placed between the two fluorescent fixtures in a line which runs down the center of the box lengthwise (table 31). The lighting system cooling fan is mounted on one end of the box at the top so that it will circulate cool air down the full length of the lighting equipment.

5. The air circulation fan is mounted as per drawing page 67.

6. Thermostats are mounted as per drawing page 66.

7. The electrical control panel, including clock, relays, etc., is mounted on the outside of the horizontal surface of the air conditioning duct (page 67).

8. NOTE: The control system is designed to operate two phytotrons independently. The refrigerator unit will easily handle two such units. The construction drawings are for one unit only. Therefore, it is necessary to multiply by two the materials needed for construction.

Table 31. Glossary and cost breakdown of equipment used in construction of growth chambers

Number needed	Model number	Specifications	Source of supply[a]	Cost[b]
2	10180	Lamp-cooling fans--60 cu.ft./min.	Dayton Electric Mfg. Co.	$ 22.00
2	20069	Circulating fans--320 cu.ft./min.	Chicago, Illinois	40.00
1	M2-5108	Time switch (clock)	Reliance Automatic Lighting Co. Racine, Wisconsin	18.00
4	Special order	Fixtures to hold eight foot super high output fluorescent lamps. Fixtures are 12" wide.	Western Lighting Corp. 3815 Medford Street Los Angeles 63, Calif.	280.00
16	F96T 12/ CW/SHO	8 foot fluorescent lamps (Westinghouse)		70.00
2	4-235	Bimetal thermoregulators	American Instrument Co. Silver Springs, Md.	40.00
2	4-5302	Supersensitive relay		114.00
3	201 O.B.	110v/24v transformer	Any electrical supply	10.00
1	PR7AY	115v 60 cy. DPDT relay (heater cutout)	Allied Radio Corp. 100 N. Western Avenue Chicago 80, Illinois	34.00
1		115v 60 cy. SPST relay (ghost relay)		
2	PR5AY	24v a.c. SPDT relay (temperature control)		
1	MP5A	230v 60 cy. SPDT (master relay)		

Table 31. Continued

Number needed	Model number	Specifications	Source of supply[a]	Cost[b]
4		Oven replacement coils (two per chamber attached in parallel--5 amps, 115v a.c.)	Sears, Roebuck and Co.	$ 6.00
14		110v porcelain lamp sockets	Sears, Roebuck and Co.	4.00
2		24v thermostats	Any electrical supply	7.00
		Lumber and hardware	Any hardware supply	165.00
		Switches	Any electrical supply	6.00
1		Refrigeration unit, 3/4 H.P. coils and installation	Used	150.00-400.00
300 ft.		Wire for installation	Any electrical supply	18.00

[a]Where obtained for current chambers.

[b]Cost of items as used for current construction.

in them on 3-inch centers. This permits a slight positive pressure under the false floor which helps to form a more even air flow up through the floor at all points. Air in the chambers is recirculated at the rate of about five times per minute. Despite the buffering effect of the false floor, the largest amount of air flows to the far end of the chamber, then up through the floor and back to the conditioning unit which decreases the magnitude of temperature frequency and amplitude changes. If the frequency and amplitude changes took place more rapidly, there would be more of a strain on the heating and cooling systems due to more rapid changes (starting and stopping of the heating and cooling units). Certainly, better control of temperature occurs when the frequency of heater-cooler activation is faster, but it is necessary to choose a frequency value which is intermediate in order to achieve a satisfactory level of accuracy in combination with reasonable frequency change periods. See heating-cooling system discussion.

The top of the chamber consists of single strength glass which is easily removed for cleaning. The lighting system is suspended above the glass. A fan (60 cubic feet per minute capacity) is used to circulate fresh air between the fluorescent lamps and the glass top of the chamber. This keeps the operating temperature around the lamps below 120 F at all times. If the temperature is allowed to greatly exceed this value, the lifetime of the ballasts is decreased.

Lighting system. The lighting system for each chamber consists of two units, each of which holds four super high output fluorescent lamps eight feet long. These lamps run the full length of the growth chamber. In addition there are seven 100-watt incandescent lamps for each chamber (table 31).

The light spectrum between wave lengths of 350 mμ is shown in

Figure 9. At 350 mμ the absorption of light by the glass top approaches 100 percent (National Appliance Co., 1959).

Growth of red kidney beans, soybeans, and tomatoes in the chamber before installation of the incandescent lighting system showed short internodes and large leaves. These symptoms are generally considered to be found under deficiencies of far-red light (VanderVeen and Meijer, 1959). With only fluorescent light this result might have been anticipated. However, with the addition of incandescent light, plant growth was expected to and actually did return to normal. The addition of the incandescent lamps, while not adding significantly to the intensity, added light in the far-red region of the visible spectrum. This compensates for the deficiency of the fluorescent lamps in that range.

By using various fractions of the total number of lamps, a range of light intensities can be obtained which varies from 2500 foot candles down to 700 foot candles, measured at plant height at a wave length of 530 mμ. If the measurement is made at the intensity peak which occurs at about 560 mμ (Figure 9) the high intensity value is about 3250 foot candles. Incident radiation was measured at 20 cm above the floor and ranged between 0.12 gram cal./cm^2/minute and 0.27 gram cal./cm^2/minute, varying with fractions of total lamps used.

Heating and cooling system. There are two main types of temperature control systems. The on-off system has the heater or refrigerator operating fully during its cycle. This system is the simplest to instrument and considerably cheaper to install and maintain than the more complex proportionate system. If a very fine degree of accuracy in combination with low capacity heating and cooling is not needed, the on-off system is satisfactory. The proportionate system employs an

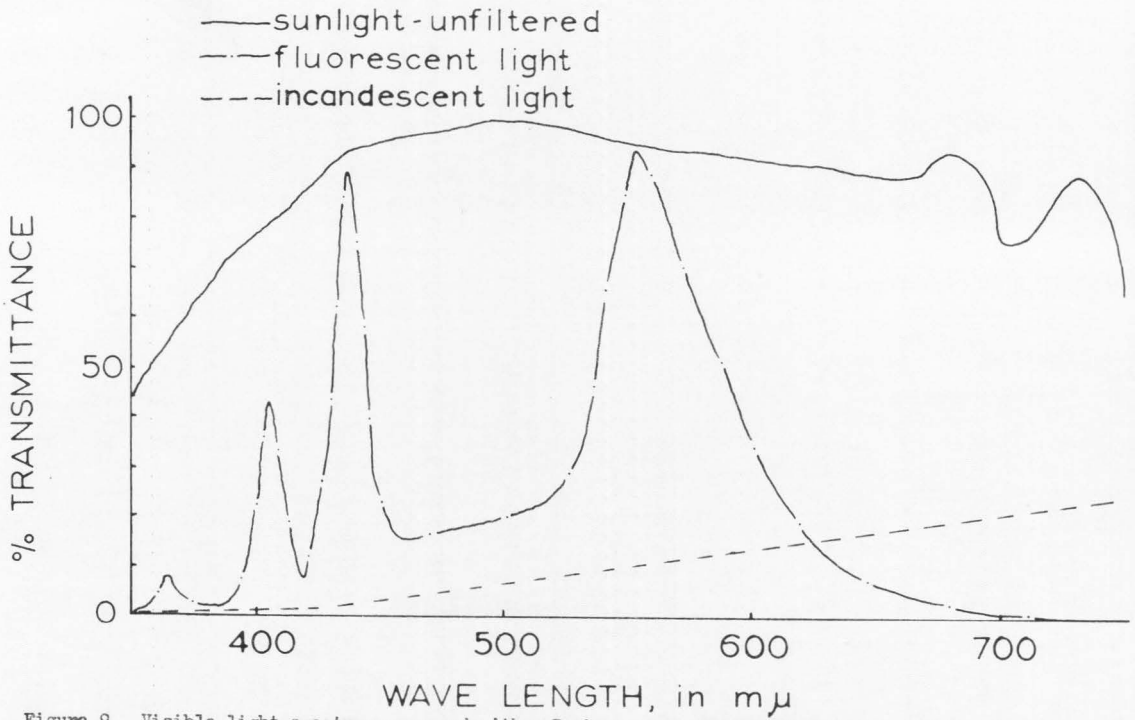


Figure 9. Visible light spectrum measured with a Beckman spectrophotometer for sunlight and actual conditions in growth chamber.

instrumentation arrangement which allows an output of heat proportionate to the difference between the actual and designed temperatures. Due to reasons of simplicity and lower expense, the on-off system is used with these chambers, and the ensuing discussion applied only to the on-off type of control.

The theory of temperature control is quite simple to understand. For example, if we first assume a chamber uniformly heated above the design temperature by the heating system (assuming a physical arrangement such as present in these chambers), the temperature sensitive relay system (thermostat) has switched off the heat and switched on the coolant. Now a time lapse occurs, due to the latent heat of the resistance coils which continues to heat the air for a short period of time after it is switched off, and due to the fact that time is required for the coolant to flow into the cooling coils and heat transfer to take place. This is called lag time. According to Evans (1959) the chamber will then cool at a rate which is proportional to the difference between the chamber temperature and the ultimate temperature which would occur should the cooling system be left on indefinitely. (If no refrigeration unit was used, the ultimate temperature would be that of the environment outside the chamber, and the proportionality constant would be affected by the efficiency of the chamber's insulation.) When the chamber is cooled to the designed temperature, the cooling system is shut off and the heating system is activated. A lag period then ensues until the heating system takes effect. The rate of heating is proportional to the difference in chamber temperature and the ultimate temperature which would occur if the heater was left on continuously. In this manner the cycle continues. The degree of accuracy of the system is affected by these facts. (a) The magnitude of temperature fluctuation is least

when the design temperature is midway between the heating and cooling ultimate temperatures. (b) The magnitude of temperature fluctuations decreases with the decrease in heater output and cooling capacity (a "happy medium" must be achieved here as the heating and cooling capacity cannot be so low that there is danger of the environment outside the chamber affecting temperatures inside). For this reason, it is desirable that the temperature of the room containing the chamber be roughly controlled. (c) The degree of humidity control (humidification decreases the ultimate temperatures of heating and cooling) also affects the degree of accuracy of the system.

The cooling system consists of a 3/4 H.P. refrigerator compressor unit using a water jacket heat exchanger. The coolant flows through a 2 1/2 inch x 12 inch x 24 inch refrigeration coil located in the conditioning chamber (one for each chamber, Figure 2). This coil can be fabricated by any good refrigeration company. The flow is separately controlled by electrically operated solenoid valves (one for each chamber). The valves are controlled by the thermostats, shutting off coolant flow when the boxes are cooled sufficiently and allowing coolant to flow when the boxes become too warm.

The heating system consists of resistance coils mounted in the conditioning unit (Figure 2). These coils are controlled by the thermostats, through relays which feed current to the coils when the chamber is cooler than the specified temperature. Since the powerful lighting system supplies a large amount of heat to the chambers during the day cycle, the heating coils are usually not needed and, therefore, disconnected completely during the day cycle by another relay (heating coil cutout relay, Figure 10). This decreases the work load for the

is needed because the day thermostats will be closed during the cooler nyctoperiod which creates a closed circuit which links the two night thermostats together. In this case, both chambers would be controlled by a single night thermostat, thus necessitating the ghost relay.

An explanation of the various switches needed is given in table 32. The numbers preceding the letters denote which of the two chambers the switch is associated with (Figure 10).

When it is desired to operate only one chamber at a time, switches A, B, C, D, E, and F of the chamber which will not be used are opened. When shutting off both chambers, it is best to shut off the whole system at the power source rather than use the switching system. Switch G is closed during operation of the humidifying unit.

Humidifying system. The humidifier consists of a plastic frame holding two parallel walls made of 1/2 inch hardware cloth, 5/8 inch apart. The area between walls is packed with excelsior (shredded wood used in packing).

Liquid is allowed to flow evenly down through the humidifier and the excess (which is necessary to prevent excessive salt precipitation) is collected in a trough at the bottom and recycled to the reservoir above the unit by means of a small pump. The humidifier is mounted in the conditioning unit in such a way that all air being circulated must pass through it. The humidifier can be removed and replaced by removing the wood screws holding the front portion of the conditioning unit in place. When this front wall is removed, the humidifier is readily accessible.

The humidity will reach a constant state within about one hour of the time the unit is placed in operation. For best results, the

Table 32. Switching mechanism of growth chambers

Key	Explanation
1 & 2 A	When opened, these switches open the control circuit to the refrigeration solenoids. This closes the solenoids and stops further cooling of the chambers.
1 & 2 B	When opened, these switches open the circuits to the temperature control relays. The relays, when not activated, assume the position which opens the heating circuits, thus deactivating the heating system.
1 & 2 C	When opened, these switches shut off the incandescent lighting system.
1 & 2 D	When opened, these switches shut off the fluorescent lighting system.
1 & 2 E	When opened, these switches shut off the fans which circulate air within the chambers.
1 & 2 F	When opened, these switches shut off the lamp cooling fans.
1 & 2 G	(normally open) When closed, these switches allow the heating coils to operate day and night, as called for by the temperature control system (used when humidifier is in operation).

excelsior should be soaked in water prior to being placed in operation. Humidity values achieved are a function of temperature, liquid composition, and geometry.

The vapor pressure of the water solution being passed over the humidifier can be changed by adding salts of various types (Hodgman, 1955, and Stokes and Robinson, 1949). Initial studies were run using water and a salt solution. Results are given in table 33. The humidity limits given are accurate with or without plants growing in the chamber.

Table 33. Humidity-salt-temperature relationships as measured in growth chambers with humidifying unit in operation

Temperature	Relative humidity	Solution
°F	percent	
80	35 ± 15	none
70	40 ± 15	none
60	45 ± 15	none
80	70 ± 2	H_2O
70	94 ± 2	H_2O
80	50 ± 2	Saturated NaCl
70	68 ± 2	Saturated. NaCl

By using various salt solutions nearly any degree of humidity can be achieved. If it is desired to have a different night humidity than that given by the salt solution being used for day humidity, it is a simple matter to set up two reservoirs, the second of which contains a

salt solution of a different concentration or different type. Switching to the second reservoir for night use could be accomplished electrically.

For an estimate of the relative humidity achieved at any temperature, the following equation can be used.

$$\text{Relative humidity} = A\ \frac{(760 - \Delta vp)}{760}$$

where:

A = the relative humidity achieved at that temperature with pure water used in the humidifier

vp = the change in vapor pressure of the liquid due to salt measured at that temperature at which A was determined.

References of vp can be found in tables of Stokes and Robinson (1949) and Hodgman (1955). Table 33 gives values found in these chambers.

This humidifying method, which may be slightly less accurate than electronically controlled methods, offers none of the complexity found in the control systems of electronic humidifiers. It is easily assembled and is reliable.

Cost analysis. The cost of these units, compared to commercial units with the same capabilities, is one of the best features. It provides a growth chamber which most research teams can afford. Cost breakdown is given in table 31. The cost of materials is approximately $1,100.00 for the two units and can be reduced considerably by eliminating the supersensitive thermostats. Cost with supersensitive thermostats replaced by household thermostats is about $950.00.

As these units were prototypes, there were no plans to follow and labor time was extensive. As no records of time spent on the job were kept, no accurate labor cost estimate can be made. However, with plans available an estimate of 300 man hours would seem to be fairly

reasonable. Some of this would be skilled labor time (refrigeration, plumbing). The control system could be assembled easily by one having only a basic knowledge of electrical applications.

Disease control. Disease control has long been a difficult problem in the greenhouse, where a large plant population is placed in a small space with moisture and heat available. The chambers, sealed inside and out with several coats of white enamel paint, are easily cleaned and disinfected after each experiment. Due to thorough cleaning and disinfecting after each experiment, outgrowth of fungi and other microorganisms has not been noticed after operation for a period of one year. It is strongly recommended that before each experiment, the glass be removed and thoroughly cleaned and the chambers swept and disinfected.

Reliability. A growth chamber must be reliable if controlled experiments are to be conducted. Temporary defects or malfunctions of the control system may kill the living system involved or, at the very least, invalidate the experiment.

The described phytotron system has been in operation for 30 months and no irregularities have been experienced. Replacement of light bulbs and normal maintenance of the refrigeration unit and electrical relays are all that have been required.