A PHYSIOLOGIC-GENETIC STUDY OF THE DIFFERENTIAL RESPONSE OF NAVY BEANS (PHASEOLUS VULGARIS L.)TO ZINC

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ABSTRACT

A PHYSIOLOGIC - GENETIC STUDY OF THE DIFFERENTIAL RESPONSE OF NAVY BEANS (PHASEOLUS VULGARIS L.) TO ZINC

by David Ernest Polson

A differential response of navy beans (Phaseolus vulgaris L.) to Zn stress was reported in 1964. Saginaw variety was tolerant to low Zn levels, while Sanilac was extremely susceptible. These two varieties were employed in a genetic and physiologic study of Zn utilization. The ultimate goal of this investigation is to determine the physiologic or anatomic mechanism(s) active in the differential response, and the genetic control of this mechanism. However this particular study has only established a foundation for further investigation pertaining to the genetic control and physiologic mechanisms related to Zn utilization.

Several lines of investigation have been carried out in this study: (1) a genetic study of differential sensitivity to low and high Zn in the field and greenhouse, respectively, as manifested by visual symptoms and growth retardation; (2) differential response to micronutrient unbalance; (3) and physiologic studies to determine the

mechanism controlling the differential response or its location in the plant.

Field experiments were conducted in 1964 and 1966 with Saginaw, Sanilac, F_1, F_2, F_3, and backcross populations grown under low Zn conditions. The degree of Zn deficiency, based on visual symptoms, was scored from one (fully tolerant) to five (fully sensitive). Saginaw, Sanilac, F_1, and F_2 populations were grown at high (5.0 ppm) Zn in the greenhouse and differential tolerance was observed in the relative reduction of growth. The segregating populations were partitioned into tolerant and sensitive phenotypes based on the performance of the parents, whose response to low Zn overlapped.

Zn (50.0, 5.0, 0.05, and 0.0005 ppm), Fe (75.0, 25.0, and 0.5 ppm), Cu (10.0, 2.0, and 0.02 ppm), and Mn (50.0 and 0.5 ppm) were utilized in the micronutrient experiments. Plants of both varieties were grown in acid washed sand and received various combinations of the above micronutrients and levels. Response to these micronutrients was determined by plant height, number of trifoliate leaves, shoot weight, and root weight. In addition, elemental concentration in various plant tissue (leaves, petioles, stems, and roots) was determined.

Reciprocal scion/root grafts, Zn content of exudate, and radioisotope (^{65}Zn) investigations were utilized in determining

location of tolerance to high Zn mechanism, Zn uptake, and Zn distribution.

The data obtained from the genetic experiments indicated that tolerance to low Zn was phenotypically dominant in the F_1. In addition, the results were indicative of two genes which interacted to give a 9:7 ratio in the F_2. In the F_3 and F_1 x Sanilac population more tolerant plants than expected (based on a 9:7 ratio) were observed. It was concluded that the two gene model giving a 9:7 ratio was extremely close to, but perhaps not the real genetic situation.

At the 5.0 ppm Zn level, it was not possible to resolve the genetic system. The F_1 generation was indicative of dominance or partial dominance of the sensitive phenotype. The F_2 generation implied dominance of the tolerant phenotype with a two gene model giving a 9:7 ratio.

In the micronutrient interaction investigations, Saginaw was tolerant of high (5.0 ppm) Zn, while Sanilac was not. This differential response was not associated with differential uptake of Zn. Although Sanilac plants did accumulate significantly higher levels of Zn in their tissue, the concentration of Zn in the tissues of both varieties was high (600-800 ppm in leaves).

The two varieties also differed in their susceptibility to a Cu-induced Fe deficiency. Saginaw was (again) tolerant while Sanilac was sensitive.

At low Zn, the physiological studies established that Sanilac often accumulated greater quantities of Zn, but translocated smaller proportions of Zn to the tops of the plants than Saginaw. This difference in distribution may be associated with greater release of Zn into the vascular tissue as evidenced by higher concentrations of Zn in the exudate of the Saginaw variety.

At the high (5.0 ppm) Zn level, reciprocal root/scion grafts of Saginaw and Sanilac established that tolerance to this level of Zn is associated with the genotype of the scion. Since differential accumulation in the leaves did not account for the response, it was postulated that there may be a difference in the distribution of extra- to intra-cellular Zn in the two varieties. Unpublished data, reported in this thesis, indicate that isolated leaf cells of Sanilac take up more Zn than Saginaw cells when the cells are obtained from plants grown at 5.0 ppm Zn and are bathed in a high Zn solution.

Three physiologic models are presented to account for the differential response of the two varieties. Two models involve differential membrane or cellular properties which account for a differential release of Zn into the vascular tissue and uptake into leaf cells. On the basis of these two models it was postulated that the same basic mechanism was responsible for the differential response of the two varieties at high and low Zn levels. The third model was based on

differential stem or root binding, which was limiting at low Zn levels, and differential leaf cell permeability, which was effective at high Zn. According to this hypothesis, different mechanisms would account for the differential response at low and high Zn and these would be under different genetic control.

Gene action and possible breeding programs for nutrient utilization were discussed.

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By

David Ernest Polson

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INTRODUCTION

Plants selectively accumulate certain ions or groups of ions to a greater extent than others, and different species or genotypes within species differentially accumulate ions or grow under conditions of either ion deficiency or toxicity. However, the mechanisms responsible for differential ion selectivity by plants have not been identified.

Varietal or species differences in ion accumulation and/or so-called differential resistance to low or high levels of a particular ion or ions provide a unique system in which to study the mechanism(s) involved. These systems can be studied from a physiological, anatomical, and genetic standpoint. Some of the physiologic or morphologic factors relating to the differential response may be identified, and then studied genetically for an understanding of the genetic control of nutrient utilization in higher plants.

In 1964, a Zn deficiency was reported (34) in navy beans (Phaseolus vulgaris) in Michigan. Deficiency symptoms were most severe when beans were grown on a heavy clay soil with a pH of 7.2 to 8.0, and occurred primarily when beans followed sugarbeets in the

crop rotation. One of the varieties, "Sanilac," was "sensitive" to the Zn deficiency, while "Saginaw" was "tolerant" (33, 66).

A genetic-physiologic study of these processes in beans was initiated in 1964. The primary purpose of this investigation has been to elucidate the physiological basis of the differential varietal response, and determine the genetic control of this mechanism.

Several lines of investigation have been followed: (1) a genetic study of the differential sensitivity to Zn deficiency as manifested by visual symptoms in the field; (2) a genetic study of differential tolerance to high levels of Zn in the greenhouse; (3) an investigation of micronutrient interaction in the two varieties; (4) and grafting, exudate, and radioisotope studies to determine the physiological mechanism(s) involved in differential tolerance to high and low levels of Zn and/or the location within the plant of this mechanism(s).

The relationship of the genetic control to the physiological mechanism of nutrient utilization in navy beans will be postulated and discussed.

REVIEW OF LITERATURE

I. History of Zn Nutrition

Thorne (98) discussed the early history of Zn in plant nutrition. He pointed out that Zn was not generally accepted as an essential nutrient until the work of Sommer (95), although evidence existed as early as 1863 that Zn was necessary for the growth of certain fungi. Research into the spectrum of flora affected by Zn deficiency and the areas where the deficiency occurred continued in the United States until, by 1962, Zn deficiency had been reported in 30 states in a large number of crops (7, 104, 106).

II. Morphological Effects of Zn Deficiency

Hewitt (54) and Thorne (98), as well as others (25, 27, 49, 105), discuss the morphological effects of Zn deficiency on plants. In general they found symptoms of Zn deficiency were a general retardation of growth and loss of chlorophyll. The leaves were often smaller than normal and often developed a yellow mottling between the veins. If Zn deficiency persisted, the mottled regions coalesced to form a continuous interveinal chlorosis and eventually

necrosis. These symptoms often affected the older leaves first. Many annual herbaceous crops are severely stunted partly due to lack of internode elongation. Also, they rapidly develop interveinal necrosis. Epinasty of leaves and petioles is common. Total seed yield is drastically reduced in many species due to poor growth, poor pollination and seed development, and/or delayed maturity.

III. Biochemical Effects of Zn Deficiency

Although Zn has been found active in several enzymatic systems, certain of the visual symptoms of Zn deficiency seem to be related to an upset auxin (indoleacetic acid) balance (54). In his review, Hewitt (54) cites the work of Skoog which indicates that between 1/10 and 1/50 of the amount of indoleacetic acid is obtained by diffusion or ether extraction from Zn-deficient stem apices or leaves of tomato than from normal tissue. In addition, auxin was inactivated by blue light more rapidly in Zn-deficient tissue than in normal tissue. Skoog and others (54) conclude that Zn is required to maintain auxin in an active condition but not for the synthesis of auxin.

In contrast, Tsui (103) found that Zn-deficient tomato plants contain significantly less tryptophan than normal plants and also less indoleacetic acid. Since tryptophan is a known precursor

of indoleacetic acid, he concluded that Zn was ultimately required for indoleacetic acid synthesis.

Thus Zn may have a dual role, one maintaining auxin in an active condition and the other affecting its synthesis.

IV. The Occurrence of Zn Deficiency in Navy Beans (Phaseolus vulgaris) in Michigan

Ellis et al. (34) indicated that Zn deficiency caused a severe reduction in yield of navy beans, when beans followed heavily P-fertilized sugarbeets in the rotation. A P-Zn interaction might have induced the Zn deficiency. Lessman (71) and Judy (65) in later work on the same problem found that P increased Zn solubility in the soil, but decreased its utilization. Lessman, utilizing nutrient solutions and radioactive Zn, found that washing the roots of beans prior to exposure to ^{65}Zn increased the amount of Zn translocated to the tops of the plants. He concluded that an insoluble complex is formed near the cell surface involving Zn and P and that washing removed this complex and allowed upward translocation of Zn.

V. Conditions Affecting Zn Deficiency

Reports conflict with respect to the effect of P on Zn availability. Two (13, 92) indicate no effect, while others (10, 11, 23, 44, 66, 110) report reduced Zn utilization in the presence of high P.

Some indicate the damaging effect of P on Zn utilization to be mainly physiological and suggest a reduced translocation of Zn within the plant (8, 72, 97). This reduced translocation was thought to be due to reduced movement in the root cell (72, 97) and Zn precipitation by P in the xylem tissue (8).

Soil compaction and high soil moisture have also been observed to decrease Zn availability (109). Martin et al. (75), in 1965, reported that raising the soil temperature from 50 to 80 F increased the availability of Zn.

Contrasting results of the effect of organic matter on Zn availability have been published. Organic matter inactivated soil Zn, which may have been due to production of chelating agents such as lignin, and microorganisms which compete with the root for available Zn (21, 21). Grimes et al. (51), in 1961, reported the opposite. They found manure to be a partial replacement for Zn.

The availability of Zn has been found to be affected by soil pH. In general, raising pH above 6.0 decreases Zn availability (20, 44).

VI. Critical Levels of Zn in the Soil and Plant

Brown and Tiffin (20) used ammoniumacetate-dithiazone to extract Zn from soils as a guide in locating geographical areas likely

to be deficient. They believed 0.5 part per million (ppm) to be a critical level below which a Zn response might be detected.

Though Steenbjerg (96) pointed out the difficulty of relating concentration to elemental status within the plant, several authors have estimated critical concentration of Zn within the plant (12, 58, 79, 89, 105, 112). These estimates range from about 10 to 20 ppm.

VII. The Interaction of Zn with Other Micronutrients

Considerable work has been done on the interaction between the micronutrients Zn, Cu, Fe, and Mn. Under some circumstances one or more of these micronutrients have an extremely detrimental effect on the utilization of others. Fe seems most subject to alteration of uptake and utilization. Chapman et al. (26), working with orange trees, obtained results suggesting that Zn prevents Fe from being translocated from the exterior root cells to the vascular system. Smith and Specht (93) observed that Cu was more effective than Zn and Mn in inducing Fe chlorosis in citrus. Excesses of Cu and Zn inhibited upward transport of Fe . Lingle et al. (72) observed that Zn reduced the translocation of Fe to soybean tops and root uptake. Fe-deficient soybeans developed a greater capacity to take up Fe than normal plants.

The relative effectiveness of several cations in inducing Fe chlorosis has been reported by Hewitt (55, 56) and Hunter and Vergnano (61). The order of severity seems to be Ni > Cu > Co > Cr > Zn > Mo > Mn.

Millikan (78) has obtained evidence of interaction between Cu and Zn in subterranean clover and alfalfa. He observed that Zn deficiency decreased the concentration of Zn within the plants and increased the copper concentration. Fuerhing (42) observed a Zn-Mn and Fe-Mn interaction, which affected the Zn concentration in corn plants. Fuering and Soofi (43) observed, in corn, that the optimum Zn concentration for grain yield was found to be dependent upon the level of Mn. Low Mn in the leaves resulted in a low Zn requirement, while higher Mn caused a higher requirement for Zn. Thus they concluded that balance between the various microelements seemed to be most important. This balance seemed to be less important when the elements were in the external solution than when they had been taken up by the plant. Micronutrient unbalance seemed to affect translocation more than uptake.

VIII. Genetic Differences in Ion Accumulation or Utilization

An early review by Collander (28) deals with preferential absorption of cations by different species of plants. He stated that

plants do not absorb elements in the same proportion as they occur in nutrient solution, and that the mechanism of selective salt absorption was obscure.

Working with 20 different plant species grown in several different nutrient solutions, Collander found that the concentrations of Na and Mn are about 20 to 60 times greater in the plant species of highest accumulation than the species of lowest accumulation. The differences in the other cations studied (Li, Mg, Ca, Sr, K, Rb, Cs) were not as great but still of a magnitude two to five times as concentrated between the upper and lower species. He observed that some plant species are invariably relatively rich in some cations and poor in others. He speculated that the root cells exert selection of cations and actively secrete them into the xylem vessels, and pointed out that "the whole problem of cation selection is an extremely intricate one."

Vose (107), in his review of varietal differences in plant nutrition, cited a report in 1863 on differential uptake of I. Subsequent reports have dealt with differential yield responses to nutrient application, differential accumulation, differential requirements to alleviate nutrient deficiency, and differential resistances to metal toxicity (37, 45, 107).

A. Qualitative Responses

A well-known example of a differential nutrient response is the requirement for Fe by two soybean lines (Hawkeye and PI-54619-5-1). This phenomenon was first noted by Weiss (111). Efficiency of utilization was found to depend upon a single dominant gene. He concluded that the relatively low soluble Fe observed in the aerial plant tissue was probably due to the high pH which may have been induced by the low K content.

Pope and Munger (83, 84), working with celery, found a differential susceptibility to low (0.01 ppm) B and to low (2.5 ppm) Mg. In both instances, efficiency was dominant and segregated 3 to 1 in the F_2 population.

A mutant (yellow-stripe = ys1) of corn has been found and described by Beadle (3) and Bell et al. (5, 6) which renders the plant Fe-inefficient when homozygous. Bell et al. (5, 6) showed that the inefficiency which was due to an inability to utilize ferric Fe was restricted to the roots. Brown (14) observed differential chlorosis of an inefficient line (ys1/ys1) and an efficient line (Pa 54) on calcareous soils. He found that a sixfold increase in P decreased the Fe concentration in the tops of the inefficient but not the efficient line.

Howell (60), working with the soybean varieties "Lincoln" and "Chief," obtained a differential response to high P. Chief

responded favorably to P concentrations as high as 3.62 mM whereas Lincoln was inhibited by concentrations as low as 1.61 mM. Foote and Howell (41), on the basis of reciprocal grafting experiments, determined that this difference was related to the genotype of the root. Tolerance was found to be due primarily to reduced P accumulation.

Recently, differential tolerance to high Al concentrations of barley (74) and cotton (39) has been observed. Al interfered with the accumulation of K, Mg, Ca, and P in barley. It was associated with shallow rooting in cotton. Foy and Brown (40) noted that differential tolerance of the buckwheat and barley species was due to the ability to absorb and utilize P in the presence of excess Al.

Two varieties of beans differentially respond to low levels of Zn encountered under field conditions in Michigan (33, 66). The Saginaw variety produced 18.7 bushels per acre without added Zn on a calcareous soil, with a pH of 7.8, while Sanilac produced 7.6 bushels. The partially reduced yield of Saginaw was due mainly to delayed maturity. Sanilac, on the other hand, was extremely stunted and chlorotic (Figure 1).

Saginaw was derived from crosses and backcrosses made between several navy bean types including Michelite. It has an

FIGURE 1. --Differential tolerance of Saginaw and Sanilac navy beans grown on a soil (Saginaw County) low in Zn.

Upper picture--Differential stunting, chlorosis, and necrosis of Saginaw and Sanilac navy beans.

Lower picture--Differential maturity of Saginaw and Sanilac navy beans [Sanilac (on right) normally 9-10 days earlier than Saginaw (on left) is approximately 10-14 days later].

indeterminate growth habit and is generally 8-10 days later than Sanilac in maturity (1). Sanilac has a complex genetic background based primarily on an X-Ray induced early bush mutant of Michelite (2).

B. Quantitative Response

Although quantitative differential mineral accumulation is more difficult to study physiologically, the evidence shows real differences between and within species.

Smith (94) observed a difference in utilization of P in maize and attributed the differential response to root type. Efficiency was genetically dominant. A high ratio of secondary to primary roots accounted for efficiency.

Harvey (53) noted that differential response of corn to N was controlled by a partially dominant gene system and that differential growth of tomatoes induced by K was due to an incompletely dominant genetic system.

Massey and Loeffel (76, 77) found differences ranging from 38.4 ppm to 15.5 ppm in Zn content of corn grain, which were attributed to higher transfer of Zn from the ear leaf to the grain in the higher accumulating lines. Butler et al. (24) found that accumulation of Zn in ryegrass (Lolium) was highly heritable (.401 ± .176 in the broad sense).

Gorsline and his associates (46, 47, 48), studying accumulation of a number of elements by maize, found additive gene action for ear leaf accumulation and in most cases for grain accumulation. They observed a strong relationship between Sr and Ca uptake suggesting that uptake of both elements is controlled by the same physiological mechanism.

Kleese (70) utilized reciprocal grafting experiments to establish whether differential accumulation of ^{89}Sr and ^{45}Ca by soybeans was a root or shoot phenomenon. The accumulation of both radioisotopes was controlled to a greater degree by the genotype of the stem.

Rasmussen and his associates (86, 87) studied differential accumulation of ^{89}Sr and ^{45}Ca and the heritability of these phenomena in barley and wheat. Large genotypic differences in both species were noted. Although accumulation was higher in barley, variation was greater in wheat. Accumulation in most cases was due to additive effects and heritability in the broad sense ranged from 40 to 70 percent in wheat and 26 to 63 percent in barley. They also observed a close relationship between Sr and Ca accumulation. Pinkas and Smith (82) found a higher concentration of Sr in the fluid from the decapitated roots from a high accumulating variety than from a low. No differences in the morphology and internal anatomy of the two

varieties were observed and they concluded that Sr transport is dependent on metabolic activity.

IX. Possible Mechanism Responsible for the Observed Differential Responses

Vose (107) categorized factors which may be responsible for differential responses as follows: nutrient absorption (root morphology, exchange absorption), active uptake, translocation, and metabolism. Where the physiological mechanisms have been investigated, an attempt has been made to eliminate various possible mechanisms as causative factors in any particular instance. Smith (94) found that a high proportion of secondary to primary roots was responsible for the tolerance to low P of some maize lines.

Reciprocal grafting experiments have been often used to separate root and shoot as regions of control (4, 18, 41, 70). Kleese (70) found the genotype of the stem to be the major factor in determining differential Sr accumulation in the tops of soybean plants. The other authors cited found the root to be the determining factor in their systems.

Brown et al. (18), utilizing stem-root grafting, related the differential ability of root genotypes of soybeans for Fe absorption to a greater sorptive capacity and a greater tolerance to interference by competing ions. In a recent paper (22) they discuss the present

status of the differential iron response. The efficient genotype has a greater ability to reduce Fe^{+++} to Fe^{++} at the root. This reduction is related to the Fe stress within the plant. Thus the efficient varieties are better able to grow under Fe stress because the available Fe is utilized to a higher degree. Following reduction of Fe, a chelation with citrate occurs and the Fe is transported as Fe-citrate. Thus, plants with a greater reducing capacity at the root surface also have a greater capacity to transport Fe-citrate.

In contrast, Wallace et al. (108) found that Fe absorption by Hawkeye (efficient), when grown in the same soil solution with PI (inefficient), was decreased. This indicated that PI exuded an inhibitory factor into the soil that decreased the absorption of Fe by Hawkeye. Another possible explanation is that Fe-deficient plants might develop an increased capacity to absorb and accumulate Fe in the roots (15) which would decrease the amount of Fe available to the efficient plant.

Tracer experiments utilizing radioautography have been useful to determine circulation patterns and distribution of some nutrients within the plant (4, 8, 9, 63, 88). Biddulph (9) reported that Zn was precipitated along the veins of plants grown in solutions high in P. Under low P, Zn was uniformly distributed through leaves and not markedly concentrated in the veins.

Jacoby (63) and Cooil et al. (29), working with Na, have reported on some of the mechanisms and peculiarities of translocation. Jacoby found that radioactive Na is retained in the basal parts of the plant at low concentration. As concentration increases, a gradual Na saturation of the stem tissue occurs and distribution becomes uniform. Cooil et al. state that Na is transported out of the root but that this is not observed because Na is recycled down the stem and back into the root.

Jyung et al. (67, 68) have developed the technique of enzymatic separation of cells to study cellular uptake per se, bypassing root absorption and translocation. Utilizing this technique they have established that Rb is actively accumulated. Fe has also been found to be actively accumulated by isolated cells (69). In addition to an isolated cell system, a cell-free system has been utilized to study the nature of Zn and Zn-containing substances within the cytoplasm (64). Zn existed in a free form and bound to a protein(s).

X. Active Uptake as a Genetically Controlled Process

Metabolic energy input must be involved in any scheme of genetic control of nutrient uptake and/or utilization. If the entire process were passive there would be little basis for assuming genetic control of ion utilization; with the exception of differential response

due to different root or vascular morphology. Brown (16) recognized this when he proposed that translocation of Fe was dependent upon oxidative phosphorylation, primarily for the production of organic acids.

Although the reduction of Fe seems to be very much involved in the differential Fe response, such a scheme cannot account for differential Zn response since Zn exists primarily in only one ionic form. The carrier hypothesis, reviewed by Hansen (52), that ions are transported across membranes via carriers, seems a plausible alternative. Epstein (1961) and others (36, 73) have established that K and Rb uptake by excised barley roots is mediated by the same mechanism. They have identified two mechanisms or systems of uptake, one dominant at a concentration of about .018 mM and the other at a concentration of about 16 mM for half-maximum velocity. Na competes with the first but not the second system. Ca seems to be necessary for the selective abilities of the systems (35, 62).

Luttge and Laties (73) proposed that system one (low concentration, high affinity) operated at the plasma membrane and system two at the tonoplast. They present their conclusion as evidence confirming the symplast theory as a means of transport of salts from the outer solution to the xylem. They differentiated between long distance transport (movement into the xylem through the intercellular

cytoplasmic strands) via symplasmic uptake and vacuolar uptake. System one had the same physical characteristics as xylem transport which was expected if the symplasmic theory is correct. System two had different characteristics and was responsible for vacuolar accumulation. Both systems required energy (36).

The work of Pardee et al. (80, 81) is another confirmation of the carrier hypothesis. They have isolated and purified a protein from Salmonella which might act as a carrier for S. This is the first report of the isolation of a carrier molecule.

METHODS OF PROCEDURE

I. Low Zn Genetic Investigations

In 1964, plants of the Saginaw and Sanilac varieties, as well as F_1, F_2, and backcross progenies from crosses between these two varieties, were grown on plots with low Zn availability located on a cooperator's farm in Saginaw County, Michigan. This farm had been used for several years in Zn deficiency experimentation by the Soil Science and Crop Science Departments of Michigan State University. The soil in this area is a Wisner clay loam; a calcareous, lake-bottom soil with a pH of about 7.9. The soil has no lime requirement and the concentrations of P, K, Ca, and Mg are about 40, 330, 9200, and 600 pounds per acre, respectively. In addition, the soil was fertilized with 200 pounds per acre of 5-20-20 (non-Zn), plus 2% Mn.

The experiment was not replicated except for parents, which were replicated twice. The various generations followed successively in a series of short 28 inch rows. The seeds were space planted six inches apart. The severity of Zn deficiency as indicated visually by chlorosis, necrosis, epinasty, stunting, and vigor was estimated on each plant at about six weeks of age. A scale from one

to five was used, one being normal and five extremely zinc deficient (Figure 2). At ten weeks of age, several F_2 plants, which received scores from three to five, were transplanted in the plant science greenhouse on the Michigan State University campus at East Lansing. These plants were fertilized with Zn and eventually overcame the deficiency and set seed.

In 1966, the parents, F_1's, F_2's, and F_3's were planted on the same farm as in 1964. In addition, F_3 seed obtained from the F_2 plants transplanted in 1964 and selfed seed from both backcrosses in 1964 was planted. In 1964, the selfed-backcross seed was obtained only from plants possessing a fair degree of tolerance to low Zn and therefore was biased. The area used in this experiment had previously received Zn at an unknown rate during normal farming operations. For this experiment, 50 pounds of P per acre was added to induce a stronger Zn deficiency.

Each generation of genetic material was replicated six times. Each replicate consisted of 100 six-foot by 28 inch rows, which had been sown with 10 seeds, if available, plus two red kidney beans at each end to remove border effect. A two-foot alley was between each range. Two and one-half ranges made up one replicate. Of the 100 rows per replicate, nine were planted to each parent, three to the F_1 generation, nine to the F_2 generation, 10 to selected

FIGURE 2. -- Navy bean plants grown in low Zn soil (Saginaw County) exhibiting various levels of Zn deficiency which illustrate the "scale" (as given by the numbers) used in estimation of degree of Zn deficiency.

F_3 plants (from 1964 transplanted F_2 plants), 21 to unselected F_3 plants, and 20 to each selfed-backcross generation. A single unselected F_3 family was planted in only two replicates; thus there was a total of 63 families.

Approximately six weeks after planting, readings of visual Zn deficiency symptoms were taken on an individual plant basis using the 1964 scale.

The analyses of the 1964 and 1966 data were, in general, the same. Plants of both the Saginaw and Sanilac parents were found in several of the classes, making interpretation of the segregating populations difficult. To determine the number of genes and mode of action, an assumption of two phenotypes in the genetic population was made, one tolerant and one sensitive to Zn deficiency. Gradation observed within each phenotype was assumed to be due to low penetrance and expressivity (85, 90) of the genes regulating the response. The analysis of the genetic populations was based on partitioning these populations into the tolerant and sensitive phenotypes, using the non-segregating populations as an estimate of the degree of penetrance. This technique, similar to Powers' and Locke's (85) partitioning technique, is illustrated in Table 1. The F_2 population (partitioned into a tolerant and sensitive phenotype) then was used to

estimate the number of genes and gene action. In addition, the means and variances of each population were calculated.

TABLE 1. -- Example of partitioning technique (fictitious data).

Generation	Number of individuals in each class of Zn reaction					
	1	2	3	4	5	
Saginaw	120	80	70	30		
Sanilac	10	10	100	130	50	
F_2	120	70	80	100	30	
	Percentage of parental types in each class					
	1	2	3	4	5	
Saginaw	40.0	26.6	23.3	10.0	0	
Sanilac	3.3	3.3	33.3	43.3	16.7	
Saginaw + Sanilac	43.3	29.9	56.6	53.3	16.7	
Proportion Saginaw type	92.4	89.0	41.2	18.8	0	
Proportion Sanilac type	7.6	11.0	58.8	81.2	100	
	Partitioning F_2 into parental types					
	1	2	3	4	5	Total
Total	120	70	80	100	30	400
Saginaw type	111	62	33	19	0	225
Sanilac type	9	8	47	81	30	175

The 1966 F_3 families were split into segregating and non-segregating families on the basis of variances and means. If the variance of a Saginaw-type family didn't exceed the variance of the Saginaw parent by over 25 percent and the means were similar, it was called a non-segregating Saginaw type. The same parameters were used to identify the non-segregating Sanilac types. The segregating families were split into a 9:7 population and a 3:1 population on the basis of their segregation pattern within the five classes of Zn reaction. The two segregating populations were then partitioned into sensitive and tolerant phenotypes, using the method illustrated in Table 1.

In 1964, a bias in the Saginaw readings tended to reduce the overlap between Saginaw and Sanilac. In order to correct this bias, the F_1 population was used as an estimate of Saginaw to partition the F_2 population.

II. High Zn Genetic Investigation

To gain an indication of the inheritance of tolerance to high Zn levels (5.0 ppm), Saginaw and Sanilac parents, F_1's and F_2's were planted in the greenhouse utilizing the same procedures as in the nutrient experiments (described in the next section) with respect to sand preparation and water and nutrient application. Saginaw and Sanilac varieties were planted in the same pots; the other genetic

material was planted two per pot, with the provision that F_1's were together and also the F_2's.

Six weeks after emergence the plants were harvested, dried, and weighed. No extensive analysis was conducted on this material due to rather limited population sizes.

III. Micronutrient Interaction Investigation

To determine the effect on Saginaw and Sanilac of various combinations of several micronutrients at normal to extremely high concentrations, two different experiments were conducted. The first (June-August, 1965) consisted of planting Saginaw and Sanilac seed on opposite sides of one gallon glazed pots containing silica sand which had been washed three times with 0.5 N HCl. In between each acid washing, the sand was rinsed with deionized water twice, and after the final washing the sand was rinsed four times.

After planting, the sand was moistened with deionized water every day until the seedlings emerged. The plants were thinned to one of each variety per pot. At this time watering with nutrient solution was initiated. The macronutrient solution used was a slightly modified Hoagland's (59) listed below:

Chemical	Quantity
$Ca(NO_3)_2 \cdot 4H_2O$	1.401 grams/liter
KH_2PO_4	0.1585 grams/liter
KNO_3	0.5284 grams/liter
$MgSO_4 \cdot 7H_2O$	0.4323 grams/liter

The treatment variables were three concentrations of each of the micronutrients, Zn, Fe, and Cu, in a factorial design. The lowest concentration of each micronutrient plus the levels given for the other micronutrients, are those normally used in a Hoagland's solution (59) (Table 2). Reagent grade chemicals were used to make up all nutrient solutions. The pH of the final solution varied between 5.3 and 5.7.

The various combinations of micronutrients gave twenty-seven different treatments. The cultures were watered three times per week (Monday, Wednesday, and Friday) with the appropriate nutrient solution and on other days with deionized water. Nutrient solution or water was added until effluent flowed from a hole in the bottom of the pot. Supplemental light was used in this and all following experiments (12 hour daylength). The plants were grown in an "air-conditioned" greenhouse chamber which maintained the temperature at 80 F ± 5°.

TABLE 2. -- Micronutrients and concentrations utilized in micronutrient interaction experiment number one.

Micronutrient	as	Concentration (parts per million)
B	H_3BO_3	0.5
Mn	$MnCl_2 \cdot 4H_2O$	0.5
Mo	$H_2MoO_4 \cdot H_2O$	0.01
Zn	$ZnSO_4 \cdot 7H_2O$	0.05
Zn	$ZnSO_4 \cdot 7H_2O$	5.0
Zn	$ZnSO_4 \cdot 7H_2O$	50.0
Cu	$CuSO_4 \cdot 5H_2O$	0.02
Cu	$CuSO_4 \cdot 5H_2O$	2.0
Cu	$CuSO_4 \cdot 5H_2O$	10.0
Fe	Fe-Citrate	0.5
Fe	Fe-Citrate	25.0
Fe	Fe-Citrate	75.0

Plant height and trifoliate leaf number were recorded two weeks, four weeks, and six weeks after seedling emergence. At six weeks, the plants were removed by washing the roots out of the sand with water under pressure. The plants were divided into shoot and root, air dried, and weighed.

An analysis of variance of root and shoot weight, number of leaves, and plant height was made by computer. Another analysis was computed on the difference in shoot weight of Saginaw and Sanilac.

The second experiment (December, 1965-January, 1966) was conducted identically to the first except that the macronutrient solution used was slightly modified in an attempt to buffer possible pH changes within the culture during nutrient uptake. The nutrients used are listed below:

Chemical	Quantity
$Ca(NO_3)_2 \cdot 4H_2O$	1.401 grams/liter
KH_2PO_4	0.0793 grams/liter
KNO_3	0.5284 grams/liter
$NH_4H_2PO_4$	0.0670 grams/liter
$MgSO_4 \cdot 7H_2O$	0.4323 grams/liter
KCl	0.0434 grams/liter

The micronutrient solution utilized Mn as an additional variable to Zn, Cu, and Fe. The micronutrients and concentrations are listed in Table 3.

Six weeks after seedling emergence the number of trifoliate leaves and plant height were recorded, the plants were then harvested as described previously, except that the roots were washed

in .5 N HCl and rinsed in deionized water. Again the plants were divided into roots and shoots, dried, and weighed.

TABLE 3. -- Micronutrients and concentrations utilized in micronutrient interaction experiment number two.

Micronutrient	as	Concentration (parts per million)
B	H_3BO_3	0.5
Mo	$H_2MoO_4 \cdot H_2O$	0.01
Zn	$ZnSO_4 \cdot 7H_2O$	0.005
Zn	$ZnSO_4 \cdot 7H_2O$	0.05
Zn	$ZnSO_4 \cdot 7H_2O$	5.0
Cu	$CuSO_4 \cdot 5H_2O$	0.02
Cu	$CuSO_4 \cdot 5H_2O$	2.0
Mn	$MnCl_2 \cdot 4H_2O$	0.5
Mn	$MnCl_2 \cdot 4H_2O$	50.0
Fe	Fe-Citrate	0.5
Fe	Fe-Citrate	50.0

The dried plant material was further divided into leaves, petioles, stems, and roots. These various plant parts were ground

in a Wiley mill (with stainless steel parts to prevent contamination) to pass through a 20-mesh screen.

Samples of one-half to one g were used except when less material was available. They were dry ashed in porcelain crucibles at 500-550 C for at least six hours. The temperature was gradually raised to prevent ignition of the plant material. The ash was taken up in five ml of 2 N HCl and filtered through a Whatman No. 2 filter paper. Four rinsings of 10 ml each were made with deionized water: the crucible twice, the filter paper once, and the funnel once. The final solution volume was made up to 50 ml with deionized water.

This solution was analyzed for Zn, Cu, Fe, and Mn using a Perkin-Elmer model 290 atomic absorption spectrometer. Occasionally, further dilution of a sample was necessary to obtain a reading on the scale of the machine. The reading was linear in the range used with 0 percent absorption equal to 0 ppm and 100 percent absorption equal to the concentration of the highest standard solution used for each element. Values were converted to ppm/g dry weight using the following equation:

$$\text{ppm/g tissue} = \frac{\text{percent absorption} \times \text{solution volume}}{\text{plant weight} + \frac{100}{\text{ppm highest standard}}}$$

Analysis of variance was computed on plant weights and ppm of each element for each plant part. Again the computer was utilized for the analysis.

IV. Grafting Experiment

To determine the portion of the plant responsible for a differential reaction of the two varieties to 5.0 ppm of Zn, a series of grafts was made. Seeds of the two varieties were sown on opposite sides of a glazed pot containing acid-washed silica sand. The sand was moistened with deionized water. Following germination, when the primary leaves were partially expanded, grafts were made in all combinations of scion and stock with the Saginaw and Sanilac plants. Diagonal cuts were made through the hypocotyl approximately half the distance between the cotyledonary node and sand surface. A short section of sterilized nylon bristle was inserted into the region of the pith, which at this time was beginning to break down, for support of the scion. The scion of one plant was placed on the stock of another. A small piece of self-adhering rubber latex was placed tightly around the graft to prevent drying out of the graft union and to hold the graft tight. The grafted plants were watered with 5.0 ppm Zn nutrient solution and the cultures placed in a plastic-lined clay saucer containing about 1/2 inch of the same nutrient solution on the bottom. A

large plastic bag was placed over the culture and saucer so that it was airtight and supported above the plants by stakes and served as an efficient moist chamber. After five days the plants were taken out of the chamber and the watering schedule of nutrient solution (containing 5.0 ppm Zn) three days per week and deionized water on the alternate days was initiated. Approximately 30 grafts were made in each possible combination (Saginaw/Saginaw, Saginaw/Sanilac, Sanilac/Saginaw, Sanilac/Sanilac). Graft success was about 50 percent. Photographs were taken when the plants were approximately one month old. At two months the plants were harvested by washing the roots out of the sand, washing the roots with .2 N HCl, segmenting at the graft union, drying, and weighing. Later the dried plant material was ground, ashed, taken up in acid, and analyzed for Zn and Cu using the techniques previously listed.

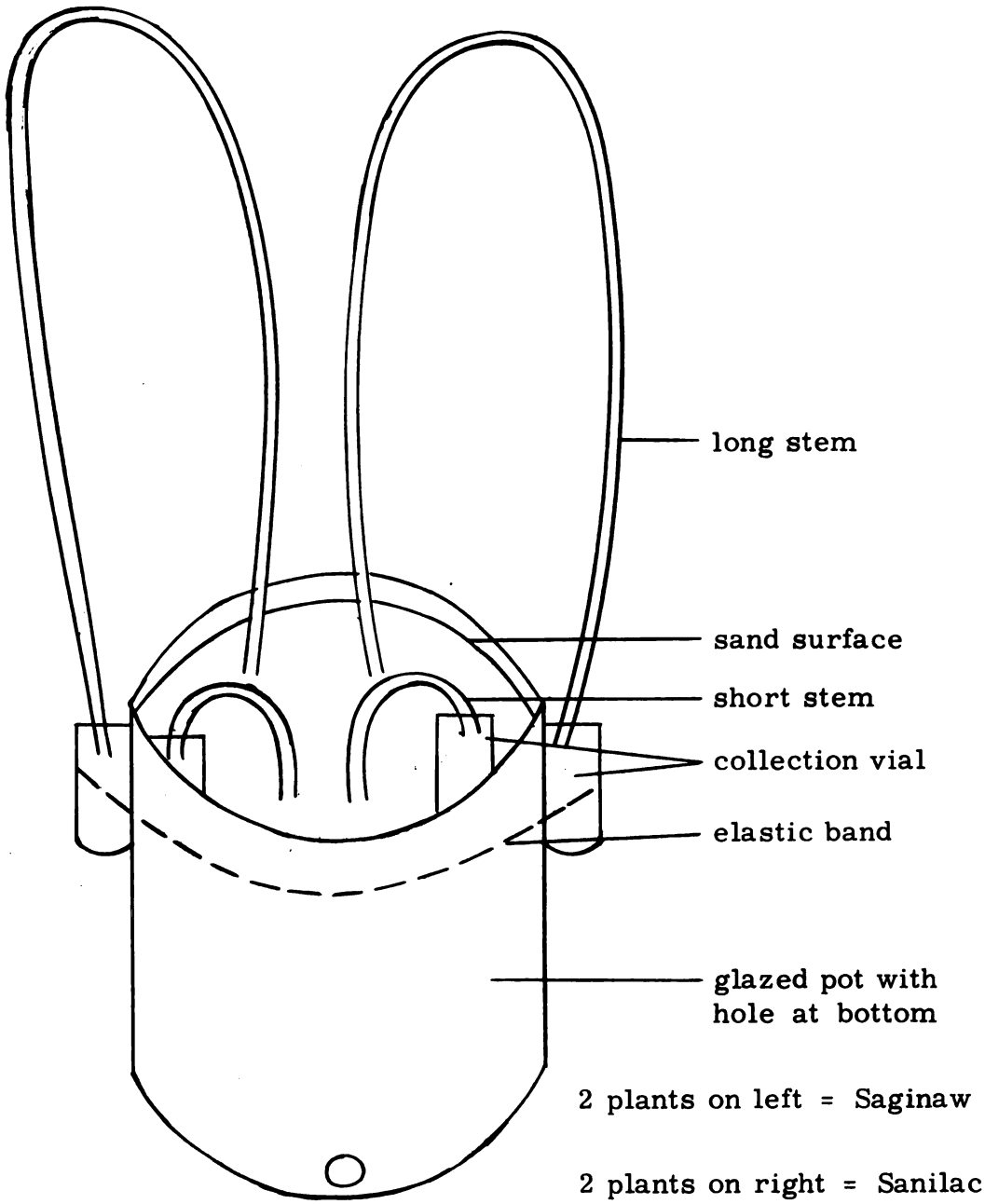
V. Estimation of Zn Bound by the Stem Using Plant Exudate

To determine whether the stems of the Saginaw and Sanilac navy beans bound Zn to the same extent, Zn content of exudate was determined. Seeds of the two varieties were sown on opposite sides of glazed pots. Following emergence, the number of plants was thinned to two of each variety per pot, and the irrigation procedure, described previously in the nutrient interaction experiment, was

initiated. The pots were divided into three groups, which received a different level of Zn in the nutrient solution. These levels were 5.0, 0.05, and 0 ppm (the zero Zn level did receive Zn as a contaminant as the plants didn't develop deficiency). At two months of age, eight cultures from each level of Zn (designated pretreatment) were selected from the groups on the basis of plant uniformity. These plants were watered for three days with deionized water to flush out Zn.

The three pretreatment groups were divided into two groups of four pots each. All leaves were removed from the plant by cutting at the base of the petiole. The cut surface was covered with vaseline to prevent exudation. Immediately after initiation of the treatment, two levels of Zn (5.0 and 0.5 ppm), one plant of each variety was cut off about three inches from the sand surface. The other plant of each variety was cut off 20-26 inches from the sand surface. The lower cutting usually left two to three nodes, while the higher left seven to eight. The stems were bent over and the cut ends placed into small (5 ml) plastic vials (Figure 3). Exudate was collected continuously for six periods of five hours each, starting at eight A. M., a new vial being placed under the end of each stem at the beginning of each 5 hour period.

FIGURE 3. -- Plant arrangement during exudate experiment.



The weight and Zn concentration of each sample of exudate was determined. The exudate was directly aspirated into the spectrometer.

Analysis of variance was computed on exudate weight, Zn concentration, and total Zn exuded.

VI. Tracer Investigation--Whole Plant

All tracer studies on the whole plant followed a similar pattern of preparation. Plants were grown in silica sand until the primary leaves were approximately 3/4 expanded (about 12 days from sowing). The roots were then washed out of the sand, rinsed off, and the plants transferred to a special plexiglass container designed for isotope-containing nutrient experiments (Figure 4).

Generally the containers were filled with Hoagland's nutrient solution up to about 1 inch from the top (1500 ml). Aeration was continuous. Supplemental light was given by fluorescent lamps (1200 foot candles) on a 12 hour daylength cycle.

Following a 12 hour acclimation period (unless otherwise specified), ^{65}Zn was added and the plants allowed to take up Zn for a period of time. Specific details concerning treatment variables and uptake period will be given for each experiment in the results section. After the uptake period, the plants were removed from the

FIGURE 4. -- Pictures of plexiglass container designed and used for exposing plants to ^{65}Zn.
(Outside dimensions = $18\frac{1}{2}$" x $2\frac{1}{2}$" x $4\frac{1}{2}$")

solution and the roots were blotted, washed in dilute HCl, blotted, rinsed in H_2O four minutes, and blotted. At this point, the plants were either pressed in order to make radioautographs or sectioned for counting. Pressing was done by placing the plants between two pieces of heavy blotter paper, usually with the primary leaves and roots severed to prevent recycling during the initial part of the pressing operation. The blotter paper with the plant in between was placed between two pieces of composition board. Several of these units (plant, paper, and boards) were stacked with a lead weight on top and placed in an oven until dry (2-4 days). The plants were removed from the blotter paper with the aid of a long-bladed spatula, mounted on a clean piece of blotter paper using rubber cement and covered with Saran wrap. Each plant and a sheet of Kodak Blue Brand Medical X-Ray film was placed between two composition boards in a dark room. These units were stacked, covered with a weight, and placed in a box for the duration of the exposure period, which was from 60 to 96 hours in various experiments. The films were then developed.

The plants were divided into leaves, petioles (in most cases), upper stem, lower stem (from the cotyledonary node down), and root for determination of ^{65}Zn content. These plant parts were placed in small wax-coated medicine cups and dried. Following

drying, the plant material was pressed into the bottom of the cup using a cork stopper covered with aluminum foil mounted on a glass rod. This was done in order to obtain uniform geometry of plant material for isotope content determination. The material was assayed for ^{65}Zn using a Geiger-Mueller Tube detector attached to a Tracerlab Versa/matic II scaler.

The analysis of these data was generally on a counts per minute basis instead of total Zn because a relative comparison between the two varieties with respect to uptake and distribution was all that was desired.

RESULTS AND DISCUSSION

I. Genetic Experiments

A. Low Zn

1. Field Data. -- The genetic populations were scored from 1 to 5 in both 1964 and 1966. In both years, the Zn deficiency readings were taken at about six weeks after emergence. Some of the plants initially scored as deficient often outgrew the deficiency. Eventually these plants appeared normal, but they matured late.

The genetic data obtained from 1964 and 1966 are summarized in Tables 4 and 5. The distribution of the various genetic populations of the two years can be seen in Figures 5 and 6. The severity of the Zn deficiency was not equal for the two years. With the exception of the Saginaw parent, all of the populations had a higher mean (greater degree of Zn deficiency symptom) in 1964 than in 1966.

In 1966, plants of the Saginaw variety were scored from 1 to 4 while those of Sanilac were scored in all classes. This distribution was probably due to the variability of the soil in the experimental plots and the threshold level of Zn differentiating sufficiency

TABLE 4. -- Summary of 1966 low Zn-genetic (Saginaw x Sanilac navy beans) population performances grown in the field (Saginaw County).

Generation	Class of Zn reaction					Total	Mean Score	Variance
	1	2	3	4	5			
Saginaw								
#	229	116	34	3		382	1.51	.476
%	60	30	9	1	0			
Sanilac								
#	14	19	76	278	56	443	3.77	.706
%	3	4	17	63	13			
F_1								
#	122	19	10			151	1.26	.326
%	81	12	7					
F_2								
#	179	98	127	83	26	513	2.37	1.563
%	35	19	25	16	5			
F_3 (selected)								
#	16	14	48	116	91	285	3.88	1.180
%	5	5	17	41	32			
F_3 (unselected)								
#	357	222	171	172	41	963	2.29	1.562
%	37	23	18	18	4			
F_1 x Sag (selfed)								
#	442	252	192	125	18	1029	2.05	1.254
%	43	24	19	23	2			
F_1 x San (selfed)								
#	368	212	235	161	12	988	2.23	1.32
%	37	21	25	16	1			

FIGURE 5. -- Distribution of Saginaw x Sanilac genetic populations grown in a soil low in Zn in 1966 (Saginaw County).

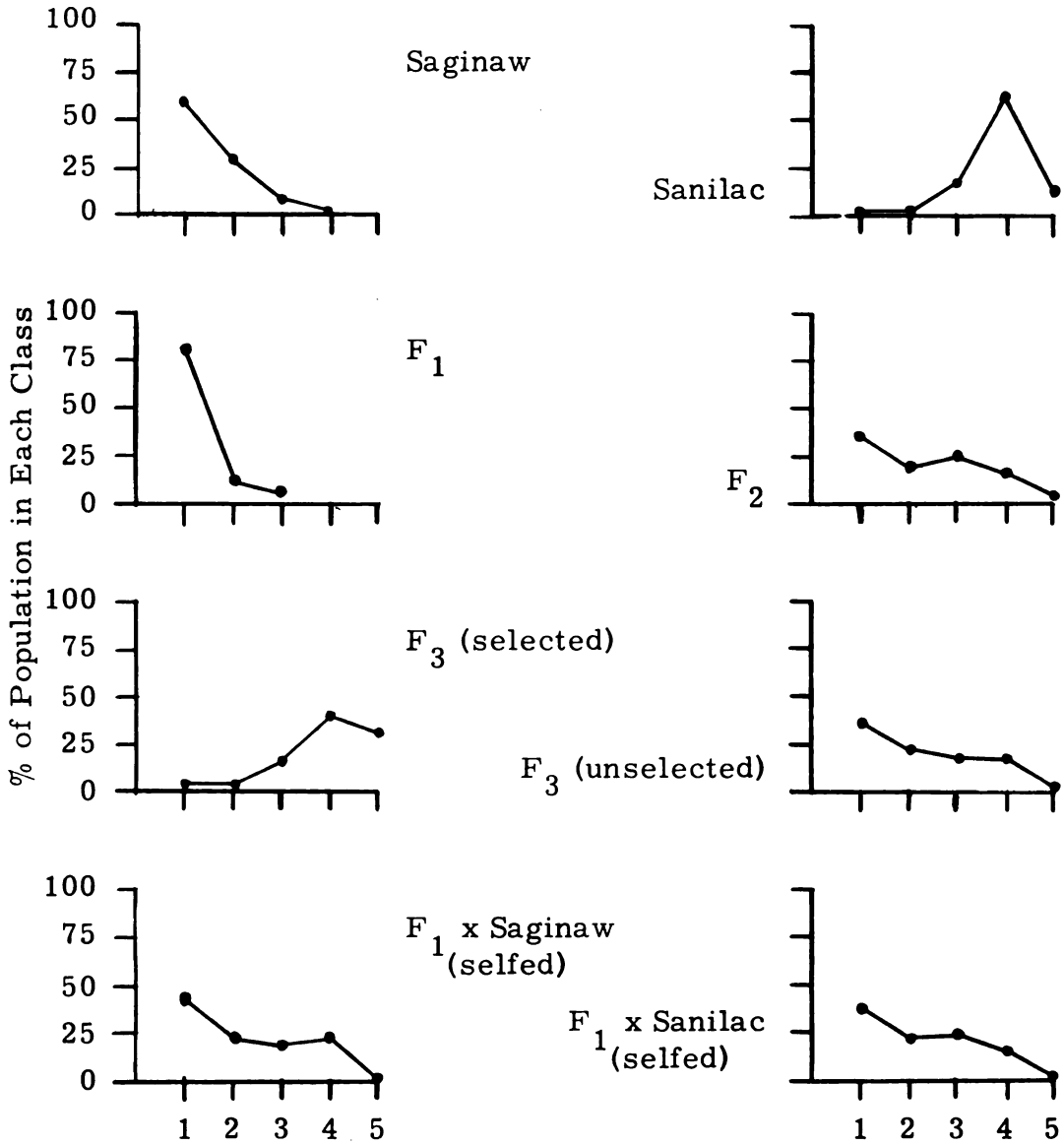
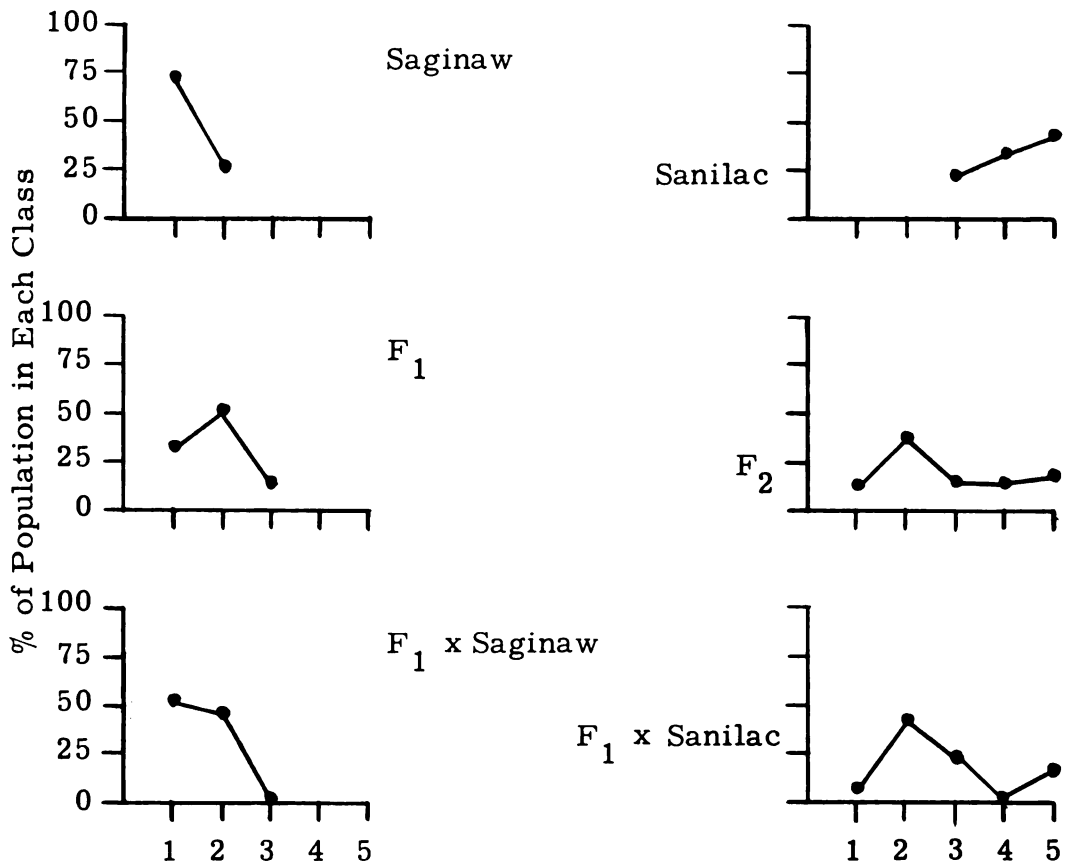


TABLE 5. -- Summary of 1964 low Zn-genetic (Saginaw x Sanilac navy beans) population performances grown in the field (Saginaw County).

Generation	Class of Zn reaction					Total	Mean Score	Variance
	1	2	3	4	5			
Saginaw								
#	49	18				67	1.27	.197
%	73	27						
Sanilac								
#			15	24	31	70	4.23	.609
%			22	34	44			
F_1								
#	20	32	9			61	1.82	.450
%	33	52	15					
F_2								
#	55	154	63	64	78	414	2.89	1.804
%	13	38	15	15	19			
BC Saginaw								
#	28	25	1			54	1.5	.283
%	52	46	2					
BC Sanilac								
#	4	21	12	2	9	48	2.81	1.553
%	8	44	25	4	19			

and deficiency within the plots. Other factors that could have contributed to the observed variability in degree of Zn deficiency symptoms are slight differences in soil moisture, soil temperature, and elemental concentration (particularly P). Environmental factors

FIGURE 6. -- Distribution of Saginaw x Sanilac genetic population grown in a soil low in Zn in 1964 (Saginaw County).



apparently act in such a manner that plants of identical genotype can shift to one side or the other of the Zn threshold level. Thus at least in this work, the genes involved in low Zn tolerance can be regarded as having relatively low penetrance (85, 90).

In 1966, 10 percent of the Saginaw population was scored in classes 3 and 4 and 25 percent of the Sanilac was scored in classes 1, 2, and 3, which emphasizes the low degree of penetrance of the genes for tolerance and sensitivity, respectively. The F_1 population in both years was similar to the Saginaw population in Zn deficiency symptoms. In 1964, the mean score of the F_1 population was 1.82 as compared to 1.27 for Saginaw and 4.23 for Sanilac. In 1966, the F_1 scored 1.26 as compared to 1.51 for Saginaw and 3.77 for Sanilac. These data suggest essentially complete dominance for the gene(s) imparting tolerance to low Zn.

The means of F_2 and F_3 populations were intermediate between the parents, although somewhat closer to Saginaw. The backcross to Saginaw exhibited full low-Zn tolerance whereas the backcross to Sanilac was intermediate. Selfed material obtained from the 1964 Saginaw backcross population was not as tolerant to low-Zn conditions as the original backcross grown in 1964 ($\bar{x}$ = 2.05 vs. 1.51). This indicated that genes for sensitivity were exposed in the backcross self population. If the trait were governed

by multiple factors, it is doubtful that a generation of selfing would expose sufficient numbers of genes conferring sensitivity to account for the loss of tolerance observed in this population.

The F_3 population obtained from F_2 plants selected in the field in 1964 for inefficient Zn utilization also indicated a small number of genes. These F_2 plants, after six weeks to two months in the greenhouse with nutrient fertilization, set seed which gave rise to a "selected" F_3 population. This population in 1966 had a mean value of 3.88 compared to 3.77 for the Sanilac parent (Table 6).

TABLE 6. --Performance of several F_3 navy bean lines in 1966 selected for Zn-deficient reaction in 1964 when grown under low Zn conditions in the field (Saginaw County).

Family	1964 value	Class of Zn reaction					Total	Mean score
		1[a]	2	3	4	5		
34	1-4[c]	8[b]	6	12	10	3	39	2.85
37	5	3	2	12	14	3	34	3.35
39	4	2	2	5	21	11	41	3.90
32	4		2	7	25	9	43	3.95
38	5	3	1	4	14	14	36	3.97
33	3		1	6	10	8	25	4.00
35	5			1	15	21	37	4.49
36	5			1	7	22	30	4.70

[a]1--highly tolerant; 5--highly sensitive.

[b]Number of individuals scored in class.

[c]Originally scored 4 but later scored 1.

This indicated that true-breeding fully sensitive plants could be selected in the F_2 population.

2. Determination of the Number of Genes and Gene Action. -- Gene number refers to the number of segregational units, while gene action is used in the classical Mendelian sense and refers to dominance, additivity, gene interaction, etc.

Though it is difficult to draw a firm conclusion with regard to a specific number of phenotypic classes present, the assumption of two seems to be the most conservative. The variability of the two varieties was used to estimate the number of individuals of the segregating populations in each class of Zn reaction which were phenotypically tolerant or sensitive. This "partitioning" method aided in splitting the F_2 and other segregating populations into tolerant and sensitive phenotypes. The probability that a plant in a given class is tolerant was computed. In 1964, the F_1 was used as an estimate of the response of tolerant phenotypes because there was no overlap in scoring of Saginaw and Sanilac parents. Scoring of both varieties, but especially Saginaw, was unconsciously biased for either a low or high rating in 1964. Using the F_1, it was possible to partition class 3 into tolerant and sensitive phenotypes.

The results of the partitioning are shown in Tables 7 and 8 for 1966 and Table 9 for 1964. These results indicated that two

TABLE 7. -- Percent distribution of Saginaw and Sanilac navy beans grown in the field in 1966 under low Zn conditions.

Variety	Class of Zn reaction					Number
	1	2	3	4	5	
Saginaw						
#	229	116	34	3		382
%	59.94	30.36	8.90	.78		
Sanilac						
#	14	19	76	278	56	443
%	3.16	4.29	17.15	62.75	12.64	
Total %	63.10	34.65	26.05	63.53	12.64	
% Saginaw	94.99	87.62	34.17	1.23	0	

TABLE 8. -- F_2 (Saginaw x Sanilac navy bean) population grown in the field in 1966 under low Zn conditions adjusted to parental type.

	Class of Zn reaction				
	1	2	3	4	5
% Saginaw	94.99	87.62	34.17	1.23	0
F_2 Field Data					
Total	179	98	127	83	26
Saginaw Type	170	86	43	1	
Sanilac Type	9	12	84	82	26

	Observed (adj.)	Expected (9:7)
Total Saginaw Type	300	289
Total Sanilac Type	213	224

x^2 prob. = .5 - .3

TABLE 9. -- Percent distribution of Saginaw and Sanilac navy beans grown in the field in 1964 under low Zn conditions and F_2 population adjustment.

Variety	Class of Zn reaction					Number
	1	2	3	4	5	
Saginaw						
#	49	18				67
%	73.17	26.87				
F_1						
#	20	32	9			61
%	32.78	52.46	14.75			
Sanilac						
#			15	24	31	70
%			21.43	34.29	44.29	
Total %	32.78	52.46	36.18	34.29	44.29	
% Saginaw (using F_1)	100	100	40.77	0	0	

		Observed (adj.)	Expected (9:7)
F_2	Total Saginaw Type	235	233
	Total Sanilac Type	179	181

genes are acting in a complementary manner to give a 9:7 ratio in the F_2 generation. A chi-square test indicated that the 9:7 ratio was compatible with the data. In 1964, the deviation of the observed from the expected 9:7 ratio was so small that a chi-square probability was not calculated.

3. Segregation Within the F3 Population. -- If a ratio of nine tolerant to seven sensitive in the F_2 population is assumed, it should be possible to predict the performance of various F_3 families. One half of the F_3 families should segregate while the other half should not segregate into tolerant and sensitive phenotypes. One half of the segregating families should segregate three tolerant to one sensitive and the other half nine to seven. Seven-eighths of the non-segregating families should be sensitive and the remaining tolerant.

The performance of the F_3 population in 1966 is given in Table 10. A given family was placed in a particular category by

TABLE 10. -- Patterns of segregation for reaction to low Zn in unselected F_3 navy bean families (1966) grown in the field.

	Segregating		Nonsegregating	
Expected	31.5		31.5	
Observed	34		29	
	Ratio--Tolerant/Sensitive		Tolerant	Sensitive
	9:7	3:1		
Expected	17	17	4	28
Observed	15	19	17	12

comparing the mean and variance of a family with that of the Saginaw and Sanilac parental populations. The partitioning values for the

families segregating 3:1 and 9:7 into tolerant and sensitive phenotypes are given in Table 11.

TABLE 11. -- Distribution of segregating unselected F_3 navy bean families grown in the field under low Zn (1966).

Ratio	Zn reaction class					Totals	
	1	2	3	4	5	Expected	Observed
9:7							
Tolerant	48	51	18	6	--	126	123
Sensitive	3	7	36	44	11	98	101
Total						224	224
3:1							
Tolerant	123	66	19	--	--	209	208
Sensitive	7	9	36	15	4	70	71
Total						279	279

When one compares the number of observed to expected F_3 families in each category (Table 10), the observed ratio of segregating to nonsegregating families closely approximates the expected. In addition, the number of families segregating 3:1 and 9:7 is close to the expected. However, there is a large discrepancy between the nonsegregating families which were tolerant and sensitive when compared to the expected number. There are many more tolerant than

sensitive plants rather than the expected reverse based on the 9:7 genetic model.

From these data, it appears that although there is some question regarding the absolute validity of the 9:7 ratio in F_2, there is also evidence supporting this interpretation. Data in Table 11 indicate how closely the partitioning method transforms the observed numbers of individuals in segregating F_3 families regarded as tolerant and sensitive to the expected numbers of each.

The distribution of the 1966 Sanilac F_1 backcross selfed population very closely approaches that of the F_2 population. If one considers the source of this backcross self population, further evidence is obtained for the 9:7 hypothesis. This population was biased due to natural selection for Zn tolerance because only tolerant plants of the 1964 F_1 x Sanilac would have set seed. The expected genotypes of this backcross are Aa Bb, Aa bb, aa Bb, and aa bb, assuming Saginaw (tolerant) to be AA BB and Sanilac (sensitive) to be aa bb. Only plants of the Aa Bb genotype would have been tolerant and would have set seed. This genotype is the same as that of the F_1 from which the F_2 generation was produced. In effect, the selfed backcross to Sanilac population was an F_2 population.

From these observations, although a two gene model may be valid, these genes may not be acting strictly in a complementary

fashion to give a 9:7 ratio. There are several indications that the genes involved are few in number. The ease with which plants were selected in the F_2 population which were true breeding for low Zn sensitivity provides evidence favoring a small number of genes (2-3). Also, the segregating populations are not distributed continuously and normally as would be expected if the trait were strictly polygenic without dominance. Instead, these populations are somewhat discontinuous and skewed toward tolerance of the low Zn condition. This indicates a low gene number with dominance controlling the trait.

There appears to be no way of resolving this question with the data presently available. Advanced generation backcrossing and intercrossing of advanced generation families which are homozygous and give specific reactions to low Zn conditions would provide more information. This information, in addition to data from segregation patterns of the existing advanced generations, should make it possible to answer the question of gene number and the mode of their action in the Mendelian sense.

B. High Zn

A summary of genetic data obtained from several populations of a Saginaw x Sanilac cross grown in nutrient sand culture with 5.0 ppm Zn is presented in Table 12. The three different lots presented were grown at two different times. Lot one responded with

somewhat greater growth of Saginaw than lots two and three, necessitating a splitting of the lots into two groups. In both instances, the populations were split into four categories on the basis of shoot weights (Table 12).

TABLE 12. -- Summary of Saginaw x Sanilac navy bean genetic experiment in sand culture at high Zn in the greenhouse.

Lot 1	Dry weight (g) of plant tops			
	0-2	2-5	5-8	8 -
Saginaw		1	1	4
Sanilac	5			
F_1	3	1		
F_2	12	12	5	1
Lots 2 and 3	0-2	2-4	4-6	6 -
Saginaw		3	5	4
Sanilac	12			
F_1	2	1	1	
F_2	41	37	11	3

Because of the relatively small population it is difficult to make an estimate of gene number and action. However, sensitivity to the high level appears to be dominant because the performance of

the F_1 generation, with a few exceptions, approximates that of Sanilac. Plants of the Sanilac variety fall only in the lowest weight range and Saginaw in the higher three ranges. If the F_2 population is split into two groups on the basis of the parental performance, the values obtained are extremely close to a 9:7 ratio of tolerant to sensitive (Table 13). The expected values are 68 to 54. Thus a conflict appears between the F_1 and F_2 data. The response of the Sanilac phenotype under high Zn is partially to completely manifested in the F_1, while in the F_2 the ratio of tolerant to sensitive plants suggests recessiveness of the Sanilac phenotype.

TABLE 13. -- Segregation of (Saginaw x Sanilac navy bean) F_2 population grown under 5.0 ppm Zn level into a Saginaw and Sanilac phenotype.

Total number of F_2 plants	(Sanilac) 0-2 grams	(Saginaw) < 2 grams
Lot 1	12	18
Lots 2 and 3	41	51
Total	53	69

A possible root and/or shoot interaction between plants within the same pot due to differential accumulation of Zn and other ions may account for this discrepancy. However, this is only speculation.

Additive gene action may be an alternative to the 9:7 ratio. The F_1 generation would be somewhat intermediate between the Saginaw and Sanilac parent with regard to growth at the high (5.0 ppm) level of Zn. The F_2 generation would also be somewhat intermediate. Both of these conditions appear to be fulfilled if the data are indicative of the real situation.

With the present data, there is no possibility of distinguishing which of the various alternatives might be correct. The determination of the true genetic action is of importance as the genetic model limits the nature of the hypothetical physiological model. If gene number and action are the same at the low and high level of Zn, a common mechanism may be postulated. If they are not the same, a common mechanism, at least in its entirety, cannot be involved.

Greater amounts of data from F_2 and backcross populations and an experimental design removing the possible competition effect but still measuring the pot to pot variability would help clarify the genetic picture with regard to the control system of high Zn tolerance.

II. Micronutrient Studies

A. Zn, Cu, and Fe

The purpose of this and the following experiment was to determine the effect of high concentrations of various micronutrients

on growth and ion accumulation of the Saginaw and Sanilac varieties of navy beans. By increasing the concentrations of certain micronutrients, deficiencies in other micronutrients might be induced and the two varieties might react differently to this unbalance. This was initially suggested by the chance observation of a differential nutrient chlorosis induced by nutrient solutions containing especially high Zn concentrations, as well as high contents of other elements. Saginaw appeared normal, while Sanilac was chlorotic under these conditions.

The effect of some of the combinations and concentrations of Zn, Cu, and Fe on the appearance of the two varieties can be seen in Figure 7. Photographs A, B, C, and D illustrate the effect of the three levels of Zn. At the normal level (A) both varieties grew well and at the 50.0 ppm level (D) both were extremely stunted. However, at the 5.0 ppm level of Zn, Saginaw grew well while Sanilac was stunted. This effect is observed in B and C, which were photographed at three weeks and six weeks after emergence, respectively. The differential stunting was observed at all levels of the other micronutrients except 10.0 ppm Cu.

Figure 8 illustrates the response due to interaction of high Cu and Fe on the two varieties. In photograph A (10.0 ppm Cu) Sanilac was chlorotic while Saginaw was normal. However, the application of high Fe (75 ppm) overcame the chlorosis as evidenced in B.

FIGURE 7. -- Saginaw (left side of pot) and Sanilac (right) navy beans grown at 0.05, 5.0, and 50.0 ppm Zn in the greenhouse.

A. -- 0.05 ppm Zn

B. -- 5.0 ppm Zn (three weeks after emergence)

FIGURE 7. -- Continued.

C. -- 5.0 ppm Zn (six weeks after emergence)

D. -- 50.0 ppm Zn

FIGURE 8. -- Saginaw (left side of pot) and Sanilac (right) navy beans grown at 10.0 ppm Cu plus two levels of Fe in the greenhouse.

A. -- 0.5 ppm Fe

B. -- 75.0 ppm Fe

This effect was observed at the two lower concentrations of Zn but not at 50.0 ppm.

The differential growth in terms of shoot weight for the two varieties at the various levels of each micronutrient can be seen in Table 14. The analysis of variance data are given in Table 15.

TABLE 14. -- Effect of various levels of Zn, Cu, and Fe on growth of Saginaw and Sanilac varieties of navy beans when grown in sand culture in the greenhouse.

Nutrient	Level	Average[a] weight (g) of shoot		
		Saginaw	Sanilac	Difference
Zn	0.05 ppm	3.9	3.2	0.7
	5.0 ppm	3.8	1.4	2.4
	50.0 ppm	0.3	0.3	0.0
Cu	0.02 ppm	3.8	2.0	1.8
	2.0 ppm	3.8	2.0	1.3
	10.0 ppm	1.0	0.8	0.2
Fe	0.5 ppm	2.7	1.6	1.1
	25.0 ppm	3.5	1.7	1.8
	75.0 ppm	2,0	1.6	0.4

[a]Average of three replications and nine treatments.

The analysis of variance of the raw data is given in Appendix Table 1. Although tabular data will not be shown, root weight, trifoliate leaf number, and plant height responded similarly to shoots to high Zn.

These data establish the fact that Saginaw is capable of withstanding much higher levels of Zn than Sanilac (Table 14).

TABLE 15. --Analysis of variance of Saginaw minus Sanilac shoot weights when grown under different levels of Zn, Cu, and Fe.

Sources of variation	d.f.	M.S.	F value
Replications	2	1.965	
Treatment	26	8.846	6.156**
Zn	2	42.85	29.819**
Cu	2	16.255	11.312**
Fe	2	13.235	9.210**
Zn x Cu	4	10.498	7.305**
Zn x Fe	4	3.388	2.358*
Cu x Fe	4	3.388	2.358*
Zn x Cu x Fe	8	2.026	1.410
Error	52	1.437	
Total	80		

*--Significant at α .05
**--Significant at α .0125

The reason for the small difference between the two varieties at either 50.0 ppm Zn or 10.0 ppm Cu is that at these concentrations neither variety grows well.

The effect of 5.0 ppm of Zn in the nutrient solution was observed as early as two weeks after emergence of the seedlings (Table 16).

TABLE 16. -- Plant height of Saginaw and Sanilac navy beans grown in sand culture in the greenhouse as affected by age and Zn levels.

Zn level	Age	Saginaw	Sanilac	% San. / Sag.
0.05	Two Weeks	11.7	15.1	129
5.0		9.8	7.5	77
50.0		2.7	2.8	104
0.05	Four Weeks	23.6	18.3	76
5.0		24.1	10.0	41
50.0		2.7	2.8	104
0.05	Six Weeks	36.3	20.0	55
5.0		36.9	11.3	31
50.0		2.7	2.8	104

B. Zn, Cu, Fe, and Mn

1. Differential Varietal Response in Terms of Growth. -- The micronutrients and concentrations (ppm) involved in this experiment were: Zn--0.005, 0.05, and 5.0; Cu--0.02 and 2.0; Fe--0.5 and 50.0; and Mn--0.5 and 50.0. In general, the results of the previous experiment were confirmed, although the magnitude of the differences between the two varieties at 5.0 ppm Zn was slightly less (Table 17). In addition to the effect of Fe and Cu, Mn tended to decrease the magnitude of the difference in growth between the two varieties. The overall analysis of variance data for trifoliate leaf number, height, and plant weights are in Table 2 of the Appendix.

TABLE 17. -- A comparison of growth characteristics of Saginaw and Sanilac navy beans grown under three levels of Zn in sand culture in the greenhouse.

Zn level	Growth Characteristic	Probability	Sanilac	Saginaw	% San./Sag.
0.005	Plant Ht. (in.)	<.05	21.1	37.9	55.7
0.05			16.9	38.9	43.4
5.0			4.7	14.3	32.9
0.005	Trifoliate Leaf No.	<.01	12.9	15.9	84.3
0.05			10.2	15.3	66.7
5.0			7.8	14.3	54.5
0.005	Shoot Wt. (g)	0.066	3.87	4.29	90.2
0.05			3.68	3.62	101.6
5.0			0.75	1.59	47.2
0.005	Root Wt. (g)	11.2	2.17	2.52	86.1
0.05			2.13	2.10	101.4
5.0			0.32	0.75	42.7

2. Elemental Concentration in Plant Parts of the Two Varieties. -- The concentration of Zn, Cu, Fe, and Mn in the leaves, petioles, stems, and roots averaged for the two varieties is given in Table 3 of the Appendix. Portions of these data were lost and estimates were made by comparing the ratios of the elemental content of Saginaw to Sanilac for the particular plant part in the other replicates. All summary and analysis of variance tables were made using these estimates. At the 5.0 ppm Zn level no values are given for

petioles and stems because of the stunting of Sanilac. The analyses of variance on these data are given in Appendix Table 4.

Roots accumulated much higher concentrations of the four elements than did the other plant parts, particularly at the higher concentrations of micronutrients. Leaves generally accumulated higher concentrations than petioles and stems, which accumulated about the same levels (Table 18).

Data in Table 19 shows two effects of Fe, Cu, and Mn on Zn content of the leaves of the two varieties. First, Zn content of the leaves is decreased significantly by increasing levels of other micronutrients only when the nutrient solution is high in Zn. Second, relatively lower concentrations of Zn are found in the leaves of Saginaw than Sanilac, in most cases and particularly, at the high level of Zn.

It appears that Saginaw may have a greater ability to maintain an internally balanced concentration of Zn with external fluctuations of other micronutrients. Saginaw also appears to have a more restricted movement of Zn into the leaves or less total uptake of Zn at the high Zn concentration in the nutrient solution.

Differential accumulation does not appear to account for the differential tolerance of the two varieties because both accumulate high levels of Zn. Therefore, either the two varieties differ in the

TABLE 18. -- Mean accumulation (in ppm tissue-dry weight) of each element summed over other nutrient treatments for each ion concentration, plant part, and variety with plants grown in sand culture in the greenhouse.

Element	Concentration in nutrient solution (ppm)	Saginaw				Sanilac			
		Leaf	Petiole	Stem	Root	Leaf	Petiole	Stem	Root
Zn	0.005	115	107	110	190	116	90	116	144
	0.05	90	107	104	151	101	84	102	143
	5.0	673	---	---	1837	777	---	---	1707
Fe	0.5	206	134	88	431	212	166	74	417
	50	693	99	165	4633	888	154	169	4122
Mn	0.5	127	44	51	61	141	44	49	59
	50	554	90	249	604	621	100	224	656
Cu	0.02	198	61	58	115	156	44	58	109
	2.0	216	83	129	512	200	44	103	536

distribution of intra- and extra-cellular Zn, or they have physiological processes within the cell or cell organelles which differ in their ability to withstand high levels of Zn.

TABLE 19. -- Zn content (ppm) of the Saginaw and Sanilac navy bean leaves at three levels of Zn in the nutrient solution and at two levels of Fe, Mn, and Cu when grown in sand culture in the greenhouse.

Element	Concentration (ppm)	Variety	Concentration Zn in nutrient solution		
			0.005	0.05	5.0
Fe	0.5	Saginaw	145	88	791
		Sanilac	123	109	812
	5.0	Saginaw	85	92	556
		Sanilac	109	93	741
Mn[a]	0.5	Saginaw	135	91	660
		Sanilac	119	88	929
	5.0	Saginaw	95	89	687
		Sanilac	113	113	624
Cu	0.5	Saginaw	102	91	803
		Sanilac	129	118	907
	5.0	Saginaw	129	89	544
		Sanilac	102	84	646

[a]Varietal differences statistically significant.

Zn may act two ways at the toxic level, either poisoning some of the physiological processes within the cell or interfering

with the utilization of other micronutrients. Several authors have pointed out the interference of Zn with the uptake and distribution of other micronutrients, particularly Fe (26, 61, 72, 93, 110). In most cases, Zn and also Cu act to induce severe Fe chlorosis. In the work reported here, high Fe (25 and 75 ppm) overcame the detrimental effects of Zn and Cu to a moderate extent. However, since increasing the concentration of other micronutrients in the nutrient solutions did not completely overcome the effects of high Zn (5.0 ppm) or high Cu (10.0 ppm), it is apparent that either some other nutritional factor was out of balance or the high level of these cations was toxic per se. It seems probable that there exists a cation level within plants of these two varieties below which certain cations induce deficiencies of other cations. Above this level, these particular cations would be toxic in and of themselves. The fact that Saginaw seems to be able to grow with relatively unbalanced nutrient solutions suggests that this variety can regulate, to some extent, the internal physiological nutritional status regardless of external nutrient concentration. Sanilac lacks this capacity. In addition, both varieties are affected equally when the cations reach toxic levels. From these data and other observations the dividing line between ion interference and toxicity per se seems to be around 5.0 ppm Zn.

III. Physiological Nature of the Differential Zn Deficiency and Toxicity Tolerance of the Saginaw and Sanilac Varieties of Navy Beans

A. Reciprocal Grafting--High Zn Level

To determine whether the roots or shoots were the plant parts active in determining tolerance to high levels of Zn, grafts in all possible combinations of Saginaw and Sanilac shoots and roots were made. The reciprocal grafting data show that the genotype of the scion determines tolerance to high Zn (Figure 9, Table 20). This

TABLE 20. -- Average dry weight of grafted plant shoots of Saginaw and Sanilac navy bean varieties grown in a media containing 5.0 ppm Zn in sand culture in the greenhouse.

	San./San.	San./Sag.	Sag./San.	Sag./Sag.
Average dry weight (g)	2.3	2.5	15.8	18.5
Total number of plants	6	11	5	4

does not imply that tolerance to low Zn is also determined by the genotype of the scion. At present, the relationship between the physiological mechanism(s) involved in differential tolerance at the two levels of Zn has not been established.

FIGURE 9. -- Scion/root grafted navy bean plants (Saginaw and Sanilac) grown at 5.0 ppm Zn in the greenhouse.

FIGURE 9. -- Continued.

The difference in tolerance to high Zn is not due to differential Zn accumulation. Although Sanilac had more Zn in the shoots than Saginaw, both varieties accumulated a high level (Table 21).

TABLE 21. -- Concentration of Zn and Cu in reciprocal root-scion grafted plants of Saginaw and Sanilac navy beans grown at 5.0 ppm Zn in sand culture in the greenhouse.

Graft	Plant part	Zn (ppm)	%[b]	Cu (ppm)	%[b]
San./San. (6)[a]	Leaves	818	19	22	22
	Stems	1148	27	33	33
	Roots	2312	54	44	45
	Total	4278		99	
San./Sag. (10)	Leaves	803	17	32	25
	Stems	841	18	35	27
	Roots	3038	65	62	48
	Total	4682		129	
Sag./San. (7)	Leaves	653	13	23	28
	Stems	573	11	18	22
	Roots	3909	76	42	51
	Total	5135		83	
Sag./Sag. (4)	Leaves	595	17	24	41
	Stems	405	12	12	20
	Roots	2424	71	23	39
	Total	3424		59	

[a]Number of plants.

[b]Percent derived from elemental concentration in each plant part given in the body of the table and not total accumulation and distribution.

Thus, some factor other than differential content of Zn in the shoots must determine tolerance or sensitivity to high Zn. Nevertheless, this factor(s) will have to be located in the shoot.

B. Quantity and Zn Concentration of Exudate

The intent of this experiment was to determine whether or not the stems of Saginaw and Sanilac retained Zn to the same degree. If the stem retains Zn throughout its length, exudate collected from a short stem and a long stem should have different amounts of Zn. If Saginaw and Sanilac stems differ in their capacity to retain Zn, this should be detected when comparing exudate from plants with short (3-4 inches) stems and long (20-30 inches) stems.

In most cases, it was possible to collect sufficient exudate for analysis within the five hours. However, in a number of instances this was not possible. In a few cases, this was due to inadvertently kinking the stem while bending it for exudate collection. Occasionally exudate leaked from the cut surface where the petiole had been removed from the stem even though the surface had been smeared with vaseline. In a few pots, none of the plants within the pot produced sufficient exudate for analysis. Estimates of missing values were made by averaging replicates for that time period; and the other time period samples for the particular plant.

The values for exudate weight, Zn concentration, and total Zn are given in Table 5 of the Appendix. Variety and cutting height were significantly different for all three variables.

The specific effect of cutting height on the two varieties when summing over all other effects is given in Table 22.

TABLE 22. -- The effect of cutting stem at 2-3 inches (short) or 18-25 inches (long) on the Zn content of the exudate obtained from Saginaw and Sanilac navy beans (averaged over six 5-hour collection periods).

Variety	Stem length	Weight of exudate (g)	Concentration of Zn of exudate ppm	Total Zn ppm x g
Saginaw	Short	1.35	0.71	1.20
Saginaw	Long	1.23	0.58	0.85
Sanilac	Short	0.73	0.45	0.49
Sanilac	Long	0.35	0.29	0.10

Weight of exudate and Zn concentration of this exudate were greater in Saginaw than Sanilac at either stem length. The difference in exudate weights suggests that either Saginaw has a larger

or more efficient root system which is capable of taking up greater quantities of water, or the xylem system of Saginaw has a greater capacity for movement of sap. There may also be a difference in osmotic pressure within the stelar tissue of plants of the two varieties. Due to the limited nature of the data, it is not possible to decide whether the root or stem is more important in determining the difference in exudate weight.

The volume of exudate delivered at a specific length may be a very complex phenomenon, affected by total cross-sectional area, the diameter of the individual vessels (which would affect the total amount of wall area and thus the amount of resistance to sap flow), and flow rate. The contribution these various factors make in determining the total volume of exudate in the two varieties is unknown. However, the difference in volume or weight of exudate obtained from the short stems and the long stems may be largely due to a difference in total cross-sectional area of the xylem at high versus low cutting height.

The difference in Zn concentration of Saginaw and Sanilac exudate indicates that either Saginaw has greater ability to absorb Zn from the medium or a greater capacity to translocate Zn from the root system into the vascular system.

The stems, apparently, retain Zn to some degree since the Zn content of the exudate decreased with increased stem length. The fact that there is a greater proportional reduction in the concentration of Zn in Sanilac exudate than in Saginaw suggests that there is a greater capacity for retention in stems of Sanilac.

This difference may be due to the greater Zn concentration in the sap of Saginaw than Sanilac. If a much higher amount of Zn is transferred into the vascular system of Saginaw, stem retention would be nearer full capacity than that of Sanilac and proportional binding would be less. In this case, the absolute binding capacity of the stem might have been the same in the two varieties, but the difference in Zn transferred into the vascular tissue could give the impression of differential binding.

The effect of pretreatment and treatment on the two varieties is given in Table 6 of the Appendix. Pretreatment had the more significant effect on the Zn content and exudate weight of the two varieties. Increasing pretreatment and treatment Zn levels increased Zn concentration and exudate weight.

C. Tracer Studies

The primary goal of the tracer experiments was to determine if there were any detectable differences between the Saginaw

and Sanilac navy bean varieties with respect to uptake and distribution of ^{65}Zn. For this reason, most of the studies were conducted from the standpoint of a comparison of Saginaw with Sanilac. These studies, in general, will be reported as a series of small experiments.

1. Uptake and Distribution. -- Plants were grown, as outlined in the methods section, and then placed into nutrient solution containing ^{65}Zn for 48 hours. The plants were removed and washed, and then dissected into leaves, upper stems, lower stems, and roots. There were four plants of each variety. A summary of the data is given in Table 23.

TABLE 23. -- Mean values for ^{65}Zn uptake and distribution by Saginaw and Sanilac navy beans for 48 hours exposure.

Plant part	Saginaw		Sanilac	
	cpm	% of total	cpm	% of total
Leaves	100	38	48	27
Upper stem	17	6	20	11
Lower stem	27	10	28	15
Roots	119	45	86	47
Total	263		182	

Varieties, plant parts, and their interaction were statistically significant. There was a real difference between the two varieties with regard to total uptake and distribution of ^{65}Zn on a cpm

basis. Saginaw took up more Zn (263 vs. 182 cpm) and translocated higher percentages of the Zn into the leaves (38 vs. 27%) than Sanilac. Differences existed between the two varieties when the cpm data were adjusted for tissue weight, but were not significant. When adjusted for weight of root only, Saginaw was far superior to Sanilac with respect to uptake and translocation of ^{65}Zn into the leaves.

2. Time Course #1 and #2. -- The previous experiment indicated that there was a difference in the distribution of ^{65}Zn in plants of the two varieties at the time the experiment was terminated. The time-course experiments were designed to establish the rate of uptake and distribution over a time period.

In the initial experiment, four plants of each variety were removed from the ^{65}Zn nutrient solution at 6, 12, 24, 48, 96, and 192 hours. Plants were removed at 48, 192, and 288 hours in the second experiment. In addition, the petioles were analyzed separately from the leaf blade and radioautographs were made of both varieties at all three time periods in the second experiment.

These radioautographs indicated that there were no striking qualitative differences in distribution pattern of Zn between the two varieties, although Saginaw did appear to have more ^{65}Zn in the smaller veins. The radioautographs also indicated a higher concentration of ^{65}Zn in the pulvinus regions of the petioles (Figure 10).

FIGURE 10. -- Radioautograph of Saginaw navy bean plant (Sanilac pattern the same) exposed to ^{65}Zn for 288 hours.

The region of heavy labeling could have been due to greater deposition of isotope or possibly due to the configuration of the xylem vessels in this region. According to Doutt (32), the vascular tissues are compressed in this region. Such a compression of label-containing tissue could give the appearance of greater concentration of label.

The data for the initial experiment are summarized in Table 24.

These data confirm that the Saginaw variety translocates a higher proportion of ^{65}Zn into the leaves than does

TABLE 24. --Uptake and distribution of ^{65}Zn by Saginaw and Sanilac navy bean varieties for six different time periods on a cpm basis.

Time (hours)	Plant part	Saginaw		Sanilac	
		cpm	% Distribution	cpm	% Distribution
6	Leaves	64	6	30	3
	Upper stem	22	2	21	2
	Lower stem	38	4	30	3
	Root	910	88	824	91
	Total	1034		905	
12	Leaves	283	16	149	13
	Upper stem	51	3	31	3
	Lower stem	80	5	57	4
	Root	1326	76	912	80
	Total	1740		912	
24	Leaves	353	21	327	18
	Upper stem	49	3	61	3
	Lower stem	80	5	101	5
	Root	1233	72	1354	73
	Total	1715		1843	
48	Leaves	490	29	650	22
	Upper stem	70	4	95	3
	Lower stem	99	6	103	4
	Root	1019	61	2086	71
	Total	1678		2934	

TABLE 24. -- Continued.

Time (hours)	Plant part	Saginaw		Sanilac	
		cpm	% Distribution	cpm	% Distribution
96	Leaves	1210	24	1689	21
	Upper stem	133	3	215	3
	Lower stem	202	4	298	4
	Roots	3452	69	5814	72
	Total	4997		8016	
192	Leaves	5666	32	5381	26
	Upper stem	460	3	550	3
	Lower stem	455	3	547	3
	Roots	10919	62	14413	69
	Total	17500		20891	

Sanilac. The difference in distribution of Zn between the two varieties becomes more apparent when one compares the ratios of ^{65}Zn contained within the various plant parts of the two varieties. The ratios in Table 25 denote the greater capacity of Saginaw to translocate ^{65}Zn into the leaves. From these ratios, it appears that the greater proportion of ^{65}Zn contained in the leaves of Saginaw results from a greater release or movement of Zn out of the roots and stems than in the case of Sanilac.

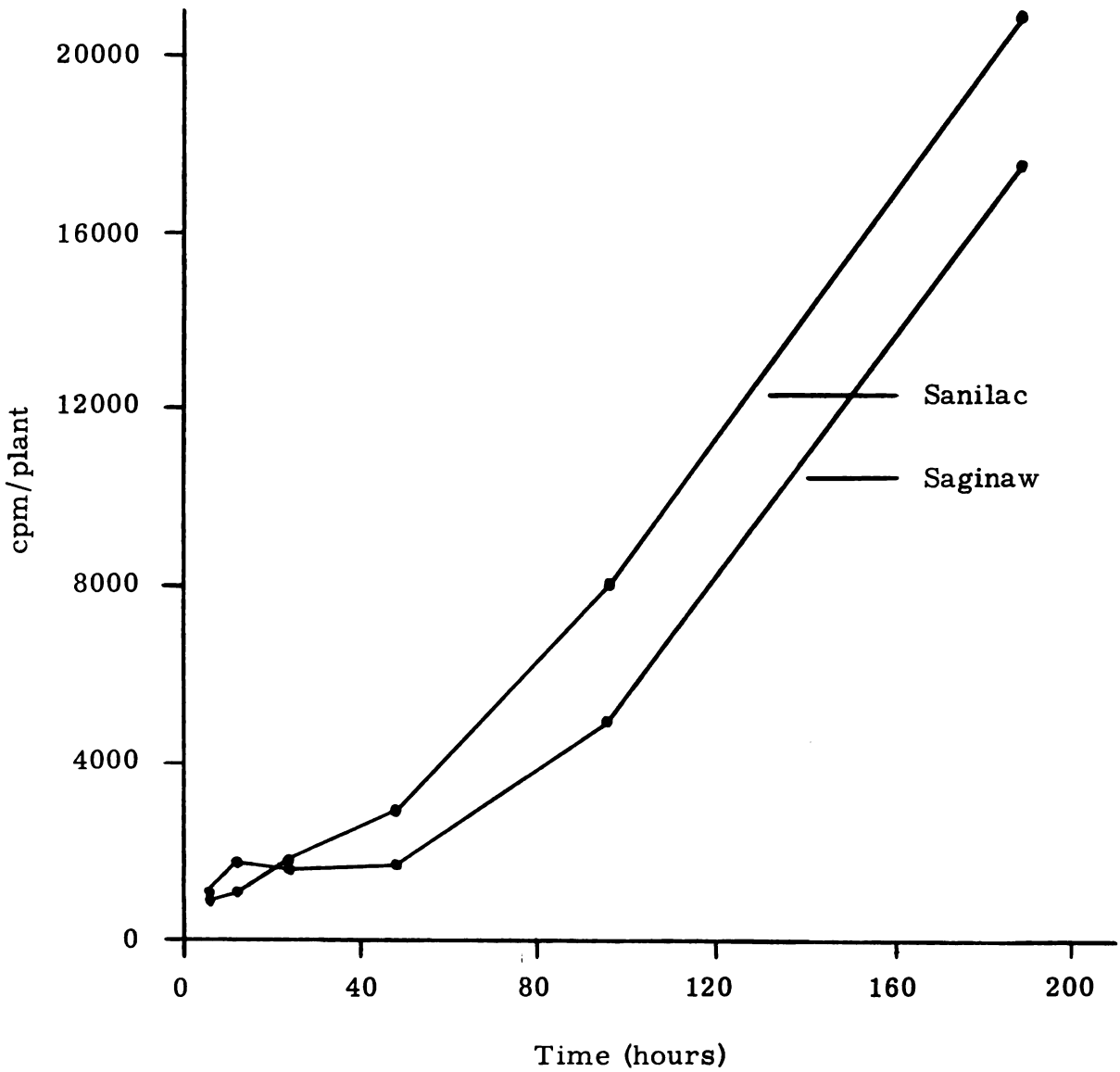
TABLE 25. -- Ratio of ^{65}Zn distributed within plant parts of Saginaw and Sanilac navy beans averaged over all time periods of exposure.

	Saginaw		Sanilac	
	cpm	cpm/g tissue	cpm	cpm/g tissue
Leaves/Stems + Roots	.29	.15	.22	.13
Leaves/Stems	3.13	.50	2.64	.48
Leaves + Stems/Roots	.42	.64	.32	.54

As opposed to the initial radioisotope study, in both time-course experiments, Sanilac absorbed more Zn than Saginaw. This is illustrated for time-course experiment 1 in Figure 11. The exposure time in the initial experiment, however, was only for about 48 hours. As can be seen in Figure 11, Saginaw takes up ^{65}Zn faster initially for about 24 hours then there is a lag period for another 24 hours, while Sanilac takes up ^{65}Zn at a slower rate, initially, but does not have a lag period. Apparently, the plants in the initial experiment were removed prior to the shift in the uptake rates of the two varieties. From 96 hours to 192 hours the rate of uptake in the two varieties is essentially the same.

In general, the data presented here have been from the first time course experiment, which ran from 6 to 192 hours. The data from the second, which ran for 48 to 288 hours, have not been

FIGURE 11. -- A comparison between Saginaw and Sanilac navy beans of uptake rate of ^{65}Zn.



presented, but the patterns of distribution and uptake in the two varieties are essentially the same.

3. Zn Concentration: Pretreatment and Treatment. -- Two experiments were conducted to determine whether there is a differential effect of concentration of Zn upon Zn uptake in the two varieties. In both experiments, plants were grown in sand culture until 12 days old. In the first experiment, 0.05 ppm Zn was used during this 12 day period. In the second, 5.0 ppm Zn was included. At the end of this period, the plants were removed and placed in nutrient solutions containing 0.0005, 0.05, and 5.0 ppm Zn for 24 hours. ^{65}Zn was added to each nutrient solution at an equivalent dilution of radioactive Zn to cold Zn.

A significant varietal response was obtained only when radioisotope activity was expressed on a root weight basis. In this instance, the concentration by variety interaction was significant. The effect of Zn concentration on the two varieties can be seen in Table 26. Greater differences may not have appeared due to the short exposure time to ^{65}Zn.

The difference in radioisotope activity of the plant material at the two pretreatment levels is partially due to differences in the amount of ^{65}Zn label added. Though there may be differences in the amount of ^{65}Zn taken up at the two pretreatment levels, these

differences cannot be detected due to the differences in radioisotope activity of the treating solutions. Comparisons between varieties and treatment levels within a specific pretreatment are valid, however.

TABLE 26. -- ^{65}Zn content expressed as cpm/g root in Saginaw and Sanilac navy beans grown under a normal (.05 ppm) and high (5.0 ppm) level of Zn prior to treatment as affected by Zn level during treatment (averaged over plant parts).

	Concentration of Zn ppm	cpm/g root weight	
		Saginaw	Sanilac
Normal pretreatment	0.0005	328	352
	0.05	296	299
	5.0	2011	2646
High pretreatment	0.0005	195	201
	0.05	146	270
	5.0	200	633

In the high Zn treatment, uptake of ^{65}Zn was significantly greater in Sanilac than Saginaw at both pretreatment levels. Pretreatment with high Zn magnified this difference, which indicates that high Zn pretreatment in some manner preconditioned plants of the Sanilac variety so that these plants absorbed greater quantities of Zn.

There were relatively small differences between the distribution of Zn in the two varieties; however, Saginaw usually translocated equal or higher proportions of ^{65}Zn into the leaves (Table 27). The increase in percent of Zn accumulated in the leaves with increasing Zn concentration in the nutrient solution may indicate a certain retention capacity in the roots and stems which becomes more and more saturated as Zn absorption by the roots increases. As root and stem retention capacity are approached, higher levels of Zn are translocated to the top.

Although Saginaw and Sanilac differ little in percent distribution of ^{65}Zn, total Zn accumulated in the plant parts differs markedly due to the greater uptake of Zn by Sanilac.

4. Other Studies. -- Several experiments were conducted on micronutrient interaction, hydrogen ion concentration and the effect of root removal. The micronutrient interaction studies were inconclusive, but offered further evidence that Sanilac absorbs greater quantities of Zn than Saginaw, yet transfers a smaller proportion of this Zn to the shoots of the plants.

The hydrogen ion experiment also indicated that Sanilac absorbs greater quantities of Zn than Saginaw. However, increasing amounts of ^{65}Zn were accumulated with increasing hydrogen ion concentration in both varieties (Table 28).

TABLE 27. -- The effect of pretreatment and Zn concentration on total uptake and distribution of ^{65}Zn by Saginaw and Sanilac navy bean varieties.

Variety	Zn level in Pretreatment ppm	Zn level in Treatment ppm	Total uptake cpm/g root	Percent distribution				
				Leaves	Petiole	Upper stem	Lower stem	Root
Saginaw	0.05	5.0	10053	23	12	18	29	19
Sanilac			13229	27	11	15	30	17
Saginaw		0.05	1482	28	17	17	16	21
Sanilac			1495	28	12	20	19	22
Saginaw		0.0005	1640	21	22	18	20	19
Sanilac			1760	18	18	22	18	23
Saginaw	5.0	5.0	1002	30	12	15	19	24
Sanilac			3162	29	13	12	19	27
Saginaw		0.05	727	22	22	16	20	20
Sanilac			1349	21	15	22	19	23
Saginaw		0.005	976	20	22	21	15	21
Sanilac			1006	18	23	21	20	18

TABLE 28. -- The effect of hydrogen ion concentration on ^{65}Zn uptake by Saginaw and Sanilac navy beans (96 hours).

pH	cpm/g tissue	
	Saginaw	Sanilac
5.0	4788	5256
6.0	2326	3956
7.0	2822	3943
8.0	370	424

The results of the root removal experiment were also inconclusive, but indicated the trend of greater translocation of Zn in Saginaw but less total uptake.

GENERAL DISCUSSION

A genetic study was conducted at low Zn levels based on the frequency of appearance and severity of Zn deficiency symptoms. A limited genetic study was conducted at toxic or high levels of Zn. Information obtained on the basis of visual symptoms may not accurately reflect the fundamental genetic control responsible for the differential appearance of those symptoms because symptoms *per se* are not primary events and may be affected by the ontological processes leading to the deficiency lesion, as well as by other characteristics such as maturity, drought tolerance, etc., which are also under genetic control. Nevertheless, the initial genetic information on differential Zn utilization should be useful in developing more specific experiments. Hopefully, when the physiological mechanism responsible for the differential Zn response of Saginaw and Sanilac plants has been determined, a genetic study can be conducted in detail at that level. Such an investigation should confirm or reject the conclusions of this study which were based on the appearance or lack of visual symptoms of Zn deficiency or toxicity.

It is clear from the literature (83, 84, 107, 111) that all the genetic work on differential varietal response to nutrients has been based on appearance of gross visual symptoms. In fact, the only differential response in which very much is known with respect to mechanism is with soybeans and Fe (14). Information regarding the mechanism is relatively recent, yet the genetic study by Weiss (111) was conducted in 1943 on the differential ability of this PI selection and the variety Hawkeye to grow under Fe stress as indicated by visual symptoms. Although no attempt has been made to correlate the genetic information gained in 1943 with recent physiological information, studies with isolines reveal that the genetic situation at the physiological level may be more complex than the early conclusions indicated (22).

The genetic investigations reported in this thesis have proven fruitful, though inconclusive. The "partition" method used to estimate the degree of overlap between phenotypes has proven valuable in trying to interpret the data. Although the specific details of the genetic model cannot be defined until more data become available, the information presently at hand indicates that very few gene pairs (2-3) regulate the differential response to low Zn. The two-gene, complementary factor model appears to be a

close approximation to the real genetic situation. Future data should clarify this situation.

Because a few genes appear to be involved in determining tolerance to low Zn, they should have major effects on the mechanism responsible. For this reason, the physiological differences between tolerant and sensitive plants should be rather discrete (as opposed to the behavior under polygenic control). Due to these discrete differences, the identification of the mechanism(s) responsible for the differential sensitivity should be possible, though perhaps difficult. A genetic study of this mechanism(s) also should be possible.

Initially when Sanilac was observed to develop chlorosis in a particular nutrient solution while Saginaw did not, the question was raised concerning the generality of the mechanism responsible for the differential tolerance of Saginaw and Sanilac under low Zn. Do these varieties differ only in degree of utilization of Zn or is the control mechanism(s) concerned with the utilization of other elements also? Two micronutrient experiments indicated that Saginaw has a greater ability than Sanilac to withstand extreme micronutrient unbalance (Figures 7 and 8). This suggested that a common physiological or anatomical mechanism(s) is responsible for the differential response of Saginaw and Sanilac plants under various micronutrient stress conditions.

The genetic investigation of the differential response to high Zn, as exhibited by stunting, was undertaken to determine whether the same physiological mechanism(s) is operative both at low and high Zn levels. If the same genetic model were applicable in both instances, this would constitute supporting evidence (though not proof) that a common mechanism is involved. Since inconclusive genetic data were obtained, it was not possible to answer this question (page 55). Even though the genetic models may be different at low and high levels of Zn, this does not imply that the mechanisms responsible for the differential response in the two varieties are not related. A mechanism at one level of Zn could be a component of a more complex mechanism which operates at the other level. This question cannot be answered until the mechanism responsible for the differential response of the two varieties at both levels of Zn is determined.

Although the specific mechanism(s) has not been identified, some of the characteristics of this mechanism(s) have been established. The grafting experiment established that shoots of the plants determine the tolerance of Saginaw to high Zn conditions (Figure 9). Grafting experiments at low Zn levels have not been successful.

One of the most persistent differences between the two varieties was the differential distribution of Zn in them. Saginaw

generally had a higher proportion of Zn in the shoots of the plants at low or normal Zn levels. This was first observed with limited data (unpublished) obtained from the two varieties grown on a low Zn soil in 1964. A summary of the data for Cu and Zn content is given in Table 29. Generally, total content of Zn or Cu was similar, but the shoots of Saginaw contained a higher percentage than Sanilac.

TABLE 29. -- Cu and Zn content (ppm) of two bean varieties grown in a low Zn soil (Saginaw County) in 1964 as determined by flame spectrophotometry.

	Saginaw				Sanilac			
	Roots		Leaves		Roots		Leaves	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
Zn →	13	24	41	41	26	44	24	28
	37		82		70		52	
	119				122			
Cu →	23.2	12	70.9	63.4	94.2	63.6	35.0	10.2
	35.2		134.3		158.2		45.2	
	169.5				203.4			

A stem exudate study (Table 22) established that Saginaw transfers greater quantities of sap which contain higher concentrations

of Zn than Sanilac. This difference in sap volume possibly could account for the greater movement of Zn into the leaves of Saginaw plants if Zn moved by a mass flow mechanism which is a function of sap-flow rate (4). Almost all of the radioisotope work has indicated that a higher percentage of radioactive Zn is transferred into the shoots of Saginaw than Sanilac. Time-course experiments indicated that Saginaw had a higher proportion of ^{65}Zn in the leaves than Sanilac (Table 24). The major proportion of this difference appeared to be due to greater transport out of the root, and to a slight degree, less binding of Zn by the stems of Saginaw (Table 25).

It is apparent that Saginaw definitely translocates more of the Zn taken up by the root into the aerial portion of the plant than Sanilac does. This difference in translocation of Zn may be related to the tolerance of Saginaw to low Zn conditions.

While Saginaw generally translocated a higher proportion of Zn to the leaves than Sanilac, there is ample evidence that Sanilac under some conditions took up more Zn than Saginaw (Table 24). When the Zn in the nutrient solution is increased, Sanilac takes up a greater quantity of Zn (Table 28). The difference in uptake between the varieties is magnified by growing the plants at initially high Zn concentrations (5.0 ppm), (Table 27). This indicates that the sensitivity of Sanilac at the 5.0 ppm level of Zn may be due to a much greater

uptake of Zn by the roots. Although reciprocal grafts indicated that the shoots of the plants determine tolerance or sensitivity to high Zn, mineral analysis of grafted plants indicated that root uptake may be affected by the upper portion of the plant (Table 21).

The difference in tolerance of the two varieties to high Zn levels could also be due to a difference in the ratio of extracellular to intracellular Zn within the leaves. ^{65}Zn uptake studies (unpublished) utilizing isolated leaf cells (67) have suggested that there may be a significant difference between membrane properties within leaf cells of the two varieties. Table 30 illustrates the greatly enhanced level of Zn uptake by cells of Sanilac from plants grown at 5.0 ppm Zn for one month.

TABLE 30. -- ^{65}Zn uptake in four hours by leaf cells isolated enzymatically from two navy bean varieties grown at two levels of Zn.

Variety	Zn level (ppm)	mM Zn/mg cells (dry weight)
Sanilac	0.05	0.57[a]
Sanilac	5.0	9.94
Saginaw	0.05	0.64
Saginaw	5.0	0.69

[a]Average of four replications.

Little physiological work has been done with these two varieties grown at low or deficient levels of Zn due to the difficulty of eliminating Zn contamination with the present facilities. Nevertheless, uptake and distribution of Zn by Saginaw and Sanilac plants grown at normal (.05 ppm) and high (5.0 ppm) Zn concentrations differ markedly. Also, there may be a great difference in distribution at low levels of Zn.

A summary of the physiological differences in Zn utilization between Saginaw and Sanilac is given in Table 31. Although some of the differences between the two varieties noted appeared small, they could be significant because most of the experiments were conducted over a relatively short period of time. In the field or greenhouse, small differences between the two varieties may be greatly magnified with increasing time.

I. Models

These differences can be used as an aid in postulating physiological models which could account for the differential tolerance of Saginaw and Sanilac at the two levels of Zn. Either a single mechanism or possibly different mechanisms may be responsible for tolerance at low and high Zn levels. Models for both possibilities will be proposed.

TABLE 31. -- A comparison of several physiological characteristics between the Saginaw and Sanilac varieties of navy beans with respect to Zn utilization.

Characteristic	Reaction		Reference
	Saginaw	Sanilac	
1. Distribution of Zn	more in leaves	less in leaves	Table 25, 27, 29
2. Cellular uptake at high Zn levels	restricted	not restricted	Table 30
3. Preconditioning of plant for high Zn uptake	low	high	Table 26, 27
4. Uptake of Zn at normal levels	lower	higher	Table 24, 26, 27, 28 Figure 11
5. Exudate volume	high	low	Table 22
6. Exudate concentration	high	low	Table 22
7. Elemental composition in micronutrient and grafting experiments (especially with high Zn)	high	higher	Table 18, 19, 21
8. Grafting	shoots tolerant	shoots sensitive	Figure 9, Table 20

MODEL I. -- Differential Membrane Permeability.

Conditions: 1. Same mechanism for resistance of Saginaw at high and low levels of Zn.
2. Same genetic control.

Characteristics:

1. Greater membrane permeability in Sanilac going from outside to inside (plasma-lemma and tonoplast) throughout the whole plant.
2. Less leakage from membranes of Sanilac (inside to outside).
3. No difference in anatomy or morphology of the two varieties.

Consequences of Model:

	Property	Saginaw	Sanilac
1.	Vascular and cytoplasmic accumulation	low	high
2.	Total Zn uptake	lower	higher
3.	Release of Zn into stelar tissue from symplasm (30, 73)	high	low
4.	Water movement into xylem (if membrane properties similar for other ions as for Zn)	high	low

End Result:

1. At low Zn levels — Sanilac has the capacity of taking up more Zn but transports much less to the top of the plant because a lower percentage of Zn is released into the xylem due to greater cellular accumulation of Zn in the roots and possibly stem. Under low levels of Zn availability, Zn uptake is low in both genotypes, but the plants of the Saginaw variety have the capacity to mobilize more Zn to the portions of the plant where there is a requirement for this ion.
2. At high Zn levels — Much greater uptake of Zn and greater cellular accumulation by Sanilac. Due to the high external concentration, relatively high levels of Zn are transported via the xylem. Due to the more limited permeability of the cells of the Saginaw variety, toxic levels of Zn do not build up within the cells of Saginaw, but do in Sanilac.

MODEL II. -- Changing Membrane Permeability.

Conditions:
1. Same mechanism acting differently at low and high levels of Zn.
2. Same genetic control.

Characteristics:

1. Membrane of Saginaw moderately permeable and relatively constant over a broad range of Zn levels.

2. The membranes of Sanilac have limited permeability at low levels of Zn. As Zn concentration increases, membrane permeability increases greatly.

Consequences:

	Property	Saginaw	Sanilac
1.	Total Zn uptake fluctuates	little	greatly
2.	Cellular accumulation of Zn fluctuates	little	greatly

End Result:

1. At low Zn levels — Saginaw plants probably take up greater quantities of Zn due to greater permeability of cortical cell membranes at low Zn concentrations. This uptake would be into the cytoplasm and movement of greater quantities of Zn into the stelar tissue would occur. Thus there results the greater ability of Saginaw to mobilize Zn into plant parts under conditions of Zn stress.

2. At high Zn levels — Zn uptake and movement into the shoots of the plants is much greater in Sanilac than Saginaw due to increased permeability of membranes within the root cells. In addition, much higher levels of Zn accumulate intracellularly in the leaves of Sanilac than in Saginaw due to the greater permeability of the membranes in the leaf cells. Higher levels of Zn build up inside the cells of Sanilac than in Saginaw due to greater uptake by cells of the roots and leaves.

MODEL III. -- Translocation and Membrane Permeability.

Conditions:
1. Two different mechanisms involved at low and high levels of Zn.
2. Two different genetic control systems.

Characteristics:

1. Limited capacity of Sanilac to transfer Zn to shoots of the plants.

2. Limited membrane transport of leaf cells of Saginaw.

Consequences:

	Property	Saginaw	Sanilac
1.	Total Zn uptake	adequate	adequate
2.	Zn transfer	adequate	inadequate at low concentrations
3.	Leaf cell accumulation of Zn	adequate, but restricted at high Zn concentrations	not restricted

End Result:

1. At low Zn levels — Both varieties take up sufficient Zn, but due to the limited capacity of Sanilac to transport Zn, only Saginaw grows at a normal or near normal level. Sanilac plants suffer from Zn deficiency.

2. At high Zn levels — Although Sanilac plants restrict Zn transport to a certain extent, toxic levels of Zn still reach the shoots of the plants. Due to high permeability of leaf cells of Sanilac, toxic levels of Zn accumulate. Saginaw plants restrict the accumulation of Zn in the leaf cells and are not affected to the same degree as Sanilac plants by the high level of Zn.

Three models are suggested by the data obtained in these studies. There may be other models which are closer to the actual mechanism(s). However, a discussion of the models presented may be useful in more clearly defining the problem and in leading to more critical experiments.

The observation that Saginaw transfers higher percentages of Zn to the shoot is in agreement with Model I. In addition, greater membrane permeability of Sanilac may account for the greater uptake of Zn that was observed in the radioactive and mineral analysis work. If there is less leakage from the symplasm, there would be lower concentrations of Zn in the exudate of Sanilac (as observed). These factors would all impart differential tolerance to low Zn conditions.

The model suggests that high Zn tolerance is located in the shoot (as was indicated by the grafting experiments). It also indicates the possibility of high Zn accumulation by Sanilac plants, as external concentration is increased, due to the higher membrane permeability. This was observed in the radioisotope studies (Tables 26 and 27).

Model II is based on the assumption that membrane permeability to Zn in Sanilac increases with increasing external Zn concentration. This could be accounted for by changes in membrane permeability to Zn per se or to increased numbers of carrier

sites or an increased rate of turnover of the carrier-Zn complex.

Zn uptake by Saginaw does not exhibit fluctuation in uptake and accumulation of Zn with external Zn concentration, at least not to the same extent as Sanilac.

If overall permeability of the cells increases in Sanilac with increasing concentrations of Zn or, perhaps, other ions, Sanilac may be subject to micronutrient unbalances while Saginaw is not (Table 29). Sanilac would also be susceptible to high external levels of Zn which may result in accumulation of very toxic concentrations of Zn in Sanilac cells but not Saginaw (Table 30).

The third model is based on the premise that there is a specific mechanism responsible for the differential reaction of the two varieties at low levels of Zn and another mechanism at high levels of Zn and that these two mechanisms are under different genetic control. The characteristics of this model include limited transport of Zn ions to the tops of Sanilac plants. Such a limited transport could be due to slower movement of sap due to less cross-sectional area of xylem, more restricted end walls in tracheids and vessels, greater restriction to movement at nodal areas, and perhaps other factors which provide greater opportunity for immobilization of Zn in root and stem tissue. Plants treated with a low amount of radioactivity

for 12 hours revealed that nodes do constitute a barrier to xylem transport of Zn (Figure 12). In addition to or instead of restricted

FIGURE 12. -- Radioautograph of Saginaw navy bean (Sanilac the same) exposed to ^{65}Zn for 12 hours.

movement of sap, root and/or stem cells of Sanilac may also possess greater binding capacity. Either possibility would decrease net movement of Zn to the shoots of Sanilac plants and could be responsible for the differential reaction of Saginaw and Sanilac at low Zn.

At high Zn levels, leaf cells of Saginaw either do not accumulate toxic levels of Zn or these cells are capable of carrying out normal metabolic functions in the presence of higher Zn levels than cells of Sanilac.

Although there may be other factors involved in the differential reaction of the two varieties, differential root uptake seems to be eliminated. Sanilac takes up nearly as much or more Zn than Saginaw at normal levels, and the genotype of the shoots has been shown to be the determining factor at toxic levels of Zn (Table 31). Differential rates of movement within the symplasm have not been discussed, but could possibly account for the greater accumulation of Zn by the roots of the Sanilac variety. Also, differential metabolism and differential accumulation of Zn within the cell organelles could be involved. In addition, the properties of the sap of the two varieties may be altered by the differential production of organic acids (19, 57). In this instance, there may be great differences between the two varieties in solubility of Zn in the sap at various levels of external Zn. None of these have been investigated in this study.

II. Gene Action

Models have been presented which explain the behavior of the two varieties of beans at low and high Zn concentrations. Regardless of the physiological and genetic models which ultimately prove correct, the question of the nature of the genetic control must be raised. It has been concluded from this investigation that at least two gene pairs interact to impart the tolerance to low Zn levels

observed in Saginaw plants. These genes either govern critical enzymatic steps in a common biosynthetic pathway leading to an end product responsible for low Zn tolerance or produce different polypeptide chains which combine to form an active enzyme which directly or indirectly confers tolerance. Another alternative is that the various genes act to produce different non-related enzymes. In this instance, the interaction of phenotypic characters which result from separate genes is necessary for tolerance.

The first two possibilities involve biochemical interaction to produce a tolerant phenotype, while the last alternative involves "gene-product" interaction to produce the tolerant phenotype. The mode of action of the genes in the first instance would give either tolerant or sensitive plants. However, if both genes are responsible for separate phenotypic characters, (as in the final alternative above), each or one of the character(s) may be responsible for a small amount of tolerance to low Zn. Under conditions of slight deficiency, these single genes for tolerance may be expressed. This may be the source of some of the confusion with the 1966 genetic data which were obtained when Zn deficiency conditions were not very severe.

III. Plant Breeding

The first prerequisite in any plant breeding program is the establishment of precise, realizable goals. With respect to

utilization of nutrients, goals have not been well established. Indeed, there has been little work on which characteristics are or are not desirable. Perhaps this is due to a lack of knowledge concerning the complexities of nutrient utilization. To oversimplify the total concept, nutrient utilization can be thought of as consisting of uptake, transport, and intracellular utilization. Ideally these three components can be brought under genetic control and manipulated so that plants may have an ability to withstand micronutrient unbalances at toxic and deficient ends of the scale. At the same time, these plants should have the ability to utilize efficiently higher quantities of nutrient to produce greater yields than they are now able to do. Simply put, the goals the plant breeder should establish are (1) a general adaptability with a high buffer capacity and (2) a specific ability to utilize high levels of nutrients when such are available.

It may not be possible to develop varieties that meet both of these specifications. Overall tolerance to micronutrient balance implies that a plant is able to maintain a favorable internal ionic relationship when the external relationship is unfavorable. Thus higher levels of nutrients and other ions do not affect these plants. For this reason, plants may not be able to utilize high levels of fertilizers. The plant breeder may have to decide whether he desires a variety with a wide range of adaptability to nutrient levels or whether he wants a variety capable of utilizing high levels of fertilizer.

With respect to a breeding program for Zn utilization, decisions regarding goals will have to depend upon the nature of the tolerance of Saginaw to low and high Zn. Certainly tolerance to low Zn levels, and ability to maintain balance in the presence of external ion unbalance, would seem to be desirable. However, the ability to withstand high levels of Zn may not be desirable. Judy et al. (66) have shown that Sanilac responds to additional Zn fertilization (in the intermediate range), as indicated by seed yield to a greater extent than Saginaw. Such a response may be related to the sensitivity of this variety to the toxic-high level of Zn. If this were the case, such a sensitivity would be desirable because toxic levels of Zn are rarely encountered in the field and the plants could utilize higher levels of Zn fertilization for higher production. Saginaw, on the other hand, due to the mechanism that conditions its ability to withstand extreme levels of Zn, is limited in its ability to utilize moderately higher levels of Zn.

The actual mechanism involved will be of significance in the formulation of the goals of the program. If there is only one mechanism at both Zn levels, with the same genetic control, the decision will have to be made whether to breed for tolerance to low Zn or for greater yield response to high Zn fertilization.

If there are two different mechanisms, it will be possible to obtain plants with low Zn tolerance and response to high Zn

fertilization. It will be necessary to select for recombinant plants having the tolerance to low Zn of Saginaw and the sensitivity to high Zn of Sanilac.

SUMMARY AND CONCLUSIONS

The differential ability of the Saginaw and Sanilac navy beans to withstand Zn stress was investigated by genetic, micronutrient balance, and physiologic experimentation. The conclusions from these investigations are summarized in the following paragraphs.

I. Genetic

A. Low Zn

Populations of F_1's, F_2's, F_3's, and backcrosses of Saginaw x Sanilac when grown on low Zn soil (Saginaw County) in 1964 and 1966 responded differentially to Zn stress. Individual plants were scored on a 1 to 5 scale on the basis of visual Zn deficiency symptoms. Due to overlap of Saginaw and Sanilac populations in several classes, the segregating generations were partitioned into tolerant and sensitive genotypes based on the performance of Saginaw and Sanilac, respectively.

The F_1 generation indicated that tolerance to low Zn was phenotypically dominant. Tolerance appeared to be mediated by two genes which gave a ratio of nine tolerant to seven sensitive in the F_2

generation. The F_3 generation was not entirely in agreement with the 9:7 ratio as there were more tolerant families than predicted. The backcross to Sanilac population was not conclusive, and more tolerant plants than expected were observed.

It was concluded that the number of genes determining tolerance to low Zn is low, probably two. These genes apparently interact to give a phenotypic ratio of 9:7 (tolerant to sensitive) in the F_2 generation. However, this ratio may not be inviolate. These genes may govern the synthesis of various product combinations which impart various degrees of tolerance to low Zn. Depending upon available Zn and the threshold level within the plant, these combinations may interact to produce an array of phenotypic ratios. The 9:7 ratio may be an average of this array. The F_3 population may have a different threshold level due to a different level of background homozygosity.

B. High Zn

In addition to differential tolerance of Saginaw and Sanilac navy beans to low Zn levels, a differential response to 5.0 ppm Zn (100 × normal) was observed in the greenhouse. It was not possible to establish the number of genes or gene action for this trait. The F_1 generation indicated that sensitivity was dominant or that there

was no dominance. The F_2 population distribution was characteristic of dominance for tolerance, or possibly indicated partial dominance. A ratio of 9 tolerant to 7 sensitive plants was observed in the F_2 which, if true, may indicate that tolerance to low and high Zn levels is mediated by the same genetic control system.

II. Micronutrient Balance Studies--Zn, Cu, Fe, and Mn

The effect of various levels of Zn, Cu, Fe, and Mn upon the growth of Saginaw and Sanilac navy beans in sand culture in the greenhouse was studied. In addition, the accumulation of these four elements in plant parts was determined. Zn at 5.0 ppm caused marked stunting of Sanilac, whereas growth of Saginaw was little affected. This differential growth did not seem to be related to differential uptake since both varieties accumulated high levels of Zn.

Cu induced a differential Fe-chlorosis which could be corrected by adding Fe. Sanilac was more sensitive to Cu in this respect than Saginaw. It was postulated that the effect of low and high Zn and high Cu may be due to the same physiological or anatomical mechanism.

III. Physiological Studies

A. Low Zn

Exudate experiments established that Saginaw transferred greater volumes of plant sap during a 30-hour period than Sanilac. In addition, the Zn concentration in the exudate was approximately three times greater in Saginaw. Whether this difference in Zn concentration was due to greater Zn uptake by Saginaw roots or to greater transfer of Zn into the vascular tissue is not known.

Radioisotope studies established that Sanilac plants normally take up higher levels of Zn but transfer small proportions of this Zn to the top of the plant. This indicates that the high concentration of Zn in the exudate of Saginaw is due to greater release of Zn into the vascular tissue.

B. High Zn

Reciprocal scion/root grafts of Saginaw and Sanilac grown in high Zn established that tolerance to this level of Zn is mediated by the genotype of the scion. Differential accumulation did not account for the differential tolerance of low Zn. Unpublished data, reported in the discussion, indicated that tolerance at high Zn may be due to a difference in the distribution of extra- and intra-cellular Zn in the

two varieties. Isolated leaf cells of Sanilac took up much more Zn at high external concentrations than did Saginaw.

IV. Discussion

Three models were presented as possible mechanisms for the differential tolerance of the two varieties of navy beans. The first two models involved differential membrane or cellular properties which account for differential release of Zn into the vascular tissue and also accumulation of different levels of Zn within the cell. On the basis of these two models it was postulated that the same basic mechanism was responsible for the observed responses at high and low Zn. The third model was characterized by differential stem and/or root binding which limited the availability of Zn to Sanilac at low external concentrations. At high Zn levels, the cells of Sanilac were postulated as being more permeable than Saginaw and thus more liable to accumulate toxic levels of Zn while the cells of Saginaw are not.

Gene action and possible breeding programs for nutrient utilization were discussed.

LIST OF REFERENCES

1. Adams, M. W. 1967. Personal communication.

2. Anderson, Axel L., E. E. Down, and Gerald Whitford. 1960. The Sanilac pea bean: Its history, development and characteristics. Mich. Agr. Exp. Sta. Quart. Bull. 43: 214-236.

3. Beadle, G. W. 1929. Yellow stripe: A factor for chlorophyll deficiency in maize located in the Pr pr chromosomes. Amer. Naturalist 63: 189-192.

4. Bell, C. W., and O. Biddulph. 1963. Transport of calcium. Exchange versus mass flow. Plant Physiol. 38: 610-614.

5. Bell, W. D., L. Bogorad, and W. J. McIllrath. 1962. Yellow-stripe phenotype in maize: 1. Botan. Gazette 124: 1-8.

6. Bell, W. C., L. Bogorad, and W. J. McIllrath. 1958. Response of the yellow stripe maize mutant (ys1) to ferrous and ferric ion. Botan. Gazette 120:36-39.

7. Berger, Kermit C. 1962. Micronutrient deficiencies in the U. S. Jour. of Agric. and Food Chem. 10: 178-181.

8. Biddulph, O. 1953. Translocation of radioactive mineral nutrients in plants. Kansas Agr. Exp. Sta. Rpt. 4: 48-58.

9. Biddulph, O., Susann Biddulph, R. Cory, and H. Koontz. 1958. Circulation patterns for phosphorus, sulfer, and calcium in the bean plant. Plant Physiol. 33:293-299.

10. Bingham, Frank T., and James P. Martin. 1956. Effects of soil phosphorus on growth and minor element nutrition of citrus. Soil Sci. Soc. Amer. Proc. 20: 382-385.

11. Bingham, F. T., and M. J. Garber. 1960. Solubility and availability of micronutrients in relation to phosphorus fertilization. Soil Sci. Soc. Amer. Proc. 24: 209-213.

12. Boawn, Louis C. 1965. Zinc concentration in hop leaves in relation to adequate and inadequate levels of zinc nutrition. Agron. Jour. 57: 523-524.

13. Boawn, Louis C., Frank G. Viets, Jr., and Carl L. Crawford. 1954. Effect of phosphate fertilizer on zinc nutrition of field beans. Soil Sci. 78: 1-7.

14. Brown, J. C. 1967. Differential uptake of Fe and Ca by two corn genotypes. Soil Sci. 103: 331-338.

15. Brown, J. C. 1966. Fe and Ca uptake as related to root-sap and stem exudate citrate in soybeans. Physiol. Plantarum. 19: 968-976.

16. Brown, J. C. 1963. Iron chlorosis in soybeans as related to the genotype of root stock: 4. Sorption and translocation as 2 separate phases of iron nutrition. Soil Sci. 96: 387-394.

17. Brown, J. C., R. S. Holmes, and L. O. Tiffin. 1961. Iron chlorosis in soybeans as related to the genotype of root stalk: 3. Chlorosis susceptibility and reductive capacity at the root. Soil Sci. 91: 127-132.

18. Brown, J. C., R. S. Holmes, and L. O. Tiffin. 1958. Iron chlorosis in soybeans as related to the genotype of root-stock. Soil Sci. 86: 75-82.

19. Brown, J. C., and L. O. Tiffin. 1965. Iron stress as related to the iron and citrate occurring in the stem exudate. Plant Physiol. 40: 395-400.

20. Brown, J. C., and L. O. Tiffin. 1962. Zinc deficiency and iron chlorosis dependent on the plant species and nutrient elements in Tulare Clay. Agron. Jour. 54: 356-358.

21. Brown, J. C., L. O. Tiffin, and R. S. Holmes. 1960. Competition between chelating agents and roots as factor affecting absorption of iron and other ions by plant species. Plant Physiol. 35: 878-886.

22. Brown, J. C., C. R. Weber, and B. E. Caldwell. 1967. Efficient and inefficient use of iron by two soybean genotypes and their isolines. Agron. Jour. 59: 459-462.

23. Burleson, C. A., A. D. Dacus, and C. J. Gerard. 1961. The effect of phosphorus fertilization on the zinc nutrition of several irrigated crops. Soil Sci. Soc. Amer. Proc. 25: 365-368.

24. Butler, G. W., P. C. Barclay, and A. C. Glenday. 1962. Genetic and environmental differences in the mineral composition of ryegrass herbage. Plant and Soil. 16: 214-228.

25. Camp, A. F. 1945. Zinc as a nutrient in plant growth. Soil Sci. 60: 157-164.

26. Chapman, H. D., G. F. Liebig, Jr., and A. P. Vanselow. 1939. Some nutritional relationships as revealed by a study of mineral deficiency and excess symptoms on citrus. Soil Sci. Soc. Amer. Proc. 4: 196-200.

27. Chesters, C. G. C., and G. N. Robinson. 1951. The role of zinc in plant metabolism. Biol. Rev. 26: 239-252.

28. Collander, Runar. 1941. Selective absorption of cations by higher plants. Plant Physiol. 16: 691-720.

29. Cooil, Bruce J., Rollo K. Le Da Fuente, and Ramon S. De La Pena. 1965. Absorption and transport of sodium and potassium in squash. Plant Physiol. 40: 625-632.

30. Crafts, A. S. 1961. Translocation in plants. Holt, Rinehart, and Winston. New York.

31. DeRemer, E. Dale, and R. L. Smith. 1964. A preliminary study on the nature of a zinc deficiency in field beans as determined by radioactive zinc. Agron. Jour. 56: 67-70.

32. Doutt, Margaret T. 1932. Anatomy of Phaseolus vulgaris L. var. Black Valentine. Technical Bulletin No. 128, Michigan State College, Agricultural Experiment Station.

33. Ellis, Boyd G. 1965. Response and susceptibility: In "Zinc Deficiency--a symposium." Crops and Soils. 17: 10-13.

34. Ellis, Roscoe, Jr., J. F. Davis, and D. L. Thurlow. 1964. Zinc availability in calcareous Michigan soils as influenced by phosphorus levels and temperature. Soil Sci. 28: 83-86.

35. Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Plant physiol. 36: 437-444.

36. Epstein, Emanuel, D. W. Rains, and O. E. Elzam. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. Proc. Natl. Acad. Sci. 49: 684-692.

37. Epstein, Emanuel, and R. L. Jefferies. 1964. The genetic basis of selective ion transport in plants. Ann. Rev. Plant Physiol. 15: 169-184.

38. Findenegg, G., and E. Broda. 1965. Mechanisms of uptake of trace elements by plant roots. Nature. 208: 196-197.

39. Foy, C. D., W. H. Armiger, A. L. Fleming, and C. F. Lewis. 1967. Differential tolerance of cotton varieties to an acid soil high in exchangeable aluminum. Agron. Jour. 59: 415-418.

40. Foy, C. D., and J. C. Brown. 1964. Toxic factors in acid soils: II. Differential aluminum tolerance in plant species. Soil Sci. Soc. Amer. Proc. 28: 27-32.

41. Foote, Beverly D., and Robert W. Howell. 1964. Phosphorus tolerance and sensitivity of soybeans as related to uptake and translocation. Plant Physiol. 40: 610-613.

42. Fuerhing, H. D. 1960. Interrelationships of the trace elements zinc, boron, iron, manganese, and copper on the growth and composition of corn. Ph.D. Thesis, University of Nebraska.

43. Fuering, H. O., and G. S. Soofi. 1964. Nutrition of corn on a calcareous soil: II. Effect of Zn on the yields of grain and stover in relation to other micronutrients. Soil Sci. 28: 79-82.

44. Giordano, P. M., J. J. Mortnedt, and R. I. Papendich. 1966. Response of corn (Zea mays L.) to zinc, as affected by placement and nitrogen source. Soil Sci. Soc. Amer. Proc. 30: 767-770.

45. Gerloff, G. C. 1963. Comparative mineral nutrition of plants. Ann. Rev. Plant Physiol. 14: 107-124.

46. Gorsline, G. W., J. W. Ragland, and W. I. Thomas. 1961. Evidence for inheritance of differential accumulation of calcium, magnesium, and potassium by maize. Crop Sci. 1: 155-156.

47. Gorsline, G. W., W. I. Thomas, and D. E. Baker. 1964. Inheritance of P, K, Mg, Cu, B, Zn, Mn, Al, and Fe concentration by corn (Zea mays L.) leaves and grain. Crop Sci. 4: 207-210.

48. Gorsline, G. W., W. I. Thomas, D. E. Baker, and J. L. Ragland. 1964. Relationships of strontium-calcium accumulation within corn. Crop Sci. 4: 154-156.

49. Gouch, Hugh G. 1957. Mineral nutrition of plants. Ann. Rev. Plant Physiol. 8: 31-64.

50. Grossenbacher, K. A. 1939. Autonomic cycle of rate of exudation of plants. Amer. Jour. Botany. 26: 107-109.

51. Grimes, D. L., L. C. Boawn, C. W. Carlson, and F. G. Viets, Jr. 1961. Zinc deficiency of corn and potatoes as related to soil and plant analysis. Agron. Jour. 53: 68-71.

52. Hanson, J. B. 1967. Roots--selecters of plant nutrients. Plant Food Review 13: 8-10.

53. Harvey, Paul H. 1939. Hereditary variation in plant nutrition. Genetics 24: 437-461.

54. Hewitt, E. J. 1963. The essential nutrient elements: Requirements and interactions in plants. In F. C. Steward, Ed. Plant Physiology--A Treatise. Vol. III. Inorganic Nutrition of Plants. Pp. 137-360. Academic Press. New York.

55. Hewitt, E. J. 1951. The role of mineral elements in plant nutrition. Ann. Rev. Plant Physiol. 2: 25-52.

56. Hewitt, E. J. 1948. Relation of manganese and some other metals to the iron status of plants. Nature 161: 489-490.

57. Hiatt, A. J. 1967. Relationship of cell sap pH to organic acid change during ion uptake. Plant Physiol. 42: 294-298.

58. Hiatt, A. J., and H. F. Massey. 1958. Zinc levels in relation to zinc content and growth of corn. Agron. Jour. 50: 22-24.

59. Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Bull. 347.

60. Howell, R. W. 1954. Phosphorous nitrition of soybeans. Plant Physiol. 29: 477-483.

61. Hunter, J. G., and O. Vergnano. 1953. Trace element toxicities in oat plants. Ann. Appl. Biol. 40: 761-777.

62. Jacobsen, Louis, Raymond J. Hannapel, David P. Moore, and Michail Schaedle. 1961. Calcium influence on ion absorption. Plant Physiol. 39: 58-61.

63. Jacoby, B. 1964. Function of bean roots and stems in sodium retention. Plant Physiol. 39: 445-449.

64. Johnson, W. J., and W. G. Schrenk. 1964. Nature of zinc containing substances in the alfalfa plant cell. Jour. of Agric. and Food Chem. 12: 210.

65. Judy, W. H. 1967. Zinc availability from soil applied zinc sulfate and zinc EDTA. Ph.D. Thesis, Michigan State University.

66. Judy, W., J. Melton, G. Lessman, B. Ellis, and J. Davis. Field and laboratory studies with zinc fertilization of pea beans, corn, and sugar beets in 1964. M.S.U. Expt. Sta. Res. Rpt. 33. Farm Science.

67. Jyung, W. H., Sylvan H. Wittwer, and Martin J. Bukovac. 1965. Ion uptake by cells enzymatically isolated from green tobacco leaves. Plant Physiol. 40: 410-414.

68. Jyung, W. H., S. H. Wittwer, and M. J. Bukovac. 1965. Ion uptake and protein synthesis in enzymatically isolated plant cells. Nature 205: 921-922.

69. Kannan, Sheshadri. 1966. Mechanisms of foliar absorption in higher plants with special reference to iron. Ph.D. Thesis, Michigan State University.

70. Kleese, R. A. 1967. Relative importance of stem and root in determining genetypic differences in Sr-89 and Ca-45 accumulation in soybeans (Glycine max L.). Crop Sci. 7: 53-55.

71. Lessman, Gary Max. 1967. Zinc-phosphorus interactions in Phaseolus vulgaris. Ph.D. Thesis, Michigan State University.

72. Lingle, John C., Lee O. Tiffin, and John C. Brown. 1963. Iron-uptake-transport of soybeans as influenced by other cations. Plant Physiol. 38: 71-76.

73. Luttge, Ulrich, and George G. Laties. 1966. Dual mechanism of ion absorption in relation to long distance transport in plants. Plant Physiol. 41: 1531-1539.

74. MacLeod, L. B., and L. P. Jackson. 1967. Aluminum tolerance of two barley varieties in nutrient solution, peat, and soil culture. Agron. Jour. 59: 359-363.

75. Martin, W. E., J. G McLean, and James Quick. 1965. Effect of temperature on the occurrence of phosphorus induced zinc deficiency. Soil Sci. Soc. Amer. Proc. 29: 411-413.

76. Massey, H. F., and Frank A Loeffel. 1967. Factors in inter-strain variation in zinc content of maize (Zea mays L.) kernels. Agron. Jour. 59: 214-217.

77. Massey, H. F., and Frank H. Loeffel. 1966. Variation in zinc content of grain from inbred lines of corn. Agron. Jour. 58: 143-144.

78. Millikan, C. R. 1953. Relative effects of zinc and copper deficiencies on lucerne and subterranean clover. Aust. Jour. Biol. Sci. 6: 164-177.

79. Nelson, Lyle E. 1956. Response of soybeans grown in the greenhouse to zinc applied to a black belt soil. Soil Sci. 82: 271-274.

80. Pardee, A. B. 1966. Purification and properties of a sulfate-binding protein from Salmonella typhimurium. Jour. Biol. Chem. 241: 5886.

81. Pardee, A. B., L. S. Prestidge, M. B. Whipple, and J. Dreyfuss. 1966. A binding site for sulfate and its relation to sulfate transport into Salmonella typhimurium. Jour. Biol. Chem. 241: 3962.

82. Pinkas, Leonard L. H., and Lawrence H. Smith. 1966. Physiological basis of differential strontium accumulation in two barley genotypes. Plant Physiol. 41: 1471-1475.

83. Pope, Daniel T., and Henry M. Munger. 1953. Heredity and nutrition in relation to magnesium-deficiency chlorosis in celery. Proc. Amer. Soc. Hort. Sci. 61: 472-480.

84. Pope, Daniel T., and Henry M. Munger. 1953. The inheritance of susceptibility to boron deficiency in celery. Proc. Amer. Soc. Hort. Sci. 61: 481-486.

85. Powers, LeRoy, and L. F. Locke. 1950. Partitioning method of genetic analysis applied to quantitative characters of tomato crosses. U.S.D.A. Technical Bulletin No. 998.

86. Rasmussen, D. C., L. H. Smith, and R. A. Kleese. 1964. Inheritance of SR^{89} accumulation in wheat and barley. Crop Sci. 4: 586-589.

87. Rasmussen, D. C., L. H. Smith, and W. M. Meyers. 1963. Effect of genotype on accumulation of Sr^{89} in barley and wheat. Crop Sci. 3: 34-37.

88. Rediske, J. H., and O. Biddulph. 1953. The absorption and translocation of iron. Plant Physiol. 28: 576-593.

89. Rosell, Ramon A., and Albert Ulrich. 1964. Critical zinc concentration and leaf minerals of sugar beet plants. Soil Sci. 97: 152-167.

90. Sang, James H. 1964. Penetrance, expressivity, and thresholds. Jour. Hered. 49: 143-150.

91. Schmid, Walter E., and Gerald C. Gerloff. 1961. A naturally occurring chelate of iron in xylem exudate. Plant Physiol. 36: 226-231.

92. Seatz, L. F., A. J. Steiger, and J. C. Kramer. 1959. Crop response to zinc fertilization as influenced by lime and phosphorus applications. Agron. Jour. 51: 457-459.

93. Smith, Paul F., and Alston W. Specht. 1953. Heavy metal nutrition and iron chlorosis of citrus seedlings. Plant Physiol. 28: 371-382.

94. Smith, Stuart N. 1934. Response of inbred lives and crosses in maize to variation of nitrogen and phosphorus supplied as nutrients. Jour. Am. Soc. Agron. 26: 785-804.

95. Sommer, Anna L. 1928. Further evidence of the essential nature of zinc for the growth of higher green plants. Plant Physiol. 3: 217-221.

96. Steenbjerg, F. 1951. Yield curves and chemical plant analyses. Plant and Soil 3: 97-109.

97. Stukenholtz, D. D., R. J. Olsen, Gerald Gogan, and R. A. Olson. 1966. On the mechanism of phosphorus zinc interaction in corn nutrition. Soil Sci. Soc. Amer. Proc. 30: 759-763.

98. Thorne, Wynne. 1957. Zinc deficiency and its control. Advances in Agron. IX: 31-65.

99. Tiffin, L. O. 1963. Plant acids and iron translocation. Plant Physiol. 38: p. ix.

100. Tiffin, L. O. 1966. Iron translocation. 1. Plant culture, exudate sampling, iron-citrate analysis. Plant Physiol. 41: 510-514.

101. Tiffin, L. O., and J. C. Brown. 1962. Iron chelates in soybean exudate. Science 135: 311-313.

102. Tiffin, L. O., J. C. Brown, and R. S. Holmes. 1960. Chelating agent and plant nutrient interactions affecting the iron nutrition of soybeans. Soil Sci. Soc. Am. Proc. 24: 120-123.

103. Tsui, Cheng. 1948. The role of zinc in auxin synthesis in the tomato plant. Amer. Jour. Botany 35: 172-179.

104. Viets, F. G., Jr. 1961. Zinc deficiency of field and vegetable crops in the western U. S. U. S. D. A. Leaflet 495.

105. Viets, F. G., Jr., L. C. Boawn, and C. L. Crawford. 1954. Zinc content of bean plants in relation to deficiency symptoms and yield. Plant Physiol. 29: 76-79.

106. Viets, F. G., Jr., L. C. Boawn, and C. L. Crawford. 1954. Zinc content and deficiency symptoms of 26 crops grown on a Zn-deficient soil. Soil Sci. 78: 305-316.

107. Vose, P. B. 1963. Varietal differences in plant nutrition. Herbage Abstracts 33: 1-13.

108. Wallace, A., R. A. Jeffreys, and V. Q. Hale. 1962. Differential ability of two soybean varieties to take up Fe^{59} from soil added to a nutrient solution. Soil Sci. 94: 111-114.

109. Ward, R. C., E. J. Langin, R. A. Olsen, and D. D. Stuckenholtz. 1963. Factors responsible for poor response of corn and grain sorghum to phosphorus fertilization: III. Effect of soil compaction and moisture levels and other properties on phosphorus zinc relations. Soil Sci. Soc. Amer. Proc. 27: 326-330.

110. Watanabe, F. S., W. L. Lindsay, and S. R. Olsen. 1965. Nutrient balance involving phosphorus, iron, and zinc. Soil Sci. Soc. Amer. Proc. 29: 562-565.

111. Weiss, Martin G. 1943. Inheritance of physiology of efficiency in iron utilization in soybeans. Genetics. 28: 253-268.

112. Wood, J. G., and Pamela M. Sibly. 1950. The distribution of zinc in oat plants. Australian Jour. Sci. Res. B3: 14-27.

APPENDIX

TABLE 1. -- Analysis of variance for plant height, number of trifoliate leaves, and plant weight for Sanilac and Saginaw navy beans in micronutrient interaction experiment number one.

Source of variance	Degrees of freedom	Mean squares	
		Plant height	No. trif.
Zn	2	14457.5946**	2636.8580**
Cu	2	4931.2613**	411.5617**
Zn x Cu	4	1949.6440	130.8641**
Fe	2	536.2119	111.7839**
Zn x Fe	4	197.5390	56.2901*
Cu x Fe	4	127.3076	6.6234
Zn x Cu x Fe	8	153.2597	13.6712
Error A	54	341.1872	17.1378
Variety (Var)	1	5373.3662**	300.2551**
Var x Zn	2	1998.5205**	88.4711**
Var x Cu	2	1479.6687**	45.9032**
Var x Zn x Cu	4	672.2304**	26.6748**
Var x Fe	2	435.5699**	18.3971*
Var x Zn x Fe	4	107.0390	14.1131*
Var x Cu x Fe	4	82.3631	9.0637
Var x Zn x Cu x Fe	8	124.4758*	9.7659*
Date	2	4069.0884**	593.2283*
Date x Var	2	2074.7551**	36.0020*
Date x Zn x Var	4	534.4465**	12.3477*
Date x Cu x Var	4	309.1779**	5.2576
Date x Zn x Cu x Var	8	96.1934	1.9032
Date x Fe x Var	4	41.4773	2.5051
Date x Zn x Fe x Var	8	13.8029	0.9758
Date x Cu x Fe x Var	8	11.5853	1.7042
Date x Zn x Cu x Fe x Var	16	10.8253	1.1262
Error B	322	58.7189	4.9095
Total	485		

Level of Significance: ** = <.01
* = .01 - .05

TABLE 1. -- Continued.

Source of variance	Degrees of freedom	Mean squares	
		Weight tops	Weight roots
Zn	2	147.5604**	40.0385**
Cu	2	62.4965**	8.2790**
Zn x Cu	4	17.2563**	2.6503**
Fe	2	7.3119	1.6605
Zn x Fe	4	1.6900	0.4804
Cu x Fe	4	2.3339	0.2762
Zn x Cu x Fe	8	1.2065	0.1216
Error A	54	2.9127	0.6298
Variety (Var)	1	43.8672**	15.3704**
Var x Zn	2	21.9579**	6.3054**
Var x Cu	2	7.3451**	1.8819**
Var x Zn x Cu	4	5.3759**	1.4424**
Var x Fe	2	5.9335**	1.6278*
Var x Zn x Fe	4	1.5570	0.7153
Var x Cu x Fe	4	1.6748	0.4287
Var x Zn x Cu x Fe	8	0.9545	0.4989
Error B	54	0.7688	0.3519
Total	161		

Level of Significance: ** = <.01
* = .01 - .05

TABLE 2(a). -- Analysis of variance of plant height, number of trifoliate leaves, and plant weights for Sanilac and Saginaw navy beans in micronutrient interaction experiment number two.

Source of variance	Degrees of freedom	Mean squares			
		Dry leaves	Dry roots	No. trif.	Height
Zn	**2**	118.3792**	**46.6669****	**135.0069***	5922.2500**
Fe	**1**	5.6406	**0.6669**	**8.0277**	1.7777
Zn x Fe	**2**	7.2552*	**0.9552**	**24.4652**	212.6944
Mn	1	4.8767	4.2025*	144.0000*	720.0277*
Zn x Mn	2	0.5134	1.6608	1.2708	152.1111
Fe x Mn	1	0.1167	0.2025	16.0000	9.0000
Zn x Fe x Mn	2	2.4529	3.1008*	8.3125	40.0833
Cu	1	7.7006*	1.0000	0.6944	240.2500
Zn x Cu	2	4.8939	1.6758	1.5486	540.7500
Fe x Cu	1	4.5156	1.5211	1.3611	136.1111
Zn x Fe x Cu	2	2.8952	1.4044	28.4236	168.6944
Mn x Cu	1	1.0850	0.1111	93.4444*	261.3611
Zn x Mn x Cu	2	0.1384	0.1169	6.7569	36.1944
Fe x Mn x Cu	1	4.4450	1.3611	106.7777*	113.7777
Zn x Fe x Mn x Cu	2	10.7888*	4.4744*	80.2152*	954.1111*
Error A	48	2.7484	1.2115	32.4305	241.0763
Variety (Var)	1	5.8000*	2.3002	841.0000**	9409.0000**
Var x Zn	2	2.4575*	0.6786	36.2708**	460.3333*
Var x Fe	1	0.2100	1.8225	11.1111	0.6944
Var x Zn x Fe	2	0.2046	0.8258	0.9236	17.6944
Var x Mn	1	0.7656	0.1736	90.2500**	693.4444*
Var x Zn x Mn	1	0.1814	0.7436	11.3125	25.8611
Var x Fe x Mn	1	1.1917	1.1736	30.2500*	14.6944
Var x Zn x Fe x Mn	2	0.3904	0.3586	20.2708*	218.3611
Var x Cu	1	0.4556	0.3600	1.0000	2.7777
Var x Zn x Cu	2	0.6231	0.8933	7.8958	136.6944
Var x Fe x Cu	1	0.0000	0.5877	0.4444	156.2500
Var x Zn x Fe x Cu	2	3.6354*	0.0719	2.1319	171.0000
Var x Mn x Cu	1	0.6267	0.1877	72.2500**	2.7777
Var x Zn x Mn x Cu	2	2.0867*	0.4477	3.9375	108.6944
Var x Fe x Mn x Cu	1	0.5017	0.0044	30.2500*	96.6944
Var x Zn x Fe x Mn x Cu	2	0.5154	0.2002	5.1458	173.6944
Error B	48	0.8540	0.8789	6.2638	102.8541
Total	143				

Level of Significance: * = .05 - .10

TABLE 2(b). -- Analysis of variance of elemental accumulation in various plant parts of Saginaw and Sanilac navy beans in micronutrient interaction experiment number two.

Source of variance	Degrees of freedom	Mean squares			
		Leaves			
		Mn	Cu	Zn	Fe
Zn	2	12498.4	189821.7**	6146996.2**	1248595.6**
Fe	1	3383.3	724.5	153860.0**	12184172.0**
Zn x Fe	2	19479.6	22038.5*	72532.6*	894994.8**
Mn	1	7402027.1**	58282.0**	91657.5*	110833.5
Zn x Mn	2	8132.8	5517.8	74993.3**	210419.9
Fe x Mn	1	11772.2	4879.3	57081.1•	16362.6
Zn x Fe x Mn	2	59286.8•	8380.1	38994.1	240298.0
Cu	1	72720.1•	34689.0*	310156.1**	647086.1•
Zn x Cu	2	16965.4	5502.2	253412.5**	124819.4
Fe x Cu	1	367.3	98648.3**	17800.0	647086.1
Zn x Fe x Cu	2	11629.0	8670.7	32179.9	153493.3
Mn x Cu	1	22801.0	20952.5	12155.0	11502.5
Zn x Mn x Cu	2	915.4	4005.3	15420.4	151833.8
Fe x Mn x Cu	1	3344.6	30537.5*	10353.0	164497.8
Zn x Fe x Mn x Cu	2	903.4	6195.5	22000.0	216571.0
Error A	46 (48)	21945.4	5494.1	19755.4	170200.3
Variety (Var)	1	59211.1**	29498.0**	52326.5*	363910.5*
Zn x Var	2	22396.5•	1432.5	38340.1*	385419.3*
Fe x Var	1	2224.6	5.8	36131.6•	325185.0*
Zn x Fe x Var	2	744.0	2199.5	26251.7•	210972.5•
Mn x Var	1	25706.7•	2508.3	73125.1•	50887.8
Zn x Mn x Var	2	28110.0*	3700.0	132095.7**	145619.8
Fe x Mn x Var	1	633.3	3.0	28420.3•	8265.8
Zn x Fe x Mn x Var	2	6862.2	2965.7	39954.5*	42871.5
Cu x Var	1	37636.0*	5890.5•	7906.1	101.6
Zn x Cu x Var	2	240.2	469.0	1988.3	11149.2
Fe x Cu x Var	1	2970.2	1813.3	91556.6**	14460.0
Zn x Fe x Cu x Var	2	44659.0**	5925.3•	146338.3**	37545.7
Mn x Cu x Var	1	28561.0•	1437.6	112616.1**	9168.0
Zn x Mn x Cu x Var	2	5961.0	2369.1	49748.4*	26670.0
Fe x Mn x Cu x Var	1	6058.0	39.0	166.8	885.0
Zn x Fe x Mn x Cu x Var	2	18589.1•	15360.2	3130.3•	9943.1
Error B	39 (48)	771.6	2452.1	11148.6	16637.1
Total	143				

TABLE 2(b). -- Continued.

Source of variance	Degrees of freedom	Mean squares			
		Petiole			
		Mn	Cu	Zn	Fe
Zn	1	356.5	22.0	270.0	1007.5
Fe	1	2330.5	104.2	32230.0**	44850.3**
Zn x Fe	1	546.2	84.4	5355.1	437.8
Mn	1	62883.8**	425.0	894.3	75.3
Zn x Mn	1	882.0	1944.0	19866.3**	7.6
Fe x Mn	1	0.5	2.0	6256.5•	4718.0
Zn x Fe x Mn	1	6.5	8.2	5535.8	110.5
Cu	1	3444.0*	2948.2	3965.5	356.5
Zn x Cu	1	16971.7	287.0	1625.3	65.0
Fe x Cu	1	90.1	1040.2	7021.3•	225.1
Zn x Fe x Cu	1	3589.3*	852.0	189.8	8456.3*
Mn x Cu	1	263.3	2301.0	364.3	23.0
Zn x Mn x Cu	1	55.5	988.2	27034.6**	2007.5
Fe x Mn x Cu	1	3516.3*	9720.4**	4718.0	102.1
Zn x Fe x Mn x Cu	1	297.5	204.2	635.5	5937.7•
Error A	28 (32)	694.1	1197.1	2113.9	1850.8
Variety (Var)	1	635.5•	18760.0**	9420.8*	13324.5**
Zn x Var	1	1935.0*	8140.2*	250.3	8493.8*
Fe x Var	1	137.8	18.4	404.3	2871.1•
Zn x Fe x Var	1	94.0	2.7	4360.5•	2330.5•
Mn x Var	1	656.3	28.2	16354.3**	14.3
Zn x Mn x Var	1	1225.5•	805.0	364.3	918.8
Fe x Mn x Var	1	128.3	150.0	1239.8	1512.1
Zn x Fe x Mn x Var	1	380.0	100.0	3255.0	3.0
Cu x Var	1	7193.3**	2795.0•	123.8	7543.8*
Zn x Cu x Var	1	250.3	2.7	1020.5	1725.5
Fe x Cu x Var	1	1113.8•	2262.0	263.3	106.3
Zn x Fe x Cu x Var	1	1725.5•	3850.7	3231.8•	6032.5*
Mn x Cu x Var	1	2593.8*	2.7	9821.3*	446.3
Zn x Mn x Cu x Var	1	4.6	40.0	742.6	2849.3•
Fe x Mn x Cu x Var	1	3026.3*	10922.7*	82.5	356.5
Zn x Fe x Mn x Cu x Var	1	0.8	5673.4•	1020.5	1560.1
Error B	16 (32)	395.6	1470.7	1681.4	1200.6
Total	75 (95)				

TABLE 2(b). -- Continued.

Source of variance	Degrees of freedom	Mean squares			
		Stem			
		Mn	Cu	Z_n	Fe
Zn	1	16907.0*	656.3	2242.7	8.2
Fe	1	27608.1*	1155.1	23688.2	178365.0**
Zn x Fe	1	18040.2*	17631.3	8740.2	170.7
Mn	1	833282.7**	3687.8	2.0	3725.0
Zn x Mn	1	5460.2	28119.3	4959.4	13.5
Fe x Mn	1	15657.0*	22478.8	1365.0	10542.0*
Zn x Fe x Mn	1	6176.0	106.3	3528.4	1633.5
Cu	1	1426.0	80562.1*	7848.2	13395.4*
Zn x Cu	1	0.1	1708.6	522.7	54.0
Fe x Cu	1	4320.2	15.8	228.2	459.4
Zn x Fe x Cu	1	20533.5*	27169.0	13160.2	1504.2
Mn x Cu	1	2053.5	1418.3	1751.0	2882.0
Zn x Mn x Cu	1	3952.7	26433.8	3105.4	1014.0
Fe x Mn x Cu	1	16801.0*	38841.3	570.4	1650.0
Zn x Fe x Mn x Cu	1	6305.0	14.3	630.4	216.0
Error A	31 (32)	4755.0	13868.0	9188.6	1958.8
Variety (Var)	1	4482.7*	4121.3	112.7	486.0
Zn x Var	1	18040.2**	0.5	368.2	247.0
Fe x Var	1	165.4	61.8	150.0	2016.7
Zn x Fe x Var	1	1335.0	2552.3	5642.7	408.4
Mn x Var	1	2970.4*	304.6	247.0	216.0
Zn x Mn x Var	1	15352.0**	8683.0*	828.4	247.0
Fe x Mn x Var	1	73.5	4523.8	135.4	1504.2
Zn x Fe x Mn x Var	1	2773.5	1811.2	22.0	1305.4
Cu x Var	1	9922.7**	4360.5	11528.2*	170.7
Zn x Cu x Var	1	3901.5*	3914.3	600.0	35.0
Fe x Cu x Var	1	12650.0**	834.3	793.5	24.0
Zn x Fe x Cu x Var	1	1552.0	2490.8	3504.2	30.4
Mn x Cu x Var	1	12285.4**	5843.8	3151.0	66.7
Zn x Mn x Cu x Var	1	3337.0*	2156.5	672.0	1785.4
Fe x Mn x Cu x Var	1	7210.6**	3372.5	6240.4	368.2
Zn x Fe x Mn x Cu x Var	1	1472.7	1211.3	8400.0	315.4
Error B	22 (32)	669.3	2614.6	3007.7	1130.8

TABLE 2(b). -- Continued.

Source of variance	Degrees of freedom	Mean squares			
		Roots			
		Mn	Cu	Zn	Fe
Zn	2	122707.8	420406.5**	41734489.6**	56371364.0**
Fe	1	396900.0*	926245.8**	2021610.0**	56260393.3**
Zn x Fe	2	217915.1*	51384.0	1222872.6**	4951362.0**
Mn	1	11697540.0**	49173.0	26082.2	5961736.1
Zn x Mn	2	110579.3	5028.8	62117.2	4006940.0
Fe x Mn	1	432087.1*	29212.5	4692.2	5223510.2
Zn x Fe x Mn	2	207675.1*	35375.0	20330.3	4317006.8
Cu	1	3778815.1*	6116141.1**	4331254.6**	24479405.4*
Zn x Cu	2	157265.6*	53094.2	3366445.8**	5073306.5
Fe x Cu	1	59780.2	750966.6**	705880.0*	23430442.2*
Zn x Fe x Cu	2	384208.1*	11530.8	677811.6*	3473530.8
Mn x Cu	1	289802.7*	11718.0	14440.0	8635761.7
Zn x Mn x Cu	2	159101.2*	862.8	23287.4	4967644.7
Fe x Mn x Cu	1	27390.2	4726.5	84584.0	6327740.2
Zn x Fe x Mn x Cu	2	399982.6**	5835.8	167626.7	6302985.1
Error A	45 (48)	61480.0	38216.3	220158.9	555942.5
Variety (Var)	1	22750.6	2747.5	135792.2*	2473280.4*
Zn x Var	2	5478.4	1436.1	47435.2	1180397.5
Fe x Var	1	58081.0*	7965.5	113232.2*	2221590.2*
Zn x Fe x Var	2	20928.8	26916.1*	133974.7**	1102600.6
Mn x Var	1	24701.3	1362.8	24596.6	3681281.7*
Zn x Mn x Var	2	9344.1	1368.2	4921.6	5373564.6**
Fe x Mn x Var	1	38155.1*	11253.6	10990.0	3896018.0**
Zn x Fe x Mn x Var	2	30831.0*	32916.2*	7000.1	5073985.1**
Cu x Var	1	1653.7	7906.1	80561.3*	5642208.4**
Zn x Cu x Var	2	10773.8	4956.9	1057.0	5120117.7**
Fe x Cu x Var	1	11628.0	25095.8	107693.3*	5442111.3**
Zn x Fe x Cu x Var	2	32091.7*	3881.3	21781.6	4475331.7**
Mn x Cu x Var	1	940.4	7014.0	88903.3*	4410000.0**
Zn x Mn x Cu x Var	2	7717.8	463.9	32413.5	1958872.5*
Fe x Mn x Cu x Var	1	13884.6	3595.8*	19090.0	3823980.2*
Zn x Fe x Mn x Cu x Var	2	33224.2*	45194.5*	4096.3	2151202.1*
Error B	35 (48)	14218.6	134608.4	23926.3	493004.8
Total	143				

TABLE 3. -- Average concentration in parts per million of elements in various plant parts of navy beans in micronutrient interaction experiment number two.

Treatment #	Treatment comb.	Concentration ratio to zinc	Leaf				Petiole				Stem				Root			
			Zn	Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn	Cu
1	[a]$Z_1F_1M_1C_1$	1-100-100-4	144	268	103	166	107	125	34	46	187	92	44	50	258	254	57	73
2	C_2	1-100-100-400	176	216	129	292	196	130	38	86	123	73	48	81	161	380	36	562
3	M_2C_1	1-100-1000-4	113	179	621	177	101	106	79	53	123	99	159	63	162	519	748	94
4	C_2	1-100-1000-400	103	257	594	242	94	127	110	54	119	67	256	96	192	315	801	707
5	$F_2M_1C_1$	1-1000-100-4	103	1366	132	356	63	150	45	78	86	148	41	57	129	3693	66	81
6	C_2	1-1000-100-400	86	1216	151	235	74	160	39	47	83	158	45	77	123	2813	59	313
7	M_2C_1	1-1000-1000-4	103	1334	544	195	82	191	106	38	82	203	261	56	190	3269	482	87
8	C_2	1-1000-1000-400	97	464	476	175	73	145	91	59	99	155	187	237	124	2617	437	303
9	$Z_2F_1M_1C_1$	1-10-10-.4	95	232	132	219	94	129	46	48	96	95	44	45	168	327	76	76
10	C_2	1-10-10-40	79	223	116	316	84	107	38	65	103	81	48	194	133	541	39	537
11	M_2C_1	1-100-100-.4	116	208	572	194	99	121	73	54	118	77	216	61	233	326	453	50
12	C_2	1-10-100-40	103	203	551	292	147	88	100	68	119	65	196	77	159	419	537	530
13	$F_2M_1C_1$	1-100-10-.4	106	844	125	258	95	152	34	66	115	162	38	70	136	3296	59	68
14	C_2	1-100-10-40	78	638	112	181	39	147	78	46	68	134	94	72	92	2526	68	294
15	M_2C_1	1-100-100-.4	101	807	691	134	84	149	93	37	128	212	313	62	144	4321	761	88
16	C_2	1-100-100-40	84	817	598	234	121	187	110	85	76	165	304	94	108	2793	404	367
17	$Z_3F_1M_1C_1$	1-.1-.1-.004	1156	252	228	104									2666	573	79	157
18	C_2	1-.1-.1-.4	689	164	121	147									1542	391	56	788
19	M_2C_1	1-.1-10-.004	790	114	623	59									2857	599	1401	269
20	C_2	1-.1-10-.4	573	190	479	131									1234	445	483	935
21	$F_2M_1C_1$	1-10-.1-.004	753	551	142	153									1876	6045	62	160
22	C_2	1-10-.1-.4	582	359	116	128									1220	5528	61	485
23	M_2C_1	1-10-10-.004	722	646	683	112									1606	10534	512	139
24	C_2	1-10-10-.4	537	445	615	127									1174	5093	541	467

[a]Z = Zn, F = Fe, M = Mn, C = Cu

TABLE 4. -- Analysis of variance computed on exudate weight, zinc content, and total zinc of exudate collected for 30 hours from Sanilac and Saginaw navy beans grown at three levels of zinc and treated with two levels of zinc during collection at two cutting heights.

Source of variance	Degrees of freedom	Mean squares		
		Exudate weight	Zinc concentration	Total zinc
Pretreatment (Pret.)	2	5.198	17.532**	21.939*
Treatment (Treat.)	1	9.152*	1.978	2.268
Pret. x Treat.	2	4.571	2.934	20.066*
Error A = Replication plus interaction with above sources	18	1.871	0.877	4.446
Variety (Var.)	1	80.876**	9.699**	75.428**
Pret. x Var.	2	4.442**	0.950	14.049**
Treat. x Var.	1	0.001	0.254	3.622*
Pret. x Treat. x Var.	2	3.810**	1.217	15.342**
Cutting Height (C.H.)	1	9.176**	3.461*	19.888**
Pret. x C.H.	2	3.143**	0.942	6.953**
Treat. x C.H.	1	0.484	0.060	0.451
Pret. x Treat. x C.H.	2	3.582**	0.297	4.620*
Var. x C.H.	1	2.448*	0.002	0.080
Pret. x Var. x C.H.	2	2.718**	3.418*	14.551**
Treat. x Var. x C.H.	1	0.067	0.148	0.001
Pret. x Treat. x Var. x C.H.	2	0.708	1.780	5.327**
Time (T.)	5	30.656**	0.207	17.982**
Pret. x T.	10	2.670**	0.732	4.661**
Treat x T.	5	0.624	0.557	4.337**
Pret. x Treat. x T.	10	0.280	0.592	2.553**
Var. x T.	5	2.454**	0.126	5.010**
Pret. x Var. x T.	10	0.650	0.178	3.054**
Treat. x Var. x T.	5	0.406	0.249	3.964**
Pret. x Treat. x Var. x T.	10	0.288	0.173	2.870**
C.H. x T.	5	0.777	0.109	0.961
Pret. x C.H. x T.	10	0.799	0.024	0.881
Treat. x C.H. x T.	5	0.495	0.006	0.679
Pret. x Treat. x C.H. x T.	10	0.202	0.101	0.579
Var. x C.H. x T.	5	0.423	0.192	1.115
Pret. x Var. x C.H. x T.	10	0.643	0.207	1.564
Treat. x Var. x C.H. x T.	5	0.424	0.065	0.645
Pret. x Treat. x Var. x C.H. x T.	10	0.135	0.141	1.085
Error B	414 (318 for zinc conc.)	0.538	0.141	1.031
Total	575 (479 for zinc conc.)			

TABLE 5. -- Weight of exudate, zinc concentration and total zinc of exudate collected from Sanilac and Saginaw navy bean varieties over a 30 hour period at various zinc pretreatment and treatment levels.

Variety	Stem length	Pretreatment zinc level added (ppm)	Treatment zinc level added (ppm)	Weight of exudate (grams)	Zinc concentration in exudate (ppm)	Total zinc (grams x conc.)
Saginaw	Short	0	.5	1.04	0.41	0.45
Saginaw	Long	0	.5	1.51	0.47	0.99
Sanilac	Short	0	.5	0.28	0.19	0.05
Sanilac	Long	0	.5	0.38	0.20	0.10
Saginaw	Short	0	5.0	1.19	0.29	0.42
Saginaw	Long	0	5.0	0.63	0.17	0.14
Sanilac	Short	0	5.0	0.54	0.10	0.06
Sanilac	Long	0	5.0	0.26	0.17	0.02
Saginaw	Short	.05	.5	1.51	0.47	0.96
Saginaw	Long	.05	.5	1.31	0.21	0.29
Sanilac	Short	.05	.5	0.69	0.13	0.09
Sanilac	Long	.05	.5	0.63	0.15	0.10
Saginaw	Short	.05	5.0	1.80	1.25	3.09
Saginaw	Long	.05	5.0	1.64	0.60	1.53
Sanilac	Short	.05	5.0	0.41	0.26	0.18
Sanilac	Long	.05	5.0	0.27	0.29	0.12
Saginaw	Short	5.0	.5	1.77	1.02	1.67
Saginaw	Long	5.0	.5	1.37	0.74	0.93
Sanilac	Short	5.0	.5	1.54	0.85	1.33
Sanilac	Long	5.0	.5	0.47	0.50	0.24
Saginaw	Short	5.0	5.0	0.81	0.81	0.60
Saginaw	Long	5.0	5.0	0.92	1.16	1.22
Sanilac	Short	5.0	5.0	0.92	1.18	1.28
Sanilac	Long	5.0	5.0	0.10	0.45	0.04

TABLE 6. -- The effect of pretreatment and treatment on exudate weight and Zn concentration of Saginaw and Sanilac navy beans.

Zn level (ppm)		Exudate			
		Weight (g)		Concentration (ppm)	
Pretreatment	Treatment	Saginaw	Sanilac	Saginaw	Sanilac
0.00	0.5	1.27	0.33	0.44	0.19
0.00	5.0	0.91	0.40	0.23	0.14
0.05	0.5	1.41	0.66	0.34	0.14
0.05	5.0	1.72	0.34	0.92	0.28
5.00	0.5	1.57	1.01	0.88	0.67
5.00	5.0	0.86	0.51	0.98	0.82