THE INFLUENCE OF ZINC FERTILIZATION UPON THE GROWTH OF AND ZINC DISTRIBUTION IN NAVY BEAN PLANT TOPS

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ABSTRACT

THE INFLUENCE OF ZINC FERTILIZATION UPON THE GROWTH OF AND ZINC DISTRIBUTION IN NAVY BEAN PLANT TOPS

By

Luke M. Mugwira

Pot experiments were conducted to study the effect of various carriers and rates of Zn on growth and Zn uptake by Sanilac navy beans (Phaseolus vulgaris L.). The effects of Zn fertilizer on the weight and Zn content of different anatomical parts of both Sanilac and Saginaw varieties were evaluated. Plants were grown in either a greenhouse or a growth chamber for 2, 4, 6 or 8 weeks using a Michigan Wisner silty clay loam which tested low in Zn.

Zinc chelate sources were applied to the soil so as to supply 0.3 ppm Zn and 1.5 ppm Zn was applied in the inorganic forms so that a 1:5 Zn chelate to inorganic Zn ratio was obtained. Plant growth responded to the Zn fertilizers except at 4 weeks after planting. Zinc sulfate, ZnZnEDTA, ZnNTA and $ZnNa_2EDTA$ generally increased plant growth more than did ZnHEIDA and $Zn(NO_3)_2$. Zinc uptake by Sanilac navy beans showed differences between the fertilizers at 6 and 8 weeks and only $ZnSO_4$ gave responses at both periods. Zinc content was not influenced by the use of Zn fertilizers.

Zinc rates began to influence plant growth at about bloom stage. Plant size was higher where $ZnSO_4$ was applied to the soil to supply 3.0 or 6.0 ppm Zn or where 0.9 ppm Zn as $ZnNa_2EDTA$ was used than where 1.5 ppm Zn

as $ZnSO_4$, 0.3 or 0.6 ppm Zn as $ZnNa_2EDTA$ was used.

In plants showing visual symptoms of Zn deficiency, the weight of the plant portion from the ground up to the primary leaves was not affected by soil Zn supply, in both Sanilac and Saginaw varieties of navy beans. Zinc became more immobilized in the stem section below the primary leaves of Sanilac beans without Zn fertilizer as the phosphate fertilizer was increased from 150 to 500 ppm P. The three oldest trifoliate leaves increased in weight, Zn content and Zn uptake where plants were fertilized with 3 ppm Zn in both bean varieties. As soil available Zn decreased under high P addition Zn was translocated from the trifoliate leaves in Saginaw but not in Sanilac. Zinc seemed to be so effectively and preferentially fixed in the lower plant parts of Sanilac beans that the quantity of Zn in the young tissues did not change with soil Zn fertilizer rates. However, there seemed to be a critical stage at which Zn uptake by all the plant parts responded to Zn in both varieties.

Zinc was so loosely bound in Sanilac bean plant tops as a whole that 87 percent of it was extractable from plant homogenate with a 0.2 M phosphate buffer at pH 7.0. In the old primary leaves, however, Zn was more strongly bound in the Sanilac than in the Saginaw beans fertilized with 3 ppm Zn. The amount of Zn from plant tissue extracted by the sodium salts of ligands increased

with the Zn chelate stability constant (log Kma); and the possibility of using chelate extractions for Zn tissue testing is considered.

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Luke M. Mugwira

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To Prisca

I dedicate this thesis to my wife. Her love, encouragement and sacrifice made this work possible.

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INTRODUCTION

Zinc deficiency in navy beans has been observed frequently in many fields in the Saginaw Valley and east central areas of Michigan. Yield responses to Zn have been most pronounced in soils with a pH of 7.2 or higher (Ellis, 1965). In the past several years field experiments in these areas conducted by several workers (Judy et al., 1964; Brinkerhoff et al., 1967; Vinande et al., 1968) have shown that 3 to 4 pounds of Zn per acre in the inorganic form or approximately one-fifth as much of a chelated form should supply enough Zn to overcome deficiency in areas where Zn deficiency is known or suspected. Zinc sulfate banded with fertilizer has generally been as good as or superior to any other Zn carriers investigated in Michigan (Davis, 1965). Chelate sources, ZnEDTA, ZnNTA and ZnHEEDTA equally increased yield and Zn uptake (Judy et al., 1965).

Bean varieties differ in their response to Zn, and both Sanilac and Saginaw varieties of navy beans have been found to respond to Zn even though the Saginaw variety may not appear to be severely Zn deficient. However, the Saginaw variety gives higher yield and Zn uptake without

Zn fertilization while the Sanilac variety produces equal or higher yields as the rate of Zn applied is increased (Judy et al., 1965).

Most of the Michigan experiments have been concerned with supplying adequate amounts of Zn fertilizer for plant growth in order to obtain high yields. Plant growth response to Zn has been evaluated, generally, only once during the growing season. Relatively little has been done on the response to Zn fertilizer by navy beans at the different stages of plant development. Investigations on the optimum amount of Zn fertilizer needed for normal plant growth and metabolism have been also limited.

The present study was conducted to investigate:

1. Zn carriers that are most effective in supplying Zn to navy beans at various stages of growth.
2. Zn rate needed for maximum growth.
3. The response to Zn application by different plant parts of Sanilac and Saginaw varieties of navy beans at various stages of growth.
4. The binding of Zn in whole, and in parts of bean plants of different ages.

REVIEW OF LITERATURE

Role of Zinc in Plants

It has been known that Zn is essential for the growth of higher plants since Brechley (1914) described Zn deficiency in higher plants. However, the specific roles of Zn in plants and animals have been assigned only in the last thirty years.

Skoog (1940) showed that Zn and auxin contents are related in higher plants. Tsui (1948) concluded that Zn is essential for the synthesis of tryptophan in tomato and, indirectly, for auxin synthesis. Nason (1950) found that Zn is needed for tryptophan formation from indole and serine. It is now recognized that the primary role of Zn is as a catalyst (Schutte, 1964). Carbonic anhydrase contains Zn in the prosthetic group (Day and Franklin, 1946). Many Zn-requiring enzymes have been described (Hoch and Vallee, 1957). It has been shown that Zn binds pyridine nucleotide to the protein portion of, and Zn atoms stabilize the structure of yeast alcohol dehydrogenase (Hagi and Vallee, 1961).

White et al. (1964) have reported that RNA synthesis is a prerequisite for protein synthesis. Kessler and Monselise (1959) found that Zn supplied to a deficient plant increases RNA and protein synthesis but decreases ribonuclease activity in citrus leaves. Wood and Sibly

(1952) have shown that tomato plants grown in Zn deficient media can provide correlations between low Zn, carbonic anhydrase activity and protein N levels. Zinc supply to deficient Neurospora increases the activity of alcohol dehydrogenase only in the presence of a N source as if protein synthesis must occur. The presence of a N source appears to be a general characteristic for the recovery of Zn-sensitive functions (Price, 1966). As Zn becomes deficient in growing organisms, metabolic lesion occurs. First there is a failure of RNA formation, followed by protein, total N and DNA (Wacker, 1962; Schneider and Price, 1962). In a severely Zn deficient Euglena the absolute amount of RNA decreases (Price, 1966). There is an increase in RNA hydrolysis with Zn deficiency in citrus leaves (Kessler et al., 1959; Kessler, 1961).

Symptoms of metabolic disorders are also expressed cytologically and morphologically. Possingham (1956) found more free amino nitrogen and amides in Zn deficient plants than in healthy ones. Inorganic phosphate also is higher in the region outside the stele and in the phloem of deficient stem tissue (Reed, 1946). In many plant species Zn deficiency is shown by interveinal chlorosis (Chapman, 1966) due to disruption of chlorophyll formation (Schutte, 1964). Seatz and Jurinak (1957) cite the following plant disorders due to Zn deficiency; the palisade cells of leaves are larger and transversely divided, rather than columnar; reduction in number of chloroplasts; the absence of starch grains; the presence of oil droplets

in the chloroplasts and the presence of calcium oxalate crystals and the accumulation of phenolic materials in the leaves.

Zinc Status in Plants

Little is known about Zn status in plants. There is evidence that Zn may be mobile in some plant species but may become immobilized in parts of other plants.

Evidence for Zinc Binding

Foliar applied Zn may move to the tips of treated citrus leaves with no indication of movement into surrounding leaves (Stewart et al., 1955). In oat plants, Wood and Sibly (1950) showed that Zn did not move from old or dead leaves nor could it be removed from macerated leaves by 24 hour dialysis against water. Johnson and Schrenk (1964) found that 87 percent of Zn in a homogenate of green alfalfa plants could be dialyzed into distilled water. Several workers have reported that although foliar applied ^{65}Zn may be absorbed by the leaf, its subsequent movement to other plant parts may be negligible or limited in extent (Leyden et al., 1960; Wittwer, 1964). Millikan and Hanger (1965) observed that injection of ^{65}Zn of high specific activity into leaves of subterranean clover resulted in its immobilization in the laminae of treated leaves.

Nature of Zinc Binding

Plant Zn is known to be closely associated with proteins (Schutte, 1964; Viets, 1966). There is evidence that Zn is bound to some enzymes. Electrophoresis of buffered plant extract showed that some Zn was bound to

proteins (Johnson and Schrenk, 1964). Sibly and Wood (1951) found that Zn was not removed by dialysis against water from plant carbonic anhydrase. Lewitt and Todd (1952) found that Zn is more concentrated in the protein fraction of Burbank Russett potato tuber than in the tuber as a whole.

Chelating agents remove or combine with Zn co-factors in enzymes resulting in loss of enzyme activity. In yeast alcohol dehydrogenase 1,10-phenanthroline does not remove Zn from the enzyme but forms a dissociable Zn-protein-chelate complex, inhibiting enzyme activity. This reaction takes place with many other pyridine-dependent Zn metalloenzymes (Vallee and Hoch, 1955; Vallee, 1956). Although Zn is so firmly bound in carboxypeptidase that it is not removed by prolonged dialysis against water, 1,10-phenanthroline removes the Zn. Carbonic anhydrase also binds Zn so firmly that its Zn does not exchange with ^{65}Zn over a period of 32 days (Hoch and Vallee, 1957).

There are two types of interaction between Zn and proteins (Ting, 1966). Zinc metalloenzymes are enzymes in which Zn atoms are specifically and firmly incorporated into the protein such that they can be considered as a single physical entity in their native state, and homogeneous metalloenzymes can be isolated and identified. In contrast, Zn metal-protein complexes are formed with enzymes which may require Zn as one of several metals for activity and are more weakly bonded with Zn and

cannot be isolated in situ. However, in carboxypeptidase, a metalloenzyme, Zn can be replaced by Fe^{+2}, Co^{+2}, Ni^{+2} and Mn^{+2} during equilibrium dialysis (Ting, 1966). The binding of Zn in plants is pH sensitive. Less Zn is bound by enzymes and by whole plant extracts (Johnson and Schrenk, 1964) as pH of the medium decreases.

Zinc Translocation within Plants

Zinc translocation within plants may be influenced markedly by other ions or compounds. Biddulph (1953) reported that high Fe concentration reduced Zn precipitation along the veins of plants grown in solution high in phosphate. Zinc was translocated under low phosphate and was uniformly distributed in the leaves, although not retranslocated from older to young leaves. Ozanne (1955) found that Zn was fixed in roots of subterranean clover under high N and low soil Zn, decreasing the Zn content of plant tops. Since environmental factors affect Zn translocation within the plants, data on Zn movement from one part to other plant parts will be variable (Thorne, 1957). Thus Wood and Sibly (1950) found that Zn was not translocated from oat leaves to other organs and during senescence and inflorescence Zn came from roots and the medium. But Williams and Moore (1952) found that 34 percent of Zn in oat leaves was translocated to other organs.

Zinc may be more mobile in some plant parts than in others on the same plant. Foliar applied ^{65}Zn did not move from citrus leaves although it was translocated to most leaves on a twig when applied to the bark of the twig

(Stewart et al., 1955). Foliar applied ^{65}Zn is absorbed and translocated more rapidly in young leaves than in old leaves of orange and lemon trees and ^{65}Zn applied near the center of the leaf is also absorbed and translocated more rapidly than when applied near the leaf margin (Wallihan and Heymann-Herschbergh, 1956). It has been shown that Zn is located mostly in and around the primary veins in corn leaf blades (Sayre, 1952).

The distribution of Zn among organelles of a plant part may differ in plant species and may be expected to influence Zn retention or release from that particular organ. Wood and Sibly (1950) found that 15 to 20 percent of leaf Zn, in oats, and 40 percent in spinach was localized in chloroplasts. Wood and Sibly (1952) in studies on oat plants at various stages of their life cycle, and on tomato plants, found that carbonic anhydrase is located only in the non-chloroplast fraction. Waygood and Clendenning (1950) have shown that in most plant species examined carbonic anhydrase is adsorbed on chloroplasts and can largely be removed from them by repeated washings with water. No carbonic anhydrase was detected in chloroplasts of oats, but 35 percent of the total carbonic anhydrase was localized in chloroplasts of spinach.

The concentration of Zn supplied to the plant may affect Zn translocation and distribution in plant parts. Wallihan and Heymann-Herschbergh (1956) found that the absorption and translocation of leaf-applied ^{65}Zn increased with ^{65}Zn dose in citrus. Millikan and Hanger (1965)

found that increasing ^{65}Zn dose resulted in the fixation of ^{65}Zn in the treated leaves. However, Leyden and Toth (1960) found that doubling the micronutrient level in the external medium, except for Zn, does not influence the absorption of foliar applied ^{65}Zn by soybean, corn or tomato plants.

Roots have exchange sites and increasing Zn concentration in the medium increases Zn absorption by roots (Lee et al., 1969). Similar exchange processes have been observed in other parts of the plant. Hewitt and Gardner (1956) have suggested that the movement of ^{65}Zn in grapevine canes is by a process of cation exchange. Millikan and Hanger 1965) obtained the following results with old leaves of subterranean clover. A high dose of ^{65}Zn applied to leaves resulted in the fixation of ^{65}Zn in the laminae of treated leaves but addition of 30, 300 and 500 micrograms of Zn to treated leaves progressively increased the movement of ^{65}Zn to other parts of the plant. Movement of ^{65}Zn from the injected leaf was also enhanced by the addition to the dose of either 0.01 M EDTA or one of various cations in amounts equivalent to 500 micrograms of Zn. The enhancement by 500 micrograms of Zn compared with that induced with EDTA, Cu^{+2}, Mn^{+2}, and Fe^{+2}; while Mg^{+2}, and Ca^{+2} were intermediate; and Na^{+2} and K^{+} were least effective.

Assessing Zinc Status in Plants

Visual growth characteristics and leaf symptoms of acute Zn deficiency are so well defined with some crops

that supplementary leaf or soil anaylses are unnecessary for diagnosis of Zn status. However, mild deficiencies in many crops cannot be easily identified and supplementary leaf or soil analysis may be necessary (Chapman, 1966). Trees particularly citrus, are very susceptible to Zn deficiency. Viets et al. (1954a) have classified 26 crops according to their susceptibility to Zn deficiency. Visual symptoms of Zn deficiency have been summarized by Chapman (1966).

Chemical analysis of plant tissue has also been used as a diagnostic tool. Zn deficiency symptoms usually develop when Zn content of plant tissue is below 15 to 20 ppm (Hiatt and Massey, 1958; Viets et al., 1954b; Melsted et al., 1969). However, Zn contents of deficient and normal plants may overlap (Viets et al., 1953). Hiatt and Massey (1958) reported that corn plants showing severe Zn deficiency had higher Zn content than plants with mild Zn deficiency symptoms. Thus plant Zn content is not necessarily diagnostic of Zn deficiency unless the deficiency has been observed or is known to occur under the existing conditions (Viets, 1966).

Tissue analysis for diagnosis of Zn status is further complicated by variation in plant Zn content caused by the amount of soil available Zn, the kind of plant, the part of the plant sampled and the stage of growth (Seatz and Jurinak, 1957). In addition, there are differences in Zn content due to variety of crop (Ellis, 1965; Judy et al., 1964; Ambler and Brown, 1969) and due to environmental

factors of climate and soil management (Wallace et al., 1969). Chapman (1966) has suggested that Zn content of leaves of known age provides a sound basis for evaluating Zn status. Recently it has been shown that the number of days to harvest maturity of Red Mexican beans increased when Zn concentration is below 20 ppm in plant tissues at or prior to bloom stage (Boawn et al., 1969).

Attempts have also been made to measure "active" Zn in plants by measuring the activity of a Zn requiring enzyme in plants supplied with varying amounts of Zn. Wood and Sibly (1952) found that carbonic anhydrase activity in oat plants was reduced by Zn deficiency. Kessler and Monselise (1959) showed that ribonuclease activity was correlated with Zn deficiency in citrus leaves. Kessler (1961) found that ribonuclease activity sharply decreased with Zn content below 15 ppm.

Soil Zn Availability to Plants

Most soils contain 10 to 300 ppm Zn but only a small fraction of the total soil Zn is available for plants (Swaine, 1955). The factors responsible for the limited availability of soil Zn for plant growth include soil temperature, clay adsorption, organic matter, pH and carbonate, P, Fe, N and soil management (Viets, 1966; Brinkerhoff, 1969).

Attempts have been made to measure the portion of soil Zn that is available for plant growth. These investigations have included soil extractions with

Aspergillus niger, weak extracting agents like water, ammonium acetate and magnesium sulfate, or stronger extracting agents such as HCl, dithizone, and EDTA. Tucker and Kurtz (1955) found that 0.1 N HCl extracted amounts of Zn that were significantly correlated with that measured by the Aspergillus niger bioassay method. Nelson et al. (1959) found that, by plotting 0.1 N HCl-soluble Zn against titratable alkalinity, they could separate Zn deficient from nondeficient soils. Melton (1968) has reported that 0.1 N HCl is a good soil test for Zn available for plant growth on some Michigan soils.

Control of Zinc Deficiency

Controls of Zn deficiency have been highly empirical and aimed at the elimination of deficiency symptoms on the crop because there is little information on the optimum amounts of Zn needed in plants. Zinc fertilizer in soil is very inefficient; the recovery of Zn by a sequence of four crops amounts to only 1 to 1.5 percent of that applied (Boawn et al., 1960a; Boawn et al., 1960b).

Chelated Zn sources are generally believed to be about five times as effective as inorganic sources in overcoming Zn deficiency (Judy et al., 1965; Wallace and Mueller, 1959). Butler and Bray (1956) found that ZnEDTA caused large increases in Zn content of ryegrass grown on fine sand soils but not on silt loam. Lucas (1964), Judy et al. (1964) and Vinande et al. (1968) have indicated that ZnEDTA is as effective as ZnNTA in overcoming Zn

deficiencies in beans grown on silty clay loam soils of Michigan. On fine sandy soils, Wallace and Romney (1970) found that Zn EDTA was more efficient than Zn NTA which was more efficient than $ZnSO_4$ in increasing uptake by corn.

MATERIALS AND METHODS

Greenhouse Procedures

Soil samples were collected from the plow layer of a Wisner silty clay loam at two locations, and samples were tested by the Michigan State University Soil Testing Laboratory. The soil test values for the Johnson soil were (ppm): Zn-2.0, P-27, K-132, Ca-2790, Mg-450, and the soil pH was 7.4; for the Schian soil, Zn-2.0, P-17, K-114, Ca-5651, Mg-455, and its pH was 7.8.

Soils were air dried and crushed with an empty acid storage bottle to pass through a 4-mesh stainless steel sieve. Three kilograms of soil were placed in one gallon tin cans lined inside with plastic bags. Fertilizer (8-32-16) to supply 150 ppm P and 20 ppm Mn was applied together with Zn treatments. The fertilizer was banded 2 inches below the surface, 1.5 inches below and one inch to the side of the seed. Two inches of soil was removed and fertilizer was applied and covered with 1.5 inches of soil. In all the experiments, 600 ml of water was added to each container before planting the bean seeds which were then covered with 0.5 inches of soil after planting. Eight seeds were planted per pot, and no water was added until after germination. Plants were thinned to four per pot three days after germination and moisture was kept at

20 percent by weighing. Harvests were made 2, 4, 6, and 8 weeks after planting. Zinc and variety treatments, and the methods of sampling plant parts are reported under the individual experiments below.

Sanilac variety navy beans were planted in the spring of 1968 in a greenhouse in order to evaluate the relative effectiveness of various Zn fertilizers as Zn sources for plant growth. The average day temperature was 70°F and the night temperature was 60°F. Zinc treatments, in four replications for each harvest, were: no Zn, 1.5 ppm Zn as $ZnSO_4.7H_2O$, and as liquid $Zn(NO_3)_2$; and 0.3 ppm Zn as ZnHEIDA, ZnZnEDTA, ZnNTA or $ZnNa_2EDTA$. Whole plant tops were harvested, frozen and freeze-dried in plastic bags and ground before Zn analysis.

The response of Sanilac navy beans to Zn rates was evaluated in plants grown in a growth chamber. The temperature at the top of the pots was maintained between 70°F and 90°F. Each of the following Zn treatments were replicated four times for each harvest: no Zn, 0.3, 0.6, and 0.9 ppm Zn as $ZnNa_2EDTA$; and 1.5, 3.0, and 6.0 ppm Zn as $ZnSO_4.7H_2O$. Whole plant tops were harvested, frozen and freeze-dried in plastic bags before Zn analysis.

Sanilac and Saginaw varieties of navy beans were used to study the reponse to Zn by the plant parts chosen for harvesting. The growth chamber temperature was maintained between 70°F and 90°F. Each of the varieties received the 150 ppm P fertilizer together with either no Zn or with 3 ppm Zn as $ZnSO_4.7H_2O$ in four replications.

Plant parts were separated as follows. Two primary leaves on each plant were harvested first, then the next three oldest trifoliate leaves along the stem were harvested, the young leaves and meristems were considered as one plant part, and their corresponding stem section and branches as another sample. The lower and middle stem sections correspond to the portions from which the primary and trifoliate leaves were harvested. Petioles were included with stems. The tissues were oven-dried at 60°C in a forced air oven for a week before analysis for Zn.

The response of the various plant parts to Zn application was also studied under high P fertilization. Sanilac and Saginaw varieties of navy beans were planted on October 14, 1969, with either no Zn or 3 ppm Zn fertilizer applied. Plants were grown in a greenhouse under artificial light and the temperature was 70°F during the day and 60°F at night on the benches. The equivalent of 500 ppm P was applied with the basic fertilizer (8-32-16) and 20 ppm Mn. Plant parts were harvested as described in the previous experiment except for the upper and branch stems, and the young leaves and meristems which were harvested as one plant part. Each of three replications was made by combining plant tissues from three pots at 4, 6 and 8 weeks; each pot was taken as a replication for two week old plants. Plant samples were frozen in plastic bags immediately at harvest by pressing them between two blocks of dry ice and then stored in a

freezer before freeze-drying.

Nutrient Culture Studies with ^{65}Zn

Sanilac navy beans were germinated in silica sand and moistened with de-ionized water in a plastic tray. Three days after planting, plants were germinated and were moistened daily with de-ionized water for 10 days before the plants were transplanted into one-liter plastic containers. One liter of the following nutrient solution was added to each container: 1 mM $NH_4H_2PO_4$, 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, and 2 mM $MgSO_4$; 2.86 ppm B, 1.81 ppm Mn, 0.22 ppm Zn, 0.08 ppm Cu, 0.02 ppm Mo, and 5 ppm Fe, and 0.1 mc ^{65}Zn. The roots were aerated by bubbling compressed air through the nutrient solution. Plant tops were harvested when they were 3 and 6 weeks old. The samples were frozen in a freezer and freeze-dried before ^{65}Zn activity was determined with a Packard liquid scintillation counter.

Laboratory Procedures

Soil Analysis

Soil pH was determined by mixing 10 grams of soil with 10 ml of water and after 15 minutes the mixture was stirred again and the pH of the suspension determined by using a Beckman Zeromatic glass electrode pH meter. Phosphorus was extracted with Bray P-1 reagent using a 1:8 soil solution ratio; available K, Ca, and Mg with 1.0 N NH_4OAC (pH 7.0) using a 1:8 soil solution ratio. Zinc

was determined by shaking 5 grams of soil to which 50 ml of 0.1 N HCl had been added for 30 minutes. The mixture was filtered through Whatman No. 1 filter paper. Zinc was determined with a Perkin-Elmer Model 303 atomic absorption spectrophotometer.

Plant Analysis

After harvest, plant samples were freeze-dried under high vacuum with thermovac freeze-dryer for three days. Freeze-dried tissues were weighed, ground with a Wiley mill through a 40-mesh stainless steel screen. A subsample of each sample was weighed in a beaker and oven-dried at 60°C for 24 hours to obtain an oven-dry weight. The ratio of oven-dried to freeze-dried weight of the subsample was used to correct the dry matter yield of the whole sample to an oven-dry basis.

Plant parts harvested when the plants were two weeks old were weighed directly into the beaker without grinding due to the small quantities of tissue obtained. All samples were ashed for 6 hours at 500°C in a muffle furnace. Five ml of 2 N HCl were slowly added to avoid vigorous effervescence before filtering the sample through Whatman No. 1 filter paper into 50 ml volumetric flasks. Zinc was determined by a Perkin-Elmer Model 303 atomic absorption spectrophotometer.

Extraction of Zinc from Plant Tissue

Data from preliminary experiments indicated that a plant tissue to solution ratio of 1:35 (w/w) was suitable for homogenization. Subsequently one gram of plant tissue

was homogenized with 25 ml of water for two minutes with a VirTis 45 high-speed homogenizer and over 90 percent plant cell breakage was obtained as viewed with a microscope.

A comparison was made between the buffering capacity and the Zn extracting ability of two buffers, 0.2 M phosphate and 0.2 M HEPES (N-2-Hydroxyethyl-piperazine-N-ethanesulfonic) a buffer with a negligible metal binding constant (Good et al., 1966). The amounts of Zn extracted by the sodium salts of chelating agents with different Zn-chelate formation constants, shown in Table 1, was also compared. The data from this experiment indicated that there was no difference in the amount of Zn extracted by the two buffers or in the amount of plant Zn removed by each chelate when buffered in either 0.2 M phosphate or in 0.2 M HEPES.

Table 1. Log of zinc chelate formation constants, log Kma, (Chaberek and Martell, 1959).

Ligand	Log Kma
HEIDA*	8.3
NTA**	10.6
EDTA***	16.5

*N-Hydroxyethyliminodiacetic acid.

**Nitrilotriacetic acid.

***Ethylenediaminetetraacetic acid.

The final procedure developed for the extraction of Zn was as follows. One gram of plant tissue was

homogenized at 2°C with 25 ml of 0.2 M phosphate buffer, pH 7.0, for two minutes with a homogenizer rheostat setting of 90. The homogenizer shaft and flask were rinsed twice with 5 ml aliguots of the buffer after each homogenization. The homogenate was transferred to a pyrex tube, and two 4 ml capacity dialysis vials were clamped together with a one inch square cellulose membrane placed between them. The homogenate was agitated with a vortex shaker and 3.5 ml of suspension was placed in one vial while 3.5 ml of the chelate solution was placed into the second vial.

Dialysis equilibrium, extractable plant Zn at different stages of plant growth, Zn recovery and percent Zn extracted by each chelate were estimated using plant tissue labelled with ^{65}Zn. Dialysis equilibrium was attained after 120 hours of shaking. The recovery of 98 percent of the ^{65}Zn in the original homogenate from the vials after equilibrium dialysis indicated that the method was reliable enough for Zn extraction.

The non-radioactive Zn samples from the greenhouse plants were dialyzed against 0.2 M phosphate, 0.01 M Na_4HEIDA, 0.01 M Na_3NTA and Na_4EDTA for 120 hours. Zinc concentration in the dialyzed homogenate was determined by weighing the homogenate in a 50 ml beaker and evaporating the solution at 40°C to obtain dry weight. The residue was digested with 2 ml of concentrated HNO_3 on a hot plate and then ashed at 500°C for hour hours. Zinc was dissolved in 0.1 N HCl in a 10 ml volumetric flask and determined by

atomic absorption as above. The Zn concentration of the sample dialyzed into the vial containing the free chelate solution was determined directly.

Statistical Methods

Statistical analyses were made using a Controlled Data Corporation (CDC) 3600 computer. Yield, concentration and uptake data were analyzed by means of the analysis of variance and significant differences between treatments were determined by Duncan's Multiple Range test (Duncan, 1955). This test is more conservative than the LSD in cases where more than two treatment means are compared.

RESULTS AND DISCUSSION

Influence of Zinc Carriers on the Growth of, Zinc Concentration in and Zinc Uptake by Sanilac Navy Bean Plant Tops

The weight, Zn concentration and Zn uptake by whole plant tops were determined in order to evaluate the effectiveness of various Zn carriers as Zn sources for Sanilac navy beans.

Plant Weight

The treatment means are shown in Table 2. The following Zn carriers increased plant weight as compared with the Zn control treatment: Zn_2EDTA, ZnHEIDA and $ZnNa_2EDTA$ at 2 weeks; none at 4 weeks; all the carriers except ZnHEIDA at 6 weeks; and all the carriers except $Zn(NO_3)_2$ at 8 weeks. Plant weight was affected equally by $ZnSO_4$, Zn_2EDTA, ZnNTA and $ZnNa_2EDTA$ except at 6 weeks where ZnNTA caused higher growth than $ZnNa_2EDTA$. The lack of consistency in plant response to Zn from the other carriers was partially due to non-uniform plant growth in different pots. $Zn(NO_3)_2$ produced 5.70 grams of plant material at 6 weeks but only 4.69 grams at 8 weeks.

The lack of differences in plant growth at 4 weeks after planting indicates that the stage of plant growth at which the plant response to Zn is evaluated may be very critical.

Table 2. Effect of various zinc carriers on the growth of Sanilac navy beans grown in a greenhouse.*

Treatment carrier	Treatment rate ppm Zn	Weeks after planting 2	4	6	8
		---------- g/4 plants ----------			
No Zn	0.0	.51a	2.29a	3.53a	5.81ab
$ZnSO_4$	1.5	.54ab	2.59a	5.40bc	9.26c
Zn_2EDTA	0.3	.59b	2.38a	5.43bc	8.48bc
ZnHEIDA	0.3	.67c	2.82a	3.52a	8.08bc
ZnNTA	0.3	.56ab	2.82a	5.74c	8.46bc
$ZnNa_2EDTA$	0.3	.59b	2.72a	4.80b	7.54bc
$Zn(NO_3)_2$	1.5	.53ab	2.74a	5.70c	4.69a

*Treatment means within a column not followed by the same letter are significantly different at the 5 percent level of Duncan's Multiple Range test.

Table 3. Effect of various zinc carriers on the zinc content of Sanilac navy beans at different stages of plant growth.*

Treatment carrier	Treatment rate ppm Zn	Weeks after planting 2	4	6	8
		ppm			
No Zn	0.0	40.0a	20.1a	17.4b	16.7bc
$ZnSO_4$	1.5	42.2a	20.0a	15.5ab	18.6c
Zn_2EDTA	0.3	35.7a	17.6a	14.1ab	15.3ab
ZnHEIDA	0.3	33.3a	14.4a	13.3a	17.4bc
ZnNTA	0.3	38.0a	17.2a	14.2ab	15.1ab
$ZnNa_2EDTA$	0.3	39.3a	17.9a	15.7ab	13.4a
$Zn(NO_3)_2$	1.5	41.7a	15.4a	21.7c	19.0c

*Treatment means within a column not followed by the same letter are significantly different at the 5 percent level of Duncan's Multiple Range test.

Table 4. Effect of various zinc carriers on zinc uptake by Sanilac navy beans at different stages of growth.*

Treatment carrier	rate ppm Zn	Weeks after planting 2	4	6	8
		----------mg/4 plants----------			
No Zn	0.0	.021a	.043a	.062ab	.097a
$ZnSO_4$	1.5	.022a	.050a	.084c	.171c
Zn_2EDTA	0.3	.021a	.042a	.077bc	.129ab
ZnHEIDA	0.3	.022a	.041a	.047a	.141bc
ZnNTA	0.3	.021a	.049a	.082c	.126ab
$ZnNa_2EDTA$	0.3	.023a	.049a	.075bc	.100ab
$Zn(NO_3)_2$	1.5	.022a	.043a	.123d	.087a

*Treatment means within a column not followed by the same letter are significantly different at the 5 percent level of Duncan's Multiple Range test.

Zinc Concentration

The data on Zn concentration are shown in Table 3. There were no differences in Zn concentration between the treatments at 2 and 4 weeks. Zinc content in 6 and 8 week old plants was generally not affected by the Zn carriers. However, the plants fertilized with $Zn(NO_3)_2$ had higher Zn content than all carriers at 6 weeks and than some carriers at 8 weeks. Zinc concentration and plant growth were not similarly affected by the Zn fertilizers as shown by the lack of differences in plant contents at 2 weeks where plant growth responded to the Zn carriers.

Zinc Uptake

There were no significant differences in Zn uptake between Zn carriers at 2 and 4 week stages of plant growth as shown on Table 4. Only $ZnSO_4$ increased the uptake at both 6 and 8 weeks. Except for $ZnNa_2EDTA$ all treatments showed a corresponding response in both plant weight and Zn uptake by 6 week old plants. Plants were at the bloom stage six weeks after planting and these data suggest that this stage of plant growth is a good time for assessing bean plant response to Zn although the Zn content of the plants remained fairly uniform. The differences in Zn uptake at 8 weeks were caused almost equally by changes in Zn concentration and plant size.

Summary and Conclusions

The weight of Sanilac navy beans increased with Zn supply from some carriers at 2, 6 and 8 weeks while Zn uptake increased only at 6 and 8 weeks. The application of

$ZnSO_4$ resulted in increased Zn uptake at 6 and 8 weeks, however, both forms of EDTA, ZnNTA and $ZnSO_4$ equally stimulated plant growth. Zinc concentration was high at 6 and 8 weeks in plants grown with $Zn(NO_3)_2$ fertilizer but at 8 weeks this was due to retarded plant growth indicating that plant Zn content may not be a good indicator of the availability of soil Zn for plant growth. Plant weight and Zn uptake at 6 weeks or bloom stage were good indicators of plant response to Zn fertilizers. Zn_2EDTA, $ZnSO_4$, ZnNTA and $ZnNa_2EDTA$ were better sources of Zn for growing Sanilac navy beans than the other Zn carriers studied. However, $Zn(NO_3)_2$ caused high Zn contents in plants at 6 and 8 weeks.

Growth of, Zinc Concentration in, and Zinc Uptake by Sanilac Navy Bean Plant Tops as Affected by Zinc Rates

This experiment was designed to study the response of Sanilac navy beans to various rates of Zn supplied from two of the best carriers studies in the previous experiment, $ZnSO_4$ and $ZnNa_2EDTA$.

Plant Weight

There were no differences in plant weights obtained with the various Zn treatments at 2 week and 4 week stages of growth as shown in Table 5. At the 6 and 8 week stages of growth 0.9 ppm Zn as ZnEDTA was as effective in increasing plant size as 1.5, 3.0 or 6.0 ppm Zn applied as $ZnSO_4$. The lower rates of ZnEDTA essentially did not influence the yield at these two stages of growth although

Table 5. Plant growth of Sanilac navy beans as affected by different rates of zinc applied to the soil as $ZnSO_4$ and $ZnNa_2EDTA$.*

Treatment carrier	Treatment rate ppm Zn	Weeks after planting 2	4	6	8
		----------g/4 plants----------			
No Zn	0.0	1.34a	5.39a	12.23a	17.01a
$ZnNa_2EDTA$	0.3	1.41a	5.66a	12.75ab	17.72ab
$ZnNa_2EDTA$	0.6	1.44a	6.48a	13.05ab	22.16bc
$ZnNa_2EDTA$	0.9	1.40a	6.45a	14.49bc	22.06bc
$ZnSO_4$	1.5	1.67a	6.13a	14.36bc	22.32bc
$ZnSO_4$	3.0	1.28a	7.07a	14.97c	21.70bc
$ZnSO_4$	6.0	1.40a	6.60a	15.55c	23.99c

*Treatment means within a column not followed by the same letter are significantly different at the 5 percent level of Duncan's Multiple Range test.

0.6 ppm Zn as $ZnNa_2EDTA$ increased plant weight at 8 weeks.

Zn Concentration

Table 6 shows Zn concentration data. All rates of zinc sulfate increased Zn concentration of navy beans during the first two weeks of growth. Three ppm Zn gave the highest increase in Zn concentration, 84.0 ppm, as compared with 23.8 ppm obtained with the control treatment. There were no significant differences between treatments at 4, 6 or 8 week stages of growth, although the Zn concentration varied from 15.1 ppm to 22.6 ppm Zn at 8 weeks. Plant weight and Zn concentration varied independently of each other at 2, 6 and 8 weeks; and this, together with the lack of differences between treatments in plant Zn concentration, indicates that Zn concentration was not a good indicator of plant response to soil Zn supply.

Zn Uptake

The data on Zn uptake by plant tops are presented in Table 7. The higher rates of ZnEDTA become more effective than $ZnSO_4$ in supplying Zn during the advanced stages of maturity as indicated by the progressive increase in Zn uptake at 8 weeks with the increasing rates of ZnEDTA. The concentration of Zn also fell from a maximum of 84.0 ppm at 2 weeks to 15.1 ppm at 8 weeks with $ZnSO_4$ as compared with 36.5 and 19.1 respectively from ZnEDTA. The amount of Zn extracted by 0.1 N HCl from pots fertilized with 6.0 ppm

Table 6. Zinc concentration in Sanilac navy beans as affected by different rates of $ZnSO_4$ and $ZnNa_2EDTA$ at various stages of plant growth.*

Treatment carrier	Treatment rate ppm Zn	Weeks after planting 2	4	6	8
		ppm			
No Zn	0.0	23.8a	22.5a	17.2a	16.1a
$ZnNa_2EDTA$	0.3	36.5abc	22.5a	17.9a	19.1a
$ZnNa_2EDTA$	0.6	30.3ab	23.9a	17.5a	20.7a
$ZnNa_2EDTA$	0.9	30.8ab	23.3a	18.4a	22.6a
$ZnSO_4$	1.5	46.4bc	27.7a	16.8a	15.8a
$ZnSO_4$	3.0	84.0d	28.8a	20.9a	15.1a
$ZnSO_4$	6.0	49.1c	25.5a	20.4a	16.3a

*Treatment means within a column not followed by the same letter are significantly different at the 5 percent level of Duncan's Multiple Range test.

Table 7. Zinc uptake by Sanilac navy beans at various stages of growth as affected by zinc rates supplied as $ZnSO_4$ and $ZnNa_2EDTA$.*

Treatment carrier	rate ppm Zn	Weeks after planting 2	4	6	8
		----------mg/4 plants----------			
No Zn	0.0	.031a	.121a	.211a	.275a
$ZnNa_2EDTA$	0.3	.051ab	.128a	.227a	.333b
$ZnNa_2EDTA$	0.6	.044a	.145a	.229a	.461cd
$ZnNa_2EDTA$	0.9	.043a	.152a	.270a	.500d
$ZnSO_4$	1.5	.079cd	.176a	.240a	.351b
$ZnSO_4$	3.0	.099d	.207a	.313b	.329b
$ZnSO_4$	6.0	.071c	.168a	.318b	.390bc

*Treatment means within a column not followed by the same letter are significantly different at the 5 percent level of Duncan's Multiple Range test.

Zn as $ZnSO_4$ also decreased between 6 and 8 weeks suggesting that some fertilizer Zn was fixed by the soil (Table 8). However, the plants grown on the soil that was supplied with 3.0 or 6.0 ppm Zn had higher Zn uptake at 2, 6 and 8 weeks than the plants grown without Zn fertilizer.

Table 8. Amount of Zn extracted by 0.1 N HCl from soil supplied with different Zn rates at various stages of plant growth.

Treatment carrier	rate ppm Zn	Weeks after planting 2	4	6	8
		ppm			
None	0.0	.38	.37	.37	.35
ZnEDTA	0.3	.36	.43	.46	.45
ZnEDTA	0.6	.59	.41	.50	.37
ZnEDTA	0.9	.43	.56	.46	.47
$ZnSO_4$	1.5	.42	.46	.70	.39
$ZnSO_4$	3.0	.67	.58	.48	.57
$ZnSO_4$	6.0	.85	.91	.92	.74

Summary and Conclusions

Plant growth only responded to Zn application at 6 and 8 weeks. The Zn concentration of the beans was higher than that of the control only at the second week harvest where $ZnSO_4$ was used. $ZnSO_4$ was a better Zn source for plant growth during the first 6 weeks but ZnEDTA increased Zn uptake by the plant much more at 8 weeks.

Increases in plant weight and Zn uptake suggested 3.0 or 6.0 ppm Zn was the best Zn fertilizer rate for plant growth.

Plant Part Development and Zinc Distribution in Navy Beans as Affected by Soil Zinc Supply at Low Phosphate Fertilizer

A preliminary investigation of the binding of Zn in plants indicated that ^{65}Zn was equally bound in the tissues of Sanilac navy bean plant tops at different stages of growth. The mixing of plant tissues of different mineral composition probably contributed to the homogeneity of the results. Consequently a comparison of the Zn status in various plant parts of beans could yield more information on the mobility of Zn in the plant as the Zn fertilizer rate was varied.

Ellis (1965) and Judy et al. (1965) have found that the Sanilac variety of navy beans is more susceptible to Zn deficiency than the Saginaw variety. The present experiment was designed to study the response to Zn rates by various plant parts of the two varieties of beans as a possible cause of their differential susceptibility to Zn deficiency.

Leaves and Meristems

Primary, trifoliate, and young leaves and meristems had a relatively high Zn content and the data on these tissues is examined together in the following tables. Stem sections had lower Zn concentration than the leaves so that their dry matter, Zn content and Zn uptake are reported separately.

Plant Weight

Zinc application increased the weight of the primary and the trifoliate leaves of the Sanilac but not those of the Saginaw beans except for the primary leaves at 4 weeks and the trifoliate leaves at 6 weeks. The growth of the young leaves and meristems responded to Zn fertilizer only at 8 weeks in Sanilac and at 4 weeks and 6 weeks in Saginaw. The data on dry matter indicate that the primary leaves matured after four weeks of plant growth and subsequently lost weight between 6 and 8 weeks. The growth pattern of the trifoliate leaves was influenced by Zn fertilization. The leaves grown without Zn fertilizer matured at 4 weeks but did not lose weight subsequently while those from the plants fertilized with 3.0 ppm Zn lost weight between 6 and 8 weeks. These differences were accounted for by the higher growth of the trifoliate leaves that resulted from Zn application. Zn fertilizer increased the weight of the trifoliate leaves from 1.62 to 2.03 grams in Sanilac and from 1.22 to 1.72 grams in Saginaw at 6 weeks but these differences were not observed at 8 weeks.

The young leaves and meristems continued to increase in weight throughout the 8 weeks. The loss of weight by the older leaves may be due to the decomposition of some compounds as the leaves dried up and photosynthesis stopped. The combined weight of all the leaves and meristems shows that these tissues were sensitive to Zn

Table 9. The growth of Sanilac and Saginaw navy bean leaves and meristems as affected by zinc sulfate fertilizer.*

Treatment Variety	ppm Zn	Weeks after planting 2	4	6	8
		----------g/4 plants----------			
			Primary Leaves		
Sanilac	0	.29a(a)	.57b(c)	.56b(c)	.37a(b)
Sanilac	3	.41bc(a)	.63b(bc)	.70c(c)	.52b(ab)
Saginaw	0	.46c(ab)	.40a(a)	.49a(b)	.38a(a)
Saginaw	3	.36ab(a)	.54ab(c)	.46a(bc)	.39a(ab)
			Trifoliate Leaves		
Sanilac	0		1.24a(a)	1.62b(a)	1.06a(a)
Sanilac	3		1.59b(a)	2.03c(b)	1.51c(a)
Saginaw	0		1.16a(a)	1.22a(a)	1.22b(a)
Saginaw	3		1.18a(a)	1.72b(b)	1.26b(a)
			Young Leaves and Meristems		
Sanilac	0		.61ab(a)	2.93a(b)	6.40a(c)
Sanilac	3		.80b(a)	3.69ab(b)	9.13b(c)
Saginaw	0		.40a(a)	3.04a(b)	8.68b(c)
Saginaw	3		.66b(a)	4.25b(b)	9.16b(c)
		Total Yield of all Leaves and Meristems			
Sanilac	0	.29	2.42	5.11	7.83
Sanilac	3	.41	3.02	6.42	11.11
Saginaw	0	.46	1.96	4.75	10.28
Saginaw	3	.36	2.38	6.43	10.81

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

application but did not show differences between the two bean varieties.

Zinc Concentration

There were no significant differences in the Zn content of the primary leaves of the two bean varieties except at 6 weeks where the Saginaw beans contained 70 and 77 ppm Zn when no Zn and 3.0 ppm Zn were applied respectively, while the Sanilac variety beans contained only 20.7 and 25.6 ppm Zn respectively.

The growth stage of the plant affected the Zn content of the leaves. The concentration decreased between 4 and 6 weeks in primary leaves of Sanilac and two weeks later in Saginaw. The Zn content of the trifoliate leaves was not affected by Zn application and did not change with plant age. The Zn content of the young leaves and meristems was not influenced by soil Zn supply but gradually decreased with plant maturity except in Saginaw without Zn fertilizer (Table 10).

Zinc Uptake

There were no differences in Zn uptake by the primary leaves of the two bean varieties except when plants were six weeks old the amount of Zn in Saginaw leaves was much higher. Zinc uptake by the trifoliate, and the young leaves and meristems of both varieties was the same. The quantity of Zn in the leaves and meristems increased with Zn rate only when plants were four weeks old as shown in Table 11.

Table 10. Zinc concentration in Sanilac and Saginaw navy bean leaves and meristems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		ppm			
		Primary Leaves			
Sanilac	0	39.8a(a)	34.7ab(b)	20.7a(a)	40.7a(b)
Sanilac	3	39.9a(b)	43.4bc(b)	25.6a(a)	38.7a(b)
Saginaw	0	31.9a(a)	31.8a(a)	70.0b(b)	34.0a(a)
Saginaw	3	42.9a(a)	46.4c(a)	77.0b(b)	48.1a(a)
		Trifoliate Leaves			
Sanilac	0		26.8a(a)	31.5a(a)	28.9a(a)
Sanilac	3		33.5ab(ab)	42.0a(b)	27.0a(a)
Saginaw	0		28.7a(a)	34.3a(a)	30.4a(a)
Saginaw	3		42.8b(a)	38.8a(a)	26.8a(a)
		Young Leaves and Meristems			
Sanilac	0		44.1a(b)	31.4a(a)	28.6a(a)
Sanilac	3		55.1b(c)	38.4a(b)	26.6a(a)
Saginaw	0		40.3a(a)	42.4a(a)	15.5a(a)
Saginaw	3		56.1b(c)	35.0a(b)	20.5a(a)
		Concentration in all Leaves and Meristems			
Sanilac	0	39.8	32.6	29.9	27.6
Sanilac	3	39.9	41.4	37.7	25.8
Saginaw	0	31.9	31.6	36.6	17.9
Saginaw	3	42.9	47.1	38.4	22.2

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

Table 11. Zinc uptake by Sanilac and Saginaw navy bean leaves and meristems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		micrograms/4 plants			
		Primary Leaves			
Sanilac	0	12a(a)	20ab(b)	12a(a)	15a(a)
Sanilac	3	16a(a)	27b(b)	18a(a)	20a(a)
Saginaw	0	15a(a)	13a(a)	34b(b)	13a(a)
Saginaw	3	15a(a)	25b(ab)	36b(b)	19a(a)
		Trifoliate Leaves			
Sanilac	0		33a(a)	50a(b)	30a(a)
Sanilac	3		54b(b)	84a(c)	41a(a)
Saginaw	0		33a(a)	42a(a)	37a(a)
Saginaw	3		50b(a)	63a(a)	34a(a)
		Young Leaves and Meristems			
Sanilac	0		26ab(a)	91a(b)	171ab(c)
Sanilac	3		44c(a)	140a(b)	228b(c)
Saginaw	0		14a(a)	98a(b)	134a(b)
Saginaw	3		37bc(a)	148a(b)	187ab(c)
		All Leaves and Meristems			
Sanilac	0	12	79	153	216
Sanilac	3	16	125	242	289
Saginaw	0	15	62	174	184
Saginaw	3	15	112	247	240

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare column; bracketed letters compare rows.

The amount of Zn in the primary leaves decreased between 4 and 6 weeks in Sanilac and between 6 and 8 weeks in Saginaw beans suggesting that some Zn was removed from these leaves. In the trifoliate leaves of Sanilac beans Zn uptake decreased between 6 and 8 weeks but not in Saginaw leaves. Zinc accumulated with plant age in the young leaves and meristems.

Lower, Middle and Upper Stems

The portions of the stem from the ground to the point where primary leaves are attached will be referred to as the lower stem; the portion from which the three trifoliate leaves were taken is the middle stem; the rest of the stem and branches is the upper stem.

Stem Weight

The lower stems of the two bean varieties increased in weight at 4 weeks, and at 8 weeks only in Sanilac. The growth of the middle and upper stems of both varieties was higher where Zn fertilizer was used. All stem sections matured at 6 weeks. Sanilac had better growth in the lower and middle stems than Saginaw (Table 12).

Zinc Concentration

Saginaw lower and middle stems had higher Zn contents than Sanilac. Concentration increased in the lower and middle stems of both varieties with Zn application during the first six weeks of plant growth except at 6 weeks in the Saginaw middle stems and Sanilac lower stems. No reponse to Zn fertilizer was obtained at 8 weeks. The

Table 12. The growth of Sanilac and Saginaw navy bean stems as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		----------g/4 plants----------			
			Lower Stem		
Sanilac	0	.23c(a)	.29a(b)	.44a(d)	.39a(c)
Sanilac	3	.14b(a)	.38c(b)	.55a(c)	.55b(c)
Saginaw	0	.14b(a)	.29a(b)	.44a(c)	.38a(c)
Saginaw	3	.12a(a)	.33b(b)	.48a(c)	.37a(b)
			Middle Stem		
Sanilac	0		.56c(a)	.97c(b)	.55b(a)
Sanilac	3		.73d(a)	1.22d(b)	.78c(a)
Saginaw	0		.32a(a)	.48a(a)	.44a(a)
Saginaw	3		.34b(a)	.71b(b)	.46a(a)
			Upper Stem		
Sanilac	0		.40b(a)	1.41a(c)	1.15a(b)
Sanilac	3		.49b(a)	1.70b(b)	1.65b(b)
Saginaw	0		.22a(a)	1.18a(b)	1.23a(b)
Saginaw	3		.43b(a)	1.69b(b)	1.54b(b)
			Whole Stem		
Sanilac	0	.23	1.25	2.42	2.09
Sanilac	3	.14	1.60	3.47	2.98
Saginaw	0	.14	.83	2.10	2.05
Saginaw	3	.12	1.20	2.88	2.37

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

Table 13. Zinc concentration in Sanilac and Saginaw navy bean stems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		ppm			
		Lower Stem			
Sanilac	0	25.5a(d)	14.5a(c)	9.3a(b)	7.1a(a)
Sanilac	3	42.8bc(c)	18.1b(b)	11.4a(a)	6.7a(a)
Saginaw	0	39.6b(b)	15.7ab(a)	11.8a(a)	11.9b(a)
Saginaw	3	51.6c(b)	19.1c(a)	41.9b(b)	12.5b(a)
		Middle Stem			
Sanilac	0		15.0a(a)	11.6a(a)	10.7a(a)
Sanilac	3		18.8b(c)	14.1b(b)	10.0a(a)
Saginaw	0		21.1b(b)	21.5c(b)	9.9a(a)
Saginaw	3		26.7c(c)	17.8bc(b)	13.2a(a)
		Upper Stem			
Sanilac	0		26.5a(b)	24.6a(b)	15.9a(a)
Sanilac	3		33.5a(b)	30.0a(b)	14.8a(a)
Saginaw	0		27.5a(b)	35.5a(c)	14.8a(a)
Saginaw	3		33.8a(b)	28.2a(b)	12.6a(a)
		Whole Stem			
Sanilac	0	25.5	17.6	21.1	12.9
Sanilac	3	42.8	23.1	21.9	12.1
Saginaw	0	39.6	21.7	28.1	13.7
Saginaw	3	51.6	24.2	28.1	12.7

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test; unbracketed letters compare columns; bracketed letters compare rows.

Zn contents of the upper stems were not influenced by Zn application.

In the lower and middle stems Zn content generally decreased with plant maturity but in the upper stems this decrease occurred only between 6 and 8 weeks as shown in Table 13.

Zinc Uptake

Zinc accumulated in the middle and upper stems between 4 and 6 weeks but it was later translocated from these stems between 6 and 8 weeks. Zinc moved from the lower stems of plants fertilized with 3.0 ppm Zn between 4 and 6 weeks in Sanilac, and between 6 and 8 weeks in Saginaw (Table 14). The pattern of Zn translocation from the stem sections suggests that Zn is removed from each stem portion as it ceases to grow except in the lower stem sections where Zn seemed to be translocated while these stems were still increasing in weight.

Table 14. Zinc uptake by Sanilac and Saginaw navy bean stems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		micrograms/4 plants			
		Lower Stem			
Sanilac	0	6a(a)	4a(a)	4a(a)	3a(a)
Sanilac	3	6a(a)	7c(b)	6a(a)	4ab(a)
Saginaw	0	5a(a)	5a(a)	5a(a)	5b(a)
Saginaw	3	6a(a)	6b(a)	20b(b)	5b(a)
		Middle Stem			
Sanilac	0		8ab(ab)	12a(b)	6ab(a)
Sanilac	3		14c(ab)	18a(b)	8b(a)
Saginaw	0		7a(ab)	11a(b)	5a(a)
Saginaw	3		9b(ab)	13a(b)	6ab(a)
		Upper Stem			
Sanilac	0		10ab(a)	35a(b)	18a(a)
Sanilac	3		16c(a)	52a(b)	24b(a)
Saginaw	0		6a(a)	43a(b)	18a(a)
Saginaw	3		14bc(a)	48a(b)	19a(a)
		Whole Stem			
Sanilac	0	6	22	51	27
Sanilac	3	6	37	76	36
Saginaw	0	5	18	59	28
Saginaw	3	6	29	81	30

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

Summary and Conclusions

The following conclusions have been drawn with respect to the plant weight, Zn concentration and Zn uptake by the various parts of Sanilac and Saginaw varieties of navy beans as they pertain to Zn response and variety differences.

Plant Weight

The growth of the primary and the trifoliate leaves of Sanilac, and the young leaves and meristems of Saginaw navy beans was a sensitive indicator of Zn supply from the soil to the plant. However, the weight of the trifoliate leaves indicated that the growth pattern of both varieties was influenced by Zn fertilizer even though these leaves did not respond to Zn in Saginaw except at 6 weeks. The trifoliate leaves from the plants fertilized with 3.0 ppm Zn lost weight between 6 and 8 weeks; the leaves of the plants grown without Zn did not change in weight.

There were no differences in the weight of the leaves as a whole between the two varieties. The growth pattern of each plant part was similar in both varieties. The primary leaves had grown to maturity at 4 weeks, the trifoliate leaves matured two weeks later, and the young leaves and meristems continued to increase in weight. The middle and upper stems responded to Zn application but the growth of the lower stems was less sensitive to the soil Zn supply.

Zinc Concentration

There were no differences in the content of the

primary leaves resulting from Zn fertilization or variety differences. The primary leaves of Saginaw beans accumulated Zn at 6 weeks but it was later translocated. Young leaves and meristems responded to Zn only at 4 weeks, but the lower and middle stems generally responded to Zn except at 8 weeks. The Zn contents of the trifoliate leaves and upper stems were not influenced by Zn fertilization.

Zinc Uptake

After the plant organs in the lower and middle sections of the plant had reached maturity Zn was translocated from them to the young leaves and meristems. This loss of Zn from the older tissues was indicated by the simultaneous reduction of dry matter and Zn content. The data shows that Zn was translocated from the plant portion from the ground up to the primary leaves after 4 weeks in Sanilac and after 6 weeks in Saginaw. The Zn loss from the middle plant portion and from the upper stems occurred after 6 weeks of plant growth in both varieties.

All the plant parts investigated responded to Zn fertilizer at 4 weeks, however, Zn uptake was not a reliable index of soil Zn supply to any of the plant parts.

Plant Part Development and Zinc Distribution in Navy Beans as Affected by Soil Zinc Supply at High Phosphate Fertilizer

Although the growth of the various plant parts of the beans grown under low P fertilizer generally responded to Zn fertilizer, the Zn contents and Zn uptake

by the various parts were not generally as sensitive to soil Zn supply. The Zn concentration in the leaves was relatively high, about 30 ppm, and no visible signs of Zn deficiency were observed. Consequently, it was concluded that the soil was not Zn deficient under these conditions.

High phosphate fertilizer has induced Zn deficiency in navy beans grown on Michigan soils (Ellis et al., 1964; Judy et al., 1964; Brinkerhoff et al., 1966). In the present experiment 500 ppm P was applied in order to induce Zn deficiency in navy beans.

Primary and Trifoliate Leaves and Young Tissues

The primary and the trifoliate leaves, and the lower and middle stems were harvested as described in the previous experiment. The remaining plant parts were labelled as young tissues.

Weight of Plant Parts

The primary leaves of both varieties did not respond to Zn fertilizer (Table 15). The growth of Sanilac primary leaves was better where no Zn was applied than where 3 parts per million Zn was applied suggesting that Zn fertilizer stimulated the growth of the younger plant parts at the expense of the primary leaves of Sanilac beans.

The trifoliate leaves of both varieties were sensitive to the soil Zn supply at all stages of plant growth. The young tissues began to respond to Zn at 4 weeks in Sanilac but not until at 6 weeks in Saginaw. The primary leaves and the young tissues of Sanilac navy beans

Table 15. The growth of Sanilac and Saginaw navy beans leaves and meristems as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		----------g/12 plants----------			
		Primary Leaves			
Sanilac	0	1.57b(a)	2.32c(c)	2.32c(c)	1.97c(b)
Sanilac	3	1.50b(a)	2.14bc(b)	1.69b(a)	1.63b(a)
Saginaw	0	.86a(a)	1.65a(c)	1.46a(bc)	1.24a(b)
Saginaw	3	.90a(a)	1.37a(a)	1.30a(a)	1.19a(a)
		Trifoliate Leaves			
Sanilac	0		3.25b(a)	3.73b(a)	3.43a(a)
Sanilac	3		5.37c(a)	5.08d(a)	5.76d(b)
Saginaw	0		2.73a(a)	4.00bc(b)	3.88b(b)
Saginaw	3		3.64b(a)	5.41d(b)	5.38d(b)
		Young Leaves and Tissues			
Sanilac	0	.66b(a)	.80b(a)	8.91b(b)	17.34b(c)
Sanilac	3	.70b(a)	3.48c(a)	11.94c(b)	29.92c(c)
Saginaw	0	.17a(a)	.27a(a)	5.30a(b)	12.36a(c)
Saginaw	3	.18a(a)	.49a(a)	9.49b(b)	32.59c(c)
		All Leaves and Young Tissues			
Sanilac	0	2.23	8.37	12.96	22.74
Sanilac	3	2.20	10.99	18.71	37.31
Saginaw	0	1.03	4.65	10.76	17.48
Saginaw	3	1.17	5.50	16.20	39.16

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

grew better than those of the Saginaw beans, however, the trifoliate leaves of both varieties had similar dry matter yields. The primary and trifoliate leaves matured at 4 weeks in Sanilac but the trifoliate leaves matured two weeks later in Saginaw. Young tissues increased in weight continously.

Zinc Concentration

Visual symptoms of Zn deficiency began to appear in the lower leaves after 4 weeks of plant growth. Zinc content in the primary and trifoliate leaves of Sanilac was generally below 15 ppm after 4 weeks indicating that the application of 500 parts per million P to the soil had induced Zn deficiency. These lower leaves had a higher Zn content in Saginaw than in Sanilac.

The Zn concentration in the primary leaves of both varieties increased with Zn supply to the soil only at 8 weeks. The trifoliate leaves had a higher Zn concentration where Zn fertilizer was used, but the young tissues had higher Zn concentration only in Saginaw (Table 16). The Zn concentration of the primary and trifoliate leaves decreased after two weeks of plant growth but it remained constant between 4 and 8 weeks in the young tissues.

Zinc Uptake

The Zn uptake data in Table 17 indicates that the primary leaves of the plants that received 3 ppm Zn absorbed Zn from the soil only during the first two weeks of growth, but the Zn was later translocated from these leaves between 4 and 6 weeks. Zinc loss from the primary

Table 16. Zinc concentration in Sanilac and Saginaw navy bean leaves and meristems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		ppm			
		Primary Leaves			
Sanilac	0	24.8a(b)	14.9a(a)	12.4a(a)	12.3a(a)
Sanilac	3	26.4a(c)	18.9a(b)	14.4ab(a)	16.9bc(ab)
Saginaw	0	27.4a(c)	14.2a(a)	17.7c(b)	19.6d(b)
Saginaw	3	29.6ab(b)	19.3a(a)	15.9bc(a)	17.8c(c)
		Trifoliate Leaves			
Sanilac	0		13.1a(a)	14.5a(a)	13.6a(a)
Sanilac	3		16.4c(a)	14.2a(a)	14.4b(a)
Saginaw	0		15.9bc(a)	16.9bc(a)	14.7b(a)
Saginaw	3		20.5d(c)	17.8c(b)	19.1c(b)
		Young Leaves and Tissues			
Sanilac	0	26.8a(b)	25.9a(b)	14.4a(a)	14.0ab(a)
Sanilac	3	38.6ab(d)	23.7a(c)	18.0a(b)	12.3a(a)
Saginaw	0	66.5b(c)	29.4a(b)	21.9a(a)	17.1bc(a)
Saginaw	3	113.2c (c)	40.3b(b)	24.4a(a)	19.0c(a)
		Total			
Sanilac	0	25.6	11.6	16.3	14.1
Sanilac	3	33.0	19.3	16.5	12.8
Saginaw	0	33.0	16.1	19.4	16.8
Saginaw	3	41.9	22.0	21.4	19.1

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

Table 17. Zinc uptake by Sanilac and Saginaw navy bean leaves and meristems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		micrograms/12 plants			
		Primary Leaves			
Sanilac	0	39c(c)	34b(bc)	29b(ab)	24bc(a)
Sanilac	3	39c(b)	41c(b)	24ab(a)	27c(a)
Saginaw	0	23a(ab)	24a(a)	26ab(b)	24bc(ab)
Saginaw	3	29b(b)	26a(b)	21a(a)	21a(a)
		Trifoliate Leaves			
Sanilac	0		43a(a)	54a(a)	47a(a)
Sanilac	3		88c(b)	72b(a)	83c(b)
Saginaw	0		43a(a)	67b(c)	57b(b)
Saginaw	3		75b(a)	96c(b)	105d(b)
		Young Leaves and Tissues			
Sanilac	0	18bc(a)	20a(a)	129ab(ab)	250ab(b)
Sanilac	3	27c(a)	83b(a)	213b(b)	369b(c)
Saginaw	0	11a(a)	8a(a)	116a(b)	212a(c)
Saginaw	3	20bc(a)	20a(a)	230c(b)	620c(c)
		Total Zn Uptake			
Sanilac	0	57	97	212	321
Sanilac	3	66	212	309	479
Saginaw	0	34	75	209	293
Saginaw	3	49	121	347	746

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

leaves of the plants that were grown without Zn fertilizer occurred after 4 weeks in Sanilac but there was no loss of Zn from the corresponding leaves of the Saginaw beans. The amount of Zn in the Sanilac trifoliate leaves did not change with plant age but increased with Zn fertilization. Zinc accumulated in the trifoliate leaves of the Saginaw beans during the first 6 weeks of plant growth but it was subsequently translocated from only those leaves of plants that were not fertilized with Zn. Under the Zn deficiency conditions obtained in this experiment, it seems that the lesser extent of Zn movement from the old trifoliate leaves of the Zn deficient Sanilac bean plants may partially account for the greater sesceptibility of the Sanilac variety to Zn deficiency.

Zinc uptake increased with plant maturity in young tissues. However, Zn uptake by these tissues increased with the Zn supply from the soil only in Saginaw. The quantity of Zn in the trifoliate leaves of both varieties of beans increased with Zn application but the primary leaves did not respond to Zn fertilization.

Lower and Middle Stems

Stem Weight

Sanilac lower and middle stems had higher yields than Saginaw stems, however, the yields increased with soil Zn supply for the plant only in the middle stems as shown in Table 18. The stems of both bean varieties increased in weight as the plants matured.

Table 18. The growth of Sanilac and Saginaw navy bean stems as affected by zinc sulfate fertilizer at various stages of plant growth.*

Treatment Variety	ppm Zn	Weeks after planting 2	4	6	8
		----------g/12 plants----------			
			Lower Stem		
Sanilac	0	.76b(a)	1.25b(b)	1.74d(c)	2.21c(d)
Sanilac	3	.71b(a)	1.31b(b)	1.57c(c)	1.91b(d)
Saginaw	0	.34a(a)	.84a(b)	1.07a(b)	1.34a(c)
Saginaw	3	.40a(a)	.84a(b)	1.19b(c)	1.48a(d)
			Middle Stem		
Sanilac	0		1.16b(a)	1.77c(b)	2.15c(c)
Sanilac	3		2.08c(a)	2.08d(a)	2.98d(b)
Saginaw	0		.67a(a)	1.08a(b)	1.33a(c)
Saginaw	3		.94b(a)	1.42b(a)	2.07c(c)
			Whole Stem		
Sanilac	0	.76	2.41	3.51	4.36
Sanilac	3	.71	3.39	3.65	4.89
Saginaw	0	.34	1.51	2.15	2.67
Saginaw	3	.40	1.78	2.61	3.55

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

Zinc Concentration

The Zn concentration in the lower and middle stems was significantly higher in the Sanilac beans with no Zn fertilizer than in the other treatments at 6 weeks, and at 8 weeks in the lower stem only. The Zn contents in the stems of Sanilac beans did not change during plant growth but where Zn was applied there was a reduction in Zn content. Zinc concentration in the stems of Saginaw beans also decreased as plants grew from 4 to 6 weeks except in the lower stem of the Zn fertilized plants where this reduction occurred between 2 and 4 weeks (Table 19).

Zinc Uptake

The highest Zn uptake by stems occurred in Sanilac lower stems with no Zn fertilizer; the highest Zn accumulation in the middle stems resulted from the same treatment. Zinc uptake increased at each stage of growth during the first six weeks in the lower stems of the Sanilac beans that were grown without Zn fertilizer (Table 20). Zinc was translocated from the lower stems of the Sanilac beans that were grown at 3 ppm Zn fertilizer between 6 and 8 weeks and from the stems of Saginaw beans without Zn fertilizer between 4 and 6 weeks. There was no evidence of translocation or accumulation of Zn in the lower stems of Saginaw beans fertilized with Zn or in the middle stems of all the plants at any stage of plant growth. These findings indicate that Zn is deposited continuously in the lower stems of deficient plants during plant growth in Sanilac.

Table 19. Zinc concentration in Sanilac and Saginaw navy bean stems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		ppm			
		Lower Stem			
Sanilac	0	18.1a(a)	35.9b(a)	36.7b(a)	34.2b(a)
Sanilac	3	22.4a(c)	14.4a(b)	15.3a(b)	9.2a(a)
Saginaw	0	33.1a(b)	32.8b(b)	12.8a(a)	8.7a(a)
Saginaw	3	52.4b(b)	15.5a(a)	13.3a(a)	10.4a(a)
		Middle Stem			
Sanilac	0		15.2a(a)	21.1b(a)	11.8bc(a)
Sanilac	3		20.0a(b)	13.3a(a)	12.1c(a)
Saginaw	0		17.9a(b)	11.9a(a)	10.4ab(a)
Saginaw	3		17.0a(b)	11.0a(a)	9.6a(a)
		Total			
Sanilac	0	18.1	26.1	28.5	23.2
Sanilac	3	22.4	18.0	14.2	11.2
Saginaw	0	33.1	26.5	12.6	10.5
Saginaw	3	52.4	16.2	10.7	9.9

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

Table 20. Zinc uptake by Sanilac and Saginaw navy bean stems at various stages of growth as affected by zinc sulfate fertilizer.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		micrograms/12 plants			
		Lower Stem			
Sanilac	0	14ab(a)	45c(b)	63b(bc)	76b(c)
Sanilac	3	16ab(a)	19ab(a)	24a(b)	18a(a)
Saginaw	0	11a(a)	28b(b)	14a(a)	12a(a)
Saginaw	3	21b(a)	13a(a)	12a(a)	15a(a)
		Middle Stem			
Sanilac	0		18a(a)	37c(a)	25c(a)
Sanilac	3		42b(a)	28b(a)	37d(a)
Saginaw	0		12a(a)	13a(a)	14a(a)
Saginaw	3		16a(a)	16a(a)	20bc(b)
		Total			
Sanilac	0	14	63	100	101
Sanilac	3	16	61	52	55
Saginaw	0	11	40	27	28
Saginaw	3	21	29	28	35

*Treatment means followed by the same letter are not significantly different at the 5 percent level of Duncan's Multiple Range test: unbracketed letters compare columns; bracketed letters compare rows.

The amount of soil Zn extracted by o.1 N HCl increased with Zn application but remained constant throughout the growth period (Table 21.). These data indicate that the loss of Zn from the old plant tissues as the plants matured was not caused by the decrease of the soil Zn that was available for plant growth.

Table 21. Amount of Zn extracted by 0.1 N HCl from Wisner clay loam after growing navy beans for 2, 4, 6, and 8 weeks in a greenhouse.*

Treatment		Weeks after planting			
Variety	ppm Zn	2	4	6	8
		ppm			
Sanilac	0	.47	.59	.37	.44
Sanilac	3	.72	.68	.67	.74
Saginaw	0	.46	.42	.47	.38
Saginaw	3	.70	.63	.67	.78

*ppm Zn extracted by 0.1 N HCl after shaking 5 grams of soil with 50 ml of solution for 30 minutes.

Summary and Conclusions

Plant Weight

The growth of the middle plant portion, the trifoliate and the middle stem, was more sensitive to the soil Zn supply than the lower plant section, the primary leaves and the lower stem. The young tissues were also good indicators of the Zn available for plant growth.

Zinc Concentration

Under Zn deficiency conditions, the Zn content of the trifoliate leaves was more sensitive to the external Zn supply but the Zn content of the primary leaves was not sensitive to Zn fertilizer. The Zn concentration in young tissues was a good indicator of available Zn during the first 4 weeks in Saginaw but not at any stage of growth in Sanilac. The young tissues received Zn from the soil and from the older plant parts since Zn content remained constant in these tissues as they continued to grow.

The Zn content of the lower and middle stems was higher in Sanilac suggesting more Zn fixation in this variety especially where no Zn was applied to the soil.

Zinc Uptake

Primary leaves absorbed Zn from the fertilizer only during the first two weeks of plant growth. Zinc was more fixed in the lower and middle plant portions of Sanilac beans than in Saginaw. This was particularly evident in the lower stems where Zn accumulated more in Sanilac without Zn fertilizer than in the other treatments; there was no indication of Zn translocation as the plant matured.

Zinc Binding in Navy Bean Plant Tissues

The binding strength of Zn in plant tissues was estimated by equilibrium dialysis of plant homogenate against different chelates. No differences were obtained in the amount of ^{65}Zn extracted by each from 3 and 6 week old Sanilac bean plant tops (Table 22). However, the amount

extracted increased with the increasing Zn chelate stability constant. These data represent only the fraction of ^{65}Zn from the original plant homogenate that was dialyzed into the chelate solution half cell at equilibrium. Consequently, these differences are only relative.

A more accurate estimation of the total amount of the ^{65}Zn extractable by each chelate was made by sequence extractions of the homogenate (Table 23). Extractable ^{65}Zn was essentially removed by the chelates after 7 extractions and the amount of ^{65}Zn extracted by each of the subsequent extractions was within the pre-determined experimental error of two percent, indicating that the equilibrium between plant Zn and the ligands had been obtained. These data indicate that Zn in whole Sanilac bean tops is so loosely bound that 87 percent of it is removed by 0.2 M phosphate buffer. Johnson and Schrenk (1964) also found that 87 percent of ^{65}Zn in an alfalfa homogenate could be dialyzed into distilled water. It is probable, however, that the values reported in this experiment were substantailly increased by mass action.

The relative amounts of Zn extracted from the primary leaves of Sanilac and Saginaw varieties of navy beans are shown in Table 24. Zinc application decreased the amount of Zn extractable with the phosphate buffer and HEIDA only in 8 week old primary leaves particularly in Sanilac. The primary leaves of Sanilac beans fertilized with Zn showed a greater reduction in weight (15), Zn concentration

Table 22. Percent ^{65}Zn extracted by various chelating agents from Sanilac navy bean plant tops of different ages of maturity.*

Extracting Solution	Age of the Plant (weeks)	
	3	6
0.2 M P buffer	19	22
0.01 M Na_4HEIDA	40	42
0.01 M Na_3NTA	43	43
0.01 M Na_4EDTA	47	47

*Percent ^{65}Zn removed by the chelating agent from the plant homogenate at equilibrium.

Table 23. Summation percent ^{65}Zn extracted from Sanilac bean tissue by various chelating agents in 10 sequence extractions.*

Extrating Solution	percent ^{65}Zn extracted									
0.2 M P buffer	20	38	56	67	74	79	82	85	86	87
0.01 M Na_4HEIDA	31	53	67	75	81	85	88	89	91	93
0.01 M Na_3NTA	33	55	70	80	86	90	92	93	94	95
0.01 M Na_4EDTA	36	60	75	84	90	93	95	96	97	98

*Percent ^{65}Zn as a fraction of the total ^{65}Zn in the homogenate subsample.

Table 24. Relative percent zinc extracted from the primary leaves of Sanilac and Saginaw varieties of navy beans of differing maturity.*

Treatment Variety	ppm Zn	Plant age (weeks)	0.2 M phosphate	0.01 M HEIDA	0.01 M NTA	0.01 M EDTA
Sanilac	0	4	33	33	49	58
Sanilac	3	4	27	37	46	54
Sanilac	0	8	32	34	49	52
Sanilac	3	8	20	21	53	57
Saginaw	0	4	32	40	48	62
Saginaw	3	4	30	40	45	55
Saginaw	0	8	37	38	47	63
Saginaw	3	8	28	30	39	51

*Percent zinc is the fraction of the total homogenate Zn that was dialyzed into the extractant dialysis vial and it is expressed on concentration basis.

**Sodium salts of the chelating agents were used.

(Table 16) and in Zn uptake (Table 17) between 4 and 8 weeks indicating that Zn was translocated from them as they began to dry. Since phosphate and HEIDA extracted only 20 and 21 percent Zn respectively from the leaves of the plants that were fertilized with Zn but 32 and 34 percent in Sanilac plants without Zn it appears that Zn was translocated from the leaves before they dried up but the residual Zn was more strongly bound than that in growing primary leaves. Phosphate and HEIDA both removed the same quantity of Zn

from the 8 week old primary leaves of both bean varieties with or without Zn fertilization suggesting also that the mature leaves bound their residaul Zn more strongly than the young primary leaves.

There were no differences in the amounts of Zn extracted respectively by NTA and EDTA from the leaves regardless of variety, Zn treatment and plant age. In general the fraction of Zn removed from the leaves increased in the following order: phosphate, HEIDA, NTA, and EDTA extracting the highest amount. It should be pointed out, however, that the data reported in the present experiment refer only to the fraction of the total Zn in the leaves that was dialyzed from the plant homogenate into the extractant solution half-cell so that the ratios between chelates are only relative.

Summary and Conclusions

Zinc was equally bound in three and six week old Sanilac beans grown in a nutrient solution but the amount of Zn extracted increased with the Zn-chelate stability constant of the extractant. Although 87 percent of the ^{65}Zn was removed from the homogenate of Sanilac bean plant tops with phosphate buffer there were increases in percent ^{65}Zn removed by the ligands with their increasing Zn-chelate stability constants up to EDTA which removed 98 percent of the plant ^{65}Zn.

The same quantity of Zn was extracted with either phosphate or HEIDA from 8 week old primary leaves but the

amount removed from Sanilac fertilized with Zn was relatively small because these leaves dried up earlier than those of the other treatments. Therefore, a portion of the Zn remaining in the old primary leaves after some had been translocated from them was so strongly bound that it could be removed only with either NTA or EDTA.

GENERAL SUMMARY AND CONCLUSION

The growth of Sanilac navy beans responded to Zn supplied to the soil by various Zn carriers. The time of sampling the plants was very critical. No plant response to Zn was obtained 4 weeks after planting, but at 2, 6 and 8 weeks. The application of 1.5 ppm Zn as $ZnSO_4$ or 0.3 ppm Zn as $ZnNa_2EDTA$, ZnNTA or as Zn_2EDTA generally increased plant growth. However, $ZnSO_4$ increased Zn uptake by plants at both 6 and 8 weeks while all the other Zn carriers increased Zn uptake at only one of these two periods. ZnHEIDA increased plant growth only at 2 weeks and $Zn(NO_3)_2$ only at 6 weeks. Zinc concentration in the plant was a poor indicator for soil Zn available for plant growth.

The investigation of the effect of Zn rate applied to the soil also indicated that the stage of plant growth at which the plant response to Zn was evaluated was critical. The dry matter yield, Zn content and Zn uptake by bean plants did not show any changes resulting from Zn application at 4 weeks. However, as the plants reached the bloom stage about 6 weeks after planting, 3.0 and 6.0 ppm Zn supplied as $ZnSO_4$ and 0.9 ppm Zn as $ZnNa_2EDTA$ stimulated plant growth much more than did 1.5 ppm Zn as $ZnSO_4$, and 0.3 ppm or 0.6 ppm Zn as $ZnNa_2EDTA$.

The growth, Zn content and Zn uptake by the

plant parts of Sanilac and Saginaw navy beans indicated that the oldest trifoliate leaves and their corresponding stem section responded to Zn at all stages of plant growth. The young tissues were also sensitive to soil Zn supply. The primary leaves absorbed Zn only during the first two weeks of growth and were not responsive to Zn fertilizer at 500 ppm P but at 150 ppm P fertilizer in Sanilac.

When 500 ppm P was applied to the soil visual Zn deficiency symptoms appeared and Zn was fixed in the primary leaves but a portion of the Zn was translocated from the leaves of the plants that had been fertilized with 3 ppm Zn. The most outstanding differences between the two bean varieties was that Zn was more extensively translocated from the older plant parts that were most responsive to Zn fertilizer in Saginaw than in Sanilac.

The estimation of Zn binding in plant tissues by extracting plant Zn with the sodium salts of the various chelates indicated that where Zn had been translocated from the leaves the residual Zn was more strongly bound in the Sanilac primary leaves than in Saginaw. However, Zn was so loosely bound in Sanilac plant tops as a whole that 87 percent of it was removed by extracting with 0.2 M phosphate buffer. The mounts of Zn extracted from all the plant tissues increased with the Zn-chelate stability constants of the ligands. The data suggested that 0.01 M Na_3NTA and 0.01 M Na_4EDTA were more effective in extracting Zn from the older plant tissues than 0.2 M phosphate buffer and 0.01 M NaHEIDA.

The most important conclusions from this study of Zn localization in navy beans were:

1. The measurement of plant growth response to Zn at only one harvest time may lead to serious errors in the evaluation of the effectiveness of Zn fertilizers as Zn sources.
2. Zinc sulfate applied to the soil at the rate of 3 ppm Zn was an optimum Zn rate for plant growth.
3. The oldest trifoliate leaves along the stems of Sanilac and Saginaw beans are the best plant parts to assess the soil Zn that is available for plant growth.
4. There is a greater translocation of Zn from the older Zn sensitive leaves of Saginaw than from those of Sanilac.
5. Although Zn is loosely bound in plant tops as a whole, tissue testing for Zn by extracting plant Zn with chelating agents may be useful if the plant parts that are most sensitive to the external Zn supply are used.

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LITERATURE CITED

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