ZINC-PHOSPHORUS
INTERACTIONS IN PHASEOLUS VULGARIS

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ABSTRACT

ZINC-PHOSPHORUS INTERACTIONS IN _PHASEOLUS VULGARIS_

by Gary Max Lessman

An investigation was conducted to: (1) study the effect of P-ZN antagonism on _Phaseolus vulgaris_ var. Sanilac (pea or navy bean type) (2) ascertain if added Zn could overcome this effect and (3) determine the site of this antagonism and its mechanism.

Field experiments were conducted for two years on the lake-plain soils of East Central Michigan where Zn deficiency was known to occur. The results indicated that high levels of applied P have a marked detrimental affect on Zn utilization. In 1963 the most effective Zn fertilizer was $ZnSO_4$ mixed with ammonium polyphosphate (APP). Zinc ethylenediaminetetraacetate (EDTA) was nearly as effective as was ZnO incorporated into APP. Zn was definitely more effective when incorporated into APP than if incorporated into ammonium orthophosphate (AOP). It was noted in 1963 that the 0.8 pound rate of ZnO incorporated into APP was better than the three pound rate, which deviates from a standard growth curve. The 1964 field trials were designed to study this phenomenon, and to compare APP and AOP as carriers of Zn. Studies of these fertilizers showed that

the solubility of Zn added to APP increased to a maximum with increasing Zn and then decreased with additional increments. Zn incorporated into AOP had a very low water solubility. It is suggested that a soluble complex is formed between Zn and polyphosphoric acid until the system becomes supersaturated. Additional Zn beyond this level is changed to an insoluble precipitate. Results of this study show that the Zn:P ratio of the fertilizers may be critical regarding Zn availability. And the characteristics of these fertilizers might depend on their method of manufacture.

A calcareous Wisner clay loam incubated with 100, 500, and 1,000 ppm P for two and one-half years and then equilibrated with Zn^{65} was successively extracted with four agents in the following order: H_2O, 0.01 M EDTA in 1 M $(NH_4)_2CO_3$, 1 M NH_4OAc buffered at pH 7.0 and 0.1 N HCl. Little water soluble Zn^{65} was extracted and the results showed no relationship between the quantity of applied P and water woluble Zn. EDTA buffer, which is regarded as one of the best extractants for evaluating available Zn, removed more Zn from the samples highest in applied P. The remaining two extractants removed small quantities of Zn^{65} and the amount removed was inversely related to applied P.

In nutrient culture studies, Zn^{65} was not translocated beyond the roots of plants produced in high P solutions. Further studies showed that P in the roots of these plants could be removed by transferring them to Hoagland's solution minus P for 24 hours. After removal of this P, ten times more Zn^{65} was translocated to the aerial portion of the plants than in plants which contained the P in the roots. After removing the P from the roots if the plants were once again subjected to a high P environment, Zn uptake was again decreased. Therefore, the location of the P-Zn antagonism was shown to be at or near the surface of the roots. It is postulated that the antagonism is due to the formation of a Zn-P complex which may also include other plant metabolites.

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INTRODUCTION

Zinc is present in most soils in adequate amounts for optimal plant growth. But mere presence alone does not foster any indication as to the availability of Zn for plants. Soil factors that influence Zn availability are: pH, P level, free $CaCO_3$ content, organic matter content, and adsorbtion by clays. Of these, the first two are regarded as the most important.

Many productive soils of the Lake-plain area of East-Central Michigan are calcareous with pH values greater than seven. Added to this factor is the presence of a high level of soil P which has resulted from high levels of fertilization carried out by the area farmers to obtain maximum yields of sugar beets. Subsequent crops of pea beans grown on these soils have shown varying degrees of Zn deficiency. Pot culture studies with pea beans have substantiated the field observations thus indicating that the P level of the soil together with the effect of alkaline soil pH values intensifies Zn deficiency.

Conflicting information has been published concerning the effect of the P level, the amount of Zn needed to correct Zn deficiency, whether the Zn deficiency was caused by precipitation of insoluble Zn compounds in the soil or if the Zn-P antagonism was of a physiological nature.

These questions prompted the following objectives of this investigation.

1. To determine the effect of some carriers and rates of Zn application on yield and uptake of Zn by pea beans (Phaseolus Vulgaris).
2. To study the interaction of P and Zn in pea bean production.
3. To elucidate the site and mechanism of P and Zn antagonism.

REVIEW OF LITERATURE

Zn has generally been recognized as an indispensable element for living organisms since 1869 when Raulin (52) showed that Zn was needed for the growth of Aspergillis niger. This observation was later confirmed by Bertrand and Javillier (6). Mazé (40), in working with Zea mays in nutrient culture solutions, was the first to show that Zn was essential for higher plants. The concept of Zn essentiality was not universally accepted until 1926, when Sommer and Lipmann (57) proved conclusively that Zn was an essential element for the growth and development of several species of higher plants.

After this time many workers found that physiological diseases such as white bud of corn, bronzing of tung trees and rosetting of citrus trees were corrected by Zn treatment. Allison et. al. (2) found that $ZnSO_4$ increased the yields of corn, peas, peanuts and millet. Later in Texas (1) pecan trees were found to respond to Zn as did citrus in California (17). It was found by these workers that the "disease" could be cured by adding Zn salts to the soil, by spraying the tree with solutions of Zn salts, or by driving pieces of galvanized iron into the trunk.

Functions of Zinc in Higher Plants

In most higher plants, less than 50 micrograms of Zn per gram of dry tissue are required for normal growth (50). Even so, deficiencies of this nutrient are widespread across the United States and almost every economic crop has been shown to respond to Zn fertilization. One of the first visual deficiency symptoms is interveinal chlorosis of the lower leaves followed by necrosis of these mottled areas. In many plants a shortening of the internodes becomes pronounced. In the case of pea beans an asymmetic development of the leaf lomina causing "sickle shaped" leaves will occur.

Possibly the most well known role of Zn in higher plants is its interrelationship with auxin. Skoag (56) found that Zn deficient tomato plants were also low in auxin. And, the addition of Zn to these plants caused an increase in auxin activity within 24 hours. Because the peroxidase activity increases in Zn deficient plants, Hewitt (27) suggested that the low level of auxin in the Zn-deficient plant was a result of the destruction of the hormone rather than a lack of synthesis. On the other hand, Tsui (61) showed that Zn was implicated with auxin synthesis because Zn deficient plants that were low in auxin were also low in tryptophan. Theoretically the enzyme system which is responsible for the formation of indole-acetic acid from the tryptophan in healthy plants is the same as that in deficient ones. Additional support was given by Nason, et. al. (43)

who showed that tryptophan synthetase, which catalyzes the formation of tryptophan from indole and serine, is significantly and specifically decreased by a Zn-deficiency.

The first enzyme in which Zn was established as a metal component was carbonic anhydrase (19) isolated from red blood cells. Although carbonic anhydrase activity has been observed to be present in plants (29) no one has been able to prove a functional relationship between Zn and the protein in plants.

Researchers (49) have found that great increases in total free amide and amino nitrogen compounds occurred in tomato plants as a result of Zn deficiency. Reed (53) observed that Zn deficient plants possessed a higher concentration of inorganic P than normal plants. But this effect was not specific for Zn. Quinlan-Watson (51) associated Zn supply with aldolase activity in subterranean clover which might explain, in part, the effect of Zn levels on the inorganic P status. Decreased aldolase could decrease the production of glyceraldehyde 3-phosphate and therefore cause decreased phosphorylation of adenosine diphosphate during triosephosphate dehydrogenase action.

Nason and Evans (42) found that Zn deficient fungi had a twentyfold increase in DPN ase concentration, but concentrations of other enzymes such as fumerase and hexokinase were unaffected. During the study of Zn deficiency and its effects on metabolism, it is of importance to note that the synthesis of vitamins, amino acids, purines or pyrimidines are unaffected. Nason and McElroy (44) have presented a theory which utilizes much known information which suggest that only those enzymes which possess a relatively simple structure appear to increase in deficient cells because they can be synthesized without the oqcurrance of several key reactions needed for the formation of complex molecules. In this situation a polypeptide pool could be built up because a single template could function as a site for formation of a single polypeptide chain. However, the secondary and tertiary structure, which is to a certain extent under nutritional control,determines the specificity of catalytic activity.

Yeast alcohol dehydrogenase has been conclusively shown to require Zn for activity (37). These workers believe that Zn serves to bind the pyridine nucleotide to the protein portion. The Zn atoms are believed to stabilize the structure of the enzyme by forming a bridge between the apoenzyme and coenzyme.

Although it can be shown that metal enzymes such as various oxidases are in lower concentration in plants deficient in a specific metal, it cannot be assumed that the deficiency in a given element such as Zn would decrease these enzymes. It must be emphasized that because a deficiency of the specific metal element caused a decrease in the concentration of the particular enzyme it does not follow that a certain enzyme contains that specific metal. Most research today is but suggestive. More extensive research is needed in this area to illustrate a specific need for the Zn ion in plant metabolism.

Geochemistry of Zinc

Zn is more uniformly distributed among rocks formed at various stages of development than other micronutrients. This fact alone perhaps accounts for the uniformly occurring Zn deficiencies in the United States. By 1962, 31 of the 50 states had reported Zn deficiencies (5). Zn is contained in most of the naturally occurring soil minerals, for example biotite and hornblende, components of acidic rocks, contains 50 ppm Zn and ferromagnesium minerals, components of basic rocks, contain approximately 100 ppm Zn (41). Other primary Zn containing minerals which occur in the soil in minute quantities are: Zn blende, ZnS; Smithsonite, $ZnCO_3$; Willemite, $Zn(FeO_2)_2$; and calamine, $Zn_2SiO_4 \cdot H_2O$. A large portion of the soil Zn is probably held as isomorphously substituted Zn in an octahedrally coordinated position normally occupied by Al and Mg. Sauconite is a montmorillonite clay mineral of this type (48).

In the soil profile, the A_1 horizon generally contains the greatest quantity of Zn because of the high organic matter content. But total Zn has been found to be highly variable in soil profiles and dependent on specific soil groups (41).

Interaction of Soil Factors and Zinc Availability

Organic Matter

There is some question as to the significance of soil organic matter in supplying Zn to the soil. Some workers (59) have found that fields which received liberal applications of manure produced plants with notable Zn deficiency symptoms. They concluded that the Zn in the soil was fixed by the organic matter. This view was supported by DeRemer, et. al. (21) who found that soils became Zn deficient when incubated with ground sugar beet tops. It was concluded that microorganisms immobilize Zn while decomposing the added organic matter. On the other hand, it has been commonly observed that a soil becomes Zn deficient when the surface soil is removed, for example during the process of leveling for irrigation.

Hodgson, et. al. (30) found that with the 20 different soils included in their investigation from 28 to 99 percent of the Zn in soil solution was complexed with organic matter. Later work by Geering and Hodgson (26) showed that adding citrate to a carbonate saturated water system increased the rate of movement of Zn dramatically. The rate of increase was found to be portional to the level of citrate added to the solution.

Wisconsin researchers (20), using infrared techniques, have found that soil organic materials make a significant contribution to the Zn complexing capabilities of the soil.

But others (62) have concluded that only very small quantities of total soil Zn could be attributed to the organic form.

It has been reported (3) that steam sterilization of Zn deficient soils released sufficient Zn to correct the deficiency. This points to the fact that some of the Zn is fixed by microbial action, at least temporarily. But it should be pointed out that steam treatment may reduce soil organic matter to a more colloidal form thus yielding more organic complexing agents in the soil solution to maintain Zn in an available form.

A possible explanation for these conflicting reports is that in soils with ample native Zn, the addition or removal of organic materials would not significantly alter plant growth. But, if the available Zn is limited, addition of an organic matter source that is low in Zn may produce Zn deficiency by microbial fixation of Zn or on the other hand, removal of soil organic matter may remove sufficient Zn to produce Zn deficiency.

Soil Reaction

It has been well documented (16, 38, 54, 55, 65, and 68) that soil reaction is the most important factor influencing Zn availability in soils. As soil pH increases as a result of liming, Zn availability decreases. Thus, calcareous soils, which inherently have high pH values, have been noted as Zn deficient for many years. Later, Ward, et. al. (66) studying several factors influencing P-Zn relations in plants and soils, reported that soil reaction alone was not a major factor governing P induced Zn deficiency, but the degree of K saturation was more important. Wear (68) studied the effect of Ca on Zn uptake by treating a Norfolk sandy loam soil with three different liming materials and reported that Zn uptake decreased as the calcium content of the plant increased, but this effect was due to pH alone. In fact, he found that 92 percent of the variation in Zn uptake from applied fertilizers could be attributed to changes in pH.

Titratable Alkalinity

Nelson, *et*. *al*. (45) reported a method of evaluating the Zn status of soils by combining acid extractable Zn and titratable alkalinity. It is a well known fact that soil pH varies little with increasing $CaCO_3$ content. The success of their method suggest that $CaCO_3$ content, as well as soil reaction, may govern Zn availability in soils.

Adsorption by Clays

A factor closely related to soil reaction is clay fixation. Studies by Elgabaly (22) showed that Zn can replace Mg and Al in the octahedral position of many clays. Also Elgabaly and Jenny (23) found that Zn was adsorbed partly as a monovalent complex ion after which it became a part of the inner layer of the electric double layer. However, Nelson and Melsted (46) found that after allowing

Zn adsorption to occur in Illinois soils the exchange capacity was not altered, thus showing in this case that Zn was not occupying cation exchange positions nor being adsorbed in the double layer as a complex ion. They further showed the following retentive relationship for the soils included in their study: $H > Zn > Ca > Mg > K$. Zn adsorbed by H-saturated soil systems could be replaced by NH_4OAc, but only a portion of Zn added to a Ca-saturated soil system could be replaced by NH_4OAc. Also, they found that the longer the period of time of contact between the Zn and the Ca-clay, the less was the Zn removed by extraction. Jurinak and Bauer (35) in studying the adsorption of Zn on carbonate materials reported about ten percent of the absorption sites on calcite are occupied by Zn where the equilibrium Zn concentration is 9×10^{-7} M at 25 C and the degree of affinity proceeded as follows: Magnesite, $MgCO_3$ > dolomite, $CaMg(CO_3)_2$ > calcite, $CaCO_3$.

Also, other researchers (10) have found that when $ZnSO_4$ was added to a soil it became to a large extent non-extractable with 0.1 N HCl. Another investigation (14) showed that when $ZnSO_4$ and ZnO was added to the top inch of a soil in a column and leached with demineralized water, very little downward movement occurred.

Soil Nitrogen

Several researchers (24) have found that Zn uptake is enhanced when a specific Zn carrier is banded with N fertilizer or incorporated into nitrogen granules. Much of the benefit of this association may be the result of an acidification effect since $(NH_4)_2SO_4$, the most acid forming N fertilizer of those tested, has been the best N material to which Zn can be added.

Soil Phosphorus

Perhaps no other facet of the soil-plant Zn relationship has been more open to controversy and to a wider variety of interpretations as that of P induced Zn deficiency. One of the first reports of this effect was made by Barnette, et. al. (4) in Florida. They reported that when $ZnSO_4$ was banded with superphosphate, corn still developed zinc deficiency symptoms but when $ZnSO_4$ was banded without P, growth was vigorous. Other investigators (11, 64) found no effects of phosphorus fertilization on Zn uptake and yield. Boawn, et. al. (11) actually doubled the P content of pea beans and found no yield decrease or zinc deficiency symptoms. A recent report from Washington (47) stated that a soil with a pH of 7.4, and fertilized with several levels of P created no Zn deficiency in wheat plants either as a visual symptom or a yield response from Zn when it was applied in combinations of N and P treatments.

In spite of these reports, numerous others (12, 13, 15, 25, 33 and 34) have found that the P level of the soil and the amount of applied P are well correlated with reduced Zn utilization.

Evidence given by Bingham, _et_. _al_. (8) favors the concept of P immobilizing Cu and Zn as relatively insoluble phosphates that he concluded were external to the root. In a later report (7) Bingham found that when soils received high rates of P the total water soluble Zn increased as compared to soils which received lower amounts of P. This information led him to conclude that the mechanism of P-Zn antagonism cannot be explained on the basis of phosphate precipitating Zn. About this same time Burleson, _et_. _al_. (15) working with corn, tomatoes and beans indicated the possibility of a P-Zn antagonism within the root but outlined no specific mechanism. Nebraska researchers (66) supported this concept. They gave two reasons for this conclusion, first if the antagonism was a simple chemical precipitation outside the root, additions of P mixed with the soil should show the greatest effects. This was not indicated by their data. Secondly, as additional Zn counteracts the detrimental effect of the P, it would indicate that an absorption phenomenon with the root cells is involved. Bingham (7) refused to support this concept. His data actually showed an increase in the Zn content of citrus leaves at the highest P level studied.

Research by Bowan, _et_. _al_. (11) has shown that $Zn_3(PO_4)_2$ may actually serve as an effective fertilizer material. Also Jurinak and Inouye (36) in studying the solubility of Zn in P systems have found that even at a pH of 8.0 a Zn concentration of 1.02 ppm was found over solid

$Zn_3(PO_4)_2$. This value is about 20 times the amount of zinc required to grow healthy plants in solution cultures (28).

Bowan and Leggett (9) offer an additional possibility. Their data on Russett Burbank potatoes does not support the view that high concentrations of P in the growth medium suppresses the movement of zinc within the plant. Instead they believe that an imbalance between P and Zn expressed as a critical P/Zn ratio of the tops is more valid and will give an overall indication of the metabolic imbalance within the plant. A critical P/Zn ratio of 400 is given at which Zn deficiency is detrimental to plant growth. Watanabe, et. al. (67) found this critical ratio to be 300 for corn.

Most recently Stukenholtz, et. al. (58) have found a definite inhibition in the translocation process as a result of an elevated P concentration which gave them a sharp reduction in the Zn concentrations of the nodal and internodal tissues. They were unable to define a critical P/Zn ratio and stated that this ratio would change greatly depending on the part of the plant analyzed. They also concluded that the antagonism is located at the root surface or in the root cells.

Although there have been variations in the results reported, some of these discrepancies might arise as a result of differences in soil properties and the kind of crop grown.

Soil Temperature

Since root development is markedly influenced by temperature, it would seem only natural that Zn deficiency symptoms would occur in a cool, wet season, since this unfavorable environment is likely to be especially pronounced and persistant in the soil. Martin, *et*. *al*. (39) showed that low soil temperatures accentuated the effect of a phosphorus induced zinc deficiency. Similar results were reported by Ellis, *et*.*al*. (25) with corn. They reported both Zn concentration and uptake were less at 55°F than at 75°F.

EXPERIMENTAL METHODS

In 1963, two experimental areas were located on calcareous lake-plain soils of Michigan, one in Midland County and one in Tuscola County. The areas were selected on the basis of (1) pH value above 7.2, (2) previous usage of high rates of P fertilizer, and (3) no previous Zn fertilization. Soil samples were collected prior to planting, air-dried, hand-crushed and analyzed. The pH of a 1:1 soil to water suspension was determined with a glass electrode in conjunction with a potentiometer. Phosphorus was extracted with 0.025 N HCl plus 0.03 N NH_4F with a 1:8 soil to solution ratio and a one minute extraction period. Phosphorus in solution was determined by the molybdenum blue reduction method. Ammonium acetate extractable K was determined by shaking one gram soil with eight ml. of 1 N NH_4OAc, pH 7.0, filtering and determining the K content of the filtrate by comparison with standards with a Coleman flamephotometer. Zinc in the soil was measured by equilibrating 5 grams of soil with 50 ml of 0.1 N HCl, filtering, and measuring the Zn content of the filtrate with a Perkin-Elmer Model 303 atomic absorption spectrophotometer. The results of the soil test from the two locations were:

Location	pH	P	K	Zn
		-------	lbs./acre	---------
Midland	7.7	16	180	9.2
Tuscola	7.5	42	206	16.8

Each experimental area was prepared for planting by the cooperator using the same cultural practices on the area as on the entire field. Beginning with planting, all cultural practices were performed by Experiment Station personnel and equipment.

A split plot design with four replications was used. The two main plots consisted of no P applied prior to planting and 350 pounds of P broadcast and disked into the soil prior to planting.

Sanilac pea beans were planted during the first week of June with a modified three row commercial planter. All plots received a planting time fertilizer consisting of 280 pounds per acre of 9-36-18 containing three percent Mn. The Zn carriers, with the exception of the ZnO incorporated into APP and AOP, were hand-mixed with the base fertilizer at planting time. No sprays were applied to the crop during the growing season.

Plant samples were collected from each experimental area at two stages of growth. Three weeks after emergence the total above ground portion of 10 plants was collected from each treatment in each replication, dried at 60°C in a forced air oven, ground in a Wiley mill, and analyzed for Zn content. Just prior to bloom stage a second sampling was made by collecting the terminal mature compound leaf and petiole from 10 plants in each plot. Zn content was determined as before.

The beans were harvested by hand-pulling, staking and then threshing with an experimental bean thresher. Plot yields were taken from one 35 foot row per plot.

Using the same basis of selection as in 1963, two different locations were obtained in 1964, one in Bay County and one in Arenac County. Soil samples were collected ana analyzed as in 1963 with the following results:

Location	pH	P	K	Zn
		--------- lbs./acre --------		
Arenac	7.9	52	312	5.6
Bay	7.8	26	184	11.6

Cultural practices were the same as used in 1963. TVA encountered difficulties in preparing the experimental fertilizer in 1963 to give uniform distribution of K. Consequently, K was omitted from the fertilizers prepared for the 1964 season. To compensate for this, 60 pounds of K per acre was broadcast over each experimental area prior to planting.

The experimental design was a split plot with a factorial arrangement of subplots. Each treatment was replicated four times.

Plant samples were taken approximately three weeks after emergence by collecting the above ground portion of 10 plants from each plot. Treatment of plant samples and harvesting of beans were the same as in 1963.

For Zn determination in 1963, plant material was dry-ashed at 500°C, the ash dissolved in HCl, and the Zn content of an alioquot determined by the "zincon" method of Johnson and Ulrich (32). In 1964, the Zn in the dry-ashed material was dissolved in 0.1 N HCl and analyzed on a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer.

Plant material was prepared for determination of P by dry-ashing after pretreatment with a $Mg(NO_3)_2$-ethyl alcohol solution. The procedures outlined by Jackson (31) for the determination of P were used with only slight modification.

An analysis of variance was conducted on all data from field experiments and treatment means were compared by use of Duncan's multiple-range test.

Laboratory Studies

The affect of applied P on Zn availability was studied in the laboratory by incubating a Wisner clay loam soil for two and one-half years after being treated with three levels of P--100, 500, and 1000 ppm P. The moisture content of the soil was held near field capacity and the temperature was 25°C during the incubation period.

After the incubation period, 25 ml. of a solution containing Zn^{65} with an activity of 12,500 counts per minute per ml. was allowed to equilibrate with five grams of soil for eight days on a reciprocating shaker. The suspension was then centrifuged, the supernatant liquid decanted and 50 ml. of distilled water added to the soil. The soil was resuspended, shaken for one hour and centrifuged. One ml. of this supernatant liquid was analyzed for gamma activity. Subsequent extractions were carried out in the following order: 0.01 M EDTA in 1.0 M $(NH_4)_2CO_3$ as reported by Trierweiler and Lindsey (60), neutral 1.0 M NH_4OAc and finally by 0.1 N HCl. The volume of all extracting agents was 50 ml.

Solution Culture Studies

Preliminary studies were conducted to determine if P level in nutrient solution would influence Zn uptake by pea bean plants. For all nutrient culture studies pea bean seedlings were germinated in quartz sand and watered with deionized water until the first true leaves began to unfold. They were then transplanted into two-quart polyethylene containers, two seedlings in each container. The nutrient solutions used throughout this study were basically normal Hoagland's solutions with the following modifications. Three levels of P (100, 250, and 500 ppm P) were employed, ferric citrate was the Fe source and no solutions contained Zn initially. Just after definite Zn deficiency symptoms appeared, Zn equivalent to normal Hoagland's solution was added to one-half of the containers.

The plants were grown in a growth chamber and constantly aerated by forcing air through gas dispersion tubes that were placed in the nutrient solution. The solution was changed only once during the growing period since the high content of P created an effective buffer.

Two ml. of a $Zn^{65}Cl_2$ solution containing 100,000 counts per minute per ml. was added to all containers approximately one week prior to harvest.

Plants were harvested at pod stage in one experiment and at bloom stage in a second experiment, separated into roots, stems, and leaves, weighed, dried in a forced air oven at 60°C and ground in a Wiley mill. Uniform weight tissue samples were pressed into a pellet in a Carver press and analyzed for gamma activity. A standard curve was developed by adding known quantities of Zn^{65} to ground plant tissue, drying and pressing the material into pellets.

Two experiments were conducted to determine if the P that prevented Zn uptake and/or translocation to leaves could be easily removed from living plants. In the first of these experiments, seedlings were grown until the onset of flowering in normal Hoaglands minus Zn except for a 500 ppm P level. At flowering, one-half of the plants were removed from their medium, the roots dipped in distilled water and then transferred to a container with normal Hoagland's minus P, minus Zn for 24 hours. Two ml. of $Zn^{65}Cl_2$ solution with an activity of 100,000 counts per minute per ml. were added to the containers at this time and after 30 hours the plants were harvested, divided into roots and tops, weighed and analyzed as before.

In the second experiment plants were grown through the initial stages as just previously outlined. Just previous to flowering, the plants were removed, dipped in distilled water for 15 to 20 seconds to remove the solution adhering to the roots, and then placed in aerated Hoagland's solution for 3, 6, 12 or 24 hours. Two treatments, used for controls, were not subjected to the latter washing procedure. After the required time, the plants were placed in normal Hoagland's solution or Hoagland's solution plus 500 ppm P, and two ml. of $Zn^{65}Cl_2$ solution with an activity of 100,000 counts per minute per ml. added to each pot. After 24 hours the plants were removed, the roots washed briefly in distilled water; divided into roots, stems and leaves; dried, ground, and weighed; and counted for gamma activity.

The solutions in which the plants were aerated to remove P were analyzed for P by the molybdenum blue reduction method.

RESULTS AND DISCUSSION

1963 Field Experiments

The results of the 1963 field experiments are given in Tables 1 and 2. The data show that at both locations a high rate of P applied prior to planting significantly decreased plant content of Zn. Yield of beans was also significantly decreased by high P in Midland County but not in Tuscola County, although high P tended to decrease yield in Tuscola County also. A high level of P accentuated the yield response to Zn as compared to Zn response at a low P level. But this was not true for Zn content. At the location in Midland County application of Zn with high P did not produce as great a yield as Zn and low P. For example, with AOP addition of high P without Zn reduced yields from 22.9 to 10.2 bushels per acre. The best treatment with Zn and high P yielded 23.6 bushels per acre. But the comparable treatment of Zn with low P yielded 29.4 bushels. Similar analogies can be drawn with APP as the planting time fertilizer. Thus, even though the application of high levels of P is clearly inducing Zn deficiency in beans, it may be also producing other adverse effects on the plants.

Of the three Zn carriers mixed with APP ($ZnSO_4$, Zn EDTA and Zn_2OSO_4), $ZnSO_4$ was the most effective in supplying Zn to the plant and increasing yield. Zn EDTA was nearly as effective and it should be noted in this regard that Zn EDTA was applied at one-fifth the rate of Zn, a quantity that would make it more competitive economically. Zn_2OSO_4 was clearly less effective, particularly when applied at the two pounds Zn per acre rate.

When ZnO was incorporated into APP, it was significantly superior to ZnO incorporated into AOP in increasing Zn content of plant tissue. It also gave higher yields of beans. In fact, ZnO incorporated into AOP, regardless of the rate of Zn application, was not appreciably different from no Zn.

Typically yield of an agronomic crop should increase with increasing fertilizer application, reach a maximum yield and perhaps decrease if excessive fertilizer is applied. Some agronomists maintain that the response follows the law of diminishing returns, i.e., the yield increases at a decreasing rate. Plant uptake of applied fertilizer should closely parallel the rate of application. But when ZnO incorporated into APP was applied to give increasing rates of Zn, the typical yield and uptake response was not realized. Thus in all but one case the 0.8 pound rate yielded greater than the 3.0 pound rate but less than the 9.0 pound rate. In addition, the Zn content data closely paralleled the yield data. While the 0.8 pound treatment seldom out-yielded the 3.0 rate by more than two bushels per acre, the consistency of the data suggest that this trend is real.

Table 1. Yield and Zn content of pea beans as affected by carrier and rate of application of Zn and source and rate of P application -- Midland County, 1963.*

Treatment		Zinc Content				Yield	
		July 2		July 18			
Zn. Rate	Carrier	Low P**	High P±	Low P**	High P±	Low P**	High P±
lbs./acre		ppm Zn		ppm Zn		bu/acre	
Source of P in starter fertilizer: Ammonium orthophosphate							
0		16.2	15.5	8.0	8.0	22.9	10.2
0.8	ZnO	19.0	15.2	9.0	10.0	29.4	23.6
3.0	ZnO	21.5	15.8	10.0	8.0	24.0	18.2
9.0	ZnO	19.0	16.8	10.0	12.0	32.0	18.5
Source of P in starter fertilizer: Ammonium polyphosphate							
0		16.7	14.7	9.0	6.0	29.4	19.6
0.8	ZnO	33.7	29.2	25.0	24.0	34.7	31.1
3.0	ZnO	25.2	25.5	18.0	14.0	32.9	29.6
9.0	ZnO	57.2	45.0	26.0	24.0	35.1	33.1
2.0	$ZnSO_4$	28.5	23.5	22.0	18.0	31.4	26.7
4.0	$ZnSO_4$	32.9	24.2	18.0	19.0	35.4	31.3
8.0	$ZnSO_4$	85.0	41.0	40.0	32.0	38.9	31.3
0.2	Zn EDTA	30.8	28.0	12.0	8.0	33.8	28.0
0.8	Zn EDTA	43.0	39.2	32.0	23.0	33.1	27.2
1.6	Zn EDTA	70.5	47.5	56.0	46.0	34.0	30.0
2.0	Zn_2OSO_4	19.2	13.8	17.0	13.0	22.9	13.1
4.0	Zn_2OSO_4	22.5	16.5	17.0	14.0	34.9	26.7
8.0	Zn_2OSO_4	22.5	17.5	22.0	14.0	34.0	28.7

*Planted June 6 and 11, harvested September 9-10.

**Low P received only starter phosphorus.

±High P received 350 pounds P per acre as 0-46-0 prior to planting.

Table 2. Yield and Zn content of pea beans as affected by carrier and rate of application of Zn and source and rate of phosphorus application -- Tuscola County, 1963.*

Treatment		Zinc Content				Yield	
		July 3		July 24			
Zn. Rate	Carrier	Low P**	High P±	Low P**	High P±	Low P**	High P±
lbs./acre		ppm Zn		ppm Zn		bu/acre	
Source of P in starter fertilizer: Ammonium orthophosphate							
0		19.5	18.0	19.0	14.0	19.1	15.6
0.8	ZnO	22.2	20.8	18.0	16.0	19.1	18.4
3.0	ZnO	31.5	33.4	20.0	17.0	22.9	18.4
9.0	ZnO	22.0	25.8	18.0	18.0	22.2	18.7
Source of P in starter fertilizer: Ammonium polyphosphate							
0		19.0	21.2	10.0	17.0	18.4	19.6
0.8	ZnO	36.0	34.8	21.0	18.0	23.1	21.6
3.0	ZnO	33.8	24.8	21.0	18.0	25.3	19.5
9.0	ZnO	66.8	55.2	31.0	27.0	24.7	27.8
2.0	$ZnSO_4$	29.5	29.2	20.0	18.0	21.4	20.7
4.0	$ZnSO_4$	40.5	47.2	30.0	24.0	24.2	27.4
8.0	$ZnSO_4$	85.0	85.0	54.0	48.0	25.8	25.2
0.2	Zn EDTA	28.2	25.8	18.0	18.0	22.5	21.1
0.8	Zn EDTA	37.0	34.2	22.0	18.0	21.3	21.1
1.6	Zn EDTA	40.5	45.6	24.0	26.0	21.3	20.0
2.0	Zn_2OSO_4	29.2	25.8	19.0	13.0	19.6	14.5
4.0	Zn_2OSO_4	27.5	26.0	18.0	17.0	20.4	18.9
8.0	Zn_2OSO_4	35.5	29.5	23.0	16.0	25.6	22.5

*Planted June 4, harvested September 11-12.

**Low P received only starter phosphorus.

±High P received 350 pounds P per acre as 0-46-0 prior to planting.

Increasing rates of $ZnSO_4$, Zn EDTA or Zn_2OSO_4 increased Zn content and yield in the expected manner. Maximum yield with $ZnSO_4$ was reached with a 4.0 rate, whereas, 8.0 pounds of Zn_2OSO_4 were required to give maximum yield. Nearly maximum yield was obtained with 0.2 pounds of Zn as Zn EDTA.

Yield increases due to application of Zn were much greater at Midland County as compared to Tuscola County. This agrees well with the soil test information and with the Zn content of the plants from the no Zn treatments. It has been suggested that the critical level of Zn in bean tissue is between 15 and 20 ppm. The value of from 14.7 to 16.7 obtained in Midland is in the lower part of this critical range. But the values of 18.0 to 21.2 from Tuscola County are near the upper part or even exceeding this range.

1964 Field Experiments

In the 1963 field trials ZnO incorporated into AOP did not appear to be a satisfactory source of Zn for pea beans. But ZnO incorporated into APP was as good as any Zn source tested. Since the response curve in the low Zn rates did not appear normal with ZnO incorporated into APP, experimental fertilizers were prepared by TVA with varying Zn:P ratios to allow study of that part of the growth response curve that is between zero and three pounds of Zn per acre.

The ZnO was added to the fine fertilizer material being recycled and added to the melt as it was transferred from the reaction chamber to a rotary granulator. The Zn was therefore uniformly distributed through the granule but was not in contact with the hot, liquid melt for extended periods of time.

The properties of the fertilizers supplied by TVA are given in Table 3. The Zn:P_2O_5 ratio varied from 1:202 to 1:7 for APP fertilizers prepared with Zn. The corresponding ratios for AOP fertilizers were 1:196 to 1:9.5. As the percentage of Zn in the APP fertilizer increased the percentage of added Zn that remained water soluble increased until the Zn:P_2O_5 ratio of 1:67 was reached. Increasing the Zn beyond this level, i.e., lower Zn:P_2O_5 ratios, markedly reduced the percent of the added Zn that remained water soluble. The percent of the added Zn that was water soluble remained very low at all levels of Zn addition to AOP suggesting that the new compounds formed were even less soluble than the ZnO added.

The total water soluble Zn in the AOP fertilizer was less than 0.63 percent in all cases. As the Zn content increased, the percentage of water soluble Zn in the APP fertilizer increased until a Zn:P_2O_5 ratio of 1:47 was reached. At this point the percentage of water soluble Zn decreased markedly but increased again at the highest Zn level. This discussion is summarized in Figure 1. This figure clearly shows that in the regions of low content of

Table 3. Chemical properties of TVA experimental fertilizers -- 1964 field trials.

		Micronutrient level			H_2O soluble, % of total sample		Micronutrient solubility, % of total element present		
Carrier	Grade	Zn	Zn:P_2O_5	Mn	Zn	Mn	Zn	Mn	pH of filtrate
		%		%	%		%		
APP	13.4-52.7-0	0.26	1:202.7	5.3	0.08	0.70	30.8	13.2	6.19
APP	13.2-53.4-0	0.40	1:133.5	5.3	0.16	0.61	40.0	11.5	6.03
APP	13.3-52.3-0	0.78	1:67.1	5.2	0.36	0.72	46.2	13.8	6.18
APP	13.0-52.2-0	1.1	1:47.5	5.2	0.47	0.10	42.7	1.9	6.20
APP	12.9-52.4-0	1.5	1:34.9	5.2	0.23	0.61	15.3	11.7	6.08
APP	11.7-46.8-0	6.6	1:7.1	4.7	0.53	0.48	8.0	10.2	6.52
AOP	11.6-51.0-0	0.26	1:196.2	5.1	0.03	0.07	11.5	1.4	5.59
AOP	11.1-51.2-0	0.52	1:98.5	5.1	0.01	0.05	1.9	1.0	5.50
AOP	11.8-50.4-0	0.76	1:66.3	5.0	0.03	0.06	3.9	1.2	5.72
AOP	11.7-50.4-0	1.1	1:45.8	5.0	0.02	0.05	1.8	1.0	5.72
AOP	11.5-50.0-0	1.7	1:29.4	5.0	0.02	0.05	1.2	1.0	5.63
AOP	11.9-47.7-0	5.0	1:9.5	4.8	0.02	0.02	0.4	0.4	6.20

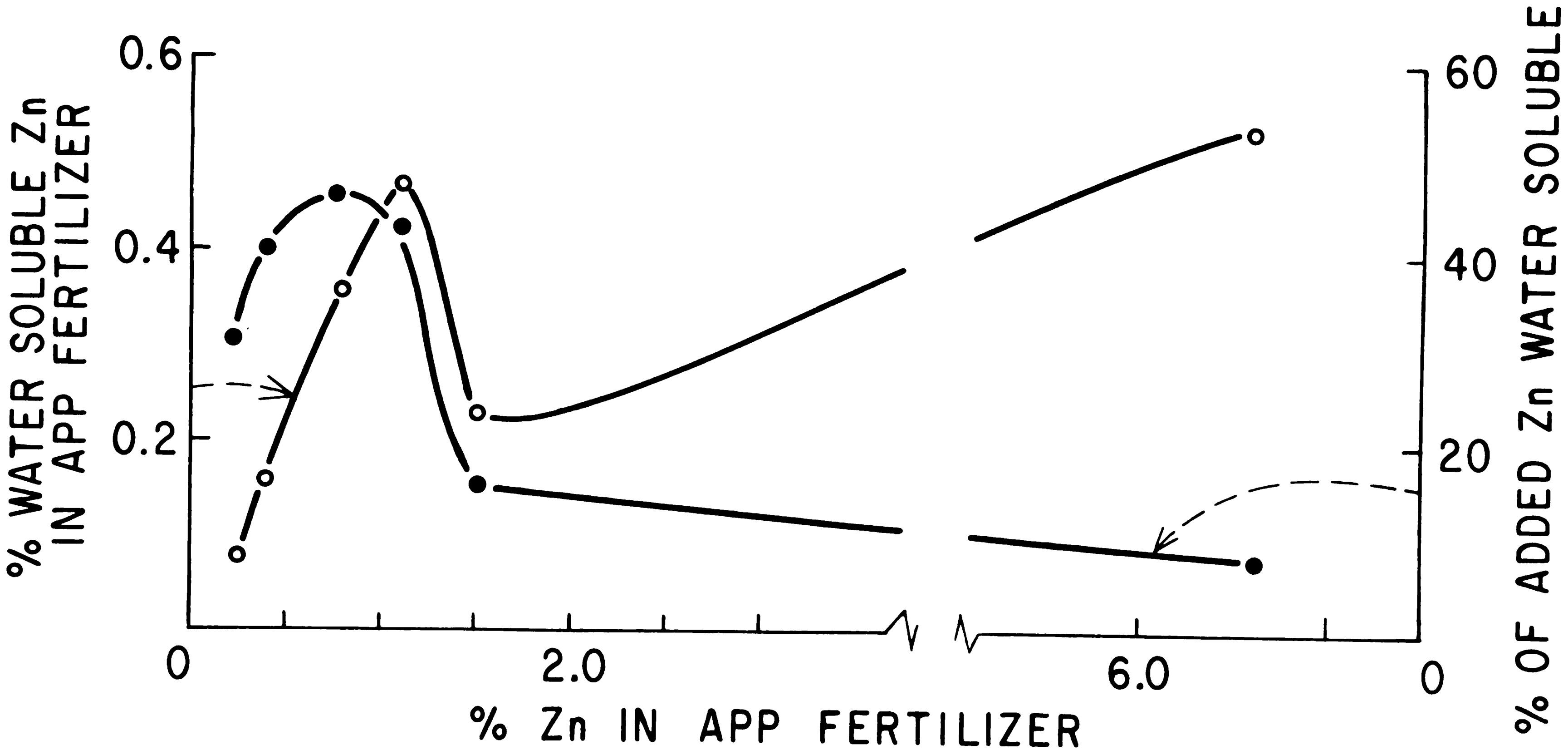

Figure 1. Relationship between total Zn content of APP fertilizer and water-soluble Zn content.

Zn in APP the water soluble Zn present goes through a maximum corresponding to about one percent Zn in the fertilizer. This phenomenon may be due to the formation of different compounds with increasing level of Zn in the fertilizer. Thus, when Zn is present in low amounts in relation to polyphosphoric acid, a relatively stable complex is formed that is water soluble. But as increasing quantities of Zn are added to this system, it becomes saturated or even supersaturated with respect to this complex. A new Zn-P compound is then formed which is much less soluble than the complex formed at low Zn levels. Once this compound is formed it may actually "mine" Zn from the complex first formed and thus total water soluble Zn may decrease for a short range.

The variables measured in the field experiments in 1964 were dry weight of young bean plants, Zn content and uptake and P content by the same plants, and yield of beans. For convenience, statistical analysis for all data are presented in Tables 9 and 10 at the end of the discussion. The data for dry weight are given in Table 4. Without exception the 8.0 pound rate of Zn incorporated with APP caused the greatest amount of early growth of any treatment at either location. Ammonium polyphosphate appeared to be the better P source at the location in Arenac County as every Zn treatment with APP produced greater plant growth than the corresponding Zn treatment with AOP as the P source. These differences did not appear at the location in Bay

Table 4. Dry weight of three-week-old Pea beans plants as affected by soil P level, planting time P source and Zn rate.

Treatment		Arenac County		Bay County	
Planting time P source	Zn rate[1]	Low P	High P[2]	Low P	High P
	lbs/acre		gms/10 plants		
Ammonium Ortho-phosphate	0	22.7	23.2	24.1	23.7
	0.4	27.6	28.7	24.5	26.7
	0.7	28.0	29.2	27.6	23.8
	1.2	28.3	30.0	28.8	27.7
	1.6	27.9	27.9	26.0	24.7
	2.7	29.8	31.4	25.3	26.0
	8.0	32.3	32.7	26.5	26.3
Ammonium Poly-phosphate	0	23.7	23.3	22.5	24.9
	0.4	27.8	27.7	26.0	26.1
	0.7	32.2	30.2	26.3	27.4
	1.2	29.4	31.1	26.2	26.4
	1.6	33.5	31.9	26.8	24.4
	2.7	32.6	32.1	28.3	25.8
	8.0	35.8	33.9	34.2	30.2

[1] Zn was applied as ZnO incorporated into either AOP or APP.

[2] Low P was zero P prior to planting; high P was 350 pounds P per acre as 0-46-0 applied prior to planting time.

County. Early growth was slowed at the latter location because of dry weather. Application of high P did not decrease plant growth at this stage of growth.

Zinc content generally followed a similar trend. The 8.0 pound rate with APP was significantly better than every other treatment. The data in Table 5 show that in Arenac County the 0.7 pound rate with APP produced plants with a higher content of Zn than any Zn rate with AOP. Results from Bay County, although somewhat inconsistent, show that plants receiving the highest rate of Zn-APP possessed the highest Zn content of any on that location. On both high and low P plots at Arenac County the Zn content was less for the 2.7 pound Zn-APP treatment than at the 1.6 pound Zn-APP rate. The P level caused a significant decrease in the Zn content at location A, and even at the highest level of applied Zn the plant content never reached the level attained on the low P plots. High P level appeared to have reduced Zn content at the Bay County location but the effect was not significant.

The 8.0 pound Zn-APP rate was again significantly better in promoting zinc uptake than the other treatments (see Table 6). Ammonium polyphosphate was the better Zn carrier on both locations, the P level appeared to have a detrimental effect on Zn uptake but the difference was insufficient for statistical significance. The AOP source did not produce much uptake at the lower rates and only the higher rate of Zn in the fertilizer made a noticeable

Table 5. Zn content of pea beans as affected by soil P level, planting time P source and Zn rate.

Treatment		Arenac County		Bay County	
Planting time P source	Zn rate[1]	Low P	High P[2]	Low P	High P
	lbs/acre		ppm Zn		
Ammonium Ortho-phosphate	0	21.4	14.4	19.2	16.9
	0.4	16.4	17.7	20.3	17.7
	0.7	18.3	16.1	17.9	19.2
	1.2	18.0	15.7	19.9	17.4
	1.6	17.4	17.7	17.4	18.7
	2.7	19.5	20.1	18.9	17.9
	8.0	19.5	21.6	20.1	20.1
Ammonium Poly-phosphate	0	16.2	16.7	19.6	18.5
	0.4	19.2	17.4	19.0	18.9
	0.7	20.1	21.8	21.5	16.5
	1.2	21.4	20.8	22.8	18.7
	1.6	23.9	24.9	20.6	21.8
	2.7	22.3	20.9	23.3	21.3
	8.0	31.0	30.1	26.9	24.4

[1] Zn was applied as ZnO incorporated into either AOP or APP.

[2] Low P was zero P prior to planting; high P was 350 pounds P per acre as 0-46-0 applied prior to planting time.

Table 6. Zn uptake by pea beans as affected by soil P level, planting time P source and Zn rate.

Treatment		Arenac County		Bay County	
Planting time P source	Zn rate[1]	Low P	High P[2]	Low P	High P
	lbs/acre		mg Zn/10 plants		
Ammonium Orthophosphate	0	0.14	0.08	0.16	0.14
	0.4	0.20	0.23	0.18	0.19
	0.7	0.21	0.22	0.21	0.15
	1.2	0.24	0.28	0.27	0.21
	1.6	0.20	0.21	0.18	0.17
	2.7	0.27	0.32	0.18	0.18
	8.0	0.32	0.37	0.22	0.21
Ammonium Polyphosphate	0	0.13	0.12	0.12	0.17
	0.4	0.23	0.21	0.20	0.25
	0.7	0.31	0.32	0.23	0.22
	1.2	0.30	0.35	0.24	0.23
	1.6	0.40	0.40	0.22	0.19
	2.7	0.36	0.34	0.30	0.22
	8.0	0.63	0.55	0.51	0.36

[1] Zn was applied as ZnO incorporated into either AOP or APP.

[2] Low P was zero P prior to planting; high P was 350 pounds P per acre as 0-46-0 applied prior to planting time.

difference. Again a decrease in Zn uptake occurs at the 2.7 pound Zn-APP rate at the Arenac County location on both P levels. This was consistent with the data on Zn content.

Phosphorus content was inversely affected by zinc rate (Table 7). In fact, the 8.0 pound rate of Zn-APP which was significantly better in increasing Zn uptake and content produced significantly lower P contents than any treatment at either location. One result which might have been anticipated was the significant increase in P content produced by increasing the P level. Both locations showed a direct relationship between P level and content. Here the APP proved to be different from the AOP, the latter seemingly a better P source. The reason for this may not be because AOP is a better source of P but as Zn was shown to be less available from the AOP, Zn deficiency might result which in turn might cause a build-up of an unusable P pool in the plant. This was discussed earlier in the literature review.

Much of the information that was found concerning seedling weight, plant content and uptake of Zn corresponds quite well when considering the yield data in Table 8. The P level caused a significant decrease in yield at both locations but the difference was more noticeable in Arenac County. A response was obtained from Zn in Bay County but with the exception of a response over the check and the highest rate correlating well with the highest yield the

Table 7. P content of pea beans as affected by soil P level, planting time P source and Zn rate.

Treatment		Arenac County		Bay County	
Planting time P source	Zn rate[1]	Low P	High P[2]	Low P	High P
	lbs/acre		1% P		
Ammonium Ortho-phosphate	0	0.46	0.59	0.55	0.74
	0.4	0.45	0.58	0.46	0.62
	0.7	0.42	0.56	0.51	0.65
	1.2	0.46	0.50	0.46	0.67
	1.6	0.44	0.54	0.47	0.63
	2.7	0.44	0.44	0.48	0.61
	8.0	0.36	0.42	0.41	0.56
Ammonium Poly-phosphate	0	0.50	0.61	0.53	0.61
	0.4	0.43	0.49	0.40	0.51
	0.7	0.34	0.43	0.40	0.57
	1.2	0.34	0.42	0.40	0.52
	1.6	0.33	0.42	0.42	0.62
	2.7	0.32	0.40	0.43	0.47
	8.0	0.29	0.33	0.38	0.43

[1] Zn was applied as ZnO incorporated into either AOP or APP.

[2] Low P was zero P prior to planting; high P was 350 pounds P per acre as 0-46-0 applied prior to planting time.

Table 8. Yield of pea beans as affected by soil P level, planting time P source and Zn rate.

Treatment		Arenac County		Bay County	
Planting time P source	Zn rate[1]	Low P	High P[2]	Low P	High P
	lbs/acre	bu/acre			
Ammonium Ortho-phosphate	0	16.9	6.2	32.0	31.1
	0.4	19.6	10.7	40.0	33.8
	0.7	20.5	15.1	38.3	32.0
	1.2	22.2	19.6	34.7	29.4
	1.6	24.9	13.3	38.3	34.7
	2.7	24.0	19.6	38.3	32.0
	8.0	29.4	19.6	42.7	33.8
Ammonium Poly-phosphate	0	9.8	5.3	36.5	31.1
	0.4	24.0	16.0	39.2	36.5
	0.7	25.8	17.8	40.9	37.4
	1.2	22.2	16.9	40.9	37.4
	1.6	29.4	19.6	41.8	40.0
	2.7	28.5	18.7	40.0	36.5
	8.0	32.0	21.4	44.5	41.8

[1] Zn was applied as ZnO incorporated into either AOP or APP.

[2] Low P was zero P prior to planting; high P was 350 pounds P per acre as 0-46-0 applied prior to planting time.

results seemed to be inconsistent. The superiority of APP over AOP as a P source in the planting time fertilizer was again significant at both locations. At Arenac County with APP a 14.6 percent increase was obtained over AOP and a 12.7 percent increase was obtained at Bay County. These data show that at both locations the yield from the 2.7 pound rate of Zn-APP was lower than the highest yield reached with lower applications of Zn. This is consistent with the results obtained with Zn uptake and Zn content.

Throughout this study it is noticed that more significant results were obtained on the Arenac County experimental area. This is undoubtedly because of the higher amounts of extractable Zn obtained from the analyses of the soil from the Bay County plot. This would be indicative of the natural ability of the soil to supply Zn to the plant.

A lucid explanation of a consistent decrease in Zn content, Zn uptake and yield with the 2.7 pound rate of Zn-APP might relate directly to Figure 1. As the percentage of water soluble Zn in the APP fertilizer increased, the values mentioned above also increased. As the water solubility decreased at the 2.7 pound rate, the measured values also decreased. Throughout the experiment APP was a significantly better carrier of Zn than AOP. In Table 1 we can see that the water solubility of the Zn in the AOP fertilizer is at a low level. From this information one might conclude that the percent water soluble Zn in a given fertilizer is a very important criteria in determining the effectiveness of the fertilizer as a supplier of micronutrients to the plant. But a direct plot of water-soluble Zn applied against yield gave little indication of a good relationship. However, if some weight is given to the total Zn present in the sample, a better relationship is obtained. An example of such a relationship is given in Figure 2. This indicated that the total value of Zn in a fertilizer is a function of both total and water-soluble Zn in the fertilizer but water-soluble is the more important factor.

Laboratory Incubation Study

From field studies of 1963 and 1964, it was clearly shown that applied P had a significant effect in inducing Zn deficiency in pea beans. As previously discussed in the review of literature, there has been considerable controversy regarding the availability of Zn in a soil high in applied P. In an attempt to clarify this enigma of whether the Zn is rendered unavailable in the soil by high P levels, or if this antagonism is a physiological effect, a calcareous Wisner clay loam was incubated with three levels of P (100, 500, and 1000 ppm) for two and one-half years. To five grams of this soil 25 ml. of a $Zn^{65}Cl_2$ solution containing 10,000 counts per minute per ml. was allowed to equilibrate for one week. Following this treatment the sample was successively extracted with four extracting solutions in the following order: H_2O, 0.01 M EDTA in 1 M $(NH_4)_2CO_3$, 1 N NH_4OAc buffered at pH

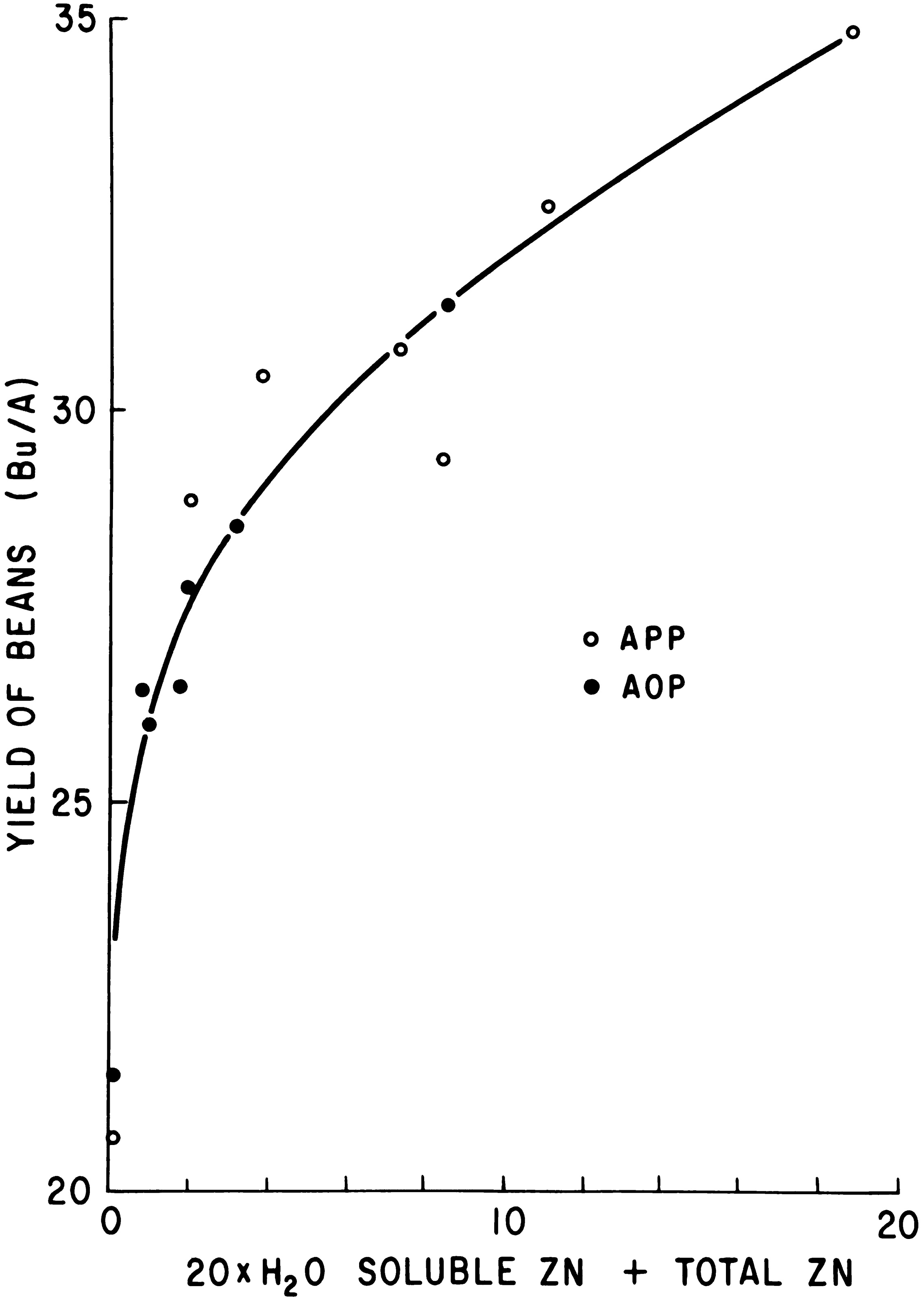

Figure 2. Relationship of water-soluble and total Zn in fertilizer to yield.

Table 9. F ratios from analysis of variance of parameters for Arenac County pea Bean plots -- 1964.

Source	d.f.	F Ratio				
		Plant Weight	Zn Content	Zn Uptake	P Content	Yield
P Level (P_L)	1	0.4	14.7*	0.0	98.1**	65.7**
Zn Rate (R)	6	35.0**	21.5**	352.9**	25.3**	58.0**
Planting Time P Source (P_S)	1	19.0**	68.5**	490.2**	62.5**	14.4**
Interaction RxP_S	6	2.3	7.6**	103.9**	4.4**	6.5**
Interaction P_LxRxP_S	6	1.0	1.6	15.2**	4.0**	4.0**

*Significant at 5% level.
**Significant at 1% level.

Table 10. F ratios from analysis of variance of paremeters for Bay County pea bean plots -- 1964.

Source	d.f	F Ratio				
		Plant Weight	Zn Content	Zn Uptake	P Content	Yield
P Level (P_L)	1	.81	5.4	0.3	1,716.7**	9.6
Zn Rate (R)	6	3.0*	5.9**	5.9**	6.8**	8.4**
Planting Time P Source (P_S)	1	2.5	34.8**	11.0**	31.0**	35.8**
Interaction RxP_S	6	.9	2.6	3.0**	0.4	1.7
Interaction P_LxRxP_S	6	9.5**	5.2**	9.4**	1.2	1.2

* Significant at 5% level.
** Significant at 1% level.

7.0, and 0.1 N HCl. Each of these solutions have been used to characterize available soil Zn. The extracting order was selected to give a sequence of decreasing suspension pH.

Very small quantities of Zn^{65} were found in the water extract. In fact this fraction included less than two percent of the Zn^{65} which was removed by the four extractions. As shown in Table 11, there is little indication that applied P was influencing the water-soluble Zn level. Certainly, if P and Zn are precipitating in the soil, the water soluble Zn^{65} should have decreased with increasing level of P.

Extraction of the soil with EDTA buffer solution removed greater than 85 percent of the recovered Zn^{65}. In addition, the quantity of Zn^{65} recovered increased with increasing P level. Thus, an additional 11,000 counts per minute were recovered in this fraction where 1,000 ppm P were added as compared to 100 ppm P. Colorado researchers (60) have shown that this extracting solution is one of the best for estimating available soil Zn. Consequently, this data show that addition of high levels of P increases rather than decreases the availability of Zn in soils.

The third extraction with 1N NH_4OAc removed about 10 percent of the Zn^{65}. Because of the lower pH of this solution it should be expected to remove more difficultly available Zn. As the amount of P applied prior to incubation increased, the quantity of Zn extracted by this reagent decreased consistently. This result can be explained because of the effect the applied P has on making Zn more easily

Table 11. Influence of P added to Wisner Clay Loam soil on availability of Zn.

Extraction number	Extracting solution	P level (ppm)		
		100	500	1,000
1.	H_2O	2,000	3,350	2,550
2.	Na-EDTA-$(NH_4)_2CO_3$	193,500	197,400	204,500
3.	NH_4OAc	23,200	20,350	17,300
4.	HCl	8,450	6,700	6,250
Total	---	227,150	227,800	230,600

available and thus extractable with the EDTA buffer. The quantity of Zn present in the more difficultly available forms is less.

Finally, the sample was extracted with 0.1 N HCl which should remove Zn which had precipitated as a basic compound when the equilibrium was established between the $Zn^{65}Cl_2$ and the soil. This fraction contained less than four percent of the Zn^{65} which was extracted. This fraction was similar to that extracted by NH_4OAc in that the quantity extracted decreased with increasing P level.

As the EDTA extracting agent is assumed to give an estimate of the readily available Zn, the results of this experiment indicate that when the P level of the soil is increased by heavy fertilizer applications, the amount of available Zn is also increased. The data actually shows a slight increase in the total Zn^{65} replaced when high levels of P had been applied, but this increase is well within experimental error. Collectively, this is strong evidence that the Zn-P antagonism does not occur in the soil due to formation of insoluble Zn phosphate compounds as has been suggested by some early work.

Nutrient Culture Studies

The hypothesis was made that the antagonistic effect of P on Zn was of a physiological nature. Solution culture studies were initiated to test this hypothesis under a controlled environment, permitting more efficient observations of P effects without interference from adsorption of P and Zn by soil.

Pea bean seedlings, which were germinated in quartz sand, were transplanted into two-quart polyethylene containers that held two seedlings each. The treatments were three levels of P (100, 250, and 500 ppm). One half of each level contained Zn in a concentration recommended by Hoagland and the other half received no Zn. Each treatment was established in duplicate.

The main objective of the research at this time was to determine if P prevented Zn uptake from solution culture and if so, the location within the plant where the Zn was blocked and made unavailable for use. The plants were allowed to set pods since it was believed that at seed formation the plants requirements for Zn was increased putting added Zn stress on the plants. The plants which were Zn deficient were extremely chlorotic and in some instances necrotic. At this time $Zn^{65}Cl_2$ was added to all pots for 48 hours. The plants were constantly aerated during this period as well as during the entire growing period by forcing air through gas dispersion tubes which were in each container. The plants were harvested, roots washed, separated into roots, stems and leaves, dried in a forced air oven at 60 C, and ground in a Wiley mill. The tissue was then weighed into one gram portions, pelleted by a Carver hydraulic press and analyzed for gamma ray activity by a scintillation crystal and counter.

The results in Table 12 show that regardless of prior Zn treatment, most of the added Zn^{65} was not translocated further than the roots. And this was also true to a large degree at all P levels. The data do indicate, however, that greater quantities of Zn^{65} were absorbed into the roots at higher P levels. Plants which were produced in normal Hoagland's solution held more Zn^{65} in their roots as compared to the Zn deficient plants. However, these differences were small except for the 250 ppm P level. The total activity found in the stems and leaves was so low that any differences between treatments may be due to experimental error. More total Zn^{65} was adsorbed by plants produced under high P levels, but this reflects little more than the data for the roots.

The experiment was repeated as before except that the plants were given $Zn^{65}Cl_2$ at the onset of flowering and at a level 10 times the previous dosage. This was done to: (1) ascertain if the age of the plant affected Zn translocation and (2) permit a more accurate measurement of the translocation pattern because of the greater activity supplied to the plants.

As in the previous experiment, the largest portion of the added Zn^{65} was blocked in the roots (see Table 13); however, a greater amount of the Zn^{65} moved from the roots and into the stem and leaves, than in the previous experiment. In this experiment, from 25 to 37 percent of the Zn^{65} absorbed was found in the stems and 8 to 13 percent

Table 12. Uptake of Zn^{65} as affected by P level and Zn content of nutrient solution cultures--pea beans harvested at pod set.

Plant Part	Zn level of nutrient solution	P level of nutrient solution		
		100 ppm	250 ppm	500 ppm
		Zn^{65} in counts/minute/gm*		
Roots	none	4853	5203	6853
Roots	Normal Hoagland	5453	6753	7053
Average		5153	5978	6953
Stems	None	153	503	53
Stems	Normal Hoagland	78	103	3
Average		115	303	28
Leaves	None	13	213	18
Leaves	Normal Hoagland	28	13	3
Average		20	113	10
Total Zn^{65} Absorbed	None	5019	5919	6924
	Normal Hoagland	5559	6869	7059

*Each value reported is a mean of two replications.

Table 13. Uptake of Zn^{65} as affected by P level and Zn content of nutrient solution cultures--pea beans harvested at bloom stage.

Plant Part	Zn level of nutrient solution	P level of nutrient solution		
		100 ppm	250 ppm	500 ppm
		Zn^{65} in counts/minute/gm*		
Roots	None	11,500	15,500	11,650
Roots	Normal Hoagland	8,500	8,500	11,500
Average		10,000	12,000	11,575
Stems	None	6,300	6,300	8,500
Stems	Normal Hoagland	3,600	3,300	5,500
Average		4,950	4,800	6,500
Leaves	None	1,700	2,100	2,730
Leaves	Normal Hoagland	1,200	1,250	2,650
Average		1,450	1,675	2,690
Total Zn^{65} Absorbed	None	19,500	23,900	22,880
	Normal Hoagland	13,300	13,050	19,650

*Each value reported is a mean of two replications.

in the leaves. With the older plants in the previous experiment from 88 to 99 percent of the Zn^{65} remained in the roots. This may have been because of a greater metabolic activity of the younger tissues. In this experiment the plants growing in normal Hoagland's solution absorbed less Zn^{65} than the Zn deficient plants. This may be explained by the competition of the Zn^{65} with nonradioactive Zn in the normal Hoagland's solution which reduced Zn^{65} uptake. This would be expected to be more evident in the younger, more metabolic plants of the latter experiment.

Results from the prior two experiments show that Zn is held in the root cells or at the surface of the cells in pea bean plants produced under high P conditions. To more closely pinpoint the exact location of this Zn-P antagonism an experiment was performed to ascertain if the Zn was precipitated within the cell or at the cell surface.

Pea bean plants were grown as before with the exception that none of the seedlings were given Zn. The plants were allowed to grow until budding when the plants from one-half of the containers at each P level were removed from their medium, their roots dipped into deionized water for 15 seconds to wash away excess P from the nutrient solution and then transferred to a normal Hoagland's solution minus P. After 24 hours, $Zn^{65}Cl_2$ was added to all containers. Forty hours later all plants were removed, dipped into deionized water for 15 seconds and divided into roots and tops. The tissue samples were treated as before. The nutrient solutions were analyzed for P at the completion of the experiment to ascertain the quantity of P which had moved from the plants to the containers.

The data from this experiment are given in Table 14. The roots that were washed for 24 hours had less Zn^{65} present in them than those which were not washed. But the most striking results are those from the tops. The Zn^{65} activity from the tops whose roots were washed was nearly ten times greater than the activity from the tops whose roots were not washed free of P. These results clearly indicate that as the P is removed from the roots by washing, the Zn is free to be adsorbed and translocated to the top of the plant. If the P remains concentrated in the roots, the Zn^{65} accumulates and very little is able to move from the root system.

From these results it is postulated that an insoluble Zn-P complex is formed in the root cortex or at the membrane surface itself making translocation of Zn difficult if not impossible. If this solid phase is dissolved because of the absence of orthophosphate ions in the medium, the Zn^{65} is released from the complex and is accumulated by the plant. Table 14 shows that roots produced in high P solutions released approximately 9 ppm P to the solution during 24 hours washing; whereas, the roots produced in low P solutions released less than 1 ppm P to the washing solution. This does show that the P accumulated by the pea bean roots in high P solutions is free to move to external solutions and

Table 14. Uptake of Zn^{65} as affected by P level of nutrient solution and washing of roots.

Treatment		P in solution at completion of Experiment	Zn^{65} Uptake		Fresh Weight	
P level	Washing time*		Roots	Tops	Roots	Tops
ppm	hours	ppm	Counts/min/gm		gm/pot	
100	none	n.a.	5,139	318	27.7	29.7
100	24	< 1	4,745	3,103	23.0	21.3
250	none	50	6,207	408	25.9	24.5
250	24	1	4,634	3,221	24.3	26.7
500	none	110	6,804	453	23.3	22.3
500	24	9	3,834	2,827	22.7	26.9

*Washing was accomplished by placing plants in aerated, Hoagland's minus P and Zn nutrient solution for 24 hours.

thus, is assumed to be external to the cell interior.

Two questions that remain unanswered from the previous experiment are: (1) was the Zn^{65} able to translocate as a result of new growth which may have been stimulated by washing and (2) does the P in solution have an effect in Zn utilization? To answer these questions, a final experiment was conducted. Pea beans were grown in high P (500 ppm) nutrient solution until one week prior to bloom stage. Zn was added at the end of the first week at a 0.01 ppm level to prevent Zn deficiency from becoming so severe as to prevent growth. The plants were showing marked Zn deficiency during the entire experiment. Plants were washed as in the prior experiment by placing them in Hoagland's solution minus P and Zn for varying periods of time (0, 3, 6, 12, and 24 hours). At the end of the washing period the plants were transferred to nutrient solution containing either 60 or 500 ppm P and $Zn^{65}Cl_2$. After a period of 36 hours the plants were removed, dipped into deionized water, separated into roots, stems, and leaves, and analyzed as before.

It was hypothesized that if new growth accounted for the increased uptake of Zn^{65}, the effect should uniformly increase with length of washing period. If on the other hand the antagonism is due to the P in existing roots as previously hypothesized, the greatest effect should be seen during the early washing periods because the rate of dissolution of the suggested complex would be greatest at that time. By precisely controlling the level of P in the nutrient solution at the time of uptake, the effect of P in the solution on uptake may be ascertained.

The results of this experiment are given in Table 15. The results for the P removed during the washing period are summarized in Figure 3. A rapid loss of P occurred from 0 to three hours, followed by a linear loss. When expressed on a percent basis (mg P×100/mg dry roots), the P lost varied from 0.6 percent in three hours washing to 1.4 percent in 24 hours washing.

With increasing periods of washing up to six hours there was a decrease in the amount of Zn^{65} in the root system when the plant was placed in a high P solution during the uptake period. And there was a corresponding increase in the translocation of Zn into the tops. Increased washing beyond six hours had little effect on the Zn^{65} content of any plant part. Little weight was given to the 12 hour points since one replication at this treatment had to be discarded because one plant in each container was quite small compared to other plants in the experiment.

Despite washing of the roots the beans placed in a normal P environment did not change in their Zn^{65} uptake. But there was a marked increase in the Zn^{65} in the tops with increased washing time with the greatest change occurring up to 12 hours washing time.

Evidence strongly indicates that as the P is washed from the root system, Zn is free to translocate to the tops.

Table 15. Uptake of Zn^{65} as affected by P level of nutrient solution and washing time of roots.

Washing time*	P level during uptake period	Zn^{65} Uptake			Dry Weight Yield		
		Roots	Stems	Leaves	Roots	Stems	Leaves
hours	ppm	counts/minute/pot			gm/pot		
none	60	24,971	12,260	9,123	0.34	0.49	0.76
none	500	27,994	11,569	6,362	0.45	0.52	0.96
3	60	25,623	10,796	8,410	0.55	0.62	1.24
3	500	26,214	13,427	8,290	0.36	0.51	0.96
6	60	25,359	12,848	11,685	0.28	0.48	0.89
6	500	24,716	13,204	10,948	0.34	0.42	0.78
12	60	26,813**	13,028**	15,900**	0.55**	0.72**	1.60**
12	500	28,142**	10,813**	12,650**	0.52**	0.62**	0.77**
24	60	25,548	12,917	13,185	0.44	0.67	1.04
24	500	24,800	10,819	10,500	0.38	0.62	0.72

*Washing was accomplished by placing plants in aerated, Hoagland's minus P and Zn nutrient solution.

**Includes one sample only. Other values are a mean of two replications.

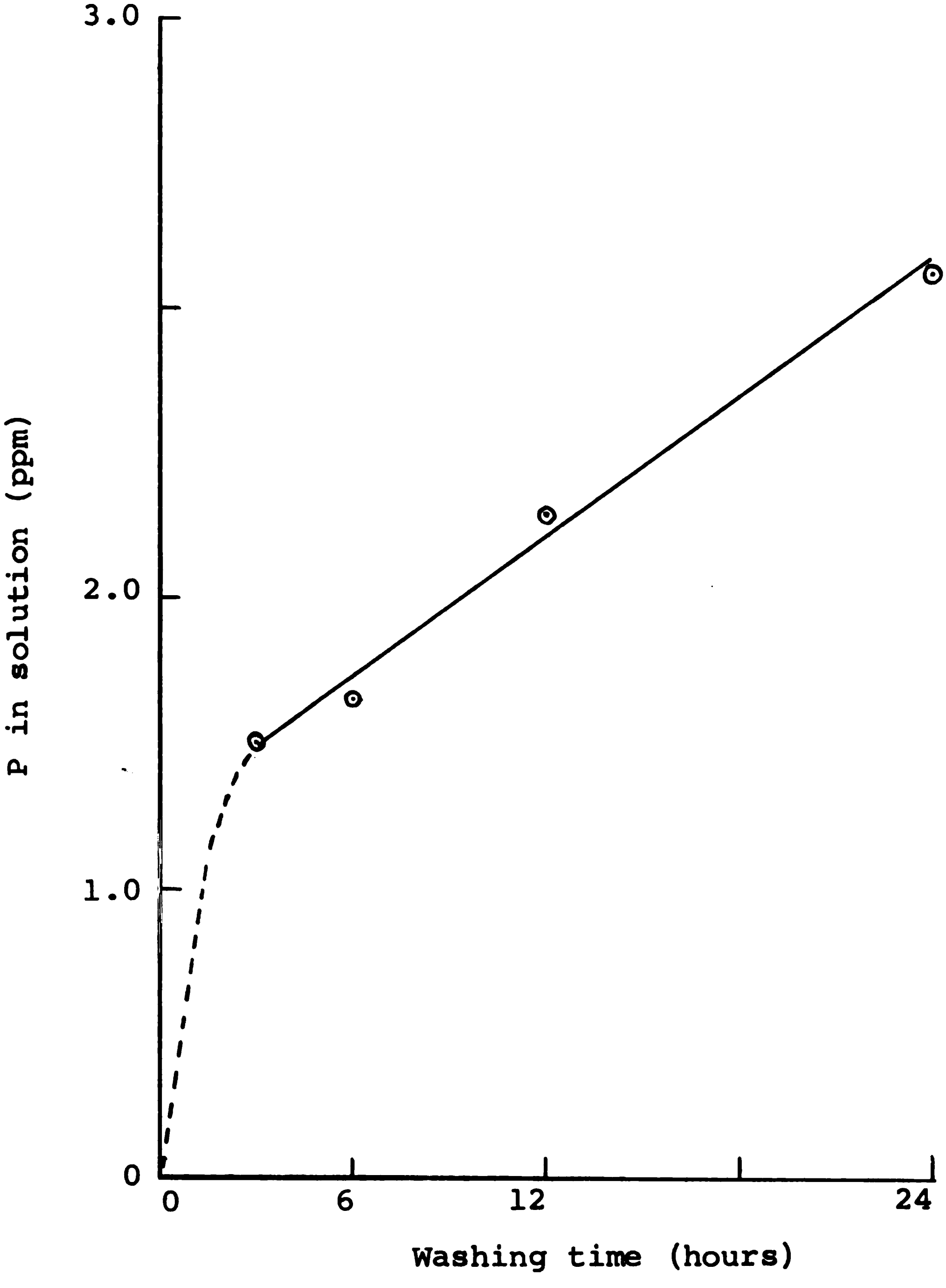

Figure 3. Effect of washing time on P removal from pea bean plants.

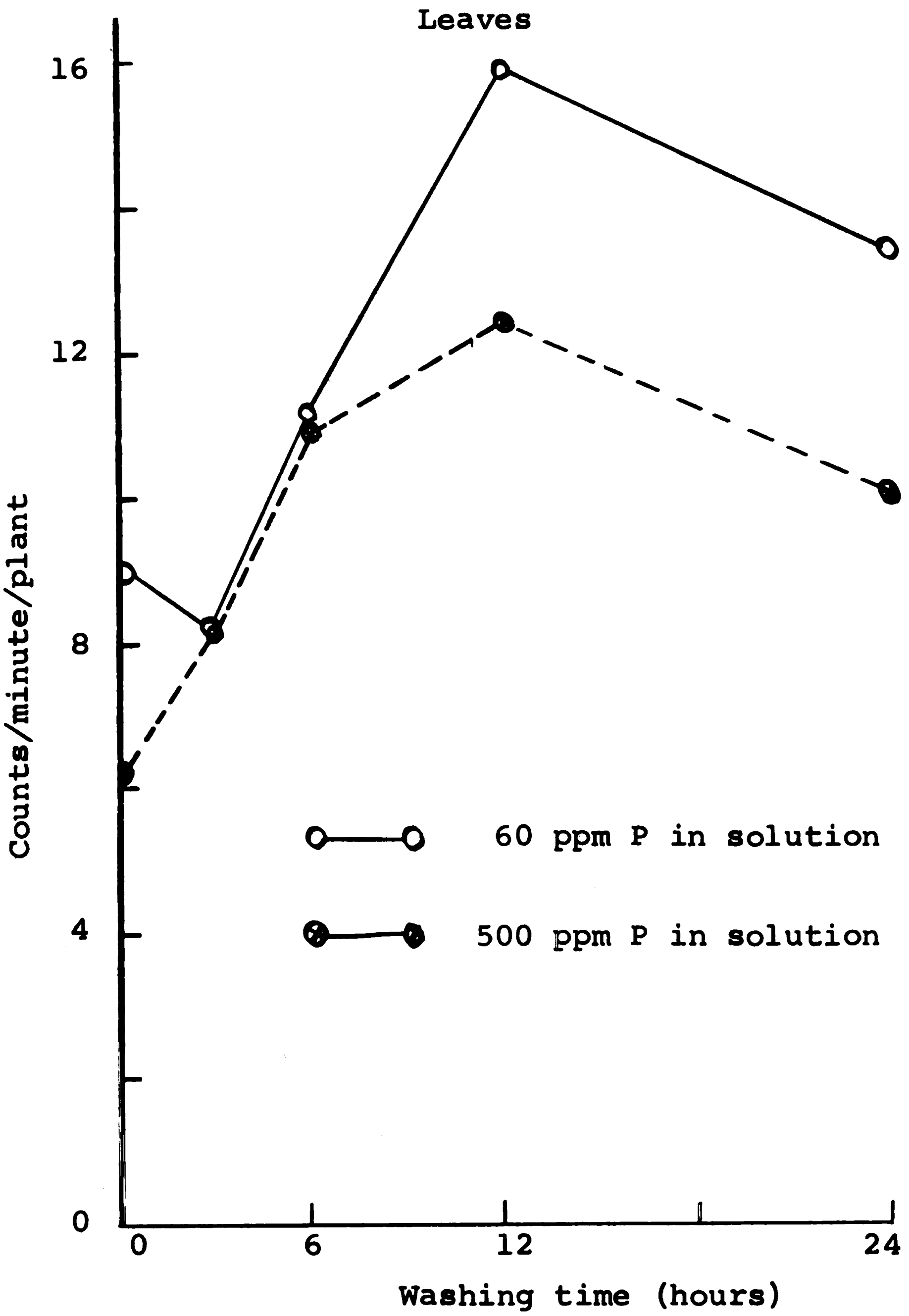

Figure 4. Effect of washing time and P content of nutrient solution on uptake of Zn^{65} by pea bean ~~leaves~~.

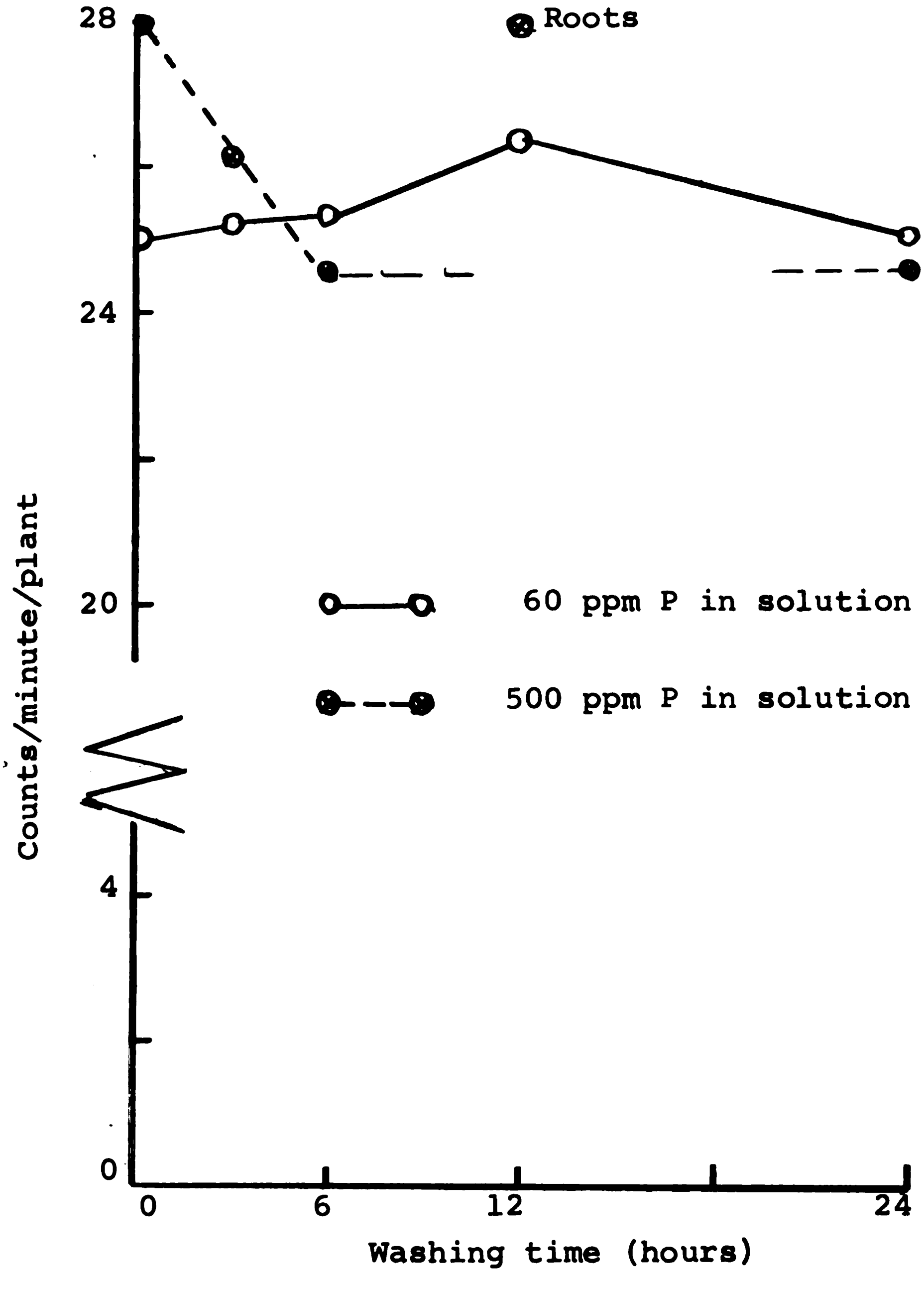

Figure 5. Effect of washing time and P content of nutrient solution on uptake of Zn^{65} by pea bean roots.

This is strong support for the hypothesis that at or near the cell membrane an insoluble Zn-P complex forms either alone or with other components of plant metabolism. A subsequent removal of this P then frees the Zn for plant use.

P content of the nutrient solution at the time of uptake was found to have a definite influence on Zn translocation. Data shown in Figure 4 indicated that up to a washing time of 6 hours the Zn^{65} translocated to the leaves was similar under both P levels. However, at the conclusion of the experiment there was a large difference between quantities of Zn^{65} translocated to the leaves. Thirty percent more was translocated to the leaves of the plants in normal Hoagland's solution at this time as compared to the plants grown in solution containing 500 ppm P. This might be expected since the longer the plants were grown in a high P environment the more likely they would be to revert to the high P condition that existed prior to the washing. The insoluble complex would certainly have adequate time to reform after a six hour period in this unfavorable environment. It does indicate that the effect of the high P in the nutrient solution is to produce high P roots, rather than to precipitate $Zn_3(PO_4)_2$ solution.

SUMMARY AND CONCLUSIONS

Zinc deficiency has long been associated with calcareous soils. More recently reports indicate that a high P soil level is usually detrimental to Zn utilization. Some reports indicated that the nature of the antagonistic effect of P was the formation of an insoluble precipitate in the soil itself while other showed indirect evidence that P-Zn antagonism is physiological in nature and that Zn is precipitated at or very near the surface of the root cells or possibly inside the root cells themselves. The latter seemed quite feasible as previous reports have indicated the presence of an abnormally large inorganic P pool as a result of an inadequate supply of Zn.

Recently Michigan farmers have found that pea beans are very susceptible to P induced Zn deficiency. Farming practices on the calcareous soils of the lake-plain region of East Central Michigan are generally characterized by a sugar beet-pea bean rotation. This resulted in high P levels that are required for high sugar beet yields and therefore in widespread Zn deficiencies.

Field experiments conducted in 1963 confirmed the fact that high P levels do accentuate a Zn deficiency in pea beans. Of the several materials used to correct the deficiency, $ZnSO_4$ mixed with APP was the most effective in both supplying Zn to the plant and in increasing yield. This was followed by Zn EDTA (applied at one-fifth the rate for inorganic carriers), and finally by Zn_2OSO_4 which was not an efficient Zn source. ZnO incorporated into granular APP was superior to ZnO incorporated into AOP. A trend was also noted which indicated that increasing increments of Zn in the APP fertilizer gave yield increases which did not follow a normal growth curve. Results also confirmed that the critical level of Zn content in pea bean tissue is 15 to 20 ppm.

Because of the striking differences obtained between ZnO incorporated into AOP as compared to APP and because the response curve with ZnO-APP appeared to be atypical in the low Zn rates, the 1964 field studies were initiated to investigate these pheonomea.

Fertilizers were supplied by TVA with variable Zn:P ratios. Water solubility studies showed that ZnO incorporated into AOP was no more than 0.03 percent water soluble at any ratio indicating that Zn is transformed into compounds less soluble than ZnO. ZnO incorporated into APP showed an increasing solubility up to a value of one percent Zn in the fertilizer and then decreased suggesting that at high Zn:P ratios a soluble complex is formed between the Zn and polyphosphoric acid. This system becomes supersaturated at the lower ratios and an insoluble precipitate is then formed. Most of the additional Zn added beyond this point is converted into an insoluble compound.

Results from 1964 conclusively showed that Zn supplied at an eight pound rate as ZnO incorporated into APP was superior to all other treatments as measured by early growth, Zn content, uptake and yield. For APP, yield and uptake data reflected the water solubility curve.

The dramatic effect of the soil P level was again noticed. High amounts of P were so detrimental to plant utilization of Zn that even at the highest rate of applied Zn, the yield at the high P level never attained the level equal to that on low P soil.

These experiments indicated that water solubility of Zn in the applied fertilizer is important in estimating fertilizer efficiency. But, a rather close relationship is obtained if the total Zn in the fertilizer is considered along with water solubility, the latter being the more important.

To clarify the question of high soil P levels affecting Zn availability in soils, a Wisner clay loam was incubated with three P levels and then allowed to equilibrate with a $Zn^{65}Cl_2$ solution. The soil was successively extracted with four agents (H_2O, 0.01 M EDTA in 1 M $(NH_4)_2CO_3$, 1 N NH_4OAc buffered at pH 7, and 0.1 N HCl) to characterize the availability of soil Zn. Very small quantities of Zn^{65} were found in the water extract. There was, in fact, little indication that applied P had any influence on the water-soluble Zn level. Extraction with EDTA buffer solution removed greater than 85 percent of the recovered Zn^{65}.

And the Zn removed increased with increasing soil P levels. This extracting solution had previously been found to be one of the best for estimating available soil Zn. The final two extractions removed Zn^{65} in an inverse relationship to the soil P level. The NH_4OAc extracts the difficulty available Zn and the 0.1 N HCl extracts the Zn which precipitated as an insoluble compound. The results indicate that with increasing levels of soil P the amount of easily available Zn also increases. This would then leave less in the fractions that represent the difficultly available and insoluble portions. This evidence points to the fact that P-Zn antagonism does not occur in the soil.

In nutrient culture studies, Zn^{65} was not translocated beyond the roots of plants produced in high P solutions. To pinpoint the exact location of the Zn-P antagonism plants were grown in Hoagland's solution with high P (500 ppm) minus Zn. Before harvest one-half of the plant roots were washed with normal Hoagland's solution minus P for 24 hours and then all plants were given Zn^{65}. Although the roots that were washed had less Zn^{65} in them than those not washed, the most dramatic effect was that the tops from the washed roots contained ten times more Zn^{65} than tops from roots which were not washed. This is strong evidence that an insoluble Zn-P complex is formed in the root cortex or at the membrane surface making Zn translocation very difficult.

To answer two additional questions--(1) was the Zn^{65}

able to translocate as a result of new growth which may have been stimulated by washing and (2) does the P level in solution have an effect in Zn utilization,--a final experiment was conducted. Pea beans that were grown in high P nutrient solution until one week before budding were washed in Hoagland's solution minus P and Zn for varying lengths (0, 3, 6, 12, and 24 hours) and then one-half were transferred back to a normal Hoagland's solution and the remainder to Hoagland's with 500 ppm P. All cultures received $Zn^{65}Cl_2$ for 36 hours. It was found that washing time had a direct influence on Zn translocation up to six hours under both P levels, i.e., the greater the P removed the more Zn^{65} translocated. At the end of 24 hours there was 30 percent more Zn^{65} translocated to the leaves of plants grown in normal Hoagland's as compared to the solution containing 500 ppm P.

From these results it is concluded that an insoluble complex is formed near the surface of the cell membrane involving Zn, P and possibly other plant metabolites. If the P is removed the complex is dissolved and then Zn is free to move into the plant. But if P is again increased in the solution the complex will again reform to its previous state.

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