THE UNIVERSITY OF MANITOBA

INTERACTIONS OF PHOSPHORUS AND ZINC IN THE NUTRITION OF CEREAL AND OILSEED CROPS AND THE MECHANISMS OF PHOSPHORUS-INDUCED ZINC DEFICIENCY IN WHEAT

by

SHIHUA TU

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Submitted to the Faculty of Graduate Studies
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ABSTRACT

A study was conducted on an Almasippi sandy loam soil to examine the interaction of P and Zn in canola and barley as affected by P and Zn fertilization, and their residual effects on wheat in two rotations in the field. Investigations of the mechanisms of P-induced Zn deficiency and the effect of vesicular-arbuscular mycorrhizal (VAM) fungus on plant nutrition and growth were also conducted in a growth chamber study.

The field experiment was conducted by using a completely randomized split-split plot design. Phosphorus, as the main factor with three rates(0, 100, 250 kg $P.ha^{-1}$), was added in the first year; Zn, as the subplot factor with two rates(0, 20 kg $Zn.ha^{-1}$ and 0, 10 kg $Zn.ha^{-1}$ respectively), was split in the first and second year; N and K were added in both years as basal fertilizers. Barley and canola were grown in the first year and wheat was selected as the subsequent crop.

The results revealed that both barley and canola showed significant response to the added P, but only canola exhibited sensitivity to low soil Zn (0.7 mg $Zn.kg^{-1}$) and hence significantly responded to added Zn. In the second year, fertilizer P had pronounced residual effect on the wheat grain yield on the barley stubble but not on the canola stubble. With respect to Zn it seemed that Zn addition was required in the second crop only when continuous cereal cultivation was practiced with no Zn fertilization in the first year. Zn fertilization enhanced P concentration and uptake in the plant in most cases, suggesting that Zn is essential to P utilization by the plant. The effects of added P on Zn concentration and uptake were complicated

in this experiment. In the first year, added P greatly reduced Zn concentration in canola and barley at all the levels of Zn fertilization. The interaction between P application and Zn uptake was negative in the canola crop but this was not manifested in the barley crop when there was concomitant use of both P and Zn fertilization. In the second year, the residual P in the soil depressed Zn concentration and uptake by wheat on canola stubble but enhanced Zn concentration in straw and total uptake by plants on barley stubble. The intriguing residual effect of P and Zn in different rotations may be ascribed to allelopathic effects arising from the interactions between plants, microbes and their assimilation products in the soil.

The results further suggest that the interaction between P and Zn was also likely to take place in the calcareous soil. Furthermore, different crops and rotations tend to change the interaction patterns both in the soil and within plant tissues even under the same environmental conditions.

The growth chamber experiment was carried out as a randomized complete block design with four rates of P (0, 50, 100 and 300 mg $P.kg^{-1}$ soil), three rates of Zn (0, 2.5 and 10 mg $Zn.kg^{-1}$ soil), two rates of VA mycorrhizae (-VAM and +VAM) with three replicates. Soils were pasteurized in an Automatic Soil Pasteurizer and the mycorrhizae were re-introduced by using VAM infected corn roots from a previous batch culture.

The results revealed that the high rates of P added to the soil were able to induce the symptoms on the wheat leaves resembling Zn deficiency in appearance but being P toxicity in nature. This

conclusion was based on the facts that both incidence and severity of the symptoms were not related to low Zn concentration but well correlated with high P concentration in the plant tops at the heading stage. The analysis further indicated that a concentration of >6.0 g $P.kg^{-1}$ top dry matter at heading tended to produce severe P toxicity symptoms in the wheat plant in this study.

Mycorrhizal infection had a striking effect on nutrient uptake and the growth of wheat. At the early growth stages of wheat, it was observed that mycorrhizal inoculation significantly depressed the number of tillers, height of the plants, dry matter yields and all the nutrients determined: Zn, Cu, Mn, Fe and especially P. Therefore, the number of leaves showing symptoms of P toxicity was greatly reduced in the mycorrhizal plants. After heading, however, the mycorrhizae-infected plants dramatically exceeded the non-mycorrhizae-infected plants in total uptake of the nutrients and growth rate, especially in grain production. The results suggested an overall beneficial impact of mycorrhizal inoculation on the wheat plant.

Mycorrhizae was also found to be involved in modifying the patterns of the P x Zn interaction in the wheat plant. At the heading stage, the concomitant addition of P and Zn increased Zn concentration and uptake when treated with P50 but without VAM, and then markedly decreased with further increase in P application. In contrast, the concentration of Zn was significantly decreased at P50 and then significantly increased with further increase in P application in +VAM treatments.

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CHAPTER ONE

INTRODUCTION

Interactions among nutrients are ubiquitous phenomena in soil fertility and plant nutrition. Of paramount interest, however, is the interaction of P and Zn. This is not only because of the unique fundamental metabolic roles of P and Zn in living organisms but also because of their mysterious interaction mechanisms having puzzled soil scientists and plant nutritionists for many years.

Interaction is generally referred to as that sum of individual influences of two or more factors being modified when they are present together. If factors in combination result in a growth response greater than the sum of their individual effects, the interaction is positive; if smaller, it is negative. Additivity indicates the absence of interaction. There is no doubt that this definition can be applied to describe the relationship between any two or more nutrients. In terms of P and Zn, however, it appears that the majority of investigators between 1930's and 1960's only observed a negative interaction, viz. increased P application reduced Zn uptake and tended to induce Zn deficiency symptoms in plants. They have, therefore, designated the interaction of P and Zn as "P-induced Zn deficiency", and these terms have been interchangeably used in literature. Unfortunately, the interchangeable use of the two terms sometimes gives rise to confusion when the negative interaction occurs but without any sign of presence of Zn deficiency, and further leads to the dilemma of whether high P is able to induce Zn deficiency when the situation of either positive or no

interaction does occur. Therefore, the work on interaction of P and Zn, if it were only for distinguishing the different terms from the confused situations, is very necessary and meaningful.

Among nutrients applied, phosphorus, required in the largest quantity second only to nitrogen, is present in all plants and animals. It plays an essential role in life processes such as photosynthesis, the synthesis and breakdown of carbohydrates, and the transfer of energy within plants. Zinc, on the other hand, although required in very minor quantity, exerts striking effects on plant growth. It acts either as a metal component of enzymes or as a functional, structural, or regulatory cofactor of a large number of enzymes. An insufficient supply of either element from a medium usually slows down the metabolic processes it involves, retarding growth of plants and rendering poor yield and quality of their products. Excessive supply, on the other hand, can also inhibit growth, leading to reduction of yield and quality of the plants due to toxicity.

Apart from this, high rates of P application can also cause problems to other nutrients in plants due to negative interactions. Zinc, as has been frequently reported in literature, is one of the most vulnerable victims to high P among the essential nutrients. It is believed that heavy dressing of P fertilizers, as mentioned above, tends to depress Zn concentration in plants and results in Zn deficiency of the plant, hence inhibiting plant growth and lowering both grain yield and quality. Consequently, with a worldwide acceptance of increasing use of P fertilizers as a measure of high production in modern agricultural cropping systems, research on the interaction mechanisms of P and Zn is desperately needed and is obviously of utmost significance

both in effective management of P and Zn to improve yield and quality of agricultural produce in practice and in understanding of their behavior in theory.

Great attention has been attracted to the subject of P-induced Zn deficiency since the 1930's. However, the interpretations of this phenomenon are still controversial and frustrated, often because the experiments can seldom show consistent results. Furthermore, the explanations of different authors are frequently fraught with many apparent contradictions, thus requiring further substantiation.

As far as the past research into P-induced Zn deficiency is concerned, attention was initially focused on the amounts of P applied, and later on extended to plant species and some environmental factors. It is obvious that the interaction of P and Zn cannot be simply generated as affairs of P and Zn alone. Instead, many other factors may contribute to the interaction and should be taken into account in research. Therefore, this study considers some additional factors, with emphasis on effect of rotations, residual effect of P and Zn, and VA mycorrhizae inoculation.

The objectives of this study were: (1) to investigate P and Zn fertilization and their residual effects on crops as well as on the interaction in field cropping systems; (2) to assess the effects of different rotations on the interaction; (3) to test whether high rates of P are able to induce Zn deficiency or not; (4) to examine the mechanisms of P-induced Zn deficiency if existing; (5) to study the impact of vesicular-arbuscular mycorrhizae on the interaction of P and Zn.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The subject of the interaction between phosphorus and zinc has been reviewed extensively by Olsen (1972), Adams (1980) and Sumner and Farina (1986). However, there is still a lack of agreement on whether P can induce Zn deficiency, and, if existing, what the mechanisms of "P-induced Zn deficiency" are.

Before the 1950's, researchers frequently reported that they were able to induce Zn deficiency symptoms in plants with high rates of P (West, 1938; Reuther, 1946; Millikan, 1946,1947; Loneragan, 1951). By contrast, Boawn et al. (1954) and Ellis et al. (1964) reported that they were unable to induce Zn deficiency symptoms in corn and beans with high rates of P application, even though P concentration in the plant tissue was doubled (Boawn et al., 1954). Furthermore, Orabi et al. (1981, 1982, and 1985) repeatedly reported that there was a positive relationship between Zn and P uptake by corn plants.

In spite of the confusion and controversy in the literature, most investigators still hold the view that high levels of P are able to induce Zn deficiency in plants. But, the dispute of whether the interaction, per se, takes place in soils or within the plant has not been made clear. Pauli et al. (1968) conducted a sand culture experiment with ^{32}P and ^{65}Zn to determine the influence of $CaCO_3$ and P on the occurrence of Zn deficiency in navy bean plants (*Phaseolus*

vulgaris). They found that adding $CaCO_3$ decreased the translocation of Zn, and increased translocation of P from roots to leaves, and decreased water extractable P and Zn in the soil. High P, on the other hand, increased water extractable Zn. Other workers (Bingham and Garber, 1960; Brown et al., 1970; Sharpe et al., 1983; Pasricha et al., 1987) also reported that applying P to the soil increased Zn intensity in the soil solution. Even so, the symptoms of the so-called "P-induced Zn deficiency" were observed in the plants, implying that the interaction may have occurred in the plant. However, Marschner and Schropp (1977) found that increased P supply substantially decreased Zn content and induced Zn deficiency symptoms in soil-grown plants, but did not affect Zn content in plants grown in nutrient solution. Similarly, Bingham (1963) observed no significant depression of Zn levels in plants grown in nutrient solution as P concentrations were increased from 1 to 100 mg $P.kg^{-1}$, and concluded that no physiological effect accounted for a P and Zn interaction. These findings seemed to have supported the view that the interaction of phosphorus and Zn took place not in the plant but in the soil (Marschner, 1986; Ghoneim and Bussler, 1980; and Loneragan et al., 1979).

2.2 The Distinction between the Simple Zn Deficiency and the "P-induced Zn Deficiency"

a. The simple Zn deficiency -- Zinc deficiency is widespread among plants grown in highly weathered acid soils and in calcareous soils (Marschner, 1986). Although the degree and pattern of Zn

deficiency symptoms vary with plant genotypes (Halim et al., 1968), a characteristic feature of all plants suffering from Zn deficiency is retardation of growth, with almost complete cessation of internode growth or "rosetting". The leaves may reach only 1/10 - 1/20 of their normal size in apple and other deciduous fruit plants (Shkolnik, 1984), and appear as "little leaves" in subterranean clover (Millikan, 1963). Quite often these symptoms are combined with chlorosis (Marschner, 1986), and further develop into necrosis (Millikan, 1963). In cereals such as maize, chlorotic bands along the midrib and a red, spotlike discoloration (caused by anthocyanins) on the leaves often occurs. On the old leaves, visual symptoms and necrosis are most likely the result of P toxicity (Marschner, 1986). In dicotyledons the symptoms may be similar in appearance to virus infection (Shmidt et al., 1972).

It is recognized that different sensitivity to Zn deficiency can be observed not only among different plant species, but also among different varieties of the same species (Shkolnik, 1984). Even so, the critical levels are usually below 15 - 20 mg $Zn.kg^{-1}$ dry weight of leaves (Marschner, 1986). Under conditions of Zn deficiency, grain and seed yield tend to suffer most, because Zn seems to play a specific role in the sexual fertilization (Polar, 1975). Pollen grains were found to contain a very high Zn content in tobacco and fodder beans. During pollen germination the zinc migrates into the pollen tube, then incorporates into the developing seed, and accumulates there in this way.

b. The "P-induced Zn deficiency" -- The "P-induced Zn deficiency" symptoms, as described by many authors, can occur in any soil or any growth media as long as P fertilization is high enough, but with an unpredictable feature. The visual symptoms of "P-induced Zn deficiency" are somewhat like the simple Zn deficiency: first appearing as interveinal chlorosis, and then developing into necrosis. However, the symptoms of "rosetting" and "little leaves" which are characteristic of Zn deficiency in many species (Chapman, 1966) do not necessarily occur in the plant (Loneragan et al., 1979, 1982; Christensen and Jackson, 1982; Halim et al. 1968). Instead, P toxicity symptoms characterized by puckering of leaves, thickening and upward curling of leaves, and grayish-brown necrosis can be often observed (Christensen and Jackson, 1982).

Zinc concentration in the plant tops, on the other hand, is not always an indicator of "P-induced Zn deficiency" (Boawn and Leggett, 1964; Halim et al., 1968), as illustrated in the table below.

Table 2-1. Zn Concentrations in the Leaves of Normal Plants and "P-induced Zn Deficient" Plants

Plant	Deficient	Normal	Medium	Authors
Okra	14-26	>22	Nutrient	Loneragan et al. 1982
Cotton	10-14	32-40	Nutrient	Cakmak & Marschner, 1986
Subterranean clover	22-49	30-37	Nutrient	Millikan, 1963
	<20	>20	Nutrient	Loneragan et al., 1979
Potato	12-16	14-25	Soil (field)	Boawn & Leggett, 1964
	17-19	16-17	Nutrient	Boawn & Leggett, 1964
	10-15	18-24	Nutrient	Boawn & Brown, 1968
	15.6-17.9	16.1-16.2	Nutrient	Christensen & Jackson, 1982
Navy beans	11-23	15-20	Nutrient	Amber & Brown, 1969
Sugar beet	8-10	>10	Nutrient	Rosell & Ulrich, 1964

It is obvious that the symptoms of "P-induced Zn deficiency" can show up on the plant at a Zn concentration either below its critical values (Loneragan at al., 1951) or as high as in the normal plant (Boawn and Brown, 1968; Loneragan et al., 1979; Christensen et al., 1982). Furthermore, this type of deficiency, unlike the simple Zn deficiency, typify a high concentration of P in the plant tissues (Table 2-2), indicating a high P concentration is most likely to be a responsible factor regardless of other interpretations. This will be reviewed later.

2.3 Interactions of P and Zn in Soils

The evidence submitted to explain the mechanisms of P-induced Zn deficiency due to interaction in the soil and the factors governing the interactions fall into two categories, viz.,

a. Formation of $Zn_3(PO_4)_2$ precipitate in the soil -- The formation of insoluble $Zn_3(PO_4)_2$ as a mechanism of "P-induced Zn deficiency" was proposed by early workers and continued until 1970's. Boawn et al. (1954) reported that a solubility for $Zn_3(PO_4)_2$ was between 1 and 2.3 mg $Zn.kg^{-1}$ at 25°C and at pH 6.7. Later on (1957) they applied $Zn_3(PO_4)_2$ as a source of Zn for sorghum in greenhouse and found its availability was equal to ZnO, $ZnCO_3$, and $ZnSO_4.7H_2O$ on a Ritzville fine sandy loam, pH 7.2. Further Jurinak and Inouye (1962) measured the solubility of $Zn_3(PO_4)_2$ at different pH's values: the lowest concentration of Zn at pH 8 was 1.02 mg $Zn.kg^{-1}$ which equals to 15.7 μM; while in a flowing solution culture maximal or near-maximal yields were obtained with a legume at 0.05 μM Zn and with cereals at 0.10 μM (Carroll and Loneragan, 1968, 1969). Furthermore, Zn concentration in the well-known Hoagland solution is only about 0.77 μM (see Appendix 1), twentyfold lower than the solubility of $Zn_3(PO_4)_2$. The evidence strongly indicates that $Zn_3(PO_4)_2$ was a good source of Zn and therefore is not involved in "P-induced Zn deficiency" (Allen and Terman, 1966; Lindsay and Norvell, 1969).

b. Reactions related to specific sorption of P and Zn on Fe and Al oxides in the soil -- The prevailing view on the interaction of P and

Zn in the soil is based on the increased Zn sorption by sesquoxides due to increased P sorption. For example, Saeed and Fox (1979) found that P fertilization increased Zn sorption in Hawaiian soils of acidic reaction (pH 5.1-6.2), in which the cation exchange capacity was known to increase upon sorption of P. They suggested that increasing the negative charge on Fe and Al oxide surfaces due to the adsorbed phosphate caused increases in the sorption of Zn. However, adding P to alkaline or calcareous soils decreased Zn adsorption (Saeed, 1977), or increased Zn intensity in the soil solution (Bingham and Garber, 1960; Pasricha et al., 1987). The reason for this is that little or no negative charge increase takes place in calcareous soils after adding P (Mengel and Kirkby, 1982). In 1970, Stanton and Burger showed that sorption of Zn by Fe and Al oxides was a function of both pH and the amounts of phosphate ions adsorbed by the oxides. In agreement with this, other workers (Shuman, 1976, 1988; Goh et al., 1986b; Ghanem and Mikkelsen, 1988) have also measured greater specific adsorption of Zn by oxides following increases in pH dependent charge and the incorporation of organic anions into the structure of oxides. Bolland et al. (1977) found that the presence of previously sorbed P enhanced Zn sorption by goethite in the slightly acid range. They too suggested that the sorption of negatively charged P reduced the positive charge of the oxide surfaces and thereby increased the sorption of Zn. Therefore, the sesquoxides, especially hydrous iron oxides, play an important role in sorption of Zn in soils and may possibly affect its availability to crops (Ghanem and Mikkelsen, 1988).

However, Loneragan et al. (1979) suggested that despite the fact

that a depression in Zn uptake as affected by P application was observed due to Fe oxide sorption in his experiment conducted in ferruginous sands, a special caution must be taken when applying the above-mentioned proposal under different situations, because it may be important in some soils but not in others. Marinko and Igue (1972) further substantiated this by the evidence that volcanic soils as well as tropical oxides with a high capacity to react with P failed to show any enhancement of Zn adsorption and any depression in the final absorption by corn when treated with large quantities of P fertilizers.

2.4 Effect of Vesicular-arbuscular Mycorrhizae (VAM) on the Interaction

It is well established that vesicular-arbuscular mycorrhizae can improve nutrient supplies, such as phosphorus, zinc, and possibly copper and potassium, to higher plants, and hence their growth in soils deficient in these nutrients, as reported in onion (Ojala et al., 1983), wheat (Kucey and Janzen, 1987), sorghum (Raju et al. 1987), soybeans (Pacovsky, 1986), barley (Jensen, 1982), and citrus (Tinker, 1975, 1978). Zinc deficiency in citrus was cured by inoculation with VAM fungus (Tinker, 1975).

Aside from these merits, it has been suggested that VAM fungi may function as a metabolic sink causing basipetal mobilization of photosynthate to roots, thus providing stimulus for greater photosynthetic activity (Frier, 1977). Moreover, elevated levels of hormones, such as auxins (Strzelczyk and Pokojska-urdziej, 1984; Slankis, 1973), cytokinins (Allen et al., 1980) and gibberellins (Allen

et al., 1982; Marschner, 1986), were found in association with VAM infection, which in turn, and especially for cytokinins, could elevate photosynthetic rates by stomatal opening (Incoll et al., 1977), further influencing ion transport (Stevenick,1976) and regulating chlorophyll levels (Richmond and Lang, 1957; Stetler and Laetsch, 1965). What is more, it has been reported that most VAM fungi can "regulate" nutrient status of plants under some adverse conditions. The reports are of great interest that nutrient status and tolerance of onion to salinity were improved in saline soils when inoculated with VAM fungus (Ojala et al., 1983), and Zn toxicity was alleviated in grasses growing in Zn-polluted soil (Dueck et al., 1986). Furthermore, with an increase in soil P status, the inflow of P was increased in non-mycorrhizal onions (Smith et al., 1986), clover (Smith, 1982), apple (Bhat, 1982), and ryegrass (Powell, 1977), but the P inflow declined in the corresponding mycorrhizal treatments, implying that VAM may possess the potential of reducing luxurious P uptake or even mitigating P toxicity.

There is little information available on the effect of VAM on interaction of P and Zn. Only a few papers have been hitherto published in this area, e.g., Lambert et al.(1979), Pairunan et al. (1980), Singh et al. (1986) and Lu and Miller (1989). Pairunan et al. showed that infected roots took up more Zn than non-infected roots, whereas Lambert et al. reported that increased P reduced Zn content in mycorrhizal infected soybean plants but hardly affected that in noninfected plants. Although the results were contradictory, it is obvious that the interesting findings above-mentioned still stimulate our enthusiasm and give us impetus to introduce VAM fungus into the experiment examining

the mechanisms of "P-induced Zn deficiency".

2.5 Interactions of P and Zn in the Plant

Numerous researchers believed that the reaction of "P-induced Zn deficiency" takes place in the plant. This view partly comes from the experiments in which soils alone provide no information to interpret the "P-induced Zn deficiency", and is partly due to the analytical data of the plants being able to illustrate the phenomenon and from the experiments conducted in hydroponics. The preponderance of evidence would seem to corroborate that the induced deficiency occurs within plants as indicated below:

a. Effect on translocation of Zn in the plant -- Many workers have reported that applied P induced or accentuated Zn deficiency symptoms in plants (Loneragan, 1951; Loneragan et al., 1979, 1982; Thorne, 1957; Burleson et al., 1961; Brown and Tiffin, 1962; Brown et al., 1970). In some cases applied P reduced the concentration of Zn in the aboveground portion of the plant and lowered the total uptake of Zn (Loneragan, 1951; Langin et al., 1962; Stuckenholtz et al., 1966). In some cases P and Zn appeared to be mutually antagonistic in the plant whenever either element exceeded some threshold value (Boawn and Leggett, 1964). Burleson et al. (1961) reported that applied P induced Zn deficiency symptoms in corn early in the growing season and lowered yield. They suggested the possibility of a P and Zn antagonism within the roots to explain their results. The addition of Zn had a pronounced effect on alleviating the symptoms of Zn deficiency in these cases.

b. Dilution effect -- In other reports, however, applied P decreased the Zn concentration, while the total uptake of Zn was the same or even increased (Millikan, 1963; Boawn and Leggett, 1964; Watanabe et al., 1965; Jackson et al., 1967; Singh et al., 1986, 1988). Under this circumstance, the authors designated it as "dilution effect". That is to say, when the rate of plant growth exceeds the rate of uptake of a particular nutrient, the concentration of that nutrient in the tissue decreases or is "diluted". For instance, Watanabe et al. (1965) demonstrated in their experiment that applied P reduced Zn concentration by 2-3 times compared to the lowest dosage of P applications, but the total uptake of Zn did not change much. The reduction in Zn concentration of the plant tissue was largely due to the more than twofold increase in yield. After reviewing a number of other similar reports, Olsen (1972) suggested that this interaction generally occurs when the soil is deficient in P and borderline or slightly deficient in available Zn. Consequently the growth rate increases because of applied P, but the uptake of Zn does not increase fast enough to maintain a sufficient concentration of Zn in the tops. In soils where available Zn is sufficient, on the other hand, further applied P often does not result in Zn deficiency arising from a dilution effect (Armbruster et al., 1975; Ellis et al., 1964; Racz et al., 1974; Smilde, 1973). Further adding P to a P sufficient soil does not enhance plant growth, but usually stimulates P uptake and may result in P toxicity (Loneragan et al., 1979; Adams, 1982).

c. Distribution of Zn between roots and tops -- A number of researchers reported that in some cases, "P-induced Zn deficiency" was due to a low Zn concentration in the tops while concentration of Zn was still normal in the roots. For example, in a greenhouse experiment with corn, Stuckenholtz et al. (1966) showed that the concentration and uptake of Zn were increased by applied P in roots but decreased in the leaves, nodes, and internodes. Carroll and Loneragan (1968) compared the percentage of Zn distribution in four legumes in a flowing culture system: 35% of the total Zn uptake was detected in the roots when growth was limited by Zn deficiency, compared to 18% under optimal Zn supply. This phenomenon was ascribed to the well-known principle that operates in the case of Zn, that is, the plant part closest to the source of supply obtains its nutrient requirement first before significant translocation to the tops takes place (Olsen, 1972).

d. Physiological effects -- In studies on "P-induced Zn deficiency", contradictions often arise. As discussed above, the key issue whether P could induce Zn deficiency or not depends upon Zn concentration of the plant tops. However, Boawn and coworkers (1954) found that Zn content of leaves is not always an indicator of Zn deficiency. In an experiment with beans, they showed no relationship between a lower yield induced by the added P and Zn content of the plant. Further they found that high P applications were associated with Zn deficiency symptoms, but not with Zn concentration in the plant (Boawn and Leggett, 1964). Using a split-root technique, Boawn and Brown (1968) could demonstrate that high P rates were able to induce Zn

deficiency symptoms in both beans and potatoes without decreasing Zn content of the plants. Thus a conclusion was drawn that the dilution effect on Zn concentration was not a factor in their study. Several other authors obtained similar results, in which P concentrations in the plant were always found to have been extremely high. Therefore, these observations have led to several suggestions that (1) excess P interferes with "normal metabolism" of Zn (Boawn and Brown, 1968), (2) increasing P concentrations in plant tissues induces a higher physiological requirement for Zn; this is termed "P-enhanced Zn deficiency" (Loneragan et al., 1979), and (3) the antagonism between P and Zn involves a physiological imbalance; this is usually called "imbalanced P/Zn ratio" (Millikan, 1951, 1963; Watanable et al., 1965; Boawn and Leggett, 1964).

Unfortunately, among the suggestions above, "P-enhanced Zn deficiency" was ruled out by Loneragan et al. (1982) in a later experiment with okra. Stuckenholtz et al. (1966) suspected that P interfered with the metabolic processes responsible for Zn absorption by root cells and for Zn translocation to leaves, in contrast to Smilde (1973) who found no such evidence.

2.6 Genotypical Differences in Plant Species

It has been well documented that different plant species and varieties vary greatly in their sensitivity to Zn deficiency and resistance to P toxicity (Foote and Howell, 1964; Amber and Brown, 1969; Clark, 1978; Shukla, 1987), as well as in their susceptibility to "P-induced Zn deficiency" (Safaya and Singh, 1977; Paulsen and Rotimi,

1967; Brown and Tiffin, 1962; Burlesen et al., 1961). Halim et al. (1968) measured Zn and P levels of corn leaves of 24 inbreds and 10 single crosses grown in growth chambers with normal nutrient solution and high phosphorus nutrient solution. They found that some lines were very sensitive to Zn deficiency, some showed early or late resistance, while only a few inbreds and single crosses showed no visible Zn deficiency symptoms in either high P or normal P solutions. Shukla and Raj (1987) evaluated the relative response of four crop species to Zn deficiency in the soil, the susceptibility being in an order of corn, cowpea, sorghum and pearl-millet, showing a remarkable difference in susceptibility to Zn deficiency among species. The studies of Safaya and Singh (1977), a pot experiment with two varieties of cowpeas (HFC-42-1 and FOS-1), demonstrated that the former variety had a higher sensitivity to P-induced Zn disorder, which appeared to be due to a nearly two times higher P concentration as compared to the latter one. Their results indicated that HFC-42-1 had a higher need for Zn because of its inherent capacity to accumulate more P. In the meantime, dry matter yield, P and Zn contents in the two varieties were also differentially affected by the application of both P and Zn. The differences may be directly related to different "feeding power" among varieties of a plant species (Millikan, 1961) or their efficiency of absorption and utilization of nutrient constituents from the soil (Gregory and Growther, 1928; Schjorring and Nielsen, 1987). The inheritance of sensitivity to high P levels was considered to be controlled by a single gene pair (Bernard and Howell, 1964). So far the genotypic differences have successfully explained some contradictory

results from experiments involving different plant species or varieties.

2.7 Phosphorus Toxicity in Plants

Since none of the hypotheses on P and Zn interaction that have been proposed could satisfactorily explain "P-induced Zn deficiency" in the plant from every aspect, there may be some other factors rather than Zn governing the occurrence of the Zn deficiency symptoms. Although P toxicity had been long reported by a number of investigators tested on several plant species such as oats (Rossiter, 1952), lupines (Warren and Benzian, 1959; Asher and Loneragan, 1967), soybeans (Foote and Howell, 1964), and wheat (Bhatti and Loneragan, 1970), it was not until 1979 that Loneragan et al. first employed it to interpret "P-induced Zn deficiency" in subterranean clover. In their study, they showed that there was no evidence that high rates of P induced Zn deficiency in the subterranean clover by depressing Zn absorption or transport in plant tissues. Instead the symptoms occurring in the plants were attributed to accumulation of P to toxic levels. The concentration of P in plants showing necrotic symptoms resulting from P toxicity was summarized by Loneragan et al. (1982) and is given in Table 2-2.

Table 2-2. Concentrations of P Reported for Shoots and Leaves of Plants with Symptoms Attributed to (A) P Toxicity, (B) Zn Deficiency, and (C) P-induced Zn Deficiency. (Loneragan et al., 1982)

Plant	P as % d.m. shoot	leaf	reference
Symptoms attributed to P toxicity			
Clover, oats	1.3-3.1	3.0-4.5	Rossiter, 1952,1955
Lupins	1.1-2.2	-	Warren & Benzian, 1959
Soybeans	-	1.2[1]	Foote & Howell, 1964
Wheat, oats	0.8-1.2	1.3-2.2	Loneragan et al., 1979
Clover, lupins, silver grass	0.9-1.8	-	Asher & Loneragan, 1967
Barley	0.8-1.2	2.2-2.8	Green et al., 1973
Clover	1.0-1.2	-	Loneragan et al., 1979
Symptoms attributed to Zn deficiency[2]			
Sugar beet	-	3.1-4.0	Rosell & Urich, 1964
Tomato	2.2	2.4	Poribok & Alekseeva - Popova, 1965
Corn	1.1-1.8	-	Clark, 1978
Beans, flax, chilies, cotton, millet, lettuce, tomato, onion	-	1.1-4.5	Rahimi & Bussler, 1979
Symptoms attributed to P-enhanced Zn deficiency[3]			
Clover	1.0-3.5	-	Millikan, 1963 Millikan et al., 1968
Potato	1.2-2.7	-	Boawn & Leggett, 1964
Beans, potato	0.8-2.4	-	Boawn & Brown, 1968
Navy bean	1.1-1.9	-	Ambler & Brown, 1969
Cowpea	0.8-2.5	-	Safaya & Singh, 1977

1. P concentration in cotyledonary leaf.

2. These studies were selected for evidence that Zn deficient plants may accumulate high concentration of P.

3. The P concentrations listed are those in plants of high P treatments which intensified symptoms resembling Zn deficiency.

Loneragan et al. (1982) further suggested that Zn deficiency interfered with P metabolism, enhancing the amounts of P absorbed by roots and transported to tops, and hence P accumulated to toxic levels in the leaves under conditions of high P supply, inducing or accentuating symptoms resembling Zn deficiency. They therefore claimed that their findings seemed to explain previously puzzling observations why high rates of P enhanced Zn deficiency symptoms without any reduction in Zn contents of the plant tops, and further discounted the existence of a mechanism by which P inactivated Zn in the leaves. Furthermore, where the P concentration was high in the leaves, added Zn failed to eliminate all the Zn deficiency symptoms or the deleterious effect on the plants induced by high P rates (Poulsen and Rotimi, 1968; Loneragan et al., 1982). On the basis of the new finding, they therefore pointed out that "P-enhanced Zn requirement" was not the cause of the "P-induced Zn deficiency" since Zn concentration of the tops was not reduced by high P. At about the same time and shortly thereafter several other authors (Christensen and Jackson, 1981; Cakmak and Marshner, 1986, 1987) obtained similar results which strongly supported the view of P toxicity as a mechanism of "P-induced Zn deficiency".

2.8 Interference of Zn with P Metabolism

A number of investigators have reported that Zn appears to "regulate" P uptake. The observations showed that high levels of Zn decreases P uptake, an optimal level of Zn increases both Zn and P uptake, and low levels of Zn or Zn deficiency considerably stimulate P uptake and transport, accumulating to toxic levels in the leaves

(Safaya, 1976; Loneragan et al., 1979; Loneragan et al., 1982; Cakmak and Marschner, 1986). The "regulation" is possibly through its effect on some macromolecular components of cell membranes (Chvapil, 1973). A suggestion has been advanced that Zn may play an important role in root cell membrane integrity, i.e., involving the structural orientation of macromolecules within the membrane or in the maintenance of ion transport mechanisms there (Welch et al. 1982), and along with Ca ions, regulates ion transport across cell membranes (Epstein, 1972). Under these functions the presence of adequate Zn ions prevents excessive P uptake by roots and transport of P from roots to leaves (Welch et al., 1982). When suffering Zn deficiency, the membrane integrity is impaired and its permeability increases (Paribok and Alekseeva-Popova, 1965). Consequently the roots lose their ability to regulate P metabolism and transport, thus increasing P uptake and transport to the tops (Welch et al., 1982).

CHAPTER THREE

PHOSPHORUS AND Zn FERTILIZATION, THEIR RESIDUAL EFFECTS AND INTERACTIONS IN CANOLA-WHEAT AND BARLEY-WHEAT ROTATIONS

3.1 Introduction

The majority of previous studies on the interaction of P x Zn have been conducted in pot or solution culture systems, because of the merits of their convenience in operation and readily controllable environmental conditions. However, pot or solution culture experiments do not lend themselves well to test any residual effects of plant nutrients on plant growth and especially to the study of any effects of crop rotations under these conditions. For this reason, a field experiment was carried out.

It is well established that different crop species or varieties vary considerably in their susceptibility to "P-induced Zn deficiency" (Safaya and Singh, 1977; Paulsen and Romiti, 1968; Brown and Tiffin, 1962; Burlesen et al., 1961), possibly resulting from their different "feeding power" or efficiency of absorption and utilization of P and Zn nutrients from soils (Millikan, 1961; Gregory and Growther, 1928). Different species may markedly affect growth and yield of their subsequent crops in a rotation system, a phenomenon usually referred to as "allelopathy" (Overland, 1966; Martin and Rademacher, 1960; Harper and Lynch, 1982). This theory considers crops, weeds and microbes in growth media as an ecological system, in which the growth of each member is influenced by phytotoxic and/or growth promoting effects of the

exudates, the decomposition of their residues and assimilation products on each other (Goh et al., 1986b). The effects of allelopathy on plant growth have attracted interest of scientists for decades. In spite of the ample knowledge on allelopathy, there is little information available concerning the relationship between crop rotations and the interaction of P x Zn. Therefore, a field experiment was conducted to examine (1) the mechanisms of P x Zn interaction in barley and canola, (2) the residual effects of P and Zn fertilization on their interaction in a subsequent crop of wheat, and (3) the impacts of different rotations on the interaction.

3.2 The First Year Experiment (1987)

3.2.1 Materials and Methods

The experiment was conducted near Haywood (SW 25-8-6 W) on an Almasippi soil (Eilers, 1985) with the physical and chemical characteristics as shown in Table 3-1. The rotations were established with three crops, barley (*Hordeum vulgare L.*) and canola (*Brassica napus*) in 1987 and wheat (*Triticum aestivum L.*) as the succeeding crop in 1988. The study was carried out by using a completely randomized split plot design with four replicates. Phosphorus, as the main factor with three rates (0, 100, 250 kg $P.ha^{-1}$), and Zn, as the subplot factor with two rates (0, 20 kg $Zn.ha^{-1}$), were applied in May, 1987; N and K were added as basal fertilizers. All the fertilizers were broadcast except Zn which was sprayed on as a solution of $ZnCl_2$. The plots were plowed after fertilization in 1987.

Plant samples were collected at the heading stage and maturity for barley and at the pod stage and maturity for canola. After air-drying, yields of the shoots, grain and seed were recorded. The plant parts were then ground to < 2 mm mesh in a Thomas-Wiley mill before further analysis for nutrient content. The digestion was performed in a perchloric-nitric acid mixture (Bowen, 1967; Chapman & Pratt, 1961). The concentration of P in the plant tissue was determined by the molybdate-blue method (Murphy & Riley, 1962) and those of Zn, Cu, Mn and Fe were analyzed by atomic absorption spectroscopy.

Soil samples were taken before fertilization in 1987. Soil P was determined by using $NaHCO_3$ extraction (Olsen et al., 1954, Murphy & Riley, 1962) and Zn, Cu, Mn and Fe by DTPA extraction (Lindsay and Norvell, 1969; Follet and Lindsay, 1971). The data are listed in Table 3-1.

Table 3-1. The Physical and Chemical Characteristics of the Almasippi Soil

Texture	VFSL
Inorg. carbonate	2.5 %
pH (H_2O)	8.0
Salinity	0.75($mS.cm^{-1}$)
Nutrient	**($mg.kg^{-1}$)**
NO_3 - N ($NaHCO_3$)	4.7
P ($NaHCO_3$)	2.7
K (NH_4OAc)	86
SO_4^{2-}	20
Cu (DTPA)	0.45
Zn (DTPA)	0.7
Mn (DTPA)	10.2
Fe (DTPA)	38

3.2.2 Results and Discussion

(1) Response of crop growth and yield to applied P and Zn

In 1987, in spite of the low level of soil available Zn, neither barley straw nor grain yield showed significant responses to added Zn but only to added P (Fig. 3.1 and 3.2).

Canola, on the other hand, was more sensitive to Zn nutrition and hence significantly responded to added Zn. Without the application of Zn, seed yield of canola did not increase regardless of amount of P added (Fig. 3.4), though the shoot yield was not affected in the same manner (Fig. 3.3). However, since added phosphorus increased the seed yields only when both Zn and P were applied, the Zn deficiency was deemed to be a limiting factor to canola production on this soil.

The metabolic requirement for P and Zn were obviously different between the two crops. Canola, compared with barley, accumulated higher concentrations of P and Zn in the tops even when its seed yield was limited by the low soil available Zn; however, in the treatments of Zn20P100 and Zn20P250, Zn concentrations were lower in canola than in barley as seen in Tables 3-2 and 3-3.

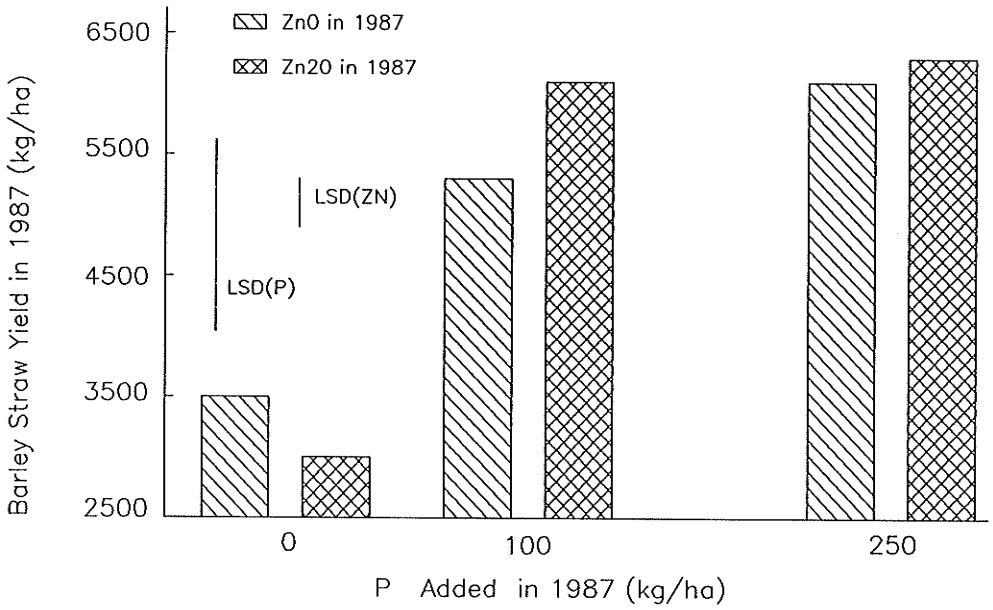

Fig. 3.1 Barley Straw Yield as Affected by P and Zn Application

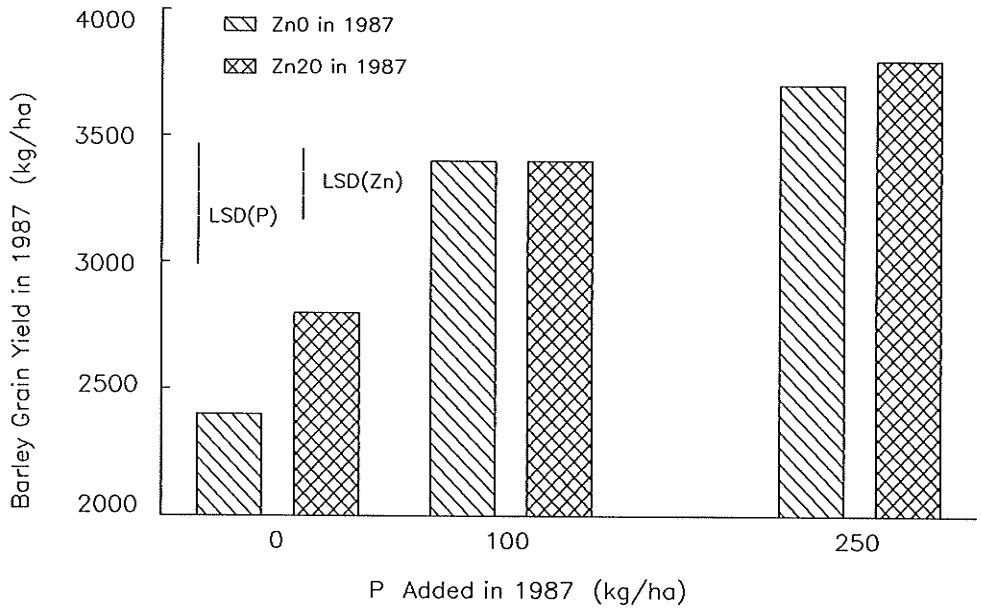

Fig. 3.2 Barley Grain Yield as Affected by P and Zn Application

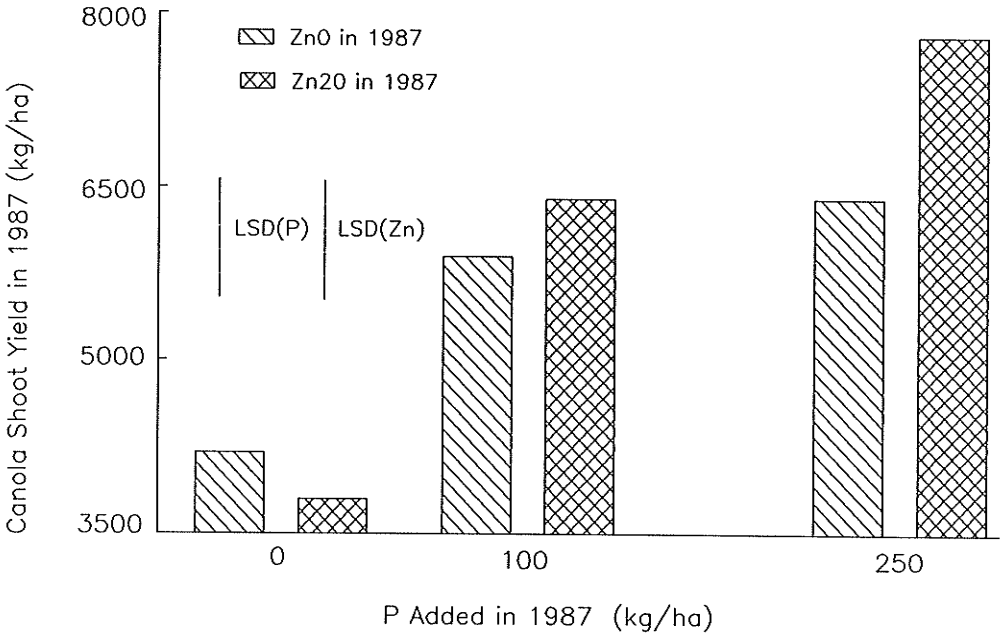

Fig 3.3 Canola Shoot Yield as Affected by P and Zn Application

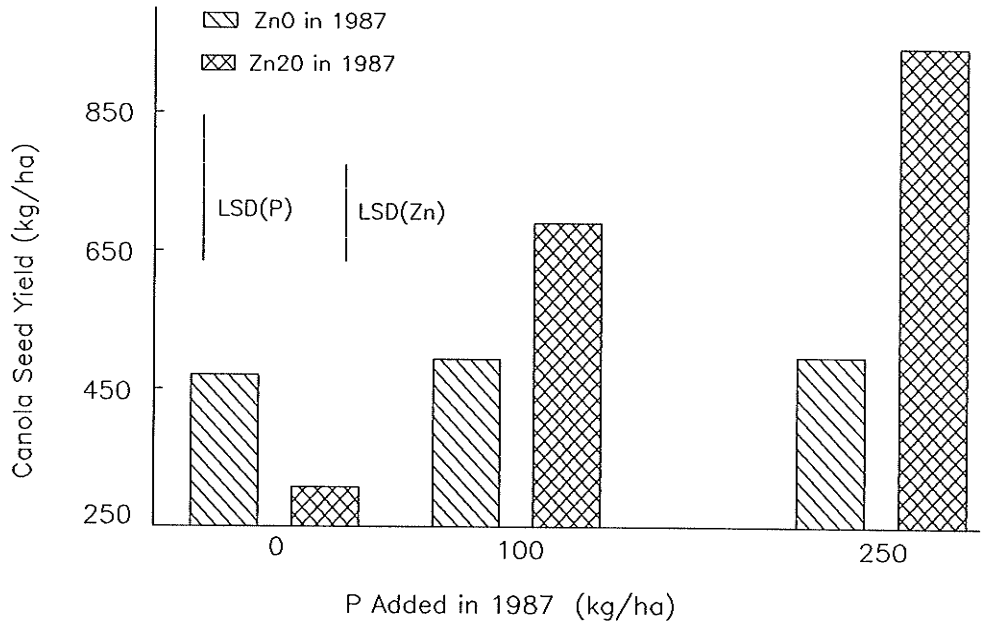

Fig 3.4 Canola Seed Yield as Affected by P and Zn Application

Table 3-2. P Concentration ($g.kg^{-1}$) in Barley and Canola Plants as Affected by P and Zn Application (1987)

Crop	Zn added ($kg.ha^{-1}$)	P added ($kg.ha^{-1}$)					
		0	100	250	0	100	250
		Straw			Grain		
Barley	0	0.6	0.8	1.5	2.4	4.4	4.6
	20	0.6	0.9	1.1	2.8	4.6	5.0
		$LSD._{05}$ = 0.2			$LSD._{05}$ = 0.3		
		Shoot			Seed		
Canola	0	0.8	1.7	2.0	6.0	8.8	9.1
	20	0.9	1.7	1.8	6.9	8.5	8.4
		$LSD._{05}$ = 0.3			$LSD._{05}$ = 0.5		

Table 3-3. Zn concentration ($mg.kg^{-1}$) in Barley and Canola Plants as Affected by P and Zn Application (1987)

Crop	Zn added ($kg.ha^{-1}$)	P added ($kg.ha^{-1}$)					
		0	100	250	0	100	250
		Straw			Grain		
Barley	0	13	6	5	32	24	23
	20	30	20	18	43	43	41
		$LSD._{05}$ = 3			$LSD._{05}$ = 3		
		Shoot			Seed		
Canola	0	15	9	10	45	43	37
	20	34	17	11	51	50	45
		$LSD._{05}$ = 3			$LSD._{05}$ = 3		

These results are in good agreement with the findings by Strong and Soper (1974) that canola had the strongest absorption ability for P among four tested crops consisting of flax, wheat, canola and buckwheat under the condition of added or non-added P. The observations by Strong and Soper (1974) lead to a deduction that different crops or cultivars have different critical values for a given nutrient in both soil and plant tissues. In other words, a species or cultivar with a high demand on a certain nutrient requires a greater supply of that nutrient in the soil or other media. For example, Asher and Loneragan (1967) conducted a flowing culture solution experiment to compare eight annual pasture species in their demands on P, and the results showed a range of 10-fold in the P concentrations needed for maximum growth between the most demanding and least demanding species.

In addition, different crop species or varieties also vary greatly in the their ability to take up nutrients from soil or growth media. The high efficiency in utilizing soil P and Zn by canola is attributed to its high rooting density and its ability to secrete solubilizing agents, such as H^+, from its roots when the soil is P deficient (Nye, 1984), which is of importance especially in calcareous soils. In some soils the soil P solubilized by canola root exudates can reach an extent that is available to the subsequent crops in the rotation (Woodbury, 1986).

Not only are the sensitivity and ability to absorb Zn different among plant species and varieties, but also the plant organs in which Zn is stored. Like copper, Zn is found to be predominantly stored in the seeds (Shkolnik, 1984), and largely concentrated in the embryo. Reed

(1942, 1944) found that Zn is necessary in the normal development of the oosphere and the embryo and, as a result of this, pea plants grown under conditions of Zn deficiency produced no seeds. A concentration of 0.01 mg $Zn.l^{-1}$ in the nutrient solution deteriorated the oosphere completely. In the light of previous studies, Shkolnik (1984) pointed out that "Zn exerted a more pronounced influence on development of the oosphere and embryo than on pollen development, and Zn deficiency affected the formation of seeds to a greater extent than it affected the growth of vegetative organs". The response of canola to Zn application in this experiment was strong evidence of Shkolnik's statement: when no Zn was added canola seed yields did not increase regardless of amounts of P applied but in contrast the shoot yields were not affected by Zn deficiency.

(2) Interaction of P and Zn

Tables 3-3 show that the added P significantly decreased Zn concentrations in both crops at both levels of Zn, suggesting a negative effect of P on Zn utilization. Added Zn, on the other hand, hardly influenced P concentrations either in barley straw or in canola shoot, but did cause an increase in P concentrations in barley grain and a decrease in canola seed at high P treatments, i.e. P100 and P250, showing an antagonistic relationship between Zn and P contents in the canola plant. Even so the total uptake of P by both crops was usually enhanced by the addition of Zn (Table 3-4). Uptake of Zn, on the other hand, was reduced by addition of P in canola but increased only under the concomitant application of P and Zn, indicating a different patterns

of interaction of P x Zn between barley and canola.

Table 3-4 Uptake of P ($kg.ha^{-1}$) and Zn ($g.ha^{-1}$) in the Barley and Canola Tops as Affected by P and Zn Application (1987)

Crop	Zn added	P added ($kg.ha^{-1}$)					
	($kg.ha^{-1}$)	0	100	250	0	100	250
		P Uptake			Zn Uptake		
Barley	0	8.1	18.5	26.6	123	109	122
	20	9.4	22.5	30.7	206	271	261
		$LSD._{05}$ = 2.5			$LSD._{05}$ = 20		
		P Uptake			Zn Uptake		
Canola	0	6.1	14.2	17.6	83	73	76
	20	5.2	16.8	21.5	143	141	122
		$LSD._{05}$ = 4.0			$LSD._{05}$ = 25		

(3) Phosphorus and Zn translocation in the plant tissues

Figs. 3.5 and 3.6 show the relationship between P and Zn translocation from barley straw to grain as affected by total uptake of Zn and P in the aboveground parts of the plant. The two figures indicate that the uptake of P and Zn only slightly increased the transport of the counterpart nutrient to the grain. In other words, the two elements worked independently of each other on their transport on their transport from straw to grain.

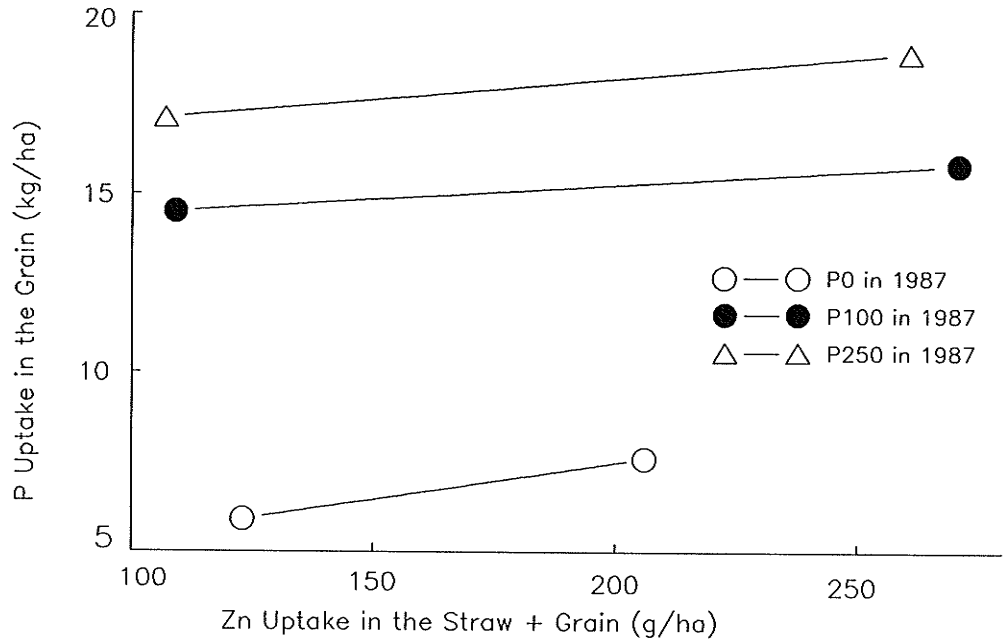

Fig. 3.5 P Translocation to the Grain as Affected by Zn Uptake in the Wheat Tops

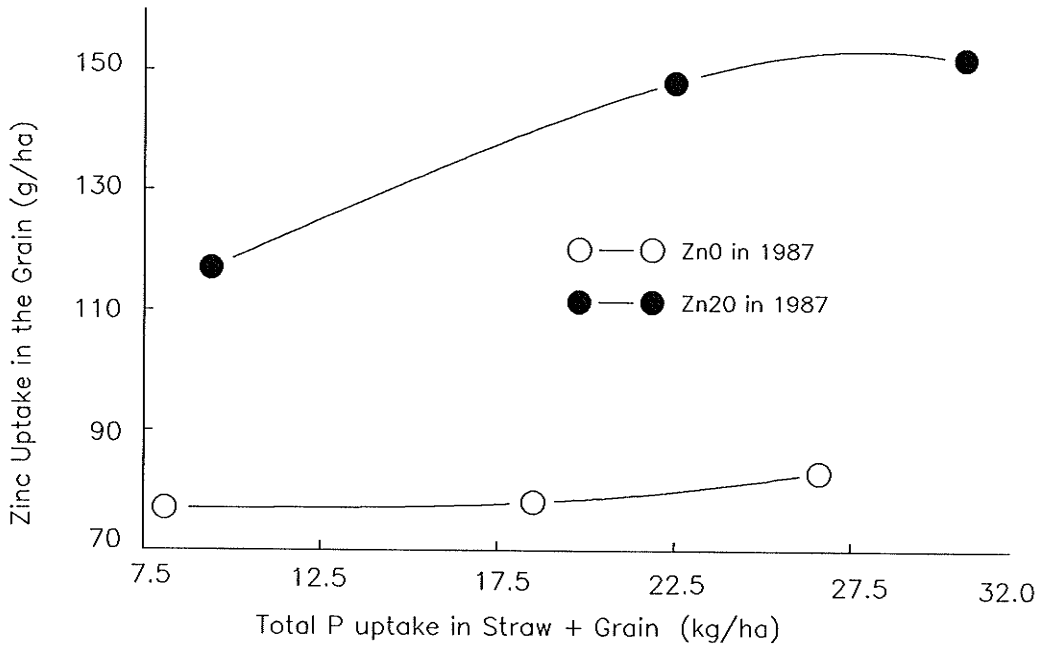

Fig. 3.6 Translocation of Zn to the Grain as Affected by Total Uptake of P in the Wheat Tops

In canola, on the other hand, these two elements exhibited very strong interactions in their transport in the plant, that is, increasing Zn uptake in the plant inhibited P transport when P was absent from application but promoted the transport when P was applied (Fig. 3.7) and the transport of P from shoot to seed exhibited the same pattern (Fig. 3.8). The results suggested that the two elements behave differently being interdependent upon each other in canola but independent of each other in barley. The interdependent relationship of these two nutrients was also observed by Mckenzie and Soper (1983). They found that both P and Zn in the plant can regulate the absorption and/or transport of its counterpart ion in blackbeans.

(4) Nutrient status in soil after the first year cropping

Soil samples were taken in May, 1988, and the pertinent nutrients were analyzed as shown in Table 3-5a, b and c. The data indicated that DTPA extractable Zn of the soil was independent of the amount of P added in the previous years when no Zn was added in 1987, ranging from 0.84 to 0.90 and 0.78 to 1.11 $mg.kg^{-1}$ on canola and barley stubble respectively (Table 3-5a). When Zn was added, however, added P significantly decreased soil Zn concentration on canola stubble but increased on barley stubble. Several workers have observed no changes and even increases in the soluble Zn or Zn intensity in the soil solution (pH 5.2-8.2) with low to high rates of applied P (Bingham and Garber, 1960; Stukenholtz et al., 1966; Brown et al., 1970; Pasricha et al., 1987). The result suggested that P-induced Zn deficiency, if existing, is unlikely to arise from the effect of P fertilization on Zn

availability in calcareous soils.

It is obvious that added Zn did not markedly affect $NaHCO_3$-extractable P on canola stubble but did cause significant increases of the extractable P on barley stubble. Concentrations of DTPA-extractable Cu was not affected by either added P or Zn on the canola stubble, but was significantly increased by both added P and Zn on the barley stubble (Table 3-5b). Manganese concentration reached the highest level at P100 and then dropped to the lowest value at P250 on canola stubble, but increased with increased P addition on the barley stubble. It is evident that different stubbles appreciably affected the status of extractable nutrients and their interactions in the soil.

Table 3-5a shows that added Zn did not markedly affect $NaHCO_3$-extractable P on canola stubble but did cause significant increases of the extractable P on barley stubble. Concentrations of DTPA-extractable Cu and Mn on the barley stubble were significantly increased by both added P and Zn (Table 3-5b). Cu concentration did not change on canola stubble by addition of either P or/and Zn, while Mn concentration reached the highest value at P100 and then dropped to the lowest points at P250. It is apparent that rotations appreciably affected the status of extractable nutrients and their interactions in the soil but little is known about why and how this occurred.

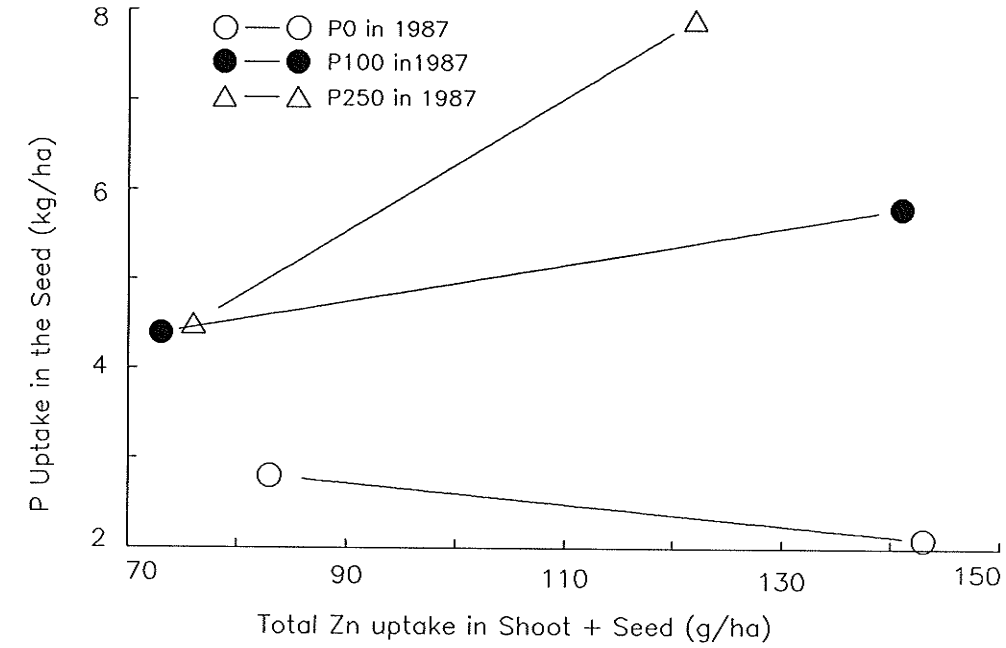

Fig. 3.7 P Translocation to the Grain as Affected by Zn Uptake in the Canola Tops

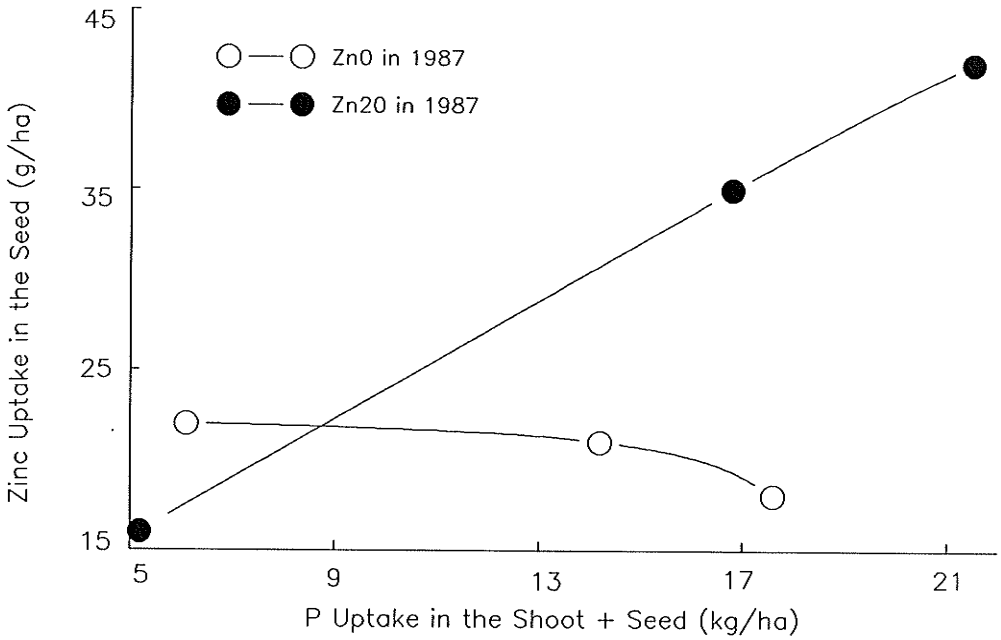

Fig. 3.8 Zn Translocation to the Seed as Affected by P Uptake in the Canola Tops

Table 3-5a. P and Zn Status after the First Year Cropping on Two Stubbles before Seeding in 1988

Stubble	Zn added ($kg.ha^{-1}$)	Nutrient Concentration ($mg.kg^{-1}$)					
		P			Zn		
		P added ($kg.ha^{-1}$)					
		0.0	100.0	250.0	0.0	100.0	250.0
Canola	0	7.3	21.1	50.4	0.88	0.90	0.84
	20	8.1	18.4	50.2	5.40	4.28	4.47
		$LSD._{05}$ =3.5			$LSD._{05}$ = 0.76		
Barley	0	6.3	21.3	50.1	0.78	0.84	1.11
	20	7.0	29.5	59.5	4.15	4.79	4.42
		$LSD._{05}$ =4.6			$LSD._{05}$ = 0.69		

Table 3-5b. Cu and Mn Status after the First Year Cropping on Two Stubbles before Seeding in 1988

Stubble	Zn added ($kg.ha^{-1}$)	Nutrient Concentration ($mg.kg^{-1}$)					
		Cu			Mn		
		P added ($kg.ha^{-1}$)					
		0.0	100.0	250.0	0.0	100.0	250.0
Canola	0	1.02	1.00	1.00	12.3	15.7	12.2
	20	0.94	1.00	1.02	12.3	18.6	10.8
		$LSD._{05}$ =0.20			$LSD._{05}$ =1.8		
Barley	0	0.50	0.58	0.80	11.7	13.1	15.8
	20	0.56	0.75	0.88	12.6	13.9	15.0
		$LSD._{05}$ =0.09			$LSD._{05}$ = 1.8		

Table 3-5c. The Average Soil Nutrient Contents after the First Year Cropping on Two Stubbles before Seeding in 1988

Stubble	Nutrient Concentration ($mg.kg^{-1}$)			
	P	Zn	Cu	Mn
Canola	25.9 b*	2.8 a	0.97 a	13.7 a
Barley	28.9 a	2.7 a	0.68 b	13.7 a

* The values followed by the same letter in a column mean nonsignificant difference between the two crops by LSD test at 5% significance level.

3.3 The Second Year Field Experiment (1988)

3.3.1 Materials and Methods

Based on the experimental design of the first year, the subplots were split once again in the second year, becoming a completely randomized split-split plot design. Since one of the objectives of this study was to examine the residual effects of different levels of added P in the first year on Zn uptake by wheat in the canola-wheat and barley-wheat rotations, P was eliminated in the second year. The usage of Zn was split into two levels: 0 and 10 kg $Zn.ha^{-1}$ on the sub-subplots. Nitrogen and potassium were applied as basal fertilizers on each plot. The zero tillage practice was adopted.

Plant sampling and the subsequent analysis of nutrients in the plant tissues followed the same procedures as described for the first year experiment.

3.3.2 Results and Discussion

(1) Plant growth and its association with allelopathy

Field observations, from the tillering stage (Zadoks 20) till maturity (Zadoks 90), clearly showed that wheat growth was much better on the canola stubble. As shown in Table 3-6, the top dry matter yield of wheat on the canola stubble at heading stage (Zadoks 50) was usually about one and a half times higher than on barley stubble. When the top dry matter yields were averaged by rotation, 851 $kg.ha^{-1}$ on the barley stubble and 1245 $kg.ha^{-1}$ on the canola resulted as seen in Table 3-8.

Table 3-6. Wheat Top Yield ($kg.ha^{-1}$) at Heading Stage on Two Rotations as Affected by Applied P and Zn (1988)

Stubble	Zn added ($kg.ha^{-1}$) in 1987	Zn0 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250	Zn10 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250
Canola	0	1025	1133	1293	1118	1313	1238
	20	1070	1155	1588	1025	1373	1695
		$LSD_{(P)\cdot 05}$ = 34			$LSD_{(Zn)\cdot 05}$ = 44		
Barley	0	735	1113	700	885	765	1030
	20	730	778	890	850	863	908
		$LSD_{(P)\cdot 05}$ = 43			$LSD_{(Zn)\cdot 05}$ = 33		

Table 3-7 shows that on the canola stubble wheat grain yield did not significantly respond to either residual P or Zn, nor to added Zn in 1988. On the barley stubble, residual P increased grain yield; however, an significant increase between minus and plus P treatments resulted only when no Zn was added in 1988. The yield was higher when Zn was added only once in the two years. In comparison between the two stubbles, the grain yields on the canola stubble always exceeded those on the barley stubble, indicating the beneficial effects of canola to the following crop. The differences in plant growth and grain yield between rotations might be ascribed to a so-called allelopathic effect that has been recognized for decades. Overland (1966) found that barley inhibited seed germination and the growth of selected plant species

including wheat, involving an inhibitory substance or substances secreted from barley roots or leached from the decomposition of its residues, as well as the allelochemical compounds or phytotoxins released from microorganisms. Among the crops, cereals seem particularly susceptible (Elliott et al., 1978). While the poor growth of the plants resembled N deficiency, it was not corrected by N

Table 3-7. Wheat Grain Yield ($kg.ha^{-1}$) at Maturity on Two Rotations as Affected by Applied P and Zn (1988)

Crop	Zn added ($kg.ha^{-1}$) in 1987	Zn0 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250	Zn10 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250
Canola	0	2330	2600	2350	2200	2230	2380
	20	2250	2350	2035	2500	2500	2780
		$LSD_{(p)\cdot 05}$ = 532			$LSD_{(Zn)\cdot 05}$ = 649		
Barley	0	1080	1580	1580	1480	1850	2350
	20	1000	1630	1730	1550	1700	2050
		$LSD_{(p)\cdot 05}$ = 433			$LSD_{(Zn)\cdot 05}$ = 495		

fertilization (Davidson and Santelmann, 1973; Duly, 1960; Kimber, 1967). Canola, however, stimulated the growth of subsequent wheat crop seedlings (Martin and Rademacher, 1960). To identify the difference in allelopathy caused by different crop species, Harper and Lynch (1982) compared four crop straws, wheat, barley, oat and rape.

They found that removal of water soluble components did not significantly change acetic acid production from cereal straws but halved the production from rape straw, demonstrating that the dominant components of cereal straws that are water-insoluble and are not rapidly released during their decomposition processes by soil microorganisms are responsible for the lasting toxicity to the subsequent crops. In the present study, canola stubble promoted whereas barley stubble inhibited the growth of the following wheat crop, which provided further evidence for the finding by Harper and Lynch.

In study of the inhibitory substances, the phytotoxic compounds isolated from fresh and decomposing crop residues and from soils belong to several chemical classes including organic acids, lactones, alkaloids, and turpenoids (Borner, 1960; Chou and Patrick, 1976; McCalla and Haskins, 1964; McCalla and Norstadt, 1974; Rice, 1984), while those from microbes included patulin and antibiotics (McCalla and Norstadt, 1974; McCalla and Haskins, 1964). Goh et al. (1986b) also found that citric, acetic and tannic acids were allelopathic to wheat seedling growth in solution media and all three organic acids accentuated aluminum toxicity if the soil conditions were right.

(2) Nutrient contents in the plant tissues

From heading to maturity, total uptake of all the nutrients measured in the wheat plant tops remained consistently significantly higher on the canola stubble except Cu (Tables 3-8 and 3-9), although

Table 3-8. The Average Top Yield and Nutrient Uptake by Wheat at Heading Stage in the Second Year Cropping on Two Stubbles (1988)

Stubble	Top yield	Nutrient Uptake ($g.ha^{-1}$)				
	($kg.ha^{-1}$)	P^1	Zn	Cu	Mn	Fe
Canola	1245 a[2]	26.8 a	5.6 a	0.39 a	2.6 a	34.8 a
Barley	850 b	19.4 b	3.6 b	0.35 a	1.7 b	28.8 b

1 - P uptake ($kg.ha^{-1}$)

2 - the values followed by the same letter in a column mean nonsignificant difference between the two crops by LSD test at 5% significance level.

Table 3-9. The Average Grain Yield and Nutrient Uptake by Wheat at Maturity in the Second Year Cropping on the Two Stubbles

Stubble	Yield ($kg.ha^{-1}$)		Nutrient Uptake ($g.ha^{-1}$)				
	Top	Grain	P^1	Zn	Cu	Mn	Fe
Canola	6000 a[2]	2400 a	11.3 a	202.7 a	8.3 a	55.9 a	311.2 a
Barley	4200 b	1630 b	7.6 b	163.0 b	8.8 a	35.6 b	255.1 b

1 - P uptake ($kg.ha^{-1}$)

2 - the values followed by the same letter in a column mean nonsignificant difference between the two crops by LSD test at 5% significance level.

the concentration of $NaHCO_3$-extractable P was significantly lower and DTPA extractable-Zn higher in the soil on the canola stubble compared to that on the barley stubble before seeding (Table 3-5a). This anomaly leads to a speculation that soil P on canola stubble may form as non $NaHCO_3$-extractable but available compounds to the subsequent wheat crop or some other unidentified substances related to canola may exist to stimulate P uptake by the crop. Evidently, Tarafdar and Claassen (1988) reported that plants were able to utilize P from all the organicsources used in their study almost as efficiently as inorganic sources as long as the organic P compounds can be hydrolysed by phosphatases secreted by plant roots or microbes in the soil. If their finding is true, it implies that in the present study, the exudates from canola roots may contain higher phosphatases than cereal crops, thus yielding a higher efficiency in utilizing soil P that even benefited its subsequent crop.

(3) Interactions of P and Zn in the plant

In relation to the nutrient concentration in the plant as affected by P and Zn fertilization, the added P in 1987 increased P concentration in both straw and grain on the two rotations. However, from a statistical point of view, it significantly increased the concentration of P in wheat straw in 1988 only under treatments with Zn20 in the canola-wheat rotation and with Zn0 in the barley-wheat rotation, and in wheat grain only under the treatment with Zn0 in the barley-wheat rotation (Table 3-10). With respect to Zn application, the concentration of P in the plant tissues were not different on either stubble with each level of P application whether Zn was added in the

first year and/or in the second year.

Table 3-11 shows that the addition of Zn significantly enhanced the concentration of Zn in the plant no matter when it was added. P added in 1987 significantly increased Zn concentration in the wheat

Table 3-10. P Concentration ($g.kg^{-1}$) in the Plant Tissues at Maturity as Affected by Applied P and Zn and Rotations (1988)

		Zn0 in 1988			Zn10 in 1988		
	Zn added						
Stubble	($kg.ha^{-1}$)		P Added in 1987 ($kg.ha^{-1}$)				
	in 1987	0	100	250	0	100	250
				Straw			
Canola	0	0.40	0.49	0.51	0.35	0.41	0.46
	20	0.31	0.53	0.47	0.38	0.51	0.45
				$LSD._{05}$ = 0.12			
				Grain			
	0	3.1	4.8	4.2	3.6	4.5	4.1
	20	4.0	4.6	4.2	3.8	4.2	4.5
				$LSD._{05}$ = 0.9			
				Straw			
Barley	0	0.38	0.52	0.63	0.32	0.52	0.54
	20	0.41	0.46	0.55	0.43	0.49	0.47
				$LSD._{05}$ = 0.12			
				Grain			
	0	2.7	5.1	4.8	2.7	4.4	3.5
	20	3.9	4.2	4.3	3.5	4.3	4.6
				$LSD._{05}$ = 1.4			

Table 3-11. Zn Concentration ($mg.kg^{-1}$) in the Plant Tissues at Maturity as Affected by Applied P and Zn and Rotations

Stubble	Zn added ($kg.ha^{-1}$) in 1987	Zn0 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250	Zn10 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250
				Straw			
Canola	0	14.3	13.1	15.5	16.0	14.5	15.8
	20	29.9	22.8	23.5	33.5	27.9	22.8
				$LSD._{05}$ = 6.2			
				Grain			
	0	68.6	42.3	34.1	60.2	47.4	28.4
	20	72.5	62.5	58.5	76.0	59.8	54.9
				$LSD._{05}$ = 12.3			
				Straw			
Barley	0	12.9	17.3	31.4	21.2	21.5	32.3
	20	29.8	30.9	44.0	34.0	41.4	48.1
				$LSD._{05}$ = 7.4			
				Grain			
	0	47.5	58.5	36.3	57.1	41.0	47.1
	20	63.6	56.4	55.3	60.9	66.0	53.5
				$LSD._{05}$ = 11.8			

straw but decreased in the grain in the barley-wheat rotation. The Zn concentrations in the plant, however, were all significantly depressed by P application on the canola-wheat rotation, except those in the straw when no Zn was added for two years.

The results demonstrated that different rotations change the availability of certain nutrients and their interactions both in the

soil and within the subsequent crop, thus yielding a stimulatory or inhibitory effect on the nutrient uptake and plant growth. These results from different rotations may shed light upon the numerous contradictory reports on the P x Zn interactions which often vary with conditions as reported by the investigators in the past.

The P uptake by the wheat plants is shown in Table 3-12. The results indicated that the applied P enhanced P uptake by the wheat plants on the two rotations, while the effect of the applied Zn on P uptake was somewhat complicated. However, Zn20P0 treatment enhanced P uptake in the wheat grain as compared to Zn0P0 treatment on the two stubbles whether Zn was applied in the second year or not; and the residual soil Zn tended to enhance P uptake in the grain but the added Zn in 1988 tended to reduce that in the grain. The results here suggested that the residual Zn and newly-added Zn affected P uptake in the wheat grain in different ways.

The effect of residual P applied in 1987 on the uptake of Zn is shown in Table 3-13. On the canola-wheat rotation, Zn uptake was considerably depressed by the P added in 1987 except that in the wheat straw at Zn0 treatment. Contrary to this, Zn uptake was remarkably enhanced in the barley plant by the residual soil P. The results revealed entirely different patterns of residual P affecting Zn uptake in the different rotations.

Table 3-12. P Uptake ($kg.ha^{-1}$) in the Plant Tissues at Maturity as Affected by Applied P and Zn and Rotations

Stubble	Zn added ($kg.ha^{-1}$) in 1987	Zn0 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250	Zn10 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250
				Straw			
Canola	0	1.31	1.70	1.68	1.27	1.63	1.73
	20	1.14	1.87	1.97	1.05	1.70	1.64
				$LSD._{05}$ = 0.12			
				Grain			
	0	7.12	12.48	9.74	7.60	9.83	9.68
	20	8.64	10.52	9.52	9.18	10.03	12.01
				$LSD._{05}$ = 1.99			
				Straw			
Barley	0	0.64	1.34	1.51	0.78	1.46	1.93
	20	0.64	1.09	1.52	1.01	1.34	1.38
				$LSD._{05}$ = 0.48			
				Grain			
	0	2.89	7.92	7.60	3.97	7.52	7.92
	20	3.83	6.76	7.33	5.25	7.13	9.14
				$LSD._{05}$ = 2.71			

Table 3-13. Zn Uptake ($g.ha^{-1}$) in the Plant Tissues at Maturity as Affected by Applied P and Zn and Rotations

Stubble	Zn added ($kg.ha^{-1}$) in 1987	Zn0 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250	Zn10 in 1988 P Added in 1987 ($kg.ha^{-1}$) 0	100	250
				Straw			
Canola	0	46.8	45.0	50.3	58.3	58.5	59.5
	20	104.0	82.3	97.0	97.5	95.3	82.5
				$LSD._{05}$ = 20.6			
				Grain			
	0	161.3	108.0	78.0	120.0	103.5	66.3
	20	157.0	144.5	133.0	184.5	146.8	152.5
				$LSD._{05}$ = 37			
				Straw			
Barley	0	23.3	44.8	75.5	51.0	64.0	117.8
	20	50.0	73.2	119.0	83.5	116.5	148.5
				$LSD._{05}$ = 65.9			
				Grain			
	0	51.8	58.5	56.3	83.3	75.5	109.0
	20	65.0	89.3	92.5	91.8	112.0	104.5
				$LSD._{05}$ = 30.4			

3.4 Anomalous Mn Nutrition in the Plant and Related Environmental Conditions

Manganese deficiency symptoms in the wheat plants were observed in the experimental site. Under field conditions, the most distinct symptom of Mn deficiency in wheat was the interveinal chlorosis on the young leaves (Plate 3.1), somewhat resembling Zn deficiency. At heading stage, almost all flag leaves were chlorotic when Mn concentration in the leaves dropped below 20 mg $Mn.kg^{-1}$ of dry matter. Accordingly, the growth of these plants was stunted, appearing to be dwarfed (Plate 3.2). Occasionally, some brownish spots were observed along the veins on the symptomatic leaves (Plate 3.3).

Plate 3.1 Interveinal Chlorosis of the Wheat Leaf Caused by Mn Deficiency at Heading in the Field Experiment in 1988

Plate 3.2 Mn Deficiency Caused Dwarfed Wheat Plants at Heading in the Field Experiment in 1988

Plate 3.3 Some Brownish Spots along the Veins on the Symptomatic Leaves of Mn Deficient Wheat Plants in the Field Experiment in 1988

The function of Mn in plant nutrition is of great importance. It is now established that manganese is required in both lower and higher plants for the Hill reaction -- the water cleavage and O_2-evolving system in photosynthesis (Cheniae and Martin, 1968). It is also believed that photosystem II contains a manganoprotein which catalyzes the early stage of O_2 evolution as seen in the following reaction:

$$2\ H_2O \xrightarrow[4\ Mn^{2+}]{4\ Mn^{3+}\ \text{enzyme}} 4\ H^+ + O_2 + 4\ e^-$$

$$4\ e^- \xrightarrow[\text{Pigment 680}]{e^-\ \ h\nu}$$

When Mn is deficient the first step of the electron transport chain of light is impaired.

Manganese is also involved in the synthesis of proteins, carbohydrates and lipids, and contributes to cell division and extension. When the levels of Mn concentration are below the critical values, net photosynthesis and chlorophyll content decline rapidly, and elongation of the cell is more sensitively inhibited than cell division. Consequently, formation of chlorotic leaves and stunted plants results.

Plant tissue analysis indicated that Mn concentrations in the leaves of deficient plants ranged from 12.5 to 16 mg $Mn.kg^{-1}$, markedly lower than those in the normal plants, which were above 20.0 mg $Mn.kg^{-1}$ as shown in Table 3-14.

Table 3-14. Mn Deficiency and Related Nutrient Contents in Wheat Plant Tissues at Heading Stage

Crop		Nutrient concentration ($mg.kg^{-1}$)				
Stubble	Tissue	P(%)	Zn	Cu	Mn	Fe
Canola	Leaves	0.19	80	4.5	12.5	83
	Stem	0.16	52	4.5	16.0	198
	Normal plant	0.21	53	4.0	22.5	247
Barley	Leaves	0.34	51	2.5	16.0	117
	Stem	0.28	45	3.0	20.0	173
	Normal plant	0.27	53	4.0	22.5	272

After comparing other investigators' work, Marschner (1986) came to a conclusion that the critical deficiency levels of manganese are between 10-20 $mg.kg^{-1}$ dry wt in mature leaves, and surprisingly consistent regardless of the plant species or cultivar or the prevailing environment conditions. The tissue Mn levels presented in this experiment provided further evidence for this conclusion. As shown in Table 3-14, with the exception of Fe, the other nutrients, such as P, Zn, Cu, were unaffected in the symptomatic plant, despite the soil DTPA-extractable Zn dropping to the marginally deficient level. This is in good agreement with the finding of Lucas and Knezel (1972) that wheat is one of the crops highly sensitive to Mn deficiency but with lower sensitivity to Zn deficiency. According to the soil test data before seeding (Table 3-5b), the soil contained 13.7 $mg.kg^{-1}$ of DTPA-extractable Mn which is considered to be adequate for plant growth. It therefore appears that the record of Mn deficiency at this site on the Almasippi sandy loam is an anomaly since the Mn content by DTPA extract indicated sufficiency of the nutrient.

A plausible explanation can however be provided. First of all, the severe drought experienced throughout the Prairies in the 1988 growing season was most likely responsible for the Mn deficiency, which appeared to restrict the plant physiologic ability to absorb Mn. In this experiment, an average concentration of Mn in the plant tops at heading stage was determined as 20.9 $mg.kg^{-1}$ of dry matter in all 96 samples, most ranging from 18 to 23 $mg.kg^{-1}$, and with extremely low and high values of 12 and 29 $mg.kg^{-1}$ respectively. However, in a following growth chamber experiment using the same wheat variety on the same soil, Mn concentration in the plant tops at the heading stage was above 40 $mg.kg^{-1}$ of the dry matter (Table 4-3, Chapter 4), with an average of 57, indicating a very strong impact of weather conditions on Mn availability to plants.

Secondly, the fixation of Mn via precipitation as MnO_2 is expected to be enhanced under hot and dry, oxidizing conditions. Behaving like Fe, Mn changes its valence, but is more redox sensitive than Fe, in responding to changes in soil pH and redox potential. The relationship can be expressed as follows:

$$\log(Mn^{2+}) = Eh_0 - 4pH - 35.7Eh$$

When Eh is high, then $\log(Mn^{2+})$ becomes low, which means that the majority of Mn^{2+} ions in the soil solution are converted into MnO_2 precipitate as illustrated by the following equation:

$$Mn^{2+} + H_2O \text{ -- } MnO_2 + 4H^{+} + 2e^{-}$$

It is obvious that for a calcareous soil, dry weather and high temperature conditions tend to dramatically raise the redox potential of the soil, and facilitate formation of MnO_2 precipitates. Lal and Taylor

(1970) found that soil drainage increased the uptake of Zn, Cu and B, but decreased the uptake of Fe, Mn and Mo by plants. As expected, Mulder and Gerretsen (1952) observed that flooding temporarily corrected Mn deficiency in fruit trees, and Childers et al. (1950) reported Mn deficiency in peach trees was usually aggravated by drought conditions in the late spring.

Finally, the plant response to P and Zn fertilization appeared to be the triggering factor to the Mn deficiency in the plant. The observation in the experimental field indicated that Mn deficiency symptoms in the plant occurred largely in P100 plus Zn and P250 treatments as if the Mn deficiency were induced by both P and Zn applications. However, from the nutrient contents determined in the plant tissues, it appeared that there was no clear relationship between them. In the Mn deficient plants, the results exhibited a surprisingly consistent phenomenon, that is, the overwhelming number of plots suffering from Mn deficiency had higher dry matter yields than the "normal" plots (Table 3-15), suggesting that the deficiency was most likely induced by dilution effect due to concomitant application of P and Zn which stimulated plant growth.

Table 3-15. Mn Deficiency as a Result of Dilution Effect: Wheat Top Yield of Normal Plants against Mn Deficient Plants in 1988 ($g.m^{-2}$)

Stubble	Treatment	Growth Status	No. plots	Yield or average
Canola	P100Zn0Zn0[1]	normal	3	532
		Mn def[2].	1	718
	P100Zn0Zn10	normal	2	500
		Mn def.	2	876
	P100Zn0Zn10	normal	1	261
		Mn def.	3	626
	P250Zn20Zn0	normal	1	464
		Mn def.	3	743
Barley	P100Zn0Zn0	normal	3	354
		Mn def.	1	600
	P100Zn0Zn10	normal	2	275
		Mn def.	2	682
	P250Zn20Zn0	normal	3	355
		Mn def.	1	719
	P250Zn20Zn10	normal	2	374
		Mn def.	2	656

1 - P and Zn are fertilizers added in $kg.ha^{-1}$; the first Zn stands for Zn added in 1987 and the second Zn in 1988 respectively.

2 - deficiency.

In conclusion, canola exhibited a positive response to Zn fertilization in the Almasippi soil and appeared to need higher tissue Zn concentration for its metabolism. Compared with barley, canola stubble benefits its following crop in nutrient uptake and growth. The different rotations affect interaction of P x Zn both in the soil and

within the plant, namely, added Zn depressed extractable P in the soil on canola stubble but increased on barley stubble, and added P decreased Zn concentration in the wheat plant on canola stubble but increased on the barley stubble. Wheat crop is sensitive to Mn nutrition. The anomalous Mn deficiency in 1988 was due to both drought and dilution effect of plant growth.

CHAPTER FOUR

THE INTERACTION OF PHOSPHORUS AND ZINC IN WHEAT AND THE EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON THE INTERACTION

4.1 Introduction

Since 1936, a number of attempts have been made to identify the "cause-and-effect" mechanisms of P-induced Zn deficiency. In spite of so many years' studies on this subject, conflicting reports occur from time to time and still perplex the soil scientists and plant physiologists who indulge their interest in this area. Some investigators (West, 1938; Millikan, 1946, 1947; Loneragan, 1951) reported that they could induce Zn deficiency symptoms with high rates of P. In contrast, some (Boawn et al. 1954) demonstrated that their attempts to induce Zn deficiency with high P failed; whereas others (Orabi et al., 1981, 1982, and 1985) found there was a synergistic rather than antagonistic relationship between the two elements. Consequently scientists cannot help but ask whether heavily applied P is able to induce Zn deficiency or not, and the subject continues to be interesting.

It has long been established that vesicular-arbuscular (VA) mycorrhizae favor most agricultural crops in absorbing nutrients, especially P, Zn, Cu, from soils low in these nutrients. Moreover, VA mycorrhizae are capable of secreting such hormones as auxins and gibberellins to the host plant and, as a result, produce significant stimulating effects on plant physiology and its growth if they are

limiting in the plant. Only when produced in excess, however, can these compounds act as a phytotoxin and as a result, inhibit plant growth (Elliott et al., 1978). By virtue of these advantageous impacts on the plants, VA mycorrhizae have been used in some agricultural crops and horticultural plants in soils low in available P. Besides, it has been reported that some species of VA mycorrhizae can "regulate" nutrient uptake under some adverse conditions. For example, Zn toxicity was mitigated in grass grown in Zn-polluted soil (Dueck et al., 1986). Nevertheless, there is little, if any, information about whether VA mycorrhizae can alleviate the P-induced Zn deficiency under this specifically imbalanced nutrient condition. Therefore, introducing VAM into an experiment seeking the mechanism of P-induced Zn deficiency is of great interest and obviously another new approach to our purpose.

In order to induce Zn deficiency in plants, unusually high dosages of P were adopted. Furthermore, a heavy rate of VA mycorrhizal inoculum was used in VA mycorrhizae treatments so as to compensate for some of the adverse effects of P imposed on the mycorrhizal growth.

The objectives of this study were to (1) examine whether high rates of P are able to induce Zn deficiency or not, (2) study the mechanisms of P-induced Zn deficiency if existing, and (3) test the effect of vesicular-arbuscular mycorrhizae on the P x Zn interaction in wheat plants.

4.2 Materials and Methods

4.2.1 Soil Preparation

The soil used for the growth chamber experiment was an Almasippi sandy loam collected from the 0 - 15 cm depth in the northeast corner of Haywood experimental field. The characteristics of the soil are listed below in Table 4-1.

Table 4-1. Selected Physical-chemical Characteristics of the Soil Used in the Growth Chamber Study in 1988

Texture	VFSL
Carbonate content	2.5 %
pH (H_2O)	7.8
Salinity	0.4 $ms.cm^{-1}$
Extractable nutrients	$mg.kg^{-1}$
NO_3-N ($NaHCO_3$)	16.4
P ($NaHCO_3$)	3.2
K (NH_4Ac)	70.0
SO_4^{2-} (H_2O)	20.0
Cu (DTPA)	0.4
Zn (DTPA)	0.6
Mn (DTPA)	5.5
Fe (DTPA)	14.0
Total content	
N	0.22%
P	0.09%
K	0.08%
S	0.08%
Ca	2.6%
Mg	0.45%
Cu	3.2 $mg.kg^{-1}$
Zn	9.0 $mg.kg^{-1}$
Mn	320 $mg.kg^{-1}$
Fe	1200 $mg.kg^{-1}$

After air-drying, the soil was passed through a 2 mm sieve and then mixed thoroughly. Prior to use, the soil was pasteurized in an Automatic Soil Pasteurizer with steam for 2 hrs at 100°C to kill indigenous mycorrhizal fungi. Five-litre pots were used in this experiment and each pot was filled with four kilogram of the soil on an oven-dried basis.

4.2.2 Vesicular-Arbuscular Mycorrhizal Culture

Montmorillonite clay, a kind of commercial floor absorbent (IMC Co. Mundelein IL.) was used as a medium, and filled in 1000 ml plastic pots. A hybrid sweet corn (*Zea mays L.*) (purchased from Stoke's Seeds, Ont.) was employed as the host plant for the mycorrhizal fungus (*Glomus intraradices*). Before seeding, the corn seeds were surface sterilized by immersion in ethanol for 30 s, and then immersed in 1% Javex for 4 m, followed by rinsing in four changes of deionized water. The seeds were placed in petri plates for germination. After germination, two seeds were placed in each pot to which four grams of fresh mycorrhizae-infected corn roots from a previous experiment had been spread at a depth of 1.5 cm from the surface. A series of controls, i.e. without mycorrhizal inoculation, were set up correspondingly. A modified Hoagland solution (Appendix 1) was used to maintain the growth of the corn plants. The plants were watered with the nutrient solution every other day in the first two weeks and every day thereafter. The corn roots were harvested at d 45. Representative subsamples were examined under microscope after staining with Phillips-Hayman procedure (Phillips and Hayman, 1970. see Appendix 2) and confirmed to be indeed well

infected with the VA mycorrhizal fungus.

4.2.3 The Experimental Design and Fertilization

The experiment was carried out by using a randomized complete block design with four rates of P (0, 50, 100 and 300 mg $P.kg^{-1}$ soil), three rates of Zn (0, 2.5 and 10 mg $Zn.kg^{-1}$ soil) and two rates of VA mycorrhizae (control and plus mycorrhizae) with three replicates. Phosphorus was added as powdered monocalcium phosphate. Zinc as $ZnCl_2$ was prepared as a nutrient solution along with the basal fertilizers: N as NH_4NO_3 (100 mg $N.kg^{-1}$) and K as KCl (50 mg $K.kg^{-1}$). Phosphorus was spread and the nutrient solution was sprayed on the soil while mixing in a plastic pan.

4.2.4 Mycorrhizal Inoculation

Mycorrhizal inoculation treatments were prepared by introducing fresh corn roots either mycorrhizal (+VAM) or non-mycorrhizal (-VAM) from the previous culture. Both +VAM and -VAM corn roots were separately cut into small pieces in two containers. After thoroughly mixing, eight grams of fresh roots were weighed and then spread as a net at a depth of 1 cm below the seeds in each pot. Then four wheat (*Triticum aestivum L.*) seedlings were planted in each pot. Owing to the light intensity difference between the two growth chambers (one with light intensity of 4500 lux at plant canopy and the other with that of 5000 lux), the pots resided in the chambers by replicate, and shifting of the pots was made regularly.

During the growing period, plant growth was measured and "P-

induced Zn deficiency" symptoms were observed.

4.2.5 Sampling and Analyses of Plant Nutrients

At about heading (42 days after seeding), one plant shoot from each pot was harvested. The final harvest was performed at maturity when above ground material and roots were collected separately. After air-drying, the yields of shoot, straw, grain and roots were weighed, ground to pass a 2 mm mesh in a Thomas-Wiley mill, and then digested in a perchloric-nitric acid mixture (Bowen, 1967; Chapman & Pratt, 1961). Concentration of P in plant tissues was determined by the molybdate-blue method (Murphy & Riley, 1962) and those of Zn, Cu, Mn and Fe were analyzed by atomic absorption. In addition, using the given factor 5.7, the protein contents were converted from total N in the grain determined by the Auto Kjeldhal method (Kjeldhal, 1883; Schuman et al., 1973). VAM infection rates of the wheat roots were examined at final harvest.

4.2.6 Statistical Analyses

Analyses of variance were performed for a randomized complete block design. Orthogonal contrasts and orthogonal polynomials were used to analyze interactions between treatment factors and to achieve appropriate response trends of wheat growth and the interaction of P and Zn to each factor. Some of the pertinent statistical analyses are listed in Appendix 3-6.

4.3 RESULTS

4.3.1 Plant Growth

Although the soil nutrient status (Table 2-1) indicated that $NaHCO_3$ extractable P (3.2 $mg.kg^{-1}$) and DTPA extractable Zn (0.6 $mg.kg^{-1}$) were deficient and marginally deficient respectively for general agricultural crops, the wheat plants significantly responded only to added P but not to Zn. The analysis of the effect of applied P on plant growth showed that a statistically significant difference between dry matter yields appeared only when comparing P0 to plus P treatments, rather than between the plus P treatments themselves (Table 4-2).

The increase in dry matter yields due to fertilizer P was higher in +VAM plants than in -VAM plants as seen in comparisons between P0 and plus P treatments. Applied Zn, despite ranging from 0 to 10 mg/kg soil, did not bring about any obvious impact on plant growth throughout the growing period. This result is in good accordance with the findings of the field experiment (Chapter 3) that this wheat crop showed low sensitivity to low available Zn. VA mycorrhizal inoculation, on the other hand, had a pronounced effect on plant growth: it significantly reduced the number of tillers, plant hight and nutrient uptake by the wheat plants (Table 4-3) as well as their growth (Table 4-2) before heading. The depression in growth due to inoculation at early stages may be ascribed to the drain effect of the mycorrhizae on photosynthetates from the host plants during its vesicular and arbuscular establishment (Tinker, 1978; Hayman, 1983). However, the cause of depressing nutrient uptake in the same period is unknown.

Table 4-2. Wheat Top Dry Matter Yields ($g.pot^{-1}$) as Affected by VA Mycorrhizae at Each Level of P and Zn Fertilization

Treatment		D.W.B.			D.W.M.			Difference		
VAM	P added	Zn added ($mg.kg^{-1}$)								
	($mg.kg^{-1}$)	0	2.5	10	0	2.5	10	0	2.5	10
-VAM										
	0	6.7	6.6	6.5	54.8	51.0	51.6	34.7	31.2	32.1
	50	8.3	7.1	8.5	59.9	59.9	57.3	35.0	38.6	31.8
	100	8.0	8.5	7.5	61.4	64.3	62.5	37.4	38.8	40.0
	300	8.5	8.3	8.3	64.1	62.9	65.1	38.6	38.0	40.2
$LSD._{05}$			1.8			5.3			5.3	
+VAM										
	0	4.9	4.9	4.7	49.2	48.7	48.6	34.5	34.0	34.5
	50	6.4	6.8	6.0	59.6	59.6	57.2	40.4	39.2	39.2
	100	6.3	7.0	6.7	60.1	59.7	60.6	41.2	38.7	40.5
	300	6.5	6.7	6.3	61.1	59.2	60.5	41.6	39.1	41.6
$LSD._{05}$			1.0			3.1			3.0	

D.W.B. - dry top yield before heading/1 plant
D.W.M. - dry top yield (straw+grain)/3 plants at maturity
Difference = D.W.M. - 3D.W.B.

In spite of these effects, the results show that VA mycorrhizae is beneficial for wheat production since they appear to have increased the efficiency of top dry matter production between heading and maturity (i.e. the difference in Table 4-2) as well as grain yield at harvest (Table 4-4) when comparing VAM to no VAM plants.

Table 4-3. Effect of VA Mycorrhizae on Wheat Growth and Nutrient Uptake until Heading Stage (d 42) (Average of 36 pots)

Treatment	P	Zn	Cu	Mn	Fe	Top yield
		Concentration		($mg.kg^{-1}$)		(g)
-VAM	0.35% a*	26.8 b	5.5 a	61.8 a	49.9 a	7.73 a
+VAM	0.31% b	29.6 a	4.8 b	51.9 b	42.7 b	6.11 b
Effect of VAM (%)	-11.4	+10.4	-12.7	-16.0	-14.4	-21.0

						Tiller No	Height (cm)
		Uptake	(mg/1 plant)				
-VAM	2.8 a	0.021 a	0.042 a	0.47 a	0.38 a	13.7 a	55.4 a
+VAM	2.0 b	0.018 b	0.029 a	0.31 b	0.26 b	12.6 a	52.1 a
Effect of VAM (%)	-28.6	-14.3	-30.9	-33.8	-32.6	-2.9	-2.3

* The means in a column followed by the same letter are not significantly different between VAM and no VAM treatments at 5% LSD level.

Table 4-4. Wheat Grain Yields ($g.pot^{-1}$) as Affected by VA Mycorrhizae at Each Level of P and Zn Application

Treatment			Zn added	($mg.kg^{-1}$)		
P added (mg/kg)	0	2.5	10	0	2.5	10
		-VAM			+VAM	
0	13.3	12.3	12.4	15.4	14.7	15.2
50	16.6	16.2	16.2	18.6	18.8	18.4
100	17.3	17.6	18.3	18.9	19.6	19.9
300	17.9	17.6	17.6	20.0	19.2	20.1
$LSD._{05}$		1.9			2.0	

4.3.2 The "P-induced Zn Deficiency" Symptoms

In this experiment, P-induced Zn deficiency symptoms commenced on the middle, fully expanded wheat leaves at high P treatments three weeks after seeding, expressed as chlorosis or interveinal chlorosis, and developed from leaf tips downward (Plate 4.1) and from chlorosis to necrosis when becoming severe (Plate 4.2). However, the appearance, incidence and severity of the symptoms varied depending upon different amounts of P and Zn application, their combination, and VA mycorrhizal inoculation as well. For instance, if the plants were treated with P100 (including some P300) but absent from Zn and VAM inoculation, interveinal chlorosis initially appeared at tips of young leaves or at margins close to tips and then expanded downward one third to one half of the leaf or even the whole leaf. With time necrotic strips or splotches developed, some of which appeared brown in color and others took on "bronzed" or "sunburned" appearance.

At P300 treatments, the necrosis appeared on one fourth of fully expanded young leaves without first showing marked chlorosis (Plate 4.3). If both P and Zn applications exceeded P300 and Zn10 respectively, the symptoms were more severe. Furthermore, chlorosis no longer necessarily occurred as the onset of the symptoms, and more often, the brown streaks were scattered on the leaves. After heading, most old leaves developed severe necrotic patches (Plate 4.2).

In contrast to -VAM treatments, the VAM inoculation had a significant effect on alleviating "P-induced Zn deficiency" (Fig. 4.1). The incidence of foliar symptoms per pot were reduced by twofold at P100

and sixfold at P300, and the severity was also lighter when VAM inoculum was added to the soil. Most symptoms in +VAM plants were without brown streaks or spots.

One phenomenon deserving of special attention is that prior to the occurrence of the symptoms, all the plants grew normally; and throughout the growing period, observations such as "little leaves" or "dwarfed plants" symptomatic of Zn deficiency did not appear in plants of any treatment; instead, puckering of leaves, which typify P toxicity, did occur.

Plate 4.1. Interveinal Chlorosis Produced by P Toxicity in Wheat Leaves Resembling Zn Deficiency before Heading in the Growth Chamber Experiment in 1988

Plate 4.2. P Toxicity Led to Necrotic Symptoms in the Lower Wheat Leaves after Heading in the Growth Chamber Experiment in 1988

Plate 4.3. Necrotic Symptoms Caused by P Toxicity in the Wheat Leaf before Heading in the Growth Chamber Experiment in 1988

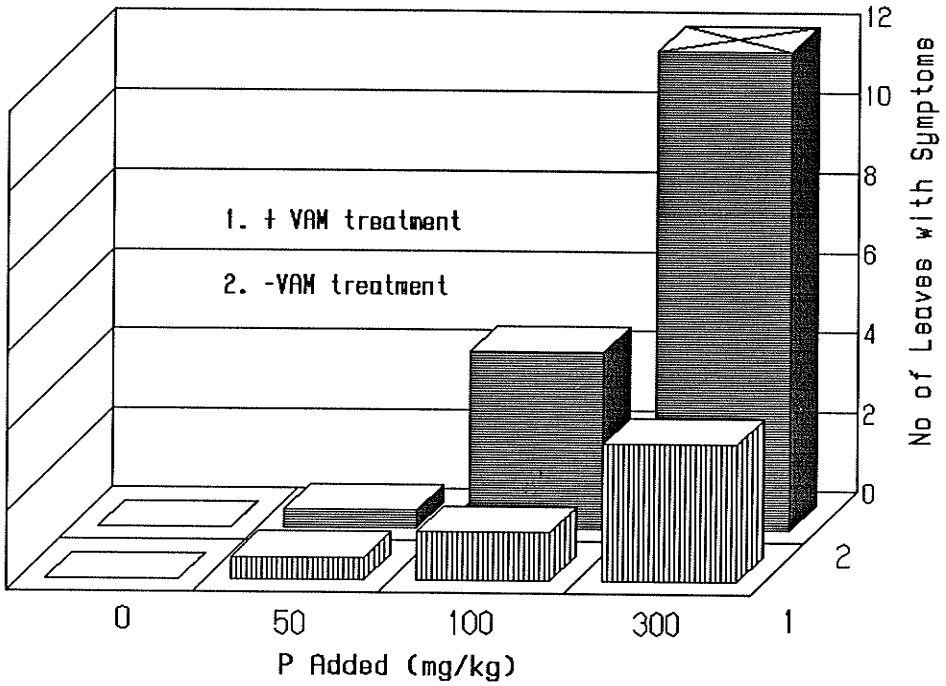

Fig. 4.1 Effect of VAM Inoculation on the Incidence of P Toxicity Symptoms

4.3.3 Zn Concentrations and Contents in Plants

At d 42, Zn concentrations of -VAM plants at Zn0 treatments were consistently reduced with increase in P application as shown in Table 4-5; while at plus Zn treatments addition of P at a concentration of 50 mg $P.kg^{-1}$ increased Zn concentrations and then gradually decreased with further increase in P addition, suggesting that 50 $mg.kg^{-1}$ of P applied to this soil be optimal for Zn nutrition in wheat in absence of VAM. In contrast, Zn concentrations in the +VAM plant were reduced by added P at P50 but then increased with further increase in P addition. The uptake of Zn by the plant showed the same trend.

Table 4-5. Zn Concentration ($mg.kg^{-1}$) and Uptake (μg/1 plant) in Wheat Tops at Heading Stage (d 42) as Affected by VA Mycorrhizae and Different Levels of P and Zn Application

Treatment		Zn added ($mg.kg^{-1}$)					
VAM	P added ($mg.kg^{-1}$)	0	2.5	10	0	2.5	10
		Concentration ($mg.kg^{-1}$)			Uptake (μg/1 plant)		
-VAM							
	0	19.1	28.8	39.3	127.7	189.0	257.0
	50	13.8	29.5	46.2	113.7	209.3	391.0
	100	12.8	24.7	35.5	105.0	208.7	263.0
	300	10.8	21.2	39.3	92.3	174.0	326.3
$LSD._{05}$			6.0			54.9	
+VAM							
	0	22.1	34.4	46.0	108.3	170.0	282.3
	50	14.2	26.0	38.5	91.0	176.7	232.7
	100	17.0	28.7	41.3	107.3	203.3	280.0
	300	20.3	33.8	42.5	133.0	228.2	267.0
$LSD._{05}$		5.3				42.5	

As far as VAM inoculation is concerned, Zn concentrations were usually higher in +VAM plants than in -VAM plants (Table 4-5). The very interesting phenomenon here is that all the Zn concentrations at Zn0 in -VAM plants were lower than 20 $mg.kg^{-1}$ which has been proposed as the critical level of Zn deficiency in plant tissue (Ohki, 1977; Boehle and Lindsay, 1969), hence it is reasonable to expect Zn deficiency symptoms occurring in those plants and being aggravated by increased P. However, at Zn2.5 and Zn10, despite the increased Zn concentrations in the plants to values meeting sufficiency criteria (Table 4-5), the symptoms of so-called P-induced Zn deficiency persisted. What is more, increased Zn concentrations in the plants along with high P concentration aggravated the symptoms (Fig. 4.2 and 4.3). In +VAM plants, on the other hand, the number of leaves with symptoms was greatly reduced by VA mycorrhizal inoculation, seemingly resulting from reduced P concentration and increased concentration of Zn in plants as well.

At maturity, P concentration in the plant tissues was increased by added P but not affected by added Zn (Table 4-8). In comparing no VAM to plus VAM treatments, P concentration in the plant parts did not differ statistically. VAM inoculation did, however, render a slightly higher P concentration in the grain.

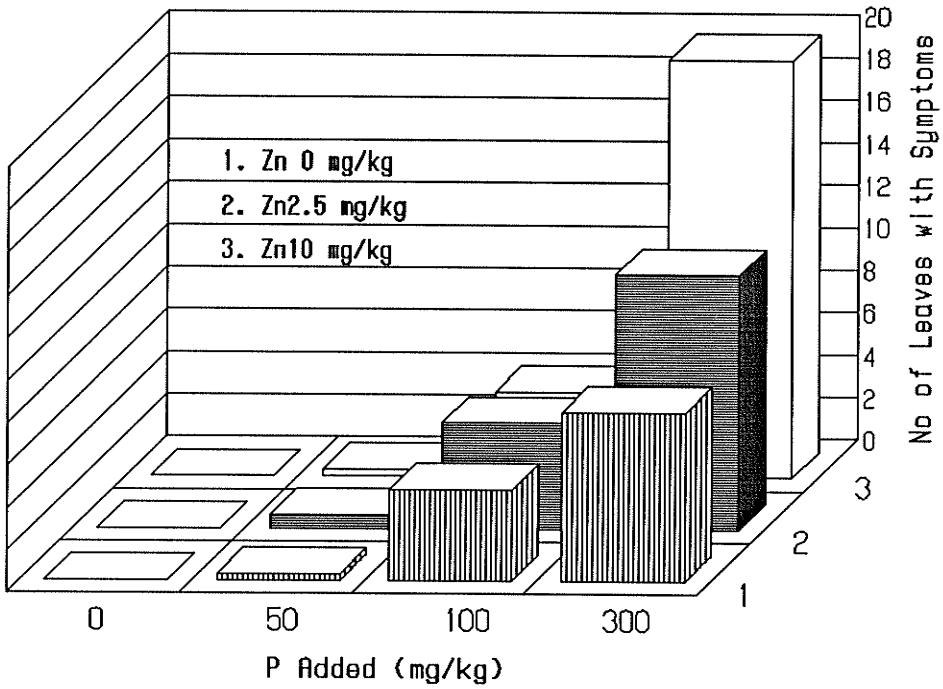

Fig. 4.2 P Toxicity Symptoms in -VAM Wheat Leaves as Affected by P and Zn Application

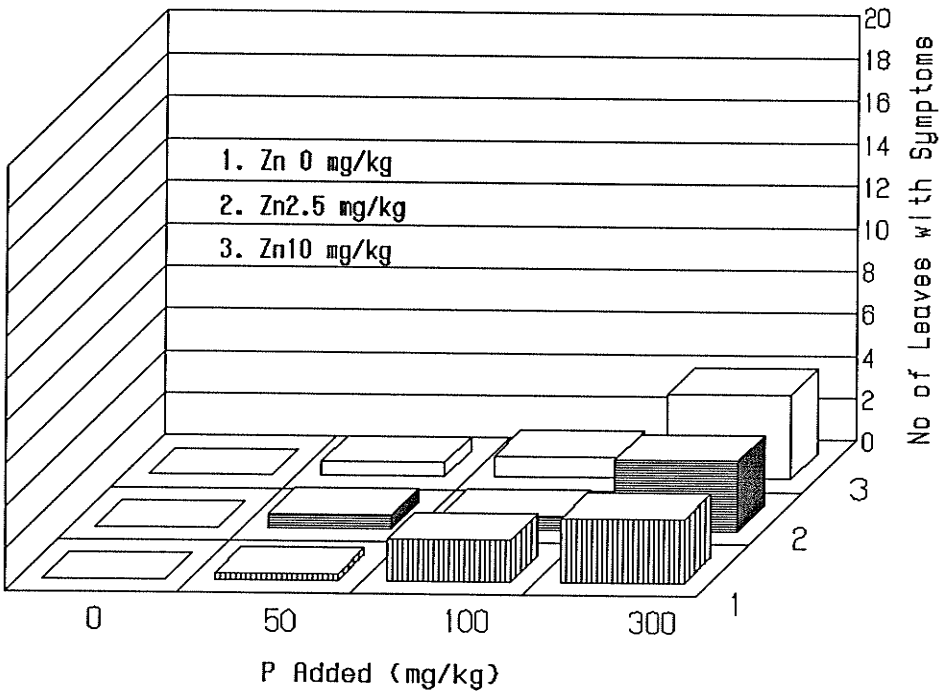

Fig. 4.3 P Toxicity Symptoms in +VAM Wheat Leaves as Affected by P and Zn Application

Table 4-6. Zinc Concentration ($mg.kg^{-1}$) in Wheat Plant Tissues at Maturity as Affected by VA Mycorrhizae and Different Levels of P and Zn Application

Treatment		Zn added ($mg.kg^{-1}$)								
VAM	P added (mg/kg)	0	2.5	10	0	2.5	10	0	2.5	10
		root			straw			grain		
-VAM										
	0	15.2	42.1	49.9	13.4	24.0	27.3	24.0	28.4	33.6
	50	14.7	27.3	43.6	10.1	19.6	27.5	16.2	24.0	29.5
	100	13.1	24.7	43.2	10.4	14.8	21.1	16.2	23.1	28.0
	300	19.2	15.0	41.2	9.2	15.0	17.1	13.4	20.3	25.0
$LSD._{05}$			6.0			3.7			3.9	
+VAM										
	0	24.8	32.6	48.8	10.2	22.8	26.3	30.2	38.8	32.8
	50	21.5	24.0	36.3	9.7	23.3	21.7	29.0	33.2	44.9
	100	19.5	23.8	35.8	8.2	12.9	19.2	27.0	30.8	37.6
	300	16.3	21.0	35.0	6.6	7.4	14.6	26.7	27.3	35.4
$LSD._{05}$			2.7			3.4			7.9	

Neither addition of P nor Zn significantly influenced concentration and uptake of Cu, Fe and Mn by the wheat plant throughout the growing period (data not shown).

4.3.4 P Concentrations and Contents in Plants

At the heading stage, P concentrations and contents in the plants were significantly increased by added P but not affected by added Zn (Table 4-7). At comparable levels of P and Zn, the concentration and uptake of P were always higher in -VAM plants than in +VAM plants except

the P0 treatments and the Zn0P50 and Zn0P100. In all the P0 treatments VAM inoculum considerably enhanced P concentration in the plant tissue. The symptoms of interveinal chlorosis on the leaves were intensified in -VAM plants with increased P concentrations and the incidence of foliar symptoms was also closely related to P concentrations in aboveground plant parts (Fig. 4.2 and Table 4-7). The leaves with faint symptoms contained 2.6-4.0 g P kg^{-1} dry top yield; those with marked symptoms had >4.0 g P kg^{-1} and those with severe necrosis had >5.0 g P kg^{-1}, indicating that the symptoms were due to P toxicity. However, how VAM reduced P concentrations in the plant at early growth stages (Table 4-7) is obscure.

At maturity, the concentration of P in roots in the -VAM treatments was higher at P0 and P50 fertilization levels but lower at P100 and P300 than the +VAM plant. However, the differences were not significant (Table 4-8). The retention of P in the straw of -VAM and P300 treatments was significantly higher than +VAM treatments, illustrating that VAM favoured P re-translocation or metabolism in the host plants. In this experiment, P concentrations of the plants were hardly affected by Zn treatments (Table 4-8). The +VAM treatments markedly enhanced concentration of P of the grain compared to the -VAM treatments. Moreover, this enhancement of P in the grain was more pronounced at either P0 or Zn0 treatment compared to -VAM plants, signifying the beneficial effects of VA mycorrhizae on P and Zn nutrition were more pronounced when available amounts of P and/or Zn was low in the soil.

Table 4-7. Concentration ($g.kg^{-1}$) and Uptake (mg/1 plant) of P in Wheat Tops as Affected by VA Mycorrhizae and Different Levels of of P and Zn Application at Heading Stage (d 42)

Treatment							
VAM	P added ($mg.kg^{-1}$)	Zn added ($mg.kg^{-1}$) 0	2.5	10	0	2.5	10
-VAM		Concentration			Uptake		
	0	1.1	1.2	1.1	7.3	8.1	7.3
	50	2.6	3.2	2.9	21.2	22.4	24.5
	100	3.9	4.5	4.1	30.6	38.0	30.7
	300	5.1	5.7	6.1	43.6	46.8	50.0
$LSD._{05}$			0.7			7.1	
+VAM							
	0	1.5	1.4	1.5	7.6	6.7	7.1
	50	2.6	2.4	2.6	16.3	16.0	15.4
	100	4.0	3.7	3.8	25.2	26.4	25.7
	300	4.6	4.8	5.0	29.7	32.1	31.2
$LSD._{05}$			0.6			5.4	

Table 4-8. P Concentration ($g.kg^{-1}$) in Wheat Plant Tissues at Maturity as Affected by VA Mycorrhizae and Different Levels of P and Zn Application

Treatment										
VAM	P added ($mg.kg^{-1}$)	Zn added ($mg.kg^{-1}$) 0	2.5	10	0	2.5	10	0	2.5	10
		root			straw			grain		
-VAM										
	0	1.0	1.1	1.0	0.3	0.3	0.3	2.3	2.2	2.2
	50	1.1	1.2	1.3	0.8	0.9	0.9	3.6	3.6	3.5
	100	1.3	1.4	1.4	1.7	1.7	1.6	3.7	3.8	3.9
	300	2.2	2.3	2.3	2.2	2.7	2.6	4.0	4.0	4.0
$LSD._{05}$			0.2			0.2			0.2	
+VAM										
	0	0.9	0.9	0.9	0.5	0.4	0.3	2.5	2.6	2.4
	50	1.0	1.2	1.2	1.5	0.9	1.0	4.1	3.5	3.7
	100	1.4	1.4	1.5	1.6	1.5	1.5	4.1	4.0	3.8
	300	2.3	2.4	2.5	2.0	2.2	2.2	4.2	4.1	4.1
$LSD._{05}$			0.3			0.2			0.3	

4.3.5 Effect of VA Mycorrhizae on the Plant Growth and Nutrient Uptake by the Wheat Plant

As discussed earlier, VAM had a marked effect on plant growth and nutrient uptake in this experiment. At the early growth stages of wheat, it significantly depressed the number of tillers, height of plants, dry matter yield and uptake of all nutrients determined, P, Zn, Cu, Fe, and Mn (Table 4-4). In terms of concentration, Zn was significantly enhanced but P was reduced in the mycorrhizal plants. The reduction in P concentration was closely associated with reducing the symptoms of the wheat in VA mycorrhizae-infected plants, and vice versa in non-mycorrhizae-infected plants. Besides, VAM inoculation exerted an influence on the interaction of P and Zn: added P at a concentration of 50 mg/kg in the soil pronouncedly enhanced Zn concentration in -VAM plants in the presence of Zn; but the same P treatment dramatically reduced Zn concentrations in the +VAM plants, irrespective of how much Zn was added.

Unlike in the early growth stages, VAM inoculation surprisingly stimulated plant growth after heading (Table 4-9). Wheat quality was also improved, yielding a significantly higher protein content. Moreover, the wheat plants treated with VAM exceeded the plants without VAM in uptake of P, Zn and Cu after heading, but reduced uptake of Mn and Fe (Table 4-9).

Table 4-9. Effect of VA Mycorrhizae on Nutrient Uptake, Dry Matter Yield of Wheat and Protein Content in the Grain at Maturity (Average of 36 pots)

	Nutrient uptake ($mg.pot^{-1}$)						grain	Protein
	P	Zn	Cu	Mn	Fe	R+S[1]	(g)	%
-VAM	117 a[2]	1.27 a	0.24 a	6.57 a	34.0 a	43.5 a	16.1 b	9.8 b
+VAM	119 a	1.31 a	0.29 a	5.74 b	27.6 b	38.8 b	18.2 a	10.3 a
Effect of VAM (%)	+2.3	+3.1	+20.8	-14.5	-34.1	-12.2	+13	+5.1

1 - yield of roots + straw

2 - the values followed by the same letter in a column mean nonsignificance difference between VAM and no VAM treatments by LSD test at 5% significance level.

4.3.6 The Relative Distribution of P and Zn in the Plant

The relative distribution of P and Zn in the different parts of wheat plants as expressed in percentage of the total uptake is shown in Tables 4-10, 4-11 and Appendix 7-8. In the non-mycorrhizal plant, low level of P supply favored transport of P from the straw to the grain in a large proportion and thus less was retained in the straw. This transport was gradually depressed with increasing P supply. At P300, it therefore resulted in the lowest recovery in the grain and the highest retention of P in the straw.

Table 4-10. Relative Distribution of P as Expressed in Percentage of Total Uptake in Wheat Plant at Maturity as Affected by P and Zn Application and VAM Inoculation

Treatment	- VAM			+ VAM		
	root	straw	grain	root	straw	grain
P0	20.1 (26.6)*	18.4 (25.4)	61.5 (51.7)	11.3 (19.6)	20.9 (27.2)	67.8 (55.4)
P50	12.9 (21.1)	29.0 (32.6)	57.8 (49.5)	9.4 (17.9)	29.5 (32.9)	61.1 (51.4)
P100	12.0 (20.3)	38.6 (38.4)	49.7 (44.8)	10.3 (18.7)	33.3 (35.2)	56.3 (48.6)
P300	14.7 (22.6)	46.4 (42.9)	38.3 (38.2)	14.2 (22.1)	38.3 (38.2)	47.6 (43.6)
$LSD_{0.05}$**	(1.6)	(2.5)	(1.5)	(1.4)	(2.9)	(1.8)
Zn0	14.1 (22.1)	33.2 (35.2)	52.7 (46.6)	10.9 (18.3)	32.7 (34.9)	56.5 (48.7)
Zn2.5	15.0 (22.8)	33.6 (35.4)	51.0 (45.6)	12.2 (20.4)	29.5 (32.9)	58.9 (50.1)
Zn10	15.6 (23.3)	32.5 (34.8)	51.8 (46.0)	11.5 (19.8)	29.4 (32.8)	59.3 (50.4)
$LSD_{0.05}$	(1.4)	(2.2)	(1.3)	(1.2)	(2.5)	(1.6)

* values in parantheses are $\sin^{-1}\sqrt{x}$ transformed data.

** $LSD_{0.05}$ for $\sin^{-1}\sqrt{x}$ transformed data.

Table 4-11. Relative Distribution of Zn as Expressed in Percentage of Total Uptake in Wheat Plant at Maturity as Affected by P and Zn Application and VAM Inoculation

Treatment	- VAM			+ VAM		
	root	straw	grain	root	straw	grain
P0	21.6 (27.7)*	48.9 (44.4)	29.4 (32.8)	20.3 (26.8)	37.4 (37.7)	43.2 (40.5)
P50	21.6 (27.7)	47.5 (43.6)	30.3 (33.4)	17.8 (25.0)	36.8 (37.4)	45.5 (42.4)
P100	26.5 (31.0)	41.2 (39.9)	32.7 (34.9)	19.8 (26.4)	29.9 (33.2)	50.1 (45.1)
P300	30.1 (33.3)	40.4 (39.5)	29.5 (32.9)	21.1 (27.4)	25.1 (30.1)	53.8 (47.2)
$LSD_{0.05}$**	(3.0)	(2.6)	(1.7)	(1.4)	(2.8)	(2.6)
Zn0	21.6 (27.7)	44.2 (41.7)	34.2 (35.8)	19.5 (26.2)	26.6 (31.1)	54.4 (47.5)
Zn2.5	23.8 (29.2)	46.1 (42.8)	30.3 (33.4)	18.8 (25.7)	35.2 (36.4)	46.1 (42.8)
Zn10	29.4 (32.8)	43.3 (41.2)	27.4 (31.6)	21.0 (27.3)	35.2 (36.4)	44.5 (41.8)
$LSD_{0.05}$	(2.6)	(2.3)	(1.4)	(1.2)	(2.4)	(2.2)

* values in parantheses are $\sin^{-1}\sqrt{x}$ transformed data.

** $LSD_{0.05}$ for $\sin^{-1}\sqrt{x}$ transformed data.

In the root, a high retention of P was obtained at two extreme supplies of P (P0 and P300), and a low retention at the medium supply. These results may suggest that when P supply from the soil is limited, the shoot/root ratio tends to decrease so that the roots could explore more soil effectively to encounter sufficient additional P to supply the plant. Indeed the shoot/root ratio for -VAM plants in P0 or Zn0 treatments is low (Appendix 9). When the plant was mycorrhizal, the root efficiency of absorption was compensated by the fungal hyphae, thus yielding the highest shoot/root ratio at P0Zn0 treatment. With increase in P supply and decrease in shoot/root ratio, percentage of P retained in the root declined. When the P in the soil was in excess, the luxurious consumption of P in the root and shoot may result.

Zn application did not show a conclusive response to P transport in the plant.

In the mycorrhizae-infected plants, the effect of P added on the transport of P in the plants demonstrated the same trend as described above. In comparison between the mycorrhizal and non-mycorrhizal treatments, a difference between them was that the former greatly enhanced P transport from the root to straw, and then to the grain. Although the percentage of P in the infected roots at P300 did not differ from the non-infected roots, the transport of P from the straw to the grain was increased to a great extent.

Table 4-11 shows the general trend of Zn distribution in the plant as affected by different treatments. When the plant was non-mycorrhizae inoculated, increasing P addition resulted in an increase in retention of Zn in the root and transport from straw to grain except at

P300. The highest P treatment led to the highest Zn retention in the root and the lowest in the aboveground portions. In contrast, added P was always favorable to the Zn transport to the grain in the mycorrhizal plant.

The effect of Zn application was unfavorable to its own transport regardless of VAM inoculation, that is, higher retention in the root and lower in the grain. Effect of mycorrhizal infection on Zn transport, like that on P transport, was always beneficial.

The results revealed an overall beneficial effect of mycorrhizae on the nutrient transport or utilization by the plant but an inhibitory effect of high levels of the nutrient supply on its own transport. However in either work (4.3.2 - 4.3.3), we found that both P and Zn concentrations were the highest in the plant at P300 and Zn10 treatment, signifying that the concentrations of P and Zn may have reached their maximal values that can be reached in the grain and thus leading to ineffective accumulation or luxurious consumption of the rest in the non-economic parts of the plant.

The mycorrhizal infection rates in the wheat roots were decreased with both increased P and Zn application in the soil and their concentrations in the plant tissue (Appendix 10).

4.4 DISCUSSION

4.4.1 The Symptoms of Zn Deficiency and P Toxicity

Several investigators have described the symptoms of Zn deficiency (Stiles, 1961; Chapman, 1966; Marschner, 1986) and of "P-induced Zn deficiency" or P toxicity (Loneragan et al., 1979, 1982; Cakmak & Marschner, 1986). It has been postulated that Zn deficiency may interfere with at least two distinct areas of plant metabolism (Loneragan et al., 1982). First of all, zinc is considered as a very important element in the synthesis of 59 enzymes catalyzing various biochemical reactions in plants (Shkolnik, 1984), such as chloroplast enzymes, RNA polymerase, the NADH-dehydrogenases, (Hewitt & Needham, 1984) as well as the metabolism of auxins (Allen et al., 1980, 1982), i.e., the synthesis of tryptophan, a precursor for the synthesis of IAA, requires Zn (Tsui, 1948). Therefore, Zn deficient plants tend to fail to elongate internodes and develop leaves, resulting in dwarfed plants as in cereals and in rosette leaves in many fruit trees (Stiles, 1961; Chapman, 1966). Zinc also plays a distinct role in sexual fertilization (Polar, 1975) and seed formation (Reed, 1943, 1944). Polar (1975) found that pollen grains contained very high contents of Zn in tobacco and fodder beans. The zinc migrates into the pollen tube during pollen germination, and then to the developing seeds. Inside the growing seeds, Zn is required in the normal development of the oosphere and the embryo, and, as a result, pea plants grown under a condition of Zn deficiency produced no seeds (Reed, 1942 and 1944). The findings elucidated why Zn is predominantly stored in the seed and largely

concentrated in the embryo (Shkolnik, 1984).

In the present experiment, however, the plants with the symptoms of abnormal growth were not dwarfed either before or after the symptoms occurred, and the yields of plants with low Zn content were not reduced either when compared to the normal plants, implying that Zn deficiency did not operate directly in producing the symptoms. Furthermore, increasing levels of Zn to high values failed to eliminate the symptoms induced by high P treatment, which is in a good agreement with the findings in soybeans (Paulsen & Rotimi, 1968) and in subterranean clover (Loneragan et al., 1979). Conversely, both high Zn and high P concentrations aggravated the symptoms. The number of leaves that suffered from toxicity was well correlated with P concentrations but not with Zn in the plant. This suggests that the symptoms occurring in the plus Zn plants in this experiment can be attributed to P toxicity, which eliminates the explanation suggested by Loneragan et al. (1979) that P-induced Zn deficiency under the situation with normal Zn contents in the plant tissues was invoked by a "P enhanced Zn requirement" due to the effect of high P concentrations in plant tissues. In Zn0 treatments, Zn concentrations of non-mycorrhizal plant tops were below 20 $mg.kg^{-1}$, which is considered as a critical level of Zn deficiency. It is interesting to note that the plants with low Zn concentrations did not suffer stunting in growth or more severe symptoms of Zn deficiency compared with plus Zn treatments; conversely, the more severe symptoms occurred in the plants containing high P combined with high Zn concentrations, further substantiating that the symptoms were due to P toxicity. It is evident that in the plant suffering from "P-induced Zn

deficiency", tissue Zn concentration is no longer an indicator of the so-called "Zn deficiency" (Boawn and Leggett, 1964; Halim et. al., 1968). Millikan (1963) reported that in a solution culture system, Zn concentrations ranged from 22 to 49 mg.kg^{-} dry matter in the subterranean clover plants suffering from P-induced Zn deficiency as compared to those from 30 to 37 mg.kg^{-1} in the normal plants. In 1982, Loneragan and coworkers observed a similar phenomenon in their experiment, i.e. some of the okra plants showing Zn deficiency symptoms had even higher Zn concentration than the healthy plants. The more important feature, however, as they pointed out, is the unusual high P concentration in the symptomatic plants.

4.4.2 Effect of Zn on P Translocation

It appears that low Zn concentration in the plant of Zn0 treatments did not enhance P uptake and translocation of P from roots to aboveground parts in the present study. This is in a good agreement with the results of Tu and Goh (1989) but discrepant with some other reports by Loneragan et al. (1979, 1982), and Cakmak and Marschner (1986), who found that low Zn concentration or Zn deficiency significantly enhanced P uptake and translocation to tops which led to P toxicity. From their work, lower levels of Zn were considered to predetermine whether P will be accumulated in tops or not. However, their experiments were almost all conducted in nutrient solution or sand culture, in which Zn concentrations can be controlled with ease and even completely eliminated; while this experiment was implemented in soil where Zn concentration, although low, can still supply the plants for

normal growth, but can be never reduced to nil content. Therefore, Loneragan et al. (1979) indicated that the enhancement will occur more easily in plants grown in sand and water culture than in plants grown in soils with some capacity to react with P.

4.4.3 The Interaction of P and Zn

The interaction of P and Zn does not always appear to be negative or antagonistic in plants. More often, the interaction is shifted or altered by amounts of P and Zn applied to the medium, by plant species, and by some other factors. When amounts of P and Zn are both applied at an optimal level or in other words, kept at a metabolic balance, they tend to mutually facilitate the utilization of each other; otherwise, when each of them is applied below or above a certain threshold, the antagonistic relationship occurs. The work of Menser and Sidle (1985) clearly elucidated this relationship of P and Zn in their experiment involving soybeans. As a whole, increased P in the present study depressed Zn concentration and uptake. However, an addition of P50 in combination with Zn yielded the highest utilization of Zn by the -VAM plants (Table 4-5). Similarly, addition of Zn2.5 enhanced P content in -VAM plants more than Zn0 and Zn10 treatments from P0 to P 100 (Table 4-7), although the difference was not significant. By contrast, when the plants were mycorrhizal, they obtained the lowest Zn at P50 and P at Zn2.5 (Table 4-7 and Table 4-5), demonstrating that VAM infection dramatically changed the P-Zn relationship. In addition, the interactions can be changed by other factors. As described in Chapter 3, the field data also revealed different patterns of P-Zn interaction

in barley and canola with the same treatments of P and Zn in the first year of the trial (Tu and Goh, 1989). In the following year, the interactions of P and Zn in the subsequent wheat crop on the different stubbles varied even under the same management practice.

4.4.4 Effect of Light

Light intensity has been reported to have quite large effects on the sensitivity of Zn deficiency (Hoagland, 1944; Ozanne, 1955). Zinc deficiency was stated to be accentuated during the summer in California (Hoagland, 1944). In the present study, a similar phenomenon was observed: five days after rotating a replicate in one chamber with light intensity of 5000 lux to another chamber with light intensity of 4500 lux, the symptoms of abnormal growth in the plants that had been transferred from high to low light intensity were reduced or even disappeared, while the symptoms became severe or accentuated in the plants that had received the opposite light shift. However, the nutrient uptake between replicates under the rotation was relatively constant. It can be speculated that the accentuation of the symptoms in the growth chamber with higher light intensity may have resulted from higher consumption of water under more intensive light, causing high osmotic pressure in plant tissues to damage leaf cell.

4.4.5 Effect of VA Mycorrhizal Infection

The beneficial effects of VA mycorrhizae on growth of many plants when soils are low in phosphorus has been confirmed repeatedly and widely accepted. Besides P, uptake of other nutrients, such as Zn, Cu,

K, are also increased by the mycorrhizal plants (Hayman, 1978; Tinker, 1975, 1978). However, depression of growth following VAM infection have also occasionally been reported (e.g. Tinker, 1978; Buwalda & Goh, 1982; Koide, 1985). As illustrated in this experiment, VAM infection significantly reduced the plant growth, numbers of tillers, height of plant, and dry matter yield up to the heading stage of the growing period. The depression on growth in most cases is considered to be caused by high available P (Mosse, 1973) and by competition for carbon between host and fungus (Cox et al., 1976; Buwalda & Goh, 1982). In the fungus-plant association, VAM infection forms a drain of photosynthetates on the plant. Therefore, the depression will continue as long as the loss of carbon owing to infection exceeds the gain in ability to enhance photosynthesis in mycorrhizal plants. As Saif (1977) and Tinker (1975) described, there are three phases observed in the development of VAM infection: a delay or lag phase, a rapid development phase and an infection plateau. The processes of mycorrhizal establishment in the plant may be responsible for the corresponding depression in plant growth. As pointed out earlier, mycorrhizae exerted a strong impact on plant nutrient uptake and the interaction of P and Zn in this study, that is, the VAM infection greatly depressed nutrient uptake before heading but enhanced nutrient uptake, translocation and plant growth thereafter. Furthermore, the interaction of P and Zn in the plants at the early growth stages of wheat growth was also entirely changed by inoculation with mycorrhizae.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS

5.1 The Interactions of P and Zn in the Plant

Although most studies in literature showed a negative relationship between P and Zn (Millikan, 1951; Watanable et al., 1965; Brown and Leggett, 1964; Loneragan et al, 1979), the interactions of P and Zn do not always appear to be negative or antagonistic in the plant. Some other studies have shown that added P did not affect Zn concentration and uptake in the plant even under the conditions when the plants exhibited symptoms of the so-called "P-induced Zn deficiency" (Millikan, 1963; Boawn and Brown, 1968) or P toxicity (Cakmak and Marschner, 1986), implying that there was no negative P-Zn interaction existing. In some cases, a positive interaction between P and Zn had been observed but nevertheless not studied in detail, a reflection, perhaps, of the investigators' preoccupation with the antagonistic effect between P and Zn nutrition. For example, Millikan (1963) conducted four experiments to study the effects of different levels of P and Zn on the growth of subterranean clover. In one of the trials, increased P fertilization from 0.1 to 1 mM P enhanced both the Zn concentrations and uptake in the plant tops at each level of Zn application, but further increases of P to 2 mM depressed both Zn in the plant. In the same year, Bingham reported a set of data in which adding P from 1 to 100 $mg.kg^{-1}$ resulted in increased Zn concentrations in the leaves of red kidney beans, sweet corn and sour orange seedlings but decreased in the tomatoes only. It

was not until 1981 that Orabi et al. first announced that there was a positive P-Zn relationship in the corn plant. Thereafter, Orabi and coworkers obtained the same results in their following experiments involving corn (e.g. Orabi and Abdel-Aziz, 1982; Orabi et al., 1985). In the present study, similar results were observed: an addition of 50 mg $P.kg^{-1}$ soil in combination with Zn obtained the highest Zn concentration and uptake in the -VAM plant (Table 4-5). However, further increasing or decreasing P application depressed Zn content in the plant. The effect of added Zn on P uptake in the -VAM plant showed a similar trend (Table 4-7). The results suggested that there must exist optimal amounts of P and Zn under which the nutrients mutually facilitate the utilization of each other. Whenever either of the nutrients applied drops below or exceeds a certain threshold, the antagonistic relationship may occur.

5.2 Phosphorus-induced Zn Deficiency

Phosphorus-induced Zn deficiency is not a synonym of the interaction of P and Zn. Instead, it is an extreme example of the interaction only when excessive amounts of P are applied to a medium where both P and Zn are low or deficient to a crop. Under such circumstances, the plant growth is greatly stimulated by the added P , but the uptake of Zn is unable to keep pace with the growth, and as a result, leads to produce Zn deficiency symptoms in the plant. Frequently, the symptoms can be easily corrected by Zn fertilization. This is therefore termed as a dilution effect mechanism of P-induced Zn deficiency as described by many workers (Millikan, 1963; Boawn and

Leggett, 1964; Jackson et al., 1967; Olsen, 1972). If, however, the soil available Zn is sufficient or the crop species is not sensitive to the soil Zn levels normally considered as low or deficient to most other crops, the high rates of P application can also induce symptoms resembling Zn deficiency but hardly affect Zn uptake by the plant, e.g. in cotton (Ghoneim and Bussler, 1980; Cakmak and Marschner, 1987), wheat (Chapter 4 of this thesis), and okra (Loneragan et al., 1982). In these experiments, Zn concentrations in the leaves or tops are no longer satisfactory indicators for the diagnosis of Zn deficiency. In such circumstance, added Zn does not efficiently overcome the symptoms; by contrast, it sometimes even accentuated the symptoms in high Zn treatments as discussed in Chapter 4.

The results from these studies indicated that the severity of the symptoms is well correlated with P concentrations in the plant but does not produce dwarfed or stunted plants. The evidence leads to the conclusion that the "P-induced Zn deficiency" is attributed to P toxicity rather than Zn deficiency (e.g. Loneragan et al., 1979, 1982; Christensen, 1981; Cakmak and Marschner, 1986, and Chapter 4 of the present study).

Since Boawn et al. (1954) reported that their attempts to induce Zn deficiency symptoms with high P (400 bl $P_2O_5.A^{-1}$ equal to 79 kg $P.A^{-1}$) failed even though phosphate was raised to approximately twice its normal concentration in the plant tissue, several authors (Ellis et al., 1964; Halim et al., 1968; Paulsen and Rotimi, 1968; Saeed and Fox, 1979; Adams, 1982; Orabi et al., 1985; and Sumner and Farina, 1986) have cited it to question whether high rates of P are able to induce Zn deficiency

or not. These authors, however, may have neglected the most important point in the paper, that is, the P fertilizer used in the experiment of Boawn et al. was contaminated with Zn at a percentage of 0.65, which may have provided the plant with an sufficient Zn supply. Therefore, the repeated citations appeared to have more or less caused some confusion in the past.

5.3 The Site of P-induced Zn Deficiency

Whether the "P-induced Zn deficiency" takes place in the plant or in the soil has long been in dispute. According to the work done in the past, it is most likely to occur in the root and visually expressed in the aboveground parts, but to be induced by many factors originating from the growth medium and environment, including plant species, soil properties, cropping systems, management practices and other factors related to the soil-plant ecosystem such as mycorrhizal infection. The support for this statement can be obtained from numerous studies done in this area. It is well known that different crop species or varieties differ in their sensitivity to Zn deficiency and resistance to P toxicity (Foote and Howell, 1964; Clark, 1978; Shukla, 1987), as well as in their susceptibility to the "P-induced Zn deficiency" (Safaya and Singh, 1977; Paulsen and Rotimi, 1967; Burlesen et al., 1961). For instance, Halim et al. (1968) examined 24 inbreds and 10 single crosses grown in growth chambers with normal nutrient solution and high P solution. They found some lines very sensitive to Zn deficiency, some with early or late resistance, but only a few with high resistance. The authors, therefore, ascribed the difference to the inherent properties

of the different species, such as the varied "feeding power" among species (Millikan, 1961) or their efficiency of absorption and utilization of nutrient constituents from the soil (Gregory and Growther, 1928; Schorring and Nielsen, 1987). The "feeding power" may be related to the cation-exchange capacity (CEC) of the root cell walls (Marschner, 1986), apparently controlled by a single gene pair (Bernard and Howell, 1964).

However, some inherent properties can be altered with changes in the environmental conditions. For example, Marschner and Scropp (1977) compared grapevines growing in a calcareous soil and in a water culture. They found that Zn deficiency symptoms were induced by high rates of P in the soil-grown plants but not in the water-grown plants even in which P uptake was much higher and Zn was lower than in the soil-grown plants. The results led them to draw a conclusion that the major P-Zn interactions took place in the soil rather than in the plant. It is obvious that this statement is contradictory to the facts that a number of experiments conducted in the nutrient or sand cultures have successfully induced Zn deficiency in the plant. Without question the soil chemical and physical constituents and the allelopathic substances from other organisms coexisting in the soil may greatly affect the interactions between nutrients and thus plant growth. It has been well documented that soil chemical properties, especially the pH, dictate a series of soil chemical reactions and availability of nutrients to plants. Absorption ability and efficiency of the plant roots to certain nutrients are drastically changed with the pH of the medium. That the maximal availability of P to plants is known to be between pH 5.0 and

6.5 and Zn deficiency more readily occurs in calcareous soils have been familiar facts to soil scientists. In addition, increased anions specifically adsorbed by sesquoxides in the acid soils result in a increase in Zn sorption (Saeed and Fox, 1979; Shuman, 1977; Goh et al., 1986a). Furthermore, the allelopathic substances from plant residues and soil microbes, acting as either inhibitors or stimulators to the plant growth, tend to have an effect on wheat germination, root development (wheat and barley straw had some phytotoxicity up to 30% root inhibition) and the absorption ability, as well as shoot growth (Elliott et al., 1978).

In the present study, we also found that the P-Zn interaction differed not only in various crops, barley and canola under the same soil and management practice but also in the same wheat crop under different rotations (Chapter 3). In the growth chamber experiment, inoculating the wheat plants with mycorrhizae, which was done with intention to change the "feeding power" of the root, remarkably reduced the "P-induced Zn deficiency" symptoms compared to non-mycorrhizal plants (Chapter 4).

5.4 Conclusions

This study has clearly shown that the relationship between P and Zn, like that between any other nutrients, can be expressed negatively, positively or additively. In most cases, the interactions are observed as antagonism, most likely attributed to the positive interaction in most crops frequently occurring in a rather narrow range of P supply.

The results suggest that high rates of P are able to induce "Zn

deficiency symptoms" in the plant. However, it is notable that there is little, if any, risk for the normally recommended rates of P fertilization to induce Zn deficiency in the plant, except under some special conditions, such as low available soil Zn and sensitive crops. In this study the symptoms were due to P toxicity despite the similarity of appearance to the simple Zn deficiency. For this reason, the severity of the symptoms was well correlated with P concentrations in the plants. Under this circumstance, the Zn concentration in the plant was normal or even higher than in the healthy plant and of course was no longer an indicator of the diagnosis and the symptoms could not be overcome by Zn application either.

The results revealed that the interaction of P and Zn as well as the "P-induced Zn deficiency" evidently take place in the plant root but are controlled by the inherent properties of plant species, soil properties, cropping systems and some other factors related to the soil-plant ecosystem. Canola exhibits high sensitivity to low soil Zn compared to barley and wheat, hence suffering from Zn deficiency and resulting in reduction in seed yield under the same environmental conditions. Canola stubble was favorable for the growth of a following wheat crop but resulted in an overall negative P x Zn interaction in both the canola and wheat plants. Barley stubble, however, was inhibitory to its subsequent wheat crop but yielded a positive interaction. The differences between the two rotations were most likely caused by the allelopathic effect of decomposing crop residues and the metabolic substances of residue-related soil microbes. Mycorrhizal infection had a beneficial effect on plant growth and grain quality, and

alleviated P toxicity by regulating the feeding power of the wheat plant.

Based on the present study, I believe that in calcareous soils, Zn application is favorable for oilseed production when the soil DTPA extractable-Zn is below 1.0 mg/kg.

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Appendix 1

Hoagland Solution

Solution 1

Ingredients	g/l	ml in a litre	Comments
1M KH_2PO_4	136*	1	Add 1 ml of solution A to
1M $KHNO_3$	101.1	5	either solution 1 or 2 and
1M $Ca(NO_3)_2$	164.1	5	bring to a 1; add solution
1M $MgSO_4$	120.3	2	B or its alternate, adjust pH to 6 with 0.1N H_2SO_4

Solution 2

1M $NH_4H_2PO_4$	132.07	1
1M KNO_3	101.1	6
1M $Ca(NO_3)_2$	164.1	4
1M $MgSO_4$	120.3	2

Solution A

Ingredients	g/l	ppm
H_3BO_3	2.86	0.5
$MnCl_2.4H_2O$	1.81	0.5
$ZnSO_4.7H_2O$	0.22	0.05
$CuSO_4.5H_2O$	0.08	0.02
$H_2MoO_4.H_2O$	0.02	0.01

Solution B

	g/l	ml in 1 litre
Iron tartrate	5.0	1

(necessary to add at regulary intervels)

Alternate Solution B - Dissolve 26.1 g ethylene-diamine tetra-acetic acid in 268 ml of 1N KOH. Then add 24.9 g $FeSO_4.7H_2O$ and dilute to 1 litre. After aerating overnight to produce the stable ferric complex, the pH should be about 5.5. A ml provides 5 ppm to 1 l of solution - one addition is enough.

* - In VA mycorrhizal culture, P was reduced to 0.31 mM in the solution.

Appendix 2

Phillips - Hayman Root Staining

Improved procedures for clearning roots and staining parasites and VAM fungi for rapid assessment of infection.

Transactions British Mycological Society. 1970. 55(1):158-161.

The Procedures:

1. Heat roots at 90°C for about 1 h in 10% KOH or NaOH, or stand for about 20 h.

2. Rinse in distilled water and acidfy with dilute HCl.

3. Stain the roots by semmering for 5 m in 0.05% trypan blue in lactophenol (glycerol), remove the excess stain in clear lactophenol (glycerol).

4. Place stained roots in a jar and fill with holding solution*.

5. Stand for a day or two before checking for fungal infection.

* Holding solution:	lactic acid	100 ml
	glycerol	100 ml
	water	200 ml

Appendix 3

Mean Squares for the Analysis of Variance for Concentrations of Nutrients Analyzed in the Non-VAM Wheat Plant at Heading Stage

	P	Zn	Cu	Mn	Fe
P effects					
0 vs others	0.6**	64.7	3.2	402.1**	65.6
50 vs (100+300)	0.3**	200.3**	0.0	220.0*	892.2**
100 vs 300	0.1**	1.4	1.7	2334.7**	23.3
P linear	0.9**	167.2**	0.1	306.3**	312.7
P quadratic	0.1**	28.8	4.8*	2189.0**	98.3
Zn effects					
0 vs others	0.02*	2860.2**	2.2	118.6	108.8
2.5 vs 10	0.0	1184.4**	1.0	266.7*	609.0*
Zn linear	0.01*	150.9**	0.1	373.2**	111.3
Zn quadratic	0.0	3893.7**	3.2	12.1	606.5*
Interactions					
P in Zn0	0.2	112.7*	2.7	1042.1**	86.2
P in Zn2.5	0.2	85.0	8.3*	692.2**	699.1**
P in Zn10	0.2	188.0**	0.5	1317.9**	3.5
P linear in Zn0	0.1	2.9	0.1	230.2**	209.4
P linear in Zn2.5	0.2	1.1	0.7	33.4	181.4
P linear in Zn10	0.2	111.5**	0.1	87.6*	7.2
P Q* in Zn0	0.0	65.0*	1.4	660.6**	46.5
P Q in Zn2.5	0.0	35.3	1.3	482.1**	142.9
P Q in Zn10	0.0	24.2	0.4	1114.2**	2.5
Zn in P0	0.0	854.7**	0.5	21.4	87.0
Zn in P50	0.0	888.9**	1.6	1.6	827.6**
Zn in P100	0.0	926.2**	4.5*	43.7	43.0
Zn in P300	0.0	852.1**	0.2	85.5	93.8
Zn linear in P0	0.0	56.6*	0.0	16.6	145.2
Zn linear in P50	0.0	44.9*	1.1	0.3	160.0
Zn linear in P100	0.0	39.4	0.3	42.3	13.9
Zn linear in P300	0.0	15.1	0.2	1.4	0.0
Zn Q in P0	0.0	798.1**	0.5	4.8	28.9
Zn Q in P50	0.0	843.5**	0.5	1.3	1495.2**
Zn Q in P100	0.0	886.8**	0.2	1.4	72.1
Zn Q in P300	0.0	836.9**	0.0	84.1	187.7

Q* - quadratic

*,** - indicates 0.05 and 0.01 significance level, respectively.

Appendix 4

Mean Squares for the Analysis of Variance for Concentrations of Nutrients Analyzed in the VAM Wheat Plant at Heading Stage

	P	Zn	Cu	Mn	Fe
P effects					
0 vs others	0.3**	250.9**	0.7	1055.3**	408.7**
50 vs (100+300)	0.2**	47.0	4.1*	634.1**	19.6
100 vs 300	0.04**	0.3	0.1	1309.0**	36.1
P linear	0.9	167.2**	0.1	704.4**	311.2*
P quadratic	0.1	28.8	4.8*	2248.4**	113.4
Zn effects					
0 vs others	0.0	2261.3**	0.1	36.4	83.6
2.5 vs 10	0.0	1171.8**	0.1	11.2	39.0
Zn linear	0.0	150.9**	0.1	36.4**	3.4
Zn quadratic	0.0	3893.7**	3.2	12.1	119.3
Interactions					
P in Zn0	0.3	112.0	1.4	1744.0**	89.2
P in Zn2.5	0.4	177.4*	3.9	1002.4**	25.9
P in Zn10	0.3	135.7	2.0	468.4*	145.0*
P linear in Zn0	0.2	75.5	0.8	479.1**	119.4
P linear in Zn2.5	0.4	8.3	0.9	325.1*	29.0
P linear in Zn10	0.3	117.2*	0.1	36.6	202.8*
P Q* in Zn0	0.0	31.0	0.6	1248.1**	46.2
P Q in Zn2.5	0.0	5.9	2.8	677.2**	2.4
P Q in Zn10	0.1	1.7	1.7	431.7**	174.0
Zn in P0	0.0	614.4**	4.0	104.4	163.9*
Zn in P50	0.0	1568.7**	1.2	279.2	15.4
Zn in P100	0.0	771.2**	0.5	246.2	32.8
Zn in P300	0.0	1249.1**	0.1	13.4	7.2
Zn linear in P0	0.0	28.9	0.2	75.0	35.9
Zn linear in P50	0.0	77.9*	0.4	277.9*	7.2
Zn linear in P100	0.0	54.1	0.0	246.2*	21.8
Zn linear in P300	0.0	9.0	0.1	5.7	5.4
Zn Q in P0	0.0	585.5**	3.8	29.4	291.9*
Zn Q in P50	0.0	1490.8**	0.8	1.3	23.7
Zn Q in P100	0.0	717.1**	0.5	0.0	43.9
Zn Q in P300	0.0	1240.0**	0.0	7.7	9.0

Q* - quadratic

*,** - indicates 0.05 and 0.01 significance level, respectively.

Appendix 5

Mean Squares for the Analysis of Variance for Top Yield of Wheat at Heading Stage

	-VAM	+VAM
P effects		
0 vs others	219.9	537.7*
50 vs (100+300)	774.5*	74.5
100 vs 300	23.3	1.6
P linear	417.0*	311.5*
P quadratic	217.5	110.9
Zn effects		
0 vs others	53.2	163.5
2.5 vs 10	609.0	157.5
Zn linear	161.9	3.5
Zn quadratic	500.3*	116.8
Interactions		
P in Zn0	267.7	681.9
P in Zn2.5	76.7	2097.4**
P in Zn10	435.1*	10.5
P linear in Zn0	119.4	369.2
P linear in Zn2.5	29.2	181.4
P linear in Zn10	202.8*	7.2
P quadratic in Zn0	46.2	230.8
P quadratic in Zn2.5	3.0	142.9
P quadratic in Zn10	174.0	2.6
Zn in P0	327.9*	358.0
Zn in P50	30.9	1802.7**
Zn in P100	63.4	86.0
Zn in P300	14.4	187.7
Zn linear in P0	35.9	355.2
Zn linear in P50	7.2	104.0
Zn linear in P100	22.5	13.9
Zn linear in P300	5.4	0.0
Zn quadratic in P0	291.9*	2.8
Zn quadratic in P50	23.7	1698.7**
Zn quadratic in P100	40.9	72.1
Zn quadratic in P300	9.0	187.7

*,** - indicates 0.05 and 0.01 significance level, respectively.

Appendix 6

Mean Squares for the Analysis of Variance for Root, Straw and Grain Yield of Wheat

	-VAM			+VAM		
	root	straw	grain	root	straw	grain
P effects						
0 vs others	36.4**	50.9**	142.5**	29.1**	135.7**	112.6**
50 vs 100+300	15.0*	3.9	11.2	0.4	0.1	9.3*
100 vs 300	0.0	6.5	0.0	0.3	0.0	0.4
P linear	31.7**	45.1**	74.1**	14.1**	40.6**	66.8**
P quadratic	0.0	6.5	0.0	0.3	0.0	0.4
Zn effects						
0 vs others	0.6	2.0	0.5	0.5	3.6	0.1
2.5 vs 10	1.6	13.3	0.2	2.3**	0.0	0.0
Zn linear	0.1	2.7	0.6	0.3	1.4	0.1
Zn quadratic	2.1	12.6	0.1	2.5**	2.2	0.0
Interactions						
P in Zn0	7.0	10.7	37.1**	15.4**	50.3**	34.5**
P in Zn2.5	34.4**	39.6	62.6	9.9**	40.1**	48.6**
P in Zn10	17.3**	49.0*	57.5	6.4**	48.3**	46.6**
P L* in Zn0	5.9*	10.0	21.3	6.3**	14.4**	21.7**
P L in Zn2.5	18.1**	8.8*	25.4	7.1**	17.3**	30.7**
P L in Zn10	9.4**	5.1	27.5	1.8*	9.5*	15.7**
P Q* in Zn0	1.1	0.5	13.9	7.6**	28.6**	9.9*
P Q in Zn2.5	13.4**	0.6	36.9	2.1*	19.3**	17.7**
P Q in Zn10	7.9**	36.9*	28.7	3.8**	25.3**	28.1**
Zn in P0	1.1	6.0	2.1	1.2	0.4	0.8
Zn in P50	0.3	22.9	0.3	1.6	3.0	3.0
Zn in P100	6.4*	21.1	1.6	1.8	1.8	1.5
Zn in P300	1.8	3.2	0.2	0.2	1.2	1.6
Zn L in P0	0.8	3.8	1.2	1.1	0.3	0.8
Zn L in P50	0.1	12.1	0.1	0.1	0.1	0.4
Zn L in P100	0.0	10.6	0.0	0.0	1.2	0.4
Zn L in P300	0.1	2.3	0.1	0.2	1.2	1.4
Zn Q in P0	0.3	2.2	0.9	0.1	0.1	0.0
Zn Q in P50	0.2	10.8	0.2	1.5*	2.9	2.5
Zn Q in P100	6.4*	10.6	1.6	1.7*	0.7	1.1
Zn Q in P300	1.7	0.9	0.1	0.1	0.1	0.2

L* -- Linear Q* -- Quadratic
*,** indicates 0.05 and 0.01 significance level, respectively.

Appendix 7

Phosphorus Distribution as Expressed in Percentage of Total Amount in Wheat Plant Tissues at Maturity as Affected by Each Level of VAM, P and Zn Application

Treatment		Zn added (mg/kg)								
VAM	P added (mg/kg)	0	2.5	10	0	2.5	10	0	2.5	10
		root			straw			grain		
-VAM										
	0	19.4	21.1	19.8	17.3	18.4	19.4	63.3	60.4	60.7
	50	11.9	12.6	14.1	29.3	30.1	27.7	58.0	57.3	58.2
	100	10.5	12.3	13.3	41.8	38.5	35.3	48.3	49.2	51.4
	300	14.7	14.1	15.3	44.3	47.2	47.7	41.0	37.0	37.0
+VAM										
	0	11.4	11.3	11.3	23.3	19.9	19.4	65.5	68.5	69.4
	50	7.8	10.6	9.7	35.3	26.6	26.7	56.9	62.8	63.6
	100	10.1	10.4	10.5	35.2	32.1	32.8	54.6	57.5	56.7
	300	14.1	14.1	14.3	36.8	39.4	38.6	49.1	46.6	47.0

Appendix 8

Zinc Distribution as Expressed in Percentage of Total Amount in Wheat Plant Tissues at Maturity as Affected by Each Level of VAM, P and Zn Application

Treatment		Zn added (mg/kg)								
VAM	P added (mg/kg)	0	2.5	10	0	2.5	10	0	2.5	10
		root			straw			grain		
-VAM										
	0	16.5	21.8	26.5	47.6	50.2	48.9	35.7	28.0	24.5
	50	19.0	20.9	25.0	45.3	49.3	47.9	35.7	29.8	27.0
	100	22.3	24.6	32.5	43.0	42.9	37.8	34.8	33.5	29.7
	300	28.7	27.9	33.7	40.7	42.0	38.5	30.4	29.9	28.3
+VAM										
	0	19.6	19.1	22.1	30.2	40.2	41.7	50.3	40.8	38.4
	50	20.1	15.0	18.2	28.4	45.9	36.1	51.4	39.5	45.7
	100	19.6	19.3	20.4	25.3	31.4	33.1	55.0	49.1	46.5
	300	18.5	21.8	23.1	22.4	23.2	29.7	59.1	55.0	47.2

Appendix 9

The Shoot/Root Ratio of the Wheat Plant as Affected by Mycorrhizae, P and Zn Application

	-VAM			+VAM		
	Zn0	Zn2.5	Zn10	Zn0	Zn2.5	Zn10
P0	4.15	4.43	4.26	5.33	4.56	4.97
P50	4.30	4.68	4.22	4.66	4.64	4.82
P100	4.33	4.16	3.24	4.52	4.90	5.20
P300	4.35	3.98	3.78	4.57	4.95	4.63

Appendix 10

The Mycorrhizal Infection (%) in the Wheat Roots at Maturity in the Growth Chamber Experiment Following Inoculation with VAM

P added ($mg.kg^{-1}$)	Zn added ($mg.kg^{-1}$)			average
	0	2.5	10	
P0	39.3	25.6	32.5	33.2
P50	31.8	21.9	16.1	23.2
P100	28.3	25.2	11.7	18.2
P300	13.9	14.6	9.4	12.7
Average	28.3	21.8	17.5	