Effects of Phosphorus and Copper on Factors Influencing Nutrient Uptake, Photosynthesis, and Grain Yield of Wheat[1]

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ABSTRACT. Application of large amounts of phosphorus fertilizers is used to enhance early plant growth and yield of wheat (*Triticum aestivum* L.). This practice can lead to an accumulation of phosphorus in soil and a reduced copper uptake by plants which may result in a reduction of plastocyanin concentrations in chloroplasts. As a result, photosynthetic rates and crop yield can be adversely affected. While copper uptake is sometimes enhanced by vesicular-arbuscular mycorrhizal (VAM) fungi, large accumulations of soil phosphorus can reduce VAM-enhanced copper uptake.

Varying amounts of phosphorus and copper were applied to research plots in order to measure their effect on the wheat leaf content of phosphorus, copper, and plastocyanin. VAM spore counts, leaf photosynthesis, and grain yield were also measured.

Application of phosphorus to soil increased leaf phosphorus content, but decreased VAM spore count and photosynthesis. Application of copper to soil decreased VAM spore count but had no other effects. The combined effects of soil applied phosphorus and copper increased both photosynthetic rates and grain yield. As expected, photosynthetic rates increased with increasing leaf plastocyanin content, but grain yield could not be related to either plastocyanin content or photosynthetic rates.

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INTRODUCTION

Copper (Cu) is one of the 16 essential chemical elements required for plant growth and reproduction. Copper uptake and concentration in plant tissue is influenced by various contributing factors such as soil applied phosphorus (P) fertilizers which, in excess, reduced copper uptake by plants (Bingham and Garber 1960, Sparling and Tinker 1978). Since it is readily fixed by soil, large quantities of phosphorus are sometimes applied at seeding in order to meet the large phosphorus requirement of plants, especially at early stages of growth (Kline and Rust 1966). This practice can lead to high soil levels of phosphorus that affect the intermediatory portion of the soil-plant system, the vesicular-arbuscular mycorrhizal (VAM) fungi. This organism provides an absorbing mechanism, and in association with roots of host plants can play an important role in uptake and transport of slowly mobile nutrients. Elevated levels of soil phosphorus reduced the VAM population and root infection rates in several studies (Khan 1975, Mosse 1981, Sparling and Tinker 1978, Tinker 1980) which led to decline in uptake, translocation, and concentration of copper in plant tissue (Hattigh et al. 1973, Rhodes and Gerdemann 1975). Chloroplasts can account for 60–80% of the total copper content of leaf cells (Hewitt 1983, Neish 1939) where it is mainly concentrated in plastocyanin (PC) and superoxide dismutase, and where the plastocyanin concentration is usually six-fold higher than that of superoxide dismutase (Hewitt 1983). The presence of plastocyanin is essential to electron transport in the photosynthetic (PS) apparatus of green leaves (Atta-Asafo-Adje and Dilley 1985, Malkin et al. 1969).

MATERIALS AND METHODS

A field study, designed to determine the effects of soil applied phosphorus and copper on soil microrrhizal spore population, leaf phosphorus and copper content, plastocyanin concentrations, photosynthetic rate, and grain yield of wheat was conducted during the 1987 growing season on a Crosby silt loam soil (fine, mixed, mesic aeric ochraqualfs). The site for this study was The Ohio State University's Don Scott Agronomy Farm in northern Columbus, OH. Triple superphosphate ($Ca[H_2PO_4]_2$) was applied at rates of 0, 560, 1680, and 3360 Kg•ha^{-1} and copper sulfate ($CuSO_4 * 5H_2O$) was applied at rates of 0, 33.6, and 67.3 Kg•ha^{-1}, and both were incorporated to a depth of 10 cm by disking the research area twice.

Treatments were arranged in a split-plot design with soil application of triple superphosphate and copper sulfate as main and subplots, respectively. The study was organized in a randomized complete block design with three replications.

Prior to conducting this experiment, a soil sample was collected from the research area and analyzed for nutrient levels using recommended chemical soil test procedures for the North Central region (Table 1). Wheat was seeded in rows spaced 18 cm apart and at a depth of 3.6 cm, on

TABLE 1

Soil test values for the field research area prior to application of P and Cu.

Soil test data	
pH	6.9
P (ug g^{-1})	27
K (ug g^{-1})	113
Ca (ug g^{-1})	1995
Mg (ug g^{-1})	238
Fe (ug g^{-1})	51.2
Zn (ug g^{-1})	5.5
Cu (ug g^{-1})	1.5

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20 October 1986. The seeding rate was 56.6 viable seeds per meter of row. At the inception, plots were 2.3 m wide and 7 m long, but were end trimmed to 6.4 m length at harvest. The level of soil nutrients other than phosphorus and copper was maintained above the minimum recommended level for maximum yield (Ohio Agronomy Guide 1985). A 28% nitrogen fertilizer solution (urea, ammonium nitrate) was used to apply nitrogen at the rates of 84 Kg•ha^{-1} on 20 March 1987 followed by an additional nitrogen application of 56 Kg•ha^{-1} on 28 April 1987. Upon appearance of the first stem node on 4 April 1987, 1.1 L of Brominal (octanoic acid, 3,5-dibromo-4-hydroxybenzonitrile ester; butyric acid 3,5-dibromo-4-hydroxybenzonitrile ester; 2-methyl-4-chlorophenoxyacetic acid, butoxyethyl ester) ha^{-1} was applied to control weeds. Upon complete emergence of the uppermost leaf (flag leaf), these leaves were sampled and analyzed for copper and phosphorus content using the AOAC Official Methods of Analysis (1975).

The plastocyanin content of 700 g of fresh leaf tissue was determined using the procedure described by Graziani et. al (1974). Soil VAM spore populations were determined by the flotation-adhesion technique described by Sutton and Barron (1971). The photosynthetic rates of intact leaves were determined using a portable LI-Cor (LI-6000) photosynthesis meter using the technique described by Dwyer et al. (1989).

Plots were harvested on 10 July 1987 and plot grain yields were determined as Kg•ha^{-1} of grain at 13% moisture. Following harvest, appropriate statistical analyses of variance (Table 3) were conducted using *F* ratios to determine significance at the 0.05 level of probability. Values of least significant differences were calculated and used to compare treatment means (Table 2). Linear regression equations were developed to: quantify the cause and effect relationships of significant treatments (Table 4) along with their coefficients of determination. Additionally, regression equations were developed to quantify the relationships of: 1) PS rate *vs.* soil applied P and Cu, 2) PS rate *vs.* leaf PC, 3) grain yield *vs.* soil applied P and Cu, and 4) leaf phosphorus and copper concentrations *vs.* leaf plastocyanin.

RESULTS

The leaf phosphorus, copper, and plastocyanin content along with photosynthetic rates and grain yield of wheat, and VAM fungi spore count are presented (Table 2). The addition of triple superphosphate to the soil increased the leaf content of phosphorous, but inversely affected both VAM fungal spore population and photosynthetic rates (Table 4). Soil triple superphosphate application had no effect on the leaf content of copper or plastocyanin nor did it effect grain yield (Table 3).

TABLE 2

Leaf P, Cu and Plastocyanin concentrations, spore count, photosynthesis rate and grain yield as affected by soil application of phosphorus and copper.

P and Cu Soil Applications (kg•ha^{-1})	Leaf P (ug•g^{-1})	Leaf Cu (ug•g^{-1})	Plastocyanin (ug•g^{-1})	Spore Count (Spores/10 g soil)	Photosynthetic Rate mole CO_2m^{-2}•s^{-1}	Grain Yield (kg•ha^{-1})
0.0 P						
0.0 Cu	3737	6.3	5.0	21.0	30.7	4685
33.6 Cu	3540	4.9	3.8	14.7	27.7	4249
67.3 Cu	3668	5.8	4.1	14.7	28.7	4241
Avg.	3648	5.6	4.3	16.7	29.0	4391
560 P						
0.0 Cu	4025	4.4	4.0	14.7	27.0	4506
33.6 Cu	4124	5.1	3.4	12.0	27.3	4812
67.3 Cu	4213	5.4	3.8	12.7	27.7	4672
Avg.	4120	4.9	3.7	13.1	27.3	4663
1680 P						
0.0 Cu	4713	5.7	3.7	13.3	27.7	4697
33.6 Cu	4795	4.7	3.4	10.3	27.0	4231
67.3 Cu	4815	4.6	3.6	7.3	27.3	4403
Avg.	4774	5.0	3.6	10.4	27.3	4444
3360 P						
0.0 Cu	4619	4.6	2.8	6.7	21.7	4160
33.6 Cu	5192	5.2	3.2	5.3	28.0	5209
67.3 Cu	5291	5.6	3.4	3.3	28.0	4518
Avg.	5033	5.1	3.1	5.1	25.8	4629
LSD .05						
Phosphorus	629	N.S.	N.S.	4.7	1.4	N.S.
Copper	N.S.	N.S.	N.S.	2.5	N.S.	N.S.
P x C	N.S.	N.S.	N.S.	N.S.	1.2	599

TABLE 3

Mean squares for wheat leaf P, Cu, PC, PS, soil spore numbers, and yield.

Source of Variations	dF	Mean Squares					
		Leaf P	Leaf Cu	Leaf PC	PS	Spores	Yield
Block (B)	2	583758.25	0.94	0.29	1.44	10.11	588939.5
Phosphorus (P)	3	3553274**	0.87	2.06	14.55*	216.9**	162436.93
Error (P x B)	6	297659.47	1.75	1.00	1.55	16.55	783775.90
Copper (Cu)	2	152574.25	0.50	0.61	4.19	61.77**	87340.53
P x Cu	6	102513.14	1.16	0.42	14.53**	5.55	398967.34*
Error	12	69619.875	0.88	0.43	2.07	8.32	119871.47

* and ** = significant at $P<0.05$ and $P<0.01$, respectively.

TABLE 4

Regression equations derived from the data in Table 2 and accompanying coefficients of determination.

Regression Equation	Coefficient
Spore Count = 15.9 - 0.003 Applied P	0.60
Spore Count = 13.5 - 0.06 Applied Cu	0.11
Leaf P = 3834 + 0.04 Applied P	0.59
Leaf PC = 1.67 - 0.0004 leaf P + 0.07 leaf Cu	0.79
PS Rate = 28.0 - 0.0008 Applied P - 0.02 Applied Cu	-.20
PS Rate = 28.5 - 0.001 Applied P	0.20
PS Rate = 22.6 - 0.0008 leaf P + 1.6 leaf Cu	0.45
PS Rate = 19.9 + 2.04 leaf PC	0.53
Grain Yield = 3108 + 0.25 leaf P + 66.4 leaf Cu	0.12
Grain Yield = 4505 + 0.04 Applied P - 0.8 Applied Cu	0.10

The addition of copper sulfate to the soil reduced the VAM fungal spore population, but had no other effects. The interaction of soil applied triple superphosphate and copper sulfate increased photosynthetic rates and grain yields, but had no other effects.

As expected, photosynthetic rates increased with increasing leaf plastocyanin content, but grain yield could not be related to either leaf plastocyanin content or photosynthetic rates using regression. Regression analysis indicated that leaf phosphorous and copper levels interacted to influence leaf PC levels, photosynthetic rates, and grain yield. The regression equation relating leaf phosphorus and copper levels to leaf plastocyanin content (Table 4) had a coefficient of determination of 0.79, which reveals the strong cause and effect relationship between leaf phosphorous and copper, and leaf plastocyanin content. The correlation coefficient for leaf copper and plastocyanin content was 0.84 and indicates that most of the leaf copper was located in the plastocyanin, which has also been indicated by Hewitt (1981). The PS rate was increased by the interaction of soil applied P and Cu, as was grain yield. PS rates also increased with increasing leaf PC levels (Table 4).

DISCUSSION

The increase in leaf phosphorus content seen in the present study was caused by the soil application of triple superphosphate and is in agreement with the findings of Timmer and Leyden (1980). Addition of triple superphosphate to the soil apparently increases the phosphorus concentration gradient between the soil and roots, enhancing phosphorus uptake by plants. The reduced number of VAM fungal spores resulting from soil applications of phosphorous and copper agrees with the findings of other researchers (Hepper and Smith 1976, Kline and Rust 1966, Timmer and Leyden 1980). When phosphorus deficiency occurs, plants may release large amounts of sugar and amino acids into the rhizosphere which are utilized by VAM fungi for growth. Such a relationship was demonstrated previously in phosphorus deficient sorghum (Graham et al. 1981). The amount of substrate leakage in phosphorus deficient plants has been negatively correlated to phospholipid levels in root cell membranes (Ratnayake et al. 1981). Therefore, elevated phosphorus concentrations in plants may reduce the substrate leakage rate, thus suppressing fungal infection of the roots. This, however, did not limit the uptake and concentration of phosphorus in the leaf tissue. An abundance of phosphorus in the soil was adequate to compensate for the reduced number of VAM fungal spores, and root uptake of phosphorus was adequate.

The addition of copper sulfate to the soil also suppressed the fungal spore population. Heavy metals have been shown to reduce or inhibit germination of VAM fungal spores (Hepper 1979, Hepper and Smith 1976). Although high levels of phosphorus in soils have been associated with reduced copper uptake by plants (Bingham and Garber 1960, Racz and Haluschak 1974, Timmer and Leyden 1980), nitrogen is known to enhance copper uptake (Loneragan 1981, Singh and Swarup 1982) by forming a soluble organic N - Cu compound that moves freely in the xylem and phloem saps and thus facilitates the translocation of copper throughout the plant (Loneragan 1981). Nitrogen also encourages root growth which facilitates additional uptake of nutrients, especially the slowly mobile nutrients such as copper. Nitrogen fertilizer was liberally applied to the field plots used in this study, and therefore, nitrogen may have facilitated the translocation of root-absorbed copper, which could account for the absence of a phosphorus-induced copper deficiency in leaves.

Within chloroplasts, preplastocyanin forms apoplastocyanin, which acts as a chelating agent with a very high affinity for copper (Garrett et al. 1984).

Plastocyanin is an essential part of the electron transport system, thus influencing photosynthetic rates. In this study, photosynthesis and plastocyanin were strongly related having a coefficient of determination of 0.53 (Table 4). Photosynthesis was, however, negatively influenced by high leaf phosphorus concentrations. For illuminated intact chloroplasts, high concentrations of orthophosphate have been shown to inhibit photosynthesis, although this effect is reversible (Walker 1976).

In our study, the photosynthesis rate did not significantly affect grain yield. This was probably the result of grain yield being established over a 30- to 34-day period, while photosynthesis was measured but once during that period. Two researchers (Gent and Kyomoto 1985, 1989) found that canopy photosynthesis measured during early stages of grain fill were not related to yield. It has been suggested that as much as half of the photosynthate may be lost during the grain fill period through respiration and abscission (Gent and Kyomoto 1989). These results help to explain the lack of a relationship between photosynthesis and grain yield found in our study. The increase in grain yield resulting from the soil application of phosphorus and copper has been confirmed by Singh and Swarup (1982), but only when nitrogen was applied along with the phosphorus and copper.

In this study, the uptake of soil nutrients by plants was not adversely affected by a suppression of the VAM spore population. Nitrogen enhanced nutrient uptake and the abundance of nutrients in the soil resulted in the absence of mineral deficiency in the leaf tissue. The quantity of a nutrient in a soil alone does not always influence the status and physiological contribution of that nutrient in the leaf tissue of the plant. In the present study, the addition of copper to the soil did not influence leaf copper content, nor did it induce an elevation of plastocyanin concentration. Even so, regression analysis indicated leaf PC was a function of leaf P and Cu levels, which in turn affected photosynthetic rates. However, a strong dependency of crop yield on photosynthesis rate was not apparent.

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