EFFECT OF APPLIED B, Cu, Mn AND Zn ON SOYBEAN YIELD AND MICRONUTRIENT CONCENTRATION

by

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INTRODUCTION

As soybean production continues to intensify, nutritional guidelines for maximum production require re-evaluation. The use of concentrated fertilizers on highly weathered soils with depleted micronutrient reserves and increased crop yields has resulted in more frequent occurrence of micronutrient deficiencies. Application of micronutrients to soybeans has received much attention in the Atlantic Coastal Plain region because of possible yield losses due to micronutrient deficiencies. Manganese deficiency of soybeans has been prevalent in this area for many years. Sandy-textured soils with low organic matter content and with pH values ranging from slightly acidic to neutral levels frequently supply inadequate Mn for soybeans. Recent studies under similar conditions indicate the possible occurrence of B, Cu and Zn deficiencies in soybeans, especially after correction of Mn deficiency. It is in this environment that the demand for other elements also increases.

The present study was undertaken to investigate the response of soybeans to B, Cu, Mn and Zn applications and to evaluate methods, rates and times of Mn application. Five field experiments were conducted for this research at four different locations in the Atlantic Coastal Plain region during the 1982 and 1983 growing seasons.

The objectives of this study were as follows:

1) to determine the effect of B, Cu, Mn and Zn applications on soybean seed yield on selected soils;
2) to compare levels of extractable soil B, Cu, Mn and Zn with soybean yield response to application of these micronutrients;
3) to investigate the effect of B, Cu, Mn and Zn applications on the concentration of these micronutrients in soybean plants; and
4) to evaluate the effect of time and rate of foliar Mn applications on soybean seed yield on selected soils.

LITERATURE REVIEW

GEOCHEMISTRY OF BORON, COPPER, MANGANESE AND ZINC

BORON

Boron is the only nonmetal among micronutrient elements. It has a constant valence of +3 and a very small ionic radius (Krauskopf, 1972). Total boron content in soil range from 2 to 1000 µg/g (Fleming, 1980). Soil formed from marine sediments and shales are highest in B; the lowest B contents occur in soils derived from acid igneous rocks and freshwater sedimentary deposits with a coarse texture and a low organic matter content (Aubert and Pinta, 1977; Gupta, 1979; Fleming, 1980).

A large portion of soil B is found in silicate minerals because B has the ability to replace Si in tetrahedrally-coordinated positions (Norrish, 1975). Fleming (1980) pointed out that there are about 30 B silicates and that only tourmaline is present in significant quantities in soils. Tourmaline, which contains 3 to 4% B, is formed from acid rocks and metamorphosed sediments (Norrish, 1975). Most of the plant-available fraction of B originates from sediments or organic materials (Bowen, 1977).

COPPER

The total Cu content in soil ranges from trace amounts to 250 µg/g (Aubert and Pinta, 1977). According to Kubota (1981), the average soil Cu content in the United State is about 9 µg/g and the highly-weathered soils in the Atlantic Coastal Plain have the least Cu with an average of about 5 µg/g. Basalt contains the highest amount of Cu, and among sedimented rocks, shales are the richest in Cu (Fleming, 1980). Copper mostly occurs as primary sulfide minerals such as bornite (Cu_5FeS_4) or chalcopyrite ($CuFeS_2$); leaching and precipitation from these minerals results in secondary sulfide minerals such as chalcocite (Cu_2S) and covellite (CuS) (Parker, 1981; Krauskopf, 1972; Fleming, 1980). The solubility of the sulfide Cu forms is low as a result of strong covalent bonds between Cu and S (Fleming, 1980).

Copper has two valences, +1 and +2, in natural compounds, and +2 is the common valency in most of the compounds found in the earth surface. The Cu^{+1} is more common in minerals formed beneath the earth's surface (Krauskopf, 1972). In soil solution, Cu exists either as a divalent ion or as a divalent complex form, but Cu^{+1} is unstable (Krauskopf, 1972; Fleming, 1980). Stable anionic forms of Cu^{+1} like $CuCl_2^-$ have been found in the soil solution (Krauskopf, 1972).

Copper content in soil is directly proportional to clay content. Since Cu is very immobile, many soil profiles show little variation in Cu content with depth, but in leached soils, the B horizon contains a higher amount of Cu than the A_2 horizon (Hodgson, 1963; Aubert and Pinta, 1977). Formation of covalent bonds between Cu and clay minerals prevents movement of Cu with water flow (Krauskopf, 1972).

MANGANESE

The amount of total Mn in soil varies considerably from <20 to >6000 µg/g (Krauskopf, 1972). Unlike other heavy metals in sedimentary rocks, Mn content is much higher in carbonate rocks like limestone and dolomite than in shales (Krauskopf, 1972). Sandstone contains a relatively small amount of Mn, which ranges from 20 to 500 µg/g (Aubert and Pinta, 1977). In common igneous rocks the ratio of Mn/Fe usually is rather constant and Mn contents in basic eruptive rocks like basalt and gabro are much higher than that in acid eruptive rocks like granite and rhyolite (Krauskopf, 1972; Aubert and Pinta, 1977). Manganese found in igneous rocks is mostly present in silicate minerals in the forms of manganous, the Mn^{2+} cation (Norrish, 1975).

Manganese in naturally occurring compounds shows one of three valences, +2, +3, or +4 (Krauskopf, 1972). Manganous

Mn is the most mobile form of these three and is easily taken up by plants (Cheng, 1973; Aubert and Pinta, 1977). The quadrivalent cation could be found only at very low pH levels and the trivalent cation is unstable in solution (Krauskopf, 1972).

Weathering of Mn minerals in a reduced environment releases Mn^{2+} into solution. Under oxidizing conditions, Mn^{2+} is oxidized and forms manganite (MnOOH), pyrolusite (MnO_2) or other forms of complex compounds (Norrish, 1975). Formation of a large number of oxides and hydroxides and substitution of Mn^{2+} and Mn^{3+} for Mn^{4+} complicate Mn mineralogy. Oxidation and reduction of Mn ions, without changing their position in the crystaline structure, results in mechanical instability by rearrangement of the structure into a new phase (McKenzie, 1977).

Oxidation of Mn is favored by alkaline conditions and, at near-neutral pH values, oxidation is slow. Once a small amount of MnO_2 is formed, the oxidation of Mn^{2+} is accelerated as MnO_2 is autocatalytic (Krauskopf, 1972; Jauregui and Reisenauer, 1982). Pyrolusite is the most stable Mn oxide (Norrish, 1975; Krauskopf, 1972). Various kinds of crystaline Mn oxides are found in soil; among them, birnessite, lithiophorite and hollandite are the most common (McKenzie, 1972). Lithiophorite mainly occurs in neutral to acid subsurface soils whereas birnessite is more common in alkaline surface horizons (Taylor et al., 1964). These complex forms of Mn oxide are present as coatings on other soil particles, as deposits in cracks and veins and as nodules of various shapes up to 2 cm in diameter (Taylor et al., 1964; McKenzie, 1977). Nodules contain oxides of both Fe and Mn as well as other soil particles and their concentric layering suggests a seasonal growth (McKenzie, 1977). Manganese oxides and hydroxides have a high sorption capacity for heavy metals and, thus, may even control the availability of these trace elements.

ZINC

The Zn content in soil ranges from 10 to 300 µg/g and averages approximately 80 µg/g (Lindsay, 1972; Fleming, 1980). Basic eruptive rocks have a higher Zn content than acid eruptive rocks, e.g., basalt contains 100 µg Zn/g, whereas granite has a Zn content of about 40 µg/g (Aubert and Pinta, 1977; Fleming, 1980).

Zinc has only one valence, +2, in natural minerals (Krauskopf, 1972; Lindsay, 1972). Most commonly, Zn^{2+} shows 4-coordination in mineral structures, but in some minerals 6-coordination occurs with oxygen (Krauskopf, 1972). The solubility of Zn compounds is generally high and, as a result, simple compounds like zincite (ZnO) and smithsonite ($ZnCO_3$) do not persist in soil (Lindsay, 1972). Sphalerite (ZnS), the most common Zn mineral in soil, forms where H_2S is produced under reducing conditions (Lindsay, 1972).

High concentrations of Zn^{2+} do not persist in basic solutions due to $Zn(OH)_2$ precipitation. The $Zn(OH)_2$ is unstable and subsequently decomposes to form zincite (ZnO) (Krauskopf, 1972). Zincite reacts with either carbonates or silicates to precipitate smithsonite ($ZnCO_3$), hemimorphite ($Zn_4(OH)_2Si_2O_7{\bullet}H_2O$) or, at higher temperatures, willemite (Zn_2SiO_4). These compounds are too soluble to control the amount of Zn^{2+} in most soil solutions. Krauskopf (1972) postulated that adsorption on clay minerals, hydroxy oxides and organic compounds controls the amounts of Zn^{2+} in soil solutions.

FACTORS AFFECTING THE AVAILABILITY OF BORON, COPPER, MANGANESE AND ZINC IN SOIL

SOIL pH AND Eh

The activity of micronutrients in soil solution is largely determined by the Eh and pH of the environment (Shuman et al., 1979; Sims and Patrick, 1978; Lucas and Knezek, 1972). Generally, higher amounts of soil B, Cu, Mn and Zn occur in the exchangeable and organic fractions at lower pH and Eh levels than at higher levels (Loneragan, 1975; McBride, 1982; Lindsay, 1972).

Several reports on B indicate that the plant available fraction of soil B is negatively correlated with soil pH (Bennett and Mathias, 1973; Bartlett and Picarelli, 1973; Wolf, 1940; Wear and Patterson, 1962; Gupta and Macleod, 1981). However, deviation from this occurs with factors such as crop species and soil characteristics (Gupta, 1977; Martens, 1968). Boron retention by soil clay particles and organic complexes is pH dependent with a maximum in the alkaline range (Berger and Truog, 1945; Peterson and Newmen, 1976; Gupta and Macleod, 1977; Hingston, 1964). Retention of B by Al and Fe hydroxides and oxides decreases B availability under alkaline conditions (Sims and Bingham, 1968; Loneragan, 1975). Loneragan (1975) reported that B adsorption by Fe oxides is strongest at pH 9.0 and becomes weaker as pH decreases. According to Fleming (1980) maximum B adsorption occurs in the pH range of 5.5 to 7.0 for Al oxides and in the range of pH 8 to 9.0 for Fe oxides and clay minerals.

Soil Mn undergoes more changes than Cu and Zn with fluctuations in soil pH (Sims and Patrick, 1978). The oxidation state of Mn varies with the Eh of the environment; Mn^{4+} and Mn^{3+} compounds occur in the solid phase in oxidized environments, while Mn^{2+} is dominant in the soil solution and in the solid phase in reduced environments (Schwab and

Lindsay, 1983). Formation of insoluble higher oxides in oxidized environments decreases the availability of soil Mn, whereas flooding increases Mn solubility (Iu et al., 1981; Chaudhry and McLean, 1963; Jugsujinda and Patrick, 1977).

It is well established that soil acidity has a pronounced effect on the availability of Mn to plants (Godo and Reisenauer, 1980; Shuman et al., 1979; Mulder and Guerretsen, 1952; Cheng and Ouelette, 1971). Berger and Gerloff (1947) corrected Mn toxicity in potatoes (Solanum tuberosum L.) by application of limestone to an acid soil. Liming decreased the extractable soil Mn by 12 to 15% and the average tissue Mn concentration from 970 to 390 µg/g (VanLierop et al. 1982). Crinkel leaf of cotton (Gossypium hirustum L.), which is caused by Mn toxicity, occurs very frequently on acidic soils in the eastern Cotton Belt. Foy et al. (1981) overcame this problem by increasing soil pH. Parker et al. (1981) observed a decrease in Mn concentration in soybean tissue and an increase in seed yield and weight from an increase in soil pH. The increased pH resulted in a three-fold decrease in exchangeable soil Mn and a two-fold decrease in plant Mn content (Cheng and Ouellette, 1971).

Under alkaline pH levels, Mn availability is decreased by direct chemical oxidation, by biological oxidation (Tierney and Martens, 1982; Bromfield, 1978) or by formation of complexes with organic compounds (Loneragan, 1975; McBride, 1982). The release of Mn^{2+} from soil organic complexes depends on the pH of the environment. McBride (1982) concluded that much Mn^{2+} is adsorbed as outer-sphere complexes by solid soil organic materials at low pH levels and that, with pH increases, more carboxylate sites are available for formation of inner-sphere complexes. There is less Mn mobility from inner-sphere than from outer-sphere complexes.

The plant available fraction of soil Cu and Zn is decreased with an increase in soil pH (White et al., 1979; Wallace et al., 1978; Lindsay, 1972; Harter, 1983). White et al. (1979) reported a 67% reduction in Zn absorption by soybean plants grown in a soil with a pH of 6.5 in comparison with plants grown in a soil with a pH of 5.5, though the same amount of Zn was applied to both soils. Limestone application corrected Zn toxicity in cotton (Lee and Page, 1967) and soybeans (Lee and Craddock, 1969) grown in peach orchard soils with low pH and high available Zn.

Retention of Cu and Zn by soil dramatically increases above pH 7.5. Zinc is mainly retained on exchange complexes and Cu may be retained on exchange complexes or as a precipitate (Harter, 1983). Soil pH and contents of soil clay, hydroxides, oxides and organic matter are the dominant properties that influence Cu and Zn sorption (Iyengar et al., 1981; Mullins et al., 1982). Even though the solubility of Cu and Zn decreases when soil pH becomes alkaline, formation of metal-organic complexes retain a considerable amount of Cu and Zn in soil solution (Lindsay, 1972; Loneragan, 1975).

Oxidation-reduction conditions in a soil either may decrease or increase the availability of micronutrients. Solubilization of Fe and Mn oxides under reduced environments releases other occluded ions, like Cu^{2+} and Zn^{2+} (Ponnamperuma, 1972; Verloo et al., 1980). Formation of insoluble sulfides may reduce the solubilties of Cu and Zn under severe reducing conditions whereas release of these elements is favored by accelerated oxidation of organic complexes under oxidizing environments (Verloo et al., 1980).

ORGANIC MATTER

Hodgson (1963) pointed out three major ways that organic matter affects Mn transformations in soil; i) reduction of Mn ions in solution by complexing agents, ii) direct or indirect decreases in oxidation potential of soil through increased microbial activity and iii) incorporation of Mn in biological tissues as a result of stimulated microbial activity. Formation of insoluble organic complexes, especially in alkaline organic soils, causes Mn deficiency in plants even though the total soil Mn content is adequately high (Mulder and Gerretsen, 1952; Christensen et al., 1950; Cheng and Ouellette, 1971). Page (1962) concluded that changes in the availability of Mn with pH were due neither to biological oxidation nor to formation of higher oxides, but were due to complexation of Mn by organic matter. In contrast, addition of organic matter into acid mineral soils increased the exchangeable and easily reducible Mn (Cheng and Ouellette, 1971; Mandal and Mitra, 1982).

Immobilization of B, Cu, Mn and Zn may occur through either solid phase complexation or formation of precipitates (Stevenson and Ardakani, 1972). On the other hand, humic and fulvic acids combine with these elements to form soluble compounds that are readily available to plants (Stevenson and Ardakani, 1972). Copper is more strongly chelated by organic matter than Mn, Zn or B (Legerwerff and Milberg, 1978; Loneragan, 1975; Stevenson and Fitch, 1981). Since Cu forms soluble organic complexes within a wide range of pH, there is little change in Cu absorption by plants with a fluctuation in soil pH (Loneragan, 1975). Most of the B in soil is held by organic matter, which partially prevents leaching loss of B (Fleming, 1980).

Application of organic fertilizers like manure usually increases the plant availability of micronutrients (Prasad and Singha, 1982). Soil organic C positively correlates with available Mn and Zn (Prasad and Singha, 1982). However, incorporation of plant residues into soil may cause micronutrient deficiencies during the early stages of decomposition (Lindsay, 1972). During later stages, mineralization of organic matter as well as solubilization of soil minerals through stimulated microorganism activity increase the amounts of micronutrients in soil solution (Baker, 1973; Lindsay, 1972).

MICROBIOLOGICAL ACTIVITY

Early workers reported that Mn deficiency occurred in oat plants (*Avena sativa* L.) on an area that was inoculated with soil from Mn "deficient" fields (Guerretsen, 1937; Timonin, 1946). This phenomena was explained as oxidation of soil Mn by bacteria and then by formation of insoluble Mn oxides.

Microorganisms may affect the availability of plant nutrients in several ways, namely, decomposition of organic compounds, immobilization of ions by incorporation into microbial tissues, oxidation, reduction, or indirectly by changing pH or Eh (Alexender, 1977). Oxidation of Mn^{2+} to Mn^{4+}, which controls the Mn^{2+} concentration in soil solution, is more related with current microbilogical activities than with physico-chemical factors (Loneragan, 1975). Lambert et al. (1979) suggested that suppression of the growth of mycorrhizae caused Zn and Cu deficiencies in corn (Zea mays L.) and soybeans under heavy P fertilization. Rapid growth of microorganisms at least temporarily immobilizes plant nutrients and therefore, may cause nutrient deficiencies (Lindsay, 1972).

Soil sterilization either decreases or increaes the plant availability of Mn. Sterilization may result in toxic levels of Mn in some soils (Boyd, 1971; Cheng and Ouellette, 1970). In contrast, Jones (1957) used soil sterilization to control the higher levels of Mn solubility caused by lime-induced microbial growth.

A number of bacteria including chromobacterium, flavobacterium and corynebacterium (Bromfield and Sherman, 1950) and certain fungi of the genera cladosporium, trirgschemia, and pleospora have the ability to oxidize Mn and, thus, to decrease Mn availability (Cheng and Ouellette, 1971). Studies by Gregory and Staley (1982) revealed the Mn oxidizing ability of a large variety of heterotropic, fresh water bacteria. Several microorganisms have the ability to utilize MnO_2 as a source of oxygen and, thereby, to release Mn^{2+} into soil solution, thus, these microorganisms indirectly reduce Mn oxides by changing Eh (McKenzie, 1977; Cheng and Ouellette, 1971; Trimble and Ehrick, 1968). Bacillus 29 and Coccus 32, isolated from ferromanganese nodules have the ability to reduce MnO_2 under anaerobic as well as aerobic conditions (Trimble and Ehrlich, 1968). Complex sulfides of Cu and Zn can be oxidized to form simple compounds such as $CuSO_4$ and $ZnSO_4$ by *Thiobacillus ferrooxidans* (Lundgren and Silver, 1980).

RHIZOSPHERE EFFECTS

Root exudates may change the chemical environment of the rhizosphere either by directly interacting with soil constituents or by influencing microbial activity. The effect of both is to change the availabilty of plant nutrients at the vicinity of the root surface (Hodgson, 1963). At a given pH, rhizosphere soil shows a greater Mn solubility than the bulk soil due to chemical reduction and chelation (Godo and Reisenauer, 1980; Mulder and Gerretsen, 1952). Solubility of soil Mn in common root exudate compounds such as citrate, malate, glutamate, and a mixture of them is much greater than that in $CaCl_2$ solution (Godo and Reisenauer, 1980). Malic acid, an important constituent of the root exudates of several plant species, has been shown to reduce Mn in MnO_2 and, thereby, to increase Mn solubility. Malate is oxidized to CO_2 and H_2O by MnO_2. Hydrogen ions are consumed and Mn^{2+} ions are released during the reaction. Acidic conditions favor the reaction, but higher pH levels in calcareous systems do not inhibit the process (Jauregui and Reisenauer, 1982).

Root extracts like organic acids, HCO_3^- and H^+ ions may affect soil colloids and result in a release of trace elements sorbed to them (Loneragan, 1975). Broomfield (1958) observed a considerable increase in Mn solubility after treating insoluble Mn oxides with wash water of oat roots. Ketogluconate excreted by microorganisms living in the root rhizosphere accelerates the release of Zn from insoluble silicate minerals (Webly et al., 1960). Studies by Wilkinson et al. (1968) with wheat (Triticum aestivum L.) and by Tiller et al. (1972) with subterranean clover (Trifolium subterranean L.) in alkaline soils revealed that 70% or more of the Zn absorbed by plants originated from specifically bound Zn due to root activities. Colonization of Mn oxidizing bacteria in oat rhizosphere causes Mn deficiency (Timonin, 1946). Attraction of these bacteria toward the rhizosphere is stimulated by Ca citrate, a secondary product of the citric acid secreted by roots.

INTERACTION WITH OTHER ELEMENTS

The availability of micronutrients is affected by the presence of other elements and by interactions among the micronutrients. Reports on these interactions are often conflicting, probably because of the contribution of other factors (White et al. 1979; Yadav and Shukla, 1982; Wallace et al. 1978; Mathur and Levesque, 1983).

Absorption and translocation of Zn by soybean plants are decreased when the available Mn content in soil is high (Reddy et al., 1978; Hauf and Schmid, 1967; Sing and Steenburg, 1974). Competition for the same absorption sites and changes in physiogical processes involved in Zn translocation at higher levels of Mn probably govern the observed low Zn content in plants (Reddy et al., 1978). White and Chaney (1980) stated that the foliar Mn level in soybeans grown in high Mn soils is increased with the application of Zn, but is decreased in low Mn soils. The interaction between Mn absorption and Zn application varies with soil pH. Root Mn in soybeans was increased linearly with the addition of Zn at pH levels of 5.5 to 6.5, but leaf Mn accumulation was increased curvilinearly at pH 5.5 and linearly at pH 6.5 (White et al., 1979). Somewhat contradictorily, Ohki (1977) reported that the Mn content in soybean leaves reached the toxic level of 375 µg/g under Zn deficient conditions.

Application of Cu fertilizer to organic soils releases Mn and Zn from organic complexes to weaker sites of adsorption or chelation (Mathur and Levesque, 1983; Kuradi and Doner, 1983). Mixing either Cu or Mn into rice (*Oryza sativa* L.) paddy soils decreases extractable Zn as well as Zn availability (Halder and Mandal, 1982). Experiments by Fuehring and Soofi (1964) show that soil Cu did not affect Mn uptake by sugarcane (*Saccarum officinarum* L.) but negatively correlated with Zn uptake. In contrast, Kuradi and Doner (1983) found a negative correlation between soil Cu content and Zn sorption by soil particles in soils low in cation exchange capacity.

It is well known that heavy rates of P application have an adverse affect on Zn absorption and translocation by plants (Wallace et al., 1978; Yadav and Shukla, 1982; Safaya, 1976; Paulsen and Rotimi, 1968; Saeed and Fox, 1979). Paulsen and Rotimi (1968) stated that P-induced Zn deficiency is caused by less translocation of Zn within the plant. According to Safaya (1976), higher levels of P decrease Zn absorption through epidermal or surface cell layers of the root and prevent the entrance to the root xylem through the endodermis. In addition, P application increases the negative charges on Fe and Al oxides (Seed and Fox, 1979; Loneragan, 1975) and, as a result, Zn adsorption is increased on these soil components. Mandal and Maldar (1980) observed precipitation of Mn phosphate with heavy rates of P application.

Correction of Mn toxicity in Medicago spp. with application of Ca is directly related to the reduction in Mn uptake by plants (Robson and Loneragan, 1970; Ouellette and Dessureaux, 1958) and to the retention of absorbed Mn in the root system (Ouellette and Dessureaux, 1958). Jauregui and Reisenauer (1982) observed a strong negative effect of $CaCO_3$ on Mn availability by immobilization of Mn^{2+} through adsorption and precipitation or formation of manganocalcite. Formation of Ca metaborates, which polymerized to form chain structures, reduces the B availability in limed soils (Colwell and Cuminings, 1944). Precipitation of $Al(OH)_3$ with liming reduces soluble B as freshly formed $Al(OH)_3$ has a high affinity for B (Hatcher et al., 1967).

Zinc concentration in soybean tissue is increased with application of low rates of elemental S (Kumar and Singh, 1979; Procopiou et al., 1976). Application of elemental S increases tissue Mn levels in soybeans. This can not be a result of reduced pH because the change in pH was only from 7.7 to 7.4 (Procopiou et al., 1976). It is possible that oxidation of elemental S may release some Mn from bound forms.

PLANT FUNCTIONS OF BORON, COPPER, MANGANESE AND ZINC

FUNCTIONS OF BORON

Unlike Mn, Cu and Zn, B has not been identified in any plant enzyme system. Death of shoot buds and roots (Gauch and Dugger, 1956; Van de Venter and Currier, 1977), obstructed translocation (Baker et al., 1956; Van de Venter and Currier, 1977; Mengel and Kirkby, 1982) and accumulation of phenolic compounds (Slack and Whittington, 1964) are the most common disorders that result from an inadequate supply of B. Gupta (1978) suggested that a continuous supply of B is required for maintenance of meristematic activity. Under B deficient conditions, there is impairment of meristematic growth, death of terminal meristems and abnormal growth of stomata (Mengel and Kirkby, 1982; Baker et al., 1956).

Boron has a major role in synthesis of uracil, an essential component of RNA synthesis (Birnbaum et al., 1977). Lack of uracil also affects the synthesis of uridine diphosphate glucose, an essential coenzyme in the formation of sucrose (Mengel and Kirkby, 1982). Translocation of assimilates is inhibited under such a condition because sucrose is the most important form of sugar transported within the plant (Mengel and Kirkby, 1982). Disorganization of cambial tissue growth in B deficient plants changes the vertical arrangement of sieve elements, and excessive callose formation plugs the sieve plates and, therefore, decreases translocation (Van de Venter and Currier, 1977). Pollard et al. (1976) suggested that membrane functions and, hence, ion selectivity of membranes depends on the B supply to plants.

FUNCTIONS OF COPPER

Copper is an essential constitute in a number of key enzymes in plants (Bussler, 1981; Nicholas, 1975; Lyszcz et al., 1976). Tyrosinase, laccase, ascorbic acid oxidase, cytochrome oxidase and plastocyanin are some of the important enzymes with Cu as a component (Nicholas, 1975). Depression of the respiration rate in sunflower (*Helianthus annus* L.) was attributed to the reduction of ascorbic acid oxidase and catechol oxidase under Cu-deficient conditions (Lyszcz et al., 1976). Plastocyanin, a non-autoxidizable Cu enzyme, mediates the photosynthetic electron transport for photosystem I (Nicholas, 1975; Bussler, 1981). Copper is strongly as well as preferentially bound to plastocyanin (Bussler, 1981). Because of these two characteristics, this enzyme functions under conditions of a very low level of plant Cu (Bussler, 1981).

Induction of chlorosis, structural malformation of leaves and inhibition of photosynthesis have been observed as a result of an inadequate supply of Cu (Bussler, 1981).

Plant chloroplasts contain a considerable amount of Cu; for example, Neish (1939) reported that 75% of the total Cu content in clover (*Trifolium repens* L.) was found in chloroplasts. Nitrogen fixation associated with legumes is affected by Cu deficiency and, as stated by Snoball (1980), Cu-deficiency induced chlorosis in subterranian clover is due to a secondary effect caused by reduced N fixation. Plant Cu influences the formation as well as the lignification of xylem vessels (Bussler, 1981). Plant growth and pollen fertility are decreased in Cu deficient plants. Studies by Dell (1981) showed that wheat, oats and barley (*Hordeum distichon* L.) were highly sensitive to these abnormalities.

FUNCTIONS OF MANGANESE

In 1937, Pirson showed for the first time that Mn deficiency decreased photosynthesis without having much of an effect on chlorophyll content or on respiration (Boardman, 1975). Studies by Kessler (1955) suggested Mn as an essential element for O_2 evolution during the photosynthetic process. Further studies by Possingham and Spencer (1962) revealed that Mn is tightly bound in grana in chloroplasts. They concluded that Mn is directly involved in the Hill reaction and that it is an essential component of the O_2 evolving sequence of chloroplasts.

When Mn is deficient, the structure of chloroplasts is markedly impaired (Possingham, 1964; Gavalas and Clark, 1971; Heath and Hinda, 1969). The number of components per grana and the number of grana per chloroplast are reduced and disorganization of intergrana membranes occurs with an inadequate supply of Mn (Possingham, 1964).

Inhibition of photosynthesis occurs in Mn deficient oat plants without a considerable decrease in chlorophyll content (Gerretson, 1949). Observations with soybeans suggested that the reduction of photosynthesis in Mn deficient plants was not entirely due to reduced chlorphyll content and that the tissue respiration rate was decreased considerably (Cooper and Girton, 1963). Manganese occurs in various respiratory enzyme systems (Gerretson, 1949), and the role of Mn in the activity of the Kreb's cycle reflects malfunctionings in most of the physiological processes under low Mn supply (Epstein, 1972). Manganese is tightly bound to the photochemically active lamellae (Possingham, 1964). Possingham proposed that Mn is a constituent of some macromolecular complexes such as manganoproteins. Two separate Mn fractions were isolated by Cheniae and Martin (1970) from chloroplasts; one was loosely bound and the other was firmly bound to the membrane. It was found that the loosely bound fraction was associated with O_2 evolution whereas the firmly bound Mn fraction was an essential part of the as yet unknown electron donor in photosystem II. Oxygen yield is linearly related with the amount of bound Mn and there are 3 to 4 functional Mn atoms per photosystem II photosynthetic unit (Cheniae and Martin, 1970). The proportion of chlorophyll-A to chlorophyll-B is changed according to Mn supply and, in Mn deficient plants, the chlorophyll-B content is relatively higher (Anderson and Pyliotis, 1969).

A low nitrate reductase activity in Mn deficient soybean plants is an indirect effect resulting from reduced photynthesis (Heenan and Cambell, 1980a). Increased activity of indoleacetic acid due to low activity of indoleacetic acid oxidase under a low supply of Mn may change the growth of plants (Tayler et al., 1968).

FUNCTIONS OF ZINC

Zinc is a component of a number of enzymes including glutamic dehydrogenase, alcohol dehydrogenase, lactic dehydrogenase, carbonic anhydrase, carboxy peptidase and DNA polymerase I (Nicholas, 1975). The level of carbonic anhydrase and the activity of the enzyme is low in Zn deficient plants (Randall and Bouma, 1973). Photosynthetic activity of Zn deficient plants is adversely affected because carbonic anhydrase is an enzyme that facilitates the diffusion of

CO_2 through the liquid phase of the cell to chloroplasts (Hatch and Slack, 1970; Zelith, 1971). Observations by Randall and Bouma (1973) suggested that the reduction of carbonic anhydrase activity has little effect on photosynthesis, but Zn deficiency decreases the biochemical capacity of the plant to fix CO_2.

An inadequate supply of Zn reduces the bacterial N_2-fixation capacity of soybean roots (Demeterio et al. 1972; Kapur et al., 1975). Zinc functions in the formation of auxins and maintains the activity of the enzyme. Auxins loosen the cellulose cross linkage of root hair tips and, thus, ease the entrance of Rhizobium through cortical and epidermal cells to root hair cells (Kapur et al., 1975). Enolase, which catalyzes the dehydration of d-2-phosphoglyceric acid to phosphenol pyruvate, is activated by either Mn or Zn (Nicholas, 1961).

DETECTION OF BORON, COPPER, MANGANESE AND ZINC DEFICIENCIES

Chlorosis and certain other growth abnormalities are similar for B, Cu, Mn and Zn deficiencies, but specific symptoms particular to each element are useful for visual diagnosis of the deficiencies. Boron deficiency causes death of bean roots and brittle petioles mainly due to changes in turgor of the plant (Van de Venter and Currier, 1977). Copper deficiency occurs in older leaves of plants. This deficiency is characterized by pale green mottling, little contrast in color between veinal and interveinal areas and stunted growth of the root and shoot systems (Barnes and Cox, 1973). Characteristically, interveinal chlorosis in younger leaves of the Mn deficient soybean plants is followed by stunted growth and by late flower initiation and, under severely deficient conditions, by early leaf senescence (Epstein, 1972; Heenan and Campbell, 1980b). Recently matured Zn deficient soybean leaves exhibit a dull green, wrinkled surface with raised interveinal areas. Scattered interveinal chlorosis occurs especially in older leaves; and flower and axillary bud growth are inhibited in Zn deficient plants (Ohki, 1977).

TISSUE ANALYSIS

Tissue analysis can be effectively used as a diagnostic tool, along with soil analysis, to confirm suspected deficiencies. Data from regression analyses indicate that seed yield is highly related with leaf and seed Mn contents and that these values can be used for determination of Mn deficiency (Robertson et al., 1973). The accuracy and validity of interpretive data from leaf analysis depends upon preparative steps such as proper sampling, washing, grinding, drying and storage (Boswell, 1972). Pubescent leaves of soybeans should be washed in deionized water to remove possible contaminants like dust and spray materials. The Mn status of the soil at the time of leaf development is indicated by the Mn concentration of the leaves. The most suitable part of the plant for chemical analysis to obtain the current status of soil fertility levels is the uppermost matured trifoliolate leaf (Kluthcouski and Nelson, 1979; Ohki, 1976).

The tissue concentration of an element below the lower critical level is considered to be insufficient for optimal plant growth (Loneragan, 1968). The sufficiency range of B concentrations in soybean leaves was 10 to 63 µg/g (Woodruff, 1979). Makarim and Cox (1983) reported that the critical Cu concentration for the whole soybean plant at the R2 growth stage is 6 µg/g. The critical Mn concentration is 13 µg/g in recently matured soybean leaf blades at the R2 growth stage and 22 µg/g during the late pod filling stage (Ohki et al., 1977; 1979). For Zn the critical level was found to be 15 µg/g in trifoliolate leaves (Ohki, 1977).

SOIL ANALYSIS

The total content of soil B, Cu, Mn and Zn does not indicate the availability of these micronutrients because the plant available fractions are highly dependent on other soil factors including pH, Eh and organic matter content (Cox and Kamprath, 1972). On the other hand, the critical soil level of an element is not a constant as it varies with soil properties (Shuman et al., 1980). Because of the variation among soils, it is not practical to formulate a general extraction method for all soils (Shuman et al., 1978). Dilute mineral acids or chelating agents are widely used for the prediction of available micronutrients in soils (Shuman et al., 1978).

The suitabiity of a Mn extractant varies with soil pH because the solubility of Mn changes with pH. Shuman and Anderson (1974) observed that the DTPA extractant gave a suitable prediction of Mn availability in the range of pH 5.8 to 6.8 and that, at pH 4.8, a water extract gave a more accurate prediction. According to the findings of Robertson et al. (1973) hydroquinone, ammonium oxiacetate or double acid extractable Mn could be effectively used to determine Mn deficiency in soybeans. Over a range of Mn applications under different pH levels, DTPA extractable Mn accounted for 72% of the variation in tissue Mn content, while double acid extractable Mn accounted only for 22% (Wilson et al., 1981). Among 18 extractants studied for 57 soils, DTPA and 0.1 N H_3PO_4 gave equally good results in prediction of Mn availability (Randall et al., 1976). The critical levels of soil Mn related to the four extractants, double acid, DTPA, Mehlich I and NH_4HCO_3-DTPA were 2.60, 0.22, 1.80 and 0.4 µg/g, respectively (Shuman et al., 1980). Manganese absorption by soybean plants was well explained by the DTPA and NH_4HCO_3-DTPA extractants, but inclusion of soil pH in a regression equation provided an equal ability of all four extractants to predict the plant Mn content (Shuman et al., 1980).

The most common extractants used for prediction of available Cu and Zn are DTPA, double acid and 0.1 N HCl (Baker and Amacher, 1982). Acid extractants are not recommended for calcareous soils; DTPA, however, could be used for soils with a wide range in pH. Hot-water soluble B contents in soil is considered to be an estimate of the B supplying capacity of soil (Gupta, 1979; Touchton and Boswell, 1975). Boron content in soybean leaves is highly correlated with hot-water soluble B content in the first 15-cm depth, but when B accumulation in soil is low and pH is high, the correlation is very low (Touchton and Boswell, 1975).

CORRECTION OF BORON, COPPER, MANGANESE AND ZINC DEFICIENCY

Sodium borates including borax, solubor, sodium tetraborate and sodium pentaborate are the most popular B fertilizers (Murphy and Walsh, 1972). Touchton and Boswell (1975) observed significant increases in soybean yield from foliar and broadcast application of the sodium borates at rates ranging from 0.28 to 1.12 kg B/ha. Either organic or inorganic forms of Cu and Zn fertilizers are used for correction of these deficiencies in field crops. Sulfates of these elements are widely used as a foliar spray and as a soil amendment (Murphy and Walsh, 1972). In studies related to Zn fertility in soybeans, $ZnCl_2$ (Wilson et al., 1983) and Zn chelates (Robertson and Thompson, 1969) have been used effectively as Zn sources. Parker et al. (1980) used sulfates of Cu and Zn as a broadcast application to correct these deficiencies.

Various application methods combined with different rates and numerous sources have been used to correct Mn deficiency in soybeans. Row application of $MnSO_4$ at the time of planting ranging from 5 to 22 kg Mn/ha controls Mn deficiency in soybeans very effectively, and row application is somewhat more effective than broadcast application (Randall et al., 1975). Broadcast application of 11.2, 22.4 and 44.8

kg Mn/ha increased soybean yield on unlimed soils, but on limed soils, only the two higher rates increased yield (Alley et al., 1978). Less than 0.5% of the broadcasted Mn was found in the aerial parts of the soybean plant and the amount of Mn removed by the plant for maximum growth was only about 59 g/ha (Wilson et al., 1981). Randall et al. (1975) observed that foliar application of Mn-EDTA was highly effective in supplying Mn, but soil application of Mn-EDTA was ineffective.

Foliar application of Mn has been widely used as a corrective measure of Mn deficiency in soybeans (Randall et al., 1975; Robertson and Thompson, 1969; Alley et al., 1978). According to Cox (1968), application of 2.2 kg Mn/ha as $MnSO_4$ was the optimum rate to be used as a foliar spray for correction of Mn deficiency in soybeans. He also suggested that a split foliar application is superior to a single foliar application. A combination treatment of 5 to 11 kg Mn/ha in the plant row before seeding along with a foliar spray during late growth was reported to be the best correction measure for moderate to severe Mn deficiency in soybeans (Randall et al., 1975). Manganese sulfate is the most common Mn source used for correction of Mn deficiency in crop plants. Shuman et al. (1979) found that $MnSO_4$ was more effective than MnO, Mn frit or Mn chelates as a Mn source.

MATERIAL AND METHODS

Five field experiments were conducted during the 1982 and 1983 growing seasons to determine soybean response to micronutrient applications. The experimental sites were selected on the basis of either low levels of soil test dilute $HCl-H_2SO_4$ extractable Mn or uniformly moderate to extremely severe Mn deficiency symptoms in soybeans. These experiments were located in the Atlantic Coastal Plain region. Phosphorus and K were applied to the five experimental areas according to VPI & SU Extension Service Recommendations (Donohue and Hawkins, 1979a).

1982 FIELD EXPERIMENTS

In 1982, field studies were conducted on Dragston, Myatt and Rains fine sandy loams. Selected chemical characteristics of the soils are given in Table 1. Studies on the Dragston and Rains soils consisted of seven treatments including foliar application of Mn and broadcast application of Mn and Zn (Table 2). Treatments were arranged in a randomized complete block design with four replications on both soils. The number of rows per plot on the Dragston soil was 6 and on the Rains soil was 8. Row width was 0.91 m and row length was 7.62 m for plots on both of the sites. Manganese sulfate was uniformly broadcast for treatment numbers one and two at the rate of 40 kg Mn/ha. The rate of $ZnSO_4$ applied on the Dragston soil was 17 kg Zn/ha and on the Rains soil was 20 kg Zn/ha. All treatments received broadcast $ZnSO_4$, except treatment number two, the Zn control. The entire experimental area received 1.0 kg B/ha as sodium borate and 6 kg Cu/ha as $CuSO_4$. Sulfur levels among the treatments were balanced by broadcast application of appropriate levels of $CaSO_4$. All of the amendments were disked into the soil to a depth of approximately 10 cm prior to planting soybeans. The soybean cultivar grown on the Dragston soil was Forrest and on the Rains soil was Essex.

Foliar treatments consisted of two rates of Mn, 1.1 kg Mn/ha and 2.2 kg Mn/ha and two different times of application on both soils (Table 2). The surfactant XL-77 was mixed with the $MnSO_4$ spray solutions (0.03%, v/v) for all foliar treatments. Treatments were applied on soybean foliage with a stainless-steel back pack pump sprayer during the V7 and R1 growth stages. The Mn solutions were applied at the rate of 52.9 liters of solution/ha.

Three treatments in the field experiment on the Myatt soil were foliar application of 2.2 kg Mn/ha, foliar application of 2.2 kg Mn/ha combined with broadcast application of 2.2 kg B/ha and a control (Table 3). The soybean culti-

Table 1. Selected chemical properties of surface and subsurface horizons of the soils under study.

Soil Type	Taxonomic Class	Soil Horizon	Soil pH	Organic Matter	Hot-Water Soluble B	DTPA Extractable Cu	DTPA Extractable Mn	DTPA Extractable Zn	$HCl-H_2SO_4$ Ext. Mn	EDTA Ext. Zn
				%	µg/g					
1982										
Dragston fsl.	Coarse-loamy, mixed, thermic Aeric Ochraquults	Ap	6.3	2.1	---	---	1.34	0.49	3.1	1.0
		B	5.8	1.5	---	---	0.72	0.16	1.6	0.5
Myatt fsl	Fine-loamy, siliceous, thermic Typic Ochraquults	Ap	6.9	1.7	0.24	---	1.03		3.1	1.0
		B	6.5	1.0	0.17	---	0.23		1.0	0.4
Rains fsl	Fine-loamy, siliceous, thermic Typic Paleaquults	Ap	6.0	3.1	---	---	2.80	0.92	3.2	2.3
		B	5.2	1.8	---	---	1.02	0.54	1.2	0.9
1983										
Myatt fsl	Fine-loamy, siliceous, thermic Typic Ochraquults	Ap	6.9	1.7	---	0.15	1.63	0.55	2.7	1.7
		B	6.2	1.0	---	0.11	0.52	0.14	1.0	1.1
Slagle fsl	Fine-loamy, siliceous, thermic Aquic Hapludults	Ap	6.0	2.6	0.12	0.22	0.68	0.68	1.1	1.3
		B	5.6	1.1	0.08	0.16	0.34	0.21	0.3	0.2

Table 2. Rates, methods and times of treatment application on Rains and Dragston soils.

Treatment No.	Broadcast Micronutrients*			Foliar Mn†	
	Mn	Zn			
		Rains Soil	Dragston Soil	V_7 Growth Stage	R_1 Growth Stage
	kg/ha				
1	40.0	20.0	17.0	---	---
2	40.0	0.0	0.0	---	---
3	0.0	20.0	17.0	---	---
4	0.0	20.0	17.0	1.1	---
5	0.0	20.0	17.0	2.2	---
6	0.0	20.0	17.0	1.1	1.1
7	0.0	20.0	17.0	---	2.2

*Broadcast micronutrients mixed into soil before planting.
†Growth stages were based on the system developed by Fehr et al. (1971).

var grown on the experimental area was York. These treatments were assigned in a randomized complete block design with four replications within a uniformly Mn deficient area. Each plot consisted of 6 rows, 6.10-m long and 0.61-m apart. During the V5 growth stage, Borate 65 was broadcasted as the source of B and $CaSO_4$ was applied to supply 36 kg S/ha. Manganese sulfate solution, which was prepared as previously described, was sprayed on foliage at the rate of 1.1 kg Mn/ha during the V5 and R1 growth stages.

Plants from a 4.6-m section of the two center rows of each plot of the three experiments were threshed at maturity for yield determination. Seed yields were calculated as Mg/ha at 13% moisture level. Sub-samples from each plot were used to determine the weight per 100 seeds. Number of seeds per hectare was calculated from seed yield and weight data.

1983 FIELD EXPERIMENTS

During the 1983 growing season, field experiments were conducted on the Myatt and Slagle fine sandy loams. Selected chemical characteristics of both soils are given in Table 1. Experimental sites at both locations were selected on the basis of uniform Mn deficiency symptoms in soybeans. The study on the Myatt soil consisted of foliar application

Table 3. The rates methods and the time of treatments application on Myatt soil (1982).

Treatment No.	Broadcast* B	Foliar Mn† V5 Growth Stage	Foliar Mn† R_1 Growth Stage
	kg/ha	kg/ha	kg/ha
1	0.0	0.0	0.0
2	0.0	1.1	1.1
3	2.2	1.1	1.1

*B was broadcast at the V5 growth stage.
†Growth stages were based on the system developed by Fehr et al. (1971).

of two rates of Mn, a foliar application of Cu combined with Mn and a control (Table 4). Two additional treatments were included in the experiment on the Slagle soil (Table 4). These treatments consisted of either broadcast B or foliar Zn with foliar Mn application. Each treatment in the two field experiments was applied as two split-applications. The sources of B, Cu, Mn and Zn used in these studies were Borate 68, $CuSO_4$, $MnSO_4$, and $ZnSO_4$, respectively. The surfactant XL-77 was mixed with all of the spray solutions at a concentration of approximately 0.03% (v/v). A stainless steel back pack sprayer was used for foliar applications and Borate 68 was uniformly broadcasted on the treatment plots. The rate of foliar spray application was 36.1 l/ha.

Four treatments on the Myatt soil were arranged in a randomized complete block design with four replications. Each plot consisted of four rows, 6.1-m long and 0.61-m apart. The soybean cultivar grown on the experimental area was York. The soybean plants were severely Mn deficient during very early growth stages and, consequently, the first foliar Cu and Mn sprays and the first broadcast B treatment were applied during the V2 growth stage. The plants recovered very rapidly after the first foliar Mn application. During the V4 growth stage, the inter-row spaces of the entire experiment were sprayed with 15 kg S/ha to avoid S de-

Table 4. The rates, methods and time of treatments application on Myatt (1983) and Slagle soils.

Treatment No.	Broadcast B	Foliar Micronutrient Cu	Mn	Zn	Time of Application* Myatt Soil†	Slagle Soil†
	------------- kg/ha ---------					
1	0.00	0.00	0.00	0.00	V2, V9	V4, R1
2	0.00	0.00	1.12	0.00	V2, V9	V4, R1
3	0.00	0.00	0.56	0.00	V2, V9	V4, R1
4	1.12	0.00	0.56	0.00	-- --	V4, R1
5	0.00	0.28	0.56	0.00	V2, V9	V4, R1
6	0.00	0.00	0.56	0.28	-- --	V4, R1

*The given rates were repeated at both of the growth stages.
†Growth stages were based on the system developed by Fehr et al. (1971).

ficiency. Sevin was sprayed on foliage of the total area at the rate of 1.1 kg/ha. The second application of treatments was made when the plants were in the V9 growth stage.

Six treatments applied on the Slagle soil (Table 4) were assigned in a randomized complete block with four replications. The plots in the study consisted of four rows, 7.6-m long and 0.91-m apart. The soybean variety grown on the experimental area was York. The experiment received the first split application of treatments when plants were in V4 growth stage. At the V6 growth stage, a sulfate solution was sprayed on inter-row spaces at the rate of 15 kg S/ha to prevent S deficiency. The entire experiment was sprayed with Sevin at the rate of 1.1 kg/ha. The balance of the treatments was applied when plants were in the R1 growth stage. Seed yields, weights and numbers on the Myatt and Slagle soils were determined by identical procedures as described for 1982 experiments.

SOIL ANALYSIS

Soil samples were collected from each of the experimental sites for determination of chemical components. When the soil samples were collected during 1982, plants on the Dragston and Rains soils were in the V7 growth stage and on the Myatt soil were in the V5 growth stage (Fehr et al.

1971). Samples were collected from the Slagle and Myatt soils, during the 1983 studies, when the plants were in the V4 and V2 growth stages, respectively. Ten random samples were collected from each plot from the 0- to 20- and 20- to 40-cm soil layers. The soil samples were air-dried and ground to pass a 10-mesh sieve. Subsamples from these soils were used for the determination of pH; organic matter content; DTPA extractable Cu, Mn and Zn; dilute $HCl-H_2SO_4$ extractable Mn and EDTA extractable Zn. In addition, hot-water soluble B content was determined for the Myatt soil in 1982 and for the Slagle soil in 1983. Only those soil samples from the plots where broadcast Mn was not applied were used for the analysis of Mn content in the Dragston and Rains soils. Similarly Zn content in the soils was determined using the samples collected from the Zn control plots.

Soil pH was determined in a 1:1 soil-to-water mixture after a 15-minute equilibration period. The sodium dichromate method described by Donohue and Friedericks (1983) was used for determination of the organic matter content in soil. The DTPA extractable Cu, Mn and Zn were determined by the procedure outlined by Lindsay and Norvell (1978). For this extracion, 10 g of soil was shaken for 2 hours with 20 ml of DTPA extraction solution in a polypropylene centrifuge tube. The suspension was filtered with Whatman No. 42 filter paper, and the concentration of Cu, Mn and Zn in the filtrate was determined by atomic absorption spectrophotometry. For the extraction of dilute HCl-H_2SO_4 soluble Mn, 5 g of soil was shaken for 15 minutes with 20 ml of a 0.05 N HCl in 0.025 N H_2SO_4. The suspension was filtered through Whatman No. 42 filter paper and the concentration of Mn in solution was determined with an atomic absorption spectrophotometer. The EDTA extractable Zn was determined by the procedure outlined by Donohue and Friedericks (1983). A subsample of 10 grams of soil was shaken with 20 ml of EDTA-$(NH_4)_2CO_3$ solution in a polypropylene centrifuge tube for five minutes, and filtered through Whatman No. 42 filter paper. Zn in the filtrate was determined with an atomic absorption spectrophotometer. Hot-water soluble B content of the soils was determined by the method outlined by Parker and Gardner (1971). Fifteen g of soil was refluxed with 30 ml of 0.02 M $CaCl_2$ in a B-free flask for 5 minutes. The solution was filtered through Whatman No. 42 filter paper, and the B concentration in the filtrate was determined by an azomethine-H procedure.

TISSUE ANALYSIS

Trifoliolate leaf samples from experimental plots were collected when the plants were in R1 growth stage. Twenty four upper most fully matured trifoliolate leaves per plot were carefully collected and rinsed twice with distilled water and two more times with deionized water. Samples were covered in polyethylene bags and stored with ice. Leaf blades and petioles were separated and dried at 70°C for 24 hours. The dried samples were ground in a stainless steel Wiley Mill to pass a 20-mesh sieve. For the determination of Cu, Mn and Zn contents in tissue, 1.0 g of dried sample was ashed in duplicate at 475°C for 5 hours. The ash from each of the samples was wetted with 5 ml of 0.5 N HCl and then dissolved by adding 20 ml of 0.5 N HCl. The mixture was filtered through Whatman No. 42 filter paper and Cu, Mn an Zn concentration in the filtrate was determined with an atomic absorption spectrophotometer. For the determination of B content, 1.0 g of tissue was ashed in duplicate at 500°C for 5 hours in a porcelain crucible. The ash was dissolved in 25 ml of 0.5 N HCl by adding 5 ml first and then 20 ml of the acid. The azomethine-H procedure outlined by Gaines and Mitchell (1979) was used for the determination of B concentration in solution.

STATISTICAL ANALYSIS

Soybean nutrient concentration and seed yield data were analyzed by analysis of variance and, to obtain mean differences, by linear contrasts and orthogonal comparisons (Little and Hills, 1978). Mean differences were claimed herein at the 0.10 level of probability. The statistical analyses were performed on an IBM 3801 computer with the SAS82 statistical analysis system (SAS Institute Inc., 1982).

RESULTS AND DISCUSSION

The dilute HCl-H_2SO_4 extractable Mn procedure is currently used in Virginia to determine if soils will supply adequate Mn for soybeans (Donohue and Hawkins, 1979a). Other extractable micronutrient procedures (Table 1) have not been calibrated to evaluate the need for micronutrient application. These extractable micronutrient data were included in Table 1 for future reference in expectation that calibration data will become available for these procedures. Based on present calibration data for the dilute HCl-H_2SO_4 extractable Mn procedure and soil pH (Donohue and Hawkins, 1979a), a yield response to Mn application was predicted on each of the soils used in the field investigations (Table 1). Early season field observations indicated that Mn deficiency symptoms occurred in soybeans on the Dragston, Myatt and Slagle soils, but not on the Rains soil.

SEED YIELD AND YIELD COMPONENTS

During 1982, Mn deficiency symptoms in soybean leaves on the Dragston soil disappeared after Mn application. The average soybean seed yield of Mn-treated plots on the Dragston soil increased by 0.87 Mg/ha over the control (Table 5). There was no difference in seed yield between broadcast and foliar Mn applications. Comparison among the foliar Mn applications revealed that there was no yield advantage of 2.2 kg Mn/ha as a single application over 1.1 kg Mn/ha as a single application. Higher soybean yield was obtained from the split Mn application than from the single Mn applications probably because of a more uniform Mn supply during the growth and reproduction cycle (Cox, 1968; Murphy and Walsh, 1972). Decreased seed weight of the Mn-deficient plants might have been due to the low photosynthetic rate of those plants since the structure of chloroplast is adversely affected when Mn supply is inadequate. The stunted growth and the reduction of fertile nodes per plant, when Mn is deficient, reduces the number of seeds per unit area, but the number of seeds per pod is not affected (Heenan and Campbell, 1980b).

Although Mn deficiency in soybeans was expected from soil test data, Mn deficiency symptoms were not observed in soybeans on the Rains soil (Table 6). Concurringly, Mn application did not increase soybean seed yield on the Rains soil in 1982. Overall, these data agree with other findings (Gettier, 1981) that the dilute HCl-H_2SO_4 extractable procedure over-predicts the incidence of Mn deficiency in soybeans. Zinc application did not increase soybean seed yields on the Dragston (Table 5) and Rains (Table 6) soils.

Table 5. Effect of foliar Mn and of broadcast Mn and Zn on seed yield, seed weight and seed number on Dragston fine sandy loam.

#	Broadcast Mn	Broadcast Zn	Foliar Mn Rate	Foliar Mn Growth Stage	Seed Yield	Seed Weight	Seed Number
	------ kg/ha	-------			Mg/ha	g/100 seeds	#/ha
1	40.0	17.0	0.0	---	3.11	14.25	1.91×10^7
2	40.0	0.0	0.0	---	3.06	13.53	1.98×10^7
3	0.0	17.0	0.0	---	2.09	11.48	1.57×10^7
4	0.0	17.0	1.1	V7	2.77	12.98	1.86×10^7
5	0.0	17.0	2.2	V7	2.94	13.15	1.95×10^7
6	0.0	17.0	1.1	V7, R1	3.15	13.53	2.04×10^7
7	0.0	17.0	2.2	R1	2.71	13.30	1.78×10^7

Orthogonal Comparisons:

	Seed Yield	Seed Weight	Seed Number
	-----------	F value	-----------
Treat. 1 and 2 vs 4, 5, 6 and 7	1.70	2.29	0.10
Treat. 1 vs 2	0.04	1.07	0.16
Treat. 3 vs 1, 2, 4, 5, 6 and 7	21.29**	13.66**	6.03*
Treat. 6 vs 4, 5 and 7	2.87†	0.45	1.27
Treat. 4 vs 5 and 7	0.06	0.17	0.00
Treat. 5 vs 7	0.94	0.05	0.78

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.
† Significant at the 10% level of probability.

Table 6. Effect of foliar Mn and of broadcast Mn and Zn on seed yield, seed weight and seed number on Rains fine sandy loam.

#	Treatment: Broadcast Mn	Treatment: Broadcast Zn	Treatment: Foliar Mn Rate	Treatment: Foliar Mn Growth Stage	Seed Yield	Seed Weight	Seed Number
	kg/ha	kg/ha	kg/ha		Mg/ha	g/100 seeds	#/ha
1	40.0	20.0	0.0	---	2.83	12.25	$2.01x10^7$
2	40.0	0.0	0.0	---	2.61	11.98	$1.88x10^7$
3	0.0	20.0	0.0	---	2.48	11.80	$1.80x10^7$
4	0.0	20.0	1.1	V7	2.74	12.20	$1.93x10^7$
5	0.0	20.0	2.2	V7	2.82	12.05	$2.02x10^7$
6	0.0	20.0	1.0	V7, R1	2.71	12.20	$1.93x10^7$
7	0.0	20.0	2.2	R1	2.82	13.00	$1.88x10^7$

Orthogonal Comparisons:	Seed Yield (F value)	Seed Weight (F value)	Seed Number (F value)
Treat. 1 and 2 vs 4, 5, 6 and 7	0.07	0.47	0.00
Treat. 1 vs 2	0.45	0.21	0.41
Treat. 3 vs 1, 2, 4, 5, 6 and 7	1.22	1.11	0.98
Treat. 6 vs 4, 5 and 7	0.09	0.20	0.01
Treat. 4 vs 5 and 7	0.07	0.40	0.01
Treat. 5 vs 7	0.00	2.55	0.55

Manganese deficiency symptoms disappeared in soybeans on the Myatt soil after Mn application in both 1982 and 1983. Soybean seed yields were increased from Mn application during both years on this soil (Tables 7 and 8). The average seed yield during 1982 was increased by 0.83 Mg/ha from foliar application of 2.2 kg Mn/ha as a split treatment (Table 7). This yield response was a 64% increase over the control. The yield response to applied Mn was much greater in 1983 than in 1982. In 1983, a split application of 1.12 kg Mn/ha resulted in higher seed yield than a split application of 2.24 kg Mn/ha (Table 8). Based on these data, it seems possible that Mn deficiency can be corrected by a lower foliar Mn application rate, than the currently recommended rate (Getter, 1981) on some soils. Seed yield increases did not occur on the soil from B application in 1982 or from Cu application in 1983.

Responses of soybeans to B, Cu, Mn and Zn applications were studied on the Slagle soil in 1983 (Table 9). Application of treatments (all of which contained foliar Mn) at the V4 growth stage corrected chlorotic symptoms within one week. Of the various micronutrients, Mn applications increased seed yield on the soil, and application of other micronutrients with Mn did not increase yield above Mn application alone. Similar to the Dragston and Myatt soils, the

Table 7. Effect of B and Mn applications on seed yield, seed weight and seed number on Myatt sandy loam (1982).

	Treatment					
#	Foliar Mn	Broadcast B	Growth Stage	Seed Yield	Seed Weight	Seed Number
	---- kg/ha	-------		Mg/ha	g/100 seeds	#/ha
1	0.0	0.0	---	1.30	14.68	$7.73x10^6$
2	1.1	0.0	V5, R1	2.08	16.63	$1.09x10^7$
3	1.1	1.1	V5, R1	2.18	16.40	$1.16x10^7$
Orthogonal Comparisons:						
				--------	F values	------------
Treat. 1 vs 2 and 3				32.26**	13.19**	11.42*
Treat. 2 vs 3				0.41	0.15	0.32

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.

Table 8. Soybean seed yield, seed weight and seed number as related to Cu and Mn applications on Myatt fine sandy loam (1983).

#	Treatment: Foliar Micronutrients: Cu	Mn	Growth Stage	Seed Yield	Seed Weight	Seed Number
	-- kg/ha --			Mg/ha	g/100 seeds	#/ha
1	0.00	0.00	---	0.95	12.57	6.63×10^6
2	0.00	1.12	V2, V9	1.80	15.27	1.03×10^7
3	0.00	0.56	V2, V9	2.17	14.29	1.32×10^7
4	0.25	0.56	V2, V9	2.01	14.89	1.19×10^7

Orthogonal Comparisons:

	Seed Yield	Seed Weight	Seed Number
	----------- F value	-----------	-----------
Treat. 1 vs 2, 3 and 4	110.89**	9.27*	55.04**
Treat. 2 vs 3 and 4	7.81*	0.75	9.83*
Treat. 3 vs 4	1.72	0.44	2.56

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.

increased seed yield due to applied Mn reflected the effect of the two yield components, the increased seed weight and the increased number of seeds per hectare (Tables 5, 7, 8 and 9). The overall lower seed yields on the Slagle soil as compared with the other soils was due to the severe drought. The low yields on the soil may be responsible for the lack of yield difference from the rate of foliar Mn application.

TISSUE MICRONUTRIENT CONCENTRATION

The effect of applied Mn and Zn was reflected on the micronutrient concentration in blades, petioles and seeds on the Dragston soil (Table 10). Broadcst application of 40 kg Mn/ha increased the leaf blade Mn concentration of the upper most trifoliolate leaf at the R1 growth stage by 17.7 µg/g. Even though seed yield was not affected by the method of Mn application, the tissue Mn concentration of the plants that received broadcast Mn was much higher than those plants that received foliar Mn (Table 10). Broadcast application of 40 kg Mn/ha supplied more Mn than was required by the plants but did not increase Mn concentration to a toxic level.

A comparison between split and single applications of foliar Mn on the Dragston soil indicates that the increase of Mn concentration in seed is greater for the split application than for single applications (Table 10). The effect

Table 9. Soybean seed yield, seed weight and seed number as related to B, Cu, Mn and Zn applications on Slagle fine sandy loam.

#	Treatment: Broadcast B	Treatment: Foliar Micronutrients Cu	Foliar Micronutrients Mn	Foliar Micronutrients Zn	Growth Stage	Seed Yield	Seed Weight	Seed Number
	kg/ha	kg/ha	kg/ha	kg/ha		Mg/ha	g/100 seeds	#/ha
1	0.00	0.00	0.00	0.00	---	0.64	13.98	3.87×10^6
2	0.00	0.00	1.12	0.00	V4, R1	1.57	18.48	7.34×10^6
3	0.00	0.00	0.56	0.00	V4, R1	1.31	18.64	6.13×10^6
4	1.12	0.00	0.56	0.00	V4, R1	1.60	18.66	7.43×10^6
5	0.00	0.28	0.56	0.00	V4, R1	1.28	17.89	6.21×10^6
6	0.00	0.00	0.56	0.28	V4, R1	1.24	17.91	6.04×10^6

Linear Contrasts:	Seed Yield (F value)	Seed Weight (F value)	Seed Number (F value)
Treat. 1 vs 2, 3, 4, 5 and 6	12.67**	49.43**	9.15**
Treat. 2 vs 3	0.89	0.04	1.07
Treat. 3 vs 4	1.07	0.00	1.23
Treat. 3 vs 5	0.02	0.91	0.01
Treat. 3 vs 6	0.07	0.84	0.01

** Significant at the 1% level of probability.

Table 10. Effect of foliar Mn and of broadcast Mn and Zn on micronutrient concentrations in soybean tissue on Dragston fine sandy loam.

	Treatment				Micronutrient Concentration					
	Broadcast		Foliar Mn		Leaf Blade		Petiole		Seed	
#	Mn	Zn	Rate	Growth Stage	Mn	Zn	Mn	Zn	Mn	Zn
	------ kg/ha ------				---------- µg/g ----------					
1	40.0	17.0	0.0	---	30.5	40.9	7.7	12.0	16.5	46.0
2	40.0	0.0	0.0	---	32.5	26.8	8.5	8.9	15.6	33.9
3	0.0	17.0	0.0	---	13.8	41.8	3.7	13.3	9.6	47.7
4	0.0	17.0	1.1	V7	21.0	40.5	4.5	12.4	10.6	47.2
5	0.0	17.0	2.2	V7	19.2	39.5	4.2	12.4	10.5	44.5
6	0.0	17.0	1.1	V7, R1	16.9	40.5	4.3	13.8	12.6	42.8
7	0.0	17.0	2.2	R1	12.3	42.9	3.6	13.2	12.4	44.5
Orthogonal Comparisons:										
					---------- F value ----------					
Treat. 1 and 2 vs 4, 5, 6 and 7					144.33**	57.42**	113.28**	16.92**	136.28**	29.39**
Treat. 1 vs 2					1.14	88.46**	1.54	9.67**	1.84	70.50**
Treat. 3 vs 1, 2, 4, 5, 6 and 7					31.46**	8.24*	13.87**	2.74	49.37**	17.33**
Treat. 6 vs 4, 5 and 7					0.18	0.17	0.10	2.48	7.75*	4.65*
Treat. 4 vs 5 and 7					10.20**	0.28	1.37	0.07	2.25	4.70*
Treat 5 vs 7					12.62**	5.22*	1.07	1.17	8.9**	0.00

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.

of the time of Mn application on leaf blade and petiole Mn concentrations does not account for application of 2.2 kg Mn/ha during the R1 growth stage because the second application was made after the collection of trifoliolate leaves for tissue analysis. However, the Mn concentration of the seeds was increased by the late foliar Mn application. It is clear that after flowering no more vegetative growth occurs and that all of the Mn absorbed is utilized for pod formation. Split application of foliar Mn probably increased seed yield due to maintenance of higher tissue Mn levels throughout vegetative and reproductive growth.

Based on data in Table 10, it seems worthwhile to reconsider the critical Mn level reported by Ohki et al. (1979) because, even at the reported critical level of 13 µg/g Mn in the uppermost fully matured trifoliolate leaf blade at the R1 growth stage (Table 10), seed yield was increased by 41% due to applied Mn (Table 5).

Zinc concentration of the leaf blades from the Zn control plot was well above the critical level reported by Ohki (1977). Applied Zn resulted in an increase of leaf blade Zn concentration by 14 µg/g. Similarly, applied Zn increased the accumulation of Zn in petioles and seeds by 4 µg/g and 12 µg/g, respectively. Tissue Zn concentration indicated an inverse relationship with the Mn concentration, which could be due to dilution of Zn where Mn application increased soybean growth. Comparisons between split Mn application and single Mn applications, revealed that the Zn concentration of seeds is decreased with increased Mn content.

Leaf blade Mn concentration on the Rains soil was almost twice as high as the critical concentration reported by Ohki (1979). Neither leaf blade nor petiole Mn or Zn were affected by applications of these micronutrients (Table 11). Accumlation of Mn as well as Zn occurred in seed from application of the respective micronutrient. Similar to the observations on the Dragston soil, Mn concentrations of the petiole and seed tended to decline with applied Zn.

Boron and Mn concentrations of the leaf blade were increased with the application of these micronutrients during the 1982 study on the Myatt fine sandy loam (Table 12). The leaf blade Mn concentration increased from 13.3 µg/g to 27.8 µg/g with the split application of 2.2 kg Mn/ha on the foliage. A very large increase in tissue B occurred from broadcast B, which indicates high availability of the applied B. The leaf blade B concentration was increased above the sufficiency range reported by Woodruff (1979). As was observed with Zn on the Dragston and Rains soils (Tables 10 and 11), Mn concentration in the leaf blades declined with the B fertilization. These results suggest that the soybean

Table 11. Effect of foliar Mn and of broadcast Mn and Zn on micronutrient concentrations in soybean tissue on Rains fine sandy loam.

	Treatment				Micronutrient Concentration					
	Broadcast		Foliar Mn		Leaf Blade		Petiole		Seed	
#	Mn	Zn	Rate	Growth Stage	Mn	Zn	Mn	Zn	Mn	Zn
	----- kg/ha ------				µg/g					
1	40.0	20.0	0.0	---	27.9	43.1	7.0	16.3	15.5	48.3
2	40.0	0.0	0.0	---	27.6	37.3	9.0	14.6	17.7	42.2
3	0.0	20.0	0.0	---	25.3	39.7	7.8	16.3	14.0	49.0
4	0.0	20.0	1.1	V7	25.3	39.6	7.0	16.0	15.4	48.0
5	0.0	20.0	2.2	V7	29.6	41.2	8.4	16.1	15.6	46.4
6	0.0	20.0	1.1	V7, R1	29.3	43.1	8.1	15.4	15.0	46.8
7	0.0	20.0	2.2	R1	21.5	45.0	7.3	16.2	15.4	46.9

Orthogonal Comparisons:

	Leaf Blade Mn	Leaf Blade Zn	Petiole Mn	Petiole Zn	Seed Mn	Seed Zn
	F values					
Treat. 1 and 2 vs 4, 5, 6 and 7	0.41	0.96	0.17	0.48	4.74*	3.83†
Treat. 1 vs 2	0.01	2.99	3.60†	1.67	5.42*	18.10**
Treat. 3 vs 1, 2, 4, 5, 6 and 7	0.36	0.53	0.00	0.25	5.99*	5.55*
Treat. 6 vs 4, 5 and 7	1.85	0.18	0.37	0.52	0.28	0.08
Treat. 4 vs 5 and 7	0.01	1.44	0.89	0.01	0.01	1.25
Treat. 5 vs 7	5.42*	1.32	1.24	0.00	0.06	0.16

**Significant at 1% level of probability.
* Significant at 5% level of probability.
† Significant at 10% level of probability.

plant has the capacity to accumulate soil B without affecting yield.

Soybean plants on the Myatt sandy loam, during 1983, exhibited Mn deficiency at very early stages. Applied Mn corrected chlorotic symptoms on the leaves within one week. Although the Cu concentration in leaf blades was below the critical level of 6 µg Cu/g (Donohue and Hawkins, 1979b), Cu application did not increase soybean seed yield on the soil (Tables 8 and 13). The foliar Cu application increased Cu concentration in the petiole and seed, but not in the leaf blade. Leaf blade and petiole Mn concentrations were increased with the foliar Mn applications. The rates of Mn applied had no influence on tissue Mn concentrations but, in all the cases, Mn in the leaf blade was lower than the critical concentration reported by Ohki et al. (1979). Tissue Cu concentration decreased with an increase in Mn concentration of the leaf blade, petiole and seed.

Similar to experimental data on the Myatt soil, a large increase in B concentration of leaf blades, petioles, and seeds occurred on the Slagle soil from broadcast B (Tables 14 and 15). As observed (Table 9), no yield response could be expected from B application on the Slagle soil because the tissue B concentration of the plants from B control plots were within the sufficiency range (Woodruff, 1979; Do-

Table 12. Effect of B and Mn applications on micronutrient concentrations in soybean tissue on Myatt Sandy Loam (1982).

	Treatment			Micronutrient Concentration					
	Foliar	Broadcast	Growth	Blade		Petiole		Seed	
#	Mn	B	Stage	B	Mn	B	Mn	B	Mn
	------ kg/ha	-------		-----------------------		μg/g		------------------------	
1	0.0	0.0	---	46.4	13.3	28.5	4.6	11.9	11.9
2	1.1	0.0	V5, R1	40.1	27.8	30.6	5.8	12.2	12.9
3	1.1	1.1	V5, R1	74.4	20.0	37.8	5.4	21.5	11.6
Orthogonal Comparisons:									
				-----------------------		F value		------------------------	
Treat. 1 vs 2 and 3				8.52*	9.68*	1.59	4.15†	15.11**	0.32
Treat. 2 vs 3				64.48**	3.89†	1.88	0.47	39.56**	2.39

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.
† Significant at the 10% level of probability.

Table 13. Effect of foliar Cu and Mn application on micronutrient concentrations in soybean tissue on Myatt fine sandy loam (1983).

Treatment				Leaf Blade		Petiole		Seed	
	Foliar Micronutrients								
#	Cu	Mn	Growth Stage	Cu	Mn	Cu	Mn	Cu	Mn
	-- kg/ha --			µg/g					
1	0.00	0.00	---	8.2	8.2	6.1	6.0	5.7	15.1
2	0.00	1.12	V2, V9	6.9	11.7	5.0	6.8	5.9	15.1
3	0.00	0.56	V2, V9	7.0	10.7	4.9	4.9	5.7	15.3
4	0.25	0.56	V2, V9	7.2	10.5	5.9	5.0	8.3	13.7
Orthogonal Comparisons:									
				F values					
Treat. 1 vs 2, 3 and 4				4.34†	18.13**	12.91**	3.52†	4.37†	0.14
Treat. 2 vs 3 and 4				0.09	2.58	2.10	2.54	5.31*	0.32
Treat. 3 vs 4				0.06	0.12	11.38**	0.38	20.98**	1.58

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.
† Significant at the 10% level of probability.

nohue and Hawkins, 1979b). Foliar Cu application increased Cu concentration in the leaf blades and seeds but not in the petioles.

Applied Mn increased the Mn concentration in the leaf blade but not in the petioles or seed on the Slagle soil (Tables 14 and 15). Application of B with 1.12 kg Mn/ha resulted in a higher seed Mn concentration over the application of Mn alone. Even though B acts on membrane functions and, hence, on ion selectivity (Pollard et al., 1976), no such advantage from applied B was expected because the soil had an adequate supply of available B.

Tissue Zn concentrations were not increased by foliar Zn application on the Slagle soil (Tables 14 and 15). Zinc concentration in soybean seeds decreased where foliar Mn was applied to soybean plants. This relationship could reflect dilution where Mn application increased seed yield. Alternatively the relationship could be explained by the physiological changes in the plant which decrease Zn translocation (Reddy et al., 1978).

Similar to B concentration, the Cu and Zn concentrations (Tables 14 and 15) in tissue from soybean plants on the control treatment were above the critical levels on the Slagle soil (Donohue and Hawkins, 1979b). Consequently, yield response to Cu and Zn would not be expected on the

Table 14. Micronutrient concentrations in soybean leaf blade and petiole as related to B, Cu, Mn and Zn applications on Slagle fine sandy loam.

	Treatment					Micronutrient Concentration							
	Broadcast	Foliar Micronutrients			Growth	Leaf Blade				Petiole			
#	B	Cu	Mn	Zn	Stage	B	Cu	Mn	Zn	B	Cu	Mn	Zn
	kg/ha					μg/g							
1	0.00	0.00	0.00	0.00	---	39.9	6.8	7.5	33.4	29.9	4.4	4.7	20.0
2	0.00	0.00	1.12	0.00	V4, R1	34.8	5.9	11.3	30.4	26.3	4.1	5.5	17.1
3	0.00	0.00	0.56	0.00	V4, R1	34.4	5.4	10.0	35.6	27.9	3.9	5.1	21.4
4	1.12	0.00	0.56	0.00	V4, R1	63.1	5.7	10.0	30.5	35.7	3.9	5.1	16.8
5	0.00	0.28	0.56	0.00	V4, R1	34.5	6.6	10.1	34.3	27.2	4.3	5.3	20.0
6	0.00	0.00	0.56	0.28	V4, R1	36.8	5.2	9.2	34.8	26.4	3.6	4.9	20.9
Linear Contrasts:													
						F values							
Treat. 1 vs 2, 3, 4, 5 and 6						0.09	4.45†	5.20*	0.01	1.24	1.85	1.81	0.18
Treat. 2 vs 3						0.01	0.52	0.78	3.01	1.23	0.32	0.65	3.45†
Treat. 3 vs 4						76.43**	0.29	0.00	2.89	32.35**	0.03	0.00	4.18†
Treat. 3 vs 5						0.00	3.69†	0.01	0.19	0.26	1.26	0.21	0.37
Treat. 3 vs 6						0.55	0.16	0.33	0.07	1.12	0.22	0.32	0.05

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.
† Significant at the 10% level of probability.

Table 15. Micronutrient content in soybean seeds as related to B, Cu, Mn and Zn applications on Slagle fine sandy loam.

#	Broadcast B	Foliar Micronutrients Cu	Foliar Micronutrients Mn	Foliar Micronutrients Zn	Growth Stage	Micronutrient Concentration in Seed B	Cu	Mn	Zn
	kg/ha					μg/g			
1	0.00	0.00	0.00	0.00	---	27.2	5.7	9.7	33.5
2	0.00	0.00	1.12	0.00	V4, R1	22.4	5.4	10.9	27.8
3	0.00	0.00	0.56	0.00	V4, R1	20.0	4.6	9.9	30.9
4	1.12	0.00	0.56	0.00	V4, R1	30.0	6.5	13.4	28.8
5	0.00	0.28	0.56	0.00	V4, R1	20.1	7.4	10.6	30.2
6	0.00	0.00	0.56	0.28	V4, R1	22.1	5.4	10.0	33.1

Linear Contrasts:	B (F Value)	Cu	Mn	Zn
Treat. 1 vs 2, 3, 4, 5 and 6	7.95*	0.04	1.23	7.93*
Treat. 2 vs 3	1.52	0.56	0.51	4.16†
Treat. 3 vs 4	26.47**	2.92	5.79*	1.79
Treat. 3 vs 5	0.00	6.22*	0.21	0.18
Treat. 3 vs 6	1.13	0.43	0.01	2.21

**Significant at the 1% level of probability.
* Significant at the 5% level of probability.
† Significant at the 10% level of probability.

soil. Concurringly, foliar application of either Cu or Zn did not increase soybean seed yield on the soil (Table 9). In contrast, the Mn concentration in soybean tissue was below the critical levels reported by Ohki et al. (1979) and Donohue and Hawkins, (179b). As could be predicted from the foliar Mn level, foliar Mn increased soybean seed yield on the soil (Table 9).

SUMMARY AND CONCLUSIONS

The response of soybeans to applied B, Cu, Mn, and Zn was investigated through five field experiments on four different soils during a two-year period. Sites low in Mn supply were selected according to the calibration of the dilute $HCl-H_2SO_4$ extractable Mn soil test and to visual Mn deficiency symptoms. Boron was applied as a broadcast application, and Cu was sprayed on foliage. Either broadcast or foliar application was used for Mn and Zn. Manganese application increased soybean seed yield on the Dragston, Myatt and Slagle soils, but not on the Rains soil. No seed yield response was obtained from applied B, Cu or Zn. An equivalent seed yield increase was obtained from broadcast and foliar Mn applications. Among foliar Mn applications, no difference was found due to the time of application, but the split foliar application was more effective than single applications. Foliar application of 1.12 kg Mn/ha as a split application was the most suitable way to correct Mn deficiency in soybeans.

Application of B, Cu, Mn and Zn increased the concentration of these micronutrients in plant tissue. A high increase in tissue B content from B broadcast application indicated litle fixation of the applied B. Broadcast application of Mn resulted in a much higher tissue Mn concentration than foliar Mn applications. Overall, the tissue Mn data indicated that the critical level of Mn concentration in the leaf blade at the R1 growth stage should be higher than the reported 13 µg/g. This was evidenced by the 40% yield increase from Mn application where the leaf blade Mn concentration was 13.8 µg/g.

The dilute $HCl-H_2SO_4$ extractable Mn content in the soils did not provide a reliable base to predict the Mn supplying capacity of soil. The present calibration for the extractant over predicts the incidence of Mn deficiency in soybeans. Hot-water soluble B concentration of 0.12 µg/g in soil was sufficient to meet the B requirement of soybeans. For DTPA extractable Cu and Zn, concentrations of 0.15 µg/g and 0.49 µg/g were indicative of adequate amounts of these micronutrients for normal soybean growth, respectively.

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EFFECT OF APPLIED B, CU, MN AND ZN ON

SOYBEAN YIELD AND MICRONUTRIENT CONCENTRATION

by

Lionel Gunaratne

(ABSTRACT)

The effect of applied B, Cu, Mn and Zn on soybean seed yield and tissue micronutrient concentration was studied under different field conditions. Manganese application increased soybean seed yield on the Dragston, Myatt and Slagle fine sandy loams, but not on Rains fine sandy loam. Seed yield was not affected by applied B, Cu or Zn. Broadcast and foliar Mn applications were similarly effective in correction of Mn deficiency and, among foliar Mn applications, split application resulted a higher seed yield than single applications. Micronutrient concentration of the leaf blades, petioles and seeds was increased with the application of B, Cu, Mn and Zn. The increase in tissue B concentration was much greater than that of the other micronutrients. Broadcast Mn application resulted in a higher tissue Mn concentration than foliar Mn applications. The critical Mn concentration in leaf blades at the R1 growth stage was above the level of 13 µg/g, which is reported in the literature. It was concluded that the present calibration of the dilute HCl-H_2SO_4 extractable Mn soil test overpredicts the incidence of Mn deficiency in soybeans.