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COPPER, NITROGEN, AND RHIZOBIUM JAPONICUM RELATIONSHIPS IN DETERMINATE SOYBEAN

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by

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ABSTRACT

A relationship among Cu, N, and *Rhizobium japonicum* was hypothesized because previous research had shown that (a) 35% or more legumes in the Atlantic Coastal Plain have Cu concentrations of 6 mg kg^{-1} or less, (b) Cu influences N fixation in some legumes, and (c) irrigated soybean (*Glycine max* L. Merr.) can accumulate most of its N through fixation. Soybean were grown on a Cu-deficient Norfolk (fine-loamy, siliceous, thermic Typic Paleudult) loamy sand with 3 fertilizer sources of Cu, 2 strains of *R. japonicum*, and with or without 336 kg ha^{-1} of N fertilizer. Application of Cu significantly increased the number of pods $plant^{-1}$ suggesting pod abortion in determinate soybean may be caused by low Cu, but seed yield was not increased. Fertilization with N increased vegetative growth, but not total biomass or seed yield. Inoculation with *R. japonicum* strain 110 significantly increased seed yield by 0.3 Mg ha^{-1} compared to strain 587. The yield increase was similar with or without fertilizer N application indicating strain response was not totally caused by improved N efficiency. There was no relationship between seed yield and nodule occupancy as measured by the ELISA technique.

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INTRODUCTION

Symbiotic N fixation is very important for legume production on southeastern Coastal Plain soils. For example, soybean (Glycine max L. Merr.) grown on Norfolk (fine-loamy, siliceous, thermic Typic Paleudult) loamy sand have been shown to accumulate 55 to 90% of its above-ground N through this process (12). Any biological, chemical or physical factor restricting this process is important.

Mengel and Kirkby (13) stated that Cu appeared to participate in both protein and carbohydrate metabolism and cited literature suggesting a specific requirement for Cu in symbiotic N fixation. In subterranean clover (Trifolium subterranean L.), Cu deficiency increased nodule number, but decreased nodule size and N fixation (3,19). However, this was not true for peanut (Arachis hypogaea L.) (14). In a recent survey, Kubota (9) found 35% or more legumes in the Atlantic Coastal Plain had Cu concentrations of 6 mg kg^{-1} or less. For soybean, Cu concentrations in youngest mature leaves of 5 to 9 mg kg^{-1} are assumed low, with concentrations below 5 mg kg^{-1} assumed deficient (4,8,15). Therefore, when chemical analyses showed an average N concentration that was approximately 1 g kg^{-1} below sufficiency range for soybean at growth stage R2 (5) and an average Cu concentration of 4.7 mg kg^{-1}, we hypothesized Cu may be limiting N fixation in determinate soybean.

We found very little information about soybean and Cu relationships, although Small and Ohlrogge (17) cited survey work in Indiana which indicated soybean yield increased as Cu or Zn concentrations increased. Phyisologically Cu deficiencies appear to affect meristems which are active when external Cu supply is depleted, but developing independent of Cu supply in older leaves (11). This includes shoot apices, young leaves, lateral buds, pollen, and floral apices. In pea (Pisum sativum L.) flower abortion and lack of pod development were listed (16) as symptoms of Cu deficiency. This may be one explanation for high flower and pod abortion rates which often occur in soybean.

Water management may influence Cu concentrations in plants grown on coarse-textured Coastal Plain soils because Cu movement .occurs primarily by diffusion (21). Water management also differentially affects performance of various R. japonicum strains (6,7). Therefore, to evaluate possible Cu and N relationships for field-grown determinate soybean, 3 Cu fertilizer sources, and 2 R. japonicum strains were evaluated with and without N fertilization in an irrigated soybean experiment.

METHODS AND MATERIALS

Three Cu fertilizer sources including broadcast $CuSO_4$ (5.6 kg Cu ha^{-1}), banded Cu-EDTA (0.4 or 0.7 kg Cu ha^{-1}), and foliar Cu-heptogluconate (0.2 or 0.5 kg Cu ha^{-1}) were compared with a control treatment (0 Cu) to determine the effects on plant growth, nutrient concentrations, and seed yield. Those 6 treatments were evaluated in the presence of two Rhizobium japonicum strains (3I1b-110 or B-587) and with or without 336 kg ha^{-1} of N. Treatments were replicated six times in a stripped, split-plot experimental design (20).

This field experiment was conducted on Norfolk (fine-loamy, siliceous, thermic, Typic Paleudult) loamy sand near Florence, S.C., during 1983. The site was previously used for tobacco (Nicotiana tabacum L.) research with no history of soybean and a very low native R. japonicum population. Water pH (1:1, soil:water) and Mehlich I extractable nutrient concentrations were measured (17) and used to determine lime and fertilizer rates. The initial pH was very low requiring 3.4 Mg ha^{-1} of dolomitic lime (21% Ca, 12% Mg) which was applied in a split application approximately 1 month before planting. The site was disked and turnplowed between lime applications. Preplant fertilization included 48, 288, 45, 1, 28, and 6 kg ha^{-1} of P, K, S, B, Mn, and Zn, respectively. Analysis of soil samples collected when soybeans were near physiologic maturity showed lime and fertilizer applications had corrected Ca and Mg deficiencies diagnosed by the initial soil test (Table 1).

Preplant herbicides consisted of 1.8 l ha^{-1} Treflan ($\underline{\alpha},\underline{\alpha},\underline{\alpha}$-Trifluoro-2,6dinitro-N, N-dipropyl-p-toluidine) and 3.5 l ha^{-1} Vernam (5-Propylid-propylthiocarbamate). For nematode control 14.0 l ha^{-1} of Nemacur (3-methyl-4-(methio)phenyl (1-methylethyl) phosphoramidate) were applied.

A group VII soybean cultivar, Coker 237, was planted at a density of 70 seed m^{-2} in twin-rows spaced 36 cm apart with each pair spaced 96 cm apart on 4 June 1983. Molybdenum was applied at 4.5 g kg^{-1} seed with the two R. japonicum strains which were placed with the seed at a

TABLE 1

Chemical characteristics of Norfolk loamy sand where Cu, N, and Rhizobium *japonicum* relationships were evaluated.

Sampling time	Water pH	Mehlich I Extractable						Mehlich III
		P	K	Ca	Mg	Mn	Zn	Cu
		mg kg^{-1}						mg dm^{-3}
Initial	4.9	84	68	119	11	6.6	1.7	0.3
Maturity	6.0	60	67	268	73	9.8	5.1	

rate of 10^6 organisms cm^{-1} of row. The N treatment was applied as urea ammonium nitrate (UAN) solution in six equal applications between emergence (VE) and full seed (R5) growth stages. Vacuum gauge tensiometers were installed at 20- and 40-cm depths and used to monitor soil-water tension. Irrigation water was applied using Rainbird (1) model 20AH sprinklers whenever soil-water tension at 20 cm exceeded 25 kPa. A total of 23 cm of water was applied during the growing season. Insects were controlled during reproductive growth stages with an aerial application of 4.7 l ha^{-1} Lanate (S-methyl-N-((methylcarbomyl) oxy) thioacetimidate).

Youngest, fully-matured leaf samples were collected at V9 and R2 growth stages, dried at 70C, ground to pass a 0.5 mm stainless steel screen, digested with sulfuric and selenous acid for N, P, and K analysis and with nitric-perchloric acids for Ca, Mg, Cu, Fe, Mn, and Zn analyses. The N and P concentrations were determined colorimetrically and other analyses were by atomic absorption spectrophotometry. Whole plant samples were collected at growth stage R6 to determine actual stand density, pod density, biomass accumulation, and nodule number and weight. Rhizobial strain occupancy was determined using enzyme-linked immunosorbent assay (ELISA) technques (2). Seed yield was measured with an Almaco plot combine on 30 November when plants were fully-matured (R8) and seed moisture averaged 171 g kg^{-1}. Yields were adjusted to a moisture content of 130 g kg^{-1} and data were analyzed statistically using procedures outlined by Steel and Torrie (20).

TABLE 2

Nitrogen fertilization effects on soybean nutrient concentrations at growth stage V9.

N-Rate	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
kg ha^{-1}	-------------g kg^{-1}-					-------mg kg^{-1}			
0	31.1	4.07	28.5	6.41	4.14	14	335	86	50
136	43.4	4.56	30.9	4.93	3.90	10	222	70	51
LSD(0.05)	1.2	0.44	0.9	0.37	NS	1	35	6	NS

RESULTS AND DISCUSSION

Vegetative growth and V9 leaf N, P, and K concentrations were increased by fertilizing soybean plants with UAN. The V9 N concentration in nonfertilized plants averaged 31 g kg^{-1} which is approximately 1 g kg^{-1} below the sufficiency range for soybean (15) (Table 2). Fertilization with N decreased Ca, Cu, Fe, and Mn concentrations, but did not influence Mg or Zn concentrations. The lower Cu concentration was presumably caused by dilution effects. There were no significant differences in nutrient concentrations at the V9 sampling because of Cu or *R. japonicum* treatments.

Leaf samples collected at growth stage R2 (full bloom) showed N fixation was sufficient to eliminate differences in leaf-N concentration observed during vegetative growth (Table 3). Plants fertilized with N had lower Cu but higher Mg concentrations than plants which received no N fertilization. Inoculation with strain 110 significantly reduced leaf Mg 0.2 g kg^{-1} at growth stage R2, but that was the only effect inoculation with *R. japonicum* had on leaf nutrient concentration.

A quantitative relationship between leaf Cu and N fixation for soybean was not identified in this experiment because in control plots, leaf Cu averaged 10 mg kg^{-1} for both V9 and R2 samplings. All

TABLE 3

Nitrogen fertilization effects on soybean nutrient concentrations growth stage R2.

N-Rate	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
kg ha^{-1}		---------g kg^{-1}----------				-------mg kg^{-1}-------			
	50.8	4.96	22.1	6.00	4.27	13	155	78	60
	51.6	4.93	21.5	6.06	4.64	10	134	82	62
LSD(0.05)	NS	NS	NS	NS	0.17		NS	NS	NS

leaf-Cu concentrations were 2 to 3 times higher than in any previous soybean experiments on similar soil in adjacent fields. Reasons for this are unknown, although using overhead sprinklers to supply supplemental irrigation water may have enhanced Cu diffusion (20) or possibly caused surface movement of Cu from treated to nontreated plots. The strongest indication of a soybean response to Cu is shown by increased leaf Mg at growth stage R2 and increased pod number at growth stage R6 (Table 4). The increased Mg may reflect the role of Cu in chloroplasts or photosynthesis (13) while the lower pod number in control plots may reflect greater abortion as diagnosed for Cu deficiencies in pea (16). These differences, however, were not reflected in seed yield which was significantly different only where Cu-heptogluconate was foliar applied. Severe leaf burn and repeated partial defoliation were presumably responsible for reducing seed yield for those two treatments.

Fertilizing soybean with 336 kg ha^{-1} N significantly reduced the number of plants and pods m^{-2} as well as the number and weight of nodules $plant^{-1}$ (Table 5). However, biomass and seed yield were not changed significantly. Inoculation with Rhizobium japonicum strain 110 significantly increased seed yield with or without fertilizer N (Fig. 1). Although the response was greater without N than where 336 kg ha^{-1} was applied, positive response to strain 110 compared to strain 587 was probably not caused by an increase in N efficiency.

TABLE 4

Copper fertilization effects on soybean seed yield and leaf nutrient concentrations at growth stage R2.

Cu Rate	Treatment Placement	Yield	N	P		Ca	Mg	Cu	Fe	Mn	Zn
·kg ha^{-1}---		Mg ha^{-1}	-----------	-----------	g kg^{-1}	-----------	-----------	----mg	kg^{-1}	----	
0		3.00	51.2	4.84	22.3	6.14	3.76	10	125	80	59
5.6	Broadcast	3.03	48.7	4.85	20.1	6.24	4.04	12	123	86	60
0.4	Banded	2.89	51.6	5.18	21.2	5.77	4.84	11	142	82	62
0.7	Banded	3.02	49.5	4.90	23.2	6.24	4.54	11	187	73	62
0.2	Foliar	2.28	52.4	4.93	21.8	6.27	2.86	10	141	80	63
0.5	Foliar	2.37	53.5	4.92	22.1	5.59	4.53	15	144	77	58
LSD(0.05)		0.29	2.4	NS	2.1	NS	0.25		NS	NS	NS

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Nitrogen fertilization effects on nodulation, biomass, and seed yield.

N-Rate	--Nodule-- No.	Wgt.	Plant --Density--	Pod	-----Biomass----- Lamina	Pods	Stems	Seed Yield
kg ha^{-1}	--per m^2--		--No. m^{-2}--		-------g	m^{-2}	-----	Mg ha^{-1}
0	40	906	68	2450	280	390	610	2.81
336	22	375	49	2060	260	380	630	2.72
LSD(0.05)	8	159		360	NS	NS	NS	NS

Perhaps there was a rhizosphere effect similar to that described for associative N fixation (10), because with residual soil N, 336 kg ha^{-1} would be sufficient to meet plant N requirements for the level of seed yield produced in this experiment.

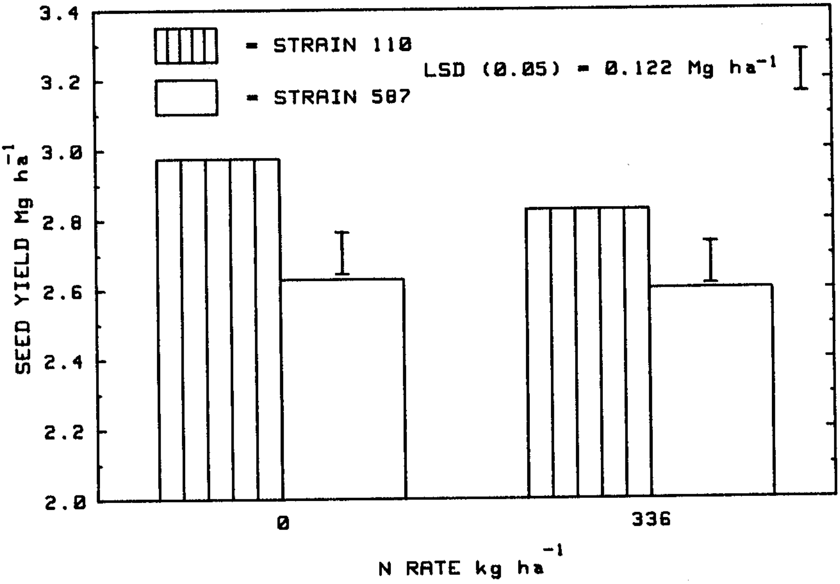

Fig. 1. Effect of *Rhizobium japonicum* strain on soybean seed yield with and without fertilizer N.

The ELISA technique confirmed that inoculation was effective in establishing significantly different *R. japonicum* populations in the nodules, but statistical correlation showed no significant relationship between strain occupancy and seed yield. There was also no significant relationship between seed yield and nodule number or weight. This further suggests that *R. japonicum* response was not caused by nitrogen.

This research suggests that Cu may be involved with pod abortion for determinate soybean, but analysis of most recently mature leaf tissue did not quanitify the relationship. Fertilization of soybean with N increased vegetative growth but not biomass or seed yield. Inoculation with *R. japonicum* strain 110 increased seed yield compared to inoculation with strain 587 with or without supplemental N applications.

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