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Effects of Soil Water on *Rhizobium japonicum* Infection, Nitrogen Accumulation, and Yield in Bragg Soybeans[1]

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ABSTRACT

Low soil water contents may be involved in the lack of success of soybean inoculation in southeastern U.S. soils having high indigenous populations of *Rhizobium japonicum*. This study was conducted to assess the effect of soil water on the inoculation, biomass, N accumulation, and yield of soybean [*Glycine max* (L.) Merr.]. 'Bragg' soybeans were inoculated with 10^8 cells/cm row of *R. japonicum* (strain 110) and grown under both irrigated and nonirrigated conditions on a Norfolk loamy sand (Typic Paleudult) in a split plot design. Rainfall maintained the surface soil above −250 mb matric potential until the late flowering stage of growth. Subsequently, a seasonal drought created treatment differences between irrigated and nonirrigated plots resulting in a twofold increase in seed yield due to irrigation (3,160 vs. 1,682 kg/ha). Inoculation increased the percentage infection by strain 110 from practically 0% background to about 20%. Inoculation increased growth and total N accumulation in pods, petioles, and leaves of Bragg soybeans under irrigated conditions. Under nonirrigated conditions, the increased infection of strain 110 appeared to induced a negative response in the vegetative growth of Bragg soybeans. Nitrogen concentration was also significantly lower in the leaves and pods of nonirrigated-inoculated plants. These differences were not observed in N content of the mature seed, and inoculation did not significantly affect seed yield. These findings indicate that *R. japonicum* strain × environment interactions may be important for soybean growth and yield in the southeast United States.

Additional index words: **Irrigation, Inoculation, Leaf N.**

WATER deficits and excesses are probably the most common limiting factors to crop yield in the southeastern Coastal Plain. Physical barriers to root development and low water holding capacities along with erratic rainfall patterns are contributing factors (Campbell et al., 1974; Martin et al., 1979; Doss et al., 1974). However, subsoiling and irrigation can alleviate water stress, and irrigation is rapidly increasing in the southeastern United States (Reicosky et al., 1976; Doty et al., 1975; and Campbell and Phene, 1977). Consequently, other yield limiting factors such as N supply and its interaction with water management will become more important. Soils of the Southeast are typically low in N; the high annual rainfall causes leaching and denitrification of both mineralized and applied N. Under these conditions, fixation of N by soybeans is quite important. Total accumulations of 225 to 450 kg/ha of N by 'Lee' soybean [*Glycine max* (L.) Merr.] in North Carolina were reported by Henderson and Kamprath (1970), while Batchelor and Scott (1979) reported 112 to 269 kg/ha of N accumulation in 'Lee 74' soybean in Arkansas. Nelson and Weaver (1980) reported greater than 300 and 75 kg/ha, respectively, for nodulating and nonnodulating Lee soybeans.

The infection of soybean by efficient *Rhizobium japonicum* strains could be an important aspect of higher soybeans yield under intensive soil and water management. Unfortunately, the formation of nodules by a new strain in a soil containing indigenous populations of rhizobia has not been successful (Weber et al., 1971; Weaver and Fredrick, 1974a, 1974b). Extreme temperatures, acidity, and moisture are possible detriments to inoculation with desired strains (Weber and Miller, 1972; Keyser and Munns, 1979a, 1979b; Osa-Afiana and Alexander, 1979; Pena-Cabriales and Alexander, 1979). The objectives of this study were to assess the interactions of irrigation with inoculation, as expressed by N accumulation and yield of 'Bragg' soybean.

MATERIALS AND METHODS

A randomized complete block design with a split plot for inoculation and three replications was used in 1978 on a Norfolk loamy sand (Typic Paleudult) with irrigation as the main plot treatment and inoculation as the split plot treatment. Main plots had six 10.7-m rows on 96-cm spacings. Soil was analyzed by the Clemson University Soil Test Laboratory, and pertinent soil characteristics are given in Table 1. The soil was fertilized with 220 kg/ha of 0-14-22 fertilizer and 45 kg/ha of Ca as calcitic limestone, disked, and planted to Bragg (group VII) soybeans on 17 June 1978. About 26 seeds/m row were placed at a depth of 2.5 cm. Frozen liquid inoculum supplied by Microlife Inc.[3] was diluted and applied directly on the seed by use of a buret with 10^8 organisms/cm of row of strain 3Ilb 110 *Rhizobium japonicum*. Seeds were then covered by use of a hoe. The background *R. japonicum* population was approximately 10^4 organism/g of soil.

Tensiometers were placed in the rows at depths of 0.3, 0.6, 0.9, 1.2, and 1.5 m, and irrigation water was applied to maintain the soil matric potential below −250 mb at the 0.6-m depth in the irrigated plots. Irrigation water was applied through trickle irrigation tubing. Rainfall and evaporation were monitored by use of a rain gauge and evaporation pan, respectively. Plant heights were measured twice per week; plant tops and roots were sampled from approximately 30 cm of row 36, 63, 78, and 104 days after planting. These dates roughly corresponded to growth states $V_{?}$, R_2, R_4, and R_6, respectively (Fehr et al., 1971). The tops were divided into stems, petioles, leaves, and pods. Sampled plants were dried, weighed, ground, digested in a block digester, and analyzed for total Kjeldahl N by use of a Technicon Auto Analyzer. Seed yields were taken from 5 m of the middle four rows of plots, and seeds were analyzed for oil and protein (Rinne et al., 1975). The root nodules were sampled by removing a core of roots and soil 25 cm in diameter

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[3] Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or the North Carolina Agricultural Research Service and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table 1. Soil nutrient status of the Norfolk loamy sand.

Horizon	Depth	pH	P	K	Mg	Ca
			kg/ha			
A_1	0–30	6.0	146	125	181	437
A_2	30–45	5.8	65	85	123	166
B	45+	5.0	9	213	255	428

Table 2. Nodule formation by strain 110 *Rhizobium japonicum* in Bragg soybeans grown on a Norfolk loamy sand soil.

Treatment	Nodule formation (%)		
	Day 36†	Day 63*	Day 104**
Nonirrigated			
Inoculated	40	25 a	25 a
Noninoculated	0	4 b	1 b
Irrigated			
Inoculated	25	14 a	19 a
Noninoculated	4	1 b	0 b

* Values for Day 63 followed by the same letter do not differ significantly at the 0.05 level (Duncan's Multiple Range Test).
** Values for day 104 are compared at the 0.01 level.
† Values for Day 36 are single observations.

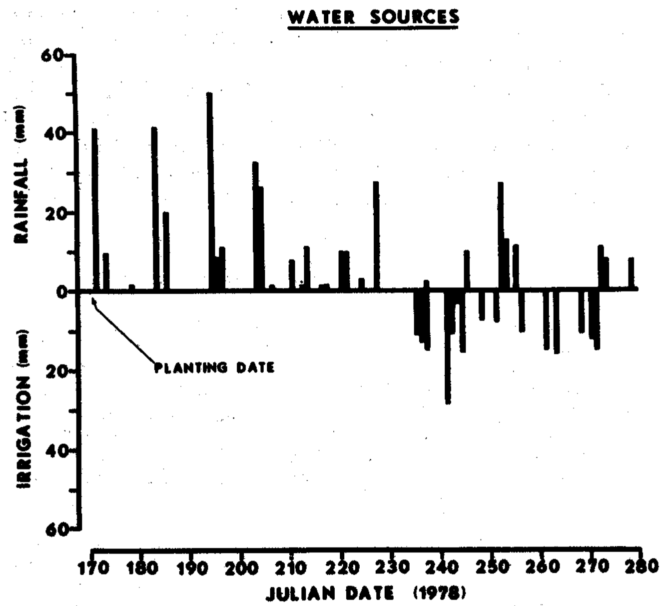

Fig. 1. Rainfall and irrigation distribution during the growing season.

with a cylinder centered on the row and driven to a 30-cm depth. Roots and nodules were gently separated from the dry sandy soil. Nodules were frozen until analyzed serologically.

The distribution of *R. japonicum* strains in nodules was evaluated using a micro-agglutination test on nodule suspensions with antisera against U.S. Department of Agriculture, strains 3Ilb24, 3Ilb31, 3Ilb46, 3Ilb62, 3Ilb76, 3Ilb94, 3Ilb110, 3Ilb122, 3Ilb123, and 3Ilb125. These strains have been reported to be in North Carolina soils (Borges and Wollum, 1980).

Twenty-four nodules were randomly selected from root systems of four plants from each of the plots, washed thoroughly with distilled water, and placed in individual 5-ml vials containing 2 ml of 0.55% saline solution (NaCl). The nodules were crushed and heated at 100 C for 30 min to eliminate nonspecific (H) flagellar antigen. Cell suspensions were adjusted to an optical density of about 0.6 at 520 nm.

The micro-agglutination tests were made by placing 0.025 ml of the antigen (nodule suspension) into the wells of Lucite plates containing an equal volume of each of the diluted antiserum of the 10 *R. japonicum* strains. The plates were sealed with plastic sealing tape, gently rotated to thoroughly mix antigen and antiserum, and incubated in a water bath at 53 C for 5 hours. After removal of the sealing tape, agglutination reactions were observed under a 6 × 10 stereo microscope.

RESULTS AND DISCUSSION

Ample but not excessive rain fell during early vegetative growth, but drought occurred during the fruiting period of the soybean growing season. There was 27.5 cm rainfall with good distribution before flowering, but after flowering, 20.5 cm of irrigation water was required to maintain desired soil water potential (Fig. 1). Water extracted from the lower profile in the nonirrigated plots delayed moisture stress into the drought period. However, by Day 100 after planting, tensiometers at the 1.5-m depth in the nonirrigated plots exceeded −800 mb.

Nodule formation by strain 110 was high for field conditions (Table 2), with the lowest residency in inoculated plots being about 14%. Infection of less than 5% with the inoculated strain has frequently been found (1977, 1978, 1979 Annual Reports of Regional

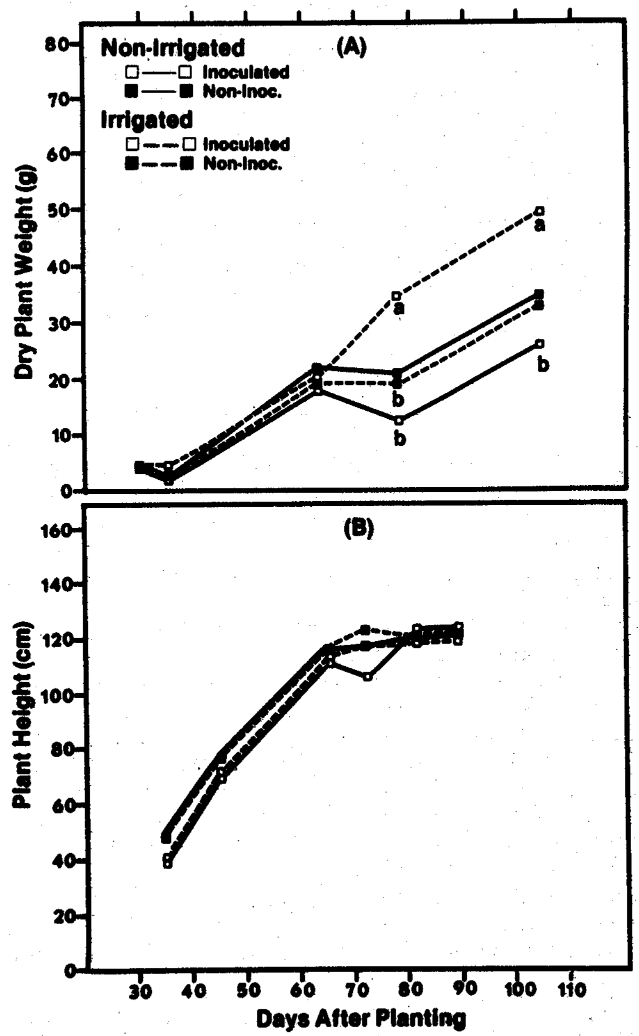

Fig. 2. Plant weight (A) and height (B) during the growing season. (Only means on the same date followed by different letters are different by the Duncan's Multiple Range Test at the 0.1 level).

Committee S-112, Enhancing biological dinitrogen fixation in soybeans and other legumes). Good conditions for early survival of strain 110 *R. japonicum* existed in both water treatments. Strain 110 represented 4% or less of the nodule infection of soybeans in noninoculated plots. Thus, the increased incidence of strain 110 in inoculated plants provided the opportunity to investigate the impact of strain 110 upon growth, N accumulation, N distribution, and yield of Bragg soybeans.

A significant F-test (0.05) interaction between inoculation and irrigation was found for plant weight. Mean biomass for the drought period was significantly higher for inoculated than noninoculated treatments under irrigated conditions (42 vs. 27 g/plant). However, under the drought stress, infection with strain 110 had a tendency to have a negative effect on biomass accumulation (Fig. 2). Biomass accumulations under noninoculated treatments were not different for irrigation. Plant heights were not different among the treatments.

Nitrogen concentrations in the stems and petioles were not statistically different at any time during the season for any treatment (data not shown). On Day 78 after planting, the leaf N content of the inoculated plants was higher in the irrigated than the nonirrigated plots at the 0.1 level, but there was no significant difference for irrigation when noninoculated (Table 3). One hundred and four days after planting, leaf N concentrations were statistically (0.05 level) higher in the irrigated and inoculated plants than in plants of either nonirrigated treatment. Also, for all dates, under irrigation, the mean leaf N in inoculated plants was statistically (0.1) higher than that of the noninoculated plants.

The difference in leaf N concentration according to Boote et al. (1978) probably affected the plant C accumulation. The N concentration of nonirrigated and inoculated soybean pods was statistically lower than that observed with all other treatments 78 and 104 days after planting. These differences in biomass and N concentration resulted in no difference in N accumulation in the stem, but large differences in accumulation in the petioles, leaves, and pods (Fig. 3). The leaf N in particular was dramatically affected by the significant (0.05 by F-test) inoculum × irrigation in-

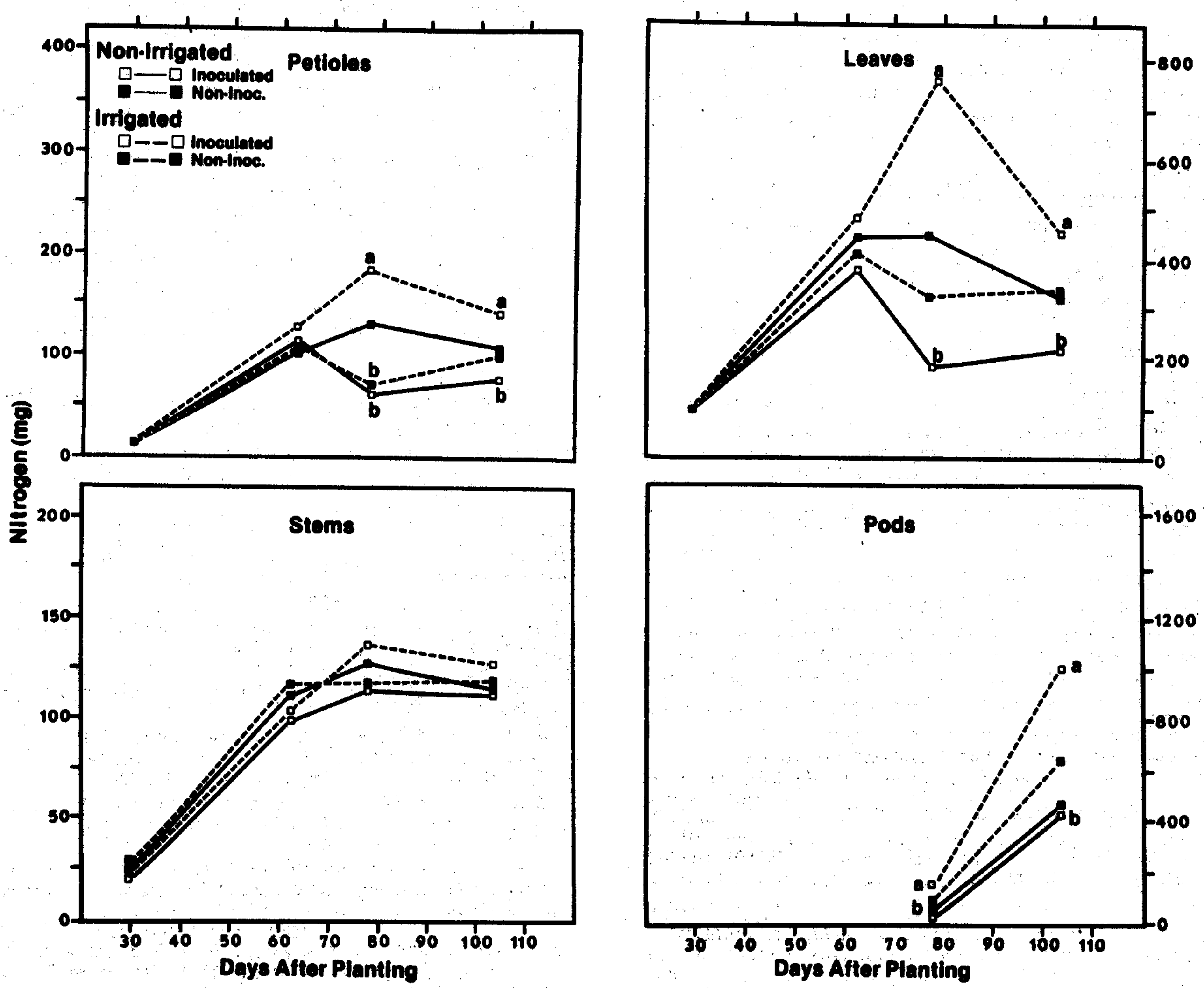

Fig. 3. Nitrogen accumulation and distribution during the growing season. (Only means on the same date followed by different letters are different by the Duncan's Multiple Range Test at the 0.1 level).

Table 3. Nitrogen concentration in Bragg soybeans grown on a Norfolk loamy sand inoculated with strain 110 *Rhizobium japonicum*.

	N concentration (%)*						
	Leaves					Pods	
Treatment	Day 36	Day 63	Day 78	Day 104	Mean	Day 78	Day 104
Nonirrigated							
Inoculated	3.93 a	5.04 a	4.22 b	3.78 c	4.27 b	3.52 b	3.85 b
Noninoculated	4.23 a	5.14 a	4.62 ab	3.95 bc	4.50 ab	4.21 a	4.57 a
Irrigated							
Inoculated	4.20 a	5.06 a	4.99 a	4.58 a	4.71 a	4.41 a	4.79 a
Noninoculated	3.39 a	5.13 a	4.58 ab	4.38 ab	4.35 b	4.34 a	4.78 a

* Column values followed by the same letter do not differ significantly at the 0.05 level according to Duncan's Multiple Range Test except leaves on Day 78 and the means are compared at the 0.1 level.

teraction. With the population of 26 plants/m, the N accumulation on Day 104 after planting was approximately 450 and 320 kg/ha for inoculated and noninoculated soybeans, respectively, when irrigated. The nonirrigated soybeans accumulated approximately 220 and 270 kg/ha of N for inoculated and noninoculated conditions, respectively. These data show a dramatic response to strain 110 inoculation under irrigated conditions. Considering the low N supplying capacity of the Norfolk loamy sand soil, these data support Nelson and Weaver's (1980) conclusions that determinant soybeans are able to supply much of their N need by fixation. The data also support the concept that more efficient strains can improve N fixation and accumulation (Weber et al., 1971). However, the lower accumulations of N under nonirrigated conditions indicate that environmental factors are very important in the response of soybeans to introduced strains of *R. japonicum*.

Although the inoculum × irrigation interaction was significant in several aspects of the vegetative growth of the plant; yields, seed protein, and oil content were not significantly affected (Table 4). Irrigation increased the yield from 1,309 to 3,012 kg/ha, but inoculation did not significantly increase yield under irrigation nor did it suppress yield under nonirrigated conditions. This lack of yield response to increased biomass and N accumulation associated with the introduction of strain 110 suggest that factors other than N limited yield. These data support the conclusions of Nelson and Weaver (1980) that determinant soybeans can supply needed N and maintain leaf N. They also suggest that factors other than the C and N limitations proposed by Sinclair and DeWit (1976) limit determinant soybean yields. However, the data showed a trend similar to that of the biomass and N accumulation: yield from the inoculated treatments was numerically higher in yield by 155 kg/ha under irrigated and lower by 64 kg/ha under nonirrigated conditions. Consequently, under inoculated conditions there was a 219 kg/ha numerically greater response to irrigation than under noninoculated conditions.

SUMMARY AND CONCLUSIONS

Liquid inoculum of strain 110 at 10^8 organisms/cm of row was sufficient to increase the percentage of strain 110 infection in Bragg soybeans on a Norfolk loamy sand soil having an indigenous rhizobia population. Vegetative growth and N accumulation responded to the increased strain 110 infection under irrigated conditions, but yield was not statistically increased.

These data point to an inoculant strain × water interaction, and indicate that strain efficiency must be assessed in relation to environment. This greatly complicates the task of selecting and recommending host-strain combinations for broad geographical areas and agronomic practices. Research is needed on the interactions of *R. japonicum* inoculant infection and performance in soybeans under different management conditions.

Table 4. Seed quality and yield of Bragg soybeans grown on a Norfolk loamy sand soil inoculated with strain 110 *Rhizobium japonicum*.

Treatment	Protein*, %	Oil, %	Yield, kg/ha
Nonirrigated			
Inoculated	41.3 a	21.1 a	1277 a
Noninoculated	40.8 a	20.8 a	1341 a
Irrigated			
Inoculated	41.4 a	20.4 a	3089 b
Noninoculated	41.4 a	20.5 a	2934 b

* Column values followed by the same letter do not differ significantly at the 0.05 level according to Duncan's Multiple Range Test.

LITERATURE CITED

1. Batchelor, J. T., and H. D. Scott. 1979. Effects of irrigation on nitrogen and potassium uptake by soybeans. Arkansas Farm Res. 28:5.
2. Boote, K. J., R. N. Gallaher, W. H. Robertson, K. Hinson, and L. C. Hammond. 1978. Effect of foliar fertilization on photosynthesis, leaf nutrition, and yield of soybeans. Agron. J. 70:787-791.
3. Borges, A. C., and A. G. Wollum, II. 1980. A field study of a soil-soybean plant-rhizobia system amended with cadmium. J. Environ. Qual. 9:420-423.
4. Campbell, R. B., and C. J. Phene. 1977. Tillage, matric potential, oxygen, and millet relations in layered soils. Trans. ASAE 20:271-275.
5. ———, D. C. Reicosky, and C. W. Doty. 1974. Physical properties and tillage of Paleudults in the southeastern Coastal Plains. J. Soil Water Cons. 29:220-224.
6. Doss, D. B., R. W. Pearson, and H. T. Rogers. 1974. Effect of soil water stress and various growth stages on soybean yield. Agron. J. 66:297-299.
7. Doty, C. W., R. B. Campbell, and D. C. Reicosky. 1975. Crop response to chiseling and irrigation in soils with a compact A_2 horizon. Trans. ASAE 18:668-672.

8. Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage of development descriptions for soybeans, (*Glycine max* (L.) Merrill). Crop Sci. 11:929-930.
9. Henderson, J. B., and E. J. Kamprath. 1970. Nutrient and dry matter accumulation by soybeans. N. C. Agr. Expt. Stn. Tech. Bull. 197. 27 p.
10. Keyser, H. H., and D. N. Munns. 1979a. Effects of Ca, Mg, and Al on growth of rhizobia in acid media. Soil Sci. Soc. Am. J. 43:500-503.
11. ———, and D. N. Munns. 1979b. Tolerance of rhizobia to acidity, Al, and Phosphate. Soil Sci. Soc. Am. J. 43:519-523.
12. Martin, C. K., D. K. Cassel, and E. J. Kamprath. 1979. Irrigation and tillage effects on soybean yield in a Coastal Plains soil. Agron. J. 71:592-594.
13. Nelson, A. N., and R. W. Weaver. 1980. Seasonal nitrogen accumulation and fixation by soybeans grown at different densities. Agron. J. 72:613-616.
14. Osa-Afiana, L. O., and M. Alexander. 1979. Effect of moisture on survival of rhizobium in soil. Soil Sci. Soc. Am. J. 43:925-930.
15. Pena-Cabriales, J. J., and M. Alexander. 1979. Survival of rhizobium in soils undergoing drying. Soil Sci. Soc. Am. J. 43:962-966.
16. Reicosky, D. C., R. B. Campbell, and C. W. Doty. 1976. Corn plant water stress as influenced by chiseling, irrigation, and water table depth. Agron. J. 68:499-503.
17. Rinne, R. W., S. Gibbons, J. Bradley, R. Seif, and C. A. Brim. 1975. Soybean, protein, and soil percentage determined by infrared analysis. U.S. Agr. Res. Serv., North Central Region, ARS-NC-26.
18. Sinclair, T. R., and C. T. DeWit. 1976. Analysis of C and N limitation of soybeans. Agron. J. 70:213-216.
19. Weaver, R. W., and L. R. Fredrick. 1974a. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. I. Greenhouse studies. Agron. J. 66:229-222.
20. ———, and ———. 1974b. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. II. Field studies. Agron. J. 66:233-236.
21. Weber, D. F., and V. L. Miller. 1972. Effect of soil temperature on *Rhizobium japonicum* seriogroup distribution in soybean nodules. Agron. J. 64:796-798.
22. ———, B. E. Caldwell, C. Sloger, and H. G. Vest. 1971. Some USDA studies on the soybean-rhizobium symbiosis. *In* T. A. Lie and E. G. Mulder (ed.) Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil Special Vol:293-304. Martinus Nighoff, The Hague.