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Interaction Between Rhizobial Inoculation and Fertilizer Nitrogen in Five Cowpea Cultivars

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Abstract. **Rhizobial inoculation with commercial cowpea 'EL' mixed strain inoculant as compared to noninoculation, and effects of four levels (0, 14, 28, and 84 kg·ha^{-1}) of fertilizer N ($CaNO_3$–15.5% N) on yield and N_2 fixation components in cowpea [*Vigna unguiculata* (L.) Walp.] were investigated in a field study. Plants were grown on a vertic albaqualf, fine, montmorillonitic, thermal soil with a pH of 6.7. Three high (H) and two low (L) N_2-fixing, indeterminate cowpea cultivars, 'H-California Blackeye No. 5', 'H-Brown Crowder', 'H-Tennessee White Crowder', 'L-Lady', and 'L-Mississippi Silver', were used. In inoculated plants, N_2 fixation was significantly reduced with increasing N levels. Although high-fixing cultivars produced more and larger nodules and expressed higher nitrogenase activity than the low fixers, no significant differences in top dry weight and total N/plant were observed between these groups at the time of flowering. Seed yield was greater in rhizobia-inoculated plants than in the noninoculated, fumigated controls. A significant linear increase in seed yield was observed with increasing N levels in the noninoculated, fumigated controls. The addition of fertilizer N to cowpeas inoculated at planting did not increase seed yield. In high-fixing cultivars, N_2 fixation did not directly influence seed yield, but increased vegetative matter was produced. Seed and biomass yield were influenced by N_2 fixation in low-fixing cultivars.**

Seed yield of cowpea, a popular food grain legume in several parts of the world, is generally lower than that of cereals (9). Nitrogen (N) deficiency, a common problem associated with tropical soils, can restrict the expression of the genetic yield potential of cowpea cultivars. Numerous investigations on the effect of combined N (NH_4 and NO_3) on nodulation have demonstrated that maximum symbiotic fixation is associated with growth media deficient in combined N (14, 17, 23). High levels of fertilizer N were found to reduce root hair infection (5, 16), nodule number (3), nodule growth (21), nitrogenase activity (10), and the total amount of biologically fixed N (1). The magnitude of inhibition was influenced by plant species (1), host genotype (10, 25), growing season (18), strain of *Rhizobium* (18), and the forms of N fertilizer (4). Synergistic effects of applied N on N_2 fixation with low levels of N were also reported in soybean (*Glycine max*) and cowpea (7). Although cowpea symbiotically fixes N_2, it has been a recommended practice to fertilize cowpea with N (11, 20). In Texas, reports indicated that cowpea showed little yield response to N fertilizer application above 20 kg·ha^{-1} (19). Addition of increasing levels of NO_3 fertilizers up to 20 kg·ha^{-1} had an adverse effect on N_2 fixation in both high- and low-fixing cultivars (14).

Although the interrelationships between N_2 fixation and seed yield in soybean have been studied extensively (2, 6), this subject has not been thoroughly investigated in field-grown cowpea. The efficiency with which biologically fixed and fertilizer N are converted into seed yield is dependent on cultivar and environment (8). Also, N_2 fixation, translocation of N, and seed production are interrelated phenomena that should be studied simultaneously. Therefore, the objective of this study was to investigate the interaction between inoculation and N levels on N_2 fixation and seed and biomass yield in cowpea.

A field study was conducted at College Station, Texas in Summer 1982 on a vertic albaqualf, fine montmorillonitic, thermal soil at pH 6.7. In an effort to eliminate the native rhizobia, the experimental site was fumigated with methyl bromide (a.i. = 100%) at 436 kg·ha^{-1}. Enumeration of rhizobia in the experimental soil at different locations by the most probable number (MPN) method (22) was made both before and two weeks after fumigation. The native rhizobial population was reduced from 1000 cells/g of soil to almost zero as a result of fumigation.

A split-plot design with three blocks was used. Two levels of inoculation, no inoculation and inoculation with rhizobia, and five cowpea cultivars, for a total of 10 treatment combinations, were allocated to main plots within each block. The three high (H) and two low (L) N_2-fixing, indeterminate cowpea cultivars used were 'H-California Blackeye No. 5', 'H-Brown Crowder', 'H-Tennessee White Crowder', 'L-Lady', and 'L-Mississippi Silver' (25). Four split plots were allocated with four fertilizer N levels, 0, 14, 28, and 84 kg·ha^{-1}. Plot size was 4 × 1 m. Shoot dry matter and percentage of N of the nonnodulating soybean isoline 'Lee', grown without fertilizer N and harvested at seven weeks, were used to measure plant-available N in the soil.

Random soil samples taken before planting and analyzed by the Texas A&M Soil Testing Laboratory indicated that extractable levels of N, P, and K were 6, 10, and 100 ppm, respectively, in the 15- to 30-cm depth. Prior to planting, $Ca(NO_3)_2$ (15.5N–0P–0K) was banded by hand, incorporated to a depth of 12 cm, and a light irrigation applied. Three seeds per hill were planted at 15-cm spacing, with 99 cm between rows. Two weeks after emergence, plants were thinned to one plant/hill and a population of 67,500 plants/ha was established. Seeds in the inoculation treatments were inoculated at planting with a peat-based cowpea 'EL' mixed strain inoculant (10^{10} cells/g) (The Nitragin Co., Milwaukee, Wis.). The plots were sprinkler-irrigated as needed. Weed control was not practiced, since fumigation effectively controlled weeds.

Seven weeks after planting, when the nitrogenase activity was high (24), two plants from each plot were harvested and the N_2 fixation variables, nitrogenase activity by C_2H_2–C_2H_4 assay (25), nodule number and dry weight per plant, and dry weight and percentage of N of the plant tops, were measured. Twelve weeks after planting, a 3-m sample of plants was cut at the soil level and the yield components, number of plants in 3-m plot, fresh and dry (70°C) weight of plant tops, and seed yield per plot (air-dried) were measured. Harvest index (seed yield/biomass yield) was derived from these data.

In order to satisfy normality assumptions, the data were transformed to either $\log_{10}$ or square root and were analyzed by multivariate and univariate analysis of variance. The differences among treatments were tested by a LSD test using the Bonferroni criterion for overall significance, which takes into account the number of tests performed on the data. The treatment means were retransformed to the original scale as reported herein.

Because multiple measurements were taken from each experimental unit, data were analyzed by using multivariate analysis of variance (MANOVA) models. The MANOVA test statistics indicated that all 3 main effects, cultivar (C), inoculation (I), and fertilizer N levels (F) were highly significant at the 0.0001% level. However, among the interactions, only I × F was significant at the 0.05% level. All other two-factor interac-

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Table 1. Cultivar means for N_2 fixation variables seven weeks after planting and yield variables at harvest, 12 weeks after planting.

Cultivar	Nodule (no./plant)	Nodule wt (g/plant)	Nitrogenase activity (μmol C_2H_4/plant per hour)	Shoot dry weight (g/plant) at flowering	N (%)	Seed yield ($kg \cdot ha^{-1}$)	Biomass yield ($kg \cdot ha^{-1}$)	Harvest index
California Blackeye No. 5	57 a[z]	0.81 a	64.2 a	36.5 a	4.0 a	1604 a	5960 a	0.22 b
Brown Crowder	33 bc	0.67 b	43.3 b	32.0 a	3.9 a	1104 b	5122 a	0.17 c
Tennessee White Crowder	37 b	0.59 b	38.1 b	25.2 b	4.2 a	1406 a	4302 b	0.24 ab
Lady	29 c	0.46 c	38.0 b	29.8 ab	4.0 a	1570 a	4727 b	0.26 a
Mississippi Silver	17 d	0.34 d	23.8 c	32.4 a	3.8 a	1510 a	4336 b	0.26 a
Non-nodulating soybean	---	---	---	18.5 [y]	4.0	---	---	---

[z]Means separated by LSD, $P = 5\%$, using the Bonferroni criterion.
[y]Indication of plant available N in the soil.

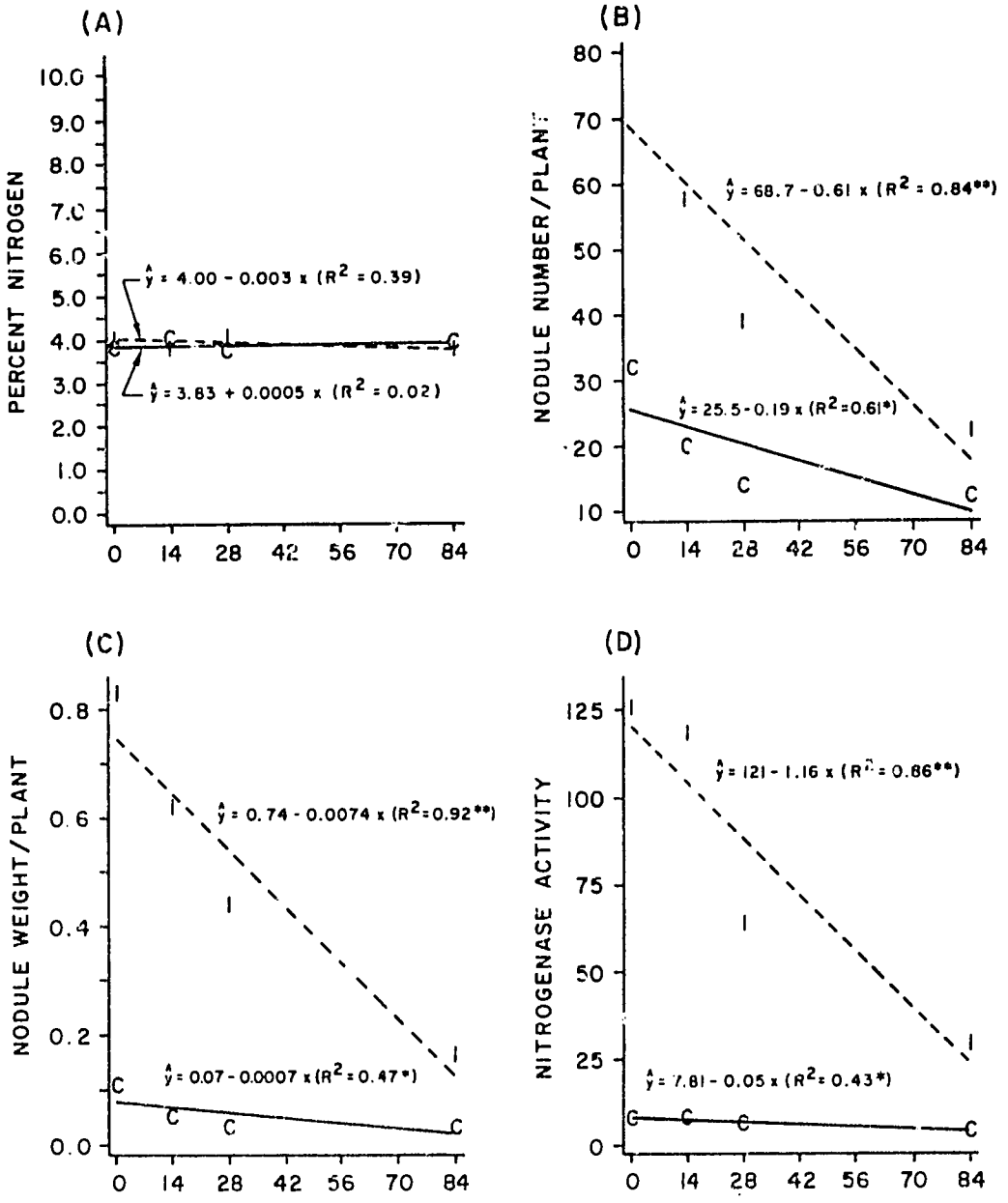

Fig. 1. The interaction between rhizobial inoculation and four levels of fertilizer N ($kg \cdot ha^{-1}$) on N_2 fixation variables [percentage of N (A), nodule number/plant (B), nodule weight (g/plant) (C), nitrogenase activity (μmol C_2H_4/plant per hour) (D)] in field-grown cowpea, where C = control and I = inoculation.

tions (I × C and C × F) and the three-factor interaction (I × C × F) were not significant. Hence, treatment comparisons were carried out for cultivars and fertilizer levels within inoculation treatments. The significance of linear and quadratic effects of fertilizer levels was also tested.

N_2 fixation variables. Because the C × F interaction was not significant, the cultivar means were computed across fertilizer levels. High-fixing cultivars, California Blackeye No. 5, Brown Crowder, and Tennessee White Crowder, produced more and larger nodules than the low-fixing cultivars, Lady and Mississippi Silver, in the inoculated, zero-

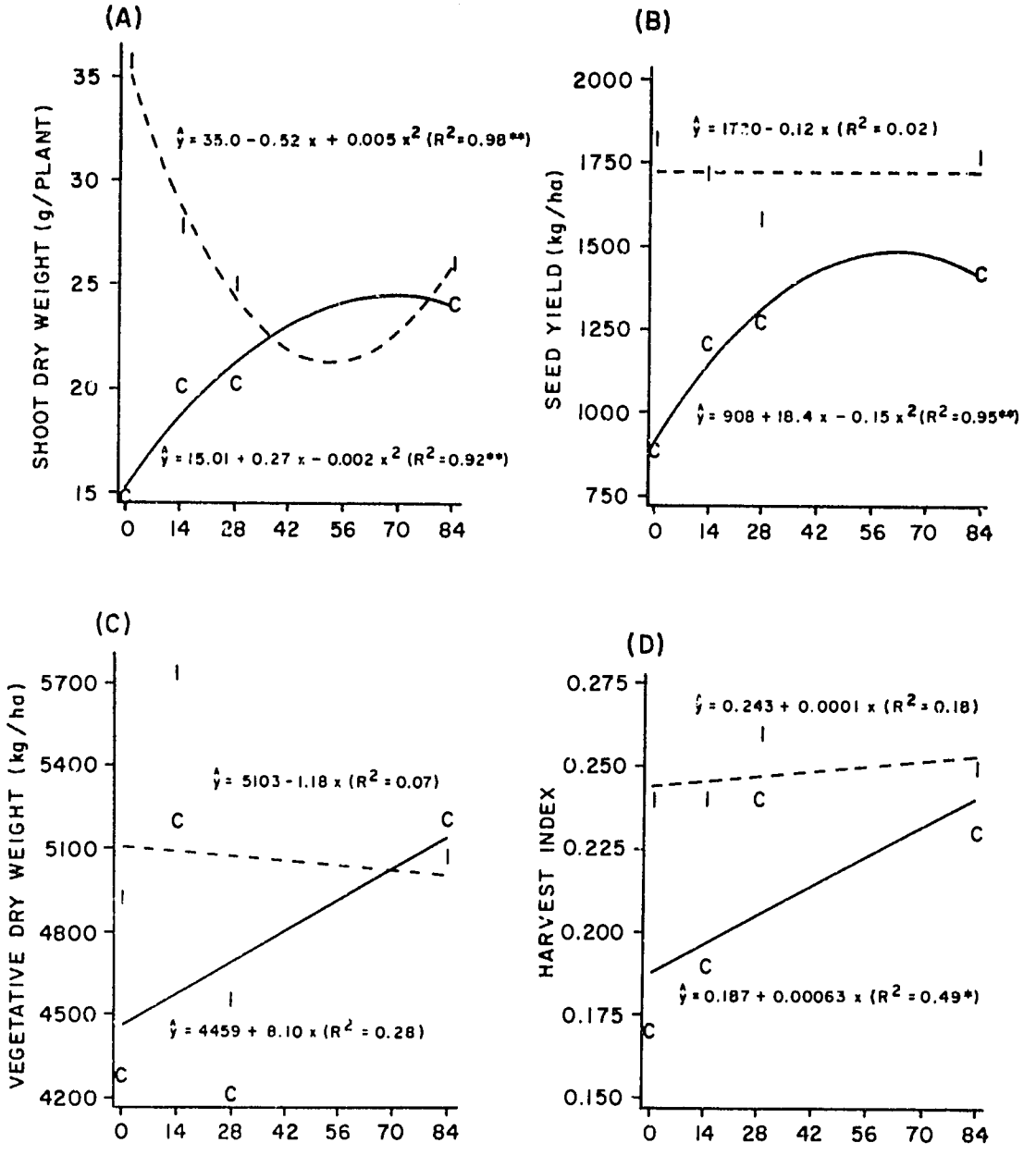

Fig. 2. The interaction between rhizobial inoculation and four levels of fertilizer N (kg·ha⁻¹) on yield variables [shoot dry weight at flowering (g/plant) (A), seed yield (kg·ha⁻¹) (B), vegetative dry weight at maturity (kg·ha⁻¹) (C), harvest index (D)] in field grown cowpea, where C = control and I = inoculation.

fertilizer N treatment (Table 1). 'California Blackeye No. 5' showed the highest nitrogenase activity, even when compared to the other two high-fixing cultivars. Shoot dry weight of the non-nodulating soybean was low, confirming the low levels of soil N and indicating the negligible contribution of soil N to plant growth. However, the low-fixing cultivars produced shoot dry weight and total N similar to that produced by the high fixers (Table 1). This indicated that differential responses in N_2 fixation measurements, i.e., nodulation and nitrogenase activity at the time of flowering, were not related to shoot dry weight and total N content of the plant.

Similarly, poor correlation between N_2 fixation measurements and shoot dry weight or total N content, the product of shoot dry weight and percent N of the tissue, in cowpea at flowering has been reported in field studies (14, 15). However, in this study, the percentage of N in the tissue was, in most cases, less variable than shoot dry weight among cultivars and treatments (Table 1; Figs. 1 and 2). Similar results have also been reported in other legumes (12, 13). Shoot dry weight is influenced primarily by several morphological, physiological, and nutritional aspects of plant growth and development. Therefore, the direct impact of N_2 fixation on shoot dry weight was not evident in cowpea at the time of flowering; however, the beneficial effects of N_2 fixation on seed yield were expressed at maturity. Variation in location, season, and experimental error might also contribute substantially to the lack of correlation between N_2 fixation and total N content in cowpea.

The interactions between rhizobial inoculation and four fertilizer N levels on N_2 fixation variables are illustrated in Fig. 1. Because the interactions between cultivar and the other two factors, inoculation and fertilizer levels, were not significant, the reported mean values of inoculation and fertilizer levels were averaged across cultivars. The influence of increasing fertilizer levels was tested for linear and quadratic effects in both control and inoculation treatments.

The results indicated that with zero N fertilizer, rhizobial inoculation significantly increased nodulation, nitrogenase activity (Fig. 1), shoot dry weight at flowering (Fig. 2), and therefore total N, above the control treatment. The observed nodulation in the control treatments was probably due to the presence of surviving native rhizobia after fumigation. Although 10–30 nodules/plant were observed in control plants, their weight and nitrogenase activity were proportionately lower than those of inoculated plants. This difference indicated that the surviving native rhizobial strains were not as effective as the "EL" strains for nitrogenase activity, probably due to low rhizobial population density. Nodule number, nodule weight, and nitrogenase activity differed significantly at the four fertilizer N treatments. Inoculated plants produced more dry matter at flowering

than the control treatments only at 0 and 14 kg·ha^{-1} fertilizer N, and dry matter production was not significantly increased by N fertilization above 20 kg·ha^{-1}.

Highly significant, negative linear relationships were observed for nodulation and nitrogenase activity with increasing fertilizer N levels in inoculated cowpea. Thus, no synergistic effects were observed for N_2 fixation, even at low fertilizer N levels (14 kg·ha^{-1}). Similar findings were also reported (14) where addition of NO_3-N reduced N_2 fixation in both high- and low-fixing cultivars. However, Eaglesham et al. (7) and Dart and Wildon (4) reported that addition of low levels of N had beneficial effects on cowpea and soybean. These inconsistencies may be due to differences in soil N levels, different methods of estimation and/or the use of different cultivars, rhizobial strains, and environments.

Significant, negative, quadratic relationships were observed for shoot dry matter harvested at flowering with increasing N levels (Fig. 2). There was a sharper decline in shoot dry matter with the addition of N at 14 kg·ha^{-1} than at the higher levels in inoculated plants. This was because the addition of low levels of fertilizer N up to 28 kg·ha^{-1} not only substantially reduced N_2 fixation, but also was not adequate for plant growth and development. However, in control treatments, addition of increasing levels of N significantly increased dry matter production. Therefore, the relationship between dry matter production and N levels was dependent on whether plants were inoculated.

Seed and biomass yield. No significant differences among cultivars for seed yield (except 'Brown Crowder') was observed (Table 1). Although 'Brown Crowder' is a high-fixing cultivar, its seed yield was lower than that of other high- and low-fixing cultivars in this study. 'Brown Crowder' was not fully matured by the time of harvest at 11 weeks, possibly causing the low yields. In the next season, when seed were harvested fully matured, 'Brown Crowder' outyielded all other cultivars (8). Although this response indicated that N_2 fixation potential of the cultivars was not directly related to seed yield, the high-fixing cultivars, California Blackeye No. 5 and Brown Crowder, generally produced more biomass than the low-fixing cultivars (Table 1). Therefore, the harvest index of the low-fixing cultivars was generally higher than that of the high-fixing cultivars. These results suggested that, although differential N_2 fixation potential among cultivars exists, without a concomitant increase in the "sink" potentials, pod set and development, an increase in seed yield might not necessarily be anticipated.

Inoculated plants yielded significantly more than the uninoculated plants in fumigated plots at four N levels (Fig. 2). Therefore, the fixation effects were biologically significant at all four N levels. In inoculated plants, addition of fertilizer N did not increase seed yield over and above the unfertilized plants. The rate of increase in seed yield was higher for the low N levels than the high levels.

Inoculated plants produced significantly more vegetative dry matter than the controls at low N levels (Fig. 2). At high N levels, the differences in vegetative matter production were not significant between control and inoculation treatments. Although the effect of inoculation was prominently shown in seed yield increase, this was not the case for vegetative growth. Harvest index (HI) is a ratio between seed and biomass yield. Therefore, an increase in HI is accompanied by an increase in seed yield. The HI of inoculated, unfertilized plants was significantly larger than the control (Fig. 2), because inoculation increased seed yield in these plants.

In conclusion, seed yield of indeterminate cowpea cultivars was greater in rhizobia-inoculated plants than in the noninoculated fumigated control. Addition of fertilizer N at planting to inoculated cowpea plants did not increase N_2 fixation or seed yield. Therefore, it might be more beneficial to inoculate cowpeas with a suitable inoculum rather than applying costly fertilizer N. A significant linear increase in seed and biomass yield was obtained with increasing N levels in noninoculated plants. Nitrogen fixation potential of host genotypes did not directly influence seed yield in cowpea cultivars; however, more vegetative matter or forage was produced in high-fixing than in low-fixing cultivars. Although differential N_2 fixation potential among cowpea cultivars exists, increases in seed yield might not be realized without an increase in sink potentials.

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