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Field Evaluation of Selected Rhizobium in an Improved Legume Inoculant[1]

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ABSTRACT

Increased grain legume production depends on effective symbiotic dinitrogen fixation through successful legume inoculation. Inoculants containing high numbers ($\geq 10^7$/g) of effective *Rhizobium* must withstand adverse field conditions. Field studies were performed to determine the effects of selected rhizobia in two different inoculant carriers on nodulation and performance of three grain legumes. The soil at the 1979 site (Vertic Haplaquept) contained $> 10^3$ cowpea *Rhizobium* and $> 10^2$ *Rhizobium phaseoli* per g of dry soil. The soil at the 1980 site (Typic Paleudalf) contained $< 10^2$ *R. phaseoli* per g of dry soil. Selected strains of *R. phaseoli* and cowpea *Rhizobium* were incorporated in peat or vegetable oil. Seeds of bean (*Phaseolus vulgaris* L.), cowpea [*Vigna unguiculata* (L.) Walp.], and peanut (*Arachis hypogaea* L.) were inoculated (ca. 10^8 *Rhizobium*/seed) and planted. Nodulation, plant growth, and yields were determined in all experiments. Strains of *Rhizobium* in oil-base inoculants generally formed a greater percentage of nodules on plants than did the same strains applied in peat-base inoculants. *Rhizobium phaseoli* 971A occupied 93% of bean nodules when applied as oil-base inoculant but only 82% when applied as peat-base inoculant. Fresh weight bean yields of 5,620 kg/ha were obtained with strain 971A (oil-base) compared to 3,370 kg/ha for the peat-base treatment. Similar significant yield increases were obtained for cowpeas and peanuts inoculated with oil-base inoculants. These experiments indicate that an improved inoculant provided high numbers of effective *Rhizobium* at planting. Through subsequent effective nodulation, oil-base inoculants increased yields and nitrogen fixation by the legumes due to increased nodulation by the superior N_2-fixing strains of *Rhizobium*.

Additional index words: **Inoculation, Intrinsic antibiotic resistance, Legumes, N_2-fixation, Nodulation, Oil-base inoculant.**

GRAIN legume production is dependent on an adequate supply of N that can be obtained from symbiotic dinitrogen fixation in the presence of effective native rhizobia or through inoculation. In many tropical and subtropical countries, as well as the southeastern United States, nodulation and increased grain production by leguminous crops are often limited by factors affecting the legume-*Rhizobium* symbiosis.

Rhizobium compatible with specific legumes may be absent from soil, especially if legumes are grown for the first time. Inoculation with *Rhizobium* can alleviate this problem and spectacular yield increases are often obtained. Inoculation of peanuts (*Arachis hypogaea* L.) grown in Lakeland fine sand with no history of peanut production resulted in a 92% yield increase compared to uninoculated plants (13). Other investigators have reported significant yield increases with inoculation on rhizobia-free soil for peanuts (1, 21, 22, 24) and soybeans [Glycine max (L.) Merr.] (19, 30).

Soils often contain rhizobia that are highly competitive against those applied in inoculants. Selected strains of peanut *Rhizobium* failed to increase yields in the presence of high populations of indigenous rhizobia (5, 26). Similarly, commercial soybean inoculants failed to increase seed yields on soils routinely cropped to soybeans (18). The studies concluded that inoculation was unnecessary under the experimental conditions since the native rhizobia appeared to be supplying adequate N for peanut and soybean growth. However, indigenous rhizobia may be ineffective in N_2-fixation. Gaur et al. (8) reported low peanut yields in India occur because of high soil populations of ineffective cowpea rhizobia. Inoculation with selected strains of peanut *Rhizobium* usually failed to elicit a yield response due to poor competition with soil rhizobia. Inoculation of legumes on soils containing ineffective rhizobia has been successful when high numbers ($\geq 10^8$ cells/seed) of selected rhizobial strains are applied using special inoculation methods (20).

Inoculants available commercially in the southeastern USA vary in numbers and effectiveness of rhizobia supplied. In Louisiana, use of commercial inoculants generally failed to increase soybean nodulation, N_2-fixation, or seed yield (6). Hiltbold et al. (14) reported that strains and numbers of rhizobia and type of carrier can account for wide variations in effectiveness of different inoculants in field studies conducted in Alabama. Skipper et al. (25) obtained similar results in greenhouse evaluations of commercial inoculants marketed in South Carolina and Georgia.

Adverse environmental conditions deteriorate inoculant quality. Hot and dry conditions at planting can cause rapid decreases in rhizobia applied to seeds (11, 23). Successful inoculation requires carriers capable of delivering high numbers of effective rhizobia under adverse conditions to insure nodulation of the host legume.

An improved inoculant was developed that enhanced rhizobial survival on seeds and promoted effective nodulation under laboratory and greenhouse conditions (16). Field evaluations were required to verify the effectiveness of the improved inoculant. The objectives of this study were to determine the effects of inoculation with selected strains of *Rhizobium* in two carriers on nodulation, N_2-fixation, and yields of three grain legumes under field conditions.

MATERIALS AND METHODS

Effects of inoculation on growth, N_2-fixation, and yield were determined in field studies conducted in 1979. Host legumes included bush bean (*Phaseolus vulgaris* L. 'Commodore'), cowpea [*Vigna unguiculata* (L.) Walp. 'Mississippi Silver'], and peanut (*Arachis hypogaea* L. 'Florunner'). The field studies were conducted at the Plant Science Research Center near Starkville, Miss. The soil was Leeper loam (Vertic Haplaquept, fine, montmorillonitic, nonacid, thermic) with a pH of 7.7 and a NO_3^-N content of 13 ppm. The site was cropped to grain sorghum (*Sorghum vulgare* L.) the previous year. In May, 225 kg/ha of sulfate of potash magnesia fertilizer (18% K, 14% S, 11% Mg) were broadcast and incorporated. Benefin (*N*-butyl-*N*-ethyl-α,α,α,-trifluoro-2,6-dinitro-*p*-toluidine) was preplant incorporated at 540 g/ha (a.i.) for weed control. In 1980, a bush bean study was conducted on Atwood silt loam (Typic Paleudalf, fine-silty, mixed, thermic) near Louisville, Miss. Soil pH was 5.5 and initial NO_3^-N content was 6 ppm. A 10-year, unfertilized stand of Bahiagrass (*Paspalum notatum* Flügge) was removed by mowing prior to seedbed preparation. Basic slag (1.3% P, 60% $CaCO_3$ equivalent) and sulfate of potash magnesia were applied at 2,040 and 670 kg/ha, respectively.

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Addition of basic slag was sufficient to raise soil pH to 6.3. Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) (granular formulation; 900 g/ha a.i.) was broadcast and incorporated with the fertilizer amendments during seedbed preparation.

The numbers of rhizobia at the field sites were determined by most probable number (MPN) procedures (27). Serial 10-fold dilutions were prepared from composite soil samples for each site. Bean or cowpea seeds, surface sterilized with H_2O_2 (27), were inoculated with 1 ml from appropriate dilutions and grown in plastic growth pouches (28). Peanuts were grown in sand-vermiculite mixtures in 473-cc pots (five replications/dilution). The frequency of positive nodulation among pouches at each dilution provided the most probable number (MPN).

Three highly effective strains each of *R. phaseoli,* cowpea *Rhizobium*, and peanut *Rhizobium* were used for inoculation. The strains were cultured in yeast extract-mannitol (YEM) broth (27). Peat-base inoculants were prepared by inoculating sterile peat with broth cultured cells of a selected strain. Broth cultures were also concentrated by centrifugation and lyophilized as previously described (16). Oil-base inoculants were prepared by suspending the lyophilized cells of an inoculant strain in peanut oil. Peanut oil was previously rendered free of rhizobia and water by heating at 120C for 12 hours. The peat-base and oil-base inoculants contained approximately 10^{10} cells/g and 10^{11} cells/ml, respectively. Inoculants were applied to seed in amounts calculated to yield about 10^8 rhizobia/seed. Seeds were inoculated immediately before planting. The seeds were placed on a sheet of hardware cloth followed by application of the appropriate amount of inoculant. Seed inoculation was accomplished by a gentle rolling motion of seed plus inoculant forward and backward on the hardware cloth. Seeds were initially moistened with water to adhere peat-base inoculants. Oil-base inoculated seeds were coated with finely ground charcoal to adsorb excess oil.

In 1979, peanuts, bush beans, and cowpeas were planted on 25 and 26 May, and 8 June, respectively. Randomized complete-block designs were employed with treatments replicated six times in plots consisting of four rows (4.5 m long with 91-cm row widths). Uninoculated controls without fertilizer N and N controls receiving 250 kg/ha of N as NH_4NO_3 were included. The N control plots were sidedressed with 125 kg/ha of N at 4 and 7 weeks after planting. Each block was bordered on either side with four border rows and was separated from adjacent blocks by a 3-m wide disked alley. The 1980 experiment was planted 9 May in plots 4.8 m long with row widths of 86 cm. The alleys were planted with 'Commodore' bush beans.

Peanut and cowpea were planted at a rate of 10 seeds/m and bush bean was planted at a rate of 13 seeds/m. Inoculated seeds were planted with a four-row planter modified to accommodate small lots of seed required for the plots. Planter boxes were disinfected with 70% ethanol after planting each plot. In addition to preplant herbicides, weeds were controlled both by hand-hoeing and mechanical cultivation during the growing season. Insects were controlled by periodic foliar spraying with carbaryl (l-napthyl *N*-methylcarbamate) insecticide.

Plant samples of each legume were taken at the full-bloom stage. Sampling was performed by excavating the whole root systems of four to six plants from the outer two rows of each plot. Plants were separated into shoot and root components. The roots were immediately placed in 473-cc glass jars, sealed with lids fitted with rubber septums and placed in soil until N_2-fixing activity could be determined. Nitrogen fixing activity was determined by the C_2H_2 reduction method (12). In 1979, the jars containing the roots were transported to the laboratory where the atmosphere of each was evacuated with a vacuum pump and replaced by injection with 10% C_2H_2 in an argon:oxygen (80:20) gas mixture. In 1980, this procedure was performed in the field before transport to the laboratory. The jars were gently shaken and allowed to incubate approximately 1 hour. Roots incubated in the absence of C_2H_2 were included as a check on background ethylene (C_2H_4) evolution. After incubation, a 250-μl sample was removed from each jar and analyzed for C_2H_4 with a Perkin-Elmer gas chromatograph by thermal conductivity. A stainless steel column packed with Porapak Q-T was used to separate C_2H_4 from C_2H_2. Peak areas were used to determine C_2H_2 and C_2H_4 concentrations based on standards prepared for each gas. Acetylene was used as an internal standard to quantitate the amount of C_2H_4 produced. After assaying, nodules were removed from the roots and fresh weight determined. Specific nitrogenase activity was expressed as μM C_2H_4 produced/g nodules/hr.

Shoots and roots free of nodules were dried at 60 C and weighed. The dried plant material was ground in a Wiley mill for total N analysis. Approximately 250 mg of the ground material were analyzed for total N by the tube digestion (17) and ammonia electrode (2) methods. Statistical comparisons for shoots and roots exhibited similar trends as those based on shoots plus roots, therefore, the results are presented as dry weight and N content of whole plants.

The identity of inoculant strains in the nodules was determined by testing for the characteristic intrinsic antibiotic resistance patterns previously reported (15). The tests were performed on subsamples of 20 nodules from a composite nodule sample of each plot. The nodules were surface-sterilized in 0.1% $HgCl_2$, crushed, and streaked on YEM agar plates (27). Isolates obtained were inoculated on YEM agar plates with and without appropriate antibiotic levels characteristic of an inoculant strain of rhizobia. Plates were incubated at 24 C for 4 and 8 days for *R. phaseoli* and cowpea group *Rhizobium*, respectively, prior to determining the antibiotic patterns.

Bush beans and cowpeas were harvested manually when the pods were at the full seed stage (pods containing green seeds that almost fill the pod cavity). All plants in a 3.05-m length of the two middle rows of each plot were harvested at 3- to 5-day intervals over a 3-week period. At each harvest fresh weights of bush bean pods were determined, dried and subsamples analyzed for total N. At each cowpea harvest, whole pods were weighed after which the pods were hand-shelled for shelled pea fresh weight determinations. The shells and shelled peas were then dried and analyzed for total N.

The two middle rows (4.57 m-long) of each peanut plot were harvested 25 October using a tractor pulled digger-shaker-inverter (Paulk Model 2200). After curing, whole pods were harvested with a peanut harvester (Lilliston Model 6000). Foliage samples were collected for moisture and total N determinations. Whole pods were weighed and yields were expressed on 10% moisture basis. Shelling percentages were determined (weight of 100 kernels/weight of 100 whole pods $\times$ 100). Kernels and shells were dried and analyzed for total N.

RESULTS AND DISCUSSION

Plate counts and MPN analyses performed on samples of unsown, inoculated seed retained after planting were closely correlated ($r = 0.91$) and indicated lower than expected numbers of rhizobia on seed inoculated with peat-base preparations (Table 1). Exposure to high temperatures at planting may have been more detrimental to rhizobia in the peat-base treatments compared to oil-base treatments. Previous work (11, 23) similarly demonstrated the detrimental effects of adverse conditions prevalent during planting on rhizobia in peat-base or liquid cultures applied to seeds. Oil-base inoculants may provide a protective environment which minimizes exposure of rhizobia to heat and dessication and enhances rhizobial survival (16).

Nodule typing by intrinsic antibiotic resistance indicated that nodule occupancy by selected rhizobia in oil-base inoculants was equal to or higher than that exhibited

Table 1. Effect of inoculant carrier and inoculation rate on recovery of inoculant strains from host plant nodules by intrinsic antibiotic resistance.

Host plant	Inoculant carrier	Strain	Inoculation rate: Plate count	Inoculation rate: MPN	Recovery of inoculant strain
			log rhizobia/seed		%
Cowpea	Oil	CB756	8.00 b*	8.14 a	71.6 c
		CV030	8.20 ab	8.21 a	90.0 a
		CV041	8.54 a	8.58 a	90.0 a
	Peat	CB756	7.00 c	6.84 b	62.5 d
		CV030	7.18 c	6.97 b	76.6 bc
		CV041	7.40 b	7.20 b	82.5 ab
	Uninoculated		--‡	--‡	3.3 e†
Peanut	Oil	CA22	8.70 a	8.22 a	80.8 ab
		C811K	8.40 a	7.96 a	84.2 a
		C870B	8.60 a	8.45 a	81.6 a
	Peat	CA22	7.18 b	7.06 b	73.3 bc
		C811K	7.48 b	6.76 b	71.6 c
		C870B	7.30 b	6.38 b	65.8 c
	Uninoculated		--§	--§	12.5 d†
Bean (1979)	Oil	B84	8.40 a	7.74 a	74.8 a
		931 A	7.60 b	7.54 ab	64.0 a
		971A	7.88 ab	7.63 a	41.6 b
	Peat	B84	7.18 c	5.90 d	36.0 b
		931A	7.60 b	6.74 bc	66.6 a
		971A	7.40 bc	6.43 c	36.0 b
	Uninoculated		--¶	--¶	0.0 c†
Bean (1980)	Oil	B84	8.54 ab	8.38 a	75.8 c
		931A	9.30 a	8.58 a	86.6 ab
		971A	8.18 b	6.97 c	93.3 a
	Peat	B84	8.30 b	7.97 b	72.5 c
		931A	8.00 b	6.97 c	78.8 bc
		971A	8.18 b	8.21 ab	82.0 bc
	Uninoculated		--#	--#	8.8 d†

* Means for each host plant followed by a common letter do not differ ($p = 0.05$) according to Duncan's Multiple Range Test.
† Percentage of soil rhizobia with antibiotic resistance similar to any of the inoculant strains.
‡ Soil contained 3.48×10^3 cowpea rhizobia per g soil.
§ Soil contained 1.61×10^3 peanut rhizobia per g of soil.
¶ Soil contained 2.00×10^2 *R. phaseoli* per g soil.
\# Soil contained 3.10×10^1 *R. phaseoli* per g soil.

by rhizobia in peat-base inoculants (Table 1). Recovery of both cowpea and peanut *Rhizobium* was higher with oil-base inoculated seeds ($\geq 10^8$ rhizobia/seed) than seeds inoculated with peat-base cultures (ca. 10^7 rhizobia/seed). Low recovery of *R. phaseoli* inoculant strains in 1979, except for strain B84 in the oil-base inoculant, may have been due to effects of saturated soil experienced during the growing season. Loss of nodules under excessively wet field conditions can occur because of limited soil oxygen (4). Recovery of *R. phaseoli* inoculant strains from nodules of beans grown in 1980 was directly related with numbers of viable *R. phaseoli* per seed. In general, higher numbers of rhizobia provided by oil-base inoculants at planting contributed to the higher nodule occupancy by the applied strains. High inoculation rates are reported to result in increased recovery of inoculant rhizobia in nodules (3, 29). High recovery of inoculant strains of both cowpea and peanut *Rhizobium* in the presence of $>10^5$ indigenous rhizobia per gram soil indicated that inoculant rhizobia were highly competitive in the infection-nodulation process.

Plant dry matter production of cowpeas at full-bloom (45 days after planting) was increased (nonsignificantly) with all inoculation treatments (Table 2). A significant ($p=0.05$) increase in plant dry matter occurred with peat-base inoculant containing strain CVO41. Plant total N

Table 2. Effect of inoculation on dry matter yield, nodulation, and N_2-fixation of Mississippi Silver cowpea at full bloom.

Inoculant carrier	Strain	Plant dry matter	Plant total N	Nodule mass	Specific activity
		g/plant	mg/plant	g/plant	$\mu M\ C_2H_4$/g/hr
Oil	CB756	15.8 b†	367 bc	2.00 b	79.0 a
	CV030	14.8 b	406 ab	2.11 b	68.8 ab
	CV041	16.4 b	443 ab	1.82 bc	77.0 ab
Peat	CB756	15.4 b	380 bc	1.98 b	72.0 ab
	CV030	12.9 b	372 bc	1.39 cd	68.0 ab
	CV041	20.1 a	494 a	2.65 a	82.2 a
Nitrogen control		16.1 b	376 bc	1.43 cd	67.9 ab
Uninoculated control		12.5 b	297 c	1.24 d	56.2 b

† Means within a column followed by a common letter do not differ ($p = 0.05$) according to Duncan's Multiple Range Test.

Table 3. Effect of inoculation on total yield and N content of Mississippi Silver cowpea.

Inoculant carrier	Strain	Fresh wt. yield: Whole pods	Fresh wt. yield: Shelled peas	N content†: Shells	N content†: Peas	N content†: Total
		kg/ha				
Oil	CB756	11,300 ab‡	5,950 a	13.4 a	92.8 a	106.2 a
	CV030	11,100 ab	5,900 a	11.6 e	78.7 b	90.3 b
	CV041	11,900 ab	5,670 ab	13.2 ab	78.1 b	91.3 b
Peat	CB756	9,800 c	4,980 bc	12.5 bc	74.4 bc	86.9 bc
	CV030	10,200 bc	5,470 ab	12.6 bc	78.3 b	90.9 b
	CV041	9,600 c	5,010 bc	10.4 f	73.5 cd	83.9 c
Nitrogen control		12,400 a	5,040 bc	11.8 de	76.3 bc	88.1 bc
Uninoculated control		10,700 bc	4,680 c	12.4 cd	71.5 c	83.9 c

† Nitrogen content expressed on dry weight basis.
‡ Means within a column followed by a common letter do not differ ($p = 0.05$) according to Duncan's Multiple Range Test.

was significantly increased by inoculation with oil-base inoculants and with strain CVO41 in the peat-base carrier as compared to uninoculated controls. The increase in plant total N with peat-base inoculant containing strain CVO41 was higher than that obtained with N-fertilized controls. Nodule mass per plant was increased by inoculation except with one peat-base treatment. Acetylene reduction activity was significantly increased by strain CB756 in the oil-base inoculant and by strain CVO41 in the peat-base inoculant.

Harvest data shown in Table 3 indicate that oil-base inoculants significantly increased the yield of edible, shelled peas and total N content. Strain CB756 was consistent in producing the highest N content for pods and peas. Conversely, strain CVO41 in the peat-base inoculant, which occupied a high proportion of nodules and promoted the highest dry matter accumulation at full bloom, failed to produce similar increases in yield (fresh weight) and total N content. Other investigators have reported high nodule occupancy by introduced rhizobia during early crop growth without subsequent yield increases (5, 26, 29). Indigenous rhizobia may be as effective as the inoculant strain in supplying N to the plant under certain field conditions. Van der Merwe et al. (26) suggested that nodule occupancy by some inoculant rhizobia decreases after flowering and results in no yield response due to inoculation.

Peanut plant dry matter, total N content, and nodule mass at full-bloom (50 days after planting) were significantly increased with oil-base and peat-base inoculants

(Table 4). Acetylene reduction activity was significantly higher for plants inoculated with oil-base inoculants containing strains CA22 and C870B and the peat-base inoculant containing strain CA22.

Oil-base inoculants were effective in significantly increasing whole pod and kernel yields of peanuts (Table 5). Yield increases with the peat-base inocula were not statistically significant. Foliage yields were variable among treatments with strain C870B resulting in both the highest and lowest yields when inoculated as peat-base and oil-base inoculants, respectively. Nitrogen content of kernels and total N produced per hectare were significantly increased by inoculation. Inoculation treatments were also somewhat more efficient in partitioning N in that 60% of the total N produced was accumulated in the kernels for inoculated plants compared to 54% for uninoculated plants.

Nitrogen fixation parameters of bush beans at full-bloom (40 days after planting) in 1979 were not increased significantly by any inoculation treatment. Samples were taken shortly after plants experienced prolonged waterlogged conditions and developed rhizoctonia root rot which probably affected these results and also contributed to limited and variable fresh pod yields. Therefore, only the 1980 results are presented (Table 6). Inoculation increased both plant dry matter and total N of bush bean plants sampled at full-bloom (44 days after planting). Very few nodules formed on plants in either of the control plots. Strain 971A applied in the oil-base inoculant significantly increased nodule mass and acetylene reduction compared to all other treatments.

Total fresh pod yields increased significantly when seeds were inoculated with oil-base preparations of strains 931A and 971A and with peat-base preparations of strains B84 and 931A (Table 7). These increases were not statistically different from those obtained for plots receiving 250 kg/ha of combined N fertilizer. The same treatments also increased total N present in the pods.

Cowpea and peanut plants inoculated with selected *Rhizobium* strains generally produced more dry matter, contained more total N, and produced higher seed yields than uninoculated plants. Selected rhizobia inoculated on grain legumes often fail to increase yields due to inability to compete with indigenous rhizobia (5, 8, 26, 30). Significant yield increases by inoculant strains can occur when soils contain a highly ineffective population of rhizobia (3, 10). Elkan et al. (7) reported that inoculation of peanut with selected strains of peanut *Rhizobium* resulted in increased nodulation and N_2-fixation on soils previously cropped to peanut. However, the method of strain identification in nodules was not reported and pod yields and plant dry weights were not measured.

Table 4. Effect of inoculation on dry matter yield, nodulation, and N_2-fixation of Florunner peanut at full bloom.

Inoculant carrier	Strain	Plant dry matter	Plant total N	Nodule mass	Specific activity
		g/plant	mg/plant	g/plant	μM C_2H_4/g/hr
Oil	CA22	20.0 abc†	414 ab	1.61 c	178 a
	C811K	23.0 ab	455 a	2.36 a	126 cd
	C870B	23.2 ab	418 ab	1.57 c	164 a
Peat	CA22	16.6 cd	304 c	1.40 cd	150 abc
	C811K	24.2 a	458 a	2.01 b	123 cd
	C870B	19.2 bcd	363 bc	1.60 c	140 bcd
Nitrogen control		18.0 cd	338 bc	1.08 de	124 cd
Uninoculated control		15.2 d	286 c	0.92 e	108 d

† Means within a column followed by a common letter do not differ (p = 0.05) according to Duncan's Multiple Range Test.

Table 6. Effect of inoculation on dry matter yield, nodulation and N_2-fixation of Commodore bush bean at full bloom (1980 field study).

Inoculant carrier	Strain	Plant dry matter	Plant total N	Nodule mass	Specific activity
		g/plant	mg/plant	g/plant	μM C_2H_4/g/hr
Oil	B84	6.04 c†	144 bcd	0.34 b	57.5 b
	931A	6.01 c	146 bcd	0.46 b	46.0 b
	971A	8.45 a	213 a	0.59 a	92.8 a
Peat	B84	5.78 cd	135 cd	0.37 b	54.8 b
	931A	7.08 bc	178 ab	0.34 b	44.7 b
	971A	6.34 c	160 bc	0.40 b	57.0 b
Nitrogen control		7.85 ab	125 de	--	--
Uninoculated control		4.86 d	94 e	--	--

† Means within a column followed by a common letter do not differ (p = 0.05) according to Duncan's Multiple Range Test.

Table 7. Effect of inoculation on total pod yield and N content of Commodore bush bean (1980 field study).

Inoculant carrier	Strain	Fresh wt	Total N†
		kg/ha	
Oil	B84	3,220 c‡	74 b
	931A	4,680 ab	110 a
	971 A	5,620 a	130 a
Peat	B84	4,750 ab	126 a
	931A	5,050 a	122 a
	971A	3,370 bc	82 b
Nitrogen control		4,650 ab	113 a
Uninoculated control		2,980 c	70 b

† Total N expressed on dry weight basis.
‡ Means within a column followed by a common letter do not differ (p = 0.05) according to Duncan's Multiple Range Test.

Table 5. Effect of inoculation on yield and N content of Florunner peanut.

Inoculant carrier	Strain	Pod yield	Kernel yield	Shelling %	Foliage yield	Shell N	Kernel N	Foliage N	Total N
		kg/ha			kg/ha				
Oil	CA22	3,490 ab†	2,720 a	78.1 a	7,640 ab	5.6 a	98.4 ab	75.4 ab	174 a
	C811K	3,400 abc	2,610 a	76.6 ab	6,700 bc	6.1 a	97.9 ab	67.7 bc	172 a
	C870B	3,680 a	2,580 a	77.2 ab	6,000 c	6.2 a	103.2 a	56.0 cd	165 ab
Peat	CA22	3,120 cd	2,400 ab	76.4 ab	5,850 c	5.6 a	86.7 b	52.5 d	144 bc
	C811K	3,150 bcd	2,440 ab	77.4 ab	6,140 c	5.3 a	93.5 ab	59.4 cd	158 ab
	C870B	3,000 cd	2,520 ab	75.4 b	8,360 a	7.2 a	93.8 ab	81.7 a	182 a
Nitrogen control		3,380 abc	2,580 a	76.3 ab	7,220 ab	5.9 a	94.0 ab	73.2 ab	173 a
Uninoculated control		2,860 d	2,180 b	76.3 ab	6,840 bc	5.2 a	70.2 c	55.0 cd	130 c

† Means within a column followed by a common letter do not differ (p = 0.05) according to Duncan's Multiple Range Test.

Yield data obtained illustrate that grain legume production can be significantly increased through inoculation. Field response to inoculation by *Phaseolus vulgaris* cultivars is variable and inoculation failures are common (9). A fresh pod yield of 5,620 kg/ha by inoculation with an oil-base preparation of strain 971A compared to 2,980 kg/ha for uninoculated plants indicates that such barriers can be overcome through the use of highly effective strains. In addition to increased plant yields, increased production of N in harvested plant parts demonstrates the potential contribution to increased protein production by legumes properly inoculated with efficient strains of rhizobia.

We have developed an oil-base inoculant which provides high numbers of lyophilized rhizobia ($>10^7$/seed) at seed inoculation, effectively nodulates the host legume and generally increases pod and/or seed yields in the field. Unprotected lyophilized rhizobia applied to seeds are susceptible to desiccation and may rapidly die after inoculation (27). Inoculants containing "microdried" rhizobia plus potentially toxic micronutrients and fungicides combined in vegetable oil have been available in the USA in recent years (14, 20). The quality of these inoculants has been limited by low rhizobial numbers ($< 10^3$/seed) and poor nodulating ability (6, 14).

Further investigations on inoculation with improved inoculants are required to determine the adaptability and persistence of applied rhizobia under a wider range of soils, host legume varieties, and environmental conditions. Studies conducted on soils with highly competitive indigenous rhizobia and where the specific legume has been previously cropped are needed.

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15