Efficacy of Bacterial or Fungal Soil Amendments on Vegetables Following Peanut

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Abstract. Amendment of soil with microorganisms during the growth cycle of one crop may affect development of succeeding crops. Species of *Rhizobium* bacteria or arbuscular-mycorrhizal fungi were added alone to, or in combination with, potting soil in pots in a greenhouse. Controls were no amendments. Seed of peanut (*Arachis hypogaea* L.) were planted and two levels of a combination NPK fertilizer, the recommended and one-fourth the recommended rate, were applied. After harvest of peanut and remoistening of soil, seed of the bell pepper (*Capsicum annuum* L.) or navy bean (*Phaseolus vulgaris* L.) were sown into the same planting medium in pots without additional inoculation with microbes. Dry weights of above-ground vegetative and edible portions of crops were determined. Inoculum type only affected peanut top and total dry weights. The recommended fertilizer level did not affect peanut yield but did cause improvement in bell pepper and navy bean yield over that of the deficient fertilizer rate. In field experiments, peanut was planted into soil receiving *Rhizobium* spp. bacteria, or arbuscular-mycorrhizal fungi alone or in combination. Controls consisted of no amendment. Only the recommended fertilizer rate was used. In the next 2 years, bell pepper or navy bean were established in plots without use of additional microbial amendment. Yields and nutrient contents of crops were determined. Type of inoculum did not affect yield or nutrient content in any crop. Bell pepper marketable yield was unaffected by year, and navy bean seed yield was higher in 2004 than in 2005. In both years, navy bean yields were below U.S. averages. Concentrations of most nutrients in edible portions of bell pepper and navy bean were lower in 2004 than in 2005. Results of the field trials were generally similar to those of greenhouse studies. Use of inocula did not provide substantial benefits to yield or nutrient content of peanut or vegetable crops that followed.

Beneficial, synergistic, and detrimental relationships can develop between plants and other organisms. Detrimental relationships are responsible for reduction in quality or quantity of yield. Beneficial or synergistic relationships can mediate effects of detrimental interactions. Arbuscular mycorrhizae (AM), beneficial fungi, colonize roots of vascular plants (Singh and Adholeya, 2002). These fungi benefit plant development

through mediation of rhizosphere conditions, improved nutrient uptake, and protection from pathogenic organisms. Some rhizosphere bacteria are used in agriculture to provide benefits to plant development (Glick, 2004; Lucy and Glick, 2004; Schulze and Pöschel, 2004; Zehnder et al., 2001). *Rhizobium* sp. bacteria take nitrogen from the air and make it available to legumes. When a medium was amended with these bacteria, foliar and root disease severity was reduced in later stages of development of cucurbits (Kokalis-Burelle et al., 2003). Other N_2 -fixing bacteria, inoculated into growth media, and later found associated with stem and leaf tissue in sugarcane (*Saccharum officinarum* L.), were associated with improved plant development; and infection was greater when supplied to the medium in the presence of an AM fungus (Muthukumarasamy and Revathi, 1999). A nitrogen-fixing bacterium was found associated with field corn (*Zea mays* L.) roots (Emitiazi et al., 2003). Other free-living and endophytic N_2 -fixing bacteria do not generally reduce the need for N-fertilizer in graminaceous crops (Andrews et al., 2003).

A beneficial *Bacillus subtilis* bacterium, in transplant mixes, improved vigor of seedlings of pepper (*Capsicum* sp.), tomato (*Lycopersicon esculentum* Mill.), strawberry (*Fragaria chiloensis* var. *ananassa* Duchesne), and several cucurbits (Kokalis-Burelle et al., 1999). *Rhizobium* sp. bacteria have been reported to be associated with asparagus (*Asparagus officinalis* L.) (Shimshick and Herbert, 1979), a nonlegume, and several other nonvegetable, nonleguminous, crops (Zahir et al., 2004). *Rhizobia* bacteria may interact synergistically with mycorrhizal fungi, increase root colonization by both, and increase nutrient content in plants (Suresh and Bagyaraj, 2002).

Portions of the southern plains are undergoing transition from cow-calf operations on perennial pasture and row crops to alternative crops, including vegetables. As this transition progresses, it is necessary to determine if the use of biologic soil inoculants can provide a benefit to producers. The inoculants can be applied annually. However, it is unclear if any benefit provided by an application of inoculants in 1 year will carry forward to other crops in subsequent years. This project was undertaken to examine the efficacy of biotic amendments on yield and nutrient content of peanut (*Arachis hypogaea* L.), and bell pepper (*C. annuum* L.) or dry bean (*Phaseolus vulgaris* L.) in rotation. This was examined under greenhouse conditions using AM fungi and *Rhizobium* spp., or fertilization rate where vegetables immediately followed peanut, and in the field where peanut in the first year was followed by vegetables in the next 2 years.

Material and Methods

Greenhouse. Pots (3.5 L) were filled with a commercial potting mix (Reddi-Earth; Scotts-Sierra Horticultural Products Co., Marysville, Ohio). To some pots, 4.4 g of a mix of AM fungi (Rhizanova Flower Rx; Becker Underwood, Ames, Iowa; 4400 propagules·g⁻¹), *Glomus brasilianum* Spain & J. Miranda, *G. clarum* Nicol. & Schenck, *G. deserticola* Trappe, Bloss and Menge, *G. intraradices*, *G. monsporum* Gerd & Trappe, *G. mosseae* (Nicol. & Gerd.) Gerd & Trappe, and *Gigaspora margarita* Becker & Hall, was added to the potting mix; to others, peanut seed was coated with a mix of commercially available *Rhizobium* spp. bacteria, *R. meliloti*, and *R. leguminosarum* biovar *trifolii* (Nitragin; Milwaukee, Wisc; 200,000,000 propagules·g⁻¹); to others, the AM and *Rhizobium* spp. bacteria were added as described before; to others, no amendments were added. Seed of peanut, var. Spanco (Clint Williams Co., Madill, Okla.), were planted in pots on 10 Oct. 2001. Beginning 2 weeks after emergence, plants were thinned to one per pot and provided biweekly with 500 mL/pot of solutions containing 1.25 g·L⁻¹ (0.25 the recommended rate) or 5 g·L⁻¹ (full recommended rate) of a soluble fertilizer, 20N–20P–20K with micro-nutrients (Peters; Spectrum Group, St. Louis,

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Mo.). Fertilization was stopped ≈ 2 weeks before harvest. Irrigation was daily through individual emitters placed in each pot. There were four amendment treatments (AM alone, *Rhizobium* alone, both, neither), two fertilizer rates, and four replications for a total of 32 pots. Pots were placed on benches in a greenhouse in a randomized complete block arrangement. Air temperature was maintained at 23 ± 1 °C, and there was at least 12 h of light augmented when necessary with artificial light (Son Agro; Phillips, Sommerset, N.J.). Chemical content and pH of the greenhouse irrigation water, potting medium, and soluble fertilizer were determined by the Oklahoma State University Soil, Water & Forage Analytical Laboratory, Stillwater, Okla.

Peanuts were harvested when pods formed closest to the crown were mature. Irrigation was withheld and plants carefully removed from pots on 10 Feb. 2002 after the soil dried for several days. Vegetative portions of plant tops were separated from roots. Pods were separated from roots that were discarded. Plant tops and pods were placed in paper bags and dried in a forced-air oven at 60 °C until top dry weights stabilized or pods were $\approx 15\%$ moisture content. Dry weights of plant tops and pods were determined. Pods were graded according to USDA, AMS (1997) standards. A visual examination of roots was done to determine if nodules were present.

Potting mix in pots was remoistened. Seed of bell pepper, cv. Jupiter (Otis Twilley Seed Co., Hodges, S.C.), were planted on 21 Feb. 2002 in 16 pots. Seed of navy bean, cv. Aspen (Idaho Seed Beans, Twin Falls, Idaho), were planted at the same time in 16 pots. One week after emergence, bell peppers were thinned to one plant per pot, and navy bean were thinned to six plants in a row across the pot diameter. Navy bean plants were separated by 5 cm. For bell pepper, there were eight pots containing AM and fertilizer at the 0.25 recommended fertilizer rate, and eight pots containing AM and fertilizer at the full recommended rate. The same was true for dry bean. After emergence, fertilizer was added to pots at the same levels and frequency as before. There was no additional amendment of the soil with AM fungi or the *Rhizobium* sp. bacterium. Irrigation was as before.

The first bell pepper harvest was on 5 May 2002 when fruit reached grade US #1 (USDA, 1989). The remaining fruit were harvested as they reached US #1 grade. A final harvest on 2 June 2003 of all remaining fruit occurred when it was obvious that fruit were not going to reach US #1 grade. Numbers of fruit and average fruit dry weight were determined as described previously. Irrigation was withheld from beans after pod fill. Pods were removed from plants on 10 May 2002 by which time 80% of leaves had dropped. Shoots and leaves accumulated on the surface of the potting soil, and leaves remaining on plants were placed in paper bags. Seeds were separated from pods and placed in separate paper bags. Bags were placed in a forced-air oven at 60 °C until dry weights stabilized or seeds

were $\approx 15\%$ moisture. Roots were visibly examined for presence of nodules.

Field. The soil, a Bernow fine-loamy, siliceous thermic Glossic Paleudalf at Lane, Okla., was prepared by disking. On 19 May 2003, fertilizer to bring N-P-K levels to 20–80–200 kg·ha⁻¹, respectively, according to recommendations for the area (Sholar et al., 1996) was applied to soil and incorporated. Nitrogen was from ammonium nitrate, P was from P₂O₅, and K was from muriate of potash. Peanuts were seeded on 20 May 2003 at the rate of 67 kg·ha⁻¹ using a Monosem vacuum seeder, plate 40 55 (ATI, Inc., Lexana, Kan.). Blocks contained plots in which: 1) AM was dispensed in the furrow in advance of the seed at the rate of 7 g/linear meter, based on label recommendations, from fertilizer hoppers on the machine; 2) seeds were coated with the *Rhizobium* spp. before sowing; 3) AM and *Rhizobium* spp. were applied as in 1) and 2); and 4) controls consisted of no biologic amendments. Plots were 3.7 m wide and 90 m long with rows on 0.9-m centers oriented north to south. Plots contained only one of the treatments. Four meters separated plots. Border rows were comprised of four rows of peanut on the east and west side of the field. Plot locations were recorded by noting distances from permanent structures near field borders. The experimental design was a randomized complete block with four treatments (AM fungi alone, *Rhizobium* alone, both, neither) and three replications. Peanuts were harvested on 26 Sept. 2003, pod yields determined, and pods were graded in accordance with USDA, AMS (1997) standards. Four-kilogram subsamples were taken from each plot and seed in the subsamples were separated from pods. After harvest, a cover crop of wheat was sown uniformly over the field on 20 Oct. 2003.

On 30 Mar. 2004, the wheat was mowed before head formation and turned under. Beginning in 2004, vegetable crops were established in plots that contained peanuts in the first year and which were amended as previously described or with no amendment. Vegetable crops were the same as in greenhouse pot studies. Row spacings were as for peanut. On 10 Apr. 2004, fertilizer to bring N-P-K levels to 56–84–112 kg·ha⁻¹ for bell pepper, and on 3 May 2004, fertilizer to bring N-P-K levels to 56–28–28 kg·ha⁻¹ for navy bean, according to recommendations for the area (Motes and Roberts, 1994), was applied to the soil and incorporated. Six-week-old greenhouse grown seedlings of bell pepper were established in the field using a mechanical transplanter (Holland Transplanter Co., Holland, Mich.) in one-half of the linear length of plots on 18 Apr. 2004. In-row spacing for bell pepper was 0.5 m. Navy beans were planted on 5 May 2004 with the vacuum seeder, plate 60 45, in the other half of the linear length of the plot with in-row spacing of 5 cm between plants in rows. Plots were 3.7 m wide by 45 m long for each vegetable. Border rows for the entire plot consisted of four rows of beans on east and west sides of plots.

Bell pepper harvest, from the middle two rows in plots, was begun on 29 June 2004, and two additional harvests took place over 14 d. Numbers and weight of green marketable (US#1 at minimum) and cull fruit were determined using the same USDA grading standards used for pot experiments. All four rows in navy bean plots were harvested on 3 Aug. 2004 with a combine (Gleaner Agro Corp., Duluth, Ga.) when $\approx 80\%$ of leaves had dropped. For yield estimation, seeds were collected in burlap bags and dried to $\approx 15\%$ moisture by passing air over them. A cover crop of wheat was sown uniformly over the field on 27 Sept. 2004. The same procedures were followed in 2005 generally around the same dates as in 2004.

Nutrient content. Representative samples of US #1 grade bell pepper from each plot were washed, air-dried, cut, and the seed and pedicels discarded. Peanut and navy bean seed and bell pepper pods were freeze-dried and then ground with a Wiley mill to pass through a 20-mesh screen. Tissues (0.1 g) were extracted for analysis with flow-injection-analysis (FIA) using the methods of Jones and Case (1990). An automated ion analyzer (AIA; Model 8000, Hach, Inc., Lachat Instruments Division, Milwaukee, Wisc.) was used to determine concentrations of nitrogen in a Kjeldahl digest (TKN) and phosphorus in a Kjeldahl digest (TKP) with FIA. Other dried samples (0.1 g) were extracted with deionized water using the methods of Russo and Karmarkar (1998) for ion chromatography (IC). Analysis was with the columns on the AIA using nonsuppressed cation IC with the methods recommended by the manufacturer (Hach). For Ca, K, Na, and Mg, the eluent was 4.5 mM methane sulfonic acid with a 1.4 mL·min⁻¹ flow rate. The eluent for suppressed anion IC, using a small suppressor to measure Cl, HPO₄, NO₂, NO₃, and SO₄, was a mixture of 1.7 mM sodium carbonate and 2.8 mM sodium bicarbonate at a flow rate of 2 mL·min⁻¹. A conductivity detector was used to determine concentrations. Samples, analyzed by FIA and IC, were compared with calibration curves for each ion and reported as $\mu\text{g}\cdot\text{g}^{-1}$ of dry weight. For total protein content in peanut seed, 0.5-g samples of freeze-dried tissue were analyzed by the method of Bradford (1976). Samples were analyzed and compared with calibration curves for known amounts of protein and reported as percent. Three replicates in each sample were analyzed for concentrations of ions and protein.

The experiment was a randomized complete block with three replications for each vegetable. Data, analyzed with the GLM procedures in Statistical Analysis System (SAS, Cary, N.C., ver. 8.1), were subjected to analysis of variance. Means were separated using the Ryan-Einot-Gabriel-Welsch multiple *F* test, or when appropriate, interactions were analyzed with least-squares means.

Results and Discussion

Greenhouse. The irrigation water (pH 7) contained small amounts of N and K, no P, B,

Ca, calcium carbonate, Mg, or SO₄ and was classified as being soft (Table 1). The potting medium (pH 6.1) contributed 155 µg·g⁻¹ of N, 8.4 µg·g⁻¹ of P, 22 µg·g⁻¹ of K and B, Ca, Cu, Fe, Na, Mg, SO₄, and Zn (Table 2). The recommended rate of the Peter's soluble fertilizer in water (pH 5.9) had the advertised amounts of N, P, and K and amounts of B, Cu, Fe, Mg, and Zn (Table 2).

Inoculum type only affected top and total dry weight of peanut and no dry weight or yield components of bell pepper or navy bean, but fertilizer rate affected navy bean and bell pepper dry weight and numbers of bell pepper fruit, but not average bell pepper fruit dry weight, and there was not a significant interaction (Table 3). Tops of peanut plants inoculated with *Rhizobium* sp. were heavier than all other treatments, which were similar; seed dry weights were similar for treatments (avg. 27.7 g); total dry weights of plants inoculated with *Rhizobium* sp. were heavier than for plants inoculated with both *Rhizobium* sp. and AM; total dry weights of plants inoculated with AM, or not receiving inoculants, were similar to each other, and intermediate to plants inoculated with only *Rhizobium* sp. or with both inoculants (Table 4). Across fertilizer rates peanut plant top, edible and total dry weights averaged 106.5, 27.7, and 134.4 g per plant.

Across inocula, average bell pepper plant top, fruit, and total dry weights were 37.8, 34.2, and 72.0 g, respectively; total numbers of bell pepper fruit averaged 11/plant, and average bell pepper fruit dry weight was 3.5 g. For navy bean, over inocula, average plant top, pod, seed, and total dry weights were 24.7, 10.8, 7.0, and 42.5 g, respectively,

per pot. Higher fertilizer rate improved plant top, fruit, and total dry weights and number of fruit, but not average fruit dry weight for bell pepper (Table 5). Heavier plant tops, seed and total dry weights were produced on navy bean plants treated with the higher fertilizer rate (Table 5). It may be that conditions in the greenhouse were conducive to plant development, as indicated by the lack of difference between inoculated and control plants. Because inocula did not provide any benefit when fertilizer levels were low, the comparison of fertilizer level was not used in the field experiments.

Field. Inocula type did not affect peanut yield, avg. 2.6 Mg·ha⁻¹, or pod rating, avg. 68.

Nodules were present on roots of peanut in all plots. For bell pepper, marketable, or cull, numbers of fruit or yield were unaffected by inoculum type; with the exception of marketable yield, all other components were affected by year; and for navy bean, marketable yield was affected by inoculum type and year (Table 6). The year × inoculum type interaction was not significant. In 2004,

for bell pepper, there were fewer, heavier, marketable fruit than in 2005. Nodules were present on roots of dry beans in all plots. Bell pepper marketable yield, unaffected by treatment, averaged 10.7 Mt·ha⁻¹ over years. In 2004, there were fewer cull fruit, higher cull yield, and cull fruit were heavier than in 2005. In 2004, navy bean yield was greater than in 2005. For navy beans, marketable yields were significantly different as a result of inoculum type in the order both > none = AM > *Rhizobium* (0.15 > 0.14 = 0.13 > 0.11 Mg·ha⁻¹). Navy bean yield in both years was below what is expected in the United States and in other navy bean-producing countries. In 2005, the yield represented a failed production season with insufficient volume to justify harvest. The reason for the failure is not immediately apparent, but the significant effects of treatment on navy bean yield must be questioned.

Nutrient content. Seed nutrient content in peanut was unaffected by inoculum type. Kjeldhal N, NO₂, NO₃, Kjeldhal P, HPO₄, K, Ca, Mg, Mn, Na, and SO₄ averaged 81.5, 1.7, 0.4, 40.8, 6.6, 758.8, 18.7, 25.9, 4.5, 36.3, and 91.8 µg/100 g, respectively, and protein content averaged 6.3%. Content of nutrients in bell pepper pods were unaffected by inoculum; some nutrient levels were affected by year; and none were affected by the inoculum × year interaction (Table 7). Levels in 2004 of TKN, Mg, and SO₄ were higher than in 2005; NO₂, NO₃, TKP, Ca, and Cl were lower than in 2005; and HPO₄, K, and Na were unaffected. Content of nutrients in navy bean seed were unaffected by inoculum; some nutrient levels were affected by year; and none were affected by the inoculum

Table 4. Effect of inoculum on dry weights of tops and seed portions of peanuts for plants grown in pots in a greenhouse.

Inoculant	Dry wt (g)		
	top	seed	total
Rhizobium	130.5a ^z	32.6a	163.1a
AM	101.6b	24.4a	126.0ab
None	98.9b	31.1a	130.0ab
Both	94.9b	22.8a	117.6b

^zValues in column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple *F* test.

Table 1. Values for constituents^z of the irrigation water used in the greenhouse.

pH	Nitrate nitrogen	K	B	Ca	Cl	Na	Calcium carbonate	Alkalinity (as CaCO ₃)	Mg	SO ₄	Hardness	EC
							µg·g ⁻¹					µmhos·cm ⁻¹
7.0	<1	2	0.07	13	15	16	41	34	2	12	39 ^y	151

^zAnalyses performed by the Oklahoma State University Soil, Water & Forage Analytical Laboratory, Stillwater, Okla.

^yhardness class is soft.

Table 2. Values for constituents^z of the Sunshine potting medium and the Peter's soluble fertilizer.

Source	pH	Nitrogen		P	K	B	Ca	Cu	Fe	Na	Mg	SO ₄	Zn	EC
		ammonium	nitrate											
Potting medium	6.1	69.7	85	8.4	22	0.1	100	1.0	14.5	29	59	90	1.2	1,425
Peters	5.9	9.0	1	10.0	10	0.01	NL	0.025	0.05	NL	0.25	NL	0.025	NL

^zAnalyses performed by the Oklahoma State University Soil, Water & Forage Analytical Laboratory, Stillwater, Okla. Results for the fertilizer are for the 1 × rate in the irrigation water; divide by 4 for the 1.25 g·L⁻¹ rate. NL = not listed.

^yTo convert µg·g⁻¹ to %, divide by 10,000.

Table 3. ANOVA table for effects of inoculum and fertilizer rate on dry weights of tops and edible portions of bell pepper and navy bean, and number of bell pepper fruit, and average bell pepper fruit dry weight for plants grown in pots in the greenhouse.

Source	Peanut Dry weight (g)			Bell pepper					Navy bean Dry weight (g)			
	top	seed	total	Dry weight (g)			No. fruit	Avg. frt. DW (g)	top	pod	edible	total
				top	edible	total						
Inoculum	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer rate	NS	NS	NS	**	**	**	**	NS	**	**	**	**

NS,***Nonsignificant or significant at *P* ≤ 0.05 or *P* ≤ 0.01, ANOVA. The interaction was not significant.

Table 5. Effect of fertilizer rate on dry weights of tops and edible portions of bell pepper and navy bean, number of bell pepper fruit/plant, and average bell pepper fruit dry weight for plants grown in pots in a greenhouse.

Fertilizer rate ^z	Bell pepper					Navy bean Dry weight (g)			
	top	fruit	total	No. fruit	Avg. frt. DW (g)	top	pod	seed	total
1.25 g·L ⁻¹	18.8b	14.9b	33.7b	3.9b	4.0a	9.6b	3.6b	0.2b	13.4b
5 g·L ⁻¹	56.8a	53.4a	110.2a	18.3a	3.0a	39.6a	18.0a	13.8a	71.4a

^zValues in column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple *F* test.

Table 6. ANOVA table for effects of inoculum on yields and pod rating of peanut, yield of bell pepper and dry bean, and effect of year on yield of bell pepper fruit and dry beans grown in the field in both years.

Source	Peanut		Bell pepper					Navy bean	
	yield (Mg·ha ⁻¹)	pod rating	number (× 1000)	yield (Mg·ha ⁻¹)	fruit wgt. (g)	number (× 1000)	yield (Mg·ha ⁻¹)	fruit wgt. (g)	Yield (Mg·ha ⁻¹)
Inoculum (I)	NS	NS	NS	NS	NS	NS	NS	NS	**
Year (Y)	— ^z	—	**	NS	**	**	**	**	**
Interaction									
I × Y	— ^z	—	NS	NS	NS	NS	NS	NS	NS
Year									
2004	— ^z	—	62.8b ^y	10.8a	151.0a	9.8b	1.4b	127.6a	0.59a
2005	— ^z	—	85.0a	10.6a	115.5b	27.5a2.8a		93.4b	0.06b

^z“—” indicates not measured for peanut which was planted only in 2003.

^yValues in a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple *F* test.

NS,*,**Nonsignificant or significant at *P* ≤ 0.05 or *P* ≤ 0.01, ANOVA.

Table 7. ANOVA table for effects of year and inoculum on nutrient content of bell pepper and navy bean in the field in both years.

Source	TKN	NO ₃ -N	NO ₂ -N	TKP	HPO ₄	K	Ca	Cl	Mg	Na	SO ₄
<i>Bell pepper</i>											
Inoculum (I)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year (Y)	**	**	**	**	NS	NS	**	**	**	NS	**
Interaction											
I × Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year					μg·g ⁻¹						
2004	198.7a	0.04b	0.01b	24.7b	8.03a	381.2a	2.41b	26.8b	12.2a	3.50a	27.8a
2005	130.4b	2.03a	0.12a	32.2a	11.64a	374.0a	6.35a	42.1a	8.3b	4.71a	11.8b
<i>Navy bean</i>											
Inoculum (I)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year (Y)	**	**	**	NS	NS	NS	**	NS	NS	**	NS
Interaction											
I × Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year					μg·g ⁻¹						
2004	169.7a ^z	0.04b	0.01b	38.0a	3.49a	140.3a	3.31b	3.29a	3.57a	2.92b	2.36a
2005	152.2b	0.25a	0.09a	35.3a	3.27a	173.7a	9.95a	4.20a	8.35a	6.75a	3.37a

^zValues in a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple *F* test.

NS,*,**Nonsignificant or significant at *P* ≤ 0.05 or *P* ≤ 0.01, ANOVA.

× year interaction (Table 7). In 2004, levels of TKN were higher than in 2005; NO₂, NO₃, Ca, and Na were lower in 2004 than in 2005; and TKP, HPO₄, Cl, Mg, and SO₄ were unaffected. Residual nitrogen from *Rhizobium*-infected legume crops is largely considered to be mostly responsible for improvement in development of crops after legumes (Hesterman et al., 1986). Populations of H₂-oxidizing bacteria increase in soil planted with legumes (Dong and Layzell, 2002; Popelier et al., 1985) and have been postulated as being, in part, responsible for improved development in plants after legumes (Dong and Layzell, 2002; Irvine and Dong, 2004). This is generally the case when *Rhizobium* sp. is specific, or at least compatible, with the crop in which they are in proximity.

Field experiments produced results similar to those in the greenhouse studies, including the formation of nodules on roots of peanut and bean. *Rhizobium* bacteria are a necessary symbiont with legumes, and

AM with leguminous and nonleguminous plants. There were effects resulting from year regarding vegetable plant development, yield, and nutrient content. However, because there was not an inoculum × year interaction, it is presumed that responses are the result of environmental conditions alone.

Although not tested, it may be that either or both types of the groups of microorganisms were present in sufficient diversity, or population, in soil so that additional populations of these organisms did not provide additional benefits. The pH of the soil was sufficient to support the biotic amendments, and there was apparently sufficient moisture and fertilizer supplied to support plant development. The experiment was undertaken to determine the efficacy of use of the biotic amendments. The reasons for the lack of positive response are not immediately clear. Mycorrhizal activity is sensitive to phosphorus levels (Koide, 1991). In the greenhouse, the majority of P was supplied by the soluble fertilizer, and plant development at the lower

fertilizer rate was significantly less than at the higher rate. It may be that the lower fertilizer rate still had P at a level that was not optimal for mycorrhizal colonization. In the greenhouse, regulation of P is generally easier than in the field. In soils that contain, or retain, relatively high P levels, mycorrhizal activity will likely be reduced. It was determined that there were no benefits to development, yield, or nutrient content of crops resulting from use of inocula. Application of inocula does not add a large amount of input cost to production and may provide a benefit if levels of beneficial microorganisms have been depleted. Producers should have soil analyzed to determine levels of AM mycorrhizae and beneficial bacteria before applying these biologic amendments. In addition, producers should have knowledge of how soil nutrient levels will affect mycorrhizal-plant interactions. Further research is needed to determine why the inoculants did not have a demonstrable beneficial effect under the greenhouse and field conditions examined.

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