

# Peanut Cultivar Responses to Bradyrhizobium Inoculation in Northeast Thailand

R. M. N. Kucey\*<sup>1</sup> and B. Toomsan<sup>2</sup>

## ABSTRACT

Effective combinations of peanut (*Arachis hypogaea* L.) cultivars and *Bradyrhizobium* strains were determined under field conditions in NE Thailand in experiments conducted in 1985 and 1986. The experiments measured yields and N<sub>2</sub> fixation (N-15 dilution) responses of peanut cultivars Tainan 9, Mokat and KAC431 to inoculation with inoculants composed of *Bradyrhizobium* strains TH205, 32HI or NC92 or mixed cultures of bacteria. Midseason parameters of nodule number and weight and acetylene reduction levels were also determined. Peanut yields varied between years and locations. Cultivar Tainan 9 was shown to be the cultivar with the best average yield for the locations tested. Inoculation with bradyrhizobia resulted in increased seed yields and amounts of N in the plants in most cases. Inoculated peanuts received an average of 71 kg N/ha from fixation; uninoculated peanuts received an average of 62 kg N/ha. Of this N, 55 to 77% remained in the stover. Midseason measurements of plant performance were of minimal value for predicting the relative performance of the three cultivars.

Key Words: *Arachis hypogaea*, N<sub>2</sub> fixation, N-15 dilution, Nodule, ARA.

The ability of peanuts (*Arachis hypogaea* L.) to withstand intermittent moisture stress and low fertility makes the crop particularly suited for northeast Thailand (10). Significant responses to inoculation with effective bradyrhizobial strains have been observed under controlled conditions or in field soils free of bradyrhizobia capable of nodulating peanuts (4, 5). Lack of response under other field conditions has been attributed to the inability of added bradyrhizobia to survive in the soil or to competition for nodulation sites by bradyrhizobia indigenous to the soil (21). Since peanuts are grown in all areas of Thailand (10), soils free of peanut bradyrhizobia are rare. Therefore, it is necessary to test the ability of a bradyrhizobial inoculant to increase N<sub>2</sub> fixation under field conditions.

The genotype of the peanut host may greatly affect the effectiveness of the nitrogen-fixing symbiosis (1, 20). The interaction of host, bacteria, soil and the indigenous bacteria makes this symbiosis very complex. Evaluation of symbiosis effectiveness based on final yield often cannot identify the host cultivar able to support the highest levels of N<sub>2</sub> fixation (21). Acetylene reduction activity (ARA) has been used for peanuts to identify superior N<sub>2</sub> fixing cultivar-strain combinations (1), but the ARA sys-

<sup>1</sup>Agriculture Canada Research Station, Lethbridge, Alberta, Canada T1J 4B1.

<sup>2</sup>Plant Science Department, Khon Kaen University, Khon Kaen, Thailand 40002.

tem measures only the activity at the time of testing. The N-15 isotope dilution technique (N-15 IDT), however, allows for measurement of N<sub>2</sub> fixation integrated over the entire growing season (16). Using the N-15 IDT, it is possible to measure levels of N<sub>2</sub> fixation to determine which cultivars are superior in N<sub>2</sub> fixation support traits, even in the absence of yield responses.

The purpose of the experiments reported in this paper was to evaluate three peanut cultivars for seed and total plant yields and responses to bradyrhizobial inoculation in northeast Thailand field soils. Measurement of N<sub>2</sub> fixation effectiveness was performed using ARA and nodule measurements and N-15 IDT methods.

## Materials and Methods

Field experiments were conducted at plots located at four sites in northern Thailand during the rainy season (July to September) in 1985 and at two sites in the rainy season of 1986. The soils at the test sites ranged in texture from loam to sandy loam, were mildly acidic (pH 5.6-6.3), and contained 0.3 to 0.6% organic matter and 0.03 to 0.05% total N. The soils at the time of planting contained approximately 1000 bradyrhizobia per gram of soil capable of nodulating peanut as determined by Most Probable Number estimates (3,19). The 1985 experiments compared three peanut cultivars for dry matter and nodule production and nitrogenase activity of the nodulated root systems when inoculated with one of three cowpea type bradyrhizobial inocula or a mixed strain inoculum composed of the three single strains tested. Peanut cultivars used were Tainan 9, Mocket and KAC 431; *Bradyrhizobium* strains tested were NC-92, 32HI and THA205. An uninoculated control treatment was included as well. In 1986, these three peanut cultivars were compared with and without inoculation with a mixed inoculum of the same bacterial strain composition used in 1985. Levels of N<sub>2</sub> fixation using the N-15 isotope dilution technique for estimating N<sub>2</sub> fixation were determined in 1986. A non-nodulating peanut cultivar was included in the 1986 tests as a non-fixing control.

Treatments in both years were arranged in a randomized complete block design with four replications at each site. Each of the strains tested was selected from previous greenhouse tests and shown to be effective when inoculated into soils free of indigenous bradyrhizobia. The bacterial inoculant was cultured in yeast extract-mannitol media and sprayed onto a sterilized peat-based carrier to produce an inoculum containing approximately 10<sup>7</sup> bradyrhizobia per gram. Field plots were 5 m long x 4 rows wide. Rows were spaced 40 cm apart and four seeds were placed in hills 20 cm apart within the rows. Inoculum was placed in seed furrows immediately before planting of seeds. Seeding and inoculum addition were done by hand. Inoculum rates were approximately 3 x 10<sup>8</sup> bacteria per 1.2 kg of seed. Phosphate (56 kg P<sub>2</sub>O<sub>5</sub>/ha) and potash (37.5 kg K<sub>2</sub>O/ha) were side-banded for all rows in each plot 15 days after planting and all plots received the equivalent of 625 kg lime per hectare prior to seeding. Plots were hand-weeded as required. The plants were thinned to two per hill after 2 wk of growth.

In the 1986 experiments, microplots within each experimental plot were established for use in determining N<sub>2</sub> fixation rates using N-15 isotope dilution methods. N-15 labelled ammonium sulphate (5% N-15 atom % abundance) was added to 1.0-m lengths of the two center rows of each plot at a rate of 10 kg N/ha soil after the plants were thinned. A nitrification inhibitor (N-Serve) was premixed with the labelled fertilizer to decrease the movement of N with the irrigation water. The N fertilizer was added adjacent to the plant roots at 10 cm depth in the soil in a dissolved form (10 mL soln./hill) using a 50-mL syringe equipped with a modified 18-gauge needle which had holes on the sides rather than the tip to prevent soil clogging the opening. This method of labelling the soil was shown to be effective for N-15 isotope dilution studies on soybeans in these soils (9,15).

### Plant Parameters Studied

Nodule number, nodule mass and ARA were determined on four plants in each plot 60 days after planting (60 DAP). The ARA procedure was performed on freshly dug root systems as described by

Boonkerd *et al.* (2). Nodules were picked by hand from the roots, washed, counted, dried (60 C for 2 days), and weighed. Final pod yields were determined for plants at harvest maturity. Pods were picked by hand from the plants, dried (60 C for 7 days) and weighed.

For the 1986 experiment, total nitrogen and N-15 content of the plant material was determined on the plants within the N-15 labelled microplots at maturity. Preparation of the plant material, Kjeldahl digestions and mass spectrometer determination of N-15 in the plant material were as described by Rennie *et al.* (14). Isotope terminology and calculations used are according to Rennie *et al.* (16).

Main effect and interactions of the data were statistically analyzed using an analysis of variance procedure. Significance of the differences between sample means was determined by calculation of Tukey's least significant difference (HSD) calculated at the P ≤ .05 level (17).

## Results

Significant differences among cultivars were observed for nodule dry weights and nitrogenase (ARA) activity at 60 DAP, but not for plant dry weight at 60 DAP or for final pod yields in the 1985 experiments (Tables 1, 2 and 3). Cultivar KAC431 showed higher nodule dry weight and nitrogenase activity than the other two cultivars.

Table 1. Nodule weights and nitrogenase activity of peanut cultivars inoculated with different *Bradyrhizobium* strains under NE Thai field conditions in 1985.

Peanut cultivar	Bradyrhizobium strain inoculum				Uninoc.
	THA205	32HI	NC92	Mixed <sup>a</sup>	
<b>Nodule dry matter at 60 DAP (mg/plant)</b>					
Tainan 9	112	92	110	114	93
Mocket	122	134	126	116	99
KAC431	145	149	120	124	127
Mean	126	125	119	118	106
HSD (P <sub>0.05</sub> ) 22					
<b>Nitrogenase activity at 60 DAP (μmol/plant/hr)</b>					
Tainan 9	7.72	6.84	9.33	8.28	6.51
Mocket	8.51	8.82	8.84	7.96	6.82
KAC431	8.54	10.3	6.68	8.30	9.22
Mean	8.26	8.65	8.28	8.18	7.52
HSD (P <sub>0.05</sub> ) 1.41					
Analysis of Variance					
		Nodule DM <sup>b</sup>		ARA <sup>c</sup>	
Location (L)		**		**	
Cultivar (C)		**		*	
Bradyrhizobium (B)		*		ns	
C x B		*		**	
C x L		*		ns	
B x L		**		*	
C x B x L		ns		ns	

Values are averages of four sites.

\*, \*\*, ns = significant at P<sub>0.05</sub>, P<sub>0.01</sub> and nonsignificant, respectively.

<sup>a</sup>Mixed inoculum composed of the three separate strains used.

<sup>b</sup>Nodule dry matter production.

<sup>c</sup>ARA = acetylene reduction assay = Nitrogenase activity.

Significant differences among *Bradyrhizobium* treatments were observed in the 1985 experiments for nodule dry weight only (Table 1). Strains THA205 and 32HI were superior to NC92 when used with cultivar

Table 2. Effect of *Bradyrhizobium* inoculation on dry matter production (DMP) of three peanut cultivars at 60 days following planting at four sites in NE Thailand in 1985.

Bradyrhizobium strain	KKU	Location			Mean
		Tha Phra	B. Moung	Samjan	
		----- Plant DMP (g/plant) -----			
Control	23.42	20.81	12.80	20.86	19.47
THA 205	23.07	17.51	12.39	27.76	20.18
32HI	20.56	17.60	13.35	23.14	18.67
NC-92	21.22	18.19	12.84	22.57	18.70
Mixed <sup>a</sup>	21.64	17.04	12.84	23.49	18.76
Mean	21.98	18.23	12.84	23.56	

HSD ( $P_{\leq 0.05}$ ) (strain x location) = 2.52

#### Peanut cultivar

Tainan 9	23.14	18.24	13.60	21.15	19.03
Moket	21.38	18.19	12.66	26.18	19.78
KAC 431	21.42	17.54	12.28	23.26	19.65
Mean	21.98	18.23	12.84	23.56	

HSD ( $P_{\leq 0.05}$ ) (strain x location) = 1.95

#### Analysis of Variance

Location (L)	**
Cultivar (C)	ns
Bradyrhizobium (B)	ns
C x B	ns
C x L	**
B x L	**
C x B x L	ns

<sup>a</sup>Mixed inoculum composed of the three separate strains used.

\*\* , ns = significant at  $P_{\leq 0.01}$  and nonsignificant, respectively.

KAC431, but not significantly different when used with the other two cultivars. All three bacterial strains increased nodule mass of Moket above that of uninoculated Moket, but inoculation did not increase nodule mass in the other two cultivars. *Bradyrhizobium* inoculation did not significantly affect nodule dry weights or ARA. Significant differences among the four locations were observed in all the parameters studied.

Peanut cultivar x *Bradyrhizobium* strain interactions ( $P_{\leq 0.05}$ ) were observed for nodule dry matter and nitrogenase activity. Strains THA205 and 32HI resulted in greater nodule mass in cultivar KAC431, but only strain 32HI increased the nitrogenase activity of this cultivar (Table 1). Strain NC92, on the other hand, increased nitrogenase activity on cultivar Tainan 9, but decreased nitrogenase activity of cultivar KAC431. These specificities, though significant, did not express themselves in plant dry matter at 60 DAP (data not shown) or in final pod yield (Table 3).

Table 3. Pod yields (maturity) of four peanut cultivars inoculated with *Bradyrhizobium* strains at four locations in NE Thailand in 1985.

Cultivar	Site			
	Khon Kaen Univ.	Tha Phra	Ban Samjan	Ban Muong
Tainan 9	1520	1840	2231	1304
Moket	1423	1795	2313	1270
KAC431	2515	1599	1497	1559
Bradyrhizobium strain				
THA205	1817	1733	2027	1373
32HI	1831	1863	2090	1344
NC92	1921	1679	1998	1404
Mixed <sup>a</sup>	1611	1770	1881	1392
Control	1979	1677	2042	1375

Cultivar	Bradyrhizobium strain inoculum				Uninoc.
	THA205	32HI	NC92	Mixed <sup>a</sup>	
Tainan 9	1722	1746	1672	1653	1803
Moket	1718	1731	1789	1706	1557
KAC431	1773	1869	1989	1632	1899
Mean	1738	1782	1817	1664	1753

#### Analysis of Variance

Location (L)	•
Cultivar (C)	ns
Bradyrhizobium (B)	ns
C x B	ns
C x L	**
B x L	ns
C x B x L	*

\*, \*\*, ns = significant at  $P_{\leq 0.05}$ ,  $P_{\leq 0.01}$  and nonsignificant, respectively.

<sup>a</sup>Mixed inoculum composed of the three separate strains used.

A location x cultivar x strain interaction ( $P_{\leq 0.05}$ ) was observed for pod yields in the 1985 experiments (Table 3). At Tha Phra, inoculation gave higher yields for cultivars Tainan 9 and Moket than for the uninoculated control, but somewhat reduced the yields of cultivar KAC431. When the Tha Phra site data were deleted, the significance of location as a main effect and in the two-way interactions was eliminated.

Cultivar x location and strain x location interactions ( $P_{\leq 0.05}$ ) were observed in the 1985 experiment (Tables 1, 2 and 3). Bradyrhizobial inoculation appeared to increase nodule dry weight at two of the sites, but no effect was observed at the other two sites (Table 4). As well, different strains appeared to be effective in the different locations, i.e., THA205 at the Samjan site, 32HI at the Khon Kaen and Ban Moung sites, and NC92 at the Tha Phra site. Plant dry weight and pod yield did not correspond to the nodule dry weights or nitrogenase activities (Tables 2 and 3).

Table 4. Average nodule weights and nitrogenase activity of peanuts at 60 days after planting at four sites in NE Thailand in 1985.

Bradyrhizobium strain	Site			
	Khon Kaen Univ.	Tha Phra	Ban Samjan	Ban Muong
<u>Nodule dry matter at 60 DAP (mg/plant)</u>				
THA205	126	120	183	76
32HI	130	114	168	88
NC92	128	113	155	78
Mixed <sup>a</sup>	125	121	155	73
Uninoc.	135	120	88	67
HSD (P <sub>≤</sub> .05)	26			
<u>Nitrogenase activity at 60 DAP (mmol/plant/hr)</u>				
THA205	7.11	7.50	11.87	6.55
32HI	8.75	8.24	10.44	7.21
NC92	7.84	9.06	10.12	6.14
Mixed <sup>a</sup>	7.48	8.89	10.03	6.32
Uninoc.	7.82	9.03	8.11	4.98
HSD (P <sub>≤</sub> .05)	2.31			

For analysis of variance, see Tables 1 and 3.

<sup>a</sup>Mixed inoculum composed of the three separate strains used.

In the 1986 experiment, plants at the Tha Phra site had greater nodule numbers, dry weights and ARA than plants at the Khon Kaen University (KKU) site (Table 5). Cultivar KAC431 had fewer nodules on the root systems than the other two cultivars at both sites. Mocket had the highest rates of ARA at both sites. Bradyrhizobial inoculation generally did not result in increases in any of the midseason parameters studied, although at Tha Phra, inoculated KAC431 plants had significantly greater ARA than uninoculated KAC431 plants.

Seed and plant yields, nodule numbers, and dry weights taken at maturity were greater at the Tha Phra site than at the KKKU site (Table 6). Cultivar Tainan 9, inoculated with *Bradyrhizobium*, had the greatest seed yields at both sites. Highest stover (Plant DMP) yields and nodule dry weights were obtained with KAC431, particularly if the plots received bradyrhizobial inoculum. Seed and stover yield responses to bradyrhizobial inoculation were positive at both sites and cultivar KAC431 gave the greatest response. Inoculation of KAC431 resulted in an average of 37% increase in seed yield and a 19% increase in stover yield. Responses to bradyrhizobial inoculation for the other two cultivars averaged 5.5% increase in seed yield and 7.7% increase in stover production.

Nitrogen yields were not different between the two sites, despite the seed and stover dry matter yield differences (Table 7). The cultivars differed in the N within the tissues. Bradyrhizobial inoculation resulted in increases in seed N yields for all cultivars at the KKKU site and for Tainan 9 at Tha Phra. Inoculation did not affect stover N yields at either site. Highest stover N yields and lowest seed N yields were obtained with cultivar KAC431 at both sites. Inoculated Tainan 9 and Mocket had nearly equivalent seed N yields at KKKU, but the

Table 5. Plant DMP, nodule number and weight and acetylene reducing activity (ARA) at 60 DAP of three peanut cultivars under field conditions in NE Thailand in the 1986 rainy season.

Cultivar	Rhiz. inoc. <sup>a</sup>	Plant DMP (g/plant)	Nodule number (/plant)	Nodule dry wt. (mg/plant)	ARA (μmol/hr)
<u>Khon Kaen University site</u>					
Tainan 9	+	16.91	137	110.8	11.97
	-	18.43	148	98.3	11.19
Mocket	+	20.11	144	126.8	15.65
	-	20.03	128	97.0	11.29
KAC431	+	15.64	88	133.0	13.01
	-	15.64	84	107.3	11.27
<u>Tha Phra site</u>					
Tainan 9	+	19.54	225	146.5	17.67
	-	19.24	234	128.3	16.92
Mocket	+	16.42	176	145.8	19.56
	-	18.88	230	150.0	17.51
KAC431	+	19.87	143	119.0	18.53
	-	19.00	153	137.5	13.62

Tukey's HSD (P<sub>≤</sub>.05)

Site	5.28	22	17.2	2.70
Cultivar	7.81	32	25.4	3.98
<i>Bradyrhizobium</i>	5.28	22	17.2	2.70

Analysis of Variance<sup>b</sup>

Site	ns	**	**	**
Cultivar	ns	**	ns	*
<i>Bradyrhizobium</i>	ns	ns	ns	ns

<sup>a</sup>+, - designate inoculated and uninoculated treatments, respectively.

<sup>b</sup>No significant interactions were observed.

\*, \*\*, ns = significant at P<sub>≤</sub>.05, P<sub>≤</sub>.01, and nonsignificant, respectively.

greatest seed N yields at Tha Phra occurred with Tainan 9.

Cultivar KAC431 generally had the highest Ndfa (N derived from atmosphere) values for seed N at both sites (Table 7). Bradyrhizobial inoculation was found to increase seed Ndfa values for Tainan 9 and Mocket at Tha Phra and for KAC431 at KKKU. At Tha Phra, Tainan 9 and Mocket seed Ndfa values were greater than those observed at KKKU; however, KAC431 seed Ndfa values were the same at both sites, resulting in a significant site x cultivar interaction. The amounts of seed N fixed were not significantly affected by cultivar or bradyrhizobial inoculations; however, the amounts of seed N fixed were greater at the Tha Phra site than at KKKU (Table 7). The % N and total N in seeds of the non-nodulating control plants were significantly lower than observed for the other cultivars.

The Ndfa values calculated for the plant stover were not affected by site differences. KAC431 had the highest values for stover Ndfa and N fixed at both sites. Bradyrhizobial inoculation decreased stover Ndfa values for KAC431 and Mocket at Tha Phra, but resulted in increased stover Ndfa values for Tainan 9 and KAC431 at

**Table 6. Plant and seed DMP and nodule numbers and weights of three peanut cultivars at maturity grown in NE Thailand in the 1986 rainy season.**

Cultivar	Rhiz. inoc. <sup>a</sup>	Seed yield (kg/ha)	Plant DMP (kg/ha)	Nodule number (/plant)	Nodule weight (mg/plant)
<b>Khon Kaen University site</b>					
Tainan 9	+	2254	2974	143	94.8
	-	2034	2944	146	76.0
Moket	+	2093	3256	180	117.3
	-	2085	2862	157	91.1
KAC431	+	2240	4127	130	167.0
	-	1541	3459	75	114.6
Non-nod		477	2354	0	0
<b>Tha Phra site</b>					
Tainan 9	+	2666	5136	175	115.2
	-	2591	4648	174	123.0
Moket	+	2431	5554	197	118.9
	-	2255	5262	172	96.4
KAC431	+	2092	6162	149	149.5
	-	1621	5204	141	150.8
Non-nod		1235	4314	0	0
Tukey's HSD (P<.05)					
Site		195	375	21	15.3
Cultivar		289	554	32	22.6
<i>Bradyrhizobium</i>		195	375	21	15.3
Analysis of Variance <sup>b</sup>					
Site	**	**	**	**	**
Cultivar	**	**	**	**	**
<i>Bradyrhizobium</i>	**	*	ns	*	*

<sup>a</sup>+, - designate inoculated and uninoculated treatments, respectively.

<sup>b</sup>No significant interactions were observed; non-nodulating cultivar excluded from analysis of variance.

\*, \*\*, ns = significant at P<.05, P<.01, and nonsignificant, respectively.

KKU, giving a significant site x *Bradyrhizobium* interaction. The amount of stover N fixed by Tainan 9 at Tha Phra was greater than the amount fixed at KKKU, and the stover N fixed by KAC431 was greater at KKKU than at Tha Phra (Table 7). As observed for seed N, stover % N and total N for the non-nodulating control plants were significantly lower than observed for the other cultivars.

## Discussion

The variability in the results, and the calculation of significant interactions between main effects, is indicative of the highly complex relationships involved in a symbiotic system such as studied here, and has also been observed under controlled conditions (11, 20). Effective combinations of bacteria and host can be selected with the potential for increased levels of N<sub>2</sub>

**Table 7. Nitrogen contents and N<sub>2</sub> fixed by three cultivars of peanuts grown in NE Thailand in the 1986 rainy season.**

Cultivar	Rhiz. inoc. <sup>a</sup>	Seed				Leaves, stems, pods			
		%N	N yield (mg/plnt)	Ndfa %	N <sub>2</sub> fixed (kg/ha)	%N	N yield (mg/plnt)	Ndfa %	N <sub>2</sub> fixed (kg/ha)
<b>Khon Kaen University site</b>									
Tainan 9	+	4.44	280.6	25.7	15.9	1.79	350.6	38.2	29.8
	-	4.11	245.6	28.1	15.1	1.85	351.7	31.7	22.7
Moket	+	4.45	287.0	32.9	22.5	1.97	345.0	37.5	31.2
	-	4.34	233.5	35.5	17.8	1.95	394.0	39.6	34.7
KAC431	+	4.30	243.2	44.1	24.0	1.92	686.7	51.1	76.5
	-	4.22	149.0	55.1	14.7	1.97	617.1	44.2	49.4
Non-nod		2.80	65.3	0	0	1.20	271.9	0	0
<b>Tha Phra site</b>									
Tainan 9	+	4.71	351.6	43.7	41.8	1.82	421.4	44.9	50.8
	-	4.56	209.7	55.2	26.4	1.77	429.7	46.4	47.0
Moket	+	4.65	213.2	36.4	22.3	1.83	344.0	30.8	31.8
	-	4.65	225.1	46.8	26.8	1.86	377.8	43.2	40.0
KAC431	+	4.40	193.4	52.4	21.3	1.75	559.6	48.7	56.3
	-	4.44	192.9	51.1	17.6	1.83	602.0	53.8	57.7
Non-nod		3.00	116.5	0	0	1.16	305.8	0	0
Tukey's HSD (P<.05)									
Site		0.14	41.7	6.5	6.4	0.10	48.3	4.9	8.1
Cultivar		0.21	61.6	9.6	9.4	0.15	71.3	7.3	11.9
<i>Bradyrhizobium</i>		0.14	41.7	6.5	6.4	0.10	48.3	4.9	8.1
Analysis of Variance <sup>b</sup>									
Site (S)	**	ns	**	*	*	ns	ns	ns	ns
Cultivar (C)	ns	*	**	ns	ns	**	**	**	**
<i>Bradyrhizobium</i> (B)	ns	*	*	ns	ns	ns	ns	ns	ns
S x C	ns	ns	*	ns	ns	ns	ns	ns	*
S x B	ns	ns	ns	ns	ns	ns	ns	*	ns

<sup>a</sup>+, - designate inoculated and uninoculated treatments, respectively.

<sup>b</sup>Only significant interactions shown; non-nodulating cultivars excluded from analysis of variance.

\*, \*\*, ns = significant at P<.05, P<.01, and nonsignificant, respectively.

fixation (21). However, the degree to which the potential may be realized can only be determined from field experiments such as those conducted in this study.

The primary criterion for the calculation of N<sub>2</sub> fixation in this study was the N-15 isotope dilution calculations using the non-nodulating peanut cultivar as a non-fixing control plant. Alternatively, we could have used a difference method for calculating the proportion of N in the plant from fixation (Ndfa = (treatment N — non-nod N) / treatment N). In calculating the contribution of N<sub>2</sub> fixation to plant N in this way, we found that the Ndfa calculated by the difference method gave higher values for N<sub>2</sub> fixed for the seed N at Khon Kaen (range 56.2% to 77.2% of seed N) than the N-15 dilution method. The difference method estimated levels of N<sub>2</sub> fixed for inoculated Tainan 9 in excess of 130 kg N/ha, which is greater than observed for soybeans grown in this area (15). The difference method also estimated lower amounts of N<sub>2</sub> fixed for stover N at both sites (range 21.2% to 60.4% of stover N at Khon Kaen, 11.1% to 49.2% of stover N at Tha Phra). Generally, the agreement between the two methods was better in treatments with lower total N than in treatments with higher N contents. Agreement between the two methods was

better for seed N at Tha Phra than at Khon Kaen.

There have been few comparisons of the difference and N-15 dilution methods for peanuts; however, several studies report comparisons made on soybeans. Generally, it has been concluded that the difference method is useful for preliminary studies, but is less precise and subject to greater errors than the N-15 dilution method (7, 8, 13, 18). One of the major errors is that a synergistic effect may occur between soil N and fixed N in soils of low fertility such as were used in this study. A plant receiving biologically fixed N is able to produce greater biomass and explore a greater volume of soil. In this way, an N<sub>2</sub>-fixing plant can absorb more soil N than a non-fixing plant, increasing the total plant N and making it appear that a greater quantity of N<sub>2</sub> was fixed. Conversely, in a soil with adequate N, the non-fixing control plant may explore a greater soil volume in order to absorb sufficient N for its needs. In this case, the fixing plant will absorb less soil N, making it appear that less N<sub>2</sub> was fixed by the difference method. Since the accuracy of the difference method depends greatly on the ability of the test plants to alter growth to compensate for increased or decreased N uptake, and the N fertility of the soil used, the reliability of the N difference method is greatly reduced.

The interaction of strain, cultivar and location is evident for both years of this study. KAC431 was the high yielding cultivar at some locations but relatively low yielding at others in both years. Overall, Tainan 9 proved to be the cultivar with the greatest yield potential. Its average yields were highest or second highest in every experiment.

Seed N yields were increased by rhizobial inoculation, but seed Ndfa values decreased as calculated by the N-15 isotope dilution method. This apparent discrepancy occurred because the Ndfa value only determines the proportion of N coming from fixation. The amount of seed N fixed was not shown to be increased by bradyrhizobial inoculation, except for KAC431 at KKU and Tainan 9 at Tha Phra, but the total N in the seeds was increased by inoculation. Therefore, since the same amount of N<sub>2</sub> was fixed and this amount was divided by a larger quantity of total N, this results in a smaller proportion of seed N derived from fractionally lower Ndfa. In the cases where inoculation did not result in increased amounts of N<sub>2</sub> fixed, but did result in increased total N yields, it is possible that native bradyrhizobia were as effective as the added inoculum bradyrhizobia when judged over the entire growing season. Inoculation may have resulted in greater early season fixation promoting more vigorous growth and resulting in greater N uptake later in the season.

When seed and stover N fixed were combined, the effect of bradyrhizobial inoculation was positive. Some combinations of bacteria and host increased seed N fixed while others increased stover N fixed. Amounts of N<sub>2</sub> fixed by inoculated plants averaged 71 kg N/ha and ranged from 46 to 101 kg N/ha as determined by N-15 dilution. Nitrogen fixed by uninoculated plants averaged 62 kg N/ha and ranged from 38 to 75 kg N/ha. Of this N<sub>2</sub> fixed by both inoculated and uninoculated plants, 55 to 77% of the N was in the stover and could

be returned to the soil where it could benefit succeeding crops.

With respect to differences between *Bradyrhizobium* strains, 32HI showed the highest nitrogenase activity at 60 DAP, while THA205 inoculation resulted in the highest plant production at 60 DAP. Final yields were greater with NC92. Elkan *et al.* (6) found strain 32HI to be effective for increasing ARA levels in a greenhouse study, and Nambiar *et al.* (12) reported NC92 to be an effective competitor in the soil and able to form a large proportion of nodules on the host roots. These studies are useful for identifying suitable strains for testing under local field conditions. However, the findings in previous studies cannot be of direct use in selection of cultivars and strains in other locations. Wynne *et al.* (20) reported that strain 32HI was a very effective strain for increasing ARA measurements under greenhouse and field conditions, and later reported that 32HI was superior to NC92 for dry matter production of two peanut varieties. Our results indicate similar results for ARA measurements, but tend to indicate the opposite with respect to final yields. This shows the importance of local testing of strains and cultivars since the soil and climate of the experimental location have a large impact on the final results. The mixed inoculum showed itself to be generally intermediate in the parameters measured, and it is thus suggested that use of a mixed inoculum would be useful under practical conditions since it would entail the least risk of poor performance.

The use of ARA and plant dry matter production measurements in midseason were of minimal value for predicting the best yielding or best N<sub>2</sub> fixing cultivars. This differs from previous reports that nitrogenase activity and nodule and plant dry matter measurements taken midseason could predict the relative performance of peanut cultivars (1, 20). In our study, plant DMP at 60 DAP would predict cultivars KAC431 and Tainan 9 to be equally good at Tha Phra while ARA measurements would rank Mocket the highest. As measured by N-15 dilution, N<sub>2</sub> fixed would agree with the plant DMP ranking at Tha Phra. The rankings do not agree for the KKU site, however, since KAC431 was the cultivar calculated to have the highest N<sub>2</sub> fixation. It appears that in a study such as ours where three cultivars that are close in performance are compared, performance predictions based on midseason measurements are not useful.

The results of this study indicate that inoculation of peanuts with bradyrhizobial inocula in northeast Thailand can result in increased yields and N uptake, even in soils that contain native bradyrhizobia. It is apparent that strains of *Bradyrhizobium* differ in their effectiveness in different soils and are also affected by the host cultivar.

## Literature Cited

1. Arunchalum, V., G. Dm. Pungle, M. Dutta, P. T. C. Nambiar, and P. J. Dart, 1984. Efficiency of nitrogenase activity and nodule mass in predicting the relative performance of genotypes assessed by a number of characters in groundnut (*Arachis hypogaea*). *Expl. Agric.* 20:303-309.
2. Boonkerd, N., C. Arunsri, W. Rungrattanakasin, and Y. Vasuvat, 1984. Effects of post-emergence inoculation on field grown soybeans. *MIRCEN J.* 1:155-161.

3. Brockwell, J. 1963. Accuracy of a plant-infection technique for counting populations of *Rhizobium trifolii*. *Appl. Microbiol* 13:481-486.
4. Burton J. C. 1976. Pragmatic aspects of the *Rhizobium* leguminous plant association. pp. 429-446. *In* Newton, W. E. and Nyman, C. J. (Eds.) *Proc. 1st Inter. Symp. on N-fixation*, Vol. 2, Wash. State Univ. Press.
5. Chomchallow, S. 1971. The effectiveness of introduced *Rhizobium* strains on "Rayong" peanut. *Thai. J. Agric. Sci.* 4:85-94.
6. Elkan, G. H., J. C. Wynne, T. J. Schneeweiss, and T. G. Isleib, 1980. Nodulation and nitrogenase activity of peanuts inoculated with single strain isolates of *Rhizobium*. *Peanut Sci.* 7:95-97.
7. Focht, D. D., and M. Poth, 1987. Measurement of biological nitrogen fixation by  $^{15}\text{N}$  techniques. Chp 11 in *Symbiotic Nitrogen Fixation Technology*. G. E. Elkan (ed), Marcel Dekker Inc., New York.
8. Henson, R. A., and G. H. Heichel, 1984. Dinitrogen fixation of soybean and alfalfa: Comparison of the isotope dilution and difference methods. *Field Crops Res.* 9:333-346.
9. Kuçey, R. M. N., P. Snitwongse, P. Chaiwanakupt, P. Wadisirisuk, C. Siripaibool, T. Arayangkool, N. Boonkerd, and R. J. Rennie, 1988. Nitrogen fixation (N-15 dilution) with soybeans under Thai field conditions. 1. Developing protocols for screening *Bradyrhizobium japonicum* strains. *Plant and Soil* 108:33-41.
10. Lampang, A. N., T. Charoenwatana, and D. Tiyawlee, 1981. Groundnut production, utilization, research problems and further research needs in Thailand. pp. 237-240. *In Proc. International Workshop on Groundnuts held in ICRISAT Center, Patancheru, India, oct. 13-17, 1980.*
11. Law, I. J., and B. W. Strijdom, 1974. Nitrogen-fixing and competitive abilities of a *Rhizobium* strain used in inoculants for *Arachis hypogaea*. *Phytolactica* 6:221-228.
12. Nambiar, P. T. C., B. S. Rao, and V. Anjaiah, 1984. Studies on competition, persistence, and methods of application of a peanut *Rhizobium* strain, NC92. *Peanut Sci.* 11:83-87.
13. Rennie, R. J., 1984. Comparison of N balance and  $^{15}\text{N}$  isotope dilution to quantify  $\text{N}_2$  fixation in field grown legumes. *Agron. J.* 76:785-790.
14. Rennie, R. J., S. Dubetz, J. B. Bole, and H. H. Muendel, 1982. Dinitrogen fixation measured by N-15 isotope dilution in two Canadian soybean cultivars. *Agron. J.* 74:725-730.
15. Rennie R. J., D. A. Rennie, C. Siripaibool, P. Chaiwanakupt, N. Boonkerd, and P. Snitwongse, 1988.  $\text{N}_2$  fixation in Thai soybeans: Effect of tillage and inoculation on  $^{15}\text{N}$ -determined  $\text{N}_2$  fixation in recommended cultivars and advanced breeding lines. *Plant and Soil* (in press).
16. Rennie, R. J., D. A. Rennie, and M. Fried, 1978. Concepts of N-15 usage in dinitrogen fixation studies. pp. 107-133. *In Isotopes in Biological Dinitrogen Fixation*, International Atomic Energy Agency, Vienna.
17. Steel, R. G. D., and J. H. Torrie, 1960. *Principles and Procedures of Statistics*. McGraw-Hill, Toronto, 481 pp.
18. Talbott, H. J., W. J. Kenworthy, J. O. Legg, and L. W. Douglass, 1985. Soil nitrogen accumulation in nodulated and non-nodulated soybeans: a verification of the difference method by a  $^{15}\text{N}$  technique. *Field Crops Res.* 11:55-67.
19. Weaver, R. W. and L. R. Frederick, 1972. A new technique for most probable number counts of rhizobia. *Plant Soil* 36:219-222.
20. Wynne, J. C., G. H. Elkan, C. M. Meisner, T. J. Schneeweiss, and J. M. Ligon, 1980. Greenhouse evaluations of strains of *rhizobium* for peanuts. *Agron. J.* 72:645-649.
21. Wynne, J. C., G. H. Elkan, and T. J. Schneeweiss, 1981. Increasing nitrogen fixation of the groundnut by strain and host selection. pp. 95-109. *In Proc. International Workshop on Groundnuts held in ICRISAT Center, Patancheru, India, Oct. 13-17, 1980.*

Accepted October 17, 1988