Field Evaluations of Peanut Cultivar-*Bradyrhizobium* Specificities[1]

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ABSTRACT

The peanut (*Arachis hypogaea* L.) generally is considered promiscuous since it forms symbioses with a diverse group of *Bradyrhizobium*. However, specific cultivar-strain combinations like Robut 33-1 and strain NC92 have resulted in significant yield increases, suggesting that host-strain combinations may be selected for superior nitrogen fixation and yield. The objectives of this study were to measure nitrogen fixation-related traits during the growing season and evaluate the interactions between host peanut genotypes and *Bradyrhizobium* strains under field conditions in North Carolina. A factorial experiment with four cultivars and five inoculants was conducted in two years at two locations (Clayton and Lewiston) in North Carolina. Traits measured during the growing season were nodule number and weight, root weight (1983 only), shoot weight, pod weight, nitrogenase activity and specific nitrogenase activity. In 1984, fruit yield was measured at harvest. Results indicated that cultivars and strains were different for most traits in 1983 at Clayton but significant host-strain interactions occurred only for nodule weight at 60 days after planting (DAP) and root weight at 132 DAP. In 1984, Clayton results indicated cultivar-strain interactions for all traits at 73 DAP and for several traits at 109 DAP. At Lewiston only cultivar differences were important. The Clayton fields had low populations of native *Bradyrhizobium* while Lewiston had a high level of the bacteria. Inoculation produced substantial yield increases at Clayton but not at Lewiston. Robut 33-1 inoculated with strain NC92 did not yield more than with other strains. Further study is needed to explain why repeated increases in yield were obtained with Robut 33-1/NC92 in tropical studies but not in North Carolina. The possibility still exists that superior cultivar-strain combinations can be identified.

Key Words: *Arachis hypogaea,* groundnut, nitrogen fixation.

Improvement of symbiotic nitrogen fixation in peanut (*Arachis hypogaea*) is complicated because the genetic systems of both the plant and the *Bradyrhizobium* symbiont are involved (15). Nitrogen fixation is influenced by the host plant, *Bradyrhizobium* strain, environment, and interactions between these factors. Variability of peanut genotypes for traits related to nitrogen fixation have been reported (1,2,4,12,15,18). *Bradyrhizobium* strains also vary in their effectiveness and efficiency in fixing atmospheric nitrogen (2,6,13,14,17). Burton (2) demonstrated a host x strain interaction even though peanut generally is considered to be promiscuous and effective with many cowpea miscellany *Bradyrhizobium* strains (5). Wynne *et al.* (16) found significant host-strain interactions for nodule number and weight, nitrogenase activity and plant color when six peanut genotypes and 10 *Bradyrhizobium* strains were used in a greenhouse study. Several other greenhouse studies at North Carolina State University have shown cultivar-strain interactions (19). Nambiar and Dart (10) reported significant pod yield increases with the peanut genotype Robut 33-1 and the strain of *Bradyrhizobium* NC92. These yield improvements were observed for several seasons and were demonstrated at several loations. Additional tests in Gujarat, India and Guiring, Cameroon have produced yield increases using the strain NC92 (10).

Objectives of this study were to measure nitrogen fixation-related traits during the growing season and evaluate the interactions between host peanut genotypes and *Bradyrhizobium* strains under field conditions in North Carolina.

Materials and Methods

The study was conducted during the 1983 and 1984 growing seasons at Clayton, NC and in 1984 at Lewiston, NC. At Clayton, fields not previously cropped in peanuts were used. A field with a history of peanut cultivation was used at Lewiston. The soil type at all locations was a Norfolk loamy sand.

A factorial arrangement of four peanut cultivars and five inoculants (four *Bradyrhizobium* strains with an uninoculated control) was used. The peanut cultivars used in 1983 were Florigiant, Robut 33-1, NC 4 and Argentine. In 1984, NC 7 was substituted for NC 4. The four *Bradyrhizobium* strains in both years were NC92, F1o1A, RP182-13 and 176A22. The cv. Robut 33-1 and *Bradyrhizobium* strain NC92 were included to determine if the yield increases reported elsewhere

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could be demonstrated in North Carolina fields.

The experimental design of all tests was a randomized complete block with four replications. At Clayton in 1983, each plot consisted of one 20-seed row with 25.4 cm between seeds and 96.5 cm between row centers. At both locations in 1984, plots consisted of four 28-seed rows with the same seed and row center spacing as in 1983 at Clayton, but 91 cm row centers at Lewiston. Two rows of each plot were used as subplots for sampling and two were reserved for yield determinations at harvest.

The experiment was planted on 19 May 1983 at Clayton and 18 and 16 May 1984 at Clayton and Lewiston, respectively. Liquid inoculum was applied at planting. Nambiar *et al.* (11) reported that liquid inoculants resulted in better nodulation. Peat with the appropriate strain of *Bradyrhizobium* was suspended in water and was dispensed in the row at the rate of 3.81×10^6 bacterial cells per cm. The rows were covered with soil using a hoe which was rinsed in a bleach solution (approximately 1% sodium hypochlorite) between plots inoculated with different strains to prevent cross-contamination. Standard cultural practices were followed during the growing season.

In 1983 samples were taken at 60, 91 and 132 days after planting (DAP). At Clayton in 1984, sampling occurred at 73 and 109 DAP with yield taken at harvest, 150 DAP. At Lewiston, sampling was done at 70 and 103 DAP with yields measured at 133 DAP.

From a sample of two competitive plants (a plant bordered on both sides by another plant) per plot or subplot per sampling date, the following traits were measured: nodule number, nodule weight (g/plant), root weight (g/plant, 1983 only), plant weight (g), fruit weight (g), nitrogenase activity (μmoles C_2H_4/plant/hour) as detailed by Hardy *et al.* (7) and yield (kg/plot, 1984 only). Specific nitrogenase activity (μmoles C_2H_4/g nodule dry weight/hour) was also determined. Entire plants were dug and then separated into plant tops with fruit and roots with nodules. All plant parts were dried prior to weighing.

Analysis of variance was performed for the traits using the General Linear Model Procedure of SAS (8).

Results and Discussion

Clayton 1983

Results from the 1983 Clayton test revealed that nitrogen fixation-related traits differed over the three sampling dates (Table 1). At the first sampling, differences among cultivars were highly significant for nodule weight, root weight, shoot weight, and nitrogenase activity. Differences among *Bradyrhizobium* strains were highly significant for nodule number, nodule weight, root weight and nitrogenase activity (Table 2). At the second sampling date, cultivar differences were highly significant for nodule weight, root weight, shoot weight, pod weight, and significant for nitrogenase activity. Strains were highly significantly different for nodule number, nodule weight, root weight, and nitrogenase activity. At the final sampling date, cultivar differences were highly significant for all traits except specific activity. Differences among strains of *Bradyrhizobium* were highly significant for all traits.

Table 1. Means of traits by cultivar and date from 1983 Clayton field evaluation.

Cultivar	Nodule number	Nodule dry wt	Root dry wt	Shoot dry wt	Pod dry wt	Nitrogenase activity (μmole C_2H_4/plant/hour)	Specific activity (μmole C_2H_4 g nod. dry wt/hour)
			(g/plant)				
				60 DAP			
Florigiant	260.8	0.475	1.318	41.82	0.02	43.02	84.92
Robut 33-1	220.7	0.279	0.977	32.05	0.06	25.73	90.97
NC 4	236.4	0.342	1.239	40.70	0.03	32.41	88.41
Argentine	195.0	0.216	0.977	32.32	0.75	24.53	114.41
$LSD_{.05}$	61.1	0.071	0.106	4.21	0.32	7.12	22.19
				91 DAP			
Florigiant	385.8	0.888	2.393	90.96	11.84	27.09	36.42
Robut 33-1	300.8	0.451	1.607	67.44	22.33	21.31	44.71
NC 4	382.4	0.814	2.578	82.86	13.01	33.79	39.70
Argentine	345.4	0.517	1.648	64.51	22.58	26.95	48.69
$LSD_{.05}$	70.9	0.153	0.241	12.63	3.34	7.54	11.35
				132 DAP			
Florigiant	651.4	1.812	3.095	136.74	73.74	65.22	37.72
Robut 33-1	417.7	0.840	1.616	69.52	62.70	35.90	42.99
NC 4	582.8	1.460	2.738	114.54	55.00	60.75	43.79
Argentine	405.5	0.964	1.862	79.93	58.79	47.84	50.07
$LSD_{.05}$	124.6	0.353	0.399	19.14	11.08	11.89	9.55

Table 2. Means of traits by strain and date from 1983 Clayton field evaluation.

Strain	Nodule number	Nodule dry wt	Root dry wt	Shoot dry wt	Pod dry wt	Nitrogenase activity (μmole C_2H_4/plant/hour)	Specific activity (μmole C_2H_4 g nod. dry wt/hour)
			(g/plant)				
				60 DAP			
NC92	349.0	0.471	1.045	34.47	0.50	43.10	95.81
Flo1A	285.2	0.368	1.053	37.80	0.52	35.94	100.21
RP182-13	273.0	0.426	1.109	38.96	0.56	42.49	104.00
176A22	229.4	0.358	1.096	36.99	0.39	34.14	98.31
Uninoc.	4.5	0.019	1.336	27.70	0.36	1.44	75.05
$LSD_{.05}$	68.3	0.080	0.119	4.71	0.36	7.97	24.81
				91 DAP			
NC92	539.8	1.110	1.871	71.86	17.42	44.94	41.31
Flo1A	450.0	0.737	1.902	80.61	19.04	33.64	47.72
RP182-13	435.4	0.850	2.029	83.56	18.54	32.82	39.94
176A22	327.6	0.567	1.830	76.82	17.25	21.24	39.77
Uninoc.	15.2	0.074	2.650	69.38	14.94	3.79	43.16
$LSD_{.05}$	79.3	0.171	0.270	14.12	3.73	8.43	12.69
				132 DAP			
NC92	799.1	2.111	2.007	102.27	62.85	62.00	31.02
Flo1A	651.0	1.403	2.328	114.01	68.18	73.07	54.44
RP182-13	596.2	1.581	2.221	113.54	67.23	64.85	41.18
176A22	471.1	1.013	1.979	102.15	68.53	54.82	56.56
Uninoc.	54.2	0.236	3.103	68.96	45.98	7.40	35.00
$LSD_{.05}$	139.3	0.395	0.446	21.40	12.39	13.29	10.68

Cultivars Florigiant and NC 4 generally performed better than Robut 33-1 and Argentine for traits indicative of nitrogen fixation (Table 1). *Bradyrhizobium* strain NC92 produced the most nodules and nodule weight. For the other traits, strain differences were negligible, but all strains produced higher means than the uninoculated control. The significant cultivar x strain interaction for nodule dry weight at 60 DAP could be explained by the fact that strain NC92 produced the greatest nodule weight with cultivars Florigiant, NC 4 and Argentine. With Robut 33-1, strain RP182-13 produced the greatest nodule weight. The interaction at 132 DAP for root weight could be accounted for by the uninoculated plots having the greatest root mass for all cultivars except Argentine. With Argentine, strain RP182-13 produced a larger root mass than the uninoculated treatment.

Clayton and Lewiston 1984

At the Clayton location in 1984, significant differences among cultivars for nodule number and weight, shoot weight, pod weight, and nitrogenase activity were found at the first sampling. Strain differences were significant for all traits except pod weight. The second sampling had significant differences for both cultivars and strains for all traits except specific activity. Differences among cultivars for all traits except nitrogenase activity at 103 DAP were significant at the Lewiston location.

Florigiant and NC 7 had higher means than Robut 33-1 and Argentine for all traits except specific activity (Table 3). Analysis of the means of the traits by strain revealed a large difference between the two locations (Table 4). At Clayton, the uninoculated plots had substantially lower means for all traits except specific activity compared to the inoculated plots. The Clayton field had never been planted in peanuts, so the natural population of *Bradyrhizobium* was low (3). In contrast, at

Lewiston, in a field with a large indigenous population of effective *Bradyrhizobium*, strains produced no significant differences and the uninoculated plots performed as well as the inoculated ones.

Table 3. Means of nitrogen fixation-related traits by cultivar, date and location from 1984 field evaluation of peanut cultivar-*Bradyrhizobium* specificities.

Cultivar	Nodule number	Nodule dry wt (g/plant)	Shoot dry wt (g/plant)	Pod dry wt (g/plant)	Nitrogenase activity (μmole C_2H_4/plant/hour)	Specific activity (μmole C_2H_4 g nod. dry wt/hour)
			CLAYTON--73 DAP			
Florigiant	280.2	1.068	134.01	6.75	41.92	38.76
Robut 33-1	204.3	0.650	81.29	8.63	29.38	41.36
NC 7	337.0	1.047	136.57	8.87	41.85	41.54
Argentine	138.3	0.362	71.71	7.93	18.71	44.60
$LSD_{.05}$	54.3	0.100	14.74	1.53	4.60	7.90
			CLAYTON--109 DAP			
Florigiant	590.8	2.246	197.14	85.56	49.71	25.19
Robut 33-1	425.4	1.437	121.40	95.91	35.56	24.62
NC 7	612.2	2.243	177.92	112.27	49.07	25.38
Argentine	262.5	0.949	113.12	63.49	26.07	25.13
$LSD_{.05}$	85.9	0.272	15.71	11.52	5.02	4.08
			LEWISTON--70 DAP			
Florigiant	519.7	0.931	76.40	3.58	31.67	37.32
Robut 33-1	415.0	0.634	53.06	3.99	26.54	42.60
NC 7	599.2	0.927	81.03	6.40	30.44	32.71
Argentine	298.4	0.455	54.99	8.02	17.51	40.92
$LSD_{.05}$	61.8	0.105	6.20	1.18	3.37	5.87
			LEWISTON--103 DAP			
Florigiant	644.6	1.593	157.96	63.89	39.21	25.07
Robut 33-1	486.6	1.122	98.39	70.81	30.97	28.43
NC 7	563.2	1.180	142.01	79.53	31.63	27.44
Argentine	348.6	0.681	102.74	59.10	18.05	26.82
$LSD_{.05}$	80.9	0.170	12.51	8.89	4.42	2.31

Table 4. Means of nitrogen fixation-related traits by strain, date and location from 1984 field evaluation of peanut cultivar-*Bradyrhizobium* specificities.

Strain	Nodule number	Nodule dry wt (g/plant)	Shoot dry wt (g/plant)	Pod dry wt (g/plant)	Nitrogenase activity (μmole C_2H_4/plant/hour)	Specific activity (μmole C_2H_4 g nod. dry wt/hour)
			CLAYTON--73 DAP			
NC92	348.2	1.076	100.30	8.29	44.24	43.66
Flo1A	398.2	1.176	120.06	7.85	46.63	44.99
RP182-13	214.2	0.789	108.39	7.93	33.07	48.26
176A22	233.8	0.833	101.32	8.05	39.86	52.02
Uninoc.	5.4	0.033	99.42	8.10	1.02	18.78
$LSD_{.05}$	60.7	0.148	16.48	1.71	5.14	8.84
			CLAYTON--109 DAP			
NC92	711.3	2.737	152.31	93.31	63.40	25.94
Flo1A	742.6	2.114	167.23	95.38	48.56	24.02
RP182-13	427.6	1.839	154.76	88.73	44.14	24.71
176A22	467.5	1.828	165.96	95.60	43.17	25.14
Uninoc.	14.6	0.076	121.73	73.52	2.15	24.10
$LSD_{.05}$	96.0	0.304	17.57	12.88	5.61	4.56
			LEWISTON--70 DAP			
NC92	45.8	0.742	67.54	5.92	27.26	41.67
Flo1A	486.6	0.784	70.67	5.40	28.36	36.52
RP182-13	445.0	0.705	64.02	5.61	24.74	37.38
176A22	451.4	0.759	63.20	5.15	25.82	36.62
Uninoc.	448.4	0.693	66.40	5.41	26.51	41.00
$LSD_{.05}$	69.0	0.117	6.93	1.32	3.77	6.57
			LEWISTON--103 DAP			
NC92	509.4	1.205	134.61	75.23	30.25	26.30
Flo1A	566.7	1.274	126.32	66.81	32.77	26.66
RP182-13	520.1	1.049	124.23	70.47	29.85	28.86
176A22	451.5	1.017	112.97	62.34	27.11	27.40
Uninoc.	506.0	1.174	128.26	66.81	29.85	25.47
$LSD_{.05}$	90.5	0.191	13.99	9.94	4.94	2.58

Analysis of the means for the factorial treatments showed that the combinations of cultivar NC 7 and strain NC92 and strain Flo1A produced the highest mean for nodule number and pod weight at Clayton at 60 DAP. Florigiant with Flo1A had the greatest root weight and shoot weight. Argentine with RP182-13 had the highest specific activity. At 91 DAP at Clayton the combination of NC 7 and NC92 had the highest nodule weight and nitrogenase activity. With Robut 33-1, strain NC92 did produce higher means for nodule number and weight, shoot weight, pod weight and specific activity, but not for nitrogenase activity. The highest pod weight recorded was for the NC 7-Flo1A combination.

Yield 1984

Highly significant differences in yield between cultivars and strains for the two locations were noted. Cultivar rankings were the same for the two locations (Table 5). NC 7 had the highest yield followed by Florigiant, Robut 33-1 and Argentine. At Clayton, the *Bradyrhizobium* strains were not different in yield, but all strains gave significantly higher yields than the uninoculated plots. At Lewiston, the uninoculated plots outyielded the inoculated ones. This may have been because the ample native population of *Bradyrhizobium* was both effective and efficient in nodulating and fixing nitrogen with these cultivars and that the inoculants were less effective. At Clayton the combination of NC 7 and RP182-13 gave the greatest yield, while at Lewiston uninoculated NC 7 had the greatest yield (Table 6).

Table 5. Mean yields by cultivars and *Bradyrhizobium* strains from 1984 Clayton and Lewiston field evaluations expressed in kg/ha.

Cultivar/strain	Clayton (kg/ha)	Lewiston (kg/ha)
Cultivar		
NC 7	3343	3279
Florigiant	3077	3076
Robut 33-1	2769	2497
Argentine	2016	2016
$LSD_{.05}$	227	159
Bradyrhizobium strain		
176A22	3021	2570
RP182-13	2943	2862
NC92	2893	2735
Flo1A	2859	2697
Uninoculated	2287	2899
$LSD_{.05}$	255	177

Previous research at North Carolina State University and at ICRISAT indicated that introduced strains of *Bradyrhizobium* did not compete effectively against native strains when the field had adequate levels of the bacterium (3,9). Since the field at Lewiston had a large population of indigenous *Bradyrhizobium* and introduced strains compete poorly for nodulation sites on the peanut root, another explanation of the yield results is possible. If the introduced strains indeed formed few of the nodules on the peanut roots at Lewiston, perhaps an interaction occurred in the soil in which the inoculants antagonized or interfered with the superior native strains. This would account for the uninoculated plots having higher yields at Lewiston, but not at Clayton.

Researchers at ICRISAT have reported that past yield increases seen with the Robut 33-1/NC92 combination may be due to a higher siderophore production by the strain NC92 which would aid the plant's iron nutrition (9). In fields with sufficient iron availability, this advan-

Table 6. Means of yield by cultivar x strain and location from 1984 field evaluation.

Cultivar	Strain	Means of yield (kg/ha) Clayton	Lewiston
Florigiant	NC92	3298	3018
	FlolA	3235	3105
	RP182-13	2949	3042
	176A22	3390	2954
	Uninoculated	2514	3260
Robut 33-1	NC92	2955	2514
	FlolA	3018	2451
	RP182-13	2769	2451
	176A22	2940	2302
	Uninoculated	2153	2769
NC 7	NC92	3484	3354
	FlolA	3158	3167
	RP182-13	3733	3220
	176A22	3547	3198
	Uninoculated	2794	3453
Argentine	NC92	1835	2059
	FlolA	2028	2069
	RP182-13	2318	2022
	176A22	2203	1820
	Uninoculated	1686	2115
$LSD_{.05}$		510	352

tage would be masked. Other factors such as soil nitrogen and moisture also may have prevented expression of a specific cultivar x strain interaction.

These results showed that the nitrogen fixation process was dynamic over the growing season and was influenced by the inoculation treatment. Although no cultivar x strain interaction was seen for fruit yields, several traits measured at the Clayton location had significant interactions. This would suggest that superior combinations of host cultivar and *Bradyrhizobium* strain may be identified and could result in higher yields.

Significant yield increases resulted from inoculation in fields with a low native population of *Bradyrhizobium*, but no yield increase resulted when a field with a high population of the bacteria was inoculated with the strains used in this study. Since this study did not duplicate the significant yield increases reported for the Robut 33-1/NC92 combination, further research is needed to determine the reason the specificity is expressed routinely at some locations but not in the two North Carolina fields.

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