Evaluation of Biological Nitrogen Fixation Capacity in *Arachis* Species and the Possible Role of Polyploidy[1]

H. T. Stalker*, M. L. Nickum, J. C. Wynne, G. H. Elkan and T. J. Schneeweis[2]

ABSTRACT

Arachis species have potential for enhancing cultivated peanut (*Arachis hypogaea* L.) germplasm as forages and cover crops. This study's objective was to evaluate a range of *Arachis* species for biological nitrogen fixation capacity. Several *Arachis* species are tetraploids, and it has been shown that tetraploidy may play an important role in nodule initiation. Species were first tested under natural field conditions and then in the greenhouse using three *Bradyrhizobium* strains that had been previously shown to be effective on peanut. Nodule number, nodule weight, nitrogenase activity determined by acetylene reduction, and shoot dry weight were measured as indicators of nitrogen fixation capacity. In the field, tetraploid species produced significantly more nodules than the diploids, but total dry matter accumulation was independent of the number of nodules or rate of fixation. In the greenhouse, no significant differences were observed among the bradyrhizobial strains. *Arachis hypogaea* and *A. monticola* showed significantly higher measures of nitrogen fixation capacity for all measured traits than the diploid species. However, autotetraploid plants of *A. villosa* did not have significantly more nodules than diploids of the same accession; the autotetraploids consistently had higher nitrogenase activity. *Arachis pusilla* never formed a symbiotic relationship with the bradyrhizobial strains used.

Key Words: *Arachis hypogaea*, wild species, nitrogen fixation, *Arachis*, tetraploid, polyploid.

Many leguminous plants have a symbiotic relationship with nitrogen-fixing bacteria of the genus *Bradyrhizobium*, resulting in sufficient supplies of nitrogen for plant growth. Peanut (*Arachis hypogaea* L.) can be nodulated by many strains of *Bradyrhizobium* isolated from an array of legume species (Date and Halliday, 1980). The capacity to fix nitrogen is significantly different among peanut genotypes (Burton, 1976; Nambiar and Dart, 1980; Wynne *et al.*, 1980) and among strains of *Bradyrhizobium* (Wynne *et al.*, 1978, 1980; Elkan *et al.*, 1980).

Symbiotic nitrogen fixation is important not only in a field crop situation, but also where legumes are grown as forages. Several *Arachis* species have potential as forage crops (IBPGR, 1990). A selection from the species *A. glabrata* Benth. was released as a summer forage legume in Florida (Prine *et al.*, 1981). Another species (*A. pintoi* Krap. et Greg. *nom. nud*) is being used as a forage in Colombia and Australia, and Asakawa and Ramirez (1989) suggested inoculation during sprigging into soil. In addition to growing wild peanuts in pure stands or mixtures with grasses, a need exists in tropical and semitropical areas for supplemental nitrogen sources for plantations and horticultural crops such as peach, coconut, palm, rubber, and grape. Although *Arachis* species have been used extensively as germplasm sources for interspecific hybridization with the cultivated peanut, comparatively little is known about potentials of *Arachis* species as forages or nitrogen sources for other crops.

Identification of *Arachis* species with the capacity to support an efficient symbiotic relationship with *Bradyrhizobium* is important for both cultivar and forage development. Ronguen *et al.* (1988) reported differences in nodulation capacities between *A. villosa* Benth. and *A. stenosperma* Greg. et Greg. *nom. nud.* for two bradyrhizobial strains (NC92 and M30). *Arachis villosa* produced almost twice the dry matter when inoculated with NC92 over the control, whereas *A. stenosperma* showed little deviation among bradyrhizobial treatments.

Several *Arachis* species are polyploids, and it has been shown that tetraploidy may play an important role in nodule initiation. Tetraploid (disomatic) cells have been reported in the roots of many angiosperms. In legumes, too, occasional tetraploid cells are found in root tissue. Wipf and Cooper (1938) observed that 28 chromosomes ($4n$) were found on somatic equatorial plates in the meristematic region of the nodule of red clover instead of the 14 chromosomes ($2n$) found in root tip cells. They reported tetraploidy in nodular tissue of field- and greenhouse-grown vetch (*Vicia sativa* L. var. *angustifolia*), garden pea (*Pisum sativum* L.), red clover (*Trifolium pratense* L.), lespedeza (*Lespedeza tomentosa* Siebold), sweet pea (*Lathyrus latifolius* L.), sweet clover (*Melilotus alba* L.), and alfalfa (*Medicago sativa* L.) (Wipf and Cooper, 1938, 1940; Wipf, 1939). They found occasional polyploid cells in root tissue, but nodule tissue was always polyploid. Wipf (1939) concluded that, when the polyploid series are considered, it appears that the absolute chromosome number is not the significant factor but rather the 2:1 relationship between infected and uninfected tissue. There are some scattered reports, all during the same era, of similar observations by other workers but none involving peanut species. Chromosome numbers associated with bacterial infection in root nodules have received little attention since these cited studies. It has not been well established whether nodules originate in a polyploid cell, or if the abnormal cell division after infection caused polyploid cells to proliferate as often happens in gall formation.

In order to answer these questions, several workers used the mitotic poison colchicine to induce doubling of chromosome numbers in various legumes (Bonnier, 1954; Migahid *et al.*, 1959; Weir, 1961; Elkan, G. H., unpubl. data). They found that colchicine-treated plants produced many more nodules at an earlier time than untreated diploid siblings. Increased nodulation did not necessarily result in increased

[1]The work was supported by the North Carolina Agricultural Research Service, Raleigh, NC and the Peanut CRSP, USAID Grant No. DAN-4048-G-SS-2065-00. Recommendations neither represent an official position or policy of NCARS nor USAID.

[2]Professor, Research Assistant (current address: Dept. of Pomology, Univ. of California, Davis, CA 95616), and Professor, Dept. of Crop Science; Professor and Research Associate, Dept. of Microbiology; respectively, North Carolina State Univ., Raleigh, NC 27695.

*Corresponding author.

nitrogen fixation per plant, however.

A large number of *Arachis* species and many *Bradyrhizobium* strains have been collected and maintained. The objectives of this study were to (1) obtain a preliminary indication of the differences among species at different ploidy levels and (2) estimate potentials for exploiting the nitrogen fixation capacity in *Arachis* species for increased forage or seed yield.

Materials and Methods

Five *Arachis* species—*A. monticola* Krap. et Rig. (K 7264), *A. batizocoi* Krap. et Greg. (K 9484), a corduroy leaf mutant of accession 9484, *A. spegazzinii* Greg. et Greg. *nom. nud.* (GKP 10038), a "small leaf" mutant of accession 10038, *A. duranensis* Krap. et Greg. *nom. nud.* (K 7988), and *A. cardenasii* Krap. et Greg. *nom. nud.* (GKP 10017) - and two cultivars (Florigiant and NC 2) were planted in the field at the Peanut Belt Research Station, Lewiston, NC. Plots consisted of five plants each planted 50 cm apart and 1.8 m between rows in a randomized complete block design with two replicates. Seeds were surface-sterilized in 30% H_2O_2 for 5 min, rinsed in water, pregerminated, planted in an autoclaved 1 sand:1 vermiculite medium, and transplanted into the field on 18 May 1977. Only *Bradyrhizobium* strains already existing in the field were available to the plant for potential infection and nitrogen fixation. No attempt was made to identify existing strains in the field. Five plants per plot were harvested during the last week of September. Roots were detached and placed in Mason jars for measurement of acetylene reduction (Isleib *et al.*, 1980). Plant tops were dried and weighed. After acetylene samples were taken, nodules were removed, counted, dried, and weighed.

Two additional experiments were conducted to evaluate nitrogen fixation capacity of 20 *Arachis* species accessions from five sections of the genus in greenhouses at Raleigh, NC during the summers of 1986 and 1988 (Table 1). Seeds were sterilized in a 40% calcium hypochlorite solution for 10 min, followed by five rinses with water. They were pregerminated and planted in Leonard jars containing a 1 sand:1 vermiculite medium as described by Wynne *et al.* (1978). The reservoirs of the assemblies received 700 mL of Bond's stock salt solution (Burton *et al.*, 1972) and 0.1 ppm Zn, 0.04 ppm Mo, and 0.001 ppm Co, but no nitrogen salts. During plant growth, deionized water was added to the storage compartments as needed.

Bradyrhizobial strains NC92 (originating from the Bolivian *A. hypogaea* cultivar Overo Colorado Blanco Grande), NC70.1 (from the diploid species *A. duranensis* accession GKBSPSc 30071 found in Bolivia), and RP182-13 (an isolate from an *A. hypogaea* cultivar of USA origin) were used as inoculum. The three strains had been previously tested and found to

Table 1. *Arachis* species evaluated for nitrogen fixation potentials.

Acc./ cultivar	Collector[a]	P.I.	Species	Ploidy	Collection site (state, country)
			Section *Arachis*		
NC 7	—	—	*A. hypogaea L.*	4x	USA cultivar
7264	K	219824	*A. monticola* Krap. et Rig.	4x	Jujuy, Argentina
30062	GKBSPSc	468196	*A. monticola*	4x	"
7830	K	261871	*A. correntina* (Burk.) Krap. et Greg. *nom. nud.*	2x	Corrientes, Argentina
10017	GKP	262141	*A. cardenasii* Krap. et Greg. *nom. nud.*	2x	Santa Cruz, Bolivia
10602	GKP	276235	*A. chacoense* Krap. et Greg. *nom. nud.*	2x	Puerto Casado, Paraguay
7988	K	219823	*A. duranensis* Krap. et Greg. *nom. nud.*	2x	Salta, Argentina
22585	Bu	298636	*A. villosa* Benth.	2x	Federacion, Argentina
409	HLK	337308	*A. stenosperma* Greg. et Greg. *nom. nud.*	2x	Parana, Brazil
9484	K	298639	*A. batizocoi* Krap. et Greg.	2x	Santa Cruz, Bolivia
10038	GKP	262133	*A. spegazzinii* Greg. et Greg. *nom. nud.*	2x	Salta, Argentina
30006	GK	468150	*A.* sp.	2x	Mato Grosso, Brazil
30008	KSSc	475986	*A.* sp.	2x	"
30093	GKSSc	468338	*A.* sp.	2x	Santa Cruz, Bolivia
			Section *Erectoides*		
10034	GKP	262142	*A. rigonii* Krap. et Greg.	2x	Santa Cruz, Bolivia
11462	KFC	—	*A. paraguariensis* Chod. et Hassl.	2x	Cordillera, Paraguay
10002	GKP	—	*A.* sp.	2x	Mato Grosso, Brazil
			Section *Ambinervosae*		
12943	GK	338452	*A.* sp.	2x	Ceara, Brazil
12946	GK	338454	*A.* sp.	2x	Pernambuco, Brazil
			Section *Caulorhizae*		
12787	GK	338447	*A. pintoi* Krap. et Greg. *nom. nud.*	2x	Bahia, Brazil
			Section *Triseminalae*		
12922	GKP	338449	*A. pusilla* Benth.	2x	Bahia, Brazil

[a]Collectors are as follows: B = D. J. Banks, Bu = Burkhart, C = C. Cristobal, F = F. Fugarazzo, G = W. C. Gregory, H = R. O. Hammons, K = A. Krapovickas, L = L. Langford, P = J. Pietrarelli, S = C. E. Simpson, and Sc = A. Schinini.

enhance yields for nitrogen fixation parameters in *A. hypogaea* (Wynne *et al.*, 1983; Nambiar and Dart, 1980). Ten mL of inoculum in YAM liquid broth (Norris and Date, 1976) were applied to each Leonard jar, delivering 109 cells per mL. Treatments consisted of 19 *Arachis* accessions plus an *A. hypogaea* check and four inocula (three bradyrhizobial strains and a sterile broth) in a factorial arrangement with four replications planted in a randomized complete block design. Experimental units were a single plant per Leonard jar. Plants were harvested 60 days after planting when plant tops were dried and weighed. The roots were cut from each plant and acetylene reduction was measured. Nodules were then removed from roots, counted, dried, and weighed.

An experiment to test the effects of ploidy on nitrogen fixation was conducted in 1990 using five replications of diploid and autotetraploid *A. villosa* (22585) and *A. hypogaea* cv. NC 7. The same procedures, analyses, and bradyrhizobial strains were used as previously described for greenhouse experiments. Data from the experiments were analyzed by the general linear model procedure of the Statistical Analysis System (SAS, 1982).

Results and Discussion

Significant differences existed between diploids and tetraploids for number of nodules and acetylene reduction rates in the field experiment (Table 2). Florigiant had the greatest number of nodules (2473 per plant), NC 2 and *A. monticola* had significantly fewer nodules, and the diploid species only ranged between 147 for *A. cardenasii* and 497 for *A. spegazzinii*. *Arachis batizocoi* and *A. cardenasii* had very low nitrogen fixation rates at the time of sampling; however, *A. spegazzinii* was not significantly different from *A. monticola* and NC 2 (Table 2). Thus, when a complex of *Bradyrhizobium* species are present in the field, both diploid and tetraploid taxa in section *Arachis* have the genetic capacity to fix nitrogen. Florigiant had an extremely low acetylene reduction rate which was likely due to the late sampling date.

Shoot dry weight was not correlated with nodulation or acetylene reduction rates. *Arachis cardenasii*, *A. duranensis*, and the normal *A. batizocoi* leaf type had approximately the same or even more dry matter accumulation as *A. hypogaea* or *A. monticola*. Either the nodules on the diploid species had a greater efficiency for nitrogen fixation during the growing season or the taxa more efficiently used the already existing nitrogen in the soil than the two tetraploids to account for the greater dry weight accumulation. These data may be skewed toward the wild species versus *A. hypogaea*, however, because pod yield was not compared since wild species have fragile pegs which make harvesting difficult. A large percentage of the total dry weight of *A. hypogaea* is partitioned into reproductive development, versus a lesser amount in other *Arachis* species which have relatively fewer and smaller pods and seeds. Regardless, the experiment gave a preliminary indication that both diploid and tetraploid species of *Arachis* have the capacity for nitrogen fixation and sufficient quantities of nitrogen can be fixed for plant growth.

To better understand interactions and potentials of nitrogen accumulation and effects of rhizobial strains on different species, two greenhouse experiments were conducted using taxa in five sections of the genus and three bradyrhizobial strains. Significant differences were not observed among strains for any measure of nitrogen fixation even though two strains originated from *A. hypogaea* plants and a third strain came from a diploid accession of *A. duranensis*. Data for measures of nitrogen fixation are thus reported as averages among strains for each genotype. Conversely, significant year interactions were observed among genotypes; thus data for the 1986 and 1988 experiments are reported separately. Most species produced nodules when bradyrhizobial strains were applied, and control plants did not produce nodules.

Twenty genotypes were evaluated during 1986 in the greenhouse. Nodule numbers per plant among diploid species of *Arachis* ranged from 0 for *A. pusilla* (12922) to 205 for for *A.* sp. acc. 30006. This latter highly nodulating species was not significantly different from NC 7. *Arachis monticola* did not have significantly greater numbers of nodules than NC 7; however, individual nodules on *A. monticola* plants were less than half the size as for *A. hypogaea*. The smallest nodules were observed on roots of the erectoid species *A. paraguariensis* (acc. 11462), which

Table 2. *Arachis* species evaluated in the field for nitrogen fixation capacity.

Species	Accession/cultivar	Nodule no.	Shoot weight (g)	Nitrogenase activity ($\mu M\ C_2H_4$/pl/hr)
A. spegazzinii	10038 large leaf	479c*	124cd	18.36bc
	10038 small leaf	352c	104d	8.65cd
A. duranensis	7988	375c	231a	6.59cd
A. batizocoi	9484	312c	177b	3.05d
"	9484 corduroy leaf	263c	128cd	1.04d
A. cardenasii	10017	147c	172b	2.12d
A. monticola	7264	1436b	183ab	39.32a
A. hypogaea	NC 2	1655b	143cd	23.32b
"	Florigiant	2473a	173b	1.60d

*Means followed by the same letter in a column are not significantly different at the 5% level of probability according to a Waller-Duncan multiple range test.

weighed only 0.16 mg/nodule. Most of the diploid species grew slower than the tetraploids as indicated by significantly less shoot dry weight at the time of harvest. This was opposed to field tests where several species accumulated as much or more dry matter over the growing season as *A. monticola* and *A. hypogaea*. The inference is that either the correct rhizobial strains were not used in the greenhouse experiment for efficient nitrogen fixation, that the diploid species maintained their capacity to fix nitrogen in the field longer than the tetraploids, or diploid species more efficiently utilize soil nitrogen. The strain RP182-13 was previously found to have a high mean for all traits in symbiosis with six *A. hypogaea* cultivars (Wynne *et al.*, 1983), but the more diverse *Arachis* species obviously react differently to this strain. Acetylene reduction values were also very low for most species, although *A. stenosperma* (acc. 409) was similar to *A. monticola* (Table 3). The generally low nitrogen fixation rate was observed in species of all four *Arachis* sections. For the 1986 experiment, correlations among number of nodules, nodule weight, plant weight, and acetylene reduction ranged between 0.71 and 0.87.

Seed germination for several accessions was poor in the 1988 experiment, and only 16 genotypes were tested for nitrogen fixation capacity (Table 4). Nodules were not observed in check plants. The tetraploid species *A. hypogaea* and *A. monticola* produced significantly greater numbers of nodules than diploid species when bradyrhizobial strains were added to the soil. A wide range of nodule sizes was again observed as estimated by nodule weight (Table 4). NC 7 had nodules which weighed nearly twice as much as other species, while the smallest nodules were observed in *A.* sp. acc. 12943. Acetylene reduction values were highest in *A. monticola* and *A. hypogaea*. Although most diploid species had very low acetylene reduction rates, *A. spegazzinii* was similar to NC 7. Sufficiently high numbers of nodules were apparently present in several species to fix nitrogen, but either nodules had very low activity levels or they were inactive at the time of sampling. For example, *A. cardenasii* averaged 160 nodules, but the acetylene reduction rate across strains only averaged 1.9 μmoles C_2H_4/plant/hour. Similar responses were observed for accessions 12943, 22585, and 30006. As was found in 1986, *A. pusilla* (acc. 12922) neither produced nodules nor fixed nitrogen.

A comparison of Tables 3 and 4 indicates that the ranking among genotypes in the two experiments is in general agreement with *A. hypogaea* and *A. monticola* at the top and *A. pusilla* at the low end for all measured traits. Variation among the entries was observed; however, *A. cardenasii* produced very few nodules during 1986 but was not significantly different from most of the other species during 1988. A similar pattern, except in reverse, was observed for *A. stenosperma*. The conflicting results are likely due to unknown environmental differences even though both tests were conducted in the same greenhouse.

The results of the combined experiments suggest that many diploid species of *Arachis* can establish a symbiotic relationship with bradyrhizobia and fix nitrogen. The amount of fixation is highly dependent upon the species, or perhaps even accessions within species. Interspecific crosses between diploid taxa and *A. hypogaea* would likely have a suppressed nitrogen fixation capacity, but the potential exists to restore high nitrogen fixation rates in advanced generation hybrids as they become more like *A. hypogaea*.

When *A. villosa* autotetraploids and diploids were compared, no significant differences were observed for number of nodules per plant (Table 5). NC 7 had significantly more nodules than *A. villosa* for strains NC70.1 and NC92, but not for RP182-13. Nitrogenase activity, however, was at

Table 3. Nodule numbers, shoot and nodule weights, and nitrogenase activity of 20 *Arachis* species averaged across three *Bradyrhizobium* strains in 1986.

Species	Accession/cultivar	Nodule no.	Nodule weight (mg)	Shoot weight (g)	Nitrogenase activity (μM C_2H_4/pl/hr)
A. sp.	30006	205bc*	211cd	5.7bcd	5.7cd
A. stenosperma	409	160cd	263bc	8.6b	14.1b
A. rigonii	10034	138c-f	109fg	3.7cde	5.7cd
A. duranensis	7988	123def	143def	8.4b	9.2c
A. sp.	30008	120def	170c-f	7.4b	8.1c
A. batizocoi	9484	100d-g	112fg	7.8bc	4.1def
A. sp.	12943	74e-h	37gh	4.5ef	1.5ef
A. paraguariensis	11462	68fgh	11h	1.7ef	0.7ef
A. chacoense	10602	64f-i	73efg	1.9ef	5.9cd
A. sp.	12946	64f-i	40gh	5.6bcd	3.7def
A. villosa	22585	60f-i	35gh	8.0ef	2.0def
A. sp.	30093	57f-i	72fgh	5.6bcd	3.7def
A. pintoi	12787	51ghi	52fgh	2.7def	3.4def
A. spegazzinii	10038 lg. leaf	43ghi	37gh	2.9def	2.4def
A. sp.	10002	41ghi	25gh	1.7ef	0.9ef
A. correntina	7830	32hi	24gh	1.6ef	1.7ef
A. cardenasii	10017	31hi	23gh	0.3f	0.1f
A. pusilla	12922	0i	0gh	0.1f	0.0f
A. monticola	30062	286a	314b	14.4a	14.1b
A. hypogaea	NC 7	227ab	608a	15.3a	19.0a

*Means followed by the same letter in a column are not significantly different at the 5% level of probability according to Waller-Duncan multiple range test.

Table 4. Nodule number, shoot and nodule weight, and nitrogenase activity of 16 *Arachis* species averaged across three *Bradyrhizobium* strains in 1988.

Species	Accession/cultivar	Nodule no.	Nodule weight (mg)	Shoot weight (g)	Nitrogeanase activity (μM C_2H_4/pl/hr)
A. sp.	30006	207b*	76def	2.9fgh	3.2f
A. duranensis	7988	181bc	125bc	10.0c	7.3cd
A. sp.	30093	168bcd	94cd	5.2de	4.9de
A. batizocoi	9484	162b-e	110c	10.4c	6.0cde
A. cardenasii	10017	160b-e	68de	1.7ghi	1.9fg
A. rigonii	10034	154b-f	64de	4.1def	7.6cd
A. correntina	7830	120c-g	66de	2.8f-i	4.0ef
A. spegazzinii	10038 lg. leaf	108d-g	56ef	4.1def	8.0bc
A. chacoense	10602	92efg	52ef	1.9g-j	3.9ef
A. stenosperma	409	82fg	47ef	3.2efg	5.2cde
A. sp.	12943	77g	25fg	1.1hij	1.3fg
A. villosa	22585	58gh	29fg	0.8ij	1.9fg
A. pusilla	12922	0h	0g	0.1j	0.0g
A. monticola	30062	338a	150b	13.0b	11.2a
A. hypogaea	NC 7	284a	243a	18.4a	10.2ab

*Means followed by the same letter in a column are not significantly different at the 5% level of probability according to a Waller-Duncan multiple range test.

least three times higher in autotetraploids versus diploids; and additional significant increases were observed for cultivar NC 7. Because nodules serve as a photosynthetic sink which can use up to 25% of the plant's energy production (Imsande, 1981), larger and more efficient nodules are desirable. This experiment indicates that some alteration of nitrogen fixation capacity may be obtained through the rhizobia-genotype interaction as, in this case, via polyploidy. An interesting observation is that strain NC70.1 produced more nodules on NC 7 than the other two strains, but it also had the lowest nitrogenase activity. On the other hand, infection from RP182-13 resulted in a nodule number of ca. 20% of that observed for NC70.1 but nearly twice the nitrogenase activity. For tetraploid *A. villosa*, the strains used neither differed in nodule number nor nitrogenase activity. Additional testing may be warranted to understand the effects of polyploidy on nodulation and nitrogen fixation in *Arachis*.

Table 5. Nodule numbers and nitrogenase activity for diploid and autotetraploid *A. villosa* for three bradyrhizobial strains.

Strain	*2x A. villosa*	*4x A. villosa*	NC 7
	Avg no. nodules observed genotype		
NC70.1	33b*	46b	251a
NC92	62b	47b	169a
RP182-13	42a	49a	52a
Control	0	0	0
	Avg nitrogenase activity (mM C_2H_4/plant/hr) observed/genotype		
NC70.1	5.6c	14.7b	46.5a
NC92	0.4c	10.2b	70.3a
RP182-13	4.7c	14.6b	89.3a
Control	0	0	0

*Means followed by the same letter in a row are not significantly different at the 5% level of probability based on the Waller-Duncan multiple range test.

Using the *Arachis* species for cover crops or grazing, with the additional benefit of supplying nitrogen in mixtures or to subsequent crops, has potential. However, the species with the highest nitrogen fixation capacity may not be well suited for grazing. For example, *A. batizocoi* and *A. duranensis* are annuals and long-term establishment would likely depend upon annual re-establishment with seed. Several other peanut species produced little nitrogen and thus would not be suitable sources for supplemental nitrogen. Fortunately, several species appeared to nodulate when exposed to a complex of rhizobial strains found in a field situation as opposed to single isolates in the greenhouse. Because of the varying results in nodulation and nitrogen fixation among species, taxa with favorable growth or foliage attributes as forage or cover crops should be also evaluated for nitrogen fixation capacity.

Literature Cited

1. Asakawa, N. M., and C. A. Ramirez. 1989. Metodologia para la inoculacion y siembra de *Arachis pintoi*. Pasturas tropicales 11:24-26.
2. Bonnier, C. 1954. Action de la colchicine sur la symbiose *Rhizobium Mesicago sativa* et *Rhizobium-Trifolium pratense*. Bull. Inst. Agron. et Stas. Recherches Gembloux. 22:167-178.
3. Burton, J. C. 1976. Pragmatic aspects of the *Rhizobium* leguminous plant association, p. 429-446. *In* W. E. Newton and C. J. Nyman (eds.), Proc. 1st Int. Symp. Nitrogen Fixation. Vol. 2. Washington State Univ. Press, Pullman.
4. Burton, J. C., C. J. Martinez, and R. L. Curley. 1972. Methods of testing and suggested standards for legume inoculants and preinoculated seed. Nitragin Sales Corp., Milwaukee, WI.
5. Date, R. A., and J. Halliday. 1980. Relationships between *Rhizobium* and tropical forage legumes, p. 597-602. *In* R. J. Summerfield and A. J. Bunting (eds.), Advances in Legume Science. Royal Botanic Gardens, Kew.
6. Elkan, G. H., J. C. Wynne, T. J. Schneeweis, and T. G. Isleib. 1980. Nodulation and nitrogenase activity of peanuts inoculated with single strain isolates of *Rhizobium*. Peanut Sci. 7:95-97.
7. Imsande, J. 1981. Exchange of metabolites and energy between legumes and *Rhizobium*, p. 179-189. *In* K. L. Giles and A. G. Atherly (eds.), Biology of the Rhizobiaceae. Vol. 13. Academic Press, New York.

8. International Board of Plant Genetic Resources (IBPGR). 1990. International crop network series 2. Report of a workshop on the genetic resources of wild *Arachis* species. Including preliminary descriptors for *Arachis* (IBPGR/ICRISAT). Rome.
9. Isleib, T. G., J. C. Wynne, G. H. Elkan, and T. J. Schneeweis. 1980. Quantitative genetic aspects of nitrogen fixation in peanuts *(Arachis hypogaea* L.). Peanut Sci. 7:101-105.
10. Migahid, A. M., A. F. El Nady, and A. M. Abd. El Rahman. 1959. The effect of gamma radiation on bacterial nodule formation. Plant and Soil 9:139-144.
11. Nambiar, P. T. C., and P. J. Dart. 1980. Studies on nitrogen fixation by groundnut at ICRISAT, p. 110-124. *In* ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Proc. Int. Workshop Groundnuts. Patancheru, A.P., India.
12. Norris, D. O., and R. A. Date. 1976. Legume bacteriology, p. 146. *In* N. H. Shaw and W. W. Bryan (eds,), Legume Bacteriology. Bull. No. 51, Commonwealth Agricultural Bureau. Alden Press, CSIRO, Oxford.
13. Prine, G. M., L. S. Dunavin, J. E. Moore, and R. D. Roush. 1981. "Florigraze" rhizoma peanut. A perennial forage legume. Univ. Florida Circ. S-275. 22 p.
14. Rongwen, J., Z. Rong, J. Moulan, and Z. Xuejiang. 1988. Evaluation of the symbiotic potential of some wild *Arachis* species with two *Rhizobium* strains. Internat. *Arachis* Newslet. 4:23-24.
15. SAS Institute, Inc. 1982. SAS User's Guide: Statistics. Cary, NC.
16. Weir, J. B. 1961. The effect of colchicine and indolyl acetic acid on diploid and tetraploid strains of red white clovers in aseptic and pot culture. Plant Soil 14:187-196.
17. Wipf, L. 1939. Chromosome number in root nodules and root tips of certain Leguminosae. Bot. Gaz. 101:51-67.
18. Wipf, L., and D. C. Cooper. 1940. Somatic doubling of chromosomes and nodule infection in certain Leguminosae. Amer. J. Bot. 27:821-824.
19. Wynne, J. C., G. H. Elkan, T. G. Isleib, and T. J. Schneeweis. 1983. Effect of host plant, *Rhizobium* strain and host x strain interaction on symbiotic variability in peanut. Peanut Sci. 10:110-114.
20. Wynne, J. C., G. H. Elkan, and T. J. Schneeweis. 1980. Increasing nitrogen fixation of the groundnut by strain and host selection, p. 95-109. *In* ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Proc. Int. Workshop Groundnuts. Patancheru, A.P., India.
21. Wynne, J. C., G. H. Elkan, T. J. Schneeweis, T. G. Isleib, C. M. Preston, and C. A. Meisner. 1978. Increasing nitrogen fixation of the peanut. Proc. Amer. Peanut Res. Educ. Assoc. 10:22-29.

Accepted April 2, 1994