Peanut Science (1983) 10:107-110

Effect of Leaf Removal on Symbiotic Nitrogen Fixation in Peanut[1]

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ABSTRACT

The effect of varying levels of leaf defoliation on the nodulation and nitrogen fixation of a virginia and a spanish-type peanut (*Arachis hypogaea* L.) cultivar was investigated in the greenhouse. Five leaf defoliation treatments – (a) control (no leaflet removal), (b) 25%, (c) 50%, (d) 75%, and (e) 100% – were carried out every 3 to 5 days throughout the growing period.

All nitrogen fixation measurements were affected by the defoliation (leaflet removal) treatments. Increased leaf defoliation reduced nodule formation and N_2 fixation. The reduction in plant dry weight, nodule number, nodule dry weight, and nitrogenase activity was most severe for the 100% defoliation treatment. Correlation coefficients (r) of N_2 fixation measurements and leaf areas and weights were highly significant.

The defoliation effect was similar in both experiments and cultivars; however, the virginia type achieved higher values for all N_2 fixation measurements.

Key Words: *Arachis hypogaea*, nodulation, defoliation, leaf area.

Nitrogen fixation influences the yield and quality of leguminous plants. Compatible bacteria and hosts can initiate nodulations, but adequate energy supplies are required for nodule growth and sustained nodule function. Carbohydrates are translocated from their source to nodules to support respiration and growth and to supply energy for N_2 fixation.

Schubert and Ryle (9) stated that N_2 fixation in legume nodules is an energy-intensive process and may utilize between 10 and 30% of the total net photosynthate produced by the host plant. Shulyndin (10) noted that infectivity of different soybean cultivars by *Rhizobium japonicum* L. largely depends on the accumulation of carbohydrates in the plant tissue. Soybeans with high carbohydrate content form larger and more numerous nodules. The data presented by Fred and Wilson (5), Herridge and Pate (7), and Minchin and Pate (8) showed a positive correlation between N_2 fixation and the photosynthetic and respiratory functions of the leguminous plant.

Hardy and Havelka (6) stated that factors that decrease the amount of photosynthate available to the nodule decrease N_2 fixation. Decreased light intensity, decreased photosynthate due to partial defoliation, high planting density and lodging, increased demand of competitive sinks during late seed development, and cessation of translocation to nodules by girdling all reduced N_2 fixation. Factors that increase the amount of photosynthate available to the nodule increased N fixation; for example, increased light intensity, increased source size by grafting additional foliage and low plant density, decreased demand of competitive sinks through pod removal, and increased photosynthesis by CO_2 enrichment of the foliar canopy.

Because the leaf is the basic photosynthetic organ and is the major source of photosynthetic assimilates to the nodules, studies have been conducted to investigate the effect of defoliation on nitrogen fixation in some legumes. The effect of severe defoliation was reported by Brun (2) in soybean. He found that partial defoliation of soybean plants after flowering reduced N_2 fixation from 125 to 100 kg N/ha.

Information is lacking on the effects of leaf defoliation on N_2 fixation in peanut (*Arachis hypogaea* L.). This study was designed to determine the effect of leaf defoliation on nodulation and nitrogen fixation.

Materials and Methods

Two peanut cultivars, NC 7 (virginia type, ssp. *hypogaea* var. *hypogaea*) and Argentine (spanish type, ssp. *fastigiata* var. *vulgaris*), were selected for this study. Plants were grown in modified Leonard jars (13). The jars and a 1:1 sand:vermiculite media were autoclaved before use to ensure freedom from rhizobial contamination and/or pathogens.

Peanuts were pregerminated and placed 25 mm below the surface of the media in the jars. Before seeds were covered, a 10-ml suspension from a four rhizobial strain mixture was applied and the seeds and inoculum were covered with sand. Strains used were NC7.1, 32H1, CB756 and NC6. The jars were watered through a glass tube into the bottom storage jar. The study was conducted twice. Treatments for each run were replicated four times, with the exception of NC 7 which was only replicated twice in the second experiment. The jars were arranged in a randomized complete block design in the greenhouse. A 250-ml volume of nutrient solution was added three times during the growing period. The nutrient solution consisted of Bond's stock salt mixture (3) supplemented with micronutrient stock solution minus nitrogen.

Fifteen days after planting the following defoliation treatments were applied to both cultivars:

(a) No leaflets removed (0% defoliation).
(b) Removal of one basal leaflet from each tetrafoliate leaf leaving about 75% of the leaf area (25% defoliation).
(c) Removal of two basal leaflets from each tetrafoliate leaf leaving about 50% of the leaf area (50% defoliation).
(d) Removal of three leaflets from each tetrafoliate leaf, leaving about 25% of the leaf area (75% defoliation).
(e) Removal of all leaves leaving only the stem and branches (100% defoliation).

Leaflet removal at the junction of the rachis and the leaflet was carried out every 3 to 5 days throughout the growing period.

After 58 to 60 days the plants were harvested. Nitrogenase activity was measured for each plant using acetylene reduction methodology. Nodules were removed and counted. Dry weight of the nodules and the plant were measured. Mean nodule weight and specific activity per nodule were calculated. Leaf weight was measured and leaf area was determined using the disc method (11). An analysis of variance was conducted for each trait and product-moment correlations among the traits were computed.

Results and Discussion

Both cultivar and defoliation (leaflet removal) influ-

[1]Paper No. 8861 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27650. This research was partially supported by USDA-SEA-CR grant 801-15-93 under A.I.D. PASA AG/TAB 610-9-76.

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enced nodulation and nitrogen fixation. The two cultivars were significantly different for both runs for nodule weight (NW), nodule number (NN), nitrogenase activity (NA), dry weight of plant (PDW), and leaf weight (LW) (Table 1). The virginia cultivar NC 7 had more and heavier nodules, greater NA and a higher plant dry weight than the spanish cultivar Argentine. These results agree with previous studies reported by Wynne *et al.* (13). PDW, NN, NW and NA of NC 7 were about twice as great as the same traits for Argentine (Table 2). Specific activity was similar for both cultivars in the first experiment but higher for Argentine in the second experiment, while mean nodule weight (MNW) was not significantly different in the two experiments. This indicated that the higher NA of NC 7 was not due to either more activity per nodule or larger nodule size, but due to the sum of the overall activity resulting from the higher number of nodules. Leaf area and leaf weight of NC 7 were at least twice that for Argentine. The better performance in nitrogen-fixing traits of NC 7 may be attributed to its greater leaf area and leaf weight and consequently better photosynthetic capacity that produced more carbohydrates to satisfy the demand of the nodules.

Table 1. Significance of traits indicative of nitrogen fixation, leaf area, and leaf weight from analysis of variance.

Source	Test	df	Nodule weight	Nodule number	Nitrogenase activity	Mean nodule weight	Specific activity	Plant dry weight	Leaf area	Leaf weight
			mg		μM C_2H_4/hr/root	mg	μM C_2H_4/hr/nodule	g	cm^2	g
Cultivar (C)	1	1	**	**	**	ns	ns	**	**	**
	2		**	**	*	ns	**	**	ns	**
Defoliation (D)	1	4	**	**	**	**	*	**	**	**
	2		**	**	**	**	**	**	**	**
Linear (L)	1	1	**	**	**	**	ns	**	**	**
	2		**	**	**	**	**	**	**	**
Quadratic (Q)	1	1	ns	ns	ns	ns	ns	*	ns	**
	2		ns	**	ns	ns	**	ns	*	*
C x D	1	4	*	*	ns	ns	ns	**	**	**
	2		**	**	ns	*	**	ns	ns	*
C x L	1	1	*	**	ns	ns	ns	**	**	**
	2		**	**	ns	*	**	ns	ns	**
C x Q	1	1	ns	ns	ns	ns	ns	ns	ns	ns
	2		ns	**	ns	ns	**	ns	ns	ns

*,**Denote significance levels at $p = 0.05$ and 0.01, respectively. ns denotes nonsignificance.

Table 2. Cultivar and defoliation means for different traits.

Cultivar	Test	Nodule weight	Nodule number	Nitrogenase activity	Mean nodule weight	Specific activity	Plant dry weight	Leaf area	Leaf weight
		mg		μM C_2H_4/hr/root	mg	μM C_2H_4/hr/nodule	g	cm^2	g
Argentine	1	142	127	5.08	0.99	0.039	3.26	237	1.34
	2	46	49	10.72	0.74	0.193	1.55	190	0.54
NC 7	1	337	263	10.18	1.14	0.045	6.40	468	2.64
	2	121	144	13.86	0.58	0.083	2.72	319	1.15
Defoliation (%):									
0	1	460	324	13.47	1.37	0.042	10.26	773	4.62
	2	148	132	24.16	1.20	0.215	4.11	676	1.88
25	1	394	317	8.07	1.20	0.028	7.16	517	2.97
	2	109	112	17.90	1.01	0.198	3.07	298	1.25
50	1	228	196	10.27	1.14	0.053	4.19	314	1.58
	2	77	101	11.67	0.96	0.207	1.57	179	0.54
75	1	106	112	6.00	0.98	0.051	2.10	158	0.73
	2	20	39	4.54	0.41	0.132	0.47	11	0.04
100	1	11	26	0.33	0.64	0.024	0.45	0	0
	2	1	20	0.56	0.04	0.029	0.48	0	0

The effects of defoliation on nitrogen-fixing traits were clearly evident (Table 1). The greatest PDW, NN, NW, NA, SA, and MNW were produced by the control treatments where no leaflets were removed (Table 2). Overall, the effects of complete leaflet removal were more drastic than those of partial defoliation where only 25, 50 or 75% of the leaves was removed. In the less severely defoliated treatments, *e.g.,* 50 or 25%, the PDM, NN, NW, NA, and MNW were higher than the 100% defoliation treatment, but were still significantly lower than the control. The nodules from the plants of the 100% defoliation treatments had far less nitrogen-fixing capacity than nodules from nondefoliated or partially defoliated plants. It seems likely that the defoliated plants lacked assimilates for nodules which resulted in lower N_2 fixation. Partially defoliated plants provided a limited amount of assimilates to their roots; therefore, their nitrogen-fixing capacity was not as reduced as that of plants of the 100% defoliation treatments.

Photosynthetic supply depends mainly on leaf area (1, 4, 12); therefore, defoliation decreases photosynthetic efficiency and assimilates translocation among plant parts. These factors affect dry matter accumulation differentially in plant tissues. The decrease in leaf area and weight by defoliation limited the supply of photosynthetic products which are used as structural compounds of developing nodules (Table 2). Thus nitrogen-fixing capacity was decreased. Boote *et al.* (1) confirmed the negative effect of defoliation in photosynthetic activity of peanuts. They found that removal of 25% of the total LA reduced CO_2 uptake 14% and the canopy carbon exchange rate by 35%. However, removal of leaflets also reduces one of the sinks for nitrogen. Thus N_2 fixation could also be limited by reduced N_2 movement out of the nodule.

Partitioning the treatments sum of squares indicated a significant linear response to defoliation for most N_2 fixation traits. A quadratic response was not significant except for PDW in the first run and NN in the second run. The linear response indicates that defoliation, no matter how small, produces an effect on N_2 fixation traits. Maximum N_2 fixation occurred at the 0 level of defoliation and the minimum was at 100% defoliation (Figures 1 and 2). The situation where both linear and quadratic response was significant (Figure 1d) suggested that the virginia-type cultivar NC 7 can sustain up to 10% defoliation without a decrease in NN. A quadratic response (Figure 2c) suggests that at a higher level of defoliation (more than 90%), dry matter production almost ceased in the spanish cultivar Argentine.

In both runs there was a significant cultivar x defoliation interaction for all traits except NA in the first run. The significant interaction for these traits resulted from the cultivars responding differently at low levels of defoliation (0, 25%), and to performing equally at high levels of defoliation (75, 100%). For NA in the first run, the difference between the two cultivars was almost constant regardless of the defoliation level (Figure 2a). Cultivar x linear interaction was found to be significant for most traits, while the cultivar x quadratic interaction was nonsignificant for almost all traits (Table 1).

The N_2 fixation traits were positively and significantly correlated indicating that these traits were highly interrelated (Table 3). The highest correlations observed were between LA and PDW (r = 0.99, 0.81), LW and PDW (r = 0.99, 0.97) and LA and LW (r = 0.99, 0.83). Positive and highly significant correlations were

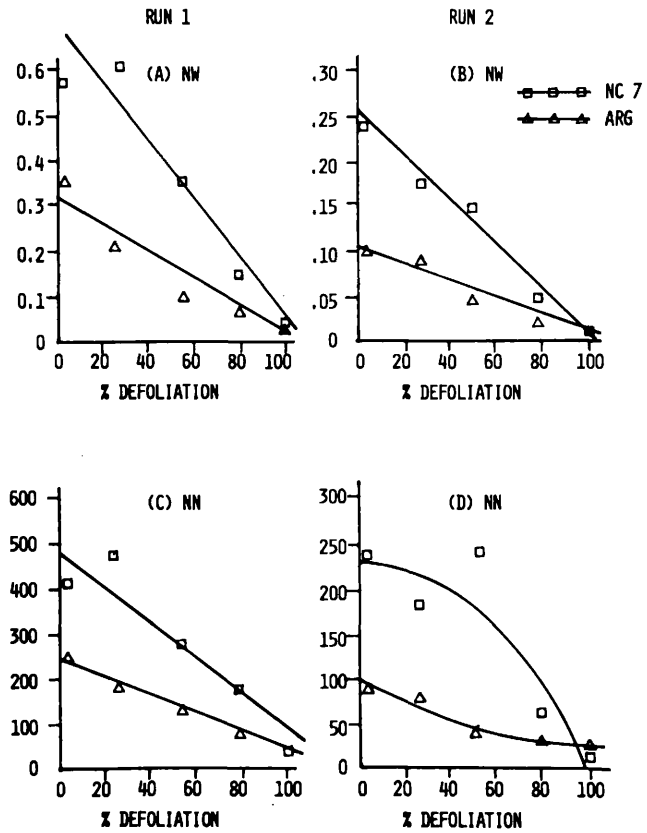

Fig. 1. Response of (A,B) nodule weight (NW) and (C,D) nodule number (NN) to defoliation.

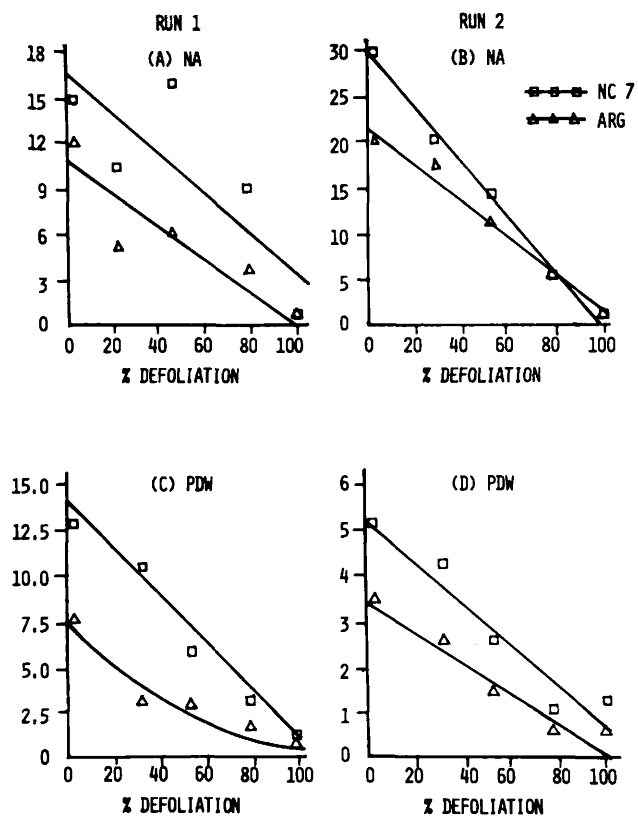

Fig. 2. Response of (A,B) nitrogenase activity (NA) and (C,D) plant dry weight (PDW) to defoliation.

observed between LA, LW and the N_2 fixation traits. NN was found to be the most highly correlated with NW followed by NA and LW, NA and MNW. These highly significant correlations between LA, LW and N-fixation traits indicated that nodulation failure or success was strongly associated with the photosynthetic system.

Table 3. Correlation coefficients among nitrogen fixation traits, leaf area, and leaf weight.

	Test	Nodule number	Nitrog-enase activity	Mean nodule weight	Specific activity	Plant dry weight	Leaf area	Leaf weight
Nodule weight	1	.94**	.62**	.72**	-.24	.88**	.82**	.85**
	2	.92**	.86**	.59**	-.31	.91**	.77**	.92**
Nodule number	1		.65**	.54**	-.22	.87**	.84**	.84**
	2		.68**	.32	-.24	.75**	.63**	.79**
Nitrogenase activity	1			.49**	.49**	.69**	.69**	.66**
	2			.69**	.48**	.92**	.78**	.95**
Mean nodule weight	1				-.05	.66**	.62**	.62**
	2				.68**	.62**	.57**	.55**
Specific activity	1					-.16	-.12	-.17
	2					-.29	-.24	-.26
Plant dry weight	1						.99**	.99**
	2						.81**	.97**
Leaf area	1							.99**
	2							.83**

**Significant at $p = 0.01$.

In order to achieve a major increase in N fixation, attention must be focused on practical approaches that lead to improvement in energy supply. In this respect, maintaining a high and efficient photosynthetic area throughout the growing period seems to be important. This could be obtained through different practices to improve photosynthetic conditions and to increase source size (LA) (*e.g.*, optimum plant density, water supply, nutrient supply, etc.). Effective control of insects and diseases also enhances the plant's ability to maximize N_2 fixation.

However, Schubert and Ryle (9) stated that the rate of nitrogen fixation is controlled by more than just the availability of photosynthate for energy. There are interactions between nitrogen availability and the portion of photosynthate into various sinks but the mechanisms of the control functions are unknown. When we better understand these complex control mechanisms in the legume-rhizobium symbiosis,we may be better able to increase nitrogen fixation and the yield potential of leguminous crops.

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Accepted December 10, 1983