Peanut Science (1980) 7, 114-119

The Effects of Irrigation, Inoculants and Fertilizer Nitrogen on Peanuts (*Arachis hypogaea* L.). I. Nitrogen Fixation.

V. M. Reddy* and J. W. Tanner[1]

ABSTRACT

Effects of irrigation, inoculants and fertilizer nitrogen (N) on $N_2(C_2H_2)$ fixation in peanuts were studied in 1976 and 1977 at Delhi, Ontario. Inoculant application increased nodulation and N-fixation in both years. Powdered peat and granular formulations containing the same strains of rhizobia resulted in almost the same amounts of nodulation and N-fixation (80 kg/ha on average). Differences in nodulation from inoculants containing different strains of rhizobia were not consistent over the two years. However, 60% difference in N-fixation resulted from the inoculants containing different strains of rhizobia in both years. Nitrogen application decreased the nodule number, nodule dry weight, and N-fixation of all the inoculated peanuts. Irrigation increased the N-fixation of the peanuts treated with granular inoculant in 1976 by an average of 45% and all the inoculated peanuts in 1977 by an average of 54% but had no effect on nodulation in either year.

Key Words: Peanuts, Nitrogen, Fixation, Irrigation.

A number of investigators have obtained nodulation responses to inoculation in soils where peanuts had not been grown previously (13, 16). Recent reports in a peanut trade journal have claimed that granular inoculants increased the yield of peanuts in locations where no response could be obtained from peat inoculants (1, 9, 10). Although the granular inoculant supplies many more bacteria per seed than peat inoculant (10) and is easy to handle (9), experimental data are lacking to compare the effects of these two forms of inoculant on nodulation and nitrogen fixation.

Moisture stress has been shown to affect the occurrence, growth, and survival of nodule bacteria, nodule formation, and the functioning of the formed nodule (17, 11, 4, 14). However, Shimshi *et al.* (14) did not find any effect of frequency of irrigation on nodulation of peanuts, and suggested that multiplication of and infection by bacteria might take place during the short periods of high soil moisture immediately after irrigation.

Combined nitrogen affects almost all of the phases of legume-*Rhizobium* symbiosis. Inhibitory effects of combined nitrogen on root hair infection, nodule initiation, and nodule development and function are well established (5, 17). However, there seem to be differences in inoculation response to combined nitrogen among strains of rhizobia, plant species, form of combined nitrogen, seasonal conditions, and the interaction among these factors (5, 17).

[1]Department of Crop Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

[2]The Nitragin Company, Milwaukee, Wisconsin.

The feasibility of growing peanuts in southern Ontario is being tested. The objectives of these experiments were to determine whether inoculation of peanuts was necessary, what form of inoculant would perform best, and how irrigation and nitrogen fertilization would affect the nitrogen fixation system in the southern Ontario environment.

Material and Methods

1976 Experiment: The experiment was conducted on a farm adjacent to the Agriculture Canada Tobacco Research Station, Delhi, Ontario, on a Fox loamy sand soil with pH 6.1 and medium to high phosphorus and potash. A basal application of 448 kg/ha of 3-9-18 analysis fertilizer was made on April 13, 1976.

The experiment was laid out in a split-plot design, with sub-sub plots containing seven rows of 7-m length, spaced at 40 cm and replicated four times. The main treatments were (a) Irrigation and (b) No irrigation. Sub-plot treatments were (a) No inoculant, (b) Commercial powdered peat inoculant[2] (Peat-I) and (c) Granular inoculant.[2] Sub-Sub-plot treatments were (a) O kg N/ha, (b) 25 kg N/ha, (c) 50 kg N/ha, and (d) 100 kg N/ha. The irrigation treatment plots were irrigated when visual observations of soil and the crop indicated that it was necessary. Inoculants (granular @ 12 kg/ha and peat @ 2 kg/ha) were applied with the seed at the time of planting. Nitrogen, in the form of ammonium nitrate, was broadcast and raked into the soil according to the treatments prior to planting.

Seed of the cultivar 'Comet' were planted on May 16, 1976 at 13 seeds/meter and at a depth of approximately 3.8 cm, using a small hand-operated V-belt seeder. Poor emergence necessitated replanting on June 9, a date which previous research would indicate to be much too late for commercial production. Weeds were controlled by two pre-emergence sprayings of 2.24 kg/ha alachlor (2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide) plus 1.12 kg/ha dinoseb (2-sec-butyl 4,6-dinitrophenol) and hand hoeing. Leaf hoppers (*Empoasca fabae* Harris) were controlled by five foliar sprays (June 25, July 9, 16, 30, and August 20) of 1.12 kg/ha carbaryl (1-naphthyl N-methylcarbamate). Leaf spot (*Cercospora* spp.) was controlled by four foliar sprays (June 25, July 9, 16, and 30) of 0.81 kg/ha chlorothalonil (tetrachloroisopthalonitrile). Gypsum ($CaSO_4$) was applied at the rate of 896 kg/ha on July 12.

Nitrogen fixation, $N_2(C_2H_2)$, was estimated at weekly intervals by means of the acetylene reduction technique (6), as modified by Hume et al. (7). Diurnal variation of nitrogen-fixing activity was determined by sampling four nodulated plants (all samples from plots with granular inoculant and no nitrogen), every 3 hours over a 24-hour period on August 18 and 19. On the basis of these results, the estimates of nitrogen fixed during 30 minutes between 10 a. m. and 12 noon on all sampling dates were converted to the amount of nitrogen fixed per day. The weekly nitrogen fixation activities were used to calculate the nitrogen fixation over the growing season using regression analysis (3). The root samples, after incubation, were stored in a freezer (4 C) for later determination of root volumes, nodule numbers, and nodule fresh and dry weights.

1977 Experiment: An experiment similar to that of 1976 was conducted in 1977 on a different field at the same location, with the following modifications: Treatments: An additional peat inoculant (Peat-II), containing the same strains of rhizobia as the granular inoculant, was added to the inoculation treatments. A split application of nitrogen treatment (25+25 kg N/ha) was added. The first half of the split was applied at the time of planting and the second 60 days later. The crop was planted on May 14, 1977. Plant protection measures during 1977 included one pre-emergence spray of alachlor + dinoseb (3.36 kg/ha and 1.12 kg/ha) on May 19, and one post-emergence spray of bentazon (3-isopropyl-1 H-2,1,3-benzothiadiazin (4)-3H-one-2,2 dioxide) (1.40 kg/ha) on June 8, for weed control. Leaf hoppers were controlled by one foliar application of disulfoton (0,0-diethyl S-(2-(ethylthio) ethyl) phosphorodithioate) granules (3.36 kg/ha) on June 6 and three foliar sprays of carbaryl (1.12 kg/ha) on July 12, 29, and August 9. Leaf spot was controlled by one foliar spray of chlorothalonil (0.81 kg/ha) on July 29. Samples for acetylene reduction were taken at two-week intervals. The crop was harvested on September 31.

Ten plants per plot were collected at harvest for recording total dry matter accumulation and nitrogen content. These samples were washed and oven-dried at 80 C to a constant weight. Kernels and the remainder of the plant were ground separately after recording the weights. About 250 mg of each sample were weighed into digestion tubes. After adding a catalyst mixture (10 gm K_2SO_4, 3 gm CU SO_4 and pumice), 7 ml of conc. H_2SO_4 and one ml of H_2O_2 the samples were digested on a Technicon BD-40 digestor. The digester samples, after dilution, were analyzed for nitrogen content on a Technicon Auto-Analyzer II (8). All the data were subjected to analysis of variance. Duncan's New Multiple Range Test was used to compare treatments irrigation *vs* no irrigation, and inoculants, while regression analysis was used to study the responses to nitrogen application (2).

Results and Discussion

1976 Experiment: The weather during the 1976 growing season was cooler and wetter than normal, with the exception of a period of dry weather during late August. As a result, the irrigated treatment consisted of two applications, each of 2.54 cm, applied on August 20 and 29. The irrigation treatment did not increase nodule number or nodule dry weight (14 nodules per plant and 45 mg/plant nodule dry weight on average). Irrigation did, however, increase N_2 fixation where granular inoculant was used (Fig. 1). The uninoculated peanuts produced few nodules (Fig. 2A), low no-

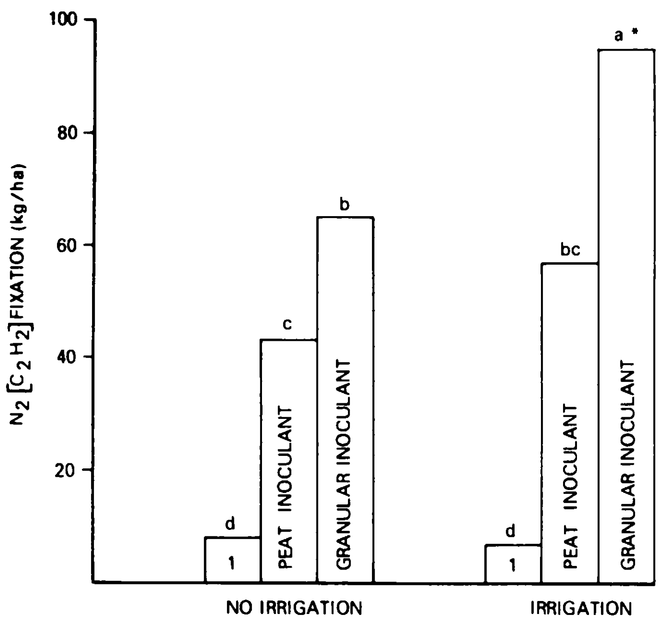

Fig. 1. Effect of irrigation and inoculants on seasonal $N_2(C_2H_2)$ fixation in peanuts in 1976 at Delhi.

* **Means followed by the same letter do not differ significantly according to Duncan's New Multiple-Range Test at the 5% level.**

1 no inoculant.

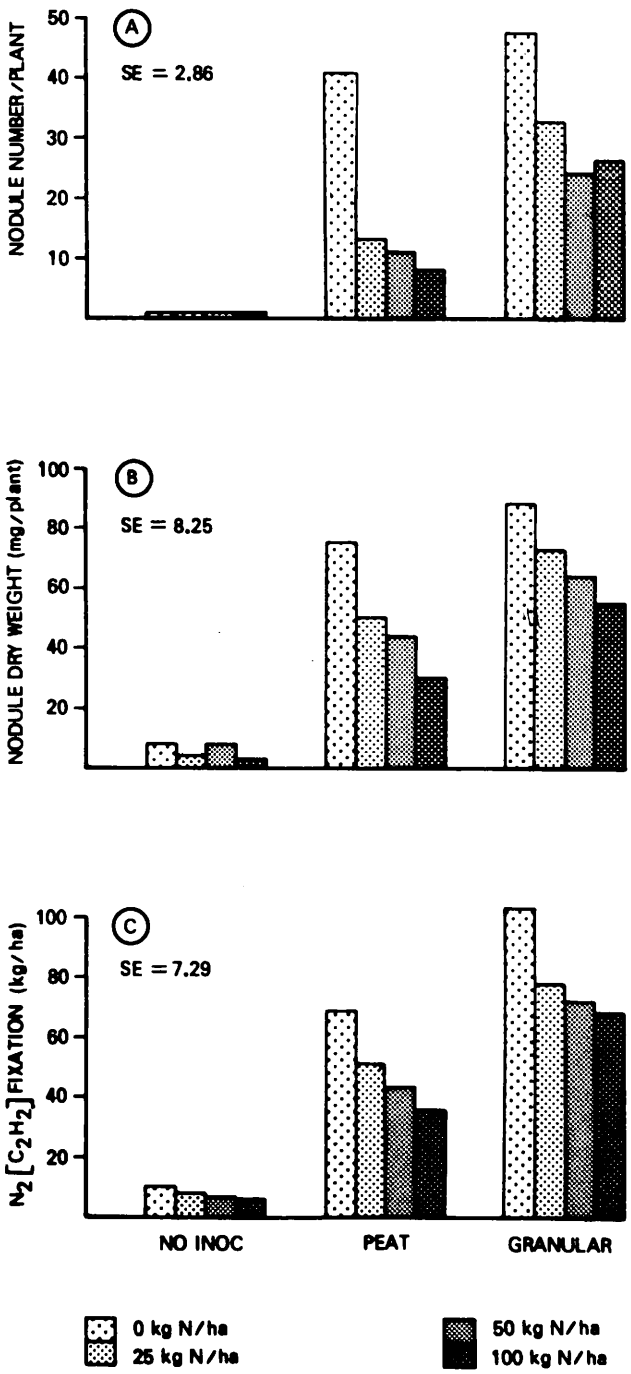

Fig. 2. Effect of fertilizer nitrogen on (a) nodule number per plant under irrigation*, (b) nodule dry weight (mg/plant) and (c) seasonal $N_2(C_2H_2)$ fixation (kg/ha) of peanuts treated with different inoculants in 1976 at Delhi. For (a) and (b), values shown are for treatment means averaged over sampling dates.

* **In order to show the significant difference in the performance of the two inoculants under irrigation, nodule number data from the no irrigation treatment was not included.**

dule dry weight (Fig. 2B), and very low N_2 fixation (Fig. 1 and 2C). In view of the fact that the experiment was conducted on a "virgin"peanut soil, it was interesting that there was any nodulation or N_2 fixation at all. Plots treated with granular inoculant were superior to those treated with peat inoculant for nodule number (Fig. 2A), nodule dry weight (Fig. 2B), and N_2 fixation (Fib. 2C) at all levels of N fertilization.

Nitrogen application decreased the nodule number of both the inoculant treatments. However, under irrigation, there was a dramatic reduction in nodule number of the peat inoculant treatment with fertilizer application, as compared to the modest reduction when granular inoculant was used (Fig. 2A). The nodule dry weight and N_2 fixation resulting from both the inoculants were decreased with the N application. However, the nodule dry weight and N_2 fixation of the granular inoculant showed a quadratic decrease in contrast to the linear decrease of nodule dry weight and N_2 fixation of the peat inoculant (Table 1). In order to help in comparison, the linear responses for granular inoculant are also given (Table 1).

Although the better nodulation with the application of granular inoculant might be attributed to the greater number of bacteria supplied by that formulation, the differential response of the two formulations to applied N cannot be explained by the higher number of bacteria alone. This observation suggested that the two formulations differed in other aspects as well. Since *Rhizobium* strains differ in their ability to nodulate and fix nitrogen in the presence of combined nitrogen (5), it was suspected that the two formulations contained different strains of rhizobia. This suspicion was confirmed upon consultation with the manufacturer. As a result, the experiment was repeated in 1977 with the inclusion of an additional peat inoculant which contained the same strains as the granular inoculant.

1977 Experiment: As in 1976, the irrigation treatment did not increase the nodule number and nodule dry weight (18 nodules per plant and 80 mg nodule dry weight per plant on average), but did increase the N_2 fixation of all the inoculated peanuts at all levels of nitrogen (Fig. 3, Table 1). The weather during the 1977 season was warmer and drier compared to the 1976 season. As a result, three irrigations, each of 2.54 cm, were applied (June 16, July 13, and 22). The higher moisture levels in the irrigated plots during the dry periods sustained greater nodule activity than the lower moisture levels in unirrigated plots.

The nodule number (one nodule per plant on average), nodule dry weight (1.6 mg/plant on average), and N_2 fixation (Fig. 3) of the uninoculated peanuts were very low, and as in 1976, were not affected by irrigation. These results indicated again that these soils contained very few rhizobia capable of nodulating peanuts. There were no differences in nodule number and nodule dry weight among the three inoculants (Fig. 4a and 4b), but the Peat-II and the granular inoculants resulted in higher N_2 fixation compared to the Peat-I inoculant, at all levels of N and under both irrigated and unirrigated conditions. The Peat-II and granular inoculants, which contained the same strains of rhizobia, produced almost the same amounts of N_2 fixation. The Peat-II and the granular inoculants also had similar yield levels and consistently outyielded the Peat-I inoculant (13). These results suggested that the differences in N_2 fixation observed among the three inoculants during 1977 were due to the differences in strains rather than differences in formulation. In light of these results, although the granular inoculant in 1976 produced better nodulation and N_2 fixation than the peat inoculant, it would appear that these differences were due to the differences in the strains contained in the two inoculants rather than the formulation differences.

Table 1. Effect of fertilizer nitrogen on a) nodule number, b) nodule dry weight, c) seasonal $N_2(C_2H_2)$ fixation (kg/ha), of peanuts treated with different inoculants in 1976 and 1977 at Delhi. For (a), (b), (d) and (e), values shown are for treatment means averaged over sampling dates.

Treatment	Regression equation	R^2
a) Nodule number per plant - 1976		
No inoculant	Non sig.	-
Peat inoculant	$Y = 18.40 + (-0.1177X)$	0.817**
Granular inoculant	Non sig.	-
b) Nodule dry weight (mg/plant) - 1976		
No inoculant	Non sig.	-
Peat inoculant	$Y = 67.28 + (-0.4087X)$	0.858**
Granular inoculant	$Y = 87.22 + (-0.6380X) + (0.003167X^2)$	0.998**
	$Y = 83.26 + (-0.3099X)$ (For comparison only)	0.912
c) Seasonal $N_2(C_2H_2)$ fixation (kg/ha) - 1976		
No inoculant	$Y = 9.38 + (-0.04297X)$	0.846**
Peat inoculant	$Y = 62.88 + (-0.3013X)$	0.843**
Granular inoculant	$Y = 101.24 + (-0.9339X) + (0.006011X^2)$	0.969**
	$Y = 93.72 + (-0.311X)$ (For comparison only)	0.725
d) Nodule number per plant - 1977		
No inoculant	Non sig.	-
Peat-I	$Y = 31.4 + (-0.1691X)$	.954**
Peat-II	$Y = 26.8 + (-0.08686X)$	.938**
Granular	Non sig.	-
e) Nodule dry weight (mg/plant) - 1977		
No inoculant	$Y = 8.5 + (-0.04857X)$	.878**
Peat-I	$Y = 145.28 + (-0.7944X)$	.930**
Peat-II	$Y = 119.36 + (-0.5219X)$	.954**
Granular	$Y = 125.06 + (-1.0348X) + (0.00552X^2)$	.999**
	$Y = 118.16 + (-0.463X)$ (For comparison only)	.886
f) Seasonal $N_2(C_2H_2)$ fixation (kg/ha) - 1977		
Unirrigated		
No inoculant	Non sig.	-
Peat-I	$Y = 47.88 + (-0.2610X)$	.829**
Peat-II	$Y = 72.88 + (-0.2458X)$	.983**
Granular inoculant	$Y = 85.02 + (-0.4496X)$	.977**
Irrigated		
No inoculant	$Y = 4.86 + (-0.02823X)$	.971**
Peat-I	$Y = 75.44 + (-0.2815X)$	.943**
Peat-II	$Y = 112.70 + (-0.4297X)$	.921**
Granular inoculant	Non sig.	-

**Significant at 1% level.

Fertilizer N, as in 1976, reduced the nodule numbers (Fig. 4, Table 1), nodule dry weight (Fig. 4, Table 1) and N_2 fixation (Fig. 3, Table 1) of all three inoculants under both irrigated and unirrigated conditons, except for the nodule numbers

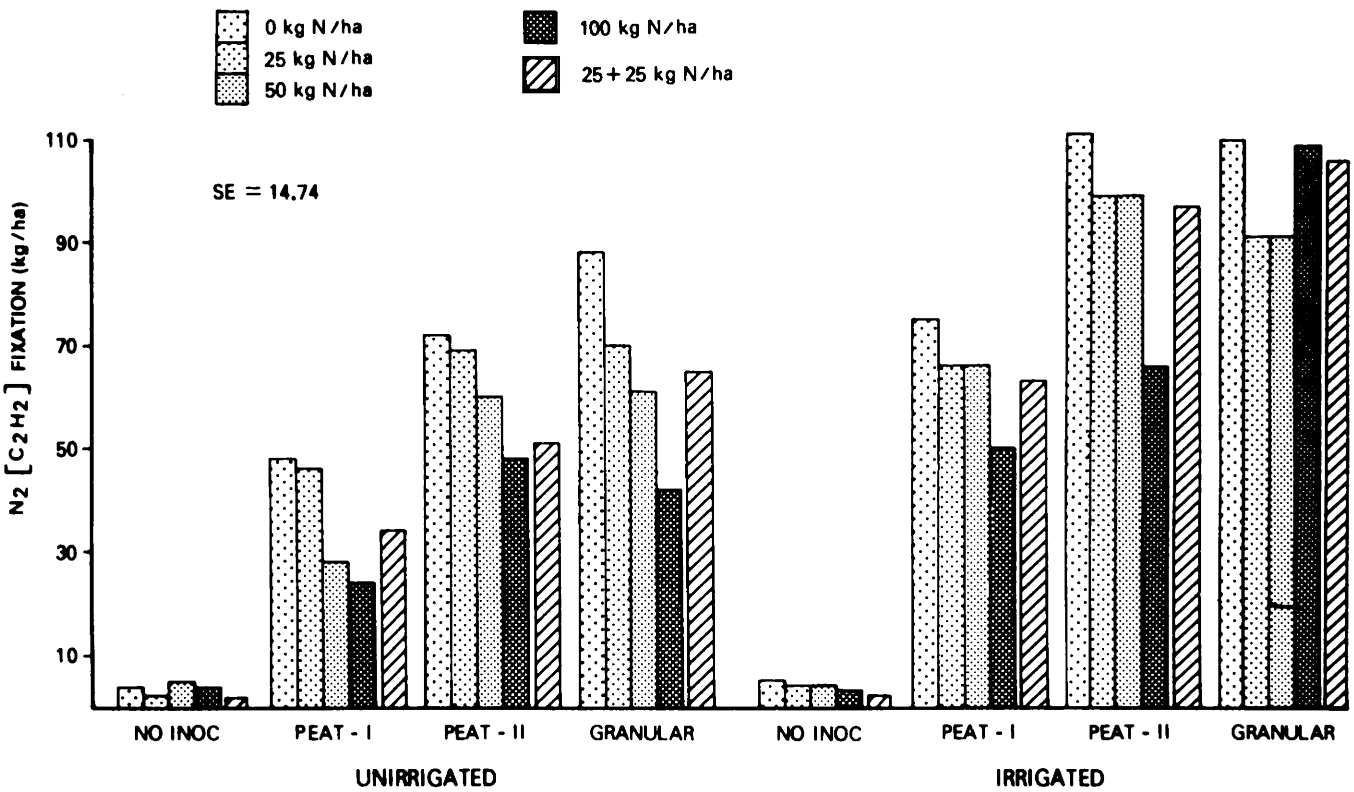

Fig. 3. Effect of fertilizer nitrogen on seasonal $N_2(C_2H_2)$ fixation (kg/ha) of peanuts treated with different inoculants under unirrigated and irrigated conditons during 1977 at Delhi.

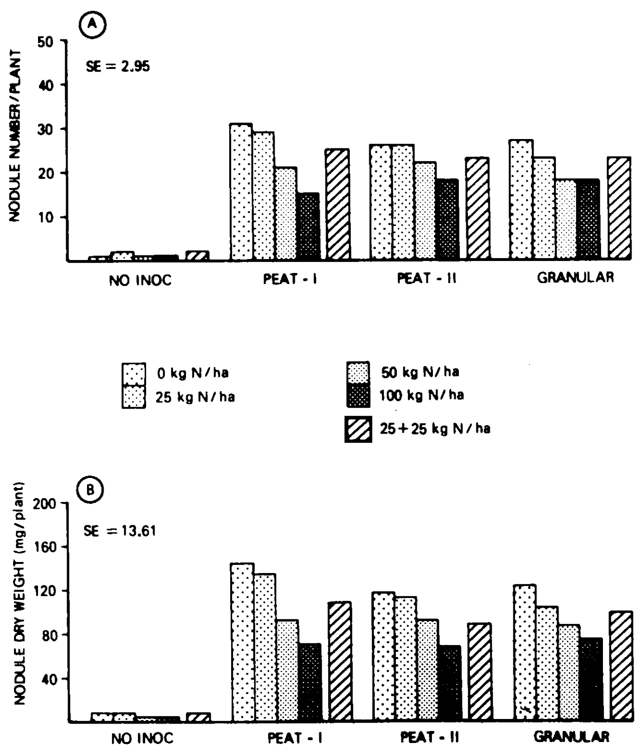

Fig. 4. Effect of fertilizer nitrogen (basal application) on (a) nodule number per plant, (b) nodule dry weight (mg/plant) of peanuts treated with different inoculants during 1977 at Delhi. Values shown are for treatment means averaged over sampling dates.

and N_2 fixation of the granular inoculant under irrigation. However, the nodule dry weight of the granular inoculant showed a quadratic decrease in contrast to the linear decrease of nodule dry weight of peat inoculants.

Total Nitrogen

Nitrogen content in the plant (2.7% on average) and protein content of the kernel (20.5% on average) at harvest were not affected by any of the treatments. The data on total nitrogen and the estimated contribution from N_2 fixation to the toal N are presented in Table 2. There were no significant differences in total N or in percentage contribution from N_2 fixation to the total N between the irrigation and no irrigation treatments. However, there was a trend toward more total N and higher percentage contribution from N_2 fixation to total N with irrigation compared to no irrigation. This trend could be attributed to the increased amount of N_2 fixed under irrigation (Fig. 3).

All three inoculant treatments had significantly greater amounts of total N than the no inoculant treatment, and also had significantly higher contributions from N_2 fixation. However, it is interesting to note that although N_2 (C_2H_2) fixation (Fig. 3) indicates very large differences, up to 100 kg N_2/ha, between inoculated and uninoculated treatments, the data on total N do not show such large differences. The acetylene reduction technique seems to have over estimated the amount of N_2 fixed. In calculating N_2 fixed, the theoretical value of 1:3 was used as the ratio between N_2 and C_2H_2. However, Bergersen (19) working with soybeans has reported that this ratio varied between 2.7 and 4.2. Therefore the factor used in this experiment might have somewhat over estimated the amount of N_2 fixed. The Peat-II and granular inoculant treatments obtained a greater proportion of the total N from N_2 fixation compared to Peat-I inoculant, which was probably a reflection of the efficiency of the strains contained in the inoculants.

The application of fertilizer N did not increase the total N, but decreased the contribution from N_2 fixation. Weber (18) indicated that the lack of soybean yield response to fertilizer N was due to the fact that the applied N replaced symbiotic nitrogen as the nitrogen source.

Conclusions

From the results of the two year study the following conclusions were drawn:

These soils lack sufficient numbers of peanut rhizobia, hence inoculation is necessary to achieve good nodulation and increase nitrogen fixation.

The differences observed among the inoculants were mostly due to the differences in strain composition rather than the formulation. However, there is still justification for using the granular formulation, particularly on soils new to peanuts. Granular inoculant application avoids seed treatment using "sticker" (sugar solution, milk, water), which may cause damage to seed coat and reduce germination, allows use of standard field equipment and also protects the rhizobia from heat and desication, as a result providing sufficient numbers of bacteria to the rhizosphere.

Fertilizer nitrogen decreased nodulation and nitrogen fixation of all the inoculated peanuts, but peanuts fixed considerable N even when supplied with 100 kg/ha N.

Acknowledgement

The authors wish to thank J. M. Elliot of the Agriculture Canada Research Station, Delhi for his assistance and the laboratory facilities. Technical assistance of Bob Roy, Peter White and Peter Gostovic is gratefully acknowledged. Financial assistance and research facilities were provided by the Ontario Ministry of Agriculture and Food and the New Crop Development Fund, Agriculture Canada.

Literature Cited

1. Barksdale, W. E. 1977. Granular inoculants produce new payoffs for growers across peanut belt. The Peanut Farmer 13: 42-45.

2. Chew, Victor. 1976. Comparing treatment means: A compendium. Hort. Science 11:348-356.

Table 2. Effects of irrigation, inoculants and fertilizer nitrogen on total nitrogen and percentage contribution from ($N_2(C_2H_2)$) fixation to total nitrogen in peanuts in 1977 at Delhi. Effect of the fertilizer nitrogen applied at the time of planting is indicated by the regression equation.

Treatments	Total nitrogen (kg/ha)	contribution from $N_2(C_2H_2)$ fixation[2] %
Irrigation	188 a[1]	34.2 a
No irrigation	168 a	24.1 a
No inoculant	148 b	2.5 c
Peat-I	173 a	29.2 b
Peat-II	193 a	40.9 a
Granular	198 a	44.1 a
0 kg N/ha	173	35.5
25 kg N/ha	172	31.5
50 kg N/ha	184	26.7
100 kg N/ha	167	24.6
25+25 kg N/ha	193	27.5
Regression equation	Non sig.	Y = (34.34) + (-0.1089X)
R^2	-	0.545**

[1] Means, within a column and factor, followed by the same letter do not differ according to Duncan's new multiple-range test at 5%

** Significant at 1% level

[2] Contribution calculated from [$N_2(C_2H_2)$ fixed (kg/ha) ÷ Total N (kg/ha)] x 100

3. Criswell, J. G., D. J. Hume and J. W. Tanner. 1976. Effects of anhydrous ammonia and organic matter on components of nitrogen fixation and yield of soybeans. Crop Sci. 16:400-404.

4. Doku, E. V. 1970. Effect of day length and water on nodulation of cowpea (*Vigna unguiculata* L. Walp.) in Ghana. Expl. Agric. 6:13-18.

5. Gibson, A. H. 1977. Influence of environment and managerial practices on the legume -*Rhizobium* symbiosis. *In* A treatise on dinitrogen fixation. Sect. IV. Agronomy & Ecology. A. H. Gibson, ed. R. W. F. Hardy, General ed. Wiley-Interscience Publ. pp. 420-450.

6. Hardy, R. W. F., R. D. Holsten, E. K. Jackson and R. C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43:1185-1207.

7. Hume, D. J., J. G. Criswell and K. R. Stevenson. 1976. Effect of soil moisture around nodules on nitrogen fixation by well watered soybeans. Can. J. Plant Sci. 567:811-815.

8. Isaac, R. A. and W. C. Johnson. 1976. Determination of total nitrogen in plant tissue, using a block digestor. J. Assoc. Off. Anal. Chem. 59:98-100.

9. Johnson, C. 1976. Soil inoculants pump yields in tests. The peanut Farmer 12:52.

10. Johnson, R. R. and L. V. Boone. 1976. Soybean inoculation: is it necessary? Illinois Research. Fall, 1976, pp. 3-4.

11. Masefield, G. C. 1961. The effect of irrigation on nodulation of some leguminous crops. Imp. J. Exptl. Agric. 29:51-59.

12. McKee, G. W. 1961. Some effects of timing, fertilization and soil moisture on seedling growth and nodulation in birdsfoot trefoil. Agron. J. 53:237-240.

13. Reddy, V. M. 1978. The effects of irrigation, inoculants, fertilizer nitrogen on nitrogen fixation and yield of peanuts (*Arachis hypogaea* L.). M. Sc. Thesis, Univ. of Guelph.

14. Shimshi, D., J. Schiffman, Y. Kost, H. Bielorai and Y. Alper. 1967. Effects of soil moisture regime on nodulation of inoculated peanuts. Agron. J. 59:397-400.

15. Sprent, J. 1972. The effects of water stress on nitrogen fixing root nodules. IV. Effects of whole plants of *Vicia faba* and *Glycine max*. New Phytol. 71:603-611.

16. Sundara Rao, W. V. B. 1971. Field experiments on nitrogen fixation by nodulated legumes. Plant and Soil, Special Vol., 287-291.

17. Vincent, J. M. 1965. Environmental factors in the fixation of nitrogen by the legume. *In* Soil Nitrogen, W. V. Bartholomew and F. C. Clark, eds., Amer. Soc. Agron., Madison, Wisconsin, pp. 384-435.

18. Weber, C. R. 1966. Nodulating and non-nodulating soybean isolines: II. Response to applied nitrogen and modified soil conditions. Agron. J. 58:46-49.

19. Bergerson, F. J. 1970. The quantitative relationship between nitrogen fixation and the acetylene-reduction assay. Aust. J. biol. Sci. 23:1015-25.

Accepted September 30, 1980