Calcium, Nitrogen, and Rhizobium Effects on Arachis Hypogaea L. Valencia C

Robert G. Taylor* and Karim Moshrefi[1]

ABSTRACT

Nutritional needs of numerous plants *(Arachis hypogaea L.)* cultivars have been extensively examined, especially responses to N fertilizer, Ca availability in the soil solution, and the use of rhizobium soil inoculum. The nutritional effects of these three factors and their interactions on particular processes at various stages of growth and development of the plant were examined. Three levels of Ca and four N treatments were tested in a greenhouse on two sets of the Valencia C peanut cultivar, one receiving a soil inoculum and the other did not. Nutritional and soil inoculum treatment effects on primary stalk length, flower production, peg and pod development, and nodulation occurrence were statistically examined.

Calcium and N were found to strongly, and independently, influence plant nodulation. Soil inoculation had no influence on flowering, peg or pod production. However, both Ca and N independently influenced these processes. Synergism between Ca and N appeared to influence peg development but not pod development. Additionally, the presence of rhizobium inoculum did not increase pod development. The presence of rhizobium soil inoculum which promoted nodule formation, was not closely related to fruit production but was related to the growth of the aerial portion of the plant.

Key Words: Flowering, nodulation, peg and pod production, calcium, nitrogen soil inoculant.

The peanut plant *(Arachis hypogaea L.)* has been extensively examined for nutritional needs related to productivity. A low response to fertilizer applications on soils with high residual fertility was noted by Sturkie and Buchanan (18). Heavy K fertilization interfered with Ca uptake by developing pegs, and increased pod diseases (7). The peanut plant is a highly efficient legume, able to provide its own N, if the proper strains of bacteria are present in the soil (19). Other investigators, have shown improved performance of the peanut plant with the application of a commercial inoculum applied to the seed furrow at planting if the field has not been planted to peanuts during the previous five years (8,9).

Nitrogen fertilization studies have been conducted in most peanut growing regions of the world. Reports vary from no yield response from increased N fertilization (3,10) to yields being directly related to N fertilizer application rates (4,5,11). However, detrimental effects of high rates of N fertilization on yield have been found (6).

Yields are often limited by a lack of Ca in the fruiting zone (2). Calcium is passively absorbed and transported almost exclusively in xylem tissue, being moved upward with the transpiration stream (12). The relationship between Ca and yield occurs as a result of the lack of Ca movement in the developing peg via the phloem. The developing fruit, consequently, depends upon the presence of adequate Ca in the soil solution (16, 17, 20).

Rhizobium bacteria enter the host plant through attraction to the plant root hairs with subsequent penetration into the deeper tissue (13). The penetration process stimulates the cortical cells of the root of the peanut plant to begin dividing and producing nodules. The establishment of a relationship between the root cells and the rhizobium bacteria initiates the fixation of N (1). Fixation effectiveness of the nodule is related to the presence of red legume hemoglobin (14).

The nutritional effects of Ca and N on particular processes at various stages in the growth and development of peanuts has not been extensively explored. This study examines stalk length, flower production, nodulation occurrence, and peg and pod development which may be influenced by availability of soil N and Ca in the phenology of the plant.

Materials and Methods

Plants were grown in washed Brownfield sand in plastic pots (15 cm. X 15 cm.) placed in a greenhouse. The experimental group of plants were divided into two subsets with one receiving 5 g per pot of a granulated microencapsulated culture of rhizobium soil inoculum, and the other receiving none. Four N concentration levels and three Ca concentration levels were examined for each subset of plants obtained from Valencia C peanut seed, not treated with fungicide, supplied courtesy of the Nunn Peanut Company of Portales, New Mexico. The peanut seed tested at 47% germination and 10 replicate plants of each Ca and N treatment level were prepared. Four seeds per pot were planted at a depth of 3.8 cm. and thinned, when necessary, to one viable plant per pot. Plants that were lost to seedling disease, and pots in which germination did not occur, were replanted and carried through a 120-day-growth cycle when possible. Some plants at each treatment level were lost and could not be replaced within the time frame of the experiment.

Plants were watered with modified Hoaglands solution containing micronutrient additions. Hoaglands was modified to remove sources of available Ca and N ions except as provided experimentally. Calcium and N experimental levels were obtained by the addition of various amounts of stock solutions prior to bringing the Hoaglands mineral solutions to final volume. The nutrient mineral solutions were prepared by use of 10mL/L of K_2SO_4 (43.565g/0.5L); 4mL/L $MgSO_4$ (60.195g/L); 2mL/L KH_2PO_4 (68.045g/L); 2mL/L FeEDTA (5mg Fe/mL); and 2mL micronutrient solution consisting of 2.86g/L H_3BO_3; 1,81g/L $MnCl_2$ $4H_2O$; 0.11g/L $ZnCl_2$; 0.05g/L $CuCl_2$ $2H_2O$; 0.025g/L Na_2MoO_4 $2H_2O$. NH_4NO_3 and $CaCl_2$ were added to the nutrient solution to provide final concentrations of 0, 70, 140, and 210 ppm available N and O, 100, and 200 ppm available Ca.

The potting medium presented little additional nutrient value. Measurements indicated an electrical conductivity of 0.28 mmho/cm, organic matter at 0.22%, N at 0.8ppm, P at 0.7 ppm and K at 13.7 ppm. The potting medium was autoclave sterilized prior to use to eliminate influence from exogenous bacteria capable of nodulating the subset of plants not receiving inoculum. The greenhouse setting of the plants included use of additional fluorescent illumination from two 40W bulbs adjusted to 0.5 m above the plants. The artificial illumination was maintained throughout the 24 hr day period. Average daily temperature of the greenhouse was 27 C plus or minus 3 C for the duration of the plant growth period.

Ten pots per treatment level, or 120 pots per subset, were employed. The number of flowers, pegs, seed pods, and nodules which developed on each plant per treatment level in each of the subsets were recorded.

Seed germination occurred within a week to ten days of planting. Senescence of the cotyledons occurred 15 to 25 days after germination and the first flowers typically appeared 40 days after planting. Each

[1]Associate Professor Microbiology, Graduate Research Assistant respectively, Eastern New Mexico University, Portales, NM.

flower was gently "thumped" with a finger to stimulate pollination. The flowers typically abscissed within a week to ten days of their appearance and the base of many flowers began growth requisite for peg formation. Each developed peg was counted regardless of its final maturity. Pod initiation was recorded when the pegs penetrated the potting medium. Other pegs developed a swollen tip without penetration and were recorded as positive for pod initiation even though their ultimate fate could not have been successful. Maturation of pods under the greenhouse environment was beyond the scope and time frame of this project. However, the primary stalk length was recorded just prior to harvest. At harvest each plant was carefully removed from its pot and washed free of sand under flowing tap water. Nodules formed on the root system were counted and recorded.

The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) was used for the statistical analyses of the data (15). The SAS package provided two methods for analyses of unbalanced data (the different methods of analysis are reflected by the way in which the total sum of squares may be partitioned), and are identified in this report as Type I and Type III analyses. If analyses by the two methods yielded the same statistical result, then the result was reported without further comment. However, when analyses by the two methods yielded different results, the conflicting results were carefully reported and indicated with daggers (†) in the summary of calculations section.

Results and Discussion

The mean values calculated for primary stalk length, numbers of flowers, pegs, pods, and root nodules from 10 replicates per treatment level in both the presence and absence of Rhizobium soil inoculum are shown in Table 1. The plant replicates of the 200/140 ppm Ca/N treatment cell not receiving soil inoculum did not reach a full 120 day maturity development as a result of experimental problems. Consequently, their mean lengths, and other values, were not included in the calculations. The probabilities of Ca, N, rhizobium, and their interactions influencing the dependent variables of root nodulations, pod production, primary stalk length, number of flowers and peg formation are presented in Table 2. A strong probability (PR) of the test factor influencing the dependent variable (PR>F) is shown by a value smaller than 0.0500. Some plants were lost at each treatment level. Therefore the collected data had to be analyzed by so called 'unbalanced data methods' since the number lost was not the same in each treatment cell. Two methods of analysis were used and are identified as Type I and Type III. The two methods of analysis yielded similar results in the majority of cases.

The presence of Ca, N, and rhizobium strongly influenced (at the 0.0001 level of confidence) the formation of bacterial nodules on the plant roots. The high probability of Ca and N, when taken together, indicated they did not act synergistically, but rather independently. However, the interactions of N x rhizobium, Ca x rhizobium, and Ca x N x rhizobium, were found well within the acceptable confidence level suggesting synergism in influencing the dependent variable of root nodulation.

Both Ca and N were found to independently influence pod production while the presence of a soil inoculum had less effect. Calcium and N were also found to strongly interact in their influence on pod production. Interactions between Ca, N, and rhizobium on pod production were not found.

Primary stalk length was influenced independently by N only. When the interaction of Ca and N were examined for influence on primary stalk length, a high level of confidence was found. Additionally, the number of flowers was strongly influenced by N. Ca did not in-

Table 1. Mean values for primary stalk length, numbers of flowers, pegs, pods, and root nodules.

Ca/N conc.	Primary Stalk Length	Flowers	Pegs	Pods	Root Nodules
		No Inoculant			
ppm	cm	Number			
0/0	54.87	4.77	4.75	3.25	5.63
0/70	56.50	0.30	0.00	0.00	0.00
0/140	69.20	0.00	0.00	0.00	0.00
0/210	63.44	0.44	0.44	0.33	4.78
100/0	71.00	5.22	1.56	0.56	12.89
100/70	63.50	1.88	0.75	0.63	2.13
100/140	53.25	2.40	0.80	0.10	4.90
100/210	51.43	0.00	0.00	0.00	1.14
200/0	81.75	2.13	0.13	0.13	4.63
200/70	53.50	5.00	1.88	1.25	2.13
200/140	-	-	-	-	-
200/210	59.50	0.00	0.00	0.00	0.00
		Inoculant Added			
0/0	43.60	8.50	3.60	3.30	129.60
0/70	41.86	7.71	0.57	0.57	85.57
0/140	70.00	2.88	1.12	0.87	62.12
0/210	66.77	1.11	0.00	0.00	15.33
100/0	59.11	5.33	2.88	2.11	70.11
100/70	69.28	2.43	0.43	0.29	7.20
100/140	60.70	2.40	1.00	0.90	25.10
100/210	71.50	0.00	0.00	0.00	6.16
200/0	81.60	0.00	0.00	0.00	24.40
200/70	38.80	0.60	0.20	0.20	5.00
200/140	64.13	0.50	0.50	0.37	12.75
200/210	59.50	0.75	0.00	0.00	0.00

Table 2. Probability levels of Ca, N and rhizobium soil inoculation influencing root nodulation, pod production, primary stalk length, number of flowers, and peg formation.

Source	Type I SS PR > F	Type III SS PR > F
Root Nodulation		
Calcium (Ca)	0.0001	0.0001
Nitrogen (N)	0.0001	0.0001
Rhizobium	0.0001	0.0001
Ca x N	0.0530	0.0544
N x Rhizobium	0.0001	0.0001
Ca x Rhizobium	0.0001	0.0001
Ca x N x Rhizobium	0.0165	0.0165
Pod Production		
Calcium (Ca)	0.0187	0.0168
Nitrogen (N)	0.0001	0.0001
Rhizobium	0.1225	0.4380
Ca x N	0.0001	0.0001
N x Rhizobium	0.2429	0.4407
Ca x Rhizobium	0.4400	0.1603
Ca x N x Rhizobium	0.5659	0.5659
Primary Stalk Length		
Calcium (Ca)	0.3730	0.2890
Nitrogen (N)	0.0486	0.0159
Rhizobium	0.3830	0.6407
Ca x N	0.0001	0.0001
N x Rhizobium	0.1257	0.1028
Ca x Rhizobium	0.2496	0.1876
Ca x N x Rhizobium	0.5110	0.5110
Number of flowers		
Calcium (Ca)	0.0764	0.0640
Nitrogen (N)	0.0001	0.0001
Rhizobium	0.0260 †	0.1673 †
Ca x N	0.0590	0.0820
N x Rhizobium	0.9592	0.9014
Ca x Rhizobium	0.0002 †	0.1438 †
Ca x N x Rhizobium	0.1438	0.1438
Peg Formation		
Calcium (Ca)	0.0532 †	0.0473 †
Nitrogen (N)	0.0001	0.0001
Rhizobium	0.6802	0.8937
Ca x N	0.0002	0.0003
N x Rhizobium	0.6461	0.7769
Ca x Rhizobium	0.6472	0.6802
Ca x N x Rhizobium	0.3395	0.3495

† indicates disagreement between Type I and Type III SS.

fluence flower numbers as an independent variable but it had a positive relationship with rhizobium. Unfortunately, discrepancies occurred between the Type I and Type III SS for this analysis. Therefore, care must be used in reaching any conclusions concerning this relationship.

Peg formation was found to be strongly influenced by soil N. The presence of Ca in the soil influenced peg formation by Type III SS but the Type I SS did not support this conclusion. Therefore inferences concerning the affect of Ca independently influencing peg formation would be questionable. Ca and N were found to strongly interact in their influence on peg formation indicating synergism. An influence of rhizobium inoculum on peg formation was strongly rejected. These data suggest that while the presence of rhizobium soil inoculum influences nodule formation on peanut plants, there is no indication that this will assist the plant in the production of pegs and pods. Responses of different plant genotypes and rhizobial strains have been found to be specific by Wynne, et al.(21). However, the interaction between the quantitative availability of the test nutrients, and the rhizobial strain, on the particular peanut cultivar used in this project, permitted isolation of the factors important in the phenology of a peanut plant.

Previous research has shown that once the peg penetrates the soil it no longer receives xylem transported Ca (16, 17, 20). Soil Ca and N, consequently, may have a greater affect on pod development once the peg enters the soil, than the ability of the plant to fix N. The data collected from our study suggests that while the influence of nodulation induction on the test cultivar may have an influence on its growth and development, it probably does not affect the nut maturation and development. If such a speculation were proven correct in the field, as indicated by the analyses of these data, then the influence of nodulation induction may affect yield primarily through its effect on the growth and vigor of the above ground parts of the plant.

Literature Cited

1. Bhuvaneswari, T.V., A.A. Bhagwat and W.D. Bauer. 1981. Transient susceptibility of root cells in four common legumes to nodulation by rhizobia. Plant Phys. 68:1144-1149.
2. Burkhard, L. and E.R. Collins. 1942. Mineral nutrients in peanut plant growth. Soil Sci. Soc. Amer. Proc. 6:272-280.
3. Dahatoude, B.N. 1978. Response of varying levels of irrigation and N P fertilization on summer groundnut. J. Maharashtra Agric. Univ. 3(2):1337-1338.
4. Georgiev, A. 1974. Effect of nitrogen and phosphorous fertilizers on the yield and quality of peanuts. Rastenievdni Nauki 11(9):71-79.
5. Gustien, Y. 1978. Differential response of two groundnut cultivars to N fertilizers.
6. Hallock, D.L. 1969. Response of Virginia peanuts to nitrogen. Virginia. J. Sci. pp 98. Abst.
7. Hallock, D.L. and K.H. Garren. 1968. Pod breakdown, yield, and grade of Virginia type peanuts as affected by Ca, Mg and K sulfates. Agron. J. 60:253-257.
8. Henning, R.J., J.F. McGill, L.E. Samples, C.W. Swann, S.S. Thompson and H. Womack. 1979. Growing peanuts in Georgia: a package approach. Bulletin 640, Univ. of Georgia pp 46.
9. Hiltbold, A.E., D.L. Hartzog, R.B. Harrison, and G. Adams. 1983. Inoculation of peanuts on farmers' fields in Alabama. Peanut Sci. 10:79-82.
10. Kolar, J.S., M.S. Tiwana, and G.S. Gill. 1976. Note on the fertilizer studies with groundnut *(Arachis hypogaea L.)* Indian J. Agric. Res. 10(4):265-268.
11. Liu, T.M. 1975. The effect of nitrogen, phosphorus, and potassium applications on the growth and production of peanuts. K'o Hsueh Nung Yeh (Taipei) 23(7-8):337-339.
12. Mengel, K. and E.A. Kirkby. 1978. Principles of plant nutrition. Int. Potash Inst., Berne, Switzerland.
13. Nambiar, R.T., S.N. Nigam, P.J. Dart, and R.W. Gibbons. 1983. Absence of root hairs in non-nodulating groundnut, *Arachis hypogaea L.* J. Exper. Botany 34:484-488.
14. Postgate, J. 1978. Nitrogen Fixation. Inst. of Biol. Stud. No. 92. Camelot Press Ltd. pp 22.
15. Ray, A.A. Ed. 1982. SAS user's guide: Statistics 1982. ed. SAS Inst. Cary, N.C. pp 139-199.
16. Skeleton, B.J. and G.M. Shear. 1971. Calcium translocation in the peanut *(Arachis hypogaea L.)* Agron. J. 63:409-412.
17. Slack, T.E. and L.G. Morrill. 1972. A comparison of large-seeded NC-2 and a small-seeded Starr peanut *(Arachis hypogaea L.)* cultivar as affected by levels of calcium added to the fruit zone. Soil Sci. Soc. Amer. Proc. 36:87-90.
18. Sturkie, D.G. and G.A. Buchanan. 1973. Cultural Practices. pp 299-326. in Peanuts: Culture and Uses. Am. Peanut Res. and Educ. Assoc., Stillwater OK.
19. Weaver, R.W. 1974. Effectiveness of Rhizobia forming nodules on Texas grown peanuts. Peanut Sci. 1:23-25.
20. Wolt, J.D. and F. Adams. 1979. Critical levels of soil-and nutrient-solution calcium for vegetative growth and fruit development of Florunner peanuts. Soil Sci. Soc. Amer. J. 43:1159-1164.
21. 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.

Accepted June 13, 1987