Nitrogen Fixation in Honey Mesquite Seedlings

ARTHUR W. BAILEY

Highlight: *Roots of honey mesquite seedlings produced nodules readily in the growth chamber. The nodulated seedlings contained more nitrogen than nonnodulated seedlings. Large plants had the largest nodules. West Texas soils were found to possess inoculum that caused nodulation in mesquite. The nodulation frequency was closely associated with soil texture and water at the time of collection of the soil inoculum source. Moist sandy soils produced the best nodulation, while dry clay soils produced the poorest nodulation.*

Honey mesquite (*Prosopis glandulosa* Torr. var. *glandulosa*) is disliked by ranchers throughout its range in the southwestern United States and Mexico. This shrub or small tree has thickened and, to some extent, encroached on many range sites during this century. Allred (1949) estimated that mesquite occurred on 55 million acres in Texas alone.

Honey mesquite is a member of the Leguminosae family, the most important nitrogen-fixing family of plants in the world. About 89% of the woody legumes examined to date are nodule-bearing (Allen and Allen, 1958). However, the Soil Conservation Service found no nodules on roots of mesquite seedlings or trees in Texas (Allred, 1949). Tiedemann and Klemmedson (1973) found increased nitrogen in the soil under velvet mesquite (*Prosopis juliflora* (Sw.) DC. var. *velutina*) trees.

This study was designed to investigate possible nitrogen-fixing characteristics of honey mesquite. The effect of soil water, texture, and inoculum source was also investigated.

The author is associate professor, Department of Plant Science, University of Alberta, Edmonton. The research was conducted in the Department of Range and Wildlife Management, Texas Tech University, Lubbock, while the author was on a sabbatical leave.

This study was supported by Texas Tech University and the National Research Council of Canada.

Manuscript received February 2, 1976.

Methods and Materials

Honey mesquite was grown from seed in a controlled growth room under 111 $\mu em^{-2}s^{-1}$ of fluorescent light for 12 hours daily. The growth room air temperature was 29°C (84°F) and the soil temperature 27°C (81°F). Mechanically scarified mesquite seed was planted in 1-gal metal cans (pots). Potting soil was 10% Amarillo fine sandy loam and 90% fine washed sand. The soil was not sterilized. All pots were fertilized with 225 kg/ha P_2O_5 and 112 kg/ha KCl. Water was added as needed to keep the soil near field capacity. The water used contained about 1,000 ppm dissolved solids. The most common constituents were calcium carbonate and bicarbonate, sodium chloride, and various sulfates. At the end of each experiment, plant tops and roots were harvested, oven dried for 48 hours at 82°C (180°F), and weighed. Nitrogen analyses were conducted in duplicate using a Coleman Model 29A, Nitrogen Analyzer II.

In the first experiment, 48 pots were seeded at the rate of 10 mesquite seeds/pot. The seed used had been collected several years before from trees growing on sandy soil of a shinnery oak site near Andrew, Texas. There were 24 noninoculated (control) pots and 24 pots inoculated with commercially available strains of *Rhizobium*. Among the inoculated pots, 12 received a strain of *Rhizobium* inoculum effective on milkvetch (*Astragalus*) and the other 12 received a strain effective on crownvetch (*Coronilla*).[1] The plants were harvested when 92 days old.

[1]Dr. J. C. Burton, The Nitragin Company, Milwaukee, Wisconsin, had run tests on mesquite seedlings in 1971 and found that several strains of *Rhizobium* gave good nodulation. Two of the better *Rhizobium* strains were those used to inoculate *Astragalus* and *Coronilla*.

The second experiment was conducted with 50 pots. Soil was collected in early June from the root zone of undisturbed or recently disturbed mesquite trees at five locations in west Texas. Small mesquite roots in the top 50 cm of soil were examined for nodules. Collections were made near Noodle (Jones County) from an upland site with sandy soil, near Lubbock from an upland site with fine sandy loam soil, near Dickens (Dickens County) from a lowland site with clay loam soil, near Post from an upland site with clay loam soil, and near Abilene from a lowland site with clay loam soil. All but the Lubbock site had been recently mechanically cleared. The field soil was thoroughly mixed with sand in the ratio of 1:9. The soil was potted, 20 mesquite seeds/pot were planted, fertilized, and the vitamins thiamine, pyridoxine, niacin, and biotin were added. The plants were harvested when 60 days old.

Results

Honey mesquite seedlings responded to adapted strains of *Rhizobium* bacteria in a manner similar to most legumes. The seedlings were usually profusely nodulated (Table 1, Fig. 1). Nodulation did occur in the controls. Control pots that had 10% of the potting soil volume as fine sandy loam from a cultivated field in Lubbock had as many nodules as pots inoculated with the *Astragalus* strain of *Rhizobium*. Only the *Coronilla* strain of *Rhizobium* caused the development of more small nodules.

In the first experiment, only 4% of the pots had plants free of nodules. Another 10% of the pots had plants with only a few nodules. These two groups were combined and compared with the pots having well-nodulated plants (Table 2). In pots having well-nodulated seedlings, plant density per pot was higher, the plants were taller and heavier, and the weight of nitrogen was greater than in pots having poorly nodulated or nonnodulated seedlings. The amount of nitrogen in mesquite seedlings was closely correlated with total plant weight (Table 3). The nodules occurred on small roots and ranged from less than 1 mm to 11 mm in length (Fig. 2). Nodules under 2 mm had little influence on plant weight and quantity of nitrogen. Larger nodules had a great influence on plant and nitrogen weight. The following multiple regression equation was developed to relate the effect of nodule size and numbers to plant weight.

$$Y = 659 + 79X_1 + 59X_2 + 8X_3$$

where Y = plant weight (mg), X_1 = number of nodules > 4 mm in cross section; X_2 = number of nodules 2 to 4 mm in cross section; and X_3 = number of nodules < 2 mm in cross section. The R^2 value was 0.78.

The source of soil (inoculum) affected nodulation frequency and nodule size (Table 4). A moist sandy soil produced the greatest number of large nodules whereas a very dry clay loam soil produced the least number of nodules. Some nodulation occurred, however, regardless of soil source.

There were some nonnodulated plants in most pots. The nonnodulated plants were about the same size as associated nodulated plants. It is not known whether these nonnodulated plants had nodules that died and decayed or whether these plants were dependent upon adjacent nodulated ones for nitrogen. It is known that where all plants in a pot lacked nodules, the plants were stunted and had very small root systems.

Discussion

Mesquite nodulated readily and profusely when an adapted strain of *Rhizobium* was present. The source of effective inoculum was open to question in experiment one. Did naturally

Table 1. Selected properties of 92-day-old mesquite seedlings according to source of *Rhizobium* inoculum (per pot).

Character	Source of inoculum		
	Control	Astragalus	Coronilla
Number of plants	4.6	3.8	4.6
Plant weight (g)	35.1	33.1	33.6
Total plant height (cm)	59.9	53.6	59.5
No. nodules	27.5 b[1]	22.6 b	49.1 a
No. nodules > 2 mm	16.9	13.4	23.6
No. nodules < 2 mm	10.6 b[1]	9.3 b	25.5 a
Nitrogen per pot (mg)	40.6	37.8	38.7
Percent nitrogen	2.1	2.1	2.0

[1]Means within a row followed by the same letter are not significantly different ($P < 0.01$). Means in all other rows are not significantly different.

Table 2. A comparison of selected properties of 92-day-old mesquite seedlings possessing many nodules with seedlings having few nodules (per pot).

Character	Many nodules	Few nodules
Number of pots	41	7
Number of plants	4.7**[1]	2.6
plant weight (mg)	1,855**	542
Total plant height (cm)	58**	23
No. of nodules > 2 mm	18**	2
No. of nodules < 2 mm	14**	1
Nitrogen weight (mg)	39**	13
% nitrogen	2.12	2.34

[1]Means within a row are significantly different (** $P < 0.01$).

Table 3. A simple correlation matrix for selected properties of mesquite seedlings.

Character	Plant weight	Nitrogen weight	Nodules >4 mm	Nodules 2–4 mm	Nodules <2 mm	% nitrogen
Weight of nitrogen	0.98**[1]					
No. nodules > 4 mm	0.81**	0.82**				
No. nodules 2–4 mm	0.67**	0.60**	0.53*			
No. nodules < 2 mm	0.28	0.18	0.11	0.40		
% nitrogen	0.12	0.31	0.28	–0.15	–0.49*	
Plant height	0.90**	0.86**	0.79**	0.69**	0.25	0.06

[1]** $P < 0.01$; * $P < 0.05$.

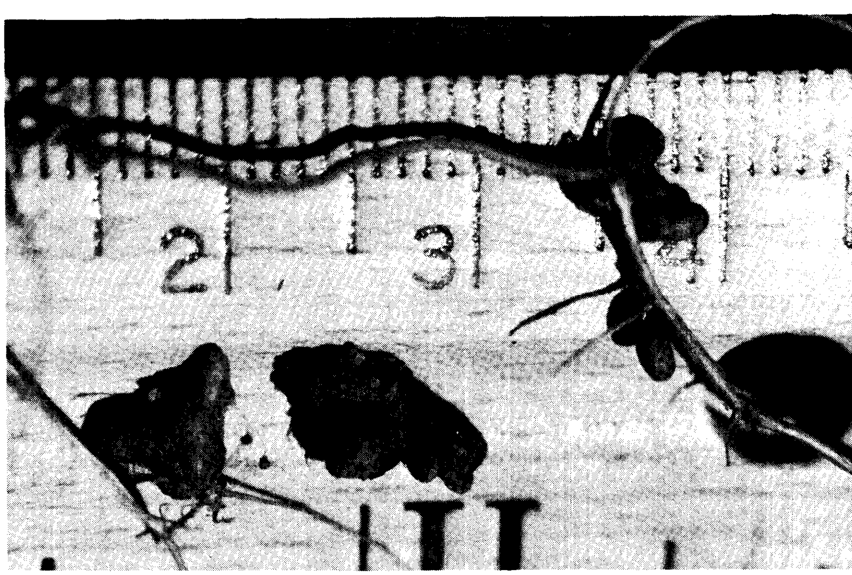

Fig. 1. *Nodules on roots of mesquite. Gradation on scale in millimeters.*

Fig. 2. *The largest mesquite nodule was 11 mm long.*

occurring *Rhizobium* cause nodulation in control and treated pots? Did the commercial strains of inoculum cause nodulation in treated pots? Did the application of commercial strains of inoculum to treated pots result in contamination of control pots? The methodology used in establishing experiment one prevented major soil contamination in control pots. These pots were prepared, seeded, and placed in the growth room prior to mixing the commercial inoculum with mesquite seeds but some air-borne bacterial contamination cannot be ruled out. Ashford and Bolton (1960) found that it was difficult to prevent air-borne contamination when attempting to grow nodule-free sweet clover and alfalfa. One of the conclusions in the review of Burton (1965) however, was that "the evidence is mounting that far greater numbers of rhizobia are needed to bring about effective nodulation than was formerly suspected." Probably only a few nodules developed as a result of air borne contamination.

The nodules in mesquite seedlings of control pots were probably caused by natural *Rhizobium* strains present in soil of the cultivated field. Nodulation in pots treated with the *Astragalus* strain may or may not have been due to the effect of the added inoculum. The *Coronilla* strain of *Rhizobium* did have a positive effect and resulted in a greater number of nodules being present.

The results of the second experiment (Table 4) are generally in agreement with studies on other legumes. Moist soils usually have higher *Rhizobium* populations than do dry soils. Waksman (1952) observed that drying is injurious to *Rhizobium* populations but it is not fully destructive.

Allred (1949) doubted the claim that mesquite put nitrogen into the soil. He reported that no nodules were found in a field examination of roots of mature mesquite trees and seedlings. The writer also examined mesquite roots in June at five sites in west Texas while collecting soil for inoculation purposes. Roots were dug to a depth of 1 m but no nodules were found. Yet every inoculum source resulted in the development of nodules on mesquite seedlings growing in the growth chamber. It is difficult to believe that nodules do not occur on mesquite trees growing in the natural environment, but it is easy to understand that a superficial examination of the root system would fail to reveal nodules. Nodules are usually present on perennial legumes for only part of the year. In the arid and semiarid areas where mesquite is found, nodules are probably only present for 1 to 3 months annually. The *Rhizobium* bacteria infect the root hairs in spring, the nodules develop and function during the growing season (Bolton, 1962), become senescent, die, and decay. The nodules on mesquite seedlings and 1-year-old plants were found on small, fragile roots usually less than 1 mm in diameter. Even with careful root washing procedures, many nodules were disengaged from roots. Meyer et al. (1971) observed that there have been few careful studies of the mesquite root system. If careful field excavations of honey mesquite are made in west Texas under the right conditions, nodules will probably be found on the young, actively growing roots. Suitable conditions would include above-average precipitation during the months of active growth (May or June) and a sandy, well-drained soil.

The significance of this study to range improvement practices in the southwestern United States and Mexico is unknown. The detrimental effects of mesquite as a water user and barrier to livestock are well known. Without extensive field research, it cannot be established whether honey mesquite is or is not an important nitrogen fixer on rangelands.

Table 4. A comparison of soil properties and nodulation of 60-day-old mesquite seedlings grown in soil collected from five sites in west Texas.

	Source of soil				
Character	Noodle	Lubbock	Abilene	Dickens	Post
Soil texture	sand	sandy loam	clay loam	clay loam	clay loam
Field moisture status	moist	medium	moist	medium	very dry
Pots with nodules (%)	100	60	80	80	70
No. nodules > 2 mm	4.8 a[1]	5.0 a	2.0 ab	1.2 b	0.9 b
No. nodules > 4 mm	1.6 a	1.2 a	1.1 a	0.4 a	0.4 a

[1]Means within a row followed by the same letter are not significantly different ($P < 0.01$).

Literature Cited

Allen, E. K., and O. N. Allen. 1958. Encyclopedia of Plant Physiology, Vol. 8, p. 48. Springer, Berlin.

Allred, B. W. 1949. Distribution and control of several woody plants in Texas and Oklahoma. J. Range Manage. 2:17-29.

Ashford, R., and J. L. Bolton. 1960. Note on a technique for the production of sterile sulphur-free pot cultures for legumes. Can. J. Plant Sci. 40:574-575.

Bolton, J. L. 1962. Alfalfa. Leonard Hill, London. 473 p.

Burton, J. C. 1965. The *Rhizobium*-legume association. *In:* Gilmour, C. M. and O. N. Allen. (Ed.) Microbiology and Soil Fertility, p. 107-134. Oregon State Univ. Press, Corvallis.

Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. Studies in Sci. No. 5, University of Wisconsin, Madison.

Meyer, R. E., H. L. Morton, R. H. Haas, E. D. Robinson, and T. E. Riley. 1971. Morphology and anatomy of honey mesquite. U.S. Dep. Agr. Tech. Bull. 1423. 186 p.

Tiedemann, A. R., and J. O. Klemmedson. 1973. Nutrient availability in desert grassland soils under mesquite (*Prosopis juliflora*) trees and adjacent open areas. Soil Sci. Soc. Amer. Proc. 37:107-111.

Waksman, S. A. 1952. Soil microbiology. Wiley, New York. 356 p.