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MESQUITE RHIZOBIA ISOLATED FROM THE SONORAN DESERT: PHYSIOLOGY AND EFFECTIVENESS

NASTARAN H. SHOUSHTARI and IAN L. PEPPER
Department of Soil and Water Science, 429 Agricultural Sciences, University of Arizona, Tucson, AZ 85721, U.S.A.

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Summary—A mesquite *Rhizobium* isolated from the Sonoran Desert (strain AZ-M1) and a commercial mesquite *Rhizobium* obtained from the Nitragin Company (strain 31A5) were chosen as superior strains from among many evaluated in a screening study of the efficiency of mesquite rhizobia. Both strains were fast-growing and acid-producing in defined media. The desert strain AZ-M1 had the shortest mean generation time of 3.5 h. Strain 31A5 grew better in broth amended with various sugars and amino acids, but generally produced less acid. The desert strain showed greater resistance to various antibiotics than did 31A5. In a greenhouse study N applied at high rates inhibited N_2 fixation when either strain was used as inoculant for mesquite seed. At low N rates, AZ-M1 fixed more N than 31A5. Total N, nodule weight, C_2H_2 reduction, mesquite shoot weight and root weight were all significantly increased when AZ-M1 was the applied inoculant. This study shows that the mesquite *Rhizobium* AZ-M1 isolated from the Sonoran Desert is infective and effective on mesquite seedlings. Fast growth rate, acid production and high resistance to antibiotics in laboratory media may indicate the adaptation of this organism to its microbial niche in the Sonoran Desert.

INTRODUCTION

Mesquite (*Prosopis*) is a tree legume which constitutes a major component of the semi-arid ecosystems of the world, yet little information is currently available regarding its N_2 fixing capabilities. Even less is known of the organisms responsible for this fixation. Bailey (1976), reported nodulation of honey mesquite (*Prosopis glandulosa torr.* var. glandulosa) in the greenhouse in response to inoculation with field soil. Nodulation of mesquite has also been reported by Eskew and Ting (1973) and Felker and Clark (1980). However, nodulation has not been observed in a natural field situation, no doubt in part because of the depth of rooting of mesquite trees. Felker and Clark (1982) reported that the presence of nodules on mesquite grown in colmns was in part dependent on the depth at which the soil became moist.

The extent of N fixation has been estimated by Kohl *et al.* (1981), Rundel *et al.* (1982) and Felker (1979). Estimates have ranged from 25–30 kg N to 150 kg N ha^{-1} yr^{-1}. These inputs of fixed N would be important in desert ecosystems, where N is often limited. Tiedemann and Klemmedson (1973) showed that soil beneath mesquite trees contained almost 3 times as much organic C and N as that outside of the mesquite's foliage cover.

Mesquites are useful for cattle forage, firewood and for enriching desert soils. Mesquite also has potential as a source of wood fuel. Nilson *et al.* (1982) recently estimated that plantation productivity could be 15,000 kg ha^{-1} yr^{-1}.

Since scant information is available on the mesquite N_2 fixing organisms, we have evaluated the characteristics of mesquite rhizobia. In this paper we report on the physiology and effectiveness of a superior strain isolated from the Sonoran Desert.

MATERIALS AND METHODS

Isolation of indigenous strains from the Sonoran Desert

To isolate indigenous rhizobia that would effectively nodulate mesquite (*Prosopis juliflora* var. velutina) an experiment was conducted in a greenhouse at the University of Arizona Experimental Farm in Tucson, Arizona in November, 1980. Mesquite seed was collected from the ground under trees located along washes in west Tucson. Seeds were scarified using sandpaper and were planted in polyvinyl chloride pipes (12 cm dia, 75 cm long) containing crystal silica sand (grade 20). One g of air-dry soil taken from beneath a mesquite tree was used to inoculate each seed, with 5 seed $column^{-1}$. Columns were replicated to allow 2 columns to be dismantled and sampled each week for the presence of nodules. N-free macronutrients (Sloger, 1969) and micronutrients (Vincent, 1970) were added at the time of watering. Plants were watered every other day during the first 3 weeks of growth, and later twice a week with 200 ml of nutrient solution $column^{-1}$.

After 8 weeks of growth nodules were observed on a few plants. A Gram-negative organism was isolated from a nodule, and maintained on a Yeast Extract Agar (YEM) slant, for future studies (Vincent, 1970). This organism indigenous to the native soil was designated AZ-M1.

Screening of strains

A second experiment in March, 1981, involved testing the native isolate from the first greenhouse study against some commercial strains of rhizobia (Nitragin Company, Milwaukee) known to form nodules on mesquite. A walk-in growth chamber (Percival, model PGW-9675) with day and night

temperatures of 28°C and 22°C respectively was used with a photo-period of 12 h. Inocula were made by growing the following rhizobia to log phase in YEM broth (YEMB): *Prosopis* spp (host), 133Z1 (strain); *Coronilia vaia*, 3188; *Coronilla cretica*, 31A5; *Baptisia alba*, 15A1 and *Prosopis juliflora*, *AZ-M1*.

Mesquite seeds were scarified using sandpaper, surface sterilized (rinsed with 95% ethanol, soaked for 3 min in 0.2% acidified $HgCl_2$, and rinsed with 5 changes of sterile water). They were then soaked on wet filter papers for 24 h, and planted into 2000 ml plastic pots containing crystal silica sand. One ml of late log phase inoculum was pipetted directly onto each seed. (10^7 cell seed^{-1}, 5 seeds pot^{-1}). They were watered with 150 ml N-free nutrient solution twice a week. Plants were examined after 70 days for nodules. Differences in the extent of nodulation among the different inocula led to the selection of two superior strains for further studies.

Physiology of selected strains

The two selected rhizobia were examined culturally in different media. The growth rates of the two strains in YEMB were determined in nephlometer flasks aerated on a rotary shaker (Labline Orbit Shaker 3590) at 100 rev min^{-1}. Turbidity of the cultures was monitored by use of a spectrophotometer (Bausch and Lomb Spec. 20) at a wavelength of 420 μm (data not reported). Concurrently, samples were taken aseptically, diluted and plate counted on YEM agar. Growth of the two strains and subsequent pH changes in a defined medium (Tan and Broughton, 1981) was also monitored. Five hundred ml of the medium was used for the growth of each strain and shaken at 25°C. Samples were periodically removed from the flasks and the viable cell number estimated by plate counts on YEM agar medium. The pH of the medium was measured on a Leeds and Northrup pH meter (Model 7415).

Growth of the two strains was also tested on different combinations of amio acids, (asparagine and glutamine) and sugars (fructose, galactose and glucose). Mannitol and yeast extract were eliminated from normal YEMB medium when testing other amino acid–sugar combinations. Sugars were added at a concentration of 60 mM and the amino acids at 6 mM final concentration (Tan and Broughton, 1981) Growth was determined by measuring the turbidity of the culture on a spectrophotometer at 650 nm.

The antibiotic disk susceptibility test (Antoun, 1982) was used to measure the intrinsic resistance of the two strains of *Rhizobium* to known antibiotics. Antibiotics were, penicillin 10 units; ampicillin 10 μg; cephalothin 30 μg; sulfadiazine 0.25 mg; carbenicillin 100 μg; chloramphenicol 30 μg; methicillin 5 μg; neomycin 30 μg; streptomycin 10 μg; tetracycline 30 μg; kanamycin 30 μg; erythromycin 15 μg. The two strains were cultured in YEM broth and grown for 4 days at room temperature on a rotary shaker. Log phase cultures of 10^7 cells ml^{-1} were diluted 20-fold. YEM agar plates were then surface inoculated in duplicate by addition of 0.5 ml diluted culture of either strain, and spread on the plates to ensure a confluent growth. Antibiotic discs (BBL Sensi-disc, Becton Dickinson and Co.) were applied on the plates, and spaced at least 3 cm apart. After 4 days at room temperature, plates were examined and strains classified according to their resistance to antibiotics (Difco Manual, 1953) by measuring the diameter of the zone of inhibition around each Sensi-disc.

Effectiveness of strains

The two selected strains of mesquite *Rhizobium*, *Coronilla cretica* 31A5 from *Coronilla cretica*, and the native isolate AZ-M1, were used in a study to compare their N_2 fixation efficiency under different rates of N fertilization. This study was performed in a greenhouse at the University of Arizona Experimental Farm in Tucson, Arizona in July 1981. Plastic buckets (11 litre) were filled with crystal silica sand. Mesquite seeds were treated as previously, before planting. Fifteen seeds bucket^{-1} were planted and later thinned to 10 bucket^{-1}. Seeds were inoculated with 1 ml of log phase culture of either strain grown in YEMB and watered with N-free nutrient solution (250 ml twice a week) or nutrient solution with different levels of N fertilizer. These were 0, 10, 20, 40, 80 or 160 mg N kg^{-1} as KNO_3. Treatments were completely randomized with 4 replicates treatment^{-1}.

Plants were harvested after 60 days when they were at least 300 mm tall. Roots were separated from shoots at the cotyledon scars. The C_2H_2 assay (Hardy *et al.*, 1968) was performed on the intact root systems with nodules to evaluate biological N_2 fixation. Shoot, root and nodule weight were determined, and their N content by use of microKjeldahl techniques (Bremner, 1965).

RESULTS AND DISCUSSION

Isolation of indigenous strains

Among the N-free nutrient treated columns in the greenhouse with soil as the inoculant, some plants had a few small nodules (2–3 mm dia) enabling us to isolate a mesquite *Rhizobium* indigenous to the native soil of Tucson. Use of the fluorescent antibody technique (Schmidt *et al.*, 1968) confirmed the presence of AZ-M1 in all nodules tested.

Screening of strains

The comparison study of nodulation in the growth chamber resulted in the selection of the native isolate designated AZ-M1 and a commercial strain isolated from nodules of *Coronilla cretica* inoculated with strain 31A5 (Nitragin Company, Milwaukee). Selection of the *Rhizobium* was based on the relative number and mass of effective nodules on the roots of mesquite seedlings. The biomass of the plants and height data were also considered in selecting the two inoculants. Nodules formed by the native-isolate were found mostly on small roots and ranged from 1 to 10 m in length and 1–6 mm dia. Nodules formed by the best selected commercial strain 31A5 were distinctly smaller in size than those from strain AZ-M1. Nodules from both strains were multi-lobed, a characteristic resulting from continuous meristematic growth (Bergersen, 1978).

Physiology of selected strains

Growth of either strain in YEMB indicated that

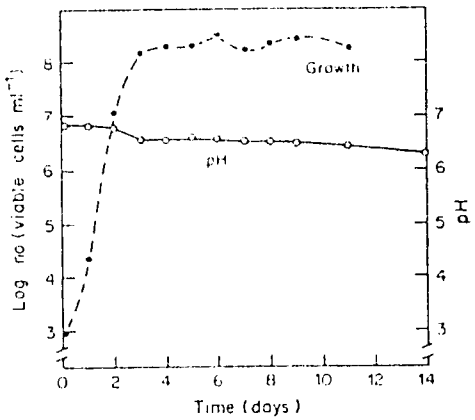

Fig. 1. Growth of native strain AZ-M1 in defined medium. Mean generation time, 3.5 h.

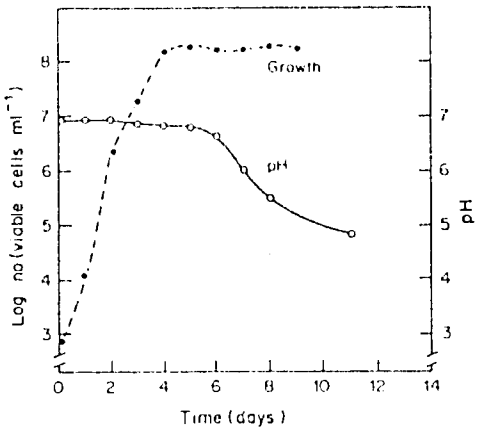

Fig. 2. Growth of strain *Coronilla cretica* 31A5 in defined medium. Mean generation time, 3.8 h.

AZ-M1 and 31A5 were both fast-growing strains. Mean generation times were 3.4 h for AZ-M1 and 4.1 h for 31A5. Growth of the rhizobia in the defined medium of Tan and Broughton (1981) resulted in mean generation times of 3.5 h and 3.8 h for AZ-M1 and 31A5, respectively (Figs 1 and 2). Strain 31A5 decreased the pH of the medium from 6.9 to 4.9. A decrease in pH to 6.3 was noted with AZ-M1. The pH decreases are characteristic of fast-growing rhizobia (Norris, 1965; Tan and Broughton, 1981). Tan and Broughton (1981) found the acidic pH change caused by fast growing rhizobia to be the result of selective utilization of substrate, which is also coupled to the secretion of various organic acids. Cooper (1982) suggested that acid or alkali production was a markedly variable strain characteristic, which is influenced by changes in the composition of growth medium. He also reported that acid or alkali can be generated by one strain in a single medium at different phases of the growth cycle. Cooper (1982) also found the pH changes to be associated with relatively high cell densities even in unbuffered media. The decrease in pH caused by strain 31A5 at the stationary phase, is in agreement with the work of Cooper (1982). Although care must be taken in assigning ecological significance to acid production in laboratory media, we feel that the potential for acid production via metabolism, may be an advantage to desert organisms existing in high pH soils and may be the result of adaptations to the desert environment. Fast-growing acid producing strains of the normally slow-growing alkali-producing *R. lupini* have also been found to prevail in the Sonoran Desert.

Growth of either strain in the modified YEMB (mannitol and yeast extract eliminated) supplemented with various amino acids and sugars was not good (Table 1). Normal (YEMB) medium was used as a control to compare growth in the modified media. Strain 31A5 grew relatively well in the combination of asparagine with galactose mannitol or glucose. Some growth was also observed in the combination of glutamine with mannitol or glucose. Tan and Broughton (1981) found their 6 strains of rhizobia to grow well on all the first 4 combinations of Table 1. They also noticed an acid reaction produced by fast growing strains in these media. The pH of the combinations in which growth was observed with strain 31A5 tended to drop only slightly, except for the combination of asparagine and glucose where the pH decreased from 7.1 to 6.5. Strain AZ-M1 grew slightly in most combinations except when fructose was the sugar. AZ-M1 also grew in the presence of yeast and galactose whereas 31A5 did not. In several instances the pH of the medium decreased dramatically with strain AZ-M1, even though growth was slight. It is worth noting that none of the combinations of amino acids or yeast with the sugar fructose provided growth for either strain.

Table 1. Growth of AZ-M1 and 31A5 on combinations of sugars and amino acids in modified YEMB

Substrate combination	Initial pH	Strain: AZ-M1 Absorbance	AZ-M1 Final pH	31A5 Absorbance	31A5 Final pH
Glutamine + fructose	6.1	0.01	6.1	0.02	6.1
Glutamine + galactose	7.2	0.04	5.7	0.01	7.1
Glutamine + mannitol	7.4	0.06	7.2	0.12	7.1
Glutamine + glucose	7.1	0.06	6.2	0.08	7.0
Asparagine + fructose	6.4	0.01	6.4	0.01	6.3
Asparagine + galactose	7.0	0.01	7.0	0.34	6.8
Asparagine + mannitol	7.5	0.06	6.0	0.45	7.1
Asparagine + glucose	7.1	0.08	5.7	0.30	6.5
Yeast + fructose	5.8	0.01	5.8	0.01	5.7
Yeast + galactose	6.9	0.36	6.6	0.02	6.3
Yeast + mannitol (control)	7.5	0.82	6.7	0.96	6.8
Yeast + glucose	6.7	0.54	6.4	0.27	6.5

Table 2. Effect of antibiotics on the growth of strains AZ-M1 and 31A5

Antibiotic disks	Strains AZ-M1	31A5
	Zone of inhibition (mm)	
Penicillin 10	0	42
Ampicillin 10	22	40
Cephalothin 30	0	46
Sulfadiazine 0.25	46	65
Carbenicillin 100	30	50
Chloramphenicol 30	31	31
Methicillin 5	0	27
Streptomycin 10	18	26
Tetracycline 30	40	75
Kanamycin 30	22	19
Erythromycin 15	20	20
Neomycin 30	14	10

Table 3. Effect of NO_3-N on N fixation by AZ-M1 and 31A5

Added N ($mg\,l^{-1}$)	Acetylene reduction ($nmol\ plant^{-1}\,h^{-1}$) AZ-M1	31A5
0	329.4	124.4
10	227.9	95.9
20	101.4	49.1
40	35.5	22.4
80	29.7	24.0
160	27.6	26.9

Each two values which are underlined do not differ significantly ($P = 0.01$)

Differences in acid production in the defined medium of Tan and Broughton (1981), may be related to utilization of sugars by strain 31A5. This medium contained lysine and glucose. Substances causing the pH change could be produced as by-products of total or partial digestion of substrates or be secreted by rhizobia during growth (Tan and Broughton, 1981). Acid production by either strain may be beneficial in the high pH soils of the arid southwest.

The antibiotic disk susceptibility test measured the variation in the intrinsic resistance of the two strains of mesquite rhizobia to 12 different known antibiotics (Table 2). Strain 31A5 was, in general, sensitive to all 12 antibiotics. This strain proved to be the least sensitive to neomycin 30 with a 10 mm zone of inhibition, and to be the most sensitive to tetracycline 30 with a 75 mm zone of inhibition. Strain AZ-M1 showed high intrinsic antibiotic resistance to penicillin 10, cephalothin 30, and methicillin. This strain was very sensitive to sulfadiazine 0.25 and tetracycline 30 as was the strain 31A5 (Table 2). Using this test, these two strains can be identified under laboratory conditions using at least 3 of the 12 antibiotics. Specific intrinsic antibiotic resistance patterns are now determined for *Rhizobium* inoculants to differentiate them from strains residing in the soil (Beynon and Josey, 1980). It is of interest that desert soils are often of high pH and at low moisture potentials. These are conditions which may promote growth of actinomycetes relative to other organisms. Actinomycetes often produce antibiotics under laboratory conditions, and this may explain the higher intrinsic resistance of the desert strain adapted to desert conditions, although it does not explain resistance to penicillin which is produced by a mold. Again care in extrapolating laboratory responses to field situations must be taken. However, Maqbul Hossain and Alexander (1984) have enhanced soybean rhizosphere colonization by using antibiotic resistant strains of *R. japonicum*.

Effectiveness of strains

In the second greenhouse study velvet mesquite seedlings were inoculated with either strain AZ-M1 or 31A5, and nodulation occurred with both strains. All variables were analyzed according to the Statistical Package for Social Sciences (1977). A 2-way analysis of variance was run to analyze the effects of NO_3-N treatments, strain differences and their interactions. Separation of the means was performed at the 1% level of significance using the Least Significance Difference (LSD) test (Steel and Torrie, 1960).

There was a significant strain by N treatment interaction with respect to N_2 fixation as shown by the C_2H_2 assay (Table 3). The desert strain fixed significantly greater N than 31A5 at the 0 or 10 mg N l^{-1} rates. At higher rates, both strains reacted similarly. As N rates increased, N fixation decreased. Thus, N fixation would be favored by the low N values usually associated with desert soils.

The effect of NO_3^- as an inhibitor of N fixation is clearly shown in Table 4 where data are averaged over both strains. C_2H_2 reduction and nodule weight decreased with increased N. However, N fixation at low applied N rates was able to promote good shoot growth. Total plant N was also similar for all treatments except the zero N rate. Generally root weights decrease with increased applied N; although this trend does occur at high N rates, the effects are confounded at low N rates by N fixation.

Dazzo and Brill (1978) reported the inhibition of nodulation of clover at the stage of infection, when the soil contained significant amounts of N. They found the degree of binding of *R. trifolii* to root hairs and the levels of lectin present decreased with increasing NO_3^- and NH_4^+ concentration. Accumu-

Table 4. Effect of NO_3-N fixation and plant biomass production

Added N ($mg\,l^{-1}$)	Acetylene reduction ($nmol\ plant^{-1}\,h^{-1}$)	Nodule weight ($mg\ plant^{-1}$)	Shoot weight ($mg\ plant^{-1}$)	Root weight ($mg\ plant^{-1}$)	Total N ($mg\ plant^{-1}$)
0	226.9a	14.95a	317.75a	189.75ab	7.16a
10	161.9b	12.10b	416.88b	225.75bc	8.80ab
20	75.3c	7.34c	411.63b	234.63c	8.66ab
40	28.9c	1.71d	403.38b	223.13bc	9.77b
80	26.8c	0.70d	400.75b	212.38abc	9.36b
160	27.3c	0.31d	361.25ab	171.50a	9.36b

Values followed by similar letter do not differ significantly ($P = 0.01$).

Table 5. Strain differences in N fixation and plant biomass production

Variables	Strains AZ-M1	31A5
Total N (mg plant^{-1})	9.51a	8.21b
Nodule weight (mg plant^{-1})	6.76a	5.61b
Acetylene reduction (nmol plant^{-1} h^{-1})	125.25a	57.11b
Shoot weight (mg plant^{-1})	423.58a	346.96b
Root weight (mg plant^{-1})	224.83a	194.21b

Values in rows followed by a different letter do differ significantly ($P = 0.01$).

lation of NO_3^- as a result of nitrate reductase activity by rhizobia and subsequent destruction of indole acetic acid may be another possible mechanism preventing infection (Arp and Zumft, 1983). Indole acetic acid produced by rhizobia from tryptophane has been implicated in the infection process (Nutman, 1977). However, Gibson and Pagan (1977) found retarded nodulation in the presence of NO_3^- even with the mutants of *R. trifolii* defective in nitrate reductase. Although the reason behind the inhibitory effect of NO_3-N is still a controversy, it is clear that the inhibition of NO_3-N in this experiment must have occurred either at the stage of infection or nodule initiation. Nitrogen also inhibited nitrogenase activity within nodular material and reduced the amount of N fixed. Examination of the data in Table 4 shows that averaged over both strains, the specific activity decreased from 15.2 when zero N was applied to 10.3 nmol mg nodule^{-1} h^{-1} when 20 mg l^{-1} of N was added.

The mean of the different variables for the two strains regardless of NO_3-N treatments are shown in Table 5. All 5 variables were significantly different for the two strains ($P = 0.01$). Although the difference is only large for C_2H_2 reduction and shoot weight, the desert isolate showed its significant superiority over 31A5 in every variable analyzed in this experiment. Interactions were only evident in the C_2H_2 data.

This study has shown that the mesquite *Rhizobium* strain AZ-M1 indigenous to desert soils is infective and effective, and capable of fixing N in the low N soils of the desert southwest. This strain exhibited physiological differences from other strains capable of nodulating mesquite. Because of its fast growth rate, potential acid production and high intrinsic resistance to antiotics in the laboratory it is possible that strain AZ-M1 has adapted to its microbial niche in the Sonoran Desert.

REFERENCES

Antoun H. (1982) Strain identification in *Rhizobium meliloti* using the antibiotic disk susceptibility test. *Plant and Soil* **66**, 45–50.

Arp D. J. and Zumft W. G. (1983) Regulation and control of nitrogenase activity. In *Nitrogen Fixation* (A. Muller and W. Newton, Eds), pp. 149–179. Plenum Press, New York.

Bailey A. W. (1976) Nitrogen fixation in honey mesquite seedlings. *Journal of Range Management* **29**, 479–481.

Benson L. and Darrow R. A. (1945) *A Manual of Southwestern Desert Trees and Shrubs*. University of Arizona Biological Science Bulletin 6.

Bergersen F. J. (1978) Physiology of legume symbosis. In *Interactions Between Non-Pathogenic Soil Microorganisms and Plants* (Y. R. Dommergues and S. U. Krupa, Eds), pp. 305–333. Elsevier, Amsterdam.

Beynon J. L. and Josey D. P. (1980) Demonstration of heterogeneity in a natural population of *Rhizobium phaseoli* using variation in intrinsic antibiotic resistance. *Journal of General Microbiology* **118**, 437–442.

Bremner J. M. (1965) Total: inorganic forms of nitrogen; organic forms of nitrogen. In *Methods of Soil Analysis* (Part 2) (C. A. Black *et al.*, Eds), pp. 1149–1254. American Society of Agronomy Monograph 9.

Cooper J. E. (1982) Acid production, acid tolerance and growth rate of *Lotus* rhizobia in laboratory media. *Soil Biology & Biochemistry* **14**, 127–131.

Dazzo F. B. and Brill W. J. (1978) Regulation by fixed nitrogen of host-symbiont recognition in the *Rhizobium*-clover symbiosis. *Plant Physiology* **62**, 18–21.

Eskew D. L. and Ting I. P. (1978) Nitrogen fixation by legumes and blue-green algal-lichen crusts in a Colorado desert environment. *American Journal of Botany* **65**, 850–856.

Felker P. J., (1979) Mesquite, an all purpose leguminous arid land tree. In *New Agricultural Crops* (G. A. Ritchie, Ed.), pp. 89–132. West View Press, Washington, DC.

Felker P. J. and Clark P. R. (1980) Nitrogen fixation (acetylene reduction) and cross-inoculation in 12 *Prosopis* (mesquite) species. *Plant and Soil* **57**, 177–186.

Felker P. J. and Clark P. R. (1982) Position of mesquite (*Prosopis* spp.) nodulation and nitrogen fixation (acetylene reduction) in 3-m long phraetophytically simulated soil columns. *Plant and Soil* **64**, 297–305.

Gibson A. H. and Pagan J. D. (1977) Nitrate effects on the nodulation of legumes inoculated with nitrate-reductase-deficient mutants of *Rhizobium*. *Planta* **154**, 17–22.

Hardy R. W. F., Holsten R. D., Jackson E. K. and Burns S. C. (1968) The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiology* **43**, 1185–1207.

Kearney T. A. and Pebbles R. H. (1960) *Arizona Flora*, 2nd edn with supplement. University of California Press, Berkeley.

Kohl D. H., Bryan B. A., Schearer G. and Skeeters J. L. (1981) Natural abundance of ^{15}N of *Prosopis* as an index of N_2 fixation in desert ecosystems. *Bulletin. Ecological Society of America* **62**, 133–134.

Maqbul Hossain A. K. and Alexander M. (1984) Enhancing soybean rhizosphere colonization by *Rhizobium japonicum*. *Applied and Environmental Microbiology* **48**, 468–472.

Nilson E. G., Rundel P. W. and Sharifi M. R. (1982) Productivity in native stands of *Prosopis glandulosa*, mesquite, in the Sonoran Desert of southern California and some management implications. *California Riparian Environment Symposium*, Sept. 17–19. Davis, California.

Norris D. O. (1965) Acid production by *Rhizobium*, a unifying concept. *Plant and Soil* **22**, 143–166.

Nutman P. S. (1977) Study frameworks for symbiotic nitrogen fixation. In *Recent Developments in Nitrogen Fixation* (W. Newton, J. R. Postgate and C. Rodriquez-Barrueco, Eds), pp. 443–447. Academic Press, London.

Rundel P. W., Nilson E. T., Sharifi M. R., Virginia R. A., Jarrell W. M., Kohl D. M. and Shearer G. B. (1982) Seasonal dynamics of nitrogen cycling for a *Prosopis* woodland in the Sonoran Desert. *Plant and Soil* **67**, 343–353.

Schmidt E. J., Bankole R. O. and Bohlool B. B. (1968) Fluorescent-antibody approach to study of rhizobia in soil. *Journal of Bacteriology* **95**, 1987–1992.

Sloger C. (1969) Symbiotic effectiveness and N_2 fixation in nodulated soybean. *Plant Physiology* **44**, 1666–1668.

Statistical Package for the Social Sciences (1977) Version 7. Vogelback Computing Center, Northwestern University, U.S.A.

Steel R. G. D and Torrie J. H. (1960) *Principles and Procedure of Statistics*. McGraw-Hill, New York.

Tan I. K. P. and Broughton W. I. (1981) Rhizobia in tropical legumes. XIII. Biochemical basis of acid and alkali reactions. *Soil Biology & Biochemistry* **13**, 389–393.

Tiedemann A. R. and Klemmedson J. O. (1973) Nutrient availability in desert grassland soils under mesquite (*Prosopis juliflora*) trees and adjacent open areas. *Soil Science Society of America Proceedings* **37**, 107–111.

Vincent J. M. (1970) *A Manual for the Practical Study of the Root Nodule Bacteria*. International Biological Programme Handbook 15. Blackwell, Oxford.

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