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Ecology of Fast-Growing and Slow-Growing Mesquite-Nodulating Rhizobia in Chihuahuan and Sonoron Desert Ecosystems

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ABSTRACT

Prosopis glandulosa **(mesquite) is effectively nodulated by both fast-growing (FG) and slow-growing (SG) rhizobia. The distribution of indigenous, free-living, FG and SG rhizobia, and their morphological and symbiotic characteristics associated with the rooting systems of mesquite were investigated. The three warm desert ecosystems studied were an arroyo, a sand dune, and a playa in the Chihuahuan Desert of New Mexico, and a sand dune and playa in the Sonoran Desert of southern California. The FG rhizobia dominated the free-living rhizobial population from 0- to 8-m depth, at the New Mexico playa. In contrast to the New Mexico playa, the surface 1 m of soil under mesquite at the California playa was dominated by SG rhizobia, otherwise FG rhizobia dominated to 9-m depth. Slow-growing rhizobia dominated the lowest depths of the mesquite rooting system in both playas. Slow-growing rhizobia dominated the entire rooting zone of both sand dune ecosystems. Fast-growing rhizobia dominated the upper 2 m and SG rhizobia dominated below 2 m in the mesquite rooting zone at the New Mexico arroyo. Regression analysis indicated that the distribution of FG and SG rhizobia was related to the concentration of total soil salts (electrical conductivity) (r^2 = 0.20). Chi-square analysis of the distribution of colony morphologies indicated distinct rhizobial populations in each ecosystem. The FG rhizobial population at the New Mexico playa was highly effective and had a high frequency of Hup$^+$ phenotypes. The FG rhizobia isolated from the arroyo were, in contrast, less effective and had a low frequency of Hup$^+$ phenotypes. The SG rhizobial population at the arroyo was highly effective and had a high frequency of Hup$^+$ phenotypes. The SG rhizobial population at the New Mexico dune system was, in contrast, mainly ineffective and had a low frequency of Hup$^+$ phenotypes. The relationship between the variable distribution of FG and SG rhizobia and environmental factors associated with the ecosystems is discussed.**

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HUMAN NEED for fuel wood, food, fodder, erosion control, and soil improvement in arid and semiarid regions has led to an increased interest in tree legumes and their use in agroforestry systems (Felker, 1979; National Academy of Sciences, 1979; Oldeman, 1983). If inoculation of tree legumes with rhizobia proves essential for their establishment in agroforestry systems, basic understanding of the ecology of the rhizobial endosymbiont would have application.

Generally, xerophytic tree legumes are deeply rooting phreatophytes (Phillips, 1963). Nodules are rarely found on surface roots of such plants in the field although several studies have attempted to find them (Beadle, 1964: Farnsworth et al., 1978; Hoegberg, 1986). Rhizobial population densities of the surface root systems can be very small or nondetectable (Beadle, 1964; Virginia et al, 1986; Jenkins et al, 1988a). Recent deep coring studies, however, have indicated that large rhizobial populations and viable root nodules occurred between depths of 2 to 10 m under the woody legume mesquite (*Prosopis glandulosa*) (Virginia et al, 1986; Jenkins et al, 1988b).

Fast-growing (FG) rhizobia develop turbid suspensions in agitated broth after 3 to 5 d and possess other genetic and physiological characteristics distinct enough from SG rhizobia (which show turbidity after 5 to 7 d or longer) to warrant their own genus, *Rhizobium* and *Bradyrhizobium* sp. respectively (Jordan, 1984). Until Dreyfus and Dommergues (1981) had reported that some Acacia spp. were effectively nodulated by both FG *Rhizobium* spp. and SG *Bradyrhizobium* spp., Acacia spp. were known to form N_2-fixing symbioses only with SG rhizobia (National Academy of Sciences, 1979). Trinick (1968), however, had reported an exception, *A. farnesiana*, which was effectively nodulated by certain FG rhizobia. Rhizobia which effectively nodulate *Prosopis* were characterized as SG cowpea rhizobia (Allen and Allen, 1981; Basak and Goyal, 1975). Hernandez and Focht (1984), however, characterized a FG cowpea *Rhizobium* strain isolated from a *Prosopis* nodule. Shoushtari and Pepper (1985) also characterized a FG rhizobial strain isolated from a mesquite seedling which had been inoculated with soil from the Sonoran Desert. Jenkins et al. (1987) reported that both FG and SG mesquite-nodulating rhizobia were components of the indigenous rhizobial population for a mesquite woodland in the Californian Sonoran Desert. They noted not only that the ratios of FG to SG rhizobia varied significantly between two rooting environments, surface and deep, but that populations of SG rhizobia differed in other characteristics as well.

The ability to utilize the H_2 constitutively evolved during symbiotic N_2-fixation (Hup) (Simpson and Burris, 1984) has been observed in strains of both *Bradyrhizobium* and *Rhizobium* sp. (Evans et al, 1987). The fraction of H_2 recycled has been defined as a measurement of a strain's N_2-fixing efficiency (Schubert and Evans, 1976). The Hup characteristic has been associated with the conservation of photosynthate, and increased N_2-fixation (Evans et al., 1987). Little is known about ecological differences between populations of Hup^+ and Hup^- phenotypes in natural ecosystems.

In this study we have approached the mesquite-rhizobia symbiosis as a model system for investigating the ecology of FG and SG rhizobia (Jenkins et al., 1987). Understanding the factors related to the distribution of FG and SG rhizobia with depth of mesquite rooting systems, and among different ecosystems occupied by the same host plant should extend our knowledge of the ecology of these two important symbiont types. The objectives of this study were: (i) to determine the ratios of FG to SG rhizobia in three warm desert ecosystem types, playa, arroyo, and coppice dune, at various soil depths; and (ii) to characterize the populations with regard to colony morphology, effectiveness, and symbiotic uptake-hydrogenase activity (Hup).

MATERIALS AND METHODS

Site Description

Three ecosystem types in which mesquite occurs were identified for this study: playa, coppice dune, and arroyo. An arroyo and a playa site were located on the NSF Jornada Long Term Ecological Research (LTER) site situated 40 km NNE of Las Cruces, New Mexico, in the northern Chihuahuan Desert. A coppice dune site was located on the adjacent USDA Jornada Experimental Range about 15 km from the above sites. A second dune and playa site were located in the Sonoran Desert of California at Borrego Sink near Borrego Springs.

Precipitation at the New Mexico sites averages 230 mm yr^{-1} with half occurring during the summer months from intense, convective thunderstorms. The frost-free period is about 200 d, and mean annual temperature is about 14 °C. At the California sites precipitation averages 65 mm yr^{-1}. From May through July there is generally <1 mm of precipitation, and mean annual temperature is about 22 °C.

Both of the playa systems are fringed with dense stands of mesquite and flood at irregular intervals. Standing water reaches the edge of these mesquite stands and recharges the soil profile. Large mesquite trees at low densities are found in the arroyo which dissects an extensive bajada. Both dune systems are dominated by mesquite which grows as low, multistemmed shrubs on dunes and entraps windblown sand to form "coppice" dunes 1- to 3-m tall. Precipitation is the main source of water to plants. The general classification of the soils at the study sites on the Jornada have been reported by Gile (1979) and Wierenga et al. (1987). The New Mexico playa soil is a Haplargid; the soils at both the New Mexico and California dune ecosystems are Torripsamments; and the soil at the arroyo in New Mexico is a Torrifluvent. The California playa consists of lacustrine deposits. Virginia and Jarrell (1983) described the general characteristics of the soils similar to those at the Borrego Sink study sites.

Sampling Methods

Undisturbed soil cores from the rooting zone of three trees in each ecosystem were removed using a split steel, continuous sampling tube, 1.56-m long with an inner diameter of 6.5 cm. This system was an updated version of the coring device essentially described by Kelley et al. (1947) and was mounted on a heavy truck. The split-tube bit fit into an outer, rotating auger bit which served as a continuous casing to prevent cave-in. As the outer bit cut through the soil, the inner, nonrotating bit was pressed into the soil. Cores were collected at the edge of the mesquite canopy. The core retainer and the two halves of the split sampling tube were cleaned of all residual soil. Their interior surface was flame-sterilized with 95% ethanol before being put together for sampling. Soil samples were removed from the surface 1 m of soil in 0.5-m increments, and thereafter in 1-m increments. Flame-sterilized trowels and spatulas were used to place each depth increment into a clean plastic bag. These were put into ice-cooled chests, and transported to the Univ. of California, Riverside, where they were subdivided for analyses.

Drilling depth for each core was determined by either the absence of roots in two consecutive 1.56-m sampling tube lengths, or the presence of coarse, dry, loose soil which could

not be retained in the tube. Sampling dates at New Mexico were in January 1986, the midpoint of the dormant season; late May 1986, during peak growth; and early October 1986 following the summer rains. Soil samples were collected in California in January 1987, when the trees were dormant.

Soil Analysis

Each bag of soil representing a depth increment was mixed thoroughly before subsampling. Using trowels and spatulas flame-sterilized with 95% ethanol, each soil sample was subdivided for various analyses. Gravimetric water content of the soil samples was determined at the time of subsampling. Soils for chemical analysis were then air-dried in a glasshouse, ground to break up clay and caliche aggregates, and passed through a 2-mm sieve. Soil saturation extracts (Salinity Laboratory Staff, 1954) were prepared, and electrical conductivity (EC) was measured using a temperature compensating conductivity meter. Soil pH was measured either using a glass electrode on soil prepared as a saturated paste or a 1:1 (wt/vol) 0.01 *M* $CaCl_2$ method as described by McLean (1982). Potassium chloride-extractable NH_4-N and NO_3-N (Keeney and Nelson, 1982), and $NaHCO_3$-extractable P (Olsen and Dean, 1965) were measured colorimetrically using a Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). Roots were separated from field-moist soil without sieving by flotation using an elutriator fitted with a 1-mm sieve (Byrd et al., 1976). Organic C was determined as the difference between total C and inorganic C. Total C was determined by dry combustion using a Leco carbon analyzer (Leco Corp., St. Joseph, MI 49085; Nelson and Sommers, 1982). Inorganic C was determined by gravimetric loss of CO_2 with the addition of 3 *M* HCl.

Isolate Collection and Characterization

Mesquite seeds, collected from the study sites in New Mexico and California, were surface-sterilized with concentrated H_2SO_4 followed by six rinses in sterile distilled water, germinated in plant tubes filled with sterile vermiculite, and were inoculated with a series of soil dilutions (Jenkins et al., 1988b). Root nodules were excised from the inoculated seedling after 10 to 12 wk. Plants were grown in a glasshouse maintained between 25 and 35 °C. All nodules for each soil inoculant treatment were pooled, surface-sterilized in acidified 0.004 *M* $HgCl_2$, and between 10 to 20 nodules were randomly chosen, and squashed on yeast-extract mannitol (YEM) agar plates, then streaked to obtain isolated colonies (Vincent, 1970). Isolates from the New Mexico ecosystems were obtained from the three sampling times. Rhizobial isolates were maintained on YEM agar slants under 20% (v/v) glycerol at −25 °C. Isolates were classified as either SG or FG types based on criteria of Jordan (1984). FG isolates developed colonies >1 mm in diameter on yeast extract-mannitol agar (YEMA) plates in 3 to 5 d at 27 °C. Slow growing types typically required more than 5 d for visual colony development. The colony morphology of isolates was categorized based on appearance shown on YEMA plates as either wet (or mucilagenous), dry (or nonmucilagenous), opaque, or translucent (Jenkins et al., 1987).

Symbiotic Effectiveness

Symbiotic function of isolates was determined by measuring N_2-fixing effectiveness (shoot N accumulation). Pure cultures of rhizobial isolates were prepared by inoculating 50 mL of YEM broth from fresh slants. Cultures were kept on a rotary shaker at 150 rpm and maintained at 27 °C until an optical density of 0.4 to 0.6 at 560 nm (midlog phase of growth) was reached. Surface-sterilized seeds were germinated in plant tubes filled with sterile vermiculite and irrigated with sterile 0.25 strength N-free Hoagland solution. At the first appearance of leaves (5 to 6 d after germination), 4 to 6 seedlings per isolate were inoculated with 1 mL of pure broth suspension having a rhizobial density of 10^8 cells mL^{-1}. Uninoculated seedlings with and without added N were included as controls (to indicate contamination) and reference treatments. The reference plants with added N received 0.008 *M* KNO_3 at each watering. The mesquite seedlings were grown in a glasshouse maintained at 25 to 35 °C. Uninoculated controls did not nodulate, indicating no contamination from exogenous rhizobia. After 8 wk, plants were harvested and shoot dry weight and shoot N (Kjeldahl) content were determined.

Uptake-Hydrogenase Assay

At the time that the mesquite plants were harvested for effectiveness determinations, the nodules were excised and stored at −25 °C. Only effective isolates were assayed for uptake-hydrogenase activity. Root-nodule uptake-hydrogenase activity was assayed using methods by Jenkins et al., (1988a). The nodules were ground in a phosphate (0.05 *M* K_2HPO_4 0.0025 *M* $MgCl_2$) ascorbic acid (0.2 *M* ascorbate) buffer, pH 7 (grinding buffer); polyvinylpyrolidone was added (1:3 wt/wt) before actually grinding with a mortar and pestle. The nodule suspension was transferred to a microcentrifuge tube and spun slowly (<1000 rpm) in a microfuge. The supernatant was saved and spun at 5000 rpm. The pellet was resuspended in the phosphate buffer and assayed for hydrogen uptake activity using a hydrogen electrode (Wang, 1980).

Statistics

The quotients of the FG rhizobia/SG rhizobia ratios for each depth increment and ecosystem were transformed by the function $\log (1 + y)$ for statistical analysis. In order to determine if the distribution of FG and SG rhizobia was related to soil factors, first-order regression models were determined using a stepwise regression search (Neter and Wasserman, 1974). The statistical program StatView (Brain-Power, Inc., Calabasas, CA) was used to determine regression models and *R* values.

RESULTS

Overall, FG rhizobia dominated the rhizobial population at both New Mexico and California playa systems; SG rhizobia dominated rhizobial populations at both the New Mexico and California dune systems; FG and SG rhizobia were equally represented in the arroyo system (Table 1). The three ecosystems of this study represent a gradient in extent of accessible water storage capacity (Jenkins et al., 1988b), and probable age since establishment is assumed to be in the following order: playa > arroyo > dune. The ratio of FG to SG rhizobia among ecosystems decreases along this gradient.

The dune ecosystems were dominated at all depths by SG rhizobia (Table 2). Fast-growing rhizobia dominated the upper 2 m at the arroyo, and SG rhizobia dominated below 2 m. In contrast to the New Mexico playa system, the surface 1 m of soil beneath mesquite in the California playa was dominated by SG rather than FG rhizobia. Otherwise, the rhizobial populations were predominantly FG rhizobia throughout the rooting zone except for depths below 8 m in New Mexico, and 10 m in California. All of the occupants of field nodules which Jenkins et al. (1988b) recovered from the playa in New Mexico were SG rhizobia.

Stepwise regression analysis of the transformed

Table 1. Ratio of FG to SG rhizobia from three desert ecosystems.

Site	Ecosystem	Ratio of FG to SG rhizobia	Chi-square†
New Mexico	Playa	2.7:1	12.96**
	Arroyo	1.4:1	0.31
	Dune	1:7.8	83.33***
California	Playa	3:1	8.81**
	Dune	All SG	NA‡

,* Significant at the 0.01 and 0.001 probability levels, respectively.
† Based on pooled ratio of FG/SG of 1.59:1 with $n = 507$.
‡ Not applicable.

quotient (y) of the ratio of FG rhizobia/SG rhizobia (per depth increment per ecosystem) against soil NH_4-N, NO_3-N, PO_4-P, electrical conductivity of the saturation extract (EC) (Jenkins et al., 1988b), percent organic C (data not shown), and total rhizobial population indicated a significant relationship only with EC. The resulting regression model, $\log (1 + y) = 0.753 - 0.162(EC)$, had a R value of 0.442 ($P < 0.001$). This model suggests that the distribution of mesquite-nodulating FG rhizobia was inversely related to EC; whereas, mesquite-nodulating SG rhizobia appeared to be more salt tolerant than their FG counterparts. Eighty percent of the variance was still unexplained since $r^2 = 0.20$.

Chi-square analysis of the distribution of colony types within FG and SG rhizobia indicates that distinct populations were associated with each ecosystem (Table 3). This was particularly evident between the two playa and dune ecosystems in which the distribution of colony types for both FG and SG populations differed greatly. These data indicate that the distribution of colony types was not a simple function of ecosystem type, but may have been affected by other factors such as seasonal rainfall distribution patterns.

Table 4. Symbiotic effectiveness of FG rhizobia from the playa, SG rhizobia from the dunes, and FG and SG rhizobia from the arroyo at New Mexico.

Ecosystem	Percent of total isolates in the following effectiveness categories			Total no. of isolates
	E_1†	E_2‡	E_3§	
Playa (FG)	97.7	0	2.3	43
Arroyo (FG)	27.3	59.1	13.6	22
Arroyo (SG)	77.4	22.6	0	31
Dune (SG)	18.0	25.6	56.4	39

† Represents those isolates producing symbiotically derived shoot tissue N that was not significantly less than the mean yield of nitrate grown plants (5.0 ± 0.9 mg) at $P < 0.05$.
‡ Represents those isolates producing symbiotically derived shoot tissue N that was significantly less than the mean yield of nitrate grown plants and significantly greater than uninoculated plant growth at $P < 0.05$.
§ Represents those isolates producing symbiotically derived shoot tissue N that was not significantly greater than uninoculated plant growth (0.9 ± 0.1 mg) at $P < 0.05$.

The uniqueness of the rhizobial populations for each ecosystem was also reflected in differences in symbiotic effectiveness between populations (Table 4). While nearly all of the FG isolates from the New Mexico playa were highly effective (E_1), most of the FG rhizobia from the arroyo were either ineffective (E_3) or only moderately effective (E_2). The majority of the SG isolates from the arroyo were highly effective (E_1), whereas the majority of SG rhizobia from the New

Table 2. Total rhizobia numbers and ratio of FG rhizobia to SG rhizobia for the New Mexico and California desert ecosystems.

Depth	Ecosystems									
	Playa (NM)		Playa (CA)		Arroyo (NM)		Dune (NM)		Dune (CA)	
meters	cells kg^{-1}	FG/SG	cells kg^{-1}	FG/SG	cells kg^{-1}	FG/SG	cells kg^{-1}	FG/SG	cells kg^{-1}	FG/SG
0–0.5	5.62E3	all FG	1.07E5	1:2.9	3.31E3	4:1	1.23E2	ND†	1.78E3	all SG
0.5–1	4.79E5	7:1	1.12E6	all SG	2.09E5	4.9:1	3.98E3	all SG	8.51E4	all SG
1–2	4.90E6	7.3:1	4.27E6	3:1	2.04E5	8:1	1.45E5	1:3	1.02E5	all SG
2–3	6.03E5	3.5:1	6.76E5	all FG	5.37E4	1:7	1.78E5	1:58	1.00E3	all SG
3–4	9.77E5	12.3:1	2.45E5	1:1.7	4.68E4	1:3	1.45E6	1:14.9	8.51E2	all SG
4–5	2.57E5	3.8:1	6.03E3	all FG	4.68E4	1:1.5	2.51E5	1:26.8	4.07E3	all SG
5–6	4.27E4	all FG	8.13E3	9:1	8.71E3	all SG	4.90E3	1:6	1.02E3	all SG
6–7	6.61E4	4.4:1	1.29E5	all FG	3.31E3	ND				
7–8	9.33E4	all FG	1.74E5	all FG						
8–9	5.37E4	1:2.3	3.98E3	all FG						
9–10	5.50E4	1:2.6	1.32E4	ND						
10–11	3.09E3	1:1	7.24E2	1:4						
11–12	7.76E2	all SG	7.24E2	ND						
12–13	1.05E3	all SG								

† No data.

Table 3. Distribution of FG and SG colony types within individual ecosystems.

Rhizobia	Location	Ecosystem	Percent of total isolates of the following colony types				Total no. of isolates	Chi-square†
			Wet, opaque	Wet, translucent	Dry, opaque	Dry, translucent		
FG	New Mexico	Playa	4.0	87.9	7.5	0.6	174	136.80**
		Arroyo	8.3	68.8	22.9	0	48	
		Dune	6.1	56.3	18.8	18.8	16	
	California	Playa	55.3	44.7	0	0	85	
SG	New Mexico	Playa	22.5	73.2	1.5	2.8	71	91.06**
		Arroyo	32.6	67.4	0	0	43	
		Dune	65.5	17.9	8.3	8.3	84	
	California	Playa	92.9	7.1	0	0	28	
		Dune	26.7	73.3	0	0	15	

** Significant at the 0.01 probability level.
† This is a chi-square test of independence, or interaction.

Table 5. Frequency of Hup+ phenotypes in populations of FG rhizobia from the playa and arroyo and SG rhizobia from the arroyo and dune ecosystems in New Mexico.

Ecosystem	Percent of total isolates		Total no. of isolates	Mean activity ± sd
	Hup+	Hup−		
	%			$mM\ H_2\ h^{-1}\ kg^{-1}$ fresh nodules
Playa (FG)	73.5	26.5	34	0.23 ± 0.16
Arroyo (FG)	8.3	92.7	12	0.40
Arroyo (SG)	58.3	41.7	12	0.34 ± 0.08
Dune (SG)	21.4	78.6	14	0.27 ± 0.17

Mexico dune system was either ineffective (E_3) or moderately effective (E_2).

Table 5 indicates that the majority of the FG rhizobia from the New Mexico playa were Hup+. In contrast, the majority of FG rhizobia from the arroyo were Hup−. Nearly 60% of the SG isolates from the arroyo were Hup+. Less than 25% of the effective SG isolates tested from the New Mexico dune system were Hup+. There were no significant differences in specific uptake-hydrogenase activity between the populations tested. The uptake-hydrogenase activities of the FG and SG rhizobia were comparable to those of Hup+ *R. leguminosarum* (Ruiz-Argueso et al., 1978; Nelson and Child, 1981).

DISCUSSION

On the assumption that FG rhizobia have a greater host specificity than SG rhizobia, Dreyfus and Dommergues (1981) argued that tree legumes effectively nodulated by FG rhizobia would respond more favorably to inoculation than tree legumes effectively nodulated by less specific SG rhizobia. Such may be the case for tree legumes which form N_2 fixing symbioses exclusively with FG rhizobia. Data reported by Jenkins et al. (1987) indicated that interactions between *Prosopis glandulosa* and FG and SG rhizobia were mediated by the soil environment. Slow-growing rhizobia dominated the nodules of mesquite seedlings inoculated with soil from a stable, continuously moist rooting environment, whereas FG rhizobia were as dominant as SG rhizobia isolated from nodules induced by inoculation of soil from a highly fluctuating surface root environment. In the present study, the distribution of FG and SG rhizobia varied among ecosystems, as well as with the depth of mesquite rooting zones.

In a glasshouse study on competition between SG and FG rhizobia from the tropics, Trinick (1983) reported that SG strains were more competitive for nodule occupancy than FG stains at temperatures of 27 to 30 °C and at high population densities, but that FG strains were better competitors at temperatures less than 25 °C and at low population densities. Although in the present study regression analysis indicated no relation between ratios of FG and SG rhizobia and the corresponding total rhizobial population density, the predominance of FG rhizobia isolated from the field nodules from the surface soil may nevertheless be attributable to a low rhizobial population comprised of both FG and SG rhizobia (Virginia et al., 1986; Jenkins et al., 1987). Summer surface soil temperatures in warm deserts are much higher than deep soil temperatures. The field data presented in this study and by Jenkins et al. (1987, and 1988a) indicate a predominance of FG rhizobia in warm surface soil, counter to the glasshouse data of Trinick (1983).

The fact that FG rhizobia were not detected in some deep soil populations does not necessarily indicate their complete absence. Jenkins et al. (1988a) found that although no rhizobial populations capable of nodulating *Psorothamnus spinosus* were detected in the surface soil of a nutrient-poor arroyo in the Sonoran Desert of California by the plant-infection method, greater than 90% of the excavated seedlings of this woody legume were nevertheless nodulated by FG rhizobia.

All of the occupants of the mesquite root nodules recovered from the playa in New Mexico (Jenkins et al., 1988b) were SG rhizobia. However, the ratio of FG to SG rhizobia trapped from soil from these same depths indicated that FG rhizobia dominated the free-living soil population. Based on competition studies inoculating *Vigna unguiculata* (L.) Walp. with FG and SG cowpea rhizobia El Hassan et al. (1986) found that the SG rhizobia consistently dominated as nodule occupants. Our data on the occupancy of field nodules recovered from the New Mexico playa suggest that, in situ, SG rhizobia may be more competitive than their FG counterparts. These results are in agreement with the studies by El Hassan et al. (1986) but contrary to Trinick's (1983).

Stepwise regression analysis indicated that the FG and SG rhizobia ratios were not related to soil nutrient concentrations (NH_4-N, NO_3-N, PO_4-P) or organic C content. Slow-growing rhizobia tended to dominate the more nutrient deficient soil environments, however, suggesting fundamental physiological differences between them and FG rhizobia (Hodgson and Stacey, 1986). In contrast to FG rhizobia, oligotrophic characteristics may enable SG rhizobia to survive in greater population densities in nutrient-poor deep soil environments (Jenkins et al., 1987). The apparent dominance of SG rhizobia over FG rhizobia in the deep rooting zones, and in the dune ecosystems, which are the most nutrient deficient of the ecosystems studied, further supports this hypothesis.

Felker et al. (1981) demonstrated the high salinity tolerance of *Prosopis* spp., and their ability to form a N_2-fixing symbiosis with a salt-tolerant (Hua et al., 1982) SG rhizobial strain isolated from a mesquite nodule (Eskew and Ting, 1978). Although early work by Graham and Parker (1964) indicated that FG rhizobia were more salt tolerant than SG rhizobia, more recently Singleton et al. (1982) reported that FG rhizobia were not more tolerant than SG rhizobia. Interestingly in our study EC, or soil salinity, was the only factor that stepwise regression analysis indicated was related to the distribution of FG and SG rhizobia.

Our data suggest that the more saline the soil environment the greater will be the likelihood of a predominance of SG rhizobia. This relationship may be exemplified by the playa ecosystem in California where the surface 1 m of soil was significantly more saline than the soil below 1 m and in which SG rhizobia predominated. However, the EC levels of the dune ecosystem in California were not significantly different

from the low EC values of the New Mexico arroyo (Jenkins et al., 1988b), yet these dunes were dominated by SG rhizobia. Salinity may be a factor affecting the distribution of mesquite-nodulating FG and SG rhizobia in warm desert ecosystems, but other environmental factors may be more important.

Rhizobia colony morphology on YEMA plates can be used as an index of genetic diversity among isolates (Jenkins et al., 1987). The differences in the distribution of colony types suggest that each SG and FG rhizobial population associated with each ecosystem is distinct. Fast-growing and SG rhizobial populations at the two playa and dune ecosystems were significantly different. The distinctions between these FG and SG rhizobial populations may reflect salient differences which exist between their respective ecosystems such as different soil types, levels of soil NO_3-N, PO_4-P, and EC (Jenkins et al., 1988b).

Data on symbiotic effectiveness among ecosystems further supports the observation that differences in the distribution of colony types between ecosystems are indicative of rhizobial populations which have developed independently. The symbiotic effectiveness of the dominant rhizobial populations at the three New Mexico ecosystems represent an effectiveness gradient, playa > arroyo > dune. This pattern parallels the water storage capacity, and age gradients. It is interesting that the young mesquite dunes, formed during the past 50 to 75 yr (Buffington and Herbel, 1965), are dominated by relatively ineffective rhizobia. This suggests that there has not been adequate time for selection and enrichment for highly effective strains in this system.

Additional evidence of genetic diversity between FG rhizobia at the New Mexico playa and arroyo, and SG rhizobia at the New Mexico dune and arroyo ecosystems was the difference in the frequency of Hup$^+$ phenotypes within their respective populations (Table 5). The genes for uptake-hydrogenase (*hup*) in FG rhizobia are generally located on large (mega-) plasmids (Brewin, 1984). Thus, differences in plasmid composition most likely exist between the FG rhizobial populations at the playa and arroyo. In the case of *Bradyrhizobium* spp., *hup* genes are generally located on the chromosome and not plasmids (Evans et al., 1987). Fundamental genotypic differences between the SG rhizobia at the arroyo and dune ecosystems in New Mexico are likely. Glasshouse studies have indicated no correlation between the Hup$^+$ characteristic and the ability to compete for nodule occupancy (Dadarwal et al., 1985). It appeared that Hup$^+$ FG rhizobia at the New Mexico playa, and Hup$^+$ SG rhizobia at the New Mexico arroyo were, however, either better competitors than their Hup$^-$ counterparts, or their numbers were significantly greater.

To our knowledge this is the first report on the frequency of Hup$^+$ SG and FG rhizobia effectively nodulating a common host-tree legume associated with different natural (nonagricultural) ecosystems. Jenkins et al. (1988a) reported that seven out of eight of the FG rhizobia isolated from field nodules of the woody legume *Psorothamnus spinosus* which had relative efficiency values (Schubert, and Evans, 1976) greater than 0.80, expressed symbiotic uptake-hydrogenase activity. Saini et al. (1987) found that 8% of *Sesbania*-nodulating FG rhizobia which were isolated from an agricultural field were Hup$^+$.

The frequency of Hup$^+$ FG phenotypes in indigenous populations is usually low, less than 25% (Brewin, 1984). Johnston et al. (1978) characterized large self-transmissible plasmids from *R. leguminosarum*, and Hooykaas et al. (1981) characterized a large self-transmissible plasmid from *R. trifolii*. A high frequency of Hup$^+$ phenotypes in natural populations of FG rhizobia such as at the New Mexico playa suggests the presence of a self-transmissible plasmid carrying *hup* genes (Fees et al., 1985; Dowling and Broughton, 1986).

The frequency of Hup$^+$ phenotypes in natural populations of *Bradyrhizobium* spp. is generally not higher than 25% (Brewin, 1984). A variation in frequency of Hup$^+$ phenotypes in SG rhizobial populations may reflect environmental influences (Hodgson and Stacey, 1986). The Hup$^+$ SG cowpea rhizobia are known to be facultatively chemoautotrophic (Stowers and Elkan, 1984). Thus, the low nutrient status of the arroyo (Jenkins et al, 1988b) combined with the soil H_2 evolved from nodules occupied by Hup$^-$ rhizobia (La Favre and Focht, 1983) and from anaerobiosis under water-saturated conditions (Lepo et al, 1980), may have enriched for the chemoautotrophic phenotypes. The high frequency of Hup$^+$ SG rhizobia at the arroyo probably does not reflect gene mobility, but rather selection for this phenotype since their *hup* genes are not generally located on plasmids (Evans et al., 1987).

The ability to survive in soil and gain access to leguminous roots is a fundamental aspect of the preinfection stage of the legume-rhizobia symbiosis (Vincent, 1980). This is the stage in which competition for nodule occupancy occurs. Interactions between mesquite-nodulating rhizobia and environmental factors will affect both rhizobial survival and subsequent competition involved in nodulation success (Dowling and Broughton, 1986). Our data indicate that such ecosystem factors as soil salinity, quantity and duration of deep water (Jenkins et al., 1988b), and age since establishment of the host plant, appear to influence the distribution of FG and SG rhizobial symbionts, and may account for the dominance of one genus over the other.

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