Spatial variability in the potential for symbiotic N$_2$ fixation by woody plants in a subtropical savanna ecosystem

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Summary

1. Root infection by symbiotic N$_2$-fixing *Frankia* and *Rhizobium* strains was quantified in relation to light and soil properties for seedlings of 12 woody species from a subtropical savanna in southern Texas, USA.

2. None of four rhamnaceous species nodulated, despite the fact that bioassays with a known actinorhizal species yielded 13 nodules per seedling. *Celtis pallida* (Ulmaceae), *Acacia greggii* and *Acacia berlandieri* (Leguminosae) also failed to nodulate even though field populations of these species were characterized by high (2.7–4.2%) foliar nitrogen concentration.

3. Infective rhizobia occurred in all soils studied regardless of soil depth, distance from a host plant or type of plant cover. Plant growth in N-free media and acetylene reduction activity suggested that all nodules were capable of N$_2$-fixation.

4. The extent of nodulation varied by species. However, nodulated seedlings were taller, produced more biomass and allocated less biomass to root systems than their non-nodulated counterparts.

5. Numbers of nodules on seedlings of *Prosopis glandulosa*, the dominant woody species in this subtropical savanna and throughout the south-western USA, were reduced by low light (15% full sunlight) regardless of soil N level; at medium and full sunlight nodule biomass expressed as a fraction of whole plant biomass decreased with increasing soil N. Nodulation of field-grown *P. glandulosa* appears to be ephemeral, apparently varying with changes in soil moisture.

6. Nodulation and N$_2$ fixation among woody legumes in subtropical savannas can occur across a broad range of soil conditions and depths with significant impacts on local and regional N-cycles.

7. Field levels of foliar N in species that failed to nodulate in the laboratory were comparable to or greater than those in species capable of nodulation, suggesting that leaf N is not a reliable indicator of N$_2$ fixation.

Key-words: *Frankia*, leaf nitrogen, Leguminosae, Rhamnaceae, *Rhizobium*.


Introduction

The abundance of woody plants has increased during the past 300 years in arid and semi-arid grassland and savanna ecosystems throughout the world (Archer 1994). In many cases, the encroaching woody species belong to families known to be capable of symbiotic N$_2$ fixation. Consequently, these changes not only affect ecosystem structure but also have the potential for profound modification of biogeochemical attributes (Langkamp, Farnell & Dalling 1982; Schlesinger et al. 1990; Schulze et al. 1991). Although a few studies have addressed nitrogen fixation by woody plants in arid and semi-arid ecosystems (Shearer et al. 1983; Lajtha & Schlesinger 1986; Vitousek & Walker 1989; Högberg 1989; Johnson & Mayeux 1990), our understanding of the extent and significance of this process remains limited.

The historic *Prosopis–Acacia–Andropogon–Setaria* savannas of the Rio Grande Plains of southern Texas (Kuchler 1964) have been largely replaced by subtropical thorn woodland in recent history (Inglis
Hypotheses proposed to account for directional shifts from grass to woody plant domination over the past century have centred around historical enrichment with atmospheric CO₂ (Johnson, Polley & Mayeux 1993), intensification of livestock grazing and changes in fire and climatic regimes (Hastings & Turner 1965; Grover & Musick 1990). In heavily grazed ecosystems, losses of soil organic matter, reductions in soil fertility and alterations in physical properties may occur together with loss of vegetative ground cover and erosion (Denevan 1967; Harrington, Oxley & Tongway 1979; Parton et al. 1987; Schlesinger et al. 1990; Thurow 1991; Walker & Steffen 1993). This could favour N₂-fixing woody plants (Bush & van Auker 1989; Cohn, van Auker & Bush 1989; van Auker & Bush 1989; Vitousek & Howarth 1991) and growth forms tolerant of low nutrient conditions (Goldberg 1982).

Shifts from grass to woody plant domination have been documented in southern Texas (Archer 1990, 1994). Of the 40 most important woody plant species in this savanna parkland–woodland system, 18 species belong to families which have been shown to be capable of symbiotic N₂ fixation, including 12 species in the Leguminosae (Allen & Allen 1981), four species in the Rhamnaceae (Bond 1983) and two species in the Ulmaceae (Trinick & Hadobas 1988). The ability to fix atmospheric N₂ may be an important factor contributing to their recent successful colonization of grassland and savanna ecosystems. Differences in their respective capabilities for N₂ fixation may also help to explain patterns of species distribution and abundance across landscapes.

As part of a larger project aimed at understanding vegetation dynamics and the influence of vegetation change on biogeochemical processes in subtropical savannas and thorn woodlands, we conducted studies to (i) assess nodule capacity for 12 important woody plant species and verify the ability of root nodules to fix atmospheric N₂ using the acetylene reduction technique; (ii) quantify soil population levels of nodule-forming bacteria and correlate them with soil characteristics and vegetative cover types; and (iii) quantify the effects of light and soil nitrogen on nodule formation and growth of seedlings.

Study area

Field work was conducted at the Texas Agricultural Experiment Station, La Copita Research Area, located c. 15 km from Alice, Texas (27°40’N, 98°12’W) in the eastern Rio Grande Plains of the Tamaulipan Biotic Province (Blair 1950). The climate of the area is subtropical with a mean annual temperature of 22.4°C and a frost-free period of 289 days. Mean annual rainfall is 680 mm with maxima in May and September. Altitudes within the study area range from 75 to 90 m a.s.l. Soils range from fine sandy loams to sandy clay loams, from slightly acid to alkaline, and include Ustolls (Calcic Chernozems) and Ustalfs (Calcic Luvisols) on uplands, and Aqualfs (Mollic Luvisols) in the lower landscape positions (Scifres & Koerth 1987). See McMahan, Frye & Brown (1984) and Whittaker, Gilbert & Connell (1979) for additional details on vegetation of the region.

Our research was concentrated on sandy loam uplands characterized by a savanna parkland physiognomy consisting of discrete multi-species woody clusters embedded within a continuous matrix of herbaceous vegetation (Archer et al. 1988). The woody clusters are typically dominated by the leguminous tree Prosopis glandulosa Torrey var. glandulosa, with a variety of broad-leaved evergreen and deciduous shrubs in the understorey. Herbaceous vegetation surrounding the clusters consists of C₄ grasses (Bouteloua rigidiseta (Steudel) A.S. Hitchc., and Chloris cocciliata Bisch., Aristida spp. and Setaria spp.) and numerous species of herbaceous dicotyledons.

Materials and methods

NODULEATION, NODULE ACTIVITY AND FOLIAR NITROGEN

Seven native species from the Leguminosae (P. glandulosa, Acacia farnesiana (L.) Willld., A. greggii A. Gray, A. rigidula Benth., A. berlandieri Benth., A. schaffneri (S. Watson) Herm. and Eysenhardtia texana Scheele), four species from the Rhamnaceae (Colubrina texensis A. Gray, Cordulia hookeri M.C. Johnston, Karwinskia hamboldtiana Zucc., and Ziziphus obtusifolia A. Gray), and one species from the Ulmaceae (Celis pallida Torrey) were screened for foliar nitrogen concentration and root nodule. Taxonomy and taxonomic authorities are based on Correll & Johnston 1979. P. glandulosa is a dominant of uplands and intermittent drainages on the site. A. farnesiana dominates fine-textured, poorly drained lake bed sites and A. rigidula dominates calcareous ridges with shallow, rocky soils. Each of these species is widespread and dominant on many landscapes throughout southern Texas and northern Mexico. The other species are typically subordinate to these in terms of stature and/or density.

Foliar samples were collected in November 1990 from at least three randomly selected plants. For each plant, leaves were collected from three canopy locations (lower, mid and upper), pooled, ground and analysed for total N by combustion-gas chromatography (Carlo Erba Model NA-1500, Fisons Instruments Inc., Danvers, MA). Soil cores (3-cm diameter x 60-cm depth) were collected beneath plants of each species in April 1990 from within 25 cm of the main stem of the target species. They were divided into 0–10, 10–20, 20–35, 35–50 and 50–60-cm depth increments. A soil coring device with a replaceable plastic liner (Forestry Suppliers, Inc., Jackson,
MS) was used to prevent bacterial contamination between cores.

Soil samples collected from beneath each species were mixed with an equal volume of sterile vermiculite and placed in two sterilized plastic containers (4-cm diameter × 20 cm). Seeds of the appropriate species were surface-sterilized in 20% H₂SO₄ for 5 minutes, then rinsed five times in sterile distilled water. As an additional check for the presence of Frankia, surface-sterilized seeds of two non-native actinorhizal species, Alnus glutinosa (L.) Gaertner (Betulaceae) and Casuarina equisetifolia L. (Casuarinaceae), were planted in soils associated with Condalia hookeri and Colubrina texensis, respectively.

Seedlings were grown in a controlled environment chamber set to a 16-hour photoperiod averaging 600 mol m⁻² s⁻¹ of photosynthetically active radiation and 30/26 °C day/night temperature. CO₂ concentration in the growth chamber averaged 500 p.p.m. Emerging seedlings were thinned so that the number of seedlings (range of two to six) and seedling size per container were similar for a given species. Seedlings were watered with 1/4-strength N-free Hoagland’s solution. Three control containers per species containing sterile vermiculite were planted to verify the absence of Rhizobium and Frankia strains in the vermiculite and nutrient solution.

Plants were harvested at 8 weeks and soil was washed gently from roots. Shoot height was measured and nodules counted. Nodules were identified with the naked eye and were assumed to be the result of a single infection, though nodules containing multiple rhizobial strains have been documented (Shoushatari & Pepper 1985). Similarly, root nodules have been observed containing multiple Frankia strains (Reddell & Bowen 1986) and infection by frankia can occur via different pathways with differential sensitivity to available nitrogen (Kohls & Baker 1989). Roots, shoots and nodules were separated, dried at 70 °C, and weighed.

Whole, nodulated root systems from seedlings of P. glandulosus, Acacia farnesiana, A. rigidula, A. schoffleri, E. texana and Alnus glutinosa were assayed for N₂ fixation using acetylene reduction (Hardy, Burns & Holsten 1973). Nodulated root systems (10 per species) were sealed in 50-mL plastic syringes with an acetylene:air atmosphere (1:10 v/v) and incubated for 60 min in the dark at 27 °C. A 5-mL gas sample was removed from the incubation syringes and collected in 7-mL Vacutainer™ (Becton-Dickinson, Rutherford, NJ). Non-nodulated root systems were incubated in an identical manner to quantify background levels of ethylene. Assays were run between 10.00 hours and 14.00 hours to minimize differences associated with diurnal variation. Ethylene was isolated and quantified using flame ionization gas chromatography. The gas chromatograph (Model 300, Antek, Houston, TX) was fitted with a stainless steel column (6.4 mm × 1.3 m) packed with 80/100 mesh Poropak N. Oven temperature was 80 °C and the flow rate of the N₂ carrier gas was 30 mL s⁻¹.

Analyses of variance for species and soil depth effects on mean number of nodules per seedling were performed with a general linear model procedure of SAS (Parker 1987). Mean values for growth and acetylene reduction rates of nodulated vs. non-nodulated seedlings were compared using Student’s t-test. Differences in leaf [N] between species classified as nodulating vs. non-nodulating were tested for significance also using Student’s t-test.

EDAPHIC INFLUENCES ON NODULATION

To determine how nodulation potential might vary within and between woody plant clusters, we inoculated seedlings with soils collected with cores (3-cm diameter; 0–25, 25–50 and 50–100-cm depth intervals) in May 1990 at the centre of discrete clusters, at the cluster perimeter and from adjoining herbaceous zones 10–15 m from the cluster perimeter. Four woody plant clusters were selected that were approximately circular in shape, 15–30 m in diameter and dominated by 10–30 P. glandulosus trees. Typical understory shrubs included Zanthoxylum fagara Sarg., Condalia hookeri, Diospyros texana Scheele, Schaefferia cuneifolia A. Gray, Z. obtusifolia, and Mahonia trifoliolata (Moricand) Fedde. Soil water content was determined gravimetrically on 25-g subsamples of soil from each depth increment. Soil acidity was quantified on 50-g subsamples in 1:1 soil:distilled water slurry with a pH meter (Model 107, Fisher, Pittsburg, PA). Soil nitrogen was determined using a Carlo Erba NA-1500 elemental analyser.

Sterile plastic containers (4-cm diameter × 20 cm) were filled with approximately 200 mL of sterile vermiculite and planted with surface-sterilized seed (see above) of P. glandulosus, Acacia farnesiana and Alnus glutinosa. The two leguminous species were selected because the study described in the previous section showed them to be capable of nodulation, and they were the dominant woody species within the study area. A. glutinosa was used because none of the native rhizomorphous species formed nodules in the experiment described in the previous section and it is relatively promiscuous in terms of the Frankia strains that will infect it (Baker 1987).

One week after germination, replicate tubes (four per soil dilution) containing three plants per tube were inoculated with 1 mL sterile distilled water containing 0-0(control), 0-1, 0-01, 0-001, or 0-0001 g of soil. Plants were grown in a controlled environment chamber under conditions identical to those described for the previous experiment. Plants were harvested after 8 weeks, vermiculite was washed gently from roots, and nodules were counted. For most-probable-number (MPN) estimates, tubes with one or more nodules were scored as positive (Vincent 1970). Mean number of nodules per seedling was determined by dividing
the sum of nodules for each of the four dilution series by the total number of seedlings.

An assessment of nodule abundance on field-grown *P. glandulosa* was conducted on 30 April 1992. Soil cores (1-8 cm diameter) were collected from beneath six *P. glandulosa* trees at distances of 0-5, 1-0 and 1-5 m from thebole. Three replicate cores at each distance were collected at 120° intervals around the bole of each tree. Cores were divided into 0–30, 30–60 and 60–100-cm depths, which corresponded to soil volumes of 76-3, 76-3 and 101-8 cm³, respectively. Soils were refrigerated until nodules could be separated by sieving and counted. On the same day as the soil cores were collected, five groups of nodules from three field-grown *P. glandulosa* trees were excavated and assayed for acetylene reduction activity. Nodules were incubated and ethylene production determined as described previously.

The effects of soil depth and location along the cluster centre-to-herbaceous zone transect on numbers of nodules per seedling were evaluated for each species using a general linear model procedure of SAS (Parker 1987). Variation in abundance of field-grown nodules with soil depth and distance from the bole of the tree were evaluated similarly. Simple correlations of numbers of nodules per seedling with MPN estimates and soil properties were determined with SAS.

**EFFECT OF VARYING LIGHT AND NITROGEN LEVEL ON NODULATION**

Pots (10-cm diameter × 75 cm) made from PVC tubing were filled with 5-5 L of a non-sterile 1:1:1 mixture of vermiculite, peat and sand, pH 6-6 ± 0-2. Five surface-sterilized seeds of *P. glandulosa*, *Celtis pallida* and *Condalia hookeri* collected from the study site were planted in each pot in June 1993 and thinned to one seedling per pot. At the time of planting, 10 g of soil (0-30 cm depth), collected from beneath conspecific adults growing at the study site, was mixed into the top 10 cm of the potting mixture of each pot. Seedlings were grown outdoors (June through November 1993) under full sunlight and under shade cloth to produce low (15% full sunlight) and moderate (70%) light levels. Pots received weekly additions of 100 mL of full strength Hoagland’s solution containing either low (25 p.p.m. N as NH₄NO₃), medium (100 p.p.m. N) or high (225 p.p.m. N) nitrogen. Over the course of the experiment the total application of mineral N for the 25 p.p.m. N treatment was comparable to rates of mineralization of N in the top 10 cm of a low-N savanna soil (Bernhard-Reversat 1982). In contrast, levels of mineral N in the 225 p.p.m. N treatment were similar to rates of mineralization under shrub canopies of semi-desert ecosystems (Charley & West 1977). Each light–nitrogen combination consisted of 12 pots with one seedling per pot. Pots were watered as needed to maintain the soil near field capacity which was monitored by weighing a subsample (*n* = 10) of the pots. After 120 days, seedlings were separated into leaves, stems and roots. Roots were refrigerated until nodules were counted and separated. All tissues were oven-dried at 70°C and weighed.

**Results**

**SPECIES NODULATION CAPACITIES AND NODULE ACTIVITY**

Of the 12 native plant species screened, only five leguminous species formed root nodules under laboratory conditions (*Prosopis glandulosa*, *Acacia farnesiana*, *A. rigidula*, *A. schaffneri*, and *Eysenhardtia texana*). Mean number of nodules per seedling ranged from 2-3 for *E. texana* to 43-1 for *P. glandulosa* (Table 1). Mean nodule biomass per seedling ranged from 6-0 mg for *E. texana* to 23-0 mg for *A. farnesiana*. The leguminous *Acacia berlandieri* and *A. greggii* and the umbraceous *Celtis pallida* did not form nodules. None of the native rhamnaceous species formed root nodules, nor did the actinorhizal *Cassuaria equisetifolia* nodulate when grown in soils from beneath the rhamnaceous *Colubrina texensis*. However, the non-native *Alnus glutinosa* did nodulate (12-8 nodules per seedling; nodule biomass of 5-2 mg per seedling), indicating the presence of at least one strain of *Frankia* in the soils associated with *Condalia hookeri*.

Nodulated seedlings were heavier and allocated less biomass to root systems than their control or non-nodulated counterparts (Table 1). Seed biomass, and hence potential levels of stored N for seedling growth varied among species. Biomass of nodulated plants was 10-83% times their seed biomass compared with less than 8 times for non-nodulated control seedlings. The growth of the non-nodulating species was similar to that of non-nodulated control seedlings of the nodulating species.

*N₂* fixation (nitrogenase activity) in root nodules was confirmed by acetylene reduction assay. Specific nitrogenase activity (C₃H₅ evolved per unit dry weight of nodule: μmol g⁻¹ h⁻¹) for leguminous seedlings ranged from 25-1 (*E. texana*) to 128-3 (*Acacia farnesiana*) (Table 1). Ethylene production by non-nodulated roots of *P. glandulosa* was 0-8 μmol g⁻¹ h⁻¹; control roots of the other species produced no detectable ethylene. Ethylene production by actinorhizal *Alnus glutinosa* (23-3 μmol g⁻¹ h⁻¹) was lower than that of leguminous species. Roots of *A. glutinosa* control plants produced no ethylene. Acetylene reduction activity among nodulated plants was significantly correlated with total nodule biomass per seedling (*r* = 0-55, *P < 0-05), but not with the number of nodules per seedling.

The relationship between number of nodules per seedling and soil depth was species-dependent (Table 2). Among the leguminous shrubs, *P. glandulosa* and *A. farnesiana* were 1-2 in rank-order of nodules per seedling at four of the five depths examined (Fig. 1).
Table 1. Nodulation and growth (mean ± SE) of 8-week-old seedlings on a 1:1 host-soil: vermiculite media (or 100% vermiculite for controls) watered with N-free Hoagland’s solution. Soils were collected beneath adult conspecifics in a south Texas savanna ecosystem, except exotic *Alamus glutinosus* which was grown on soils from beneath *Condalia hookeri*. Data presented are means across soils from all depths; biomass values are in mg

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Nodules plant⁻¹</th>
<th>Biomass</th>
<th>Biomass allocation ratio</th>
<th>Nitrogenase activity†</th>
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<td></td>
<td></td>
<td></td>
<td>Nodule</td>
<td>Plant</td>
<td>Plant:seed Shoot:root</td>
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<tr>
<td>Nodulated species</td>
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<td><em>Prosopis glandulosa</em></td>
<td>40</td>
<td>43.1* ± 2.9</td>
<td>19.8 ± 1.1</td>
<td>606 ± 30</td>
<td>17.3 ± 0.8  2.9 ± 0.03</td>
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<td><em>Prosopis</em> control</td>
<td>27</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>188 ± 7.7</td>
<td>4.7 ± 0.2  1.3 ± 0.1</td>
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<td><em>Acacia farnesiana</em></td>
<td>18</td>
<td>20.8 ± 0.8</td>
<td>23.0 ± 0.9</td>
<td>882 ± 35</td>
<td>15.0 ± 0.6  2.3 ± 0.07</td>
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<td><em>A. farnesiana</em> control</td>
<td>7</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>166 ± 2.3</td>
<td>2.8 ± 0.04  1.1 ± 0.04</td>
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<td><em>Acacia rigidula</em></td>
<td>10</td>
<td>9.7 ± 1.4</td>
<td>17.0 ± 2.1</td>
<td>294 ± 46</td>
<td>10.6 ± 1.6  2.0 ± 0.3</td>
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<td>6</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>39 ± 12</td>
<td>1.4 ± 0.4  0.7 ± 0.04</td>
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<td><em>Acacia schaffneri</em></td>
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<td>13.0 ± 1.2</td>
<td>19.5 ± 0.7</td>
<td>405 ± 10</td>
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<td>0 ± 0</td>
<td>84 ± 12</td>
<td>3.0 ± 0.04  1.4 ± 0.2</td>
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<td>2.3 ± 0.6</td>
<td>6.0 ± 1.4</td>
<td>79 ± 30</td>
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<td>0 ± 0</td>
<td>29 ± 1.3</td>
<td>8.5 ± 0.4  0.3 ± 0.04</td>
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<td><em>Almus glutinosus</em></td>
<td>58</td>
<td>12.8 ± 1.6</td>
<td>5.2 ± 0.5</td>
<td>100 ± 8.9</td>
<td>83.3 ± 7.3  0.8 ± 0.1</td>
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<td>0 ± 0</td>
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<td>Non-nodulated species</td>
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<td><em>Acacia berlandieri</em></td>
<td>50</td>
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<td>0 ± 0</td>
<td>694 ± 27</td>
<td>1.3 ± 0.05  1.35 ± 0.14</td>
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<td><em>Acacia greggii</em></td>
<td>20</td>
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<td>0 ± 0</td>
<td>280 ± 14</td>
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<td>30</td>
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<td>0 ± 0</td>
<td>7.5 ± 3.1</td>
<td>0.4 ± 0.2  5.5 ± 1.97</td>
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<td><em>Celtis texensis</em></td>
<td>23</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>171 ± 22</td>
<td>3.4 ± 0.4  0.59 ± 0.03</td>
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<td><em>Condalia hookeri</em></td>
<td>48</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>19.4 ± 1.0</td>
<td>1.3 ± 0.07  1.1 ± 0.09</td>
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<td><em>Karinwischia</em></td>
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<td><em>Humboldtiana</em></td>
<td>17</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>72 ± 18</td>
<td>8.3 ± 2.1  0.43 ± 0.28</td>
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<td><em>Ziziphus obtusifolia</em></td>
<td>16</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>69 ± 29</td>
<td>3.9 ± 1.6  2.11 ± 0.75</td>
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*Within-species means in italics are significantly different from controls at P < 0.05.
†Specific nitrogenase activity by acetylene reduction assay as C₂H₄ evolved per unit nodule of dry weight (μmol g⁻¹ h⁻¹).
‡Nodulation status not reported in literature.

Nodulation of *A. farnesiana* and *A. rigidula* seedlings were statistically comparable for soils from all depths. In contrast, nodulation of *A. schaffneri* generally increased with depth, nodulation of *A. glutinosus* decreased markedly with depth, and nodulation of *P. glandulosa* was greatest with soils from 35 to 50-cm depths.

**ESTIMATION OF MPN FOR RHIZOBIUM AND FRANKIA**

Most-probable-number (MPN) values of *Rhizobium* strains infecting *A. farnesiana* seedlings ranged from 170 to 6900 infective cells per gram of soil; with a mean (± SD) of 1408 ± 2161 cells per gram of soil. These values were significantly greater than those obtained for *P. glandulosa* (range = 184–1855; mean = 870 ± 1449). MPN estimates were significantly (P < 0.05) correlated with numbers of nodules per seedling (summed over the four dilutions for each soil sample) for both species (r = 0.83 for each). Our MPN estimates for *Rhizobium* strains nodulating *P. glandulosa* were in the same range as those reported for *P. glandulosa* plants and soils from a Sonoran Desert site (Virginia, Jenkins & Jarrell 1986). MPN of *Frankia* strains nodulating *Almus glutinosus* ranged from 0 to 17 infective propagules g⁻¹ of soil with an average of 3 ± 6. These values were significantly lower than mean MPN of *Rhizobium* strains nodulating either *P. glandulosa* or *A. farnesiana*. *A. glutinosus* seedlings nodulated with only three of the 12 soils tested. There were no significant main effects or interactions with respect to soil depth or distance from cluster centre on MPN estimates for *Frankia*.

**SPATIAL VARIABILITY AND EDAPHIC INFLUENCE ON NODULATION**

Nodulation of *A. farnesiana* seedlings inoculated with soils collected at various depths (to 100 cm) and at various locations relative to the tree bole ranged from 10-5 (herbaceous zones, 0–25 cm depth) to 46-0 nodules per seedling (drip line, 50–100 cm depth: Fig. 2b),
but did not differ significantly with depth or location (Table 3). The number of nodules on *P. glandulosa* seedlings inoculated with soils from the 50–100 cm depth (16·8 ± 11·3 nodules per seedling) was significantly greater than that for seedlings inoculated with (25–50 cm) soils (10·1 ± 8·2; Fig. 2a). Location relative to tree bole also significantly affected numbers of nodules on *P. glandulosa* (Table 3).

Soil pH ranged from 6·9 ± 1·2–8·0 ± 0·8 (Table 4) and was not correlated with MPN or nodulation potential for either *P. glandulosa* or *A. farnesiana*. Field moisture content of soils at the time of collection ranged from 6·3 ± 4·4% to 10·0 ± 0·4% and was correlated with numbers of nodules on *P. glandulosa* seedlings (*r* = 0·65, *P* < 0·05), but not with *A. farnesiana* nodule numbers. Mean soil nitrogen ranged from 0·032% at 50–100 cm depths to 0·073% for 0–25 cm depths near tree boles. Soil N was negatively correlated with nodule numbers on *A. farnesiana* seedlings (*r* = −0·59, *P* < 0·009), but not with numbers of nodules on *P. glandulosa* seedlings. None of these soil characteristics was correlated with nodule numbers on *A. glutinosa* seedlings or MPN estimates for *Frankia* strains.

Nodule abundance on field-grown *P. glandulosa* ranged from 0 to 4·4 nodules per litre of soil with a mean of 1·5 ± 0·4 L⁻¹ (Table 5). Nodule abundance was generally greatest 1·5 m from the tree bole and decreased with soil depth. All nodules collected appeared to have begun senescing and no ethylene production was detected. Nodule abundance was low compared with reports for *P. glandulosa* from other regions (0–17 nodules per litre of soil: Johnson & Mayeux 1990). A visual inspection of moist soil samples collected 2 weeks earlier for another study suggested that nodule abundance at the time of our quan-

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**Table 3.** Analysis of variance for the effects of location (tree bole vs dripline vs herbaceous zone) and depth (0–25 vs 25–50 vs 50–100 cm) of soil on nodulation of 8-week-old seedlings of *Prosopis glandulosa* and *Acacia farnesiana*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>A. farnesiana</th>
<th>P. glandulosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>F</em></td>
<td><em>P</em></td>
</tr>
<tr>
<td>Depth</td>
<td>2</td>
<td>1·13</td>
<td>0·37</td>
</tr>
<tr>
<td>Location</td>
<td>2</td>
<td>0·29</td>
<td>0·76</td>
</tr>
<tr>
<td>Depth × location</td>
<td>4</td>
<td>0·70</td>
<td>0·61</td>
</tr>
</tbody>
</table>

titative sampling had declined markedly, apparently in response to soil drying (e.g. Miettinen et al. 1988).

**FOLIAR N**

Concentrations of foliar N in leaves collected from plants on the study site ranged from 2·1% to 4·2% (Table 6). Highest foliar [N] occurred among species which did not form nodules in our study (Acacia berlandieri = 4·2%; Celtis pallida = 3·9%). Eysenhardtia texana, which had significantly lower numbers of nodules per seedling in relation to Prosopis glandulosa and A. farnesiana (Table 1), also had a relatively high foliar [N] of 3·7%. As a group, species classified as nodulated based on our results (Table 1) had foliar [N] similar to those classified as non-nodulated.

**EFFECTS OF LIGHT AND NITROGEN ON NODULATION**

Nodulation of Prosopis glandulosa occurred under all light and nitrogen combinations (Fig. 3a), but the number of nodules per seedling at 15% full sunlight was less than 15% that of seedlings grown under higher illumination. Celtis pallida and Condalia hookeri failed to nodulate under any of the treatment combinations. Fertilizer N concentration over the range used in this study had little effect on numbers of nodules per seedling. However, when nodule biomass was expressed as a fraction of total seedling biomass there were significant decreases in relative nodule biomass per seedling with increasing soil N addition under all light levels (Fig. 3b). Maximum mean total seedling biomass occurred in the high light, high N treatments for all species (C. pallida = 9·5 g; C. hookeri = 8·8 g; P. glandulosa = 16·5 g). Total seedling biomass for non-nodulating C. pallida and C. hookeri was reduced by 68% and 84%, respectively, in the low N treatment compared with the medium N treatment under medium and high light (data not shown). In contrast, total seedling biomass of nodulated P. glandulosa seedlings was comparable in the low and medium N treatments under medium and high illumination (Fig. 3c), suggesting that N₂ fixation was significantly compensating for low soil mineral N.
Table 4. Variation in soil properties (mean ± SD) with depth along a gradient extending from the centre of Prosopis-mixed species woody plant clusters to the cluster perimeter and into adjacent herbaceous zones (n = 4). Values within rows and within columns were statistically comparable, except for soil moisture at 50–100 cm in the herbaceous zone which exceeded that in the cluster centre.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Location</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cluster centre</td>
<td>Cluster perimeter</td>
<td>Herbaceous zone</td>
</tr>
<tr>
<td>Acidity (pH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–25</td>
<td>6.9 ± 1.2</td>
<td>6.9 ± 0.9</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>25–50</td>
<td>7.0 ± 1.2</td>
<td>7.4 ± 0.8</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>50–100</td>
<td>8.0 ± 0.2</td>
<td>7.9 ± 0.2</td>
<td>8.0 ± 0.8</td>
</tr>
<tr>
<td>Water content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–25</td>
<td>7.7 ± 4.2</td>
<td>6.3 ± 4.4</td>
<td>8.1 ± 7.0</td>
</tr>
<tr>
<td>25–50</td>
<td>7.5 ± 2.6</td>
<td>6.5 ± 2.9</td>
<td>7.5 ± 3.6</td>
</tr>
<tr>
<td>50–100</td>
<td>6.9 ± 0.1</td>
<td>7.7 ± 1.4</td>
<td>10.0 ± 0.4</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–25</td>
<td>0.073 ± 0.018</td>
<td>0.055 ± 0.011</td>
<td>0.057 ± 0.011</td>
</tr>
<tr>
<td>25–50</td>
<td>0.044 ± 0.001</td>
<td>0.045 ± 0.004</td>
<td>0.049 ± 0.011</td>
</tr>
<tr>
<td>50–100</td>
<td>0.032 ± 0.004</td>
<td>0.033 ± 0.005</td>
<td>0.038 ± 0.015</td>
</tr>
</tbody>
</table>

Table 5. Effect of soil depth and distance from the bole of field-grown Prosopis glandulosa trees on P. glandulosa nodule frequency on 30 April 1992 (frequency given as number of nodules per unit soil volume, in units of L⁻¹ ± SE)

<table>
<thead>
<tr>
<th>Distance from bole (m)</th>
<th>0–5</th>
<th>1.0</th>
<th>1.5</th>
<th>0–1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–30</td>
<td>2.9 ± 1.3</td>
<td>4.4 ± 2.5</td>
<td>4.4 ± 2.7</td>
<td>3.6 ± 1.1</td>
</tr>
<tr>
<td>30–60</td>
<td>0</td>
<td>1.0 ± 1.0</td>
<td>2.2 ± 2.0</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td>60–100</td>
<td>0</td>
<td>0</td>
<td>3.3 ± 0.8*</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>0–100</td>
<td>0.9 ± 0.4</td>
<td>1.6 ± 0.8</td>
<td>3.3 ± 1.9</td>
<td>1.5 ± 0.4</td>
</tr>
</tbody>
</table>

Means in *italics* are significantly greater than for other depths within a distance; means followed by an asterisk are significantly greater than other distances at the same depth, P < 0.05.

Table 6. Mean (± SD) nitrogen concentration (dry weight basis) in foliage collected from woody plants growing in a south Texas savanna. Species are categorized as nodulated or non-nodulated according to their ability to produce nodules when inoculated with soils from the study site (Table 1)

<table>
<thead>
<tr>
<th>Species</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodulated</td>
<td></td>
</tr>
<tr>
<td><em>Prosopis</em> glandulosa</td>
<td>2.9 ± 0.45</td>
</tr>
<tr>
<td><em>Acacia</em> farnesiana</td>
<td>3.8 ± 0.38</td>
</tr>
<tr>
<td><em>A. rigidula</em></td>
<td>2.3 ± 0.17</td>
</tr>
<tr>
<td><em>Eysenhardtia</em> texana</td>
<td>3.7 ± 0.16</td>
</tr>
<tr>
<td>Group mean</td>
<td>3.2 ± 0.71</td>
</tr>
<tr>
<td>Non-nodulated</td>
<td></td>
</tr>
<tr>
<td><em>Acacia</em> berlandieri</td>
<td>4.2 ± 0.46</td>
</tr>
<tr>
<td><em>A. greggii</em></td>
<td>2.7 ± 0.28</td>
</tr>
<tr>
<td><em>Celtis</em> pallida</td>
<td>3.9 ± 0.77</td>
</tr>
<tr>
<td><em>Colubrina</em> texensis</td>
<td>3.1 ± 0.20</td>
</tr>
<tr>
<td><em>Condalia</em> hookeri</td>
<td>2.1 ± 0.17</td>
</tr>
<tr>
<td><em>Karwinskia</em> humboldtiana</td>
<td>2.8 ± 0.06</td>
</tr>
<tr>
<td><em>Ziziphus</em> obtusifolia</td>
<td>2.2 ± 0.11</td>
</tr>
<tr>
<td>Group mean</td>
<td>3.0 ± 0.80</td>
</tr>
</tbody>
</table>

Discussion

Nodule formation on non-native *A. glutinosa* seedlings indicated the presence of viable, infective and effective *Frankia* strains in the soils of the study site, primarily in the upper 35 cm of the soil (Fig. 1). Lack of nodule formation on native rhizobial seedlings (Condalia hookeri, Colubrina texensis, Karwinskia humboldtiana and Ziziphus obtusifolia) suggests that these species may not be capable of forming nodules under natural or experimental conditions. Seedling size or age does not appear to have been a factor, since seedlings of *Celtis pallida* and Condalia hookeri grown for 18 weeks under varying light and nutrient levels also failed to nodulate. Although actinorhizal and rhizobial infection may be limited by environmental factors such as soil nitrogen and pH (Jenkins, Virginia & Jarrell 1988; McConnell & Bond 1957; Zitzer & Dawson 1992), these should not have been a factor in our study.

*Frankia* strains are spore-forming soil saprophiles and facultative symbionts (Smolander & Sundman 1987; Paschke & Dawson 1992), which may explain
Fig. 3. Effects of varying light and nitrogen levels on *Prosopis glandulosa*: (a) nodules per plant; (b) nodules as a fraction of whole plant biomass; and (c) whole plant biomass. Means ± SE are not significantly different if labelled with the same letter.

their presence and dominance in the surface soils of our study site despite the apparent absence of known host species. *Rhizobium* strains are also facultative symbionts, though populations of host-specific strains are often correlated with the presence of host plants (Woomer, Singleton & Bohlool 1988). However, their ability to survive under adverse soil conditions is strain-specific (Graham 1992), which may account for some of the variation in nodule numbers per seedling and MPN estimates for those host species that did nodulate. The lack of nodule formation on rhamnaceous and ulmaceous species surveyed from this savanna ecosystem may partially explain why leguminous woody species such as *P. glandulosa* and *A. farnesiana* are the more aggressive invaders of low-N soils and why rhamnaceous and ulmaceous shrubs typically appear subsequent to establishment of N$_2$-fixing species (Archer et al. 1988).

Foliar N concentration of *C. pallida* at our savanna study site was comparable to or higher than that of *P. glandulosa*, a well-known N$_2$-fixing species (Shearer et al. 1983; Lajtha & Schlesinger 1986; Johnson & Mayeux 1990). This fact, coupled with reports of nodulation and N$_2$ fixation by other species in the Ulmaceae (Trinick & Hadobas 1988), led us to anticipate that nodulation might occur. The failure of *C. pallida* to nodulate in this study suggests that factors other than N$_2$ fixation (effective mycorrhizae or its common association with N$_2$-fixing species such as *P. glandulosa*) are more likely explanations for the high tissue N concentrations observed in field plants. Two of the leguminous shrubs examined, *Acacia greggii* and *A. berlandieri*, also failed to nodulate. Lack of nodulation in *A. greggii* has also been noted by Garcia-Moya & McKell (1970), Allen & Allen (1981) and Felker & Clark (1981). Lack of nodulation of *A. berlandieri*, not previously documented, was initially surprising, given the high concentrations of
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foliar N in field plants (Table 6). The fact that many of the species which did not nodulate and had foliar N concentrations comparable to or higher than that of plants capable of nodulation indicates that foliar N concentration is not a reliable indicator of N$_2$ fixation in field populations.

Nodulation occurred in five of the native leguminous shrub species and the exotic actinorhizal *Alnus glutinosa*. These nodules produced significant amounts of ethylene (Table 1), indicating that effective *Rhizobium* and *Frankia* strains were present in the soils of our study site (Baker & Torrey 1988; Turk & Keyser 1992). Furthermore, the rates of ethylene production per unit dry weight, at 23–128 μmol g$^{-1}$ h$^{-1}$ (Table 1), were comparable to those reported for other woody legumes and actinorhizal species (van Kessel et al. 1983; Zitzer & Dawson 1989).

High levels of N in surface soils beneath woody plant canopies may produce local spatial patterns, whereby nodulation increases with depth and distance from adult canopies (Virginia et al. 1986; Jenkins et al. 1988; Jenkins, Virginia & Jarrell 1989; Johnson & Mayeux 1990). In our study potential differences in soil characteristics associated with depth in the profile or distance from centre of woody clusters (Table 4) could not explain the significant differences in numbers of nodules per seeding for *P. glandulosa* (Fig. 2). However, we observed a slight but significant negative correlation between soil N and numbers of nodules on *A. farnesiana* seedlings. Increasing levels of N did not reduce the number of nodules on 18-week-old *P. glandulosa* seedlings (Fig. 3a), but did reduce the node density of whole seedling biomass: an effect also observed for several *Acacia* species (Sun, Sands & Simpson 1992). This suggests that the soil N encountered in the soils of our study site (Table 4) would be unlikely to prevent infection of the nodulating species by *Rhizobium* strains.

We found no evidence to suggest that vertical or horizontal spatial availability of *Rhizobium* or *Frankia* strains might limit nodule formation in upland soils at this savanna parkland site. Extent of nodule formation under field conditions will depend on light levels, nitrogen availability (Fig. 3a; van Auker & Bush 1989; Lal & Khana 1993) and other factors that influence plant–microbe interactions. Field observations at our sites and others (Miettinen et al. 1988; Johnson & Mayeux 1990) suggest that nodule formation and persistence is dynamic and ephemeral, and that it fluctuates in response to soil moisture.

Our results add to a growing body of literature which indicate that nodulation in many dryland woody legume species can occur across a broad range of soil characteristics and depths (Virginia et al. 1986; Jenkins et al. 1988, 1989). The dynamics of nodulation and the extent of N$_2$ fixation under field conditions at our study site awaits further documentation. Such determinations should improve our understanding of the influence of symbiotic N$_2$ fixation on carbon and nitrogen cycling, species interactions, successional processes and responses to disturbance, as well as facilitating the development of sustainable land management in this region.

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