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SHORT COMMUNICATION

Nodulation dynamics and nodule activity in leguminous tree species of a Mexican tropical dry forest

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Biological N fixation by symbiotic micro-organisms plays a key role in making atmospheric N largely available to other organisms and nodule formation by rhizobia in roots of many legume species represents a very important pathway (Postgate 1998). Estimates of the contribution of symbiotic fixation to total N content in soils range from 44 to 200 Tg y^{-1} (Bøckman 1997).

Many studies of biological N fixation in tropical ecosystems have been conducted either in crops, plantations or pastures (España *et al*. 2006, Räsänen *et al*. 2001, Zurdo-Piñeiro *et al*. 2004). Fewer studies on nodulation have been conducted in seasonally dry tropical ecosystems where legume species have established very successfully (Crews 1999) and these include the Miombo woodlands of Africa (Högberg & Alexander 1995) and the semi-arid caatinga in Brazil (Teixeira *et al*. 2006).

Tropical dry forests cover about 42% of intratropical vegetation worldwide (Murphy & Lugo 1995). Floristically, the Leguminosae represent the dominant family in these forests (Gentry 1995). The high relative abundance of legumes in tropical floras may be greatly responsible for the high N availability generally found in tropical forests (Vitousek 1984). Both litter (Sprent *et al*. 1996) and soil have high N concentrations, and N circulates at higher rates in tropical than in temperate and boreal forests (Vitousek & Sanford 1986).

Studies on nutrient cycling in the tropical dry forest of the Chamela region on the Pacific coast of Mexico have shown high ecosystem N stocks (Jaramillo *et al*. 2003) and dynamic fluxes of NO and N_2O in response to water availability (Davidson *et al*. 1993, García-Méndez *et al*. 1991). A recent study of trehalose contents in roots and nodules of leguminous trees in the Chamela dry forest (Altamirano-Hernández *et al*. 2004) showed that nodulation occurred in the field and suggested nodules may be involved in N fixation as well. Given the high number of potentially N-fixing legumes in this forest (Lott 1993), the understanding of the N cycle requires an assessment of nodule activity in the field. Thus, we selected leguminous species to establish the presence of nodules, to determine the seasonal dynamics of nodulation, and to quantify nitrogenase activity under field conditions.

The study was conducted in the Chamela Biological Station of the Universidad Nacional Autónoma de México, near the Pacific coast in the state of Jalisco, Mexico (19°30′N, 105°03′W). The climate is highly seasonal with a pronounced dry season. Precipitation averages 741 mm y^{-1} (1983–2002; García-Oliva pers. comm.), distributed mostly from June to October; on average, about 31% of the total annual precipitation falls in September (García-Oliva *et al*. 1995). Total precipitation during the study year (1999) was 1132 mm, well above the average. Mean temperature is 25 °C, with less than a 5 °C difference between the coolest and warmest months. The landscape is of low hills (50–160 m elevation) with steep convex slopes. Soils are sandy-clay-loams, poorly developed, classified as Eutric Regosols in the FAO system, with a pH of 6–6.5 (Cotler *et al*. 2002).

The flora of the Chamela region is composed of at least 1149 vascular plant species, 572 genera and 125 families (Lott 1993). Plant families with the greatest species richness are the Leguminosae, Euphorbiaceae,

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Rubiaceae and Bignoniaceae, with more than 10% of the species endemic to the states of Jalisco and Colima. We examined eight tree and one shrub species of legume. Some of the tree species are among the dominant in the dry forest at the Chamela Reserve (Durán *et al.* 2002). The number of individuals sampled per species varied according to their availablity in the area and are indicated below in parentheses, next to their taxonomic identity. Five species were in subfamily Mimosoideae: *Albizia occidentalis* Brandegee (5), *Chloroleucon mangense* (Jacq.) Britton & Rose var. *leucospermum* (Brandegee) Barneby & Grimes (3), *Lysiloma microphyllum* Benth. (5), *Piptadenia constricta* (Micheli) Macbr. (13) and the shrub *Pithecellobium platylobum* (DC.) Urb. (1). Four species belonged to subfamily Papilionoideae: *Erythrina lanata* Rose (10), *Lonchocarpus eriocarinalis* Micheli (13), *Lonchocarpus constrictus* Pitt. (4) and *Pterocarpus orbiculatus* DC. (2). One previous report concerning the presence of nodules in their root systems in the Chamela dry forest involved five of these (*A. occidentalis*, *E. lanata*, *L. eriocarinalis*, *L. microphyllum* and *P. constricta*; Altamirano-Hernández *et al.* 2004), but nitrogenase activity was not documented. *Chloroleucon mangense*, *A. occidentalis* and *L. microphyllum* have also been reported elsewhere as nodulating (Sprent 2001). The other three species have not been yet reported in existing reviews (Allen & Allen 1981, Brewbaker *et al.* 1990, de Faria *et al.* 1989, Halliday 1984, URL: http://www.ars-grin.gov/npgs/sbml/jhw/public_html/cgi-bin/nodulation.pl) or for the Chamela dry forest.

We looked for nodules in the plants' rhizosphere to a maximum depth of 15 cm and in a 50 cm radius from the trunk. Nodules were separated from the roots to determine their size, colour and type (determinate or indeterminate) and were placed in incubation flasks to measure nitrogenase activity through acetylene reduction. Observations were made during the dry (April), rainy (July, August), and towards the end of the rainy (October, November) seasons in 1999. Five soil samples were collected from the 0–15-cm depth at each sampling date to determine gravimetric soil moisture. Nitrogenase enzyme activity was measured in the field during August and October with the acetylene reduction assay. Nodules of each species were placed in a glass jar and were incubated for 1 h. Incubation flasks contained 10% acetylene by volume. After incubation, a gas sample was taken in a 7-ml vacuum tube and was analysed in a gas chromatograph with a Poropak column and a flame ionization detector. Settings were 30 ml min^{-1} nitrogen flux, 35 ml min^{-1} H_2, and 450 ml min^{-1} air; detector and injector temperatures were 120 °C and oven temperature was 100 °C. The amount of acetylene reduced during incubation was calculated according to Drevon (1995) and Somasegaran & Hoben (1985) and was expressed as the enzyme specific activity in micromoles of ethylene (C_2H_4) produced per g of nodule (dry weight) per hour.

Table 1. Physical features of nodules collected from legume tree species of the tropical dry forest in Chamela, Mexico. I = indeterminate, D = determinate, B = brown, P = pinkish, Y = yellow.

Species	Nodule type	Maximum size observed (mm)	External colour	Number of nodules examined
Mimosoideae				
Albizia occidentalis	I	4 × 3	B, P	22
Lysiloma microphyllum	I	4 × 2	B, Y	17
Piptadenia constricta	I	3 × 2	P, Y	41
Pithecellobium platylobum	I	3 × 1	B	5
Papilionoideae				
Erythrina lanata	I	4 × 3	B, Y	29
Lonchocarpus constrictus	I	2.5 × 4.4	Y	6
Lonchocarpus eriocarinalis	I	5.7 × 2.3	B	32
	D	1.6	Y	

Nodules were observed in seven of the nine species. Only *P. orbiculatus* (Papilionoideae) and *C. mangense* (Mimosoideae) lacked nodules, which is in contrast to previous reports on *C. mangense* (Sprent 2001). Nodules were predominantly indeterminate, ranged between 1.6 and 5.7 mm in size, and external coloration varied from yellow to brown (Table 1). Colour and size changes were observed in the sampling months. During the rainy season, reddish to brownish colours and larger sizes were commonly observed, whereas whitish to yellowish colours and small nodules were observed towards the end of the rainy season.

Rainfall quantity and distribution determine soil moisture, which in turn regulates rhizobium proliferation and survival, as well as root colonization (Zahran 1999). Nodule abundance in the Chamela dry forest was markedly seasonal and clearly followed soil moisture variation (Figure 1). Nodules were absent from all individual trees assessed at the peak of the dry season (April), whereas a maximum 77% of individual trees were nodulated during July. The number of nodulated trees decreased considerably towards the end of the rainy season in November. The apparent synchrony between soil moisture and nodule presence suggests that biological N fixation is primarily controlled by soil water availability in this dry forest. Fine roots (< 1 mm), upon which nodules form, also respond to seasonal water availability in the Chamela dry forest, with an increase in production after the onset of rains (Castellanos *et al.* 2001). Another study in tropical dry forest, but in the semi-arid region of Brazil, has also suggested water limitation to biological N fixation (Teixeira *et al.* 2006).

Nitrogenase enzyme activity was detected in the nodules after *in situ* application of the assay in six species (Table 2). Activity varied within and among species, which is not uncommon in other studies with species from

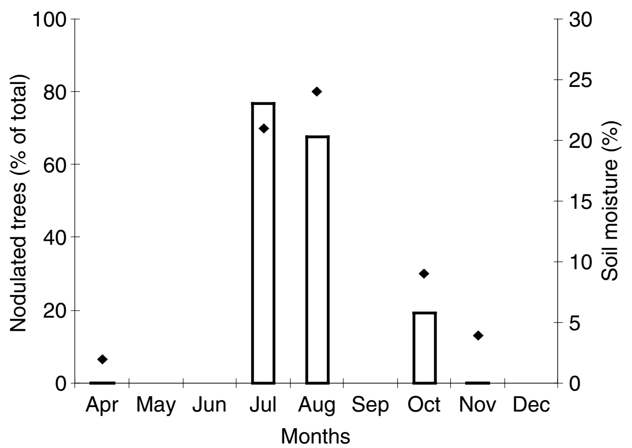

Figure 1. Seasonal variation in the percentage of nodulated leguminous trees (bars) and gravimetric soil moisture (black diamonds) in the tropical dry forest of Chamela, Jalisco, Mexico. No samples were taken in May, June and September.

Table 2. *In situ* acetylene reduction activity in nodules of legume tree species of the tropical dry forest in Chamela, Jalisco, Mexico. Values are mean and 1 ± SD. The number of individual trees per species used to perform the assay at each date is shown in parentheses. Lack of replication for some species indicates no active nodules were found except for that individual. NN = no active nodules were found.

Species	Specific activity (μmol C_2H_4 g^{-1} dry weight h^{-1}) August	October
Mimosoideae		
Albizia occidentalis	2.9 ± 1.9 (4)	NN (5)
Lysiloma microphyllum	3 ± 2.2 (3)	NN (5)
Piptadenia constricta	1.9 ± 1.4 (4)	0.3 ± 0.03 (2)
Papilionoideae		
Erythrina lanata	4.2 ± 1.9 (4)	0.4 (1)
Lonchocarpus constrictus	1.3 (1)	5.1 ± 4.7 (3)
Lonchocarpus eriocarinalis	3.4 ± 2.4 (4)	3.2 (1)

the same (Frioni *et al.* 1998) or different (Saur *et al.* 1998) genera. Nodule activity may also vary with soil age, with higher activity in younger than in older soils (Pearson & Vitousek 2002). Seasonal variation in activity was also evident in that few or no active nodules could be found for some species late in the growing season (Table 2). Nodule activity in legumes of the Chamela TDF indicates symbiotic N-fixation is occurring in this ecosystem.

The positive effect of N-fixing legumes on soil nutrient cycling rates has been shown in tropical forest plantations (Binkley & Giardina 1997). Nodule mineralization and fine-root decomposition or root-exudate liberation may release available N to the soil solution (Zahran 2001). The high percentage of legume species in the flora of the Chamela Reserve (Lott 1993) and the high abundance of some of them in the vegetation (*Lonchocarpus* and *Piptadenia*; Durán *et al.* 2002), may explain the high soil N_2O and NO fluxes at the onset of the rainy season (Davidson *et al.* 1993, García-Méndez *et al.* 1991), the high ecosystem N stocks (Jaramillo *et al.* 2003), and the high N concentrations in leaves and litterfall (Jaramillo & Sanford 1995, Rentería *et al.* 2005) in this tropical dry forest. Thus, forest conversion for agriculture and pasture as currently practiced, represents the removal of a key N source with potential implications for sustainable land use and for ecosystem recovery after abandonment.

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