Oecologia (1989) 80:27–31

Oecologia

Aerial root nodules in the tropical legume, *Pentaclethra macroloba*

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Summary. Symbiotic nitrogen fixation in angiosperms normally occurs in buried root nodules and is severely inhibited in flooded soils. A few plant species, however, respond to flooding by forming nodules on stems, or, in one case, submerged roots with aerenchyma. We report here the novel occurrence of aerial rhizobial nodules attached to adventitious roots of the legume, *Pentaclethra macroloba*, in a lowland tropical rainforest swamp in Costa Rica. Swamp saplings (1–10 cm diameter) support an average 12 g nodules dry weigth per plant on roots 2–300 cm above water, and nodules remain in aerial positions at least 6 months. Collections from four swamp plants maintained linear activity rates (3–14 μmoles C_2H_4/g nodule dry weight/hr) throughout incubations for 6 and 13 hrs; excised nodule activity in most legumes declines after 1–2 hrs. Preliminary study of the anatomy and physiology suggest aerial nodules possess unusual features associated with tolerance to swamp conditions. High host tree abundance and nodulation in the swamp compared to upland sites indicate the aerial root symbiosis may contribute more fixed nitrogen to the local ecosystem than the more typical buried root symbiosis.

Key words: N fixation – Legume-*Rhizobium* symbiosis – Aerial nodules – *Pentaclethra macroloba*

Field investigators have documented a diversity of associations between nitrogen fixing microorganisms and plant roots, stems, and leaves (Nutman 1975); the most abundant and well-known association is the legume-*Rhizobium* symbiosis. In almost all published cases of this mutualism, rhizobia nodulate buried legume roots (Allen and Allen 1981). One of the authors (CW) discovered rhizobial nodules on aerial adventitious roots of the legume, *Pentaclethra macroloba*, (Willd.) Ktze. (Mimosaceae). This tropical tree species forms rhizobial nodules on buried roots in a variety of habitats in lowland rainforest at La Selva Field Station, a research site of the Organization for Tropical Studies in Costa Rica. Aerial roots and nodules were observed only in swamps where the soil was flooded for 8–12 months/yr. The following report documents the anatomy, physiology, and ecology of aerial nodules in response to swamp conditions.

Methods

Histological study compared cross sections of several small nodules that developed solely in aerial conditions and were fixed in formalin and stained with periodic acid-Schiff's and/or toluidine blue. A typical cross section with both stains is shown in Fig. 2.

Nitrogenase activity was measured using acetylene-reduction (Hardy et al. 1968). Several nodules (total dry weight 0.5–2.5 g) were detached from roots of each sampled plant and immediately sealed in serum-capped vials, filled with 10% acetylene in air, and incubated at normal swamp air temperatures. Gas aliquots were removed and assayed for acetylene and ethylene using flame ionization in a Varian 3000 gas chromatograph. Nodules were dried at 50° C and weighed after each assay.

Nodule activity was monitored for several hours for nodules collected from 8 swamp plants and 2 upland plants. Nodule activity in short-term incubations (30–90 min) was compared using collections from 20 swamp plants and 3 upland plants. Nodules from both aerial and submerged roots were obtained from 9 of the 20 swamp plants. Recovery of nodule activity following submersion was studied using samples of aerial and submerged nodules from one swamp plant, an additional sample of aerial nodules from a second swamp plant, and one collection of buried nodules from an upland plant. Excised nodules were assayed for h, flushed with air, flooded with swamp water for 1 h, gently dried, and reassayed for 1 h.

Host tree abundance and nodulation were measured for seedlings (<1 cm diameter and <1 m height), saplings (1–10 cm and rarely 10–20 cm diameter), and adults (>20 cm diameter) of *P. macroloba* sampled in 1 swamp (approximately 90 m^2), 10 tree-fall gaps with open canopies (average area of 78 m^2), 10 adjacent understory areas (average area 78 m^2), and 10 randomly selected 78 m^2 plots in the surrounding forest. The random plots, by chance, did not fall in or near recent tree fall gaps. Swamp plants were completely censused by measuring all stems and visually estimating the abundance of aerial nodules in three diameter classes: <5 mm, 5–10 mm, and 10–25 mm. Nodule dry weights from the acetylene reduction assay were used to estimate weights of nodules in each size class. Aerial nodules were observed on *P. macroloba* in several swamps at

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La Selva but all quantitative work focused on the swamp closest to the field station laboratory. Upland plots were within 1.5 km of the study swamp. In upland census plots, all *P. macroloba* were measured, roots of small plants (<2 m height) were completely excavated, and two troughs 10 cm wide 10 cm deep and 2 m long were dug radially from large host trees to estimate nodule abundance. All nodules from upland plants were dried and weighed.

Results and discussion

Aerial nodules develop above the water line (2–35 cm and occasionally as high as 3 m above water), and form indeterminate clusters 0.2–2.5 cm in diameter (Fig. 1).These nodules have a firm texture, orange-brown exterior pigmentation similar to the aerial roots, and red interior pigment, indicating leghemoglobin. Nodules located below the water line are generally smaller than aerial nodules and spongy with black exteriors and interiors, indicating senescence. Nodules buried in upland soils range from 0.1–1.0 cm diameter, have a firm texture with light brown exteriors and white or pink interiors.

Interior anatomy of aerial nodules (Fig. 2) includes a central zone of enlarged infected cells and small uninfected interstitial cells surrounded by a zone of cortex with occasional vascular traces, and finally epidermis, including surface lenticels. The lenticel in Fig. 2A may be collapsed due to partial dessication of outer cells (Pankhurst and Sprent 1975). These zones are observed in most legume nodules, such as *Gycine max* (Bergerson and Goodchild 1972), *Medicago sativa* (Vance et al. 1979), and *Neptunia oleracea* (Schaede 1940).

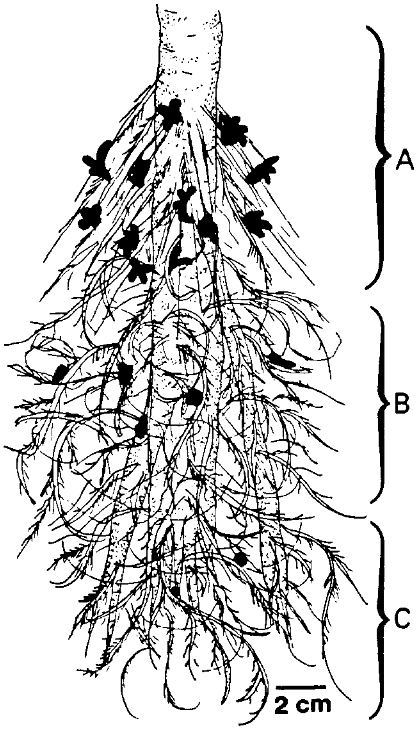

Fig. 1. Roots and nodules along a small *P. macroloba* stem, redrawn from photographs. The nodules are depicted in relative abundance as they occurred on aerial roots (*A*), on submerged roots (*B*), and within the swamp muck (*C*)

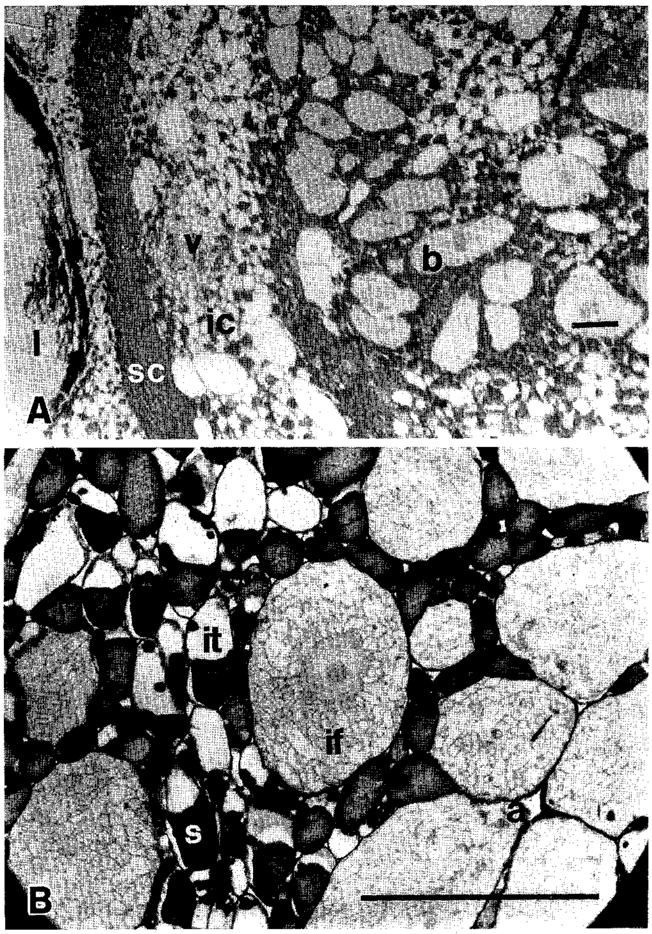

Fig. 2. A, B Photomicrographs of an aerial nodule collected from swamp *P. macroloba*. **A** Cross section: *l* lenticel, *sc* schlerid zone, *ic* inner cambium, *v* vascular trace, *b* bacterial zone. **B** Detail of bacterial zone: *it* interstitial cell, *s* starch grain, *if* infected cell, *a* air space between infected cells. Scale bars, 100 um

Aerial nodule anatomy appears to differ from these and other published legume descriptions in three features: infected cells do not fill the central zone, rather they are surrounded by many uninfected cells; these uninfected interstitial cells contain abundant starch granules; and, the schlerid layer surrounding the inner cortex is especially dense and thick. These features may not be unique to aerial nodules in *P. macroloba*, but they are likely to support sustained activity of nodules in aerial positions.

Though nitrogen (N) fixation activity varied, it was consistently highest for aerial nodules, absent or low for submerged nodules, and moderately low for nodules buried in the soil of nearby upland plants (Fig. 3). Samples of isolated roots, soils, and swamp water produced no ethylene in short-term (1–2 h) or long-term (10–14 h) incubations.

Aerial nodules from four out of eight swamp plants sustained linear activity for a minimum of 6 h (Fig. 3A). The two most active samples were extended to 13 h during which they maintained rates of 10 and 14 µmoles C_2H_4/g D.W./h. Acetylene reduction rates of detached nodules from legumes and non-legumes usually decline after 1–2 h (Hardy et al. 1973; Huss-Danell 1978; Wych and Rains 1978). Sprent (1969) observed linear rates of activity for 8 h when excised soybean nodules were maintained at high

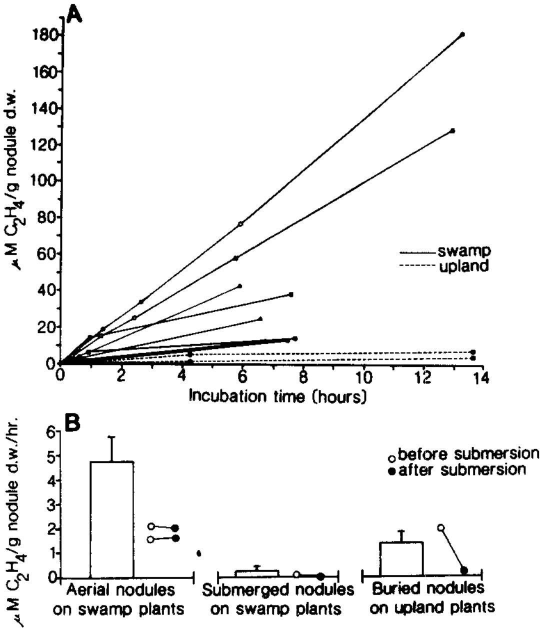

Fig. 3 A, B. Nitrogenase enzyme activity of nodules. **A** The μmoles C_2H_4 produced per gram nodule dry weight during long-term incubations. *Solid lines*: swamp plants with aerial nodules on adventitious roots; *dashed lines*: upland plants with nodules buried in soil. Each line represents a separate nodule collection. Lines with the same symbol represent the same plant. **B** Bars indicate average acetylene reduction rates and standard errors in short-term (1–2 h) incubations of nodules collected from three situations: aerial nodules on swamp plants ($N = 20$), submerged nodules on swamp plants ($N = 9$), and buried nodules on upland plants ($N = 3$). Each line indicates activity for a collection of fresh nodules in a dry vial before (open circles) and after (closed circles) nodules were submerged for 1 h and dried on the surface

Table 1. Abundance and Nodulation of *Pentaclethra macroloba*. Value are means ($\bar{x}$) and standard deviations (s)

	Swamp	Tree fall gap	Adjacent understory	Random forest plots
		$\bar{x}$ (s)	$\bar{x}$ (s)	$\bar{x}$ (s)
# *P. macroloba*/ha				
seedlings	1666	2949 (1666)	1794 (1667)	1269 (1282)
saplings	7435	218 (192)	64 (128)	26 (51)
adults	0	0	40	40
# nodulated/ha				
seedlings	897	737 (560)	161 (200)	355 (406)
saplings	7435	139 (91)	64 (128)	26 (51)
adults	0	0	40	40
nodule D.W. (g/ha)				
seedlings	1	910 (1153)	154 (205)	256 (295)
saplings	89227	885 (1038)	231 (583)	231 (449)
adult	0	0	3200	3200

moisture content and aeration. The thickened schlerid tissue in aerial nodules may protect inner cells from water loss, and abundant starch reserves in uninfected interstitial cells may provide metabolic support for the longevity of activity in aerial nodules excised from *P. macroloba*.

Individual aerial nodules appeared healthy and active on swamp plants for 6 months after their initial census until an unusually severe dry season. In one swamp, which completely dried, many nodules shrank and dropped off. Six months after the swamp regained normal levels of standing water, new aerial nodules, 1–3 mm in diameter, developed on new adventitious roots. Aerial nodule growth appears to depend on the growth of new adventitious root tissue stimulated by inundation of older roots.

Most legumes do not nodulate or proliferate in flooded soils; nevertheless, several species have adapted to swamp conditions. Rhizobial nodules form on stems of two tropical swamp legume genera, *Aeschynomene* (Albrecht et al. 1982) and *Sesbania* (Dreyfus and Dommerguess 1981), and aerenchymal roots of *Viminaria juncea* (Walker et al. 1983) when these plants are partially flooded. Flooding stimulates nodulation on submerged stems of *A. scabra*, but inhibits nodulation of submerged roots of this species (Eaglesham and Szalay 1983). Schaede (1940) reported cuttings of a garden specimen of *Neptunia oleracea* increased nodulation on flooded "water roots", but he could not measure N fixation. Aerial nodules of *P. macroloba* differ from stem nodules and "water root" nodules because nodules of *P. macroloba* develop on aerial roots and senesce when submerged for long periods of time.

Flooding directly limits N fixation in most root symbionts due to a lack of oxygen required for cellular respiration (Bennett and Albrecht 1984). In the swamp plant, *Myrica gale* (Myricaceae), nodulated roots of intact, flooded plants lost nitrogenase activity when the exogenous oxygen concentration dropped; activity recovered 50 min after oxygen increased (Tjepkema 1978). Aerial nodules of swamp *P. macroloba* performed similary after short-term submersion and redrying (Fig. 3 B). Both samples of aerial nodules maintained activity rates at presubmersion levels, while nodules from submerged and buried conditions lost activity after flooding. Based on earlier observations, the latter two types of nodules are likely to lose activity 3 h after excision, regardless of temporary submersion. Further experiments are needed to document the responses of all types of nodules to submersion, and establish the mechanisms through which aerial rhizobial nodules tolerate both short-term submersion and the opposite condition, atmospheric dessication.

The high humidity present in swamps may help maintain metabolic function in aerial nodules. Root nodules on crop plants lose nitrogenase activity when nodule water potential drops (Bennett and Albrecht 1984). Aerial nodules tolerate only temporary periods of dessication and/or submersion, and must regenerate when either condition is prolonged. Precipitation patterns at La Selva normally maintain moderately flooded swamps throughout the year; such patterns favor the development of aerial nodules. These swamps contain abundant saplings and upright branches of fallen *P. macroloba* with extensive adventitious roots.

Light gaps appear to play an important role in the pattern of *P. macroloba* abundance. The forest at La Selva appears to undergo relatively rapid turnover due to frequent tree falls and *P. macroloba* requires light gaps to mature (Hartshorn 1975). Swamps dominated by this species have an insecure soil matrix, and probably experience extremely frequent tree-falls, thus maintaining a partially open canopy.

Host tree abundance differs widely among four major habitat types: swamps, light gaps from recent tree falls, adjacent understory areas, and the rest of the forest which is a mosaic of regenerating tree-fall gaps and understory (Table 1). The study swamp supported 300 times the number of saplings compared to random plots, while tree fall gaps contained only 8 times the random sapling density. Saplings in most habitats are nodulated, but the average swamp sapling possesses 2–3 times the nodule mass of an average upland sapling. Acetylene reduction data indicate aerial nodules sustain higher rates of N fixation compared to upland buried nodules, but limited measurements of upland nodules prohibit extrapolating data in Fig. 3 to whole forest estimates.

Despite problems associated with extending small scale activity rates to large scale annual measurements, it is important to compare the potential level of N fixation with annual estimates of other ecosystems. The potential contribution of symbiotic N fixation in swamp and upland plants can be calculated using a single intermediate activity level of 3 μmoles C_2H_4/hr/g nodule D.W., a ratio of 4 to 1 for C_2H_2 to N_2 (Schubert and Evans 1977), and 200 ten-hour days per year. Given these values, the N fixed (in kg/ha/yr) is as follows: swamp, 3.4; tree fall gap, 0.06; adjacent understory, 0.12; and random plots, 0.18. The highest value, 3.4 kg/ha/yr, is low compared to agricultural systems which range from 10–200 kg/ha/yr (LaRue and Patterson 1981); other tropical forests, however, fix at similar rates. Forests on clayey latisols of central Amazonia, for example, fix 2.45 kg N/ha/yr (Sylvester-Bradley et al. 1980). Most estimates of symbiotic N fixation depend on small scale, destructive assays. The occurrence of aerial nodules on adventitious roots extends the opportunities for monitoring N fixation in undisturbed plants using methods developed for long-term assays of intact nodulated roots in potted plants (Wych and Rains 1978). Realistic estimates of the role of symbiotic N fixation in individual plants and community N budgets requires improved knowledge of the physiology of intact nodules in field environments.

Soil conditions in different habitats at La Selva influence the legume-*Rhizobium* symbiosis. Alluvial soils support high rates of nitrification assumed to be derived from high ammonium levels (Robertson 1984), and drained soils derived from residual basalt have high levels of available N and low phosphorous (Vitousek and Denslow 1986, 1987). These conditions may repress N fixation in upland habitats at La Selva. In contrast, swamps typically have low available N (Black 1968; Freney et al. 1981). Bourgeois et al. (1972) noted high levels of undecomposed organic matter and lower total N in the surface horizon of flooded soils compared to drained soils at La Selva. Decreased oxygen in inundated soils hinders N mineralization as well as normal nodule development and activity (Bennett and Albrecht 1984). *P. macroloba* has evolved the ability to form adventitious roots in response to flooding and support nodules with the anatomy and physiology to function in the aerial swamp environment. The high abundance of aerial nodules in swamps suggests symbiotic N fixation provides a potentially significant contribution to the N input of this habitat.

Field studies of the physiology and ecology of N-fixing symbioses show high spatial and temporal variation in N fixation, primarily because nodule abundance and activity decrease when available N increases. Each symbiotic association, however, is the result of responses to many variables in addition to N level. This preliminary study of aerial root nodules documents a novel adaptation of the legume-*Rhizobium* symbiosis to swamp conditions.

Acknowledgement. We thank the Organization for Tropical Studies for field support, F. Rickson for nodule sections and negatives, A.K. Walter for translations, J.M. Atherton, J. Denslow, L. Kapustka, T. Kursar, E. Leigh, F. Rickson, and A.M. Wood for helpful criticism. This work was supported by the following grants to C.A.W.: NSF–DEB79–23400, NIH–GM07183, the O.T.S. NSF-RIAS grant, and the Univ. of Chicago Hinds Fund, and a grant to B.L. Bentley, NSF-DEB80–10676.

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Received September 16, 1988