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Aerial Nodules in *Casuarina cunninghamiana*

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A complete survey of La Réunion Island showed that, in 40- to 50-year-old *Casuarina cunninghamiana* plantations located in the northeast at an altitude above 400 m, some trees bore aerial nodules as high as 6 to 7 m up the trunk. The nodules exhibited a significant specific acetylene reduction by the ARA method (0.77 μmol of C_2H_4 per h/g [dry weight] of nodule) at the time of sampling (June 1990). Aerial nodules were also found on a *Casuarina glauca* trunk. Preliminary observations show that anatomically aerial and underground nodules do not differ significantly. In addition to host plant genetic determinants, aerial nodule formation is assumed to require sufficient rainfall, an abundance of *Frankia* spp. in the soil and air, and rhytidome on the tree trunk.

On leguminous plants, N_2-fixing nodules are generally formed on the roots and function in the soil. Aerial (stem) nodules also exist and have been described on the stems of species belonging to the genera *Sesbania* (7), *Aeschynomene* (1, 2, 14), and *Cassia* and *Parkinsonia* (13). Walter and Bien (12) reported that another type of aerial nodule had been discovered on the adventitious roots of *Pentaclethra macroloba*, a tropical leguminous tree that grows in swamps in Costa Rica, but that these aerial nodules are located on the adventitious roots at some distance from the trunk and, consequently, do not adhere to it.

Until now, no aerial nodules on the trunks of actinorhizal trees had been reported, which explains why the recent discovery by one of us (Y.R.D.) of profuse aerial nodulation on the trunk of a number of *Casuarina cunninghamiana* trees in 40- to 50-year-old plantations in La Réunion Island was unexpected.

This note describes our first results of field and laboratory investigations on this new and unusual type of N_2-fixing nodules and suggests different hypotheses to explain their development.

La Réunion Island is located in the Indian Ocean, to the east of Madagascar. In June 1990 a survey was conducted throughout the island with a focus on five stations: Station A, Hauts de Moka (altitude [alt.], 800 m); Station B, Hauts de Moka (alt., 750 m); Station C, Hauts de Moka (alt., 700 m); Station D, Hauts de Moka (alt., 670 m); and Station F, Hauts de la Ressource (alt., 800 m).

At four stations two types of nodules were collected from *C. cunninghamiana*: (i) aerial nodules, collected at different heights on the trunk, and (ii) underground nodules, collected from the roots of homologous trees. Aerial nodules were also collected from a *C. glauca* tree trunk at Station D. The samples were immediately incubated in situ under C_2H_2 to assess their nitrogenase activity by using the acetylene reduction method (ARA) (9). Each nodule sample, 1.0 to 1.2 g, was placed in a serum vial containing 10% acetylene in air and incubated for 45 min at 20 to 22°C. Gas samples were collected in triplicate in air-rinsed 5-ml Venoject tubes (Polylabo, Strasbourg, France) and taken to the laboratory for analysis. Ethylene levels in each tube were estimated by using a Delsi C330 gas chromatograph (Delsi Instruments, Suresnes, France). After each assay, the nodules were air dried and then weighed. Comparative ARA estimations were made from a nodule-free piece of *C. cunninghamiana* bark (Station A) and from underground nodules of *Acacia decurrens* (Station A).

Scanning electron microscope examination of the aerial nodules was done on an ISI 60 microscope (International Scientific Instruments, Paris, France) after the nodules had been fixed in 3% glutaraldehyde (Agar Aids, Evry, France) in 0.1 M sodium cacodylate buffer (Merck, Nogent, France) and dehydrated by using a critical point drying apparatus (Polaron Equipment Limited, Watford, United Kingdom).

C. cunninghamiana that bore aerial nodules were only found in plantations in the northeast of La Réunion Island, at an altitude of over 400 m, generally less than 10 km from the sea. In this area, the climate is characterized by relatively high air humidity (the only dry months are August and September) and by heavy though erratic rains (cyclonic regimen), with an annual rainfall of up to 3,000 mm, which cause abundant stem flow. Aerial nodules seemed to develop only on the trunks of less than 10% of the trees and were generally located along a large vertical strip on that side of the trunk which conveyed most of the stem flow. Whether the percentage of nodulated trees varied with sampling station or within the station has not been explored. On trees with aerial nodules, nodules were found at a height of up to 6 to 7 m and covered about 5 to 20% of the lowest 2 m of the trunk (where the nodules were more numerous).

At one of the sites where *C. cunninghamiana* with aerial nodules was found (Station D), we found that a *C. glauca* tree also bore aerial nodules; this finding indicated that aerial nodule formation was not restricted to *C. cunninghamiana*.

Aerial nodules are uneven, roughly circular protuberances of the tree's rhytidome (the dead outer tissue of the bark in a mature tree [10]), 20 to 30 cm in diameter and 4 to 8 cm thick. The protuberances adhere tightly to the trunk (Fig. 1A and B) and are composed of dead rhytidomal tissues embedding adventitious roots intermixed with typical coralloid (Fig. 1C), dichotomously branched actinorhizal nodules whose lobes bear a nodular root at their apex. During the

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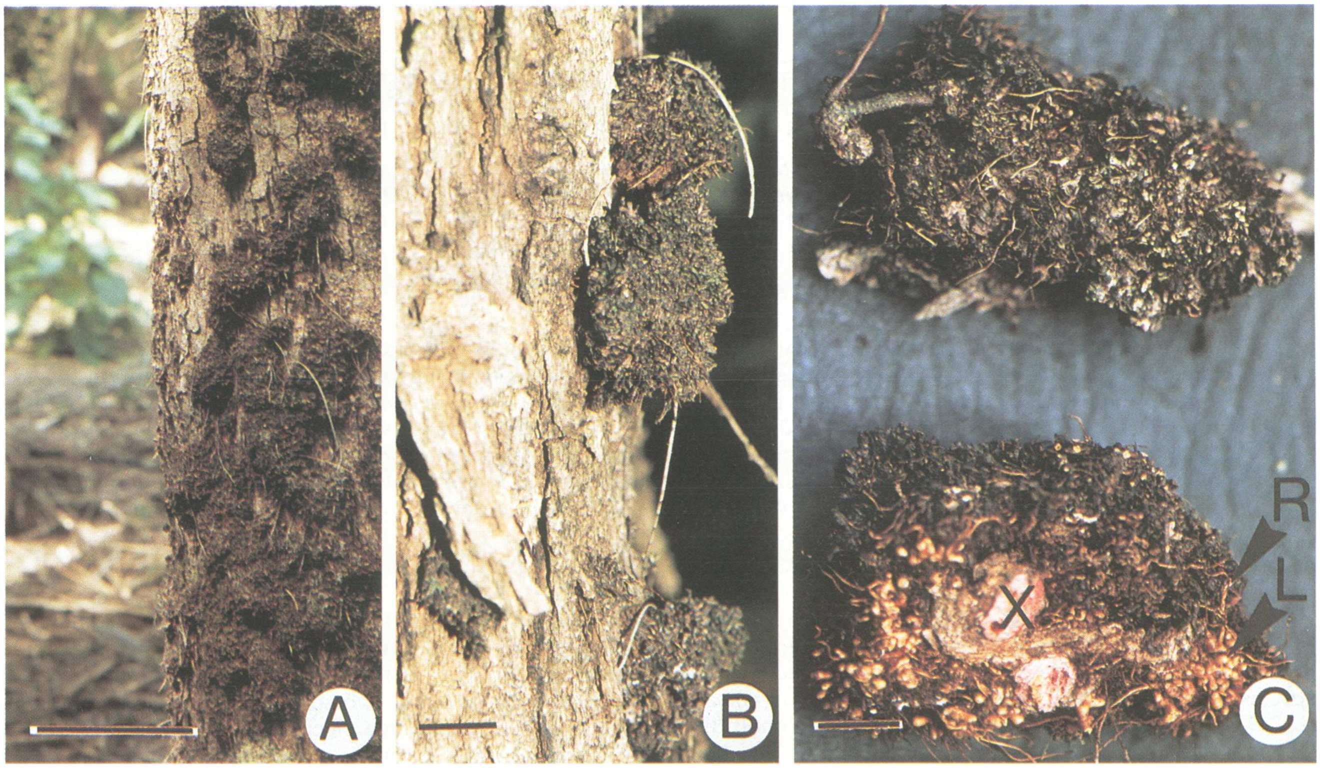

FIG. 1. (A) Trunk of *C. cunninghamiana* with profuse aerial nodulation at 130 to 150 cm above the ground. Bar, 10 cm. (B) Detailed photograph of aerial nodules, which appear as roughly circular protuberances that adhere tightly to the trunk. Bar, 3 cm. (C) Aerial nodule detached from the trunk. Bar, 1 cm. In panel C, upper nodule, the outer face made of rhytidomal tissues; in the lower nodule is the inner face, showing the typical yellow actinorhizal lobes (L) with adventitious roots (R) embedded in rhytidomal tissues. The pink spots (X) are sections of tissues, including xylem, that connect the nodule with the trunk.

season of heavy rains (December to February), the lobes of the actinorhizal nodules are yellow and healthy looking, whereas in the drier, cooler season (June) large portions of the nodules are desiccated or even decayed and thus contribute to the accumulation of dead organic matter inside the protuberance. Under the right environmental conditions, new nodule tissues are expected to form within the protuberance; in other words, there should be an annual turnover of the nodule lobes inside the protuberance.

Specific ARA values of aerial nodules at different heights and underground nodules are shown in Table 1. In the four stations where they were sampled, aerial nodules had a mean specific ARA value of 0.77 μmol of C_2H_4 per h/g (dry weight) of nodule, whereas the mean specific ARA value of underground nodules was 2.88 μmol of C_2H_4 per h/g (dry weight) of nodule. The specific ARA value of *C. cunninghamiana* nodule-free bark samples was 0, while that of underground nodules of a young *A. decurrens* was 15.4. The specific ARA of aerial nodules was lower than that of underground nodules for the following reasons. (i) Specific ARA estimations were made in June, when hygrometry and temperature were low and most of the nodule lobes were senescent. Nodule activity was probably far from maximum since the aerial location makes the nodules particularly sensitive to drought and low temperatures. Underground nodules enjoy a more buffered environment. (ii) Specific ARA estimations were made on samples in which fresh nodule lobes were mixed with dead tissue that could not be discarded before incubation. Since underground nodules are easier to clean, their specific ARA is higher. As a typical highland species, *A. decurrens* may have high specific ARA values because it is well adapted to the low ambient temperature prevailing at the time of sampling (June).

The absence of ARA in the *C. cunninghamiana* nodule-

TABLE 1. Specific ARA values of aerial and underground nodules of *C. cunninghamiana* and *C. glauca* that were sampled in June 1990 at La Réunion Island[a]

Nodule type	Station(s)	Specific ARA[b]
Aerial nodules of *C. cunninghamiana* at a ht above ground of:		
40 cm	A	1.2
115 cm	A	0.6
130 cm	B, C	0.7
160 cm	A, F	0.5
280 cm	A	0.9
450 cm	A	1.6
Aerial nodules of *C. glauca*[c]	D	0.7
Underground nodules of *C. cunninghamiana*	A, B, C, F	2.8
Nodule-free bark of *C. cunninghamiana*[c]	E	0
Underground nodules of *A. decurrens*	A	15.4

[a] Comparative specific ARA estimations made from nodule-free bark of *C. cunninghamiana* and from underground nodules of *A. decurrens*.

[b] Micromoles of C_2H_4 per hour per gram (dry weight) of nodule.

[c] 130 cm above the ground.

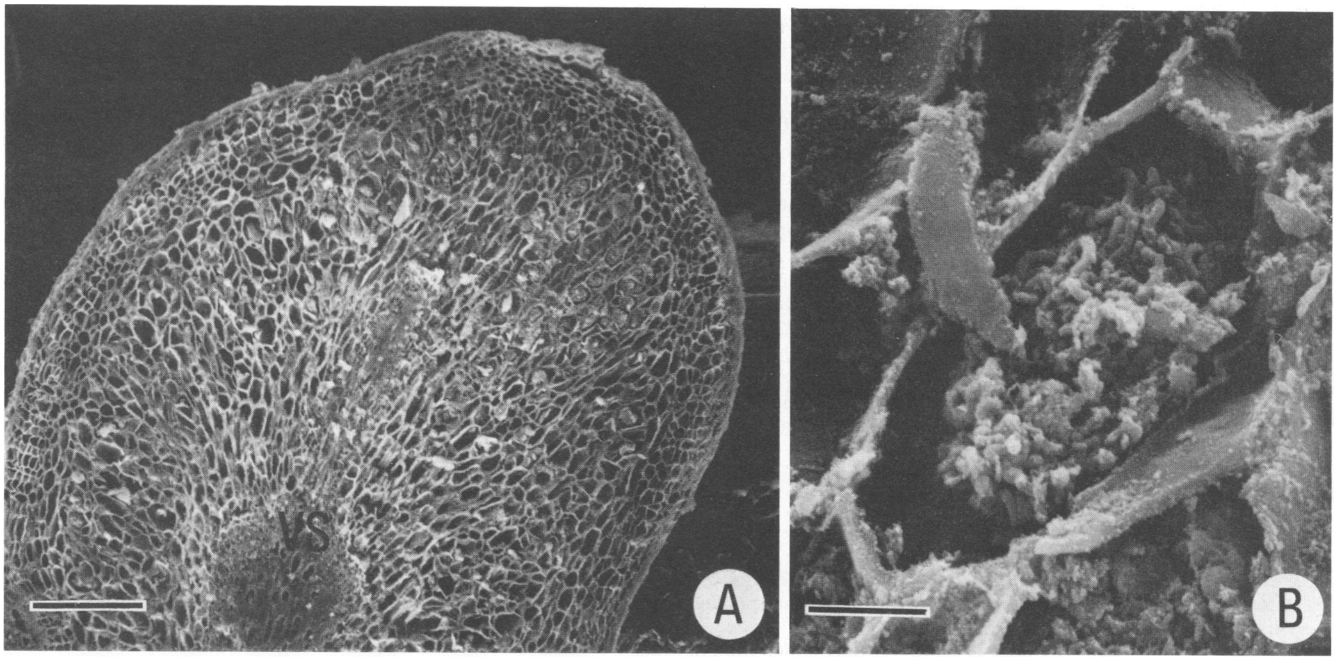

FIG. 2. (A) Scanning electron micrograph of an aerial nodule section showing the central vascular system (VS) and numerous parenchymal *Frankia*-infected cells. Bar, 200 μm. (B) High magnification of an infected cell showing a characteristic cluster of endophyte hyphae. Bar, 10 μm.

free bark sample indicates that N_2 fixation occurred only in the aerial nodules, not in the bark itself.

Histological examinations of sections of aerial nodules revealed the presence of numerous infected cells in the parenchyma (Fig. 2A). The infecting organism had the aspect of a filamentous bacterium and was not differentiable from typical *Frankia*-infected cells (4). Preliminary observations do not show significant differences in the anatomy of aerial and underground nodules. Scanning electron microscope observations (Fig. 2B) clearly confirmed the filamentous appearance of the infecting bacteria and the absence of typical vesicles. More investigations are obviously required to elucidate the taxonomic position, physiology, and ecology of the *Frankia* strain(s) involved in this aerial nodule symbiosis.

The following factors are probably of paramount importance in inducing aerial nodulation. (i) Sufficient rainfall is distributed through a relatively long rainy season, and air humidity is high throughout the year; this climate is found only in the northeast portion of the island, which explains why *C. cunninghamiana* there has aerial nodules. (ii) *Frankia* cells are present several meters up the tree trunk, which implies that *Frankia* cells are present not only in the soil but also in the air. In field surveys throughout the island, *Frankia* cells were found in all of the soils, including soils formed in 1977 from the lava emitted from the Piton de la Fournaise. *Frankia* cells probably are wind borne and transported to the trees as spores in dust or are transported up tree trunks by insects or other vectors. (iii) A rhytidome is present. Nodules were observed only on the trunks of older trees, which had well-developed rhytidome, which is a niche with specific chemical, physical, and biological characteristics that stimulate the formation of adventitious roots, facilitate their infection by *Frankia* cells, and thus favor the subsequent development of the nodules. The rhytidome functions in the same manner as a sponge retaining water and is a microsite where chemical stimuli, possibly synthesized by fungi or other organisms that thrive in the bark, are produced and induce the emission of adventitious roots. (iv) The host plant is able to produce aerial nodules. The fact that, regardless of site, only a small proportion of the trees bore aerial nodules strongly suggests that host genetic determinants affect the establishment of the symbiosis.

The infection sites for stem-nodulated legumes (7) and for *C. cunninghamiana* (and *C. glauca*) are similar, viz., the lateral adventitious roots. But there are four major differences between stem nodules of legumes and actinorhizal nodules. (i) The adventitious roots of stem-nodulated legumes are preformed as latent meristematic primordia; the adventitious roots of *Casuarina* spp. are not preformed. (ii) In *Casuarina* spp., *Frankia* cells, rather than *Rhizobium* cells, are the infection agents. (iii) For *Casuarina* spp. to develop aerial nodules requires the existence of the niche created by the rhytidome, the role of which is speculated upon above. (iv) Within the niche formed by the rhytidome, the *Casuarina* nodule lobes grow for some time on the adventitious roots, dry or decay in the course of the (relatively) dry and cold season, and resume growth when environmental conditions are favorable again; this suggests successive turnover of nodule lobes.

Like stem nodulation in legumes (3, 5, 6), aerial nodulation in *C. cunninghamiana* (and in *C. glauca*) probably confers to the host plant (i) high N_2-fixing potential and (ii) high levels of actual N_2 fixation in the field because of its independence from soil constraints, especially acidity and an excess of combined N. Since their N_2-fixing ability would be reduced only slightly by soil combined N, trees with aerial nodules are assumed to fix N_2 for most of their life and to be very efficient soil improvers, whereas trees with only root nodules (underground nodules) usually fix N_2 only during the first years after being planted in the field, until the soil N exceeds a certain threshold.

Aerial nodulation of members of the family *Casuarinaceae* should be produced artificially in the near future since preliminary laboratory experiments have shown that adventitious roots can be induced easily on the stems of *C. cunninghamiana* and *Casuarina equisetifolia*. Once the *Frankia* strains from La Réunion aerial nodules have been isolated, further research should lead to the development of a device to simulate the rhytidomial niche on the stem of young trees and to select (11) and vegetatively propagate (8) *Casuarina* clones with the capacity to bear aerial nodules.

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