

ROOT NODULES ON *PODOCARPUS LAWRENCEI* AND THEIR ECOLOGICAL SIGNIFICANCE

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Summary

Nodulated roots from *P. lawrencei* growing on a scree on Mt. Ginini, near Canberra, have been shown by the isotopic method to fix atmospheric nitrogen. The importance of this in a plant which is a pioneer of exposed, rocky situations is discussed. Although significant, the amount of nitrogen fixed by the detached nodulated roots was low: this may have been due to the small proportion of nodules with active tissue, but it is also consistent with the slow growth observed for this species. The nodulated roots also evolved hydrogen as observed during nitrogen fixation by legume nodules. Anatomical studies of the *Podocarpus* nodules confirmed early accounts of their general structure and mode of development but the symbiont was clearly a non-septate filamentous organism: no intracellular bacteria were observed.

I. INTRODUCTION

The existence of nodules on the roots of non-leguminous species of plants both in the gymnosperms and angiosperms has been known for more than 100 years (cf. McKee 1962; Bond 1963). Among the gymnosperms the species of *Podocarpus* have received most attention. Most species are nodulated and the nodule structures are similar, but the nature of the symbiont is in doubt and critical evaluation of the ability of the nodules to fix atmospheric nitrogen is lacking (McKee 1962).

Podocarpus lawrencei Hook f. (syn. *P. alpinus* R. Br. ex Mirb.) is usually a prostrate shrub of exposed subalpine and alpine habitats in New South Wales, Victoria, and Tasmania, but under favourable conditions it may attain small tree height [up to 30 ft in East Gippsland (Ewart 1930)]. It occurs mostly on rocky soils and is commonly one of the pioneer species of screes and exposed mountain-top debris (Costin 1954; Curtis 1956). The roots are almost invariably nodulated. The *Podocarpus* communities occur typically in a mosaic pattern with patches of *Podocarpus* heath separated by unvegetated rock or scree. Many of these patches are found to have originated from a single plant, from which colonization has extended by layering from the branches [cf. *P. nivalis* Hook in New Zealand (Fisher 1952)]. As the branches spread laterally, fines from washed and blown-in plant and mineral matter are able to accumulate, and the soil gradually becomes more suitable for herbaceous species. This type of colonization occurs both in the alpine and subalpine heaths (Plate 1, Figs. 1 and 2) of which this species forms one of the main associations (Costin 1954), and in subalpine woodland and upper montane sclerophyll forest in which *Podocarpus* may form a local understorey. In some of the latter communities the disparity between the age of the *Podocarpus* and the eucalypts suggests that the *Podocarpus* has pioneered the site and the eucalypts have followed. The growth rate of *P. lawrencei* is very slow under natural conditions:

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for example one 200-year-old specimen from Etheridge Range, Kosciusko, had a diameter increment of 0.01 in. per annum (Costin, unpublished data). This is one of the longest lived mountain species and in the absence of fires probably reaches an age of several hundred years.

The present study deals with nodulation in *P. lawrencei* in the Australian Alps.

II. MATERIALS AND METHODS

The site selected for study and sampling was the stabilized scree on the eastern face of Mt. Ginini, as exposed by the Upper Cotter road, just south of the Mt. Ginini ski run (lat. 35° 32'S., long. 148° 46½'E., elevation 5350 ft). The central (and presumably most recently stabilized) portion of the scree carries a *Podocarpus* heath which

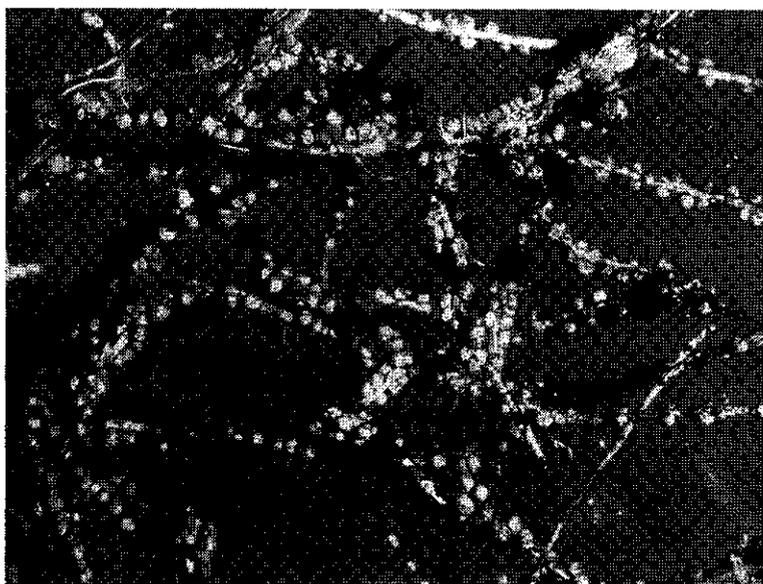


Fig. 1.—Nodulated roots of *P. lawrencei* from Mt. Ginini, A.C.T. $\times 2$.

extends towards the edges of the scree into a subalpine woodland (here reduced to scrub) of *Eucalyptus niphophila* Maiden & Blakely. The *Podocarpus* has colonized the scree from a few individuals which have spread by branch layering. In early May 1963, when the following examinations were made, nodules up to 1 mm in diameter were abundant on the smaller roots (Fig. 1).

Samples of nodulated roots were packed in ice and transported to the laboratory where tests with $^{15}\text{N}_2$ were initiated within about 2 hr from taking the samples. The surrounding soil (stones with some fines) was also sampled and analysed for nitrate and ammonia (Bremner and Shaw 1955) and for total nitrogen by Kjeldahl digestion.

$^{15}\text{N}_2$ Fixation Tests: The nodulated roots were washed free of soil and humus and blotted dry before being divided into 5-g samples and placed in 100-ml flasks which were closed with gas sampling attachments (Bergersen 1963). The flasks were

evacuated and filled with a gas mixture consisting of nitrogen (10%, 90.6 atoms % ^{15}N), oxygen (20%), and argon (70%), and then incubated for 4 hr at 23°C. At the end of this period gas samples were collected from the flasks and their composition analysed in an M86 mass spectrometer (Atlas-Werke, Bremen). The roots were then removed, ground in 3N HCl, and centrifuged. Both the deposit and the soluble material were digested and analysed for ^{15}N as previously described (Bergersen 1962).

Nodule Weights: Some nodule roots were weighed and then the nodules were removed and weighed, enabling calculation of the proportion of nodules: roots.

Nodule Structure: Portions of nodule roots were fixed in Flemming's solution overnight and after dehydration and embedding in paraffin wax microtome sections were prepared. These were stained in Heidenhain's haematoxylin or rose bengale-light green as used by Brenchley and Thornton (1925) to stain bacteria in plant tissue.

TABLE 1
FIXATION OF NITROGEN BY DETACHED NODULATED ROOTS
OF *PODOCARPUS LAWRENCEI*

Samples of nodule roots (5 g fresh wt.) bearing 1.9 g nodules were incubated 4 hr at 23°C in flasks containing 10% $^{15}\text{N}_2$ (90.6 atoms %), 20% oxygen, 70% argon

Fraction	Nitrogen in Fraction (mg)	Atoms % Excess ^{15}N in Fraction	Nitrogen Fixed* (μg)
Acid-soluble	0.70	0.078	0.60
	0.70	0.074	0.57
Acid-insoluble	8.35	0.002	n. s.
	7.65	0.002	n. s.

* Calculated from:

$$\left[\frac{\text{atoms \% excess } ^{15}\text{N}}{100} \times \frac{100}{90.6} \times \mu\text{g nitrogen in fraction} \right]$$

III. RESULTS

(a) Nitrogen Fixation by Nodulated Roots

The results of these tests are summarized in Table I from which it is seen that significant incorporation of molecular nitrogen occurred and that this newly fixed nitrogen was initially located in the acid-soluble portion of the tissues. The amount of nitrogen fixed, about 0.5 μg in 4 hr by 1.9 g (fresh wt.) nodules, is low compared with fixation by excised soybean nodules which would fix about 10 μg nitrogen per gram fresh weight under similar conditions (calculated from Bergersen 1963). Mass-spectrometric analysis of the gas in the flasks showed that the nodulated roots also evolved small amounts of hydrogen, the mass 2 peak of a sample at an inlet pressure of 6 mm Hg being 2.15 mV above background (grid leak resistance 10^{11} ohms).

(b) *Extent of Nodulation on the Roots*

Figure 1 shows the prolific nodulation of the roots. Nodules averaged 1 mm in diameter and 38% of the root weight consisted of nodules. No plants were found without nodulated roots which made it impossible to use root controls in the ^{15}N experiment. In many cases the roots did not penetrate the humus at all but hung free in spaces between stones.

(c) *Soil Nitrogen Status*

The soil in which the *Podocarpus* plants were growing was high in humus with finely weathered rock and stones of various sizes. The nitrogen analysis after removal of stones and root fragments was as follows: total nitrogen 0.43%; ammonia nitrogen, 9 p.p.m.; nitrate nitrogen, less than 1 p.p.m.

(d) *Microscopic Structure of the Nodules*

The nodules were spheroidal bodies borne on the ends of peg-like lateral branches of the root stele (Plate 2, Fig. 1). They were enclosed in a layer of intact cortical cells containing heavy tannin deposits. Beneath this outer layer were varying numbers of layers of degenerated tissue formed from the remains of successive nodule contents as these had been compressed between newly developing contents and the outer containing shell. Plate 2, Figure 2, shows new nodule contents forming from the cap of cells at the tip of the vascular branch and the previous degenerated contents about to be compressed to the periphery. In the course of expanding to fill the nodule, the cap of cells somehow becomes infected, a process not observed in these studies, and each cell is ultimately filled with non-septate mycelium convoluted to varying degrees (Plate 2, Fig. 3). The mycelium bore regularly placed red-staining (rose bengale) granules, which may have been nuclei, along its length, and it could be traced from cell to cell. The convoluted portions arose from lateral branches of the main mycelial threads. In no section were intracellular bacteria seen.

In the material examined, only about one nodule in eight had central tissue containing the symbiont. Other nodules were in various stages of the regenerative process (Plate 2, Fig. 2).

IV. DISCUSSION

Early experiments of Nobbe and Hiltner (1899) showed that healthy growth of *Podocarpus* was not possible in nitrogen-free sand in the absence of nodulation, and fixation of atmospheric nitrogen was therefore assumed. The results of the tests with $^{15}\text{N}_2$ reported here are the first proof of fixation by nodulated roots of any *Podocarpus* species. The description and drawings by Spratt (1912) of the anatomical features of various *Podocarpus* nodules closely resemble the *P. lawrencei* material used in the present study; however, Spratt and also McLuckie (1923) considered that the endophyte was identical with legume nodule bacteria. On the other hand, Nobbe and Hiltner (1899) considered that *Podocarpus* nodules contained a non-septate filamentous fungus. The nodules used in the present study clearly contained this type of filamentous endophyte.

The low fixation rates found for the nodules studied here were probably largely due to the small proportion containing well-developed endophyte. In addition, the

delay between collecting the material and initiating the $^{15}\text{N}_2$ tests would also tend to reduce nitrogen fixation. However, the observed rates of fixation are still low even after allowing for these factors, and may be related to the slow growth of this species of *Podocarpus*.

The observations that the newly fixed nitrogen is located in the acid-soluble fraction and that hydrogen is evolved represent two features in common with legume root nodules (Aprison, Magee, and Burris 1954; Hoch, Schneider, and Burris 1960), thus pointing to some degree of uniformity in symbiotic nitrogen fixation even when the details of nodule form are quite different. Similarly Bond (1961) has shown that some non-legume nodules respond to oxygen in much the same way as do legume nodules.

P. lawrencei is of considerable ecological significance as a stabilizing and pioneering species of exposed rocky situations. The ability to fix atmospheric nitrogen must convey considerable advantages to a pioneer species such as this, and although the measured fixation was low it is probably sufficient in such a long-lived species to account for the observed nitrogen levels of the soil.

V. REFERENCES

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ECOLOGICAL SIGNIFICANCE OF ROOT NODULES ON *PODOCARPUS*



Fig. 1.—*Podocarpus* heath (dark patches) colonizing a large scree on a steep slope of Mt. Loch, Vic.
Fig. 2.—A patch of *P. lawrencei* at the eastern edge of the Dargo High Plains, Vic., in which considerable invasion of herbaceous species has followed initial colonization of a stony site by *Podocarpus*.

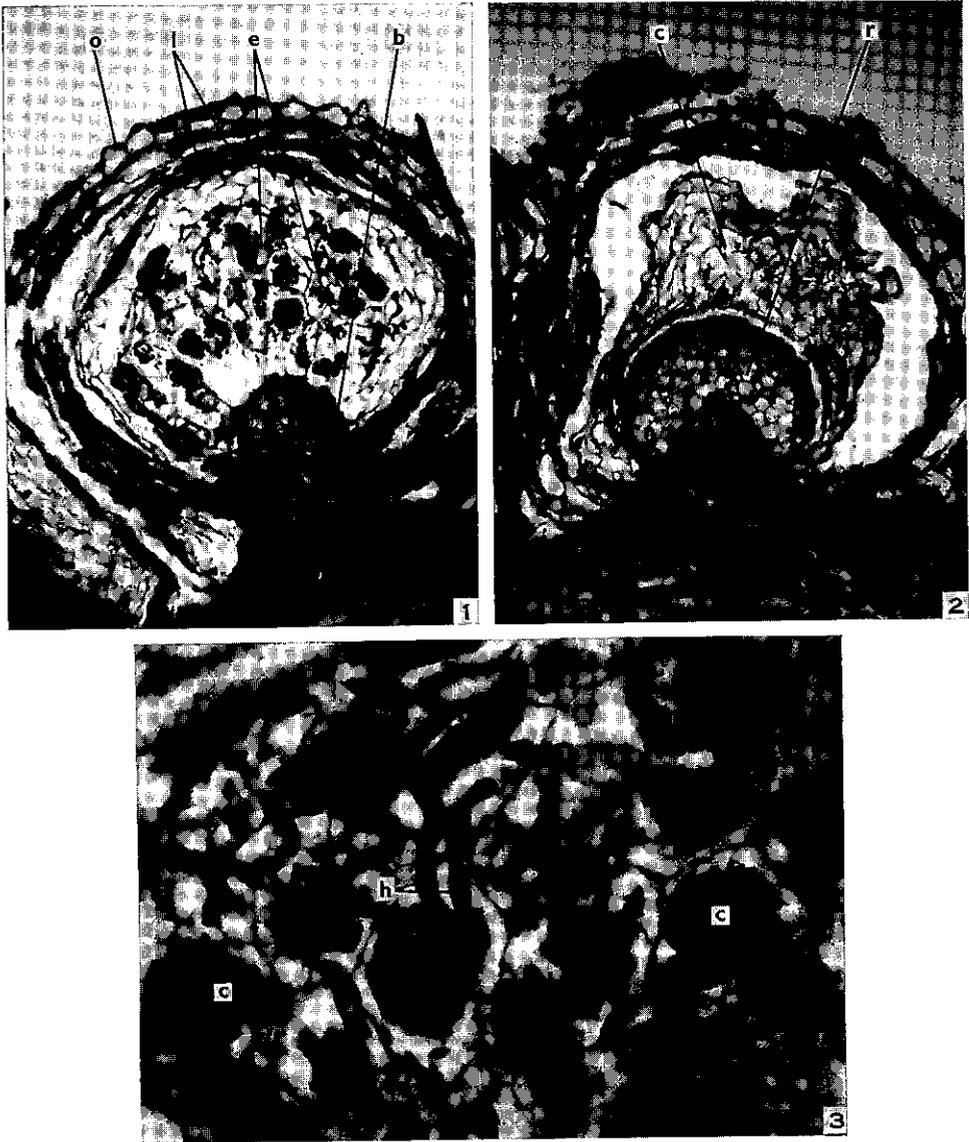
ECOLOGICAL SIGNIFICANCE OF ROOT NODULES ON *PODOCARPUS*

Fig. 1.—Section of a *P. lawrencei* nodule showing central cells containing endophyte (*e*), outer cell layer (*o*), layers of old compressed contents (*l*), vascular branch (*b*). $\times 65$.

Fig. 2.—Section of a *P. lawrencei* nodule showing regenerating contents (*r*) forming at the tip of a vascular branch, and in process of compressing the previous nodule contents (*c*) to the periphery of the nodule. $\times 65$.

Fig. 3.—Endophyte-filled central cells of a *P. lawrencei* nodule showing convoluted masses of mycelium (*c*) and hypha passing from cell to cell (*h*). $\times 260$.