

Podocarpus Root Nodules in Sterile Culture

THE morphology of the root nodules of *Podocarpus* has attracted more attention than that of any other non-leguminous plant, and the nodules have been regarded as a response to infection by bacteria¹⁻⁴ or by non-septate fungi⁵⁻¹⁰. The endophyte is considered not to be causal by recent workers^{11,12}, but the nodules are thought to be arrested lateral roots with the cortex renewed annually. Attempts by Baylis *et al.*¹¹ to maintain *Podocarpus* seedlings in sterile culture long enough to permit nodule development were unsuccessful, although nodules were formed in cultures which proved to be entirely free from fungi. We have investigated the production of root nodules in sterile culture.

Shoot cuttings from *Podocarpus lawrencei* Hook., after surface sterilization, were planted in pots of autoclaved sand and peat (equal parts) in completely sterile conditions. The structures (Fig. 2b) of the nodules produced on the adventitious roots which arose from the cuttings were the same as those which have been described before, although in contrast to nodules on plants grown in the field (Fig. 2a) no endophyte could be seen. Staining of smears and sections for bacteria gave negative results. Malt-'Marmite' agar plates inoculated with crushed surface-sterilized nodules remained free of micro-organisms on incubation for 3 weeks at 20° C, and there was no evidence of any endophyte in sections examined by electron microscopy.

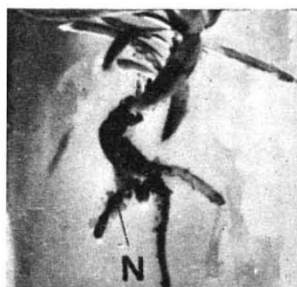


Fig. 1. Seedling (inside the culture tube) developed from excised embryo of *Podocarpus falcatus*. Note the root nodules (N). ($\times 0.5$)

When the nodules were collected, however, the medium in which cuttings had been rooted was no longer sterile, a result found before¹¹. Thus to determine whether or not nodulation is dependent on micro-organisms, excised embryos from full-size green seeds of *P. falcatus* R.Br. were grown *in vitro* on Raghavan and Torrey¹² tissue culture medium. When the chlorophyll had developed in the cotyledons, the embryos were transferred to a mineral agar medium. The seedlings were transferred every 4 weeks to fresh media. When 1 yr old, the roots of the seedlings nodulated (Fig. 1). The structure (Fig. 2c) of the nodules

was similar to that of field grown nodules except that no endophyte was detected in the cortical cells. Malt-'Marmite' agar plates incubated from the crushed nodules remained free of micro-organisms.

These observations suggest that unless nodulation is induced by some undetected endophyte transmitted by the embryo, nodules are a normal feature of the root system of *Podocarpus*, and that their formation is not induced by any microbiological factor. This does not exclude the possibility, suggested before¹¹, that such nodules on which the cortex is renewed annually represent an adaptation which allows the mycorrhizal symbiont to be retained after the long roots have shed their cortex.

The idea that nodules are arrested lateral roots^{11,12} is not supported by a comparison of the histological features of the young nodule with those of the young lateral root. Both arise endogenously but differ in their cellular configuration. The lateral root, before emergence, clearly shows a recognizable root type apical meristem with the usual open ended endodermis. The nodule, in contrast, shows, before emergence and later, a cell pattern unlike the apical meristem of any root and its new endodermis is not open-ended but over-arches the vascular system of the nodule from the beginning. Thus histologically the nodules do not seem to be modified arrested lateral roots but to be morphological features in their own right.

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Holographic Visualization of Plant Movement

HOLOGRAPHIC techniques have been applied to many fields with results which are adequately reported in the literature¹⁻³. This communication describes an application of the so-called "frozen" fringe method of holography⁴ to the visualization of plant movement or

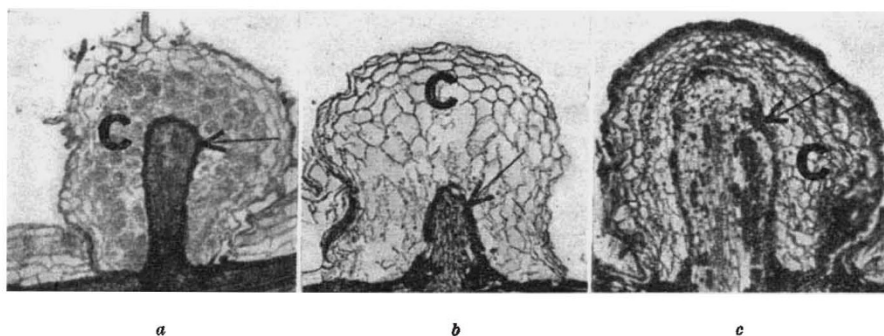


Fig. 2. Longitudinal section of root nodules of species of *Podocarpus*. Arrows indicate over-arching endodermis. C, Cortex. (a) *P. lawrencei*, field grown nodule, with fungal endophyte in the cortex. ($\times 65$.) (b) *P. lawrencei*, sterile nodule from adventitious roots of cutting; absence of endophyte from cortex. ($\times 65$.) (c) *P. falcatus*, root nodule from sterile seedling; absence of endophyte. Note the regeneration of nodule at the tip of the vascular branch underneath the endodermis. ($\times 80$.)