

Histogenesis in Roots of *Nothofagus solandri* var. *cliffortioides* (Hook. f.) Poole

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AN INDIGENOUS EVERGREEN TREE, *Nothofagus solandri* var. *cliffortioides*, forms forests which dominate mountainous regions of New Zealand. The character of the root system varies according to the degree of mycorrhizal infection (Arnold, 1960). Mycorrhizal roots are much branched and stunted by comparison with uninfected roots (Fig. 1). In cross-section mycorrhizal roots are seen to be enveloped by a mantle of hyphae which penetrate in the form of a Hartig net between the radially elongated epidermal cells (Fig. 2).

Maximum development of mycorrhizas is found where leaf-mold, moss, and humus are abundant on the forest floor, and the highest incidence of fleshy non-mycorrhizal roots is found in boggy soil, or when the tree is grown in cultivation in heavy garden loams.

The present investigation was undertaken to determine whether or not the apical organization of *Nothofagus* mycorrhizas differed from that of uninfected roots, and to compare the histogenetic pattern of *Nothofagus* roots with that of the European beech *Fagus sylvatica*.

I am indebted to the University Grants Committee for a research grant in aid of this work.

METHODS AND MATERIALS

Uninfected roots and mycorrhizas were fixed at fortnightly intervals throughout the year in the following solutions: tannin fixative (Johansen, 1940); chromium sulphate fixative (Johansen, 1940); cytoplasmic fixative (Marengo, 1952); formo-acetic alcohol (Johansen, 1940); Bouin's fixative (Baker, 1950); acetic alcohol (Darlington and La Cour, 1947).

Dehydration was carried out in a closely graded series of ethyl alcohol; clearing was done

in alcohol-benzene mixtures; the specimens were embedded in paraffin; and serial sections were cut at 10 μ .

The following stains were employed: anilin blue + safranin (Johansen, 1940); methyl violet + erythrosin (Johansen, 1940); methyl violet + eosin (Johansen, 1940); Crystal violet, chromic method (Darlington and La Cour, 1947); Feulgen technique for slides (Darlington and La Cour, 1947); Chlorazol black E + Aceto carmine (Nebel, 1940); Chlorazol black E (Cannon, 1941); Iron-alum ammonium sulphide (Wigglesworth, 1952).

This wide range of fixatives and stains was employed in an attempt to determine whether the hypodermis of mycorrhizas contains living substance or whether it is in fact relatively empty of protoplasmic content.

OBSERVATIONS

In uninfected roots of *Nothofagus solandri* var. *cliffortioides* the meristematic regions which give rise to epidermis, cortex, stele, and rootcap are readily distinguishable (Fig. 3) and their arrangement is similar to that reported for *Fagus sylvatica* (Clowes, 1961). While it is convenient to refer to these tracts of meristematic cells as dermatogen, periblem, plerome, and calyptragen, respectively, I have been unable to conclude whether or not they represent entirely discrete histogens in the original sense of the word.

Despite the very considerable histological modification of root structures in mycorrhizas, including a reduction in the size of the promeristem, it is possible to identify in them a dermatogen, periblem, plerome, and vestigial calyptragen in much the same relationship as in uninfected roots. The maturation of derivatives of the promeristem of mycorrhizas is greatly accelerated, and the derived cells are often reduced in number.

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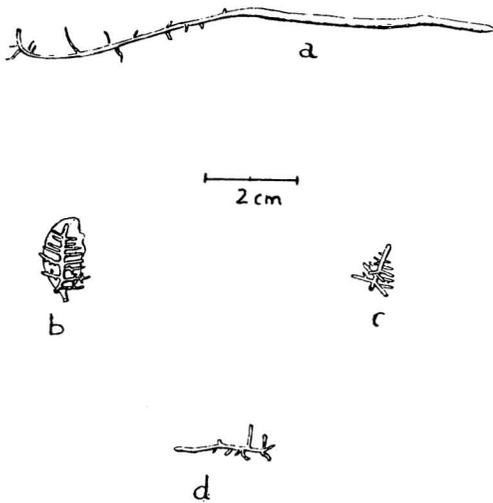


FIG. 1. *a*, An uninfected root, *b*, *c*, and *d*, mycorrhizas. All life size.

The rootcap of mycorrhizas is usually reduced to a single layer of cells confined on the outside by the fungal mantle which sheathes the whole apex and penetrates the later-formed epidermal cells.

Differentiation of the epidermis of mycorrhizas is initiated very much closer to the promeristem than is the case in uninfected roots, and is characterized by crowded divisions in the radial plane, the walls being as close together as 2.5μ in fine mycorrhizas, and slanting in a forward direction (Fig. 4). Clear vacuoles appear in the developing epidermal cells but are later replaced by an opaque mass which fills the cells completely. This opaque mass absorbed all of the stains to which the sections were subjected.

In large mycorrhizas the cortex may contain two or three layers of cells, but in finer mycorrhizas it may be reduced to a single layer or even replaced by the hypodermis (Fig. 5). Vacuolation of precursor cells of the cortex takes place earlier in mycorrhizas than in non-mycorrhizal roots. The vacuoles are at first clear, as is best shown by fixation in Marengo's cytoplasmic fixative (Marengo, 1952). Later the cortical cells of mycorrhizas become filled with an opaque homogeneous material which absorbs stains avidly. Millon's test for protein in mature cortical cells was positive and strong.

The staining reactions of the endodermis are similar to those of the cortex and epidermis but more intense. Vacuolation in the endodermis of mycorrhizas is precocious, as it is in the other tissues, and there is an early deposition of tannin in the endodermis.

Maturation of stelar tissues occurs much closer to the promeristem in mycorrhizas than in mycorrhiza-free roots.

All the foregoing histological characteristics of mycorrhizas of *N. solandri* var. *cliffortioides* are closely similar to those of mycorrhizas of *F. sylvatica* as reported by Clowes (1951), but one outstanding feature of *Nothofagus* mycorrhizas which has not been reported for *Fagus* is the peculiar histogenetic pattern of the hypodermis.

Vacuolation of the precursor cells of the hypodermis in mycorrhizas of *Nothofagus* takes place well in advance of vacuolation of the stelar cells.

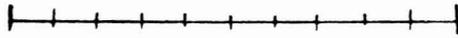
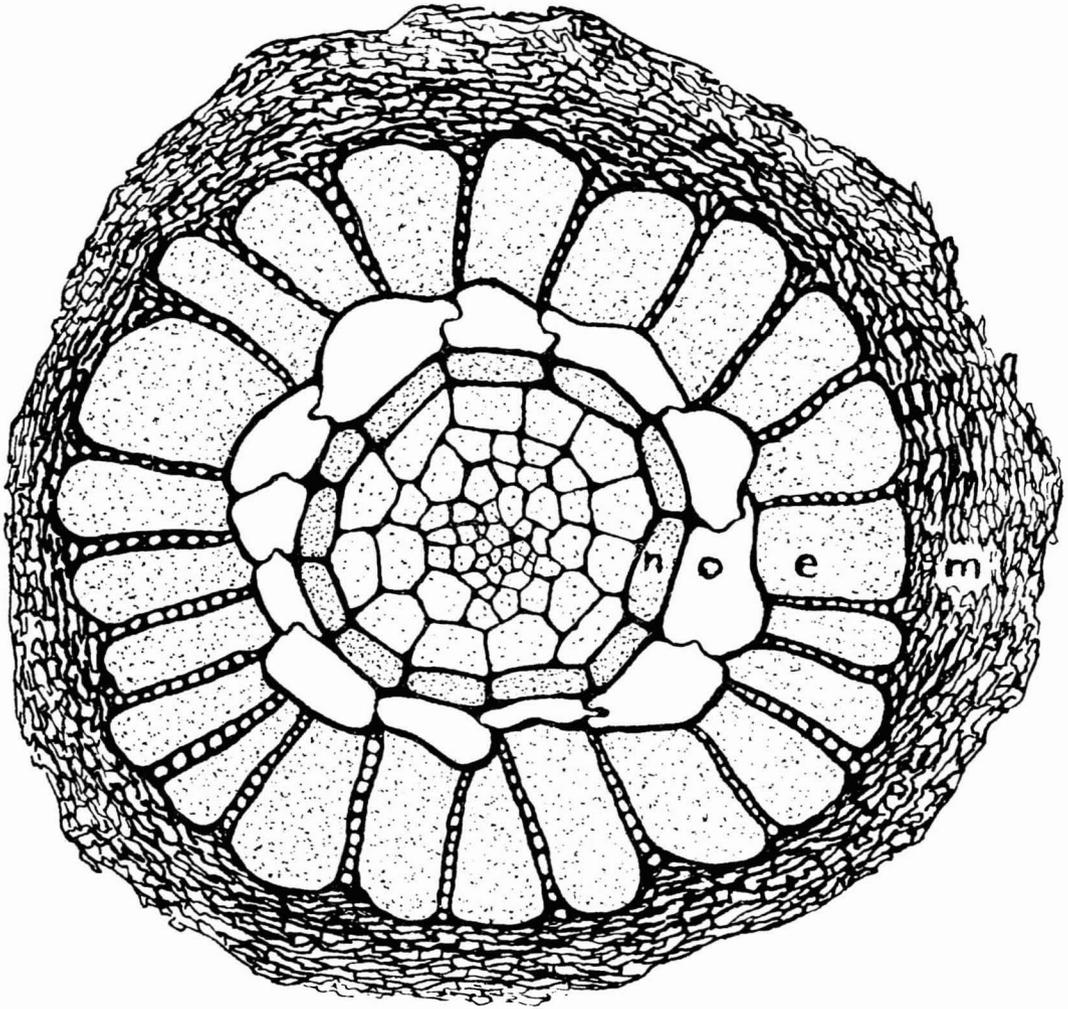
The first formed cells of the hypodermis are conspicuous extremely close to the promeristem. The cytoplasm shrinks against the walls to a lens-shaped blob in which the nucleus is embedded, and finally the entire protoplasmic content disappears. In longitudinal section an irregular line of clear cells can be seen leading back to a definite file of cells with thin buckled walls (Fig. 4).

Despite the use of a wide range of fixatives and stains, the mature hypodermis appeared quite devoid of protoplasmic content.

Hyphae were not found at any stage to enter this clear-layered hypodermis, which appears to be a barrier to further fungal invasion of the root.

DISCUSSION

Notwithstanding the undecided question of whether the meristematic layers which give rise to epidermis, cortex, stele, and rootcap in *N. solandri* var. *cliffortioides* are discrete histogens in the original sense of the term (Clowes, 1961), it is clear enough that in roots of *Fagus* and *Nothofagus* there exists the same fundamental type of organization of the apical meristem, which is consonant with the phylogenetic relationship of the two genera.



100 μ

FIG. 2. Cross section of a mycorrhiza. *n*, Endodermis; *o*, clear hypodermal cells; *e*, epidermis; *m*, mantle.

Furthermore, it is evident that mycorrhizal infection does not alter the basic disposition of dermatogen, periblem, plerome, and calyptragen in *Nothofagus* roots. The promeristem of mycorrhizas is reduced but not damaged or incapacitated. Not infrequently the apical meristem regains sufficient vigor to break through the fungal mantle and give rise to non-mycorrhizal roots, indicating a dynamic balance of growth of root and fungus.

It has been suggested earlier (Arnold, 1959, 1960) that the histological modifications found in *Nothofagus* mycorrhizas are consistent with the hypothesis that the mycorrhizal fungus exudes auxins or auxin-like substances which are a dominant factor in the morphogenesis and growth of *Nothofagus* roots. No doubt there are other accompanying effects of the presence of the fungal mantle over the tissues of the infected roots.

In the very thorough study of mycorrhizas of *Fagus sylvatica* made by Clowes (1951), no mention is made of the structure and differentiation of the hypodermis. Morrison (1956), who

studied the mycorrhizal condition in *Nothofagus menziesii* (Hook. f.) Oerst., noted without further comment that in uninfected roots "the hypodermal layer consists of thin walled clear cells while the inner layer of the cortex consists of thick walled cells." No mention was made of the presence or otherwise of a hypodermis in mycorrhizas.

In uninfected roots of *Nothofagus solandri* var. *cliffortioides* a hypodermis of clear cells has been seen in transverse sections of some specimens but not in others, and it is presumed that Morrison's observations were based on transverse sections probably somewhat remote from the apex. This presumption is made from observations on longitudinal sections of uninfected roots of *N. solandri* var. *cliffortioides* in which no instances of a clear hypodermis were found even as far back as 1.5 cm from the promeristem. Vacuolation in the hypodermis of non-mycorrhizal roots of *N. solandri* var. *cliffortioides* is very much more gradual than in mycorrhizas.

On the basis of the present observations on the histology and developmental pattern of the

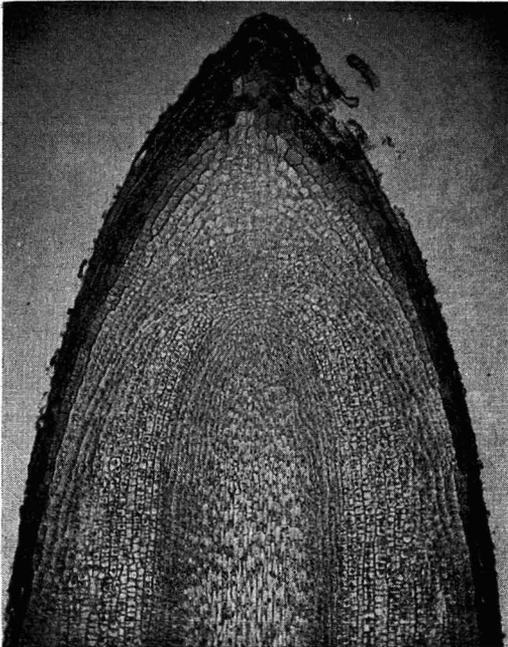


FIG. 3. Longitudinal section of uninfected root, $\times 80$. There is no premature differentiation of tissues as shown in Figure 4.

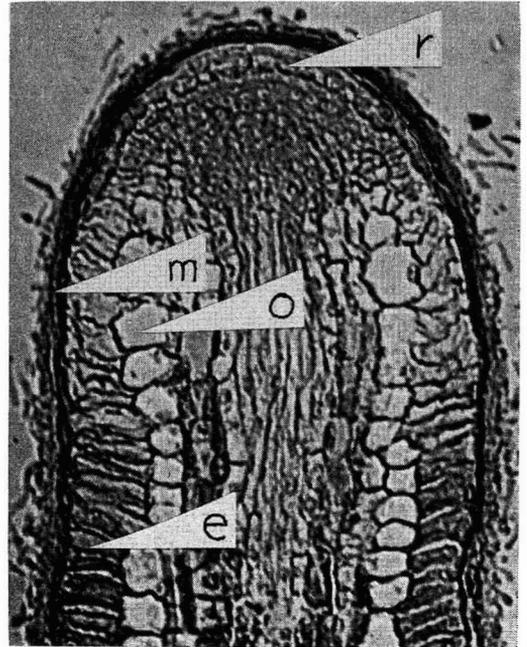


FIG. 4. Longitudinal section of a fine mycorrhiza, $\times 120$. *m*, Mantle; *e*, epidermis; *o*, hypodermis; *r*, rootcap.

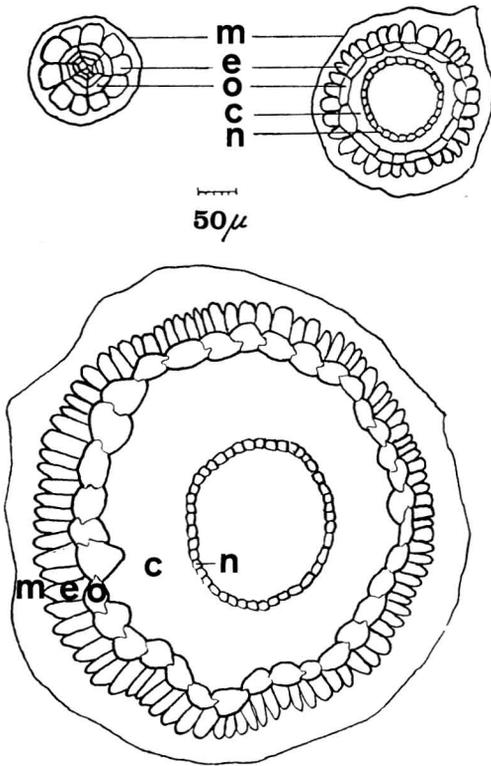


FIG. 5. Outline sketches of transverse sections of three mycorrhizas showing range in reduction of diameter and cell number. *m*, Mantle; *e*, epidermis; *o*, hypodermis; *c*, cortex; *n*, endodermis.

hypodermis of mycorrhizas of *N. solandri* var. *cliffortioides* it is suggested that the hypodermis plays an important role in preventing the further penetration of the fungus into the inner tissues of the root.

SUMMARY

The root apical meristem of *Nothofagus solandri* var. *cliffortioides* (Hook. f.) Poole is similar to that of *Fagus sylvatica*.

In both genera the organization of meristematic cells which give rise to epidermis, cortex, stele, and rootcap is basically unaltered by mycorrhizal infection.

However, the subsequent maturation of tissues in mycorrhizas and non-mycorrhizal roots differs considerably.

A noteworthy feature of mycorrhizas of *N. solandri* var. *cliffortioides* is the lack of protoplasmic content of the hypodermis, and its striking mode of differentiation from the promeristem.

It is suggested that the hypodermis plays an important role in excluding mycorrhizal fungi from further penetration of *Nothofagus* roots.

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