ECTOMYCORRHIZAS OF SOME AUSTRALIAN PLANTS

by

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DECLARATION

I hereby declare that this thesis contains no material which has been accepted for award of any other degree or diploma in any university. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except when due reference is made in the text.

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HARRY H. KOPE

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SUMMARY

The morphology and development of ectomycorrhizas formed by Peziza whitei, Labyrinthomyces sp. (Ascomycetes) and Laccaria ohiensis (Basidiomycete) on a range of hosts including Eucalyptus maculata (tree), Pultenaea obovata, Gompholobium latifolium (shrubs) and Poranthera microphylla, Angianthus tomentosus, Waitzia citrina and Stylidium graminifolium (herbs) have been studied in pure culture synthesis in a soil mix low in available phosphate.

The associations formed varied from mycorrhizas with well defined sheaths enclosing root apices on E. maculata, P. obovata and G. latifolium to less defined ones on the herbs where associations varied from partial sheathing of roots to discrete patches of fungal sheath to no sheath at all in the association A. tomentosus with P. whitei. A Hartig net was formed in most associations though Laccaria ohiensis formed an irregular one with E. maculata and none with P. microphylla, A. tomentosus and W. citrina. The results showed that the form of a mycorrhiza depended on both partners, not fungus or higher plant alone.

Compatability of some plant-fungus combinations was indicated by study of the development of mycorrhizas over time. A high percentage of roots of all orders became mycorrhizal and the percentage remained high as root numbers and orders increased.

All plant-fungus combinations, except A. tomentosus with L. ohiensis, showed a significant growth response compared with uninoculated plants which indicated mycorrhizal effectiveness, irrespective of mycorrhizal form, in the growth conditions employed.

INTRODUCTION

Certain fungi are known to form anatomical associations with the roots of higher plants. A German botanist, Frank, in 1885 called the composite organ of fungus and root, mycorrhiza. Frank described the ectomycorrhiza of beech and pine and demonstrated that mycorrhizal seedlings grew faster than uninfected ones (see Harley,1978). Experimental work by Hatch (1937) on Pinus sp. showed that mycorrhizal seedlings were larger and contained greater quantities of macronutrients than non-mycorrhizal controls. This increase in plant size was attributed to an increase of the root surface absorption area by fungal hyphae acting as fine "root hairs".

Much of the descriptive work on ectomycorrhizas has been with northern hemisphere plants such as members of the Fagaceae, Pinaceae and Betulaceae. This work has been summerized by Harley (1959,1969). Commonly occurring ectomycorrhizas are reported as having a complete fungal ensheathing of lateral roots or rootlets, with intercellular penetration by hyphae of the epidermal, and in some cases cortical cells, in the formation of a Hartig net. The colour of the fungal mantle (Marks, 1965), mantle ornamentation (Dominik, 1959), radially elongated epidermal cells and changes in root branching and morphology are characteristics often listed in defining a classical ectomycorrhiza. Harley and Smith (1983) noted that considerable variation in the formation of a fungal sheath, the development of a Hartig net and the extent of intercellular penetration, could be explained as stages of development or infection of different ages and orders of roots.

Samuel (1926) found ectomycorrhiza on Eucalyptus and suggested that many more Australian plants might be mycorrhizal. Other than the detailed descriptions of ectomycorrhizas of Eucalyptus species (Chilvers and Pryor, 1965; Anderson, 1966, 1967; Chilvers, 1968; Tandy, 1975; Ashton, 1976; Mullette, 1976), very little has been described of the morphology of ectomycorrhizas on plants of other Australian families. Chilvers and Pryor (1965) described the structure of ectomycorrhizas on Eucalyptus species and found that many ectomycorrhizas shared similar properties with those plants of northern hemisphere genera. Ashton (1976), using pure culture isolates of basidiomycetes found in association with Eucalyptus regnans F. Meull., recorded classically described ectomycorrhizas from inoculated seedlings.

Warcup (1980 a) and Warcup and McGee (1983) noted many other families of Australian indigenious plants which formed ectomycorrhizal associations as well as endomycorrhizal (VA) ones. The plants forming ectomycorrhizal associations ranged from forest trees to shrubs to small non-woody herbs. Warcup (1980 a) noted that, with some of these associations there was considerable variation in the amount of sheath formed, from partial to a complete ensheathing of roots and in the presence or absence of a Hartig net. As marked growth responses of infected seedlings were recorded over the types of fungal associations formed he considered them all to be cases of ectomycorrhizas.

The work described here has stemmed from these observations by Warcup (1980 a) that some ectomycorrhizal associations on Australian herbs and shrubs have partial sheaths with or without a Hartig net. While he showed that plants with such partial associations gave a host growth response he did not examine the morphology of the associations in detail. The morphology of ectomycorrhizas formed by several different

fungi on some Australian herbs and shrubs and on Eucalyptus have been compared. Growth responses of some host plants associated with the fungi are also reported.

MATERIALS AND METHODS

The Fungi

The fungi used are recorded in Table 1. All originated from sporocarps and those cultures provided by Dr. J.H. Warcup had been shown to be mycorrhizal with Eucalyptus (Warcup, personal communication). In isolations from sporocarps, the sporocarp was split with a sterile scalpel and pulled apart. Several small portions (2 - 4 mm) were cut away from the exposed interior and laid on a nutrient agar. Streptomycin sulphate ($100\mu gml^{-1}$) was added to the agar medium before plates were poured to minimize growth of bacteria. The plates were inverted and placed in a 20°C incubator and checked daily for growth of the fungus. Small disks (+_3 mm), cut from the edge of actively growing colonies, were subcultured on to a maintenance medium with addded streptomycin. A subsequent subculture from these plates on to the same medium without the antibiotic was done to determine if the colony was free of bacteria. The composition of the maintenance media, NDY/6 (Warcup, 1955), 2% Malt Extract media and Melin-Norkans modified media (Marx, 1969), are given in Appendix 1. Stock cultures were maintained on slopes in Kimble tubes. For pure culture synthesis of mycorrhizas, fungi were subcultured on to NDY/6 plates.

Seed

Table 2 lists by family the plants used, the source of seed and their approximate germination times.

Seeds were sown on moistened, autoclaved sand, in plastic containers and covered with a glass petri dish to retain moisture. Before planting, seed of Gompholobium latifolium and Pultenaea obovata, were surface sterilized for 10 minutes in a 20.0% solution of 'Milton', a 1.0% commercial preparation of sodium hypochlorite, rinsed 3 times in

Table 1

Identity, host, locality and date of isolation of fungi used in mycorrhizal studies

Fungus	Associated host and area	Year of isolation
Amanita grisea Mass. et Rodway (sensu Cleland, 1976)	Eucalyptus maculata, Kuitpo, S.A.	7/76, J.H.Warcup
Amanita muscaria (Fries) Hooker	Pinus radiata, Delamere, S.A.	4/83, H.Kope
Labyrinthomyces sp.	Acacia, Adelaide, S.A.	8/81, J.H.Warcup
Laccaria ohiensis (Mont.) Singer	Pinus radiata, Kuitpo, S.A.	5/73, J.H.Warcup
Peziza whitei (Gilkey) Trappe	Eucalyptus obliqua L'Her., Ironbank,S.A.	5/73, J.H.Warcup
Rhizopogen luteolus Fr. and Nordh.	Pinus radiata, Williamstown, S.A.	8/82, H.Kope
Suillus luteus (L. ex Fr.) Gray	Pinus radiata, Williamstown, S.A.	8/82, H.Kope

Table 2

Host plants, their form, germination time and source, used in mycorrhizal studies

Host and plant form		Germination (days)	Source
Compositae *Angianthus tomentosus* Wendl.	annual herb	12–18	Donggali Conservation Park, S.A.
Waitzia citrina (Benth.) Steetz	annual herb	8–12	Nindethana Seed Service, Naririkup, W.A.
Euphorbiaceae *Poranthera microphylla* Brongn.	annual herb	8–12	Grampians, Vic., J.H.Warcup
Stylidiaceae *Stylidium graminifolium* Sw.ex Willd.	perennial herb	10–14	South Hobart, Tas., J.H.Warcup
Leguminosae *Gompholobium latifolium* Sm.	shrub	8–12	National Botanic Gardens, Canberra, A.C.T.
Pultenaea obovata Benth.	shrub	8–12	National Botanic Gardens, Canberra, A.C.T.
Myrtaceae *Eucalyptus maculata* Hook.	tree	10–14	Woods and Forest Dept., Adelaide, S.A.
Pinaceae *Pinus radiata* D.Don	tree	14–16	Woods and Forest Dept., Adelaide, S.A.

distilled water and nicked with a scalpel. Seed of Pinus radiata, was stratified at 3 - 5°C for 30 days, surface sterilized in a 0.5% mercuric chloride solution, rinsed twice in sterile distilled water and germinated on 2% Malt agar. The majority of seeds germinated in the time spans indicated and were transplanted and inoculated, preferably when the primary root was less than 2 cm long.

Soil Mix

A potting media of 9:1 (V/V) sand to soil was used. The soil was collected from Kuitpo forest, South Australia (Warcup, 1980 b). Soil was passed through a 1 cm sieve to remove larger roots and stones and then through a 3 mm sieve to break down particle size. Sand and soil, separately, were spread in shallow trays (72 x 32 x 4 cm) and autoclaved for 1 hour at 121°C. Nutrient status and pH (Adelaide and Wallaroo Fertilizers Ltd., Adelaide, South Australia) of Kuitpo soil and of the sand:soil mixture, after autoclaving, are recorded in Table 3. The low nutrient status of the sand:soil mixture suggests that plants would make little growth unless they became mycorrhizal or were supplied with additional nutrients. The mildly acidic value of 6.1 does not suggest a constraint to plant growth and the value falls within the range (3.0 - 7.0) of pH optima of ectomycorrhizal fungi (Harley and Smith, 1983). Soils or growth media are described as saline if they contain excess soluble salts. Excess soluble salts in general, and sodium in particular, are undesirable for several reasons and if present, may limit plant growth (Flegman and George, 1975). Comments given for conductivity, chloride and sodium indicate no salinity problems in the soil and sand:soil mixture used. All growth experiments were conducted in a controlled environment growth room with a 12 hour day with a day temperature of 24° and 18° during darkness.

Table 3

Nutrient compostion of 9:1 mixture and Kuitpo soil after autoclaving

	9:1 mixture	Comment	Kuitpo soil	Comment
Organic Carbon %	0.40	very low	2.10	moderately high
Nitrate Nitrogen mg/kg	1.5	very low	5.0	very low
Phosphorus (Bicarb) mg/kg	7.0	very low	5.0	very low
Potassium mg/kg	19.5	very low	124.8	low
Sodium mg/kg	43.7	low	92.0	low
Chloride mg/kg	80.0	low	140.0	low
Conductivity Mhos/cm	0.07	low	0.15	low
pH (1:5 water)	6.1		4.7	

A light intensity, from a bank of 15 Phillips TFL 65/80W 33RS white tubes, of 245 microeinsteins m^{-2} sec^{-1} was measured at plant height.

Inoculation

Seedlings were transplanted from germination containers into tins of sand:soil mixture and inoculated with an appropriate fungus. Tins (90 mm diameter x 110 mm deep) were lined with plastic bags, filled with 520 grams of sand:soil mixture, and moistened to 15% Water Holding Capacity (WHC) with 40 grams of distilled water. Inoculation of the seedlings, with either a sterile agar plug or an agar plug of an ectomycorrhizal fungus (5 mm^3 maximum), was by laying the agar in contact with the tap root. Seedlings were watered to weight every third or fourth day.

Harvesting

For observations of morphology and the anatomy of mycorrhizas, plants were harvested at 4, 6 and 8 weeks after inoculation. Plants being observed for the determination of a growth response and for mycorrhiza formed on different orders of roots, were harvested at 6, 10 and 14 weeks. At harvest all tins were flooded with water to loosen the sand:soil mixture and the contents gently poured on a coarse sieve (4 mm). Any adhering soil was loosened and removed by bathing the whole root system in a large (15 cm diameter) water filled petri dish. Examinations were made under a stereomicroscope and compound light microscope in determining areas of fungal colonization and the morphology and anatomy of the mycorrhiza. Roots were stained in Toluidine Blue O (Feder & O'Brien, 1968). Toluidine Blue O stained the cells walls of the fungus and the epidermal cell walls of the roots. This accentuated hyaline and thin walled fungi against the epidermal cells.

Terminology and definitions employed

The terms employed to describe the morphology of mycorrhizas are defined below. Variations in sheath and Hartig net of the associations observed, prompted the need to draw up definitions considering the different aspects. Terminology previously used has been employed in the decription of the fungal tissues.

A classical ectomycorrhiza is described as an absorbing organ of a fungal-ensheathed-root or rootlet with the growth of hyphae between epidermal and sometimes cortical cells in the formation of a Hartig net.

Types of fungal sheath encountered in these studies are listed as Complete, Patches or Partial and are defined below;

Complete - the formation of a fungal sheath which encloses the mycorrhizal organ, including the root apex, in a layer of compact tissue. A sheath may vary greatly in thickness.

Patches - fungal colonization of single epidermal cells or small areas of no more than 10 epidermal cells, on a length of root of any order and separated by areas of uncolonized cells is referred to as patches of fungal sheath.

Partial - sheath formation of less than 15 mm, but usually 5-10 mm in length on a root of any order is referred to as a partial sheath.

Root apices were not found to be ensheathed in either patches or partial sheathing.

Fungal tissue in the mantle or sheath is described using the terminology of Chilvers (1968);

Prosenchyma is a moderately compact tissue in which the hyphal elements can be distinguished clearly, and interhyphal spaces may be relatively large.

Felt prosenchyma consists of sparingly branched hyphae with "long

cells" generally similar in appearance to individual hyphae of the same fungus growing in the soil.

Net prosenchyma consists of "shorter-celled" hyphae branching frequently at wide angles.

Synenchyma is any thoroughly compacted tissue with little obvious interhyphal space, in which the hyphal basis is difficult or impossible to discern.

Irregular Synenchyma usually consists of wide hyphae subdivided into short "cells" and is probably frequently branched, but the degree of branching and to a large extent the identity of the constituent hyphae are impossible to resolve owing to dense compaction. The overall appearance is reminiscent of a"jig-saw" puzzle.

Regular synenchyma consists of approximately isodiametric cells with fairly straight walls.

Hartig net

Fungal penetration of epidermal cells and the separation of the cells with intercellular growth up to the next layer of cells, is termed a Hartig net.

Fungus present only in the cell junctions or compacted in the depressions between epidermal cells without coming in contact with next layer of cells, is not considered to constitute a Hartig net.

Periepidermal and Paraepidermal penetration of hyphae in the formation of a Hartig net follows Godbout and Fortin (1983);

Periepidermal - the Hartig net has completely encircled the epidermal cells.

Paraepidermal - the fungus did not penetrate beneath the epidermal cells, so that these cells remain in direct contact with the cortical cells.

MORPHOLOGICAL STUDIES OF MYCORRHIZAS

Often ectomycorrhizas can be visually discerned from uninfected roots by morphological and anatomical characteristics. The morphology and anatomy of ectomycorrhizas have been described by many workers (Clowes, 1951; Robertson, 1954; Harley 1959; Marks and Foster, 1973; Ashton, 1976; Harley and Smith, 1983) from hosts which support very obvious examples.

Proliferation of root tips, either the dichotomous branching of Pinus ectomycorrhizas (Wilcox, 1963, 1968) or the pyramidal root branching of ectomycorrhizas on Fagus (Clowes, 1951) and Eucalyptus regnans (Ashton, 1976) are microscopically visual characteristics of many ectomycorrhizas. The swollen appearance of root apices and a difference in their colour can also be used to quickly screen for the presence of "classical" ectomycorrhizas.

The fungal sheath is recognized as one of the most distinct morphological features of an ectomycorrhiza (Harley and Smith, 1983). Classification of ectomycorrhizas by morphological type has incorporated features of mantle structure and hyphae (Dominik, 1959). Types of fungal tissue, mantle ornamentation and the presence or absence of rhizomorphs have also been used as taxonomic criteria (Dominik, 1959; Chilvers, 1968).

Fungal tissue of the sheath has been shown to be similar to the tissue in fungal fructifications (Marks and Foster, 1973; Harley and Smith, 1983). Mycorrhizas formed with basidiomycetous fungi show dolipore septa or clamp connexions in the mantle and Hartig net (Foster and Marks, 1966, 1967; Chilvers, 1968; Hofsten, 1969; Duddridge and

Read, 1982; Godbout and Fortin, 1983).

In transverse and longitudinal sections Hartig net formation has been shown to vary from penetration between the first epidermal cells to intercellular penetration of cortical cells up to the endodermis (Harley, 1959). Transverse sections of some ectomycorrhizas of Alnus show no intercellular penetration or only an irregular Hartig net is formed (Godbout and Fortin 1982).

The process of formation of an ectomycorrhiza has been suggested to follow either one or the other of two courses. Nylund and Unestam (1982) with Picea abies (L.) Kaist. in vitro, described the infection process to form a Hartig net first and at a later stage for the mantle to form. Robertson (1954) also described infection in this sequence in Pinus sylvestris L. Chilvers and Gust (1982 a,b), however, working with Eucalyptus st-johnii T. Bak. considered that the formation of the fungal mantle preceded Hartig net formation.

Before studying the anatomy and development of mycorrhizas on the herbs, some comparative studies were made of the mycorrhiza produced by various test fungi on woody hosts, mostly Eucalyptus and occasionally Pinus (Appendix II). Two pots of each plant-fungus combination were prepared as described previously. After harvest, transverse and longitudinal sections, 25 - 40 µm thick, of mycorrhizal roots were made using a freezing microtome and examined microscopically.

ECTOMYCORRHIZAS OF EUCALYTUS MACULATA

Ectomycorrhizas of Eucalyptus are, in form and function, similar to those of the more intensively studied northern hemisphere genera such as

beech and oak (Chilvers and Pryor, 1965). The fungi used in this study were found in Eucalypt woodlands or where Eucalypts had been replaced by exotic pines. This prompted examination of their mycorrhizal associations with Eucalyptus maculata as reference associations for comparison with the mycorrhizas formed on the herbs and shrubs which are the main subject of the study.

Eucalyptus maculata and Labyrinthomyces sp.

The mycorrhizas were white in colour under reflected light. Although the root system was relatively large only a small proportion was colonized by the fungus. Non-mycorrhizal higher order roots were covered by abundant root hairs ~~but hairs~~ were not seen on mycorrhizal roots.

The fungal sheath was smooth and composed of a moderately compact felt prosenchyma tissue. No mantle ornamentation or loose hyphae were seen. Transverse sections showed the mantle to be 10 - 15 µm thick (Fig. 1). Paraepidermal intercellular penetration formed the Hartig net. No swollen or radially elongate epidermal cells were seen.

Eucalyptus maculata and Peziza whitei

Mycorrhizas were a light brown colour becoming dark grey when older. Mycorrhizas occurred over the whole root system. Some uninfected second order roots gave rise to pyramidal mycorrhizal branches of higher order roots. The higher order roots were found to be completely ensheathed up to the point of branching. Root hairs were present on all roots that were not colonized.

A tightly bound compact irregular synenchyma tissue formed the fungal sheath. Transverse sections of mycorrhizal roots (Fig. 2)

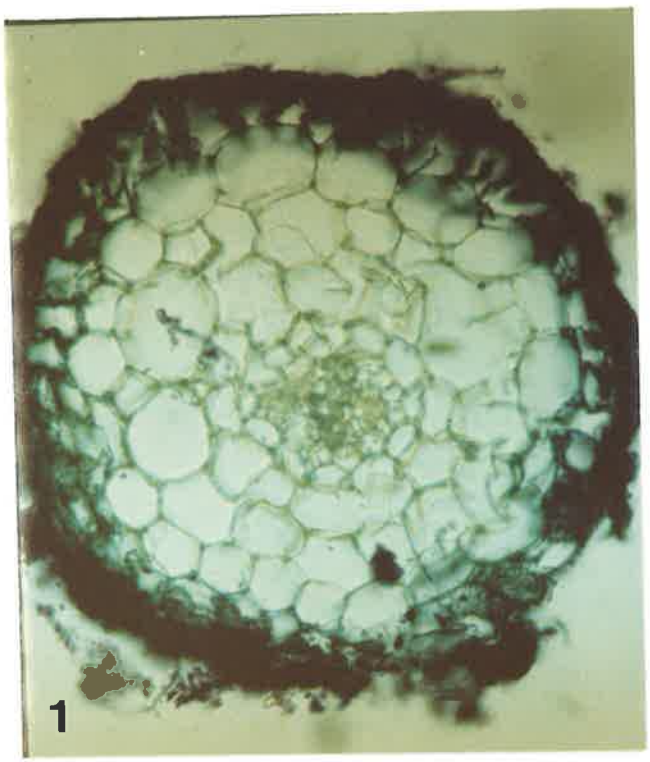
Transverse sections of *Eucalyptus maculata* ectomycorrhizas

Fig. 1 *Eucalyptus maculata* + *Labyrinthomyces* sp. x250

Fig. 2 *Eucalyptus maculata* + *Peziza whitei*. x250

Fig. 3 *Eucalyptus maculata* + *Amanita grisea*. x250

Fig. 4 *Eucalyptus maculata* + *Laccaria ohiensis*. x250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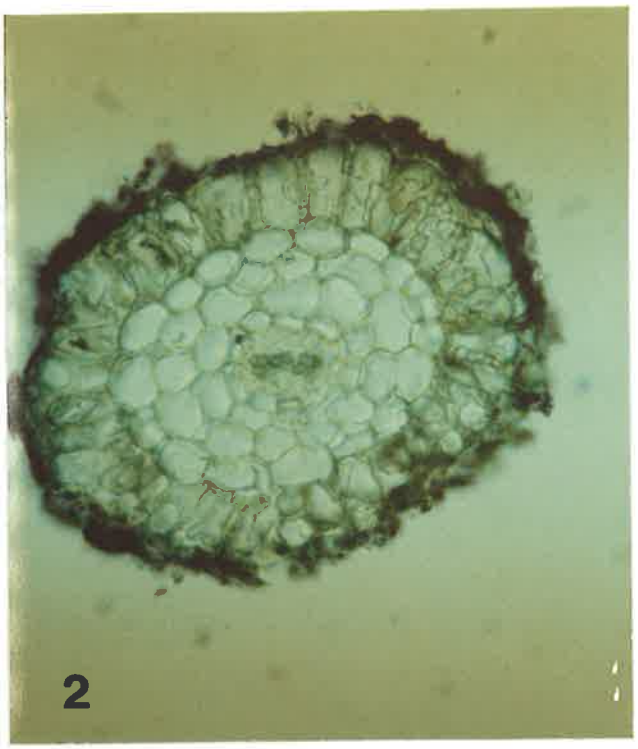
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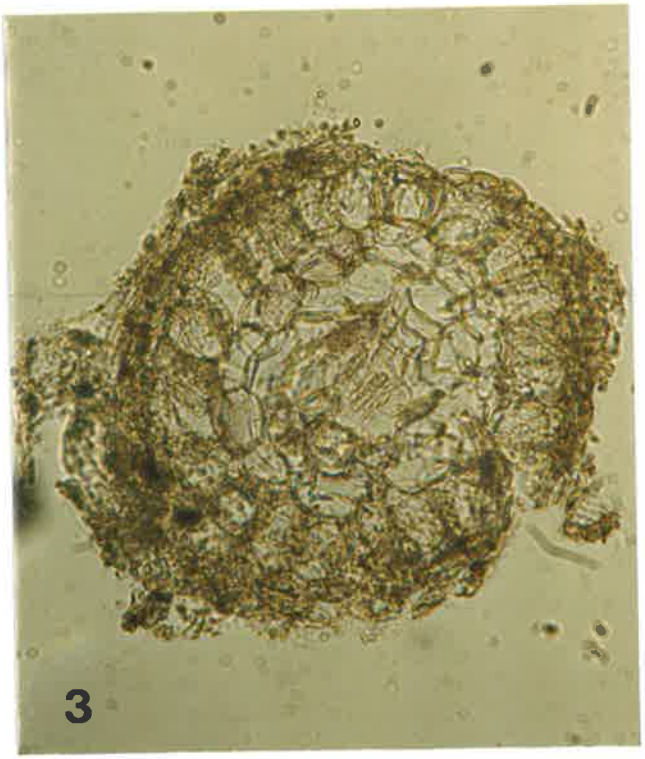
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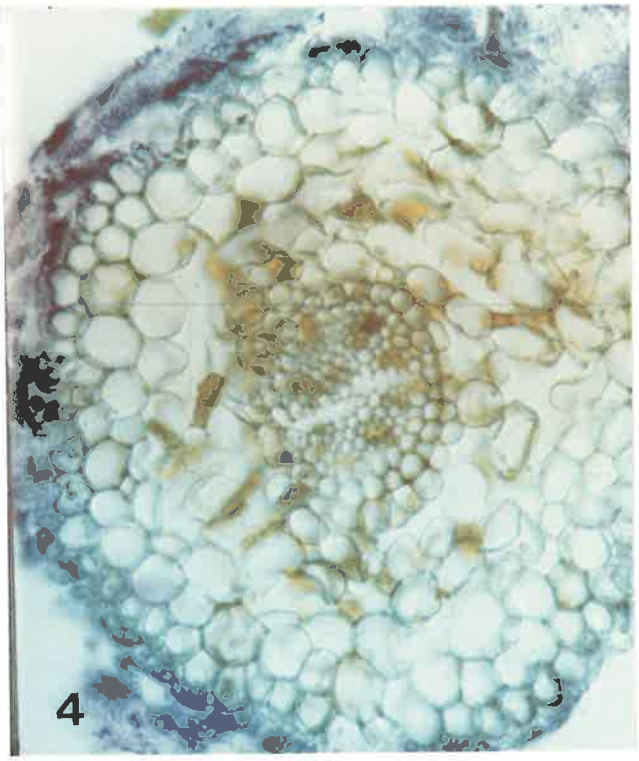
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showed a fungal mantle 10 - 15 µm in thickness. Periepidermal intercellular penetration of epidermal and cortical cells formed the Hartig net. Longitudinal sections showed radially elongated epidermal cells.

Eucalyptus maculata and Amanita grisea

Under reflected light the mycorrhizas appeared light yellow in colour becoming dark brown on older roots. Mycorrhizas were present on only a few roots of the total root system. Short (1 - 2 mm) mycorrhizal roots were found completely ensheathed. Mycorrhizal second and third order roots supported fourth and fifth order mycorrhizal roots. Root hairs were present on uncolonized, young, higher order roots and root hairs were absent from mycorrhizal roots.

The sheath was composed of a tightly compacted irregular synenchyma tissue forming a "jig-saw" puzzle pattern. In transverse section (Fig. 3) the mantle measured 20 - 25 µm. Periepidermal intercellular penetration of epidermal and cortical cells formed a Hartig net. Longitudinal sections showed radially elongate epidermal cells.

Eucalyptus maculata and Laccaria ohiensis

The mycorrhizas appeared a creamy white colour under reflected light. Colonized roots were found over the whole root system with most third and fourth order roots being mycorrhizal. Root hairs were present on uncolonized roots of all orders, but were absent on mycorrhizal roots.

The complete ensheathing of higher order roots was seen. Over the whole length of the sheath, free hyphae branched from the surface and extended to an indeterminate length into the soil. The sheath was

composed of a tightly compact irregular synenchyma tissue. Transverse sections of mycorrhizas (Fig. 4) showed a range of mantle thickness of 20 - 50 µm. Hartig net formation was irregular and varied from areas where there was paraepidermal penetration of epidermal cells to the remainder of the root where the epidermal cells supported hyphae only in the cell junctions. Radially elongate or swollen epidermal cells were not seen.

These results indicate that the fungi used readily formed ectomycorrhizas on E. maculata under the experimental conditions used. Table 4 summarizes the type of ectomycorrhiza formed. All mycorrhiza formed as complete sheaths over the growing root apices. Branching of mycorrhizal roots into simple pyramidal systems, the absence of root hairs and the presence of intercellular penetration, even if irregularly so with L. ohiensis, indicate "classical" types of ectomycorrhiza (Harley and Smith, 1983). Mycorrhiza colour, mantle thickness and its ornamention, and the composition of the sheath tissue and presence of radially elongated epidermal cells varied with the different fungi used.

Chilvers (1968) described eight distinct types of eucalypt ectomycorrhiza based on morphological and anatomical detail. A comparison of the mycorrhizas described above and those described by Chilvers showed many similarities even though most of the descriptions given by Chilvers were of associations formed by basidiomycetous fungi.

The mycorrhiza formed by Labyrinthomyces sp. compares well with the description of eucalypt mycorrhiza type 7 which appears to have been formed with an ascomycete. The mycorrhiza formed by Peziza whitei was

not comparable with any of the descriptions given. Based on a number of criteria, sheath colour, tissue type, appearance in transverse and longitudinal section, the mycorrhiza formed by Amanita grisea closely resembled that of eucalypt mycorrhiza type 2. Variation in the abundance of mycorrhizal roots could be the result of Chilvers' mycorrhiza being from field samples, where possibly a greater amount of inoculum is available to infect more root apices. The mycorrhiza of L. ohiensis and eucalypt mycorrhiza type 3 compared well, with major differences being an irregular Hartig net and no radially elongate epidermal cells.

Although similarities were seen between the descriptions of the mycorrhizas studied and those types described by Chilvers, it is unlikely that the mycorrhizas were caused by the same fungi, but more probably that in the absence of specialized hyphae the number of features available to classify mycorrhiza morphologically is limited.

Table 4 MYCORRHIZAS OF *EUCALYPTUS MACULATA*

FUNGUS	MYCORRHIZA ABUNDANCE	SHEATH	SHEATH SURFACE	COLOUR	SHEATH THICKNESS(µm)	HARTIG NET	EPIDERMAL CELLS	ROOT HAIRS
L. ohiensis	abundant	complete	free hyphae	creamy white	20 - 50	irregular paraepidermal	normal	uninfected only
P. whitei	abundant	complete	smooth	light brown	10 - 15	periepidermal	radially elongate	uninfected only
Labyrinthomyces sp.	moderate	complete	smooth	white	10 - 15	paraepidermal	normal	uninfected only
A. grisea	few	complete	smooth	light yellow	20 - 25	periepidermal	radially elongate	uninfected only

ECTOMYCORRHIZAS OF HERBS AND SHRUBS

Initially it was planned to use the four fungi, Laccaria ohiensis, Amanita grisea, Labyrinthomyces sp. and Peziza whitei (two Basidiomycetes and two Ascomycetes), used as ectomycorrhizal partners with Eucalyptus maculata, as partners on a range of Australian herbs and shrubs belonging to families that are not usually considered to have members that form ectomycorrhizas. However, the culture of Amanita grisea was slow growing and proved difficult to use to establish mycorrhizas on the herbs and shrubs. Whether this was a methodological problem or whether A. grisea does not, in fact, form ectomycorrhizas with these herbs and shrubs was not resolved. Choice of host plants used was governed by availability of seed and its percentage germination.

Poranthera microphylla (Euphorbiaceae)

P. microphylla (Fig. 5) is a small, slender, glabrous, annual herb to 15 cm long, but commonly 6 - 10 cm. The flower heads are in small, leafy corymbs with small, white monoecious flowers. The herb is usually found in shade beneath shrubs and occurs in all states of Australia.

Poranthera microphylla and Laccaria ohiensis

Under reflected light, mycorrhizas are an olivaceous colour becoming grey when old. Sheath formation was not continous over the length of a root, patches of sheath developed (Fig. 6). ~~Continuity of~~ the sheath was continuous over only a short length, ranging from a few epidermal cells up to 3 mm of the root. Between the patches of fungal sheath, were areas of root with a few hyphae on the surface. No

particular order or pattern of sheath formation was identified. All orders of roots supported sheath formation.

In comparison to a control, an acropetal succession of root branching with more orders and numbers of roots was seen on the inoculated plants, but no other specialized root branching was seen.

A predominate feature of this plant-fungus combination was the swelling of some epidermal cells. These hypertrophied cells were not continous over the length of a root but rather gave a pinched or contorted appearance to the root. Swollen cells were not found on the primary root but were present on all other orders of roots. The shape of the enlarged cells was oblong to almost spherical and their size varied in the range 77 - 165 µm in length by 44 - 77 µm in width. Most of the swollen cells appeared singly but 3 - 5 swollen cells have been found making up spherical shaped clusters (Fig. 7). Swollen cells were almost always covered by a fungal sheath although a few exceptions were noted. Normal epidermal cells adjacent to a swollen cell were either colonized or had only a few hyphae on their cell surface with no regular pattern being discernable. Root hairs were present on all orders of roots whether a sheath was present or not, but were less abundant if there was a sheath.

Initially the fungus appeared to grow in the cell junctions of epidermal cells. The fungus grew around the whole perimeter of an epidermal cell and then branched at right angles and crossed the surface of the cell. Further branching from the hyphae in the cell junctions and hyphae on the cell surface formed the mantle. Elongated hyphal cells, 3.0 - 4.0 µm wide by 15.0 - 18.0 µm long, described as irregular synenchyma tissue, and isodiametric cells, 7.0 - 8.0 µm wide by 9.0 - 10.0 µm long, described as regular synenchyma tissue made up the surface

Fig. 5 Flowering herb Poranthera microphylla. x3/4

Fig. 6 Patches of mantle and free hyphae of Poranthera microphylla + Laccaria oiensis. x250

Fig. 7 Swollen cells of a root of Poranthera microphylla + Laccaria ohiensis. x100

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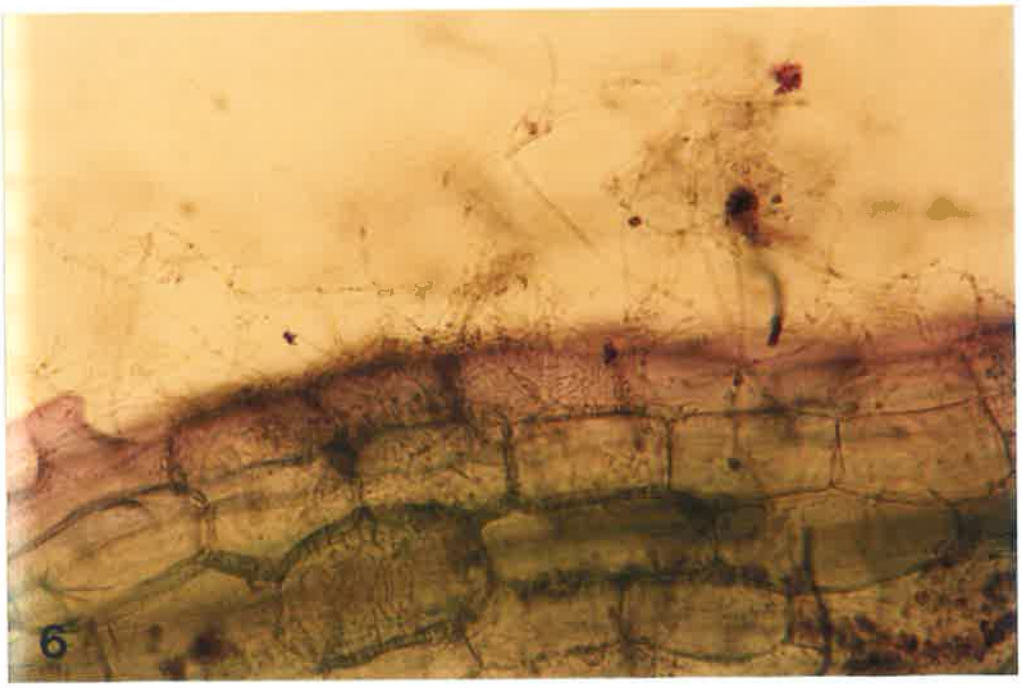

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tissue. Mantle thickness was made up of the formation of prosenchyma tissue, which had large hyphal cells tending to round off and becoming almost spherical in shape, 12.0 - 14.0 µm in diameter, and with large interhyphal spaces. Clamp connexions were present. No mantle ornamentation was seen.

Mantle thickness varied from 10.0 - 65.0 µm. Since sheath formation was not always complete around the circumference of the root in some transverse sections, mantle thickness varied markedly. Intercellular penetration of the epidermal cells in the form of a Hartig net did not occur. Hyphae only grew in the upper depressions between the epidermal cells. Cross sections of swollen epidermal cells which supported a fungal mantle did not show intercellular penetration. A regular layer of radially elongated cells was not seen in longitudinal sections.

Poranthera microphylla and Peziza whitei

Mycorrhizas were a light grey colour under reflected light. Partial sheaths, 1.0 - 6.0 mm in length, had formed on all orders and ages of roots. The fungus formed a much more complete sheath, in overall coverage of the root, than did either L. ohiensis or Labyrinthomyces sp. Completely ensheathed roots or root apices were not seen. On the older second order roots a more complete length of root was ensheathed. Partial sheaths over a few epidermal cells appeared on the higher order roots as a few mm of colonized epidermal cells. Swollen epidermal cells did not occur. Compared with an uninoculated plant, more roots and orders of roots were seen on the inoculated plant root system. An acropetal succession of root formation was seen in both root systems.

Free hyphae, branching from the fungal sheath, were regularly septate, sparsely branched and of an indeterminate length. Hyphal

diameter measured 2.0 - 3.5 µm. Sand and soil particles were often tightly bound to the free hyphae or particles were found tightly bound to the surface of the fungal sheath.

Root hairs were present over the whole length of a root and on all orders of roots. Mycorrhizas also contained root hairs. Root hairs were not colonized by the fungus but on higher order roots the fungus colonized root hair basal cells after the hairs had formed. It appeared as if the root growth and root hair formation was quicker than the fungal colonization or that the root and hairs had formed before the root was colonized.

Initial colonization by the fungus was in the epidermal cell junctions and branching at right angles from these hyphae formed a sheath over the cell surface. The outer fungal layer of the sheath was made up of felt prosenchyma tissue. Hyphal cells measured 6.5 - 8.0 µm long by 2.0 -2.5 µm wide. The fungal mantle was composed of prosenchyma tissue which was a moderately compact tissue with hyphal cells almost isodiametric to spherical in shape with large interhyphal spaces. Focusing through the outer layer of tissue, hyphae could be seen in the form of an irregular synenchyma tissue under the epidermal cells. This tightly compacted tissue had a"jig-saw" puzzle appearance. It appeared as if intercellular penetration had occurred after the surface of the epidermal cells had been colonized. Older mycorrhizas had a fungal mantle up to 5 hyphal cells thick (15µm). The additional layers were made up of prosenchyma tissue.

Mantle thickness varied from 3.0 - 15.0 µm. Transverse sections showed varations in mantle thickness over the circumference of the root. A multiseriate Hartig net formed between epidermal cells, but some cases of intercellular penetration of the epidermal cells by a uniseriate

Hartig net were seen (Fig. 8). Periepidermal penetration was seen in this association.

Poranthera microphylla and Labyrinthomyces sp.

The mycorrhizas appeared a light yellow brown in colour under reflected light. Patches of sheath were seen on the root surface. The patches varied from 3 - 4 epidermal cells to 2.0 - 3.0 mm of root surface. Uncolonized cells between mycorrhizal areas varied from a few epidermal cells, some with hyphae present in the cell junctions, to a few mm with no fungal hyphae present. Complete ensheathing of roots or root apices was not seen.

Mycorrhizas formed on all orders of roots, but in comparison with an uninoculated plant, no differences in root branching were apparent, although the total number of roots on the mycorrhizal plant was greater. Swollen cells very similar to those of P. microphylla and L. ohiensis, were found and occurred on all orders of roots. The swollen cells were mycorrhizal and somewhat spherical in shape, measuring 60.0 - 150.0 µm in length by 30.0 - 60.0 µm in width. The number of swollen cells making up a cluster was 3 - 5.

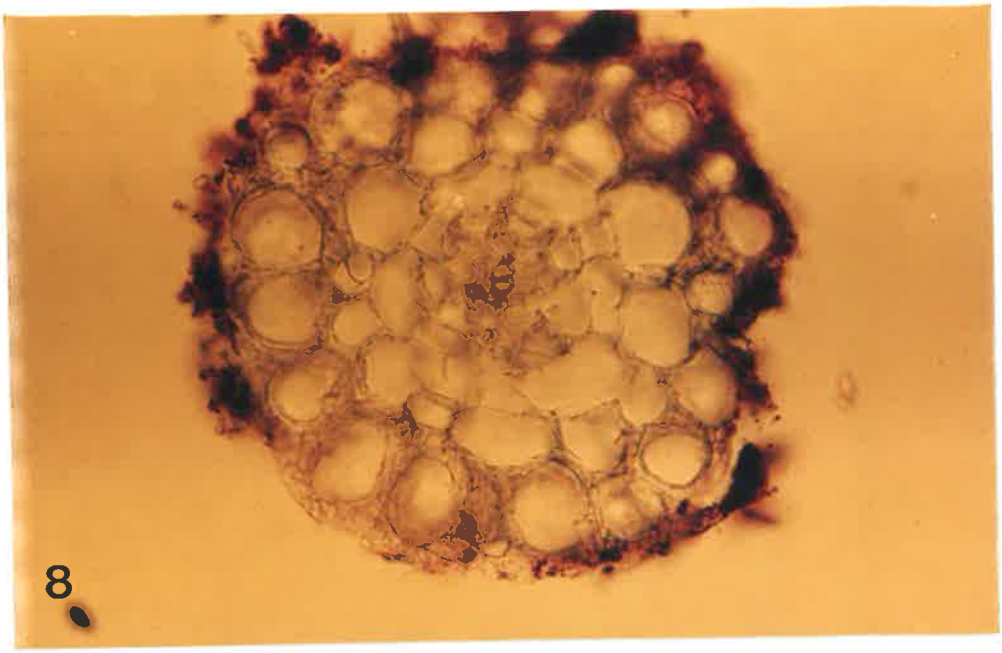
Root hairs were present over the whole root system and over the whole length of a root. Mycorrhizal sections of a root supported, visually, the same amount of root hairs as did uncolonized roots.

Colonization of epidermal cells appeared to begin with hyphae in the cell junctions. Branching from these hyphal cells gave rise to branches forming in a "jig-saw" pattern and also as a circular pattern on the cell surface (Fig. 9). This densely packed tissue formed initially from hyphae growing in the cell junctions, and was irregular synenchyma tissue with hyphal cells measuring 7.5 - 2.2 µm

Fig. 8 Transverse section of ectomycorrhiza of Poranthera microphylla + Peziza whitei. x250

Fig. 9 Fungal mantle of Poranthera microphylla + Labyrinthomyces sp. x250

Fig. 10 Transverse section of ectomycorrhiza of Poranthera microphylla + Labyrinthomyces sp. x250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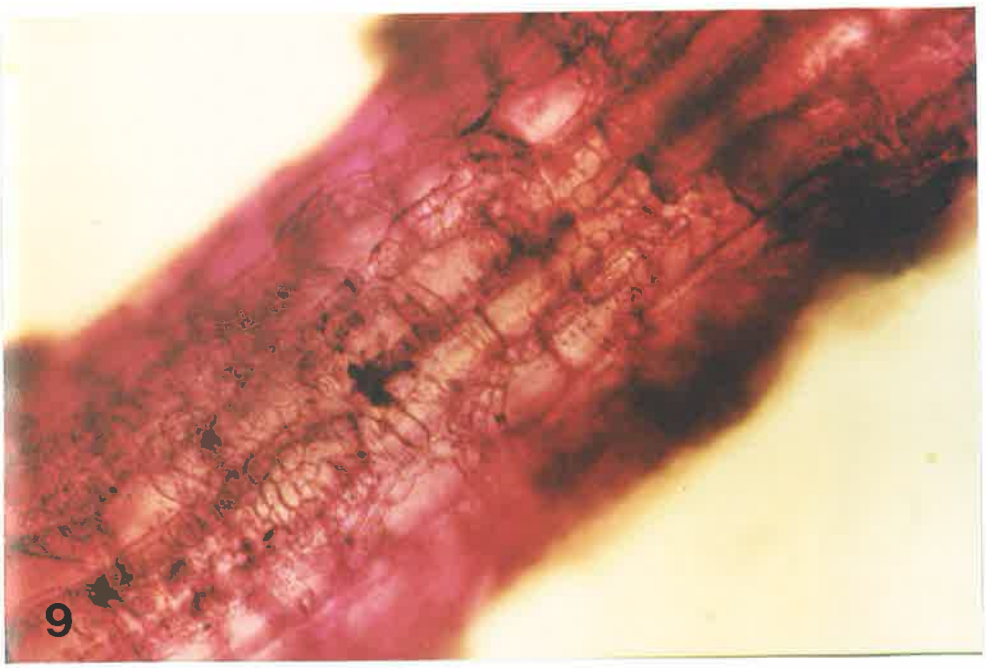
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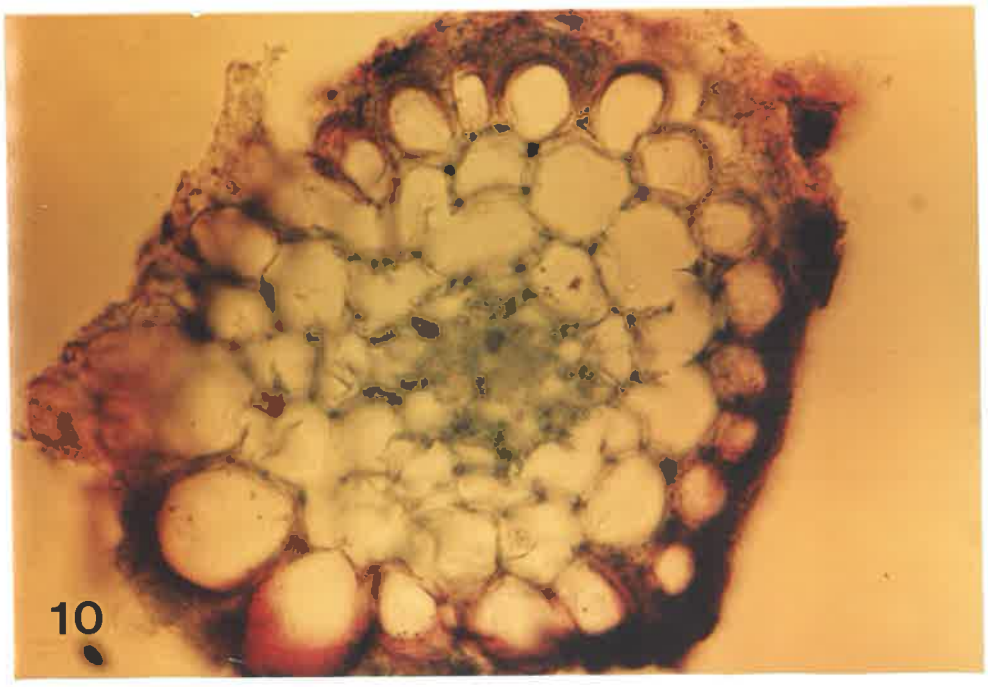
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long by 2.2 - 3.0 µm wide. Successive layering by the hyphae formed a felt prosenchyma tissue with hyphal cells measuring 2.2 - 3.3 µm in diameter.

Free hyphae, in the form of many short lengths of hyphae, 110 - 140 µm long by 6.0 - 2.0 µm in diameter, branched from the mantle surface. They appeared like short fine root hairs. Septa were visible in hyphae of the mantle and in free hyphae.

The fungal mantle was from 20.0 - 25.0 µm thick. Complete ensheathing or encircling of the diameter of the root was not often seen. A Hartig net was present, with intercellular penetration up to the first cortical cells. Paraepidermal penetration of the hyphae was seen (Fig.10).

Angianthus tomentosus(Asteraceae)

A. tomentosus (Fig.11) is an annual herb which is endemic to southern Australia. The stems are erect or ascending, 5 - 30 cm high. Compound heads are ellipsoid to ovoid. This species is common in a wide range of habitats, varying from the edge of clay pans and saline depressions to scrub, shrubland or woodland formations.

Angianthus tomentosus and Laccaria ohiensis

The mycorrhizas appeared an opaque brown colour under reflected light. The main, second and third order roots were colonized. Small patches of colonized epidermal cells appeared as the predominant feature of colonization on roots close to the point of inoculation. Where sheath covered 2 - 3 mm of root it appeared to be coalesced patches of sheath. Colonization was present in an approximate radial area from the

point of inoculation. Prolific root hair formation, which was characteristic of this plant, also occurred in areas colonized by the fungus. Free hyphae branching from the surface of the sheath measured 3.0 - 4.5µm in diameter, were of indeterminate length and were seen entangled in the root hairs. Visually, the inoculated and uninoculated plants were the same size and had the same amount of foliage. The root systems of both were very small.

Branching from hyphae in the cell junctions formed a loose felt prosenchyma tissue with large interhyphal spaces. The hyphal cells measured 6.0 - 9.0 µm in width and 12.0 - 17.5 µm in length. Mantle thickness was made up of more layers of felt prosenchyma tissue.

Variations in the total circumference of a root being ensheathed gave varying thickness of the mantle (6.0 - 10.0 µm). A Hartig net did not form between the epidermal cells with hyphal penetration being limited to the upper depressions of the cell junctions (Fig.12). Longitudinal sections showed fungal hyphae on the surface of the epidermal cells only (Fig.13).

Angianthus tomentosus and Peziza whitei

Mycorrhizas appeared a brownish yellow under reflected light. Hyphae occurred between but not over the surface of epidermal cells. Focusing down on the root and through the epidermal cells, fungal tissue on the surface of the first layer of cortical cells could be seen. Continuity in the colonization of cortical cells was over a distance of 3 - 15 mm of the root, but no roots were found that were completely ensheathed by the fungus. On the surface of the epidermal cell, a few free hyphae, usually entangled in the root hairs, could be seen. Root hairs were present over the whole of the root system and over the whole

Fig. 11 Flowering herb Angianthus tomentosus. x3/4

Fig. 12 Transverse section of ectomycorrhiza of Angianthus tomentosus + Laccaria ohiensis. Arrows show hyphae in the epidermal cell depressions only. x250

Fig. 13 Longitudinal section of ectomycorrhiza of Angianthus tomentosus + Laccaria ohiensis. Arrows show hyphae on the surface of the epidermal cells only. x250

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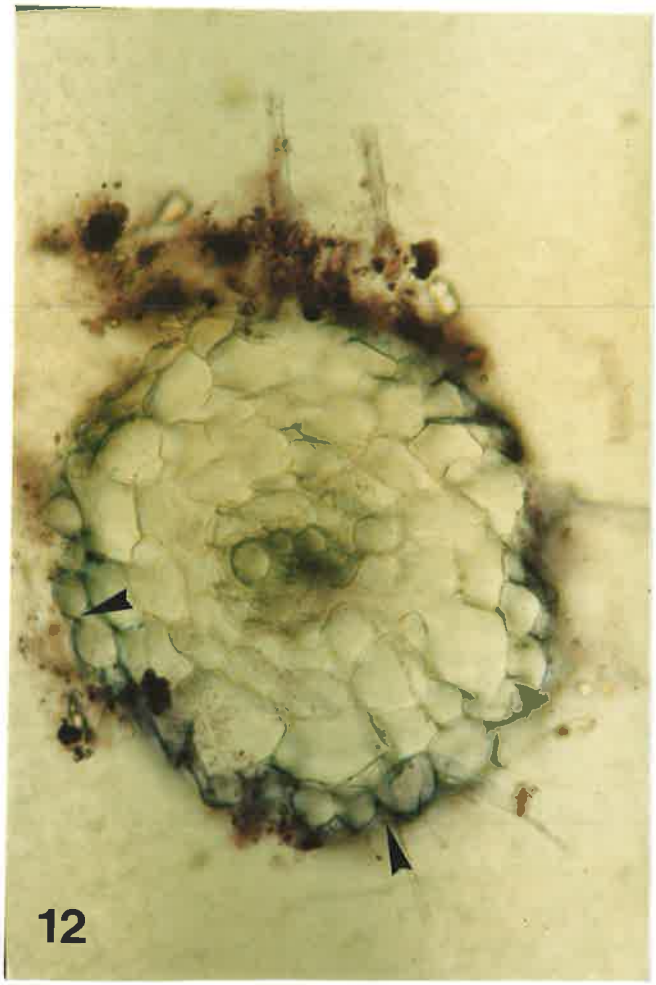

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length of a root. Mycorrhizal roots formed on all orders of roots. Root branching occurred more frequently on inoculated plants, with the formation of more roots, than on the root system of an uninoculated plant of the same age.

Sections showed hyphal growth forming a compact irregular synenchyma tissue between the epidermal and cortical cells (Fig.14) forming a periepidermal "Hartig net" between the lower surface of epidermal cells and the upper surface of cortical cells. Hyphal cells measured 5.0 - 7.0 µm in diameter. This compact tissue, had the appearance of a "jig-saw" puzzle. Intercellular hyphal growth was seen in longitudinal section (Fig.15).

Angianthus tomentosus and Labyrinthomyces sp.

The mycorrhizas appeared light brown under reflected light. The fungus was present on very little of the root system. Loosely bound hyphae were present over the surface of the epidermal cells. Patches of sheath or the colonization of single epidermal cells was recorded as the only feature of sheathing (Fig. 16). The sheath was smooth with no free hyphae branching from its surface. Ensheathed single cells were seen on the main root as well as on adjacent secondary roots. A network of hyphae in the cell junctions surrounding individual epidermal cells was also seen. Root hairs formed over the whole root system and over the whole length of a root whether it was colonized or not. The root systems of inoculated and uninoculated plants were very similar. Visually they both appeared to have few root branches and supported the same order of roots.

Growth of the hyphae in the cell junctions branched at right angles to form a tightly compacted irregular synenchyma tissue with hyphal

Fig. 14 Transverse section of ectomycorrhiza of Angianthus tomentosus + Peziza whitei. Arrow shows intercellular hyphae. Note: no fungal mantle present. x250

Fig. 15 Longitudinal section of ectomycorrhiza of Angianthus tomentosus + Peziza whitei. Arrow shows intercellular hyphae. Note: no fungal mantle present. x250

Fig. 16 Fungal mantle on a few epidermal cells of roots of Angianthus tomentosus + Labyrinthomyces sp. x250

Fig. 17 Transverse section of ectomycorrhiza of Angianthus tomentosus + Labyrinthomyces sp. Arrow shows fungal mantle on a single epidermal cell only. x400

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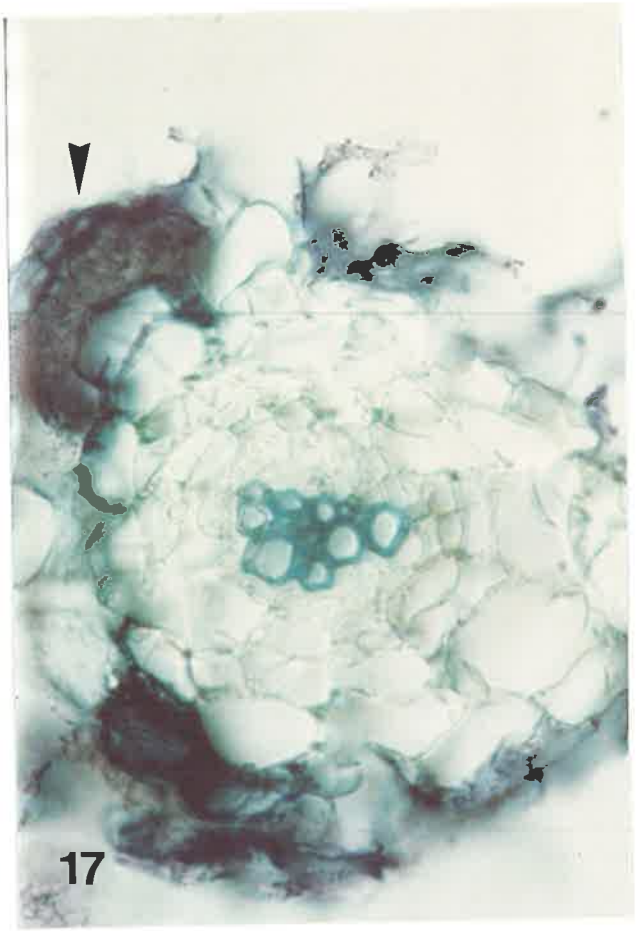

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diameters of 2.5 - 3.5 µm, over the surface of a single epidermal cell. More layers of irregular synenchyma tissue made up the sheath.

Single epidermal cells that were colonized supported a mantle of 30.0 - 40.0 µm in thickness. Intercellular penetration between the colonized cell and the adjacent uncolonized cells formed to the first cortical cell layer (Fig.17). The Hartig net was paraepidermal in form.

Waitzia citrina (Asteraceae)

W. citrina (Fig.18) is an annual herb which is endemic to Western Australia, Northern Territories and South Australia. It is an erect or ascending glabrous or slightly woolly annual. The heads are hemispherical, white or yellow in colour. The species is found on sand-plains, sand dunes and stoney outcrops.

Waitzia citrina and Laccaria ohiensis

The mycorrhizas were light yellow under reflected light. Mycorrhizas formed on all orders of roots including the tap root. A much more continuous ensheathing of the roots was seen. These partial sheaths formed completely around the circumference of the root. Complete ensheathing of short lengths of roots, 10.0 - 15.0 mm, was seen near the point of inoculation. Swelling of epidermal cells was not seen. Root hairs were present on all orders of roots. Mycorrhizal portions of root also supported root hairs. Free hyphae, branching from the surface of the sheath, were of indeterminate lengths and often tightly bound to soil particles. Both the uninoculated and inoculated plants supported an acropetal succession of roots with a greater number and order of roots on inoculated plants. Hyphal colonization of epidermal cells, ahead of an ensheathed portion of root, was along the

cell junctions. Hyphae formed around the perimeter of an epidermal cell surface, then by branching at wide angles initiated colonization of the epidermal cell surface. An irregular synenchyma tissue formed on the cell surface with hyphal cells measuring 4.8 - 6.0 µm wide by 9.5 - 14.3 µm long. Layers of a loosely bound felt prosenchyma tissue, 2.4 - 3.6 µm in diameter, formed the mantle, with the mantle thickness varying with the age of the mycorrhiza. Clamp connexions were present on the free and sheath hyphae. Complete sheathing of a root showed a fungal mantle of an approximate equal thickness around the root circumference. Mantle thickness measured 6.0 - 12.0 µm. A Hartig net was not observed in transverse section, with hyphae penetrating only the upper depressions of the epidermal cell junctions (Fig.19).

Waitzia citrina and Peziza whitei

The mycorrhizas were olivacious-brown under reflected light. Partial sheathing of the root was not often seen. Patches of sheath or growth of hyphae in the cell junctions was found in this association. The growth of the fungus in the cell junctions was not complete over the length of a root. Small colonized areas, 1 - 5 mm in length, were separated by large areas of uncolonized root surface. These small areas of colonized cells were also separated by uncolonized epidermal cells. A loose network of hyphae with large interhyphal spaces formed from branches of hyphae in the cell junctions to form the partial sheath. All orders of root except the main root were colonized. Complete ensheathing of roots or root apices was not seen. Focusing through the epidermal cells, a fungal tissue intercellularly on the surface of the cortical cells could be seen (Fig.20). In comparison, the uninoculated

Fig. 18 Flowering herb Waitzia citrina. x1/2

Fig. 19 Transverse section of ectomycorrhiza of Waitzia citrina + Laccaria ohiensis. x400

Fig. 20 Fungal mantle on root of Waitzia citrina + Peziza whitei. Irregular synenchyma tissue. x250

Fig. 21 Transverse section of ectomycorrhiza of Waitzia citrina + Peziza whitei. Arrow shows intercelluar hyphae. x250

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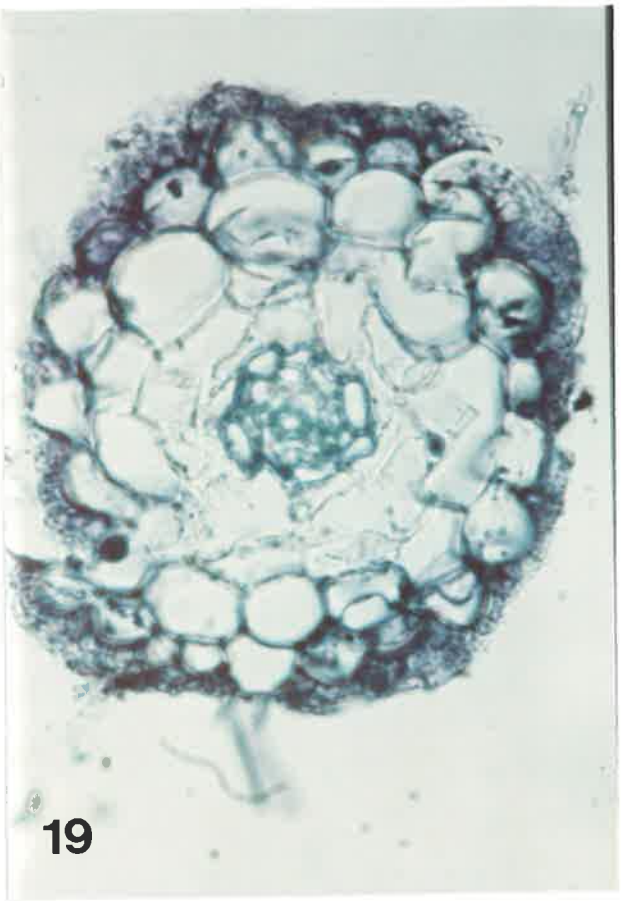

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plant had fewer roots than the inoculated plant. No specialized root branches or branching of mycorrhizal roots were seen. Root hairs were present on all orders of roots over the whole length of a root. Root hairs were also seen on roots colonized by the fungus. Some free hyphae of indeterminate length arising from hyphae in the cell junctions were seen.

Colonization of the cell junctions with intercellular growth was well established on all orders of roots. Hyphae present on the epidermal cell surfaces but not tightly appressed to the surface were mainly long celled, sparingly branched, measuring 2.0 - 4.0 µm in diameter. The partial sheath ~~that did form~~ was a loose prosenchyma tissue not colonizing an area of more than 5 - 7 epidermal cells. The hyphal diameter of the tissue measured 4.0 - 5.0 µm. Intercellular hyphae, viewed through the epidermal cells, that formed on the top of cortical cells made up an irregular synenchyma tissue of tightly compacted hyphal cells measuring 2.4 - 4.5 µm wide by 8.4 - 11.7 µm long. In most areas where the fungus had formed a sheath, mantle thickness of 1 - 3 layers (4.5 - 15 µm) of hyphal cells were seen. Both multiseriate and uniseriate hyphae formed intercellularly (Fig.21). A periepidermal Hartig net between cortical cells was seen in transverse section. Intercellular penetration between epidermal cells and on top of the first layer of cortical cells was seen in longitudinal section.

Stylidium graminifolium (Stylidiaceae)

S. graminifolium (Fig.22) is a perennial herb occurring throughout Australia except for Western Australia. The leaves occur in whorl-like tufts and are grasslike. The flowers are irregular, bisexual and pale pink to white in colour. The species is found on sandy soils.

Stylidium graminifolium and Labyrinthomyces sp.

The mycorrhizas appeared light brown under reflected light. Patches of fungal sheath were mainly seen in this association. Only a few epidermal cells were colonized nearer the point of inoculation. The fungus formed mainly in the cell junctions, forming a network along the root up to 3 mm in length. Focusing through the epidermal cells, intercellular fungal tissue could be seen on the surface of the cortical cells. Colonized epidermal cells usually occurred singly or in small groups of 2 - 3 epidermal cells. Complete ensheathing of roots or root apices was not seen.

Fungal colonization occurred on all orders of root. Both uninoculated and inoculated plants had adventitious root formation. Adventitious roots of the inoculated plants were colonized by the fungus. Very few root hairs were seen on either the uninoculated or inoculated plants and those seen occurred behind the root apices. No root hairs were present in areas colonized by the fungus nor were hairs present on the adventitious roots. Hyphal strands were seen, (Fig.23), measuring 15.0 -17.0 µm in diameter, and consisted of many single hyphal lengths in the form of a strand. Branches from the strand, of a determinate length, measured 20.0 - 30.0 µm.

Hyphae present in the cell junctions formed mainly as a network around epidermal cells over large areas of root. Hyphae of irregular shape measured 3.0 - 6.0 µm in diameter to the spherical shaped cells being 9.0 - 12.0 µm in diameter. Branching at right angles, from hyphae in the cell junctions formed a loose felt prosenchyma tissue on the epidermal cell surface. ~~The majority~~ Most of the hyphae present appeared to

be loosely attached to the root without forming a fungal sheath.

A fungal mantle, where formed, was measured one fungal cell in thickness, 3.0 - 6.0µm. Ensheathing of the complete circumference of a root was not seen. Intercellular hyphal growth was seen. In transverse section a Hartig net and periepidermal penetration was seen to have formed up to the first cortical cells (Fig.24). Longitudinal sections showed hyphal growth intercellularly between the epidermal cells (Fig.25).

Fig. 22 Flowering herb Stylidium graminifolium. x1/2

Fig. 23 Hyphal strand on root of Stylidium graminifolium + Labyrinthomyces sp. x250

Fig. 24 Transverse section of ectomycorrhiza of Stylidium graminifolium + Labyrinthomyces sp. Arrow shows intercellular hyphae. x250

Fig. 25 Longitudinal section of ectomycorrhiza of Stylidium graminifolium + Labyrinthomyces sp. showing fan-like hyphae, intercellularly of epidermal cells, making up the Hartig net. x400

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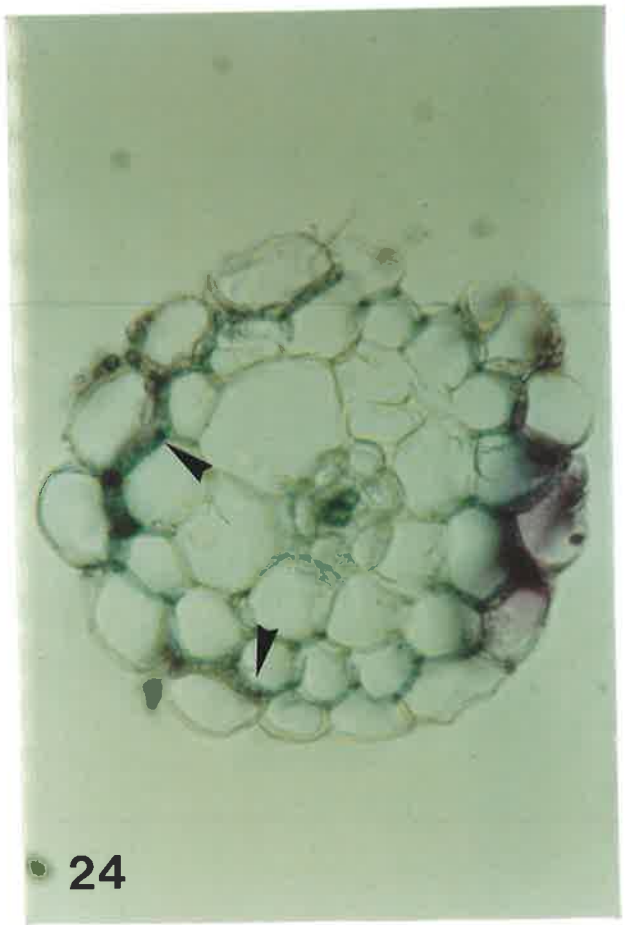

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Besides the herbs, two shrubs belonging to Fabaceae were tested with the same range of fungi. Unfortunately fewer seed of Gompholobium latifolium germinated than initially expected so Laccaria ohiensis was the only fungus used with that species.

Pultenaea obovata (Fabaceae)

P. obovata is a woody shrub which is endemic to N.S.W. It forms bushy shrubs to 2 m high with spathulate leaves to 9 mm long and bears yellow pea-flowers in spring. Plants may be found in alpine and subalpine regions, particularly near swamps and bogs, in well-drained montane forests along tablelands and in coastal woodlands, plains and heathlands in high rainfall localities.

Pultenaea obovata and Laccaria ohiensis

The mycorrhizas were a light yellow under reflected light. The upper third of the root system supported the majority of the mycorrhizas. Completely ensheathed tertiary roots and ensheathed root apices were found. Fungal colonization appeared to have spread radially from the point of inoculation. Root hairs were common on the uncolonized roots of the lower part of the root system but were absent from roots that were completely ensheathed by the fungus. The formation of branched short mycorrhizal roots gave a different appearance to the root system of inoculated compared uninoculated plants. Both root systems displayed an acropetal succession of roots with the inoculated plant supporting pyramidal branches of mycorrhizas. Few free hyphae were seen arising from the mycorrhizal roots.

The area ahead of a fungal mantle, on a short tertiary root, was examined to see the initial stages of colonization. Initially hyphal

growth was in the epidermal cell junctions with branches forming across the root surface, crossing other hyphae, and forming a tightly compacted tissue with no interhyphal spaces. Hyphae 5.5 - 10.0 µm in diameter, formed the synenchyma tissue. Focusing through the epidermal cells, where hyphal growth was only in the cell junctions and no fungal tissue had formed on the surface of the cells in the form of a sheath, intercellular hyphae between the epidermal cells to the first cortical cells were seen. Diameters of the intercellular hyphae measured 2.0 - 3.5 µm. A loosely bound tissue of net prosenchyma with interhyphal spaces formed over the synenchyma tissue (Fig.26). Regular clamp connexions were seen.

Mantle thickness measured 15.0 - 25.0 µm in the various sections studied. Intercellular penetration forming a periepidermal Hartig net was seen (Fig.27). Some intercellular penetration of the cortical cells was also seen. Longitudinal sections showed intercellular growth of hyphae between the epidermal cells as a tightly compacted synenchyma tissue (Fig.28).

Pultenaea obovata and Peziza whitei

The mycorrhizas appeared yellowish brown under reflected light. Fungal colonization formed as partial sheaths. On secondary and tertiary roots, close to the point of inoculation, only partial sheathing of the root occurred. Complete ensheathing of the roots or root apices was not seen. The root system, in comparison to an uninoculated plant, had the same morphology but supported a greater number and order of roots. Root hairs appeared on all orders of root and over the whole length of a root including those portions with partial sheaths. Hyphae branching from the sheath were of an

indeterminate length, and often tangled in the root hairs, suggesting that they ramified through the surrounding rhizosphere.

The sheathing appeared to be preceded by hyphal growth in the cell junctions. Hyphal cells in the cell junctions were spherical and varied in size between 6.0 - 14.5 µm with most 9.0 - 12.0 µm in diameter. Branching from these hyphae formed a net prosenchyma with cells 2.0 - 3.0 µm wide by 6.0 - 8.0 µm in length with many large interhyphal spaces. Focusing through the epidermal cells showed a sheathing of the cortical cells with intercellular hyphal growth.

A fungal mantle of 4.0 - 5.0 µm thick was recorded from sections of mycorrhizal root. A periepidermal Hartig net showed in the sections made (Fig.29). Longitudinal sections showed intercellular hyphae in the form of an irregular synenchyma tissue.

Pultenaea obovata and Labyrinthomyces sp.

The mycorrhizas appeared dark brownish yellow under reflected light. Mycorrhizal roots including apices were completely ensheathed. A greater number of root branches, many of which were mycorrhizal, were seen near the point of inoculation. A radial and acropetal spread of mycorrhizal roots formed from the point of inoculation. Root hairs were seen on all orders of non-mycorrhizal but not mycorrhizal roots. Short roots, number of roots and number of order of roots were greater in the inoculated plants. Few free hyphae were found branching from the sheath.

Growth of hyphae in cell junctions formed a network of encircled epidermal cells ahead of a fungal mantle. Intercellular penetration was seen at this stage. Growth in the cell junctions was 4 - 6 epidermal cells ahead of the formation of a mantle. Branching from the hyphae

Fig. 26 Complete fungal mantle of Pulteaea obovata + Laccaria ohiensis. Compacted synenchyma tissue on the surface of the epidermal cells. x250

Fig. 27 Transverse section of ectomycorrhiza of Pultenaea obovata + Laccaria ohiensis. x250

Fig. 28 Longitudinal section of ectomycorrhiza of Pultenaea obovata + Laccaria ohiensis. Arrow shows intercellular hyphae. x250

Fig. 29 Transverse section of ectomycorrhiza of Pultenaea obovata + Peziza whitei. x250

Fig. 30 Transverse section of ectomycorrhiza of Pultenaea obovata + Labyrinthomyces sp. Arrow shows intercellular hyphae. x250


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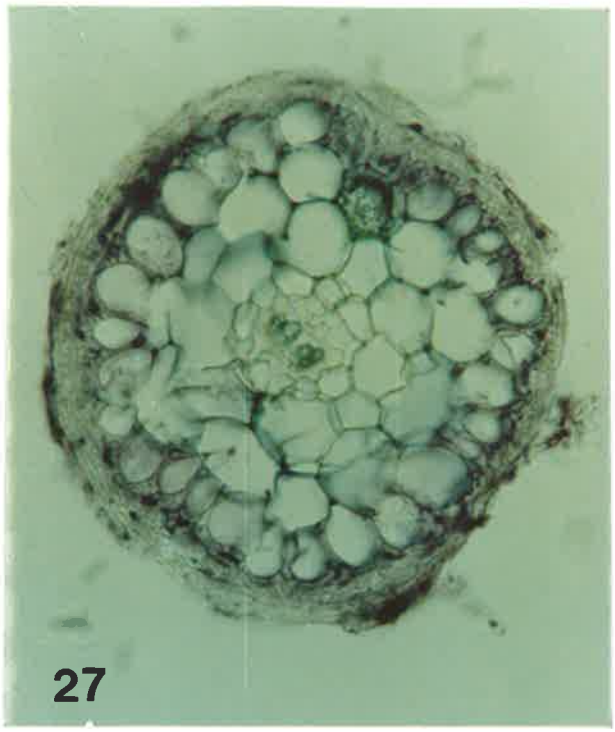
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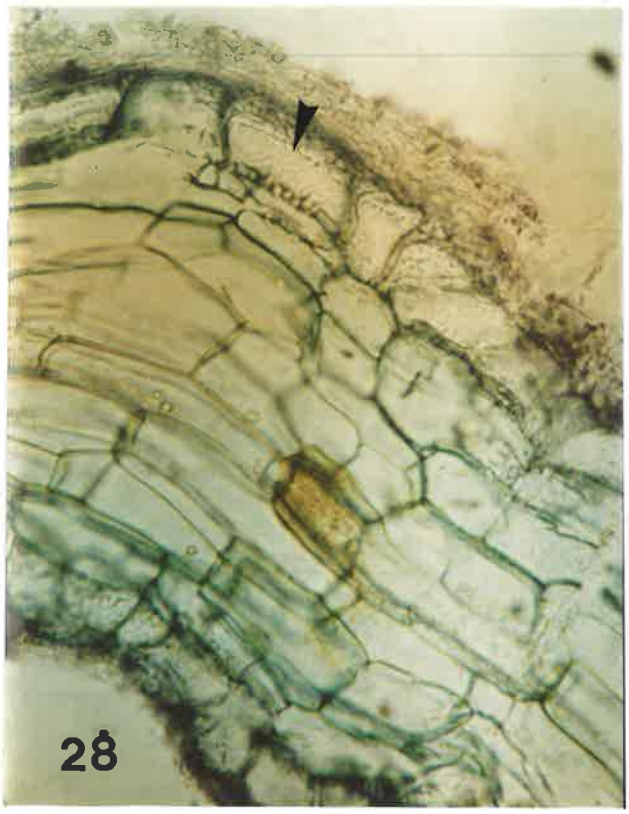
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over the surface of the epidermal cells formed a tightly compacted irregular synenchyma tissue with hyphal diameters of 10.0 - 14.5 µm. Growth of subsequent layers of hyphae formed a felt prosenchyma tissue with hyphal diameters of 5.0 - 7.5 µm.

A thin fungal mantle 10.0 - 20.0 µm thick formed. Periepidermal Hartig net formation was seen in transverse section, (Fig.30), and longitudinal section showed intercellular hyphae of irregular synenchyma to the first cortical cells.

Gompholobium latifolium (Fabaceae)

G. latifolium (Fig.31) is an Australian endemic. It forms as an open, glabrous shrub, 1 - 3 m high which occurs scattered in eastern Queensland, N.S.W. and Victoria in near-coastal open forests on poor grey sandy soils and sandstones. The flowering period is August to November and the flowers are large and a clear yellow.

Gompholobium latifolium and Laccaria ohiensis

The mycorrhizas appear yellowish brown under reflected light, Completely ensheathed roots of all orders were seen. Ensheathed second order roots supported third order root branches, most of which were mycorrhizal, and these roots made up most of the root system. In comparison to an uninoculated plant the root system of an inoculated plant had many more third order root branches present. This branching of third order mycorrhizas from second order mycorrhizal roots occurred in a pyramidal pattern. Root hairs were not present on mycorrhizas. There were fewer root hairs on uncolonized roots of mycorrhizal plants as compared to uninoculated plants. Free hyphae, of an indeterminate

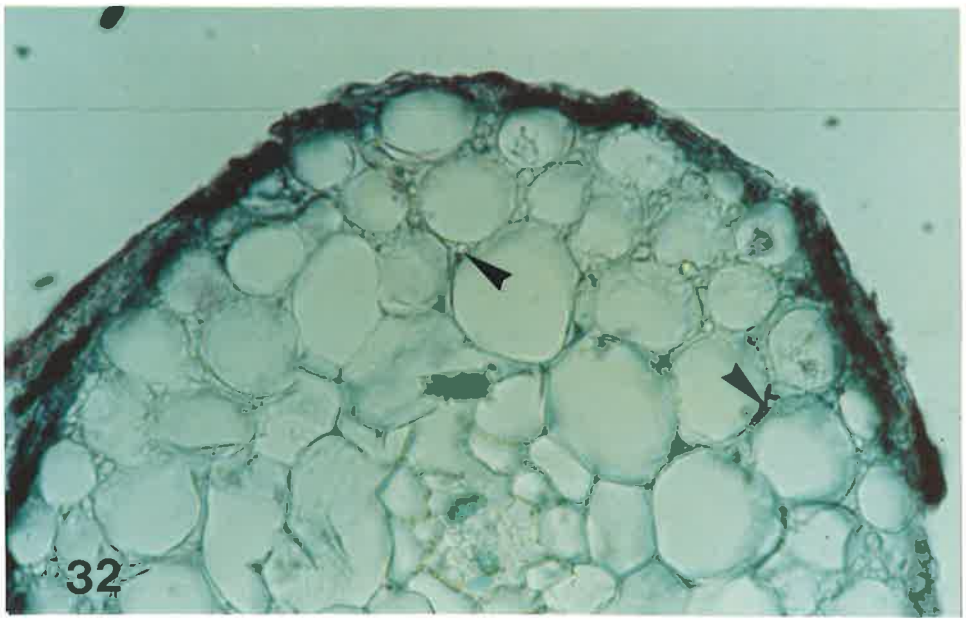
length branched from the sheath.

Fungal colonization ahead of a mantle on a second order root had hyphae forming a loose disorganized network over the root surface. Successive stages of mantle formation progressed through a pattern of a more organized growth of hyphae in the cell junctions and the formation of a loose felt prosenchyma tissue over the epidermal cell surface. Hypahe cells of the mantle tissue measured 2.5 - 4.0 µm in diameter. A mantle thickness of 20.0 - 30.0 µm was measured. A periepidermal Hartig net was found around epidermal cells as well as intercellularly between the first and second layers of cortical cells (Fig.32).

Fig. 31 Flowering shrub Gompholobium latifolium. x1/2

Fig. 32 Transverse section of ectomycorrhiza of Gompholobium latifolium + Laccaria ohiensis. Arrow shows intercellular hyphae of the cortical cells. x250


31


32

COMPARISON OF THE MYCORRHIZAS

For ease of comparison of the preceeding data, they are summarized in relation to host (Table 5) and fungus (Table 6). The most notable feature of the mycorrhizas formed by the different plant-fungus combinations examined is their variation, ranging from complete or classical mycorrhizas with sheaths enclosing root apices and with definite Hartig nets to associations without a Hartig net (P. microphylla + L. ohiensis) or without a sheath (A. tomentosus + P.whitei).

A mycorrhiza is a composite organ and reflects the interaction of both host and fungus in any association. The data in Table 5 where there is variation in many of the characters examined for each host suggest an influence of fungus rather than host. Two exceptions are apparent. All associations on E. maculata formed mycorrhizas with sheaths that covered root apices and had Hartig nets though the Hartig net of the association E. maculata + L. ohiensis was irregular and absent in some areas. However, examination of Eucalypt mycorrhizas (with unknown fungi) from the field has shown cases of mycorrhizas with thin sheaths that did not cover root apices so complete or typical mycorrhizas do not always occur on Eucalyptus. The other host reaction is the tendency of Poranthera microphylla to form swollen epidermal cells. These occurred with both an Ascomycete (Labyrinthomyces sp.) and a Basidiomycete (Laccaria ohiensis) suggesting it is a host reaction, presumably to hormone production by certain fungi. Such swollen epidermal cells were not seen with any of the other hosts.

The data in Table 6 show that the colour of mycorrhizas formed by a fungus on different hosts might vary. Young mycorrhizas were usually a

Table 5 SUMMATION BY HOST OF THE SHEATH FEATURES OF THE MYCORRHIZAS EXAMINED

HOST	PLANT FORM	FUNGUS	MYCORRHIZA ABUNDANCE	SHEATH FORM	SHEATH SURFACE	COLOUR	SHEATH THICKNESS (µm)	HARTIG NET	EPIDERMAL CELLS	ROOT HAIRS
P. microphylla	Herb	L. ohiensis	abundant	patches	free hyphae	olivacious to grey	10 - 65	absent	swollen cells	over all
		P. whitei	abundant	partial	free hyphae	light grey	3 - 15	periepidermal	normal	over all
		Labyrinthomyces sp.	moderate	partial	free hyphae	yellowish brown	20 - 25	paraepidermal	swollen cells	over all
A. tomentosus	Herb	L. ohiensis	abundant	patches	free hyphae	opaque brown	6 - 10	absent	normal	over all
		P. whitei	abundant	absent	free hyphae	brownish yellow	absent	periepidermal	normal	over all
		Labyrinthomyces sp.	few	patches	free hyphae	light brown	30 - 40	paraepidermal	normal	over all
W. citrina	Herb	L.ohiensis	abundant	partial	free hyphae	light yellow	6 - 10	absent	normal	over all
		P. whitei	moderate	patches	free hyphae	olivacious-brown	5 - 15	periepidermal	normal	over all
S. graminifolium	Herb	Labyrinthomyces sp.	few	patches	smooth	light brown	3 - 6	periepidermal	normal	few present
P. obovata	Shrub	L.ohiensis	abundant	complete	free hyphae	light yellow	15 - 25	periepidermal	radially elongate	uninfected roots only
		P. whitei	abundant	complete	free hyphae	brownish yellow	10 - 20	periepidermal	radially elongate	uninfected roots only
		Labyrinthomyces sp.	few	partial	free hyphae	yellowish brown	4 - 5	periepidermal	normal	over all
G. latifolium	Shrub	L.ohiensis	abundant	complete	free hyphae	yellowish brown	20 - 50	periepidermal	normal	uninfected roots only
E. maculata	Tree	L.ohiensis	abundant	complete	free hyphae	creamy white	20 - 50	paraepidermal	normal	uninfected roots only
		P. whitei	abundant	complete	smooth	light brown	10 - 15	periepidermal	radially elongate	uninfected roots only
		Labyrinthomyces sp.	moderate	complete	smooth	white	10 - 15	paraepidermal	normal	uninfected roots only
		A. grisea	few	complete	smooth	light yellow	20 - 25	periepidermal	radially elongate	uninfected roots only

Table 6

SUMMATION BY FUNGUS OF THE SHEATH FEATURES OF THE MYCORRHIZAS EXAMINED

FUNGUS	HOST	PLANT FORM	MYCORRHIZA ABUNDANCE	SHEATH FORM	SHEATH SURFACE	COLOUR	SHEATH THICKNESS (µm)	HARTIG NET	EPIDERMAL CELLS	ROOT HAIRS
L. ohiensis	P. microphylla	herb	abundant	patches	free hyphae	olivacious to grey	10 - 65	absent	swollen cells	over all
	A. tomentosus	herb	abundant	patches	free hyphae	opaque brown	6 - 10	absent	normal	over all
	W. citrina	herb	abundant	partial	free hyphae	light yellow	6 - 12	absent	normal	over all
	P. obovata	shrub	abundant	complete	free hyphae	light yellow	15 - 25	periepidermal	radially elongate	uninfected roots only
	G. latifolium	shrub	abundant	complete	free hyphae	yellowish brown	20 - 30	periepidermal	normal	uninfected roots only
	E. maculata	tree	abundant	complete	free hyphae	creamy white	20 - 50	paraepidermal	normal	uninfected roots only
P. whitei	P. microphylla	herb	abundant	partial	free hyphae	light grey	3 - 15	periepidermal	normal	over all
	A. tomentosus	herb	abundant	absent	free hyphae	brownish yellow	absent	periepidermal	normal	over all
	W. citrina	herb	moderate	patches	free hyphae	olivacious brown	5 - 15	periepidermal	normal	over all
	P. obovata	shrub	abundant	complete	free hyphae	brownish yellow	10 - 20	periepidermal	radially elongate	uninfected roots only
	E. maculata	tree	abundant	complete	smooth	light brown	10 - 15	periepidermal	radially elongate	uninfected roots only
Labyrinthomyces sp.	P. microphylla	herb	moderate	partial	free hyphae	light yellowish brown	20 - 25	paraepidermal	swollen cells	over all
	A. tomentosus	herb	few	patches	free hyphae	light brown	30 - 40	paraepidermal	normal	over all
	S. graminifolium	herb	few	patches	smooth	light brown	3 - 6	periepidermal	normal	uninfected roots only
	P. obovata	shrub	few	partial	free hyphae	yellowish brown	4 - 5	periepidermal	normal	over all
	E. maculata	tree	moderate	complete	smooth	white	10 - 15	paraepidermal	normal	uninfected roots only

white or a light colour but as mycorrhizas aged it usually became darker. As all the fungi had hyaline hyphae the colour of mycorrhizas could have been from the refraction of light from different textures of the mycorrhizas as they matured together with any colour produced in the roots themselves.

The abundance of mycorrhizas formed on the roots of different hosts was relatively constant for different fungi. The abundance of mycorrhiza on the roots was a subjective term and could well have been improved, especially when comparing large and small root systems, by making quantitative measurements.

The complete fungal ensheathing of roots and rootlets, usually from point of branching to the root apex is considered a distinctive characteristic of an ectomycorrhiza (Harley, 1959). The classical ectomycorrhizal association has the fungus forming an organized or uniform interweaving of fungal mycelium on the surface of the root and easily distinguishable from the mycelium of a saprophytic fungus on the root surface. The ectomycorrhizas of E. maculata generally conformed to this type. But the roots of the herbs studied were not found to be completely ensheathed and root apices were never enclosed by a fungal sheath - though this has been reported for an unidentified Ascomycete on Podolepis rugata Labill. (Warcup and McGee, 1983). It is suggested that generally the roots of the herbs grew quicker than the fungi and stayed ahead of the development of the fungal sheath. The presence of root hairs with the fungal sheaths also suggests root development before sheath formation.

The partial sheaths or patches of sheath that formed on the roots of the herbs developed from hyphae in the epidermal cell junctions. These hyphae branched to ensheath a few large areas of epidermal cells.

The association A. tomentosus with P. whitei had no sheath but had a well developed periepidermal Hartig net with organized fungal tissue present on the surface of the cortical cells below the epidermis. Patches did not appear to follow any particular order or to form on one part of a root in preference to another.

The thickness of the fungal sheath varied greatly in different host-fungus combinations, from 3 µm (a one-celled sheath) to 50 µm. Reasons for the variation in thickness are not immediately apparent. The thickness of the sheath did not vary greatly between younger areas of colonization compared with that at the point of inoculation. Any variation seen was usually of one hyphal cell thickness. There was often great variation in the thickness of the sheaths produced by the same fungus on different hosts, ie. L. ohiensis on Angianthus tomentosus or Eucalyptus maculata, or Labyrinthomyces sp. on A. tomentosus or P. obovata (Table 6).

The fungal sheaths of mycorrhizas of E. maculata were formed of a compact irregular synenchyma tissue which is a tight knit tissue in which interhyphal spaces were absent or difficult to see. In contrast, the mycorrhiza of the herbs had, either as the outermost layer of tissue or throughout the sheath, prosenchyma tissue, a loose fungal tissue with large interhyphal spaces. The mycorrhizas of P. microphylla with L. ohiensis, P. microphylla with Labyrinthomyces sp., W. citrina with L. ohiensis and A. tomentosus with Labyrinthomyces sp. had in part a layer of tissue forming on the epidermal cell surface which was made up of a compact irregular synenchyma suggesting that in the patches where colonization had occurred, conditions existed for a strongly compatable symbiotic association.

The intercellular penetration of the epidermal cells, and in some

cases cortical cells, is described as a Hartig net. With most host-fungus associations there was either no Hartig net, with the fungus only occurring in the epidermal cell junctions, or the Hartig net was limited to between epidermal cells. An exception was G. latifolium where Hartig net also occurred between outer cortical cells. This is unusual as, outside the Pinaceae, Hartig nets are usually confined to between epidermal cells. Whether paraepidermal or periepidermal penetration occurred seemed to be a host-fungus character not a fungal one (Table 6).

Harley (1959) considered that hypertrophy of the epidermal cells was an important criterion of a mycorrhizal association giving an increased area of contact between host and fungus. While radially elongate epidermal cells are common in Eucalypt mycorrhizas (Chilvers, 1968) they were only seen in one of the mycorrhiza, E. maculata with P. whitei examined here. They also occurred in Pultenaea obovata with L. ohiensis or P. whitei. No radially elongate epidermal cells were seen in any of the mycorrhizas of the herbs.

DEVELOPMENTAL STUDIES AND GROWTH RESPONSES OF SOME ECTOMYCORRHIZAL HERBS

Little attention has been given to the development of ectomycorrhizas over time. Chilvers and Gust (1982 a) recorded quantitative changes in the number and postion of ectomycorrhizas on roots of Eucalyptus st-johnii. Successive observations of colonized roots showed that the formation of patches of fungal tissue preceded the formation of completely ensheathed roots. In a subsequent study of ectomycorrhiza formation of E. st-johnii, Chilvers and Gust (1982 b) showed that the quicker growing first and second order roots usually exceeded the growth rate of the fungus, but the slower growth and the later appearance of tertiary roots meant that most were converted to mycorrhizas. Sohn (1981), working with Pinus resinosa Ait. observed negative correlations between the growth rate of long root mycorrhizas and the extent of infection. He suggested a threshold growth rate of long roots above which mycorrhiza formation was progressively inhibited and this inhibition would produce less and thinner Hartig net. At root growth rates below the threshold, Hartig net could develop throughout the mycorrhiza.

In this study, the development and distribution of ectomycorrhizas of some herbs was followed as was the growth response to inoculation with ectomycorrhizal fungi. Root systems were sampled at 6, 10 and 14 weeks to determine a sequence of mycorrhiza development and to observe the morphological consistency of ectomycorrhizas on roots of different ages and orders. The relative growth rate of roots of different orders and its influence on ectomycorrhizal formation was ascertained. Plant

tops were also harvested to record any incremental growth response to inoculation.

Species of which sufficient seed was available were used for quantitative assessements. Five replicates of each combination were inoculated and grown as previously. On harvesting each root system was washed out and placed in its entirety on a large glass slide, 20 x 10 cm, to facilitate the observation and counting of mycorrhizas and uninfected root tips. Roots or rootlets that broke off from the root system (1-5 percent of the total number of roots) were replaced either to the root from which they had become detached or matched to incomplete roots. Orders of mycorrhizas and the number in each order as well as total numbers were recorded. Plant tops were dried in a 90°C oven for 48 hours and then weighed.

DISTRIBUTION OF MYCORRHIZAS ON ROOT SYSTEMS

Distribution of the mycorrhizal roots of all plant species followed a similar pattern. A radial progression of colonization of roots from the point of inoculation outwards with acropetal spread of colonization along the length of developing second order roots, to the colonization of higher order branches. Some higher order root branches were seen that were colonized but the supporting branch was non-mycorrhizal. Here the development of mycorrhizas would be the result of root growth near another colonized root or through growth of the fungus in soil.

The results of two associations Poranthera microphylla and Angianthus tomentosus both inoculated with Laccaria ohiensis are discussed here representing two developmental and distribution trends of mycorrhiza formation and different growth responses. Of the other

associations, A. tomentosus, P. microphylla and W. citrina inoculated with P. whitei and W. citrina with L. ohiensis and P. microphylla with Labyrinthomyces sp. were similar to P. microphylla with L. ohiensis. A. tomentosus with L. ohiensis represented the other trend. These results are given in Appendices III and IV.

The percentage of uncolonized roots of different orders compared with the percentage of roots of those orders which are mycorrhizal for P. microphylla with L. ohiensis are recorded in Fig. 33 and for A. tomentosus with L. ohiensis in Fig. 34. The root system of P. microphylla was relatively large. At 6 weeks second and third order roots were common but higher order roots were present only in small numbers. However, at ten and fourteen weeks higher order roots were present and a high percentage were mycorrhizal. The bulk of roots were third order at all three sampling times.

Figure 34 of A. tomentosus with L. ohiensis indicates a much smaller root system with only a few third or fourth order roots appearing by the later sampling times. The high percentage of mycorrhizal roots and the fact the percentage remained high throughout the experiment suggests a strong compatability between fungus and host.

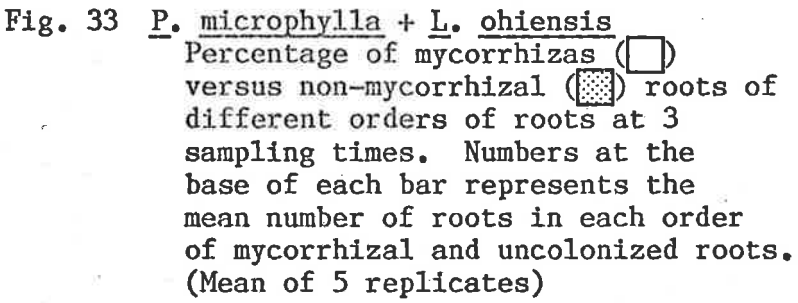


Fig. 33 P. microphylla + L. ohiensis
Percentage of mycorrhizas (□) versus non-mycorrhizal (▒) roots of different orders of roots at 3 sampling times. Numbers at the base of each bar represents the mean number of roots in each order of mycorrhizal and uncolonized roots. (Mean of 5 replicates)

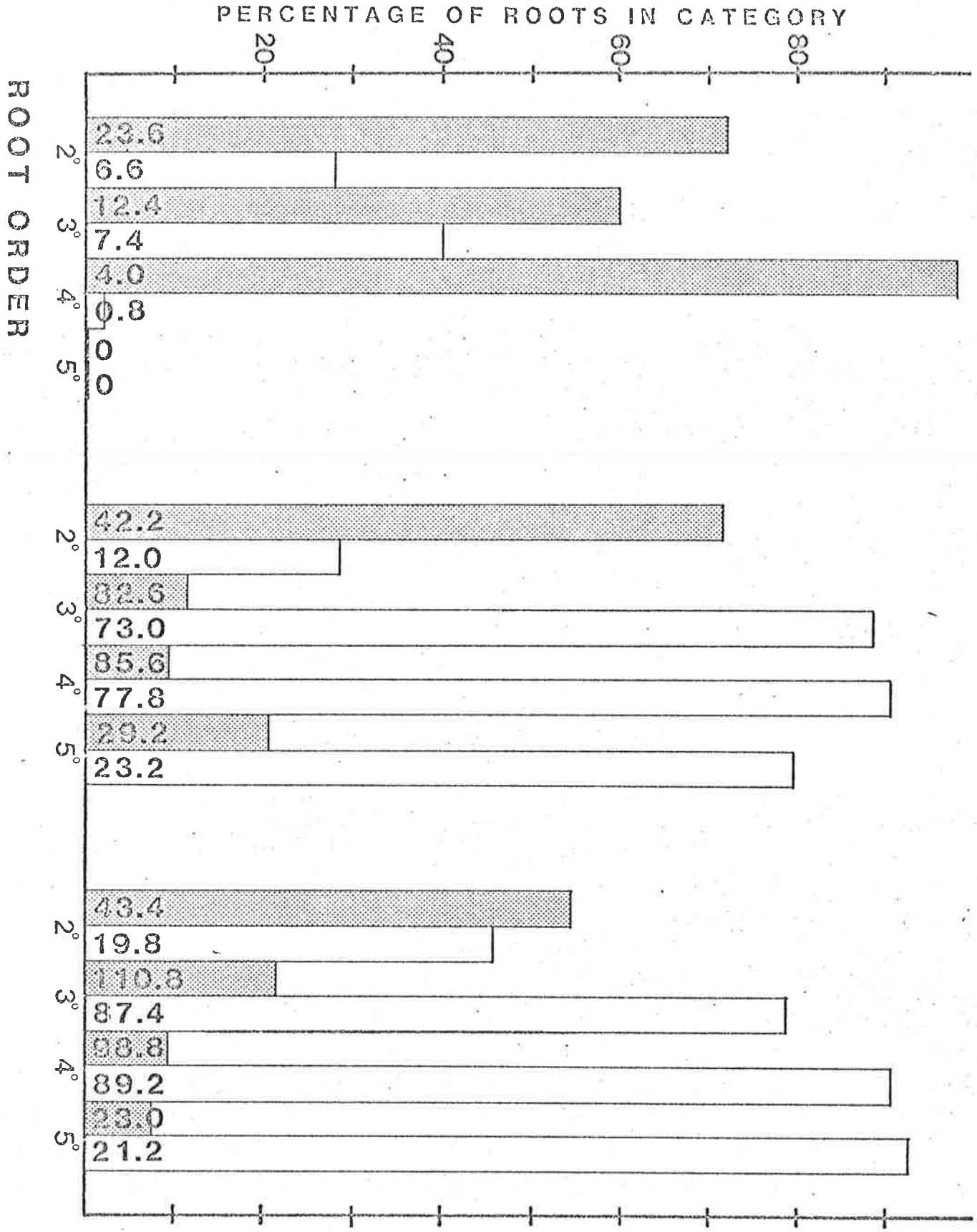
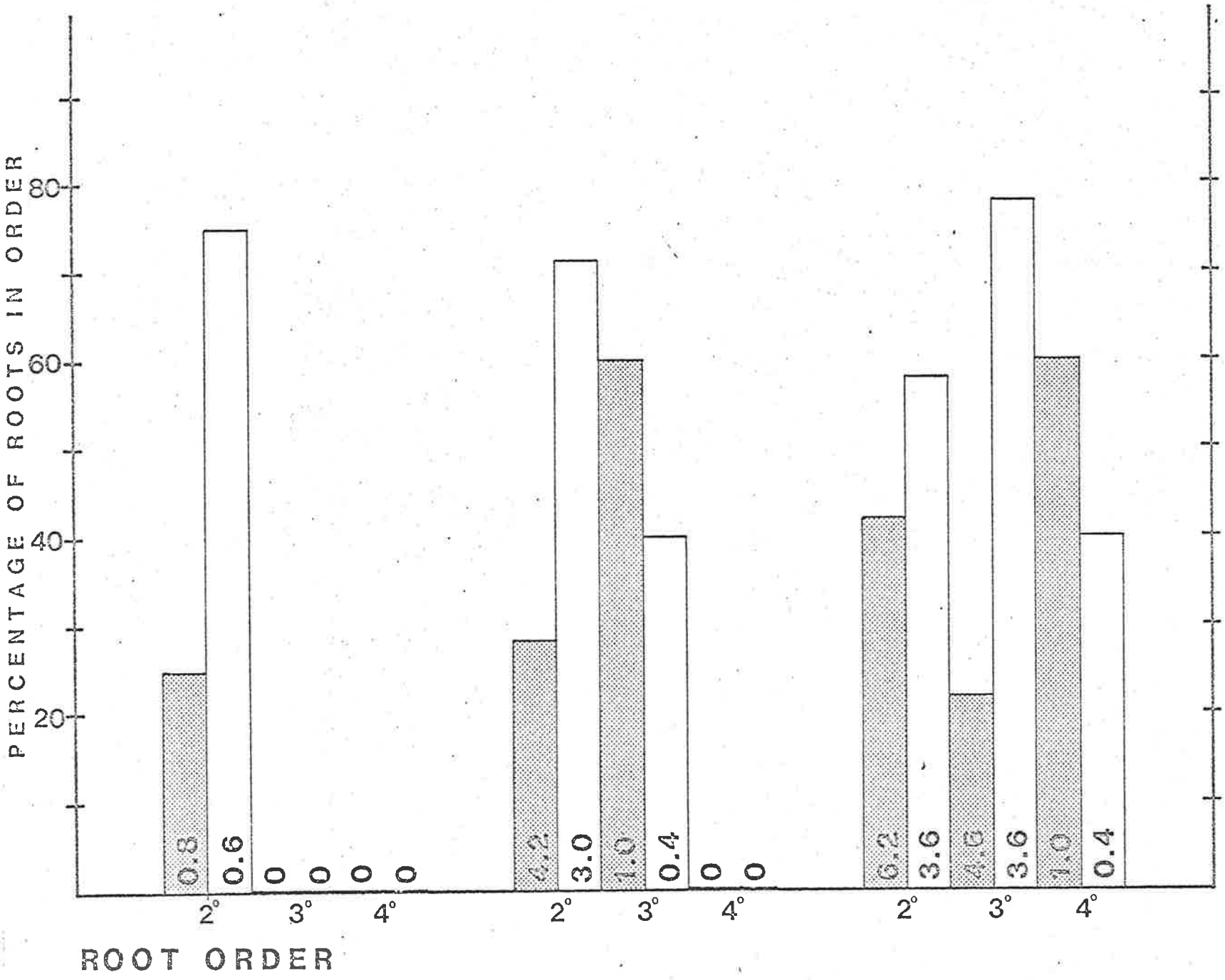

PERCENTAGE OF ROOTS IN CATEGORY
20
40
60
80
ROOT ORDER
2°
3°
4°
5°
23.6
6.6
12.4
7.4
4.0
0.8
0
0
42.2
12.0
82.6
73.0
85.6
77.8
29.2
23.2
43.4
19.8
110.8
87.4
98.8
89.2
23.0
21.2

Fig. 34 A. tomentosus + Labyrinthomyces sp.
Percentage of mycorrhizas (□) versus non-mycorrhizal roots (▨) of different orders of roots at 3 sampling times. Numbers at the base of each bar represents the number of roots in each order of mycorrhizal and non-mycorrhizal roots. (Mean of 5 replicates)


PERCENTAGE OF ROOTS IN ORDER
80
60
40
20
0.8
0.6
0
0
0
0
4.2
3.0
1.0
0.4
0
0
6.2
3.6
4.5
3.6
1.0
0.4
2°
3°
4°
2°
3°
4°
2°
3°
4°
ROOT ORDER

GROWTH RESPONSES OF MYCORRHIZAL PLANTS

Table 7 records the top dry weights of the combinations used over the three sampling times. All associations, except A. tomentosus with L. ohiensis, showed significant growth differences ($p < 0.001$, $p < 0.05$) compared with their respective uninoculated plants, indicating a positive growth response. Figures 35 and 36 are of compatible plant-fungus combinations, but with different growth responses. The values of the mean of 5 replicates at each sampling time of the top dry weight is represented by the solid triangles and the data transformed to the logarthmic value is represented by open circles with the corresponding line of best fit.

P. microphylla with L. ohiensis (Fig. 35) illustrates a positive growth response. Shoot dry weight between 6 and 10 weeks increased, with an exponential increase between 10 and 14 weeks indicating an active stage of plant growth. The inoculated plants differed significantly ($p < 0.001$, Table 7) from the uninoculated plants. Visual observation of the replicates of inoculated and uninoculated plants showed large differences in shoot height and size. A. tomentosus with L. ohiensis (Fig. 36) illustrates no significant difference, (Table 7) in growth response as compared to uninoculated plants. The shoot dry weight curve for the inoculated plants had values equalling those of the uninoculated plants at 6 and 10 weeks with a slight increase in top dry weight at 14 weeks and there was no significant difference in top dry weights (Table 7).

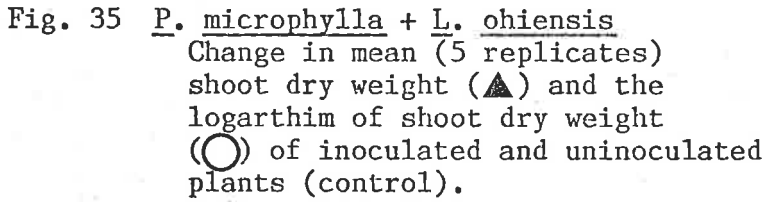


Fig. 35 P. microphylla + L. ohiensis
Change in mean (5 replicates) shoot dry weight (▲) and the logarithim of shoot dry weight (◯) of inoculated and uninoculated plants (control).

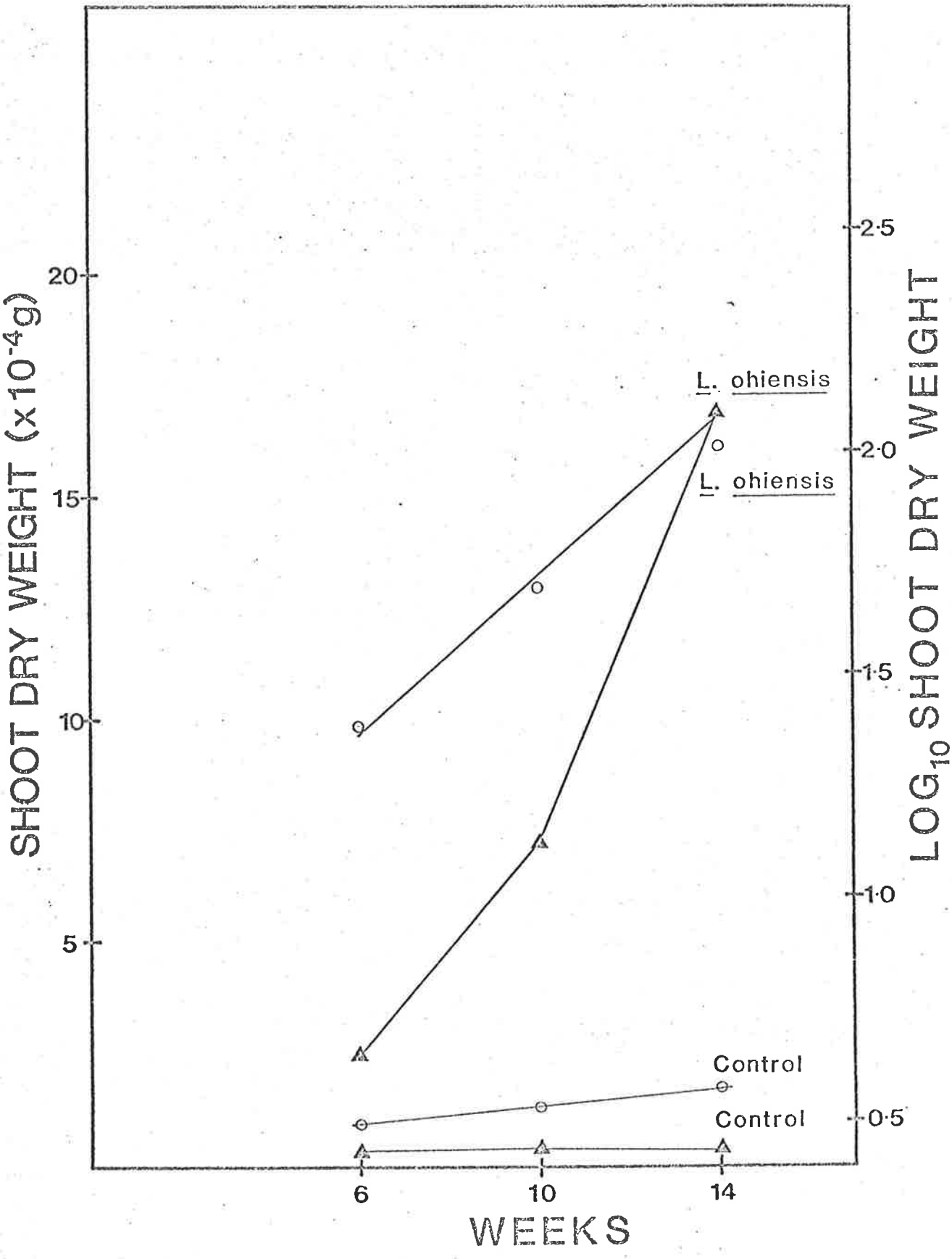

SHOOT DRY WEIGHT (x10⁻⁴g)
LOG10 SHOOT DRY WEIGHT
20
15
10
5
2·5
2·0
1·5
1·0
0·5
L. ohiensis
L. ohiensis
Control
Control
6
10
14
WEEKS

FIG. 36 A. tomentosus + L. ohiensis
Change in mean (5 replicates) shoot dry weight (▲) and the logarthim of shoot dry weight (◯) of inoculated and uninoculated plants (control).

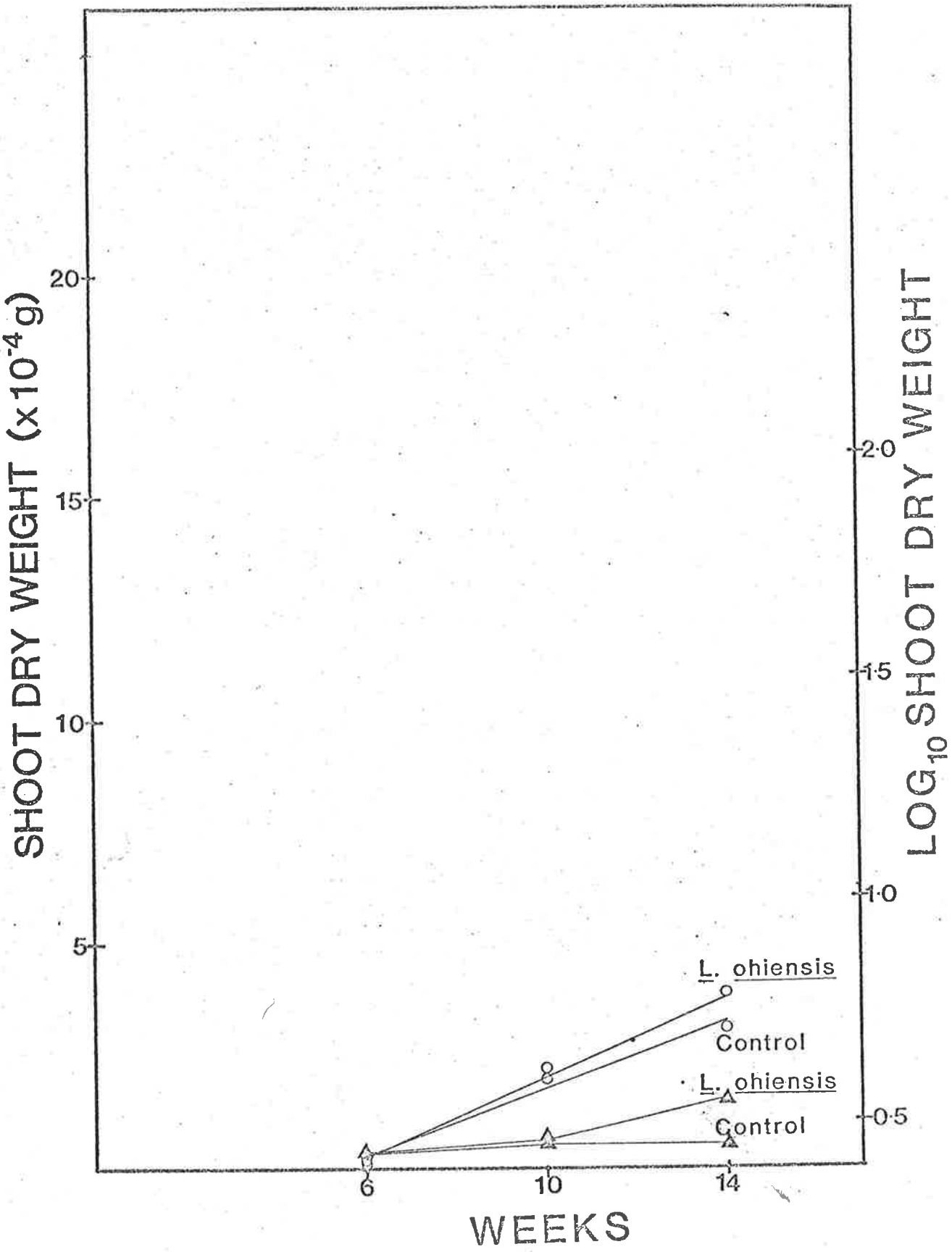

SHOOT DRY WEIGHT (x10⁻⁴ g)
LOG10 SHOOT DRY WEIGHT
20
15
10
5
2·0
1·5
1·0
0·5
L. ohiensis
Control
L. ohiensis
Control
6
10
14
WEEKS

Table 7

Effect of inoculation with ectomycorrhizal fungi on top dry weight of various herbs at 3 sampling times (mean of 5 replicates)

Plant	Inoculation	Top dry weight ($x10^{-4}$ g) Sampling time (weeks) 6	10	14	
P. microphylla	*L. ohiensis*	24.9	76.4	159.0	A
	P. whitei	19.5	79.0	133.7	A
	Labyrinthomyces sp.	17.9	16.4	33.5	B
	Uninoculated	4.6	3.9	5.5	
W.citrina	*L. ohiensis*	18.3	23.2	226.1	B
	P. whitei	74.2	172.3	449.3	A
	Uninoculated	5.2	4.8	4.9	
A. tomentosus	*L. ohiensis*	3.5	4.0	15.5	N.S.
	P. whitei	98.7	177.4	249.3	B
	Uninoculated	2.6	5.5	5.2	

Significant differences A ($p<0.01$)
B ($p<0.05$)
N.S. Not significant

DISCUSSION

An advantage of mycorrhizal observation in a controlled environment system, ~~in the study of mycorrhizas,~~ is that most stages of early root growth are present. Thus the sequence of root initiation, elongation and maturation may be studied by sequential sampling. The observations made have showed no differences in mycorrhizal sheath or Hartig net development of the mycorrhizal plants at 6 weeks compared with those examined at 14 weeks. This was interpreted to mean that under the conditions employed the associations formed were consistent over the sampling times and over the orders of roots that were formed by the seedlings. Whether this consistency would extend throughout the life of the plant, especially when mature, remains uninvestigated.

A growth response, shown by the increase in top dry weight as compared to an uninoculated plant remains one of the most striking and effective methods of demonstrating a beneficial plant-fungus combination.

That the associations which had partial sheaths or patches of sheath , with the exception of Angianthus tomentosus + L. ohiensis, showed significant growth responses is further evidence that they are part of a spectrum of associations that constitute ectomycorrhizas. It is to be noted that even the association without a sheath (A. tomentosus + Peziza whitei) gave a highly significant growth response (Table 7).

Why A. tomentosus + L. ohiensis did not show a growth response is not known. It may be that the growth conditions were unsuitable for that host-fungus combination. Certainly L. ohiensis gave positive growth responses on other herbs such as Waitzia citrina and A. tomentosus gave a growth response with Peziza whitei so that there is no intrinsic reason why fungus or host should not give a growth response.

GENERAL DISCUSSION

The associations considered in this preliminary study represent some of the variations that may be considered as ectomycorrhizas. The main features of an ectomycorrhiza are considered to be; presence of a fungal sheath, presence of a Hartig net, and generally absence of intracellular penetration of host cells. In the associations examined here, some lacked a Hartig net, one lacked a fungal sheath, but none showed intracellular growth of hyphae. In this they differed from the ectendomycorrhizas of Pinus sylvestris described by Mikola and Laiho (1964) that have a thin or absent fungal sheath, a coarse Hartig net and intracelluar penetration of cells. Similarly the pseudomycorrhizas of Melin which had no or a thin sheath, no Hartig net but some intracellular hyphae (Harley and Smith, 1983) would appear a different kind of association.

One of the major differences between the mycorrhizas studied has been in the amount and continuity of sheath formed. Laiho (1965), Chilvers and Pryor (1965), Rambelli (1973) and Nylund and Unestam (1983) observed a progression of sheath formation from an initial incomplete sheathing of the root followed shortly by the complete enveloping of the root. Observations made of the mycorrhiza of the herbs did not show the same progression through to the complete ensheathing of roots.

The formation of hyphal strands or rhizomorphs has been considered to be similar to the formation of a mycorrhizal sheath (Harley and Smith, 1983). Watkinson (1979) described the formation of hyphal strands as follows: a leading hypha will exude nutrients as it grows through a nutrient deficient zone and its branches and other lateral

hyphae will run parallel to and adhere to the leading hypha . The morphogensis of these hyphal structures includes the differentiation of the hyphae and their adhesion together. The adhesion is often associated with the production of an extra-hyphal fibrillar material analogous to that found in fungal sheaths of ectomycorrhizas (Harley and Smith, 1983). The aggregation of epiphytic mycelium into mycelial sheets (Garrett, 1970) is also dependent upon the exudation of nutrients from a main hypha which encourages lateral growth and binding. This is found to occur in low nutrient areas where the leading hypha is maintained by translocation of nutrients from a distant food source. Nylund (see Harley and Smith, 1983) suggested from the growth sequence of sheath formation after Hartig net formation, that the sheath might be controlled by a sheath-forming factor. The sheath-forming factor would be the result of a fungal-host by-product with specificity towards ectomycorrhiza forming fungi. The formation of incomplete or patches of fungal sheath on the herbs could be seen as insufficient formation of the sheath-forming factor.

Changes in the morphology of mycorrhizal root systems or roots have been attributed to the physical (Clowes, 1951) and hormonal (Slankis, 1973) properties of the colonizing fungus. The roots of classical ectomycorrhiza display a changed branching habit and maturation of root tissue nearer the root tip (Marks and Foster, 1973). Warren-Wilson and Harley (1983) re-examined these structural changes and found that the uninfected root tips of Fagus sylvatica L. had passed through a developmental sequence of a decline in the rate of elongation of the root, decrease in root diameter and the size of the root cap and differentiation of tissue nearer the apex of the root. These morophological changes had previously been thought to be the consequence

of colonization by the fungus. Warren-Wilson and Harley pointed out that some changes to the gross morphology of the roots were probably caused by the presence of the fungus.

Of the changes in morphology of mycorrhizas of Eucalyptus, herbs and shrubs studied, the mycorrhiza of E. maculata, P. obovata and G. latifolium appeared the same as the descriptions given for classical ectomycorrhiza of the northern hemisphere whereas no gross morphological changes of roots of the herbs, colonized by the fungi, were seen.

The same fungus on different hosts formed different types of mycorrhiza with a consistency of type found on the herbs studied here. The herbs appeared to remain the same in providing one nutrient base for fungal colonization with variations noted being a fungal response. The colonization by the fungus of epidermal cells in the formation of a sheath, the layering of fungal tissue (mantle thickness) the shape of the hyphal cells and intercellular penetration (Hartig net) are seen as a fungal response. Swollen epidermal cells and the presence of root hairs is seen as a host response. Warren-Wilson and Harley (1983), state that the formation of a mycorrhiza is a fungal response when in contact with the host and that fungal and host characteristics would determine the sheath type and the basic construction of the host cell.

While the mycorrhizas studied showed variation in morphological form and in their effect on root systems, all but one gave a host growth response hence it is difficult to consider them in any way but as variations of a common association. Thus the benefits of the symbiotic association was expressed as an increase in plant growth with the fungus affecting the uptake of nutrients from the soil. In the simple cultural system used in these studies, a growth response was seen as an effective symbiotic association. Where a growth response was absent, but

compatability between fungus and roots was found in the form of mycorrhizal roots, the nutrient status of the soil was considered insufficient for that particular plant-fungus combination. Experiments on nutrient levels of soil are needed to see if that is the correct explanation.

Further studies of these plant species and others in the same genera and families both in the field and in the laboratory would help elucidate their occurrence and importance of the variations in form of mycorrhizas in the environments in which they are found.

APPENDICES

Appendix I

Formulae for media

NDY/6 (Warcup, 1955)

$NaNO_3$	0.33 g
KH_2PO4	0.16 g
$MgSO_4$, $7H_2O$	0.08 g
KCl	0.08 g
$FeSO_4$, $7H_2O$	0.001g
Yeast Extract (Difco)	0.08 g
Sucrose	5.0 g
Agar	10.0 g
Distilled water	1 l

2% Malt Agar

Malt Extract (Difco)	20.0 g
Agar	15.0 g
Distilled water	1 l

Melin-Norkans modified media (Marx, 1969)

$CaCl_2$	0.05 g
NaCl	0.025g
KH_2PO_4	0.5 g
$(NH_4)_2HPO_4$	0.25 g
$MgSO_4$, $7H_2O$	0.15 g
$FeCl_3$ (1%)	1.2 ml

Appendix I (cont'd)

Thiamine HCl	100µg
Malt Extract (Difco)	3.0 g
Sucrose	10.0 g
Agar	15.0 g
Distilled water	1 l

APPENDIX II

MORPHOLOGY OF THE ECTOMYCORRHYZA OF *PINUS RADIATA* WITH *AMANITA MUSCARIA*

Mycorrhizas appeared a light brown when young to dark brown on older mycorrhiza under reflected light. Mycorrhizal roots were found over the whole of the root system. Completely ensheathed third order roots branching dichotomously formed the bulk of mycorrhizas mainly as short (2 - 3 mm) roots.

Root hairs were absent from mycorrhizas but were present on uncolonized roots. Loose hyphae were seen branching from the surface of the mantle which appeared to be composed mainly of a tightly compacted irregular synenchyma tissue.

In transverse section a fungal mantle of 10 - 25 µm thickness was measured. A Hartig net with periepidermal and cortical cells was present. Longitudinal sections showed radially elongate epidermal cells.

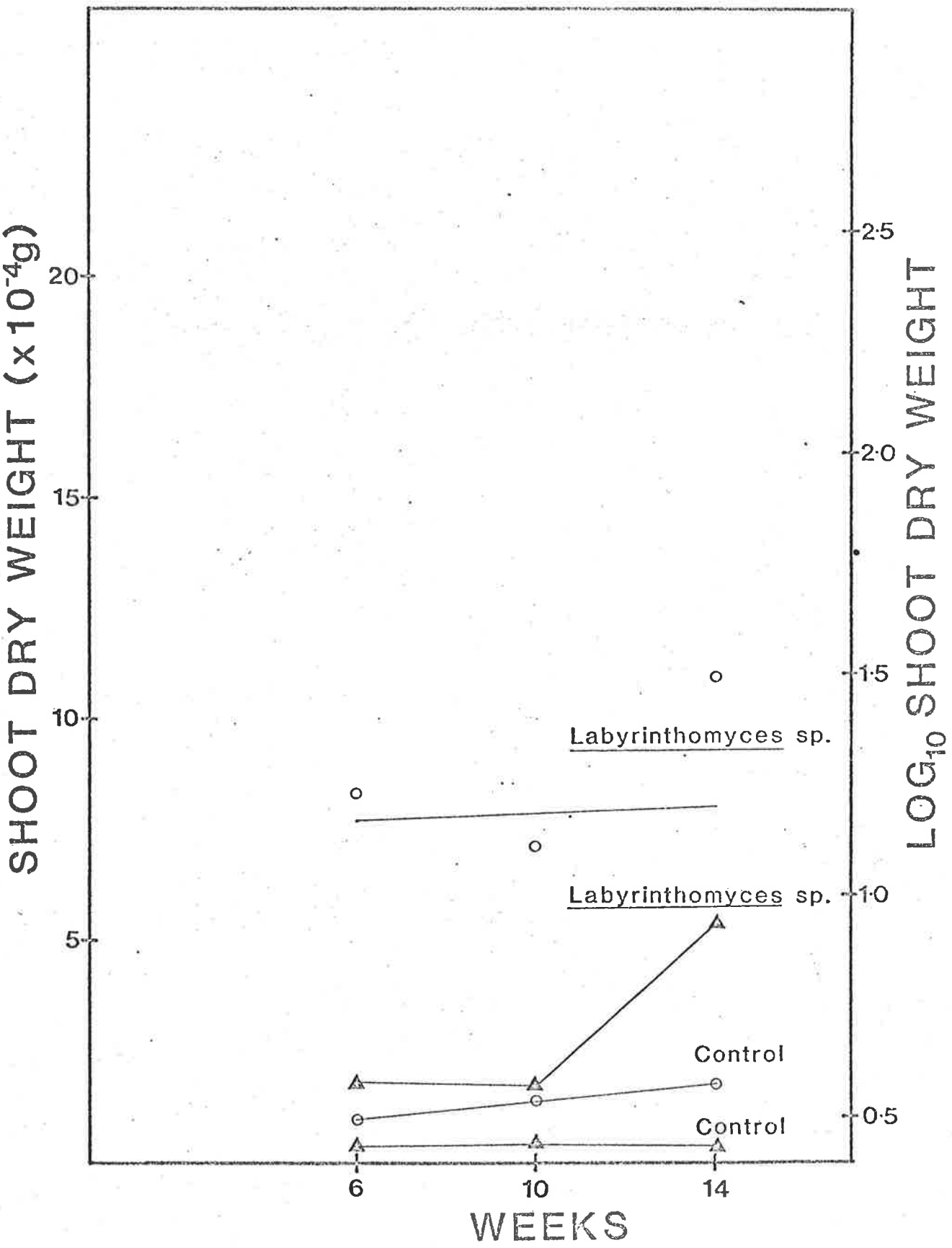
The two fungi *Rhizopogen luteolus* and *Suillus luteus* failed to initiate formation of ectomycorrhiza on *Pinus radiata*. *R. luteolus* has been reported in field observations (Theodorou, 1971, Theodorou and Bowen, 1970, 1973) and in pure culture synthesis (Chilvers, 1973) as forming ectomycorrhiza on *P. radiata*. *S. luteus* in field observations (Theodorou and Bowen, 1970) and in pure culture synthesis (Chu-Chou, 1979) have also been reported. The difficulty experienced with these cultures not forming mycorrhizas could have been cultural synthesis techniques or growth conditions but they were not further investigated.

APPENDIX III

Change in the mean (5 replicates) shoot dry weight (▲) and the logarithm of shoot dry weight (◯) of inoculated and uninoculated plants (control).

IIIA

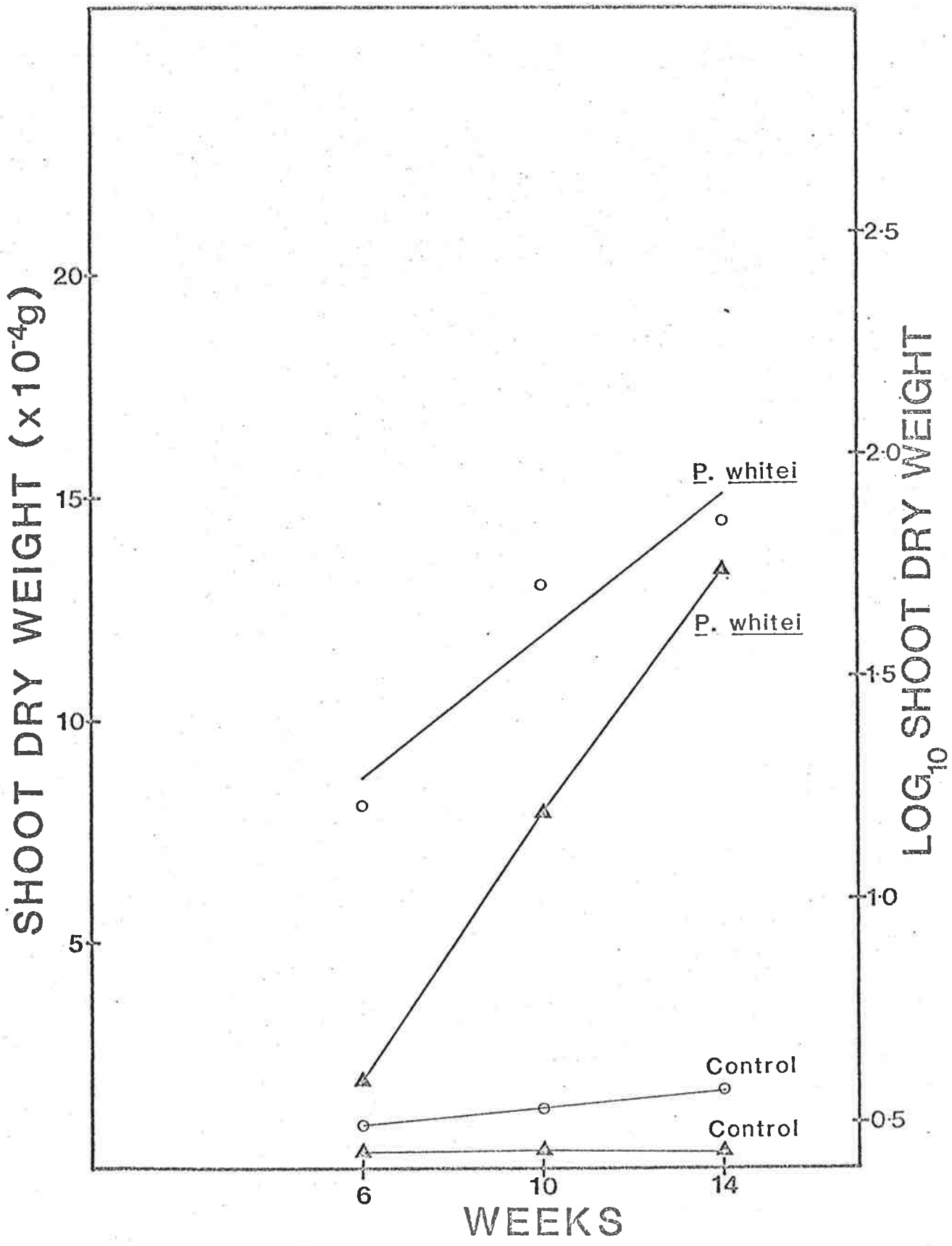
P. microphylla + *Labyrinthomyces* sp.


SHOOT DRY WEIGHT (x10⁻⁴g)
20
15
10
5
2·5
2·0
1·5
1·0
0·5
LOG10 SHOOT DRY WEIGHT
Labyrinthomyces sp.
Labyrinthomyces sp.
Control
Control
6
10
14
WEEKS

Appendix III (cont'd)

IIIB

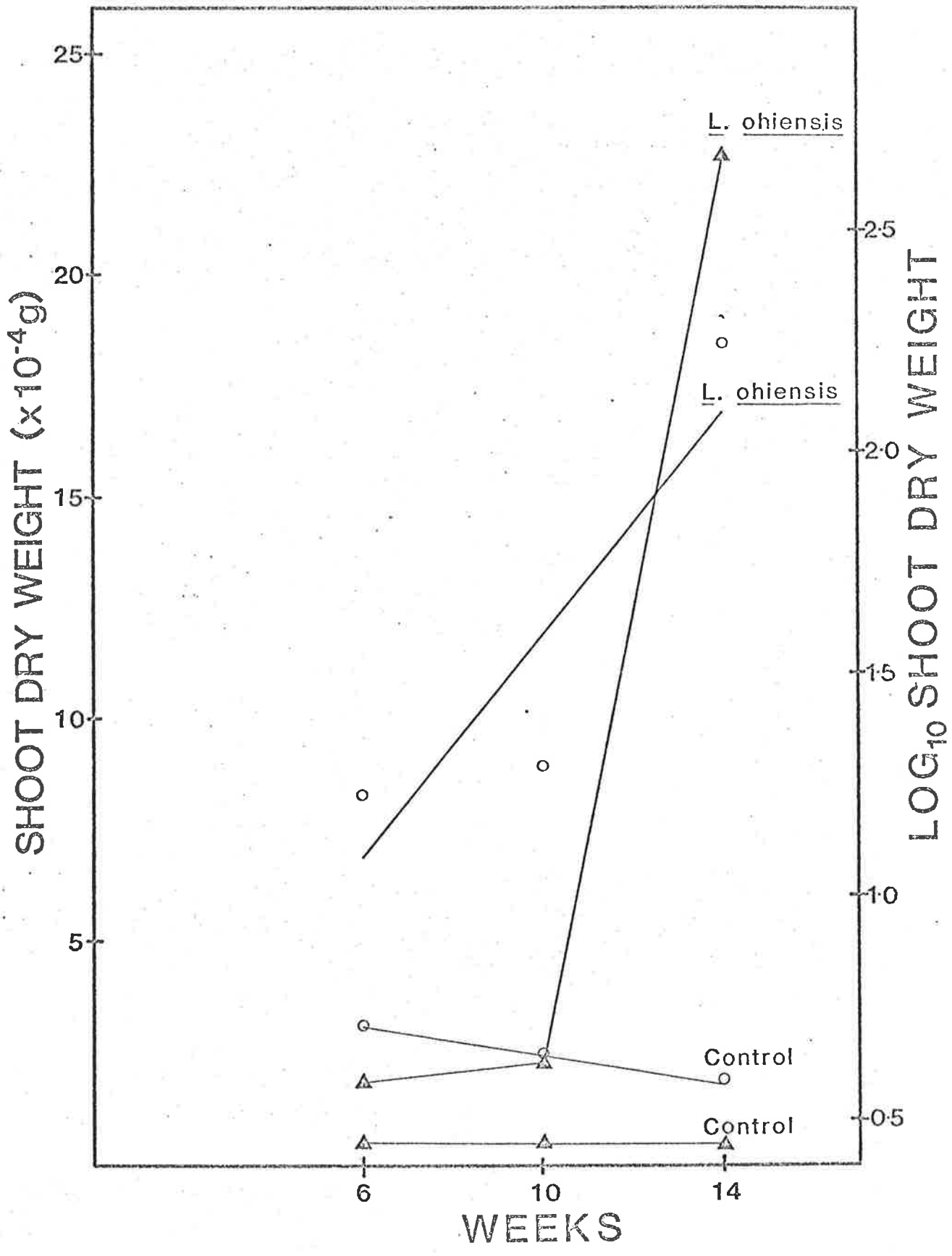
P. microphylla + *P. whitei*


SHOOT DRY WEIGHT (x10⁻⁴g)
LOG10 SHOOT DRY WEIGHT
20
15
10
5
2·5
2·0
1·5
1·0
0·5
P. whitei
P. whitei
Control
Control
6
10
14
WEEKS

Appendix III (cont'd)

IIIC

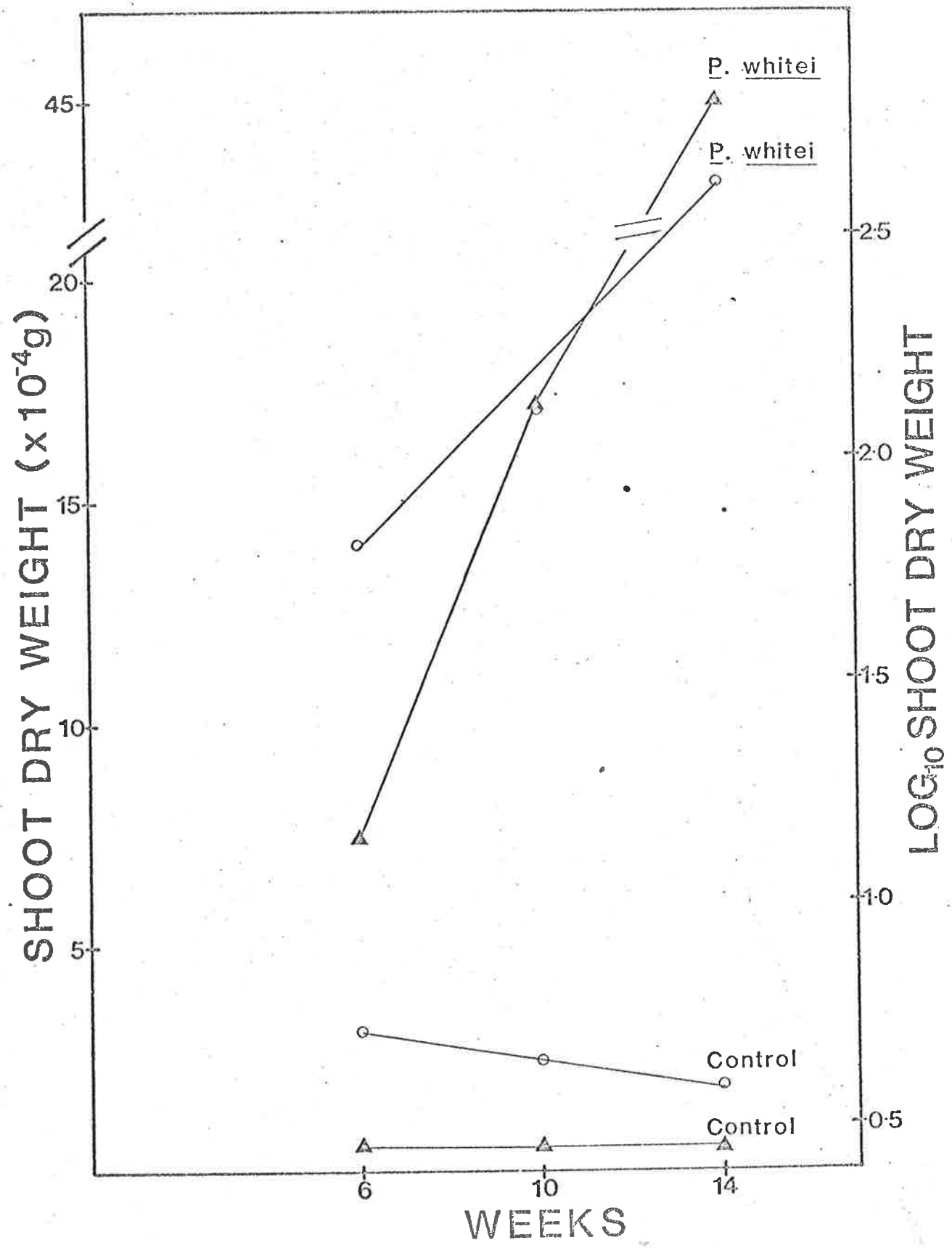
W. citrina + *L. ohiensis*


SHOOT DRY WEIGHT (x10⁻⁴g)
LOG10 SHOOT DRY WEIGHT
25
20
15
10
5
2·5
2·0
1·5
1·0
0·5
L. ohiensis
L. ohiensis
Control
Control
6
10
14
WEEKS

Appendix III (cont'd)

IIID

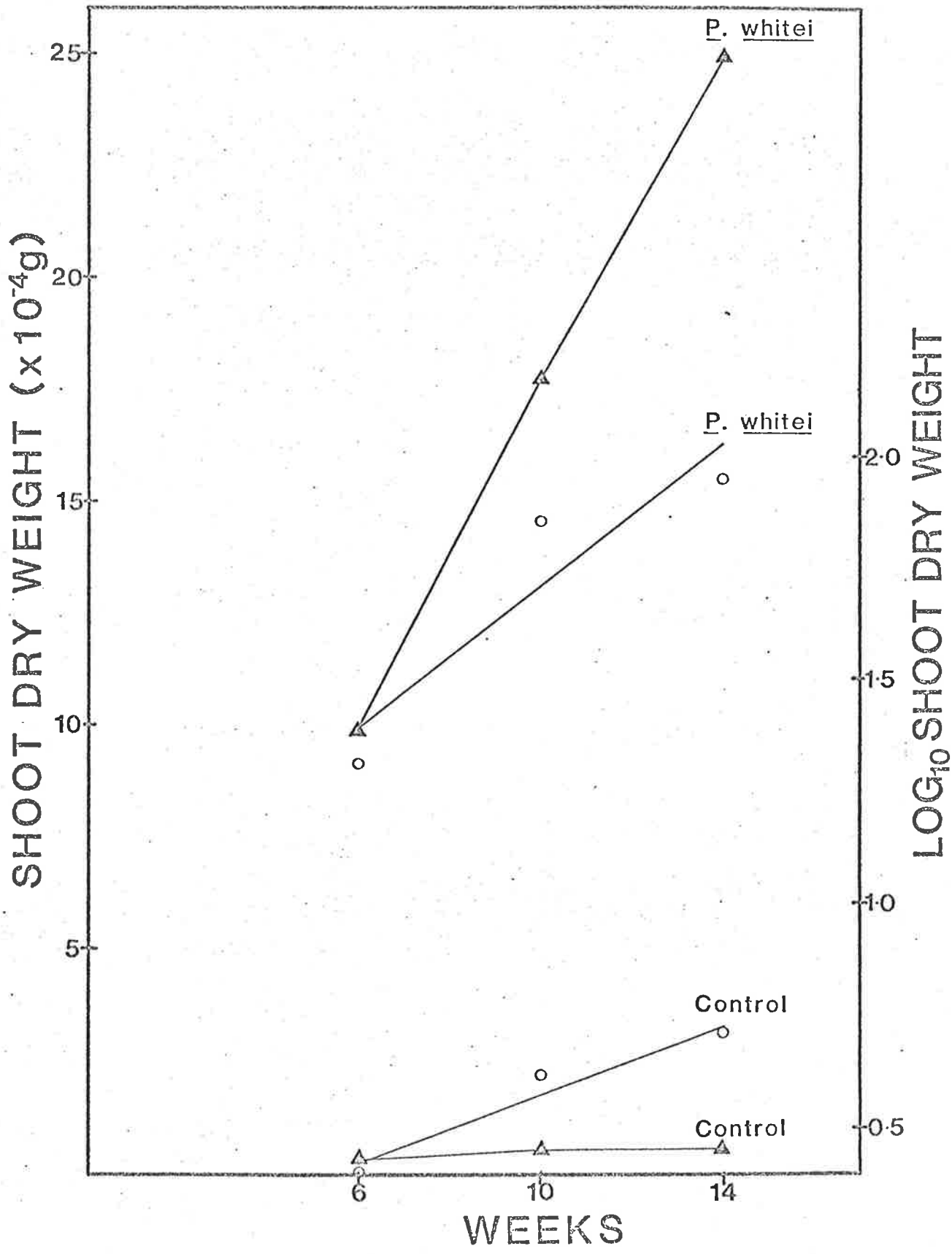
W. citrina + *P. whitei*


P. whitei
P. whitei
Control
Control
45
20
15
10
5
2·5
2·0
1·5
1·0
0·5
6
10
14
WEEKS
SHOOT DRY WEIGHT (x10⁻⁴g)
LOG10 SHOOT DRY WEIGHT

Appendix III (cont'd)

IIIE

A. tomentosus + *P. whitei*

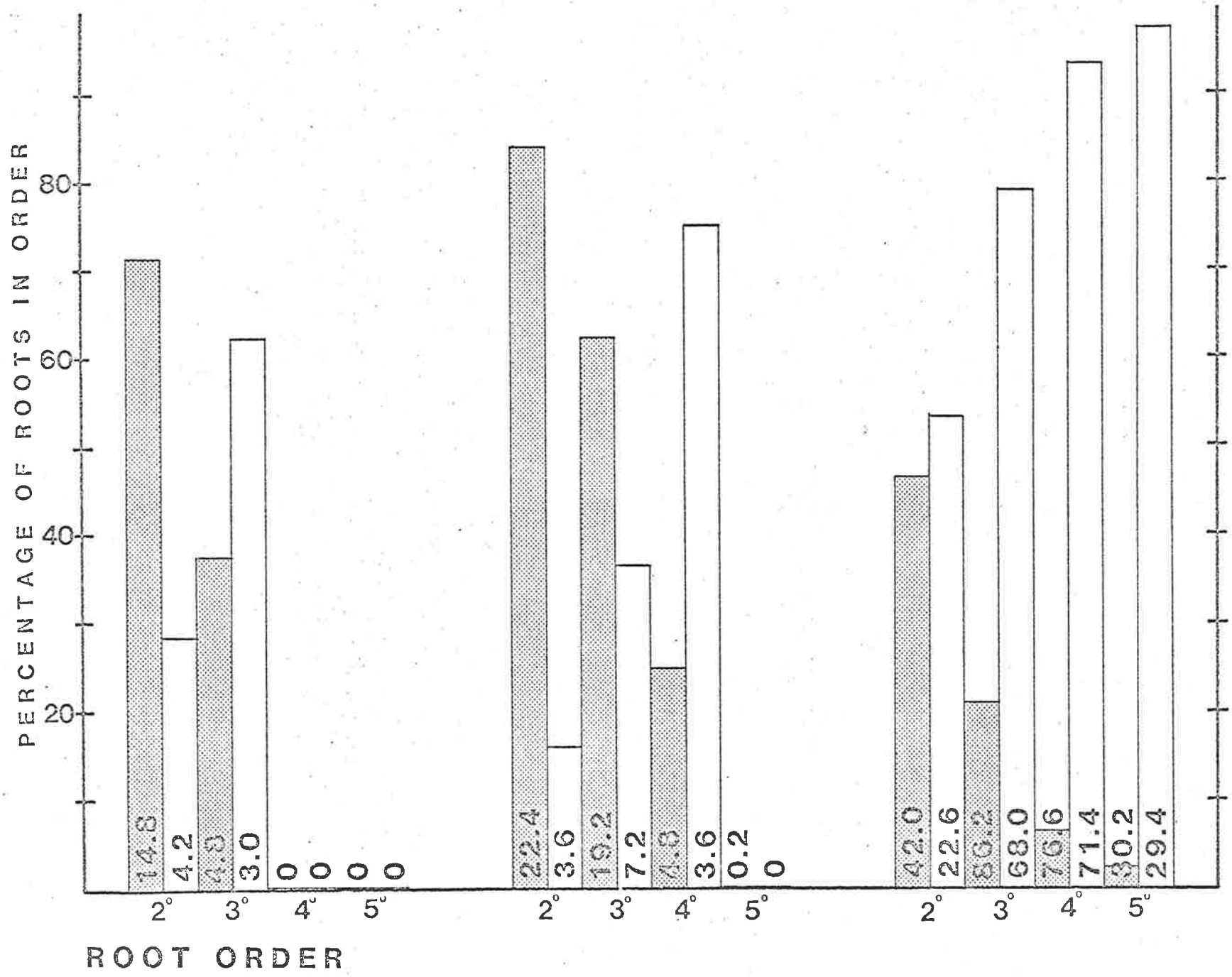

P. whitei
P. whitei
Control
Control
25
20
15
10
5
2·0
1·5
1·0
0·5
6
10
14
WEEKS
SHOOT DRY WEIGHT (x 10⁻⁴g)
LOG₁₀ SHOOT DRY WEIGHT

APPENDIX IV

Percentage of mycorrhizal (□) versus non-mycorrhizal (▨) roots of different orders at three sampling times. Numbers at the base of each bar represents the mean (5 replicates) number of roots in each order of mycorrhizal and non-mycorrhizal roots.

IVA

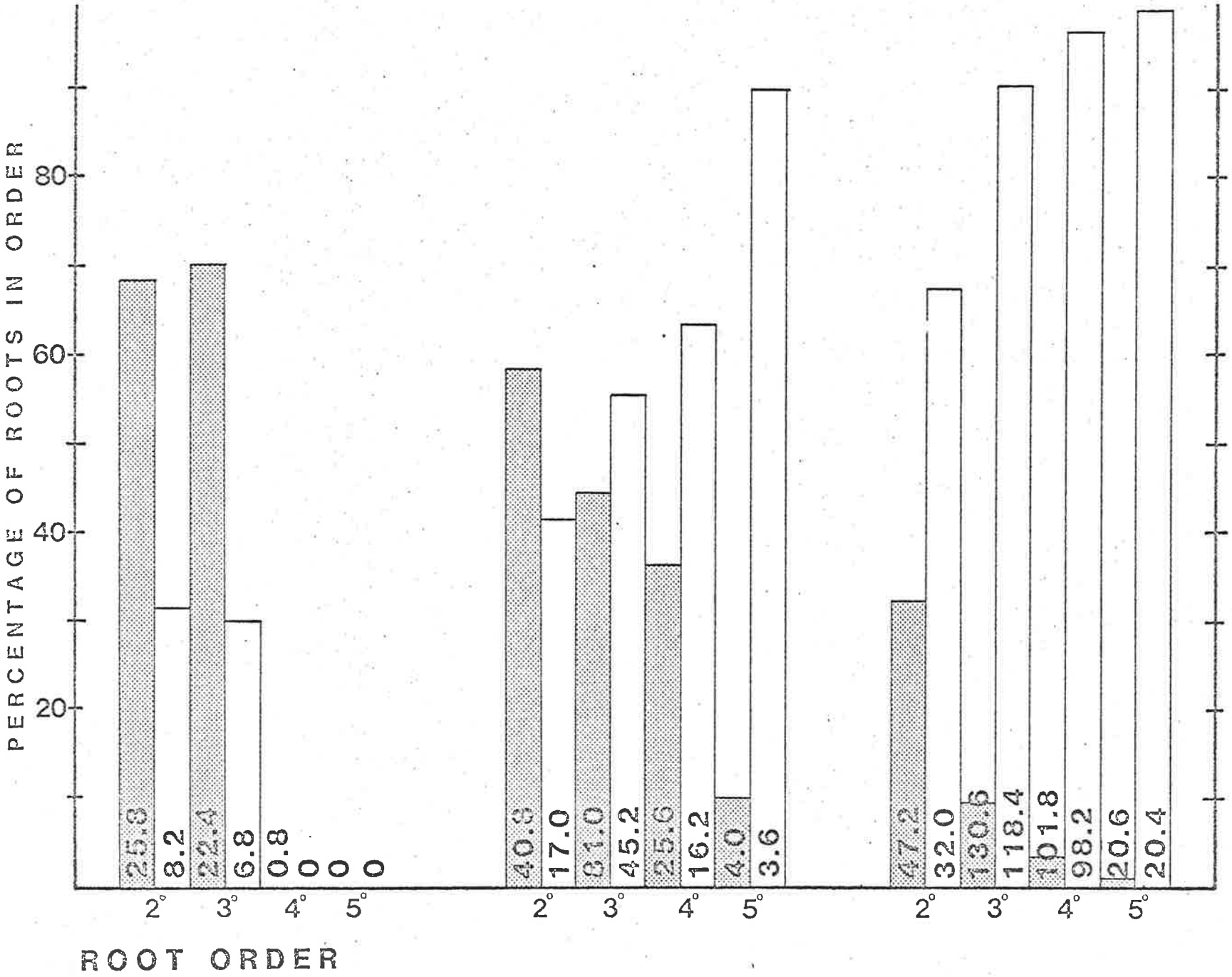
P. microphylla + *Labyrinthomyces* sp.


PERCENTAGE OF ROOTS IN ORDER
80
60
40
20
14.8
4.2
13.3
3.0
0
0
0
0
22.4
3.6
19.2
7.2
3.8
3.6
0.2
0
42.0
22.6
56.2
68.0
76.6
71.4
80.2
29.4
2°
3°
4°
5°
2°
3°
4°
5°
2°
3°
4°
5°
ROOT ORDER

Appendix IV (cont'd)

IVB

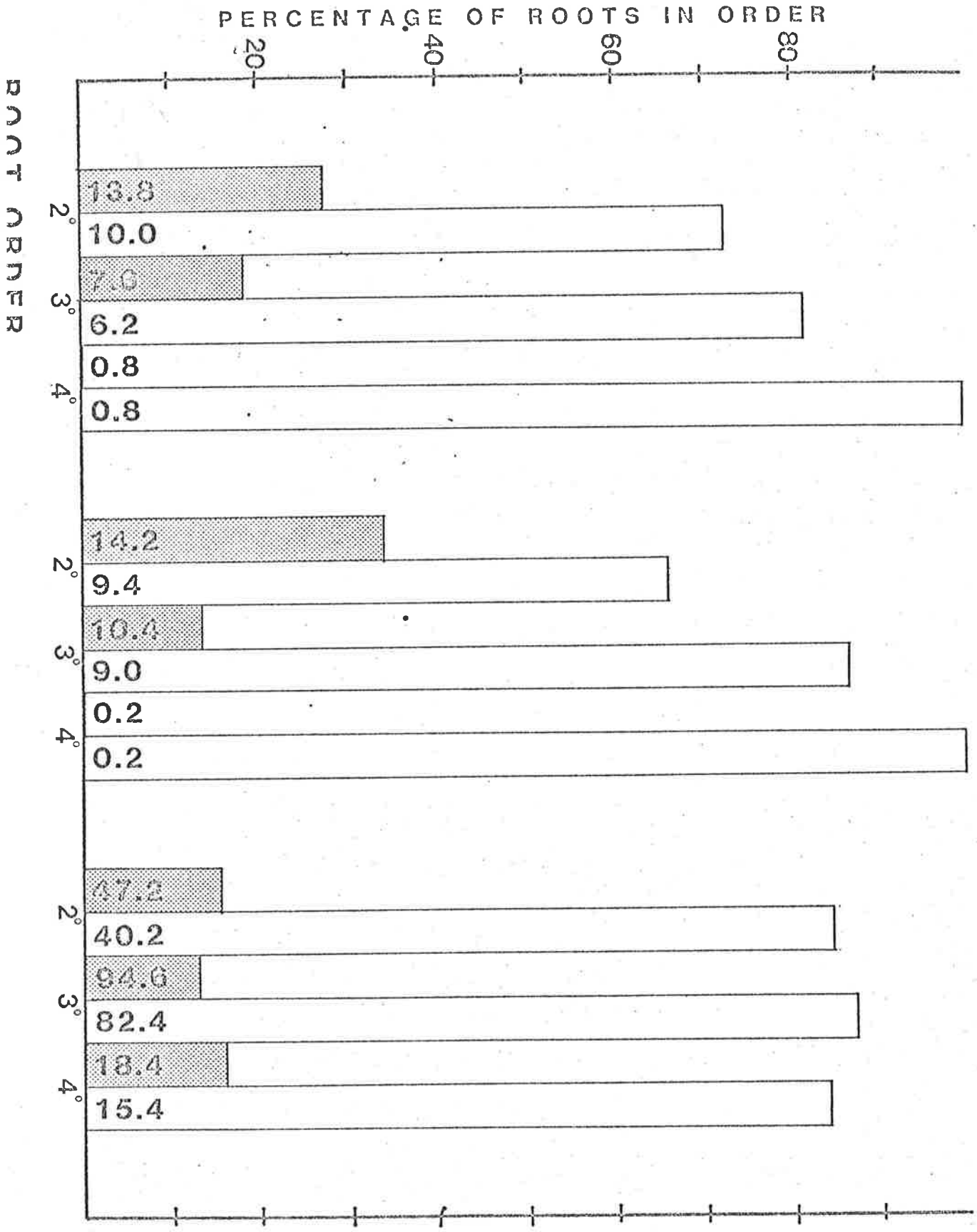
P. microphylla + *P. whitei*


PERCENTAGE OF ROOTS IN ORDER
80
60
40
20
25.3
8.2
22.4
6.8
0.8
0
0
0
40.3
17.0
31.0
45.2
25.6
16.2
4.0
3.6
47.2
32.0
130.5
118.4
101.8
98.2
20.6
20.4
2°
3°
4°
5°
2°
3°
4°
5°
2°
3°
4°
5°
ROOT ORDER

Appendix IV (cont'd)

IVC

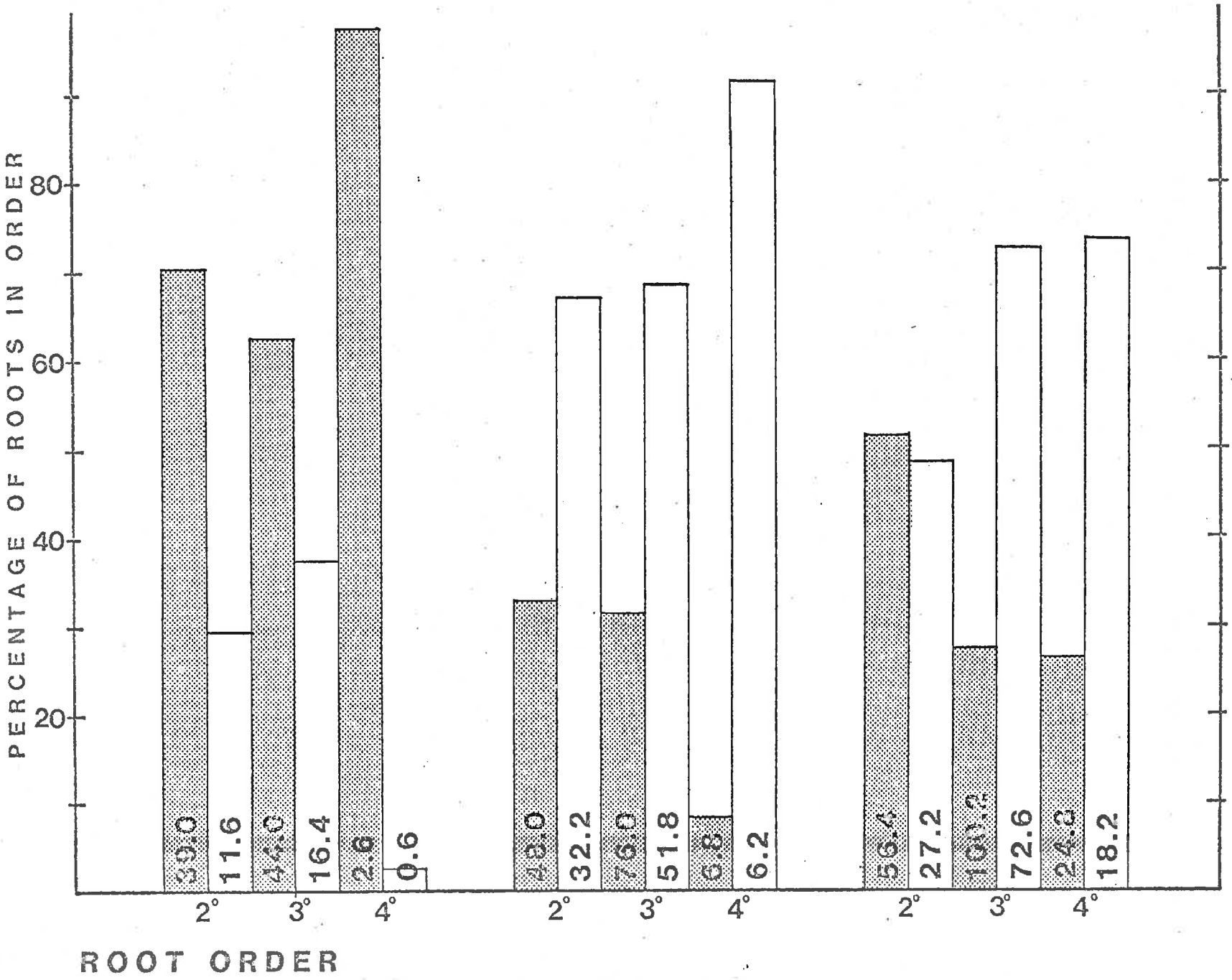
W. citrina + *L. ohiensis*


PERCENTAGE OF ROOTS IN ORDER
20
40
60
80
ROOT ORDER
2°
3°
4°
13.8
10.0
7.0
6.2
0.8
0.8
14.2
9.4
10.4
9.0
0.2
0.2
47.2
40.2
34.6
82.4
18.4
15.4

Appendix IV (con't)

IVD

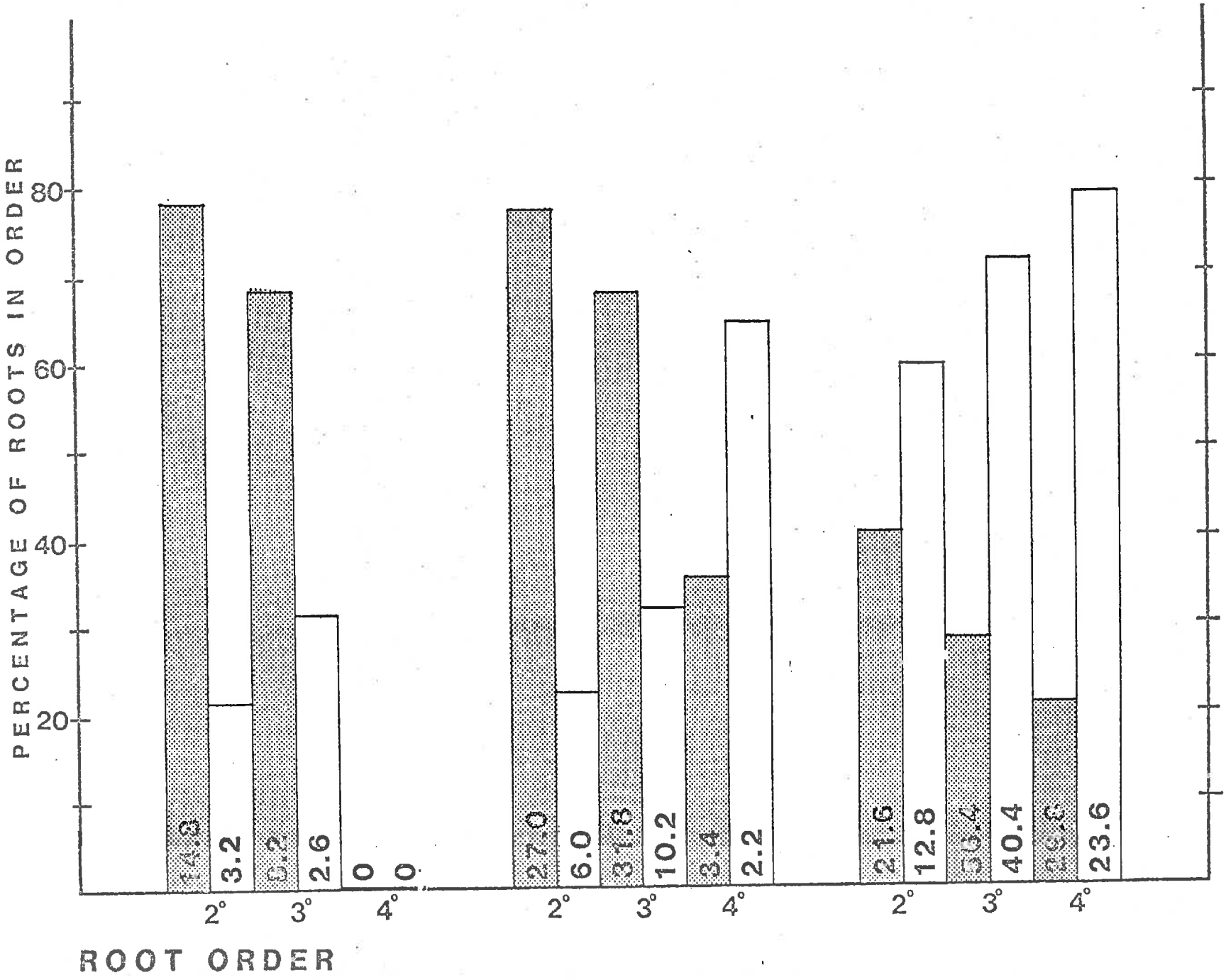
W. citrina + *P. whitei*


PERCENTAGE OF ROOTS IN ORDER
80
60
40
20
39.0
11.6
44.0
16.4
2.6
0.6
32.2
76.0
51.8
6.8
6.2
56.4
27.2
72.6
24.3
18.2
2°
3°
4°
2°
3°
4°
2°
3°
4°
ROOT ORDER

Appendix IV (cont'd)

IVE

A. tomentosus + *P. whitei*


PERCENTAGE OF ROOTS IN ORDER
80
60
40
20
41.3
3.2
0.2
2.6
0
0
27.0
6.0
31.8
10.2
3.4
2.2
21.6
12.8
30.4
40.4
29.3
23.6
2°
3°
4°
2°
3°
4°
2°
3°
4°
ROOT ORDER

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