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# *Leptodontidium orchidicola* (*Mycelium radicis atrovirens* complex, Fungi Imperfecti): conidiogenesis and interaction with some subalpine plants in culture

by

ANALIE A. FERNANDO

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

in

MYCOLOGY

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Edmonton, Alberta

Fall 1995

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Date: September 29th, 1995

To my husband, Danny D. Fernando,
and my mother, Lourdes F. Ammuyutan.

# ABSTRACT

*Leptodontidium orchidicola* is a common species in the *Mycelium radicis atrovirens* (MRA) complex. Its cultural and vegetative morphology is similar to that of *Phialocephala fortinii*. In sporulating strains of *L. orchidicola*, single-celled conidia are produced blastically along lateral or terminal walls of conidiogenous hyphae. In axenic culture with species of *Picea*, *Betula*, and *Potentilla*, *L. orchidicola* colonized epidermal and cortical cells without causing discernible pathogenic effects. In axenic culture with *Salix glauca*, it caused a marked increase in root length but also invaded the stele causing extensive cellular lysis while *P. fortinii* formed a Hartig net and thin, patchy mantle. In pot monocultures with *Potentilla fruticosa*, *Dryas octopetala*, *Salix glauca*, and *Picea glauca*, four *L. orchidicola* isolates had strain- and host-specific effects on host dry weight; the effects of *P. fortinii* were also host-specific. *Leptodontidium orchidicola* formed a range of symbiotic associations from mycorrhizal to parasitic whereas the effects of *P. fortinii* were amensal, parasitic, or neutral. In pot combination cultures, the *P. fortinii*-*Potentilla* symbiosis resulted in a significantly positive effect on plant growth in contrast with the results of the same symbiosis in monoculture resynthesis. The resynthesis experiments demonstrated that the effects of both MRA species vary according to culture conditions.

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# Chapter 1
# GENERAL INTRODUCTION

Fungi are common associates of plants and they are found in every organ and tissue. Their role, in symbiosis with plants, is diverse and ranges from mutualistic to pathogenic. Theoretically, the mutualistic symbioses between certain fungi and the roots of higher plants are called mycorrhizas. In many cases, however, it is difficult to measure the benefits of the association to both organisms involved. So, in practice, if a fungus forms a consistently recognizable and physically distinct association with the roots of a higher plant without causing any perceivable negative effect, then the symbiosis is considered mycorrhizal.

Ectomycorrhizas result when the fungus envelops the root with a sheath of hyphae, referred to as a mantle, and grows between its cortical cells or epidermal cells to form a Hartig net. Nearly all fungi involved in this type of association are members of the Division Dikaryomycota - most are from Subdivision Basidiomycotina but some also come from Ascomycotina (Kendrick 1992). The plant hosts include some of the most important forest trees.

Endomycorrhizas are established when a fungus does not form a mantle and penetrates between the plasma membrane and cell wall of the root cortical cells. The most common endomycorrhiza is the vesicular-arbuscular mycorrhiza (VAM) wherein the fungus forms arbuscules (finely-branched intracellular interfaces for nutrient exchange) and vesicles (intra- and intercellular sac-like cells for lipid storage that also act as resting spores). The VAM fungi are from the Division Zygomycota and the plant partners include more than 300,000 herbaceous and woody plant species in 380 families (Kendrick 1992).

Two other types of endomycorrhizas are orchid and ericoid mycorrhizas. In orchid mycorrhizas, several groups of basidiomycetes are able to form pelotons inside the cells of orchid roots, and even in their rhizomes and protocorms (Zelmer 1994). In ericoid mycorrhizas, several ascomycete taxa form hyphal coils in the cortical cells of its ericaceous hosts (see Stoyke and Currah 1991).

When roots exhibit combined inter- and intracellular fungal infection, the symbiosis is termed ectendomycorrhiza. The plants involved are mostly conifers and the fungi are ascomycetes in the order Pezizales and Fungi Imperfecti with dematiaceous hyphae (Mikola 1965; Wilcox et al. 1974; Wang and Wilcox 1985; Wilcox and Wang 1987a; 1987b; Egger et al. 1991). An ericaceous plant was also reported to form this type of association with a wide range of normally ectomycorrhizal fungi (Zak 1974; Molina and Trappe 1982).

Melin (1921) used the term pseudomycorrhiza to refer to an association formed by certain fungi and roots of pine and spruce. Hartig net and fungal mantle are not formed and the fungus leads an "exclusively" intracellular existence. Pseudomycorrhizas, according to Melin, are harmful to their plant hosts. Fungi that have been observed to establish pseudomycorrhizal associations are *Mycelium radicis atrovirens* (MRA), a name coined by Melin (1921) to accommodate sterile fungi with dematiaceous hyphae isolated from pine and spruce roots. These fungi that appeared dark olive-green in culture caused limited development of rootlets, absence of root hairs, and eventual pathogenicity to conifer seedlings.

Since 1921, pseudomycorrhizas have been reported by many investigators but not always as characterized by Melin (Wilcox 1983), e.g. Wilcox and Wang (1987a) used the term to refer to fungi that could form ectomycorrhiza-like associations that were also weakly pathogenic to the host. In a similar manner, the name MRA has acquired a broader definition. To date, any fungi with olive, dark brown to black hyphae, isolated from roots, and not sporulating readily are called MRA.

MRA are widely distributed in roots and soils in forests (Robertson 1954; Gams 1963; Wilhelm et al. 1969; Richard et al. 1971; Kowalski 1973; Richard and Fortin 1973; Livingston and Blaschke 1984; Summerbell and Malloch 1988). Melin (1921) reported that MRA hyphae in roots of seedlings could spread to the leaves through the hypocotyl. Richard et al. (1971) also observed MRA infection of the hypocotyl of some conifer seedlings.

Some researchers, such as Danielson and Visser (1989), consider MRA to be mycorrhizal while others (Melin 1921; Wilhelm et al. 1969; Richard et al. 1971; Livingston and Blaschke 1984; Summerbell and Malloch 1988) reported that they are pathogenic.The broad host range and geographic distribution of MRA suggested that there must be a considerable range of genetic diversity among strains (Kowalski 1973). By testing for anastomosis groups among different isolates, Gams (1963) concluded that MRA are genetically heterogeneous. Kowalski (1973) designated two groups of MRA based on the type of association produced with their host plants and in their behaviour on agar. From among his isolates, he also determined that six were pseudomycorrhizal and four were ectomycorrhizal.

Although Melin (1921) originally described MRA as sterile, Gams (1963) identified two sporulating MRA strains as *Phialocephala dimorphospora* Kendrick. Similarly, Richard and Fortin (1973) reported that 15 of 41 MRA isolates produced conidiophores and conidia that corresponded to descriptions of *P. dimorphospora.* Wang and Wilcox

(1985) later observed the production of conidia in three of their isolates which "resembled MRA" and named these *Phialophora finlandia*, *Phialocephala fortinii*, and *Chloridium paucisporum*. They also believed that the *P. dimorphospora* strains reported by Richard and Fortin (1973) were actually *P. fortinii*. They concluded that MRA is comprised of an assemblage of fungi from different taxa. Currah et al. (1987) named five MRA isolates from orchids *Leptodontidium orchidicola* Sigler and Currah, bringing, to date, the total to at least four identified MRA species (excluding *P. dimorphospora*) from four genera. Considering the taxonomic diversity of the group, it is not surprising that consistent results concerning the nature of the relationship between MRA and host plants have not been found.

In Alberta, two common species in the MRA complex are *L. orchidicola* and *P. fortinii*. Both taxa are regularly isolated from roots of native plants but non-sporulating strains of these species are almost indistinguishable using routine diagnostic techniques.

*Phialocephala fortinii* was first described from a *Pinus sylvestris* root (Wang and Wilcox 1985). In subsequent years, it has been reported as a common isolate from roots of plants growing in alpine-subalpine habitats that are characterized by cool and humus-rich soils. Its plant sources include a wide range of families, e.g. Asteraceae, Caryophyllaceae, Ericaceae, Orchidaceae, Poaceae, Primulaceae, and Rosaceae (Currah et al. 1987; 1988; Stoyke and Currah 1991; Stoyke et al. 1992). With the use of culture characteristics and restriction fragment analysis of ribosomal DNA, Stoyke et al. (1992) determined that most fungi isolated from the roots of alpine plants were conspecific or closely related to *P. fortinii*.

The cultural, vegetative, and reproductive morphology of *P. fortinii* has been described by several authors (Wang and Wilcox 1985; Currah et al. 1987; 1988; Currah and Tsuneda 1993). When cultured on corn meal agar (CMA), *P. fortinii* colonies attain a diameter of 40-60 mm after 21 days, are mouse gray to dark gray and felt-like, plane or sulcate from above, and greenish black to black from below (Currah and Tsuneda 1993). Colony margins are submerged, glabrous or whitish, and entire or slightly fibrillose. Hyphae of *P. fortinii* are septate, 2-5 µm in diameter, hyaline, thin-walled and smooth when young, and melanized and thick-walled when older. Aerial hyphae are either smooth or asperulate and occasionally form strands and loops. Submerged hyphae are olivaceous brown to greenish, made up of toruloid cells, and commonly form sclerotia. On potato dextrose agar (PDA), *P. fortinii* colonies reach 80-85 mm in diameter after 23 days and have a morphology as in the CMA-grown (Currah et al. 1987). Conidiogenesis in *P. fortinii* is phialidic and requires cold treatment for induction.

Like MRA in general, the ecological role of *P. fortinii* is not clearcut. It has exhibited pathogenicity in *Pinus resinosa* and *Picea rubens* (Wang and Wilcox 1985; Wilcox and Wang 1987a), but had no significant effect on *Menziesia ferruginea* (Stoyke and Currah 1991), *Rhododendron brachycarpum* (Currah et al. 1993), and *Pinus contorta* and *Lupinus latifolius* (O'Dell et al. 1993).

Since *L. orchidicola* was first described (Currah et al. 1987), it has been isolated frequently from orchid mycorrhizas (Currah et al. 1988; 1990; C. Zelmer, personal communication). Like *P. fortinii*, it was classified as MRA because hyphae and cultures are dematiaceous and most isolates do not readily sporulate. The cultural and vegetative characteristics of *L. orchidicola* are so similar to *P. fortinii* that, without conidia, their identities could be easily confused. The mycelium of *L. orchidicola* on CMA is mostly submerged, hyaline to olivaceous brown with sparse grayish aerial mycelium, becoming mostly hyaline with repeated transfers. The margin is submerged and whitish or very pale. Colonies attain a diameter of 66-84 mm in 22 days. Aerial hyphae are mostly smooth, sometimes finely asperulate, hyaline to olivaceous brown, 2-3 μm in diameter and may form chains of toruloid cells which are irregularly-shaped to globose. Occasionally, the hyphae also form loops and strands. Sclerotic masses of globose to irregularly-shaped or toruloid cells and chlamydospores are formed from submerged mycelium. On PDA, cultures are plane or sulcate from above and usually white at first, becoming olivaceous gray or brownish gray with a white margin. The reverse is pale to dark olivaceous gray or grayish brown to black. In 22 days, colonies reach 34-56 mm in diameter. *Leptodontidium orchidicola* forms solitary lateral conidia or small clusters of terminal conidia produced sympodially. The ecological role of *L. orchidicola* has not been examined.

In nature, fungi may play vital roles besides participating in the exchange of nutrients (Currah et al. 1993). In association with plants, fungi may benefit their hosts by reducing the frequency or intensity of grazing by soil microfauna or by providing a competitive advantage to the host plant by parasitizing other seedlings or the roots of competing plant species (Currah et al. 1993). The latter hypothesis may also be called pathogen-mediated competition and *L. orchidicola* and *P. fortinii* may be involved in this type of interaction among plants.

## Research objectives

The general objectives of this study were to: 1) examine the occurrence of *L. orchidicola* in alpine-subalpine and boreal plants; 2) describe the conidia and conidiogenesis of *L. orchidicola* in more detail to afford better means of distinguishing it from *P. fortinii*; and 3) determine the ecological function of this common dematiaceous root endophyte and compare it with that of *P. fortinii.*

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# Chapter 2

# *LEPTODONTIDIUM ORCHIDICOLA* (*MYCELIUM RADICIS ATROVIRENS* COMPLEX): ASPECTS OF ITS CONIDIOGENESIS AND ECOLOGY[1]

## INTRODUCTION

Dematiaceous fungi are commonly isolated from roots of plants growing in cool, humus-rich soils (Wang and Wilcox 1985; Currah et al. 1987; Stoyke and Currah 1991; Stoyke et al. 1992; Currah and Tsuneda 1993). Many of these fungi are sterile or produce inconspicuous conidiogenous cells and conidia that are easily overlooked. Melin (1921) grouped these dematiaceous root associates under the name *Mycelium radicis atrovirens* (MRA). Three MRA taxa (*Phialophora finlandia*, *Chloridium paucisporum*, and *Phialocephala fortinii*) were described by Wang and Wilcox (1985) and a fourth (*Leptodontidium orchidicola* Sigler and Currah) was described by Currah et al. (1987).

The genus *Leptodontidium* de Hoog encompasses dematiaceous fungi that produce conidia in a more or less sympodial order at the apex of conidiogenous cells (de Hoog 1977; 1979). The type specimen of *L. orchidicola* was derived from an isolate from the root of a native Alberta orchid, *Platanthera hyperborea* (L.) Lindl. This fungus has been reported from the mycorrhizas of a wide range of terrestrial species of Orchidaceae. Ultrastructurally, *L. orchidicola* has a simple septum with a single central

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pore flanked on either side by Woronin bodies which indicates that it is ascomycetous (Currah and Sherburne 1992). Currah et al. (1987) noted that among the species of *Leptodontidium*, conidium development in *L. orchidicola* is most similar to *L. boreale* and *L. obscurum*. *Phialocephala fortinii* resembles *L. orchidicola* in cultural (refer to Figs. 2.1 and 2.2) and vegetative morphology but conidiogenesis in the former involves incubation in the cold for about 3 months and formation of phialides with the subsequent release of dimorphic conidia. The vegetative and reproductive morphology of *P. fortinii* has been characterized by Currah and Tsuneda (1993) but similar work with *L. orchidicola* has not been done.

The objectives of this study were to: 1) characterize the conidiogenesis of *L. orchidicola* using light and scanning electron microscopy; and 2) make an initial assessment of its ecological niche by observing its effect on some host plant species when grown together in axenic culture.

# MATERIALS AND METHODS

Provenance for the strains of *L. orchidicola* is provided in Table 2.1. Strains were grown on corn meal agar (CMA) at 23°C in a dark incubator. Specimens from two strains (UAMH 5422 and F-AaN1A) were prepared for scanning electron microscopy (SEM) following the procedure of Currah and Tsuneda (1993) and examined with a Cambridge Stereoscan SEM. Slide cultures were also prepared using thin, 5 x 5 mm CMA blocks as sporulation media in 2% tap water agar (TWA) culture plates. These were incubated at 23°C in the dark. Lactofuchsin-stained mounts of the slide cultures and of fresh pieces of fungal mycelium were examined with phase contrast and Nomarski Interference microscopy.

Seeds of *Potentilla fruticosa* L. and *Betula pumila* L. collected from the treeline in Cadomin, Alberta, and of *Picea glauca* (Moench) Voss collected from the University of Alberta campus were surface-sterilized with a 20% solution of commercial bleach, rinsed three times with sterile distilled water, plated onto TWA media in Petri plates, and allowed to germinate at room temperature. Seedlings were transplanted into test tubes containing TWA and inoculated with the strain F-AaN1A. Some seedlings were left uninoculated as controls. The roots were harvested at 46 to 88 days, cleared with 10% potassium hydroxide (KOH), stained with chlorazol black, and examined under a light microscope. The production of polyphenol oxidases was tested by putting plugs of mycelia from four-week-old cultures on tannic acid media (Davidson et al. 1938) and reading the reaction within 24 hours.

# RESULTS AND DISCUSSION

Light and scanning electron microscopic observations of the eight isolates of *L. orchidicola* indicate that single-celled conidia develop blastically in an apparently random pattern along the lateral and terminal walls of hyaline or pigmented conidiogenous hyphae (Figs. 2.3-2.13). Swollen or unswollen conidiogenous cells may also occur on the same hyphae or on short lateral branches (Fig. 2.9). Lateral conidia are mostly solitary (Figs. 2.3-2.13) and terminal conidia may also be solitary (Fig. 2.13) but are often in clusters produced sympodially (Figs. 2.3-2.5, 2.8, 2.9, and 2.12). The production of solitary lateral conidia on conidiogenous hyphae is a characteristic of *L. orchidicola* and makes this species distinct from others in the genus (Currah et al. 1987). In the early stage of conidiogenesis, lateral conidia are observed more often than the terminal sympodial type. Strains that produce few conidia, i.e. UAMH 5420, F-PsN4A, and Z45.OR.a(2), rarely form clusters of terminal sympodial conidia whereas strains that sporulate more abundantly, i.e. Z63.NR.2A, UAMH 5422, and Z107.Rh.a, often produce clusters of terminal sympodial conidia. As observed in slide cultures, the onset of conidiogenesis in these more prolifically sporulating isolates is within 7 to 9 days.

Mature conidia are 1-3 x 3-6 µm and may be globose, subglobose, ellipsoidal, oval, ovate, obovate, pyriform, clavate, or spathulate (Figs. 2.3-2.13). Each one of the isolates of *L. orchidicola* exhibits most or all of the different shapes of conidia. However, a particular shape can predominate in some isolates, e.g. the type UAMH 5422 has mostly pyriform (Figs. 2.3 and 2.7) whereas F-CxN1A most often has globose to subglobose conidia (Figs. 2.4, 2.6, and 2.9). The conidia are mostly sessile but some have short stalks (Fig. 2.10). Some conidia are able to form secondary spores (Figs. 2.3, 2.6, and 2.7). These are formed blastically, usually on the apex of existing conidia, and are morphologically similar to the parent conidium.

The liberation of conidia may be schizolytic or by disintegration of the stalk or part of the subtending structure (Fig. 2.13) leaving indistinct scars. Detached conidia were rare; this is another characteristic of *L. orchidicola* which distinguishes it from other species of *Leptodontidium* (Currah et al. 1987).

Conidiogenesis in *Leptodontidium* is similar to that of *Rhinocladiella* Nannf. *sensu stricto*, *Acrodontium* de Hoog, and *Ramichloridium* Stahel ex de Hoog but the conidia of the latter genera are borne on denticles (de Hoog 1977). *Leptodontidium aciculare* Rao and de Hoog is the only reported species of the genus which produces conidia on minute denticles but is different from the above-mentioned genera on the basis of its formation of hyaline and often intercalary fertile apparatus in later transfers (Rao and de Hoog 1986).

In axenic culture, *L. orchidicola* formed associations with the roots of seedlings from three different plant taxa studied. *Leptodontidium orchidicola* invaded the proximal region of the roots, specifically the cortical and epidermal areas. A network of intra- and intercellular hyphae formed throughout the epidermal and cortical areas of *Picea* roots after 46 days. With seedlings of *Potentilla*, intracortical branched, swollen and lobed fungal cells were observed after 54 days (Fig. 2.14). In *Betula*, intracellular sclerotia filled some cortical cells within 64 days (Fig. 2.15). These structures formed by *L. orchidicola* on the roots of *Picea*, *Potentilla*, and *Betula* were morphologically similar to those formed by *P. fortinii* on the roots of *Menziesia ferruginea* as reported by Stoyke and Currah (1993). The presence of *L. orchidicola* in and on the roots did not inhibit root elongation and it did not have any apparent effect on seedling growth and survival. Currah et al. (1990) observed no indication of pathogenicity in orchids colonized by this root endophyte in nature.

*Leptodontidium orchidicola* produces polyphenol oxidases, the group of enzymes produced by white rot fungi which are involved in the breakdown of lignin. The presence of these enzymes is manifested by a dark brown to almost black zone around the plug of fungal mycelium on tannic acid medium within 24 hours (Davidson et al. 1938). Hutchison (1990) reviewed the possible ecological significance of polyphenol oxidase production in fungi, particularly laccase. Besides lignin breakdown, an indicator of potential saprobic ability, it could also function in confering resistance to antifungal phenolic compounds. Such compounds are apparently found in the humus layer of certain soils and also produced by the roots of trees as defense barriers. The aforementioned significance of polyphenol oxidase is in agreement with the report of Currah and Tsuneda (1993) regarding the relationship of polyphenol oxidase production in *P. fortinii* and its specialized niche as a root endophyte. Since they are both commonly isolated from roots of plants in alpine and subalpine habitats, *L. orchidicola* and *P. fortinii* may share a similar ecological niche.

Table 2.1. Strains of *Leptodontidium orchidicola* used in the study.

| Strain | Host | Location |
|---|---|---|
| UAMH 5422 (ex-type) | *Platanthera hyperborea* (L.) Lindl. | Devonian Botanic Garden, AB |
| UAMH 5420 (authentic) | *Coeloglossum viride* (L.) Hartm. | Devonian Botanic Garden, AB |
| F-AaN1A*[a] | *Artemisia norvegica* Fries | Cadomin, AB |
| F-CxN1A*[b] | *Carex* sp. | Cadomin, AB |
| F-PsN4A*[c] | *Pedicularis bracteosa* Benth. | Cadomin, AB |
| Z107.Rh.a | *Corallorhiza trifida* Chatelain | Obed Summit,AB |
| Z63.NR.2a*[d] | *Spiranthes lacera* (Rafinesque) Rafinesque | Whiteshell Prov. Forest, MB |
| Z45.OR.a(2) | *Piperia unalascensis* (Spreng.) | West Castle River Falls, AB |

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*These strains have been deposited at the University of Alberta Microfungus Collection and Herbarium.

[a] Accession no. UAMH 8151

[b] Accession no. UAMH 8149

[c] Accession no. UAMH 8152

[d] Accession no. UAMH 8150

Figures 2.1-2.9. Cultural morphology and phase contrast photographs of conidiogenous hyphae of *Leptodontidium orchidicola.*

Fig. 2.1. Top view of *L. orchidicola* culture grown on cornmeal agar at 23°C. The culture is plane and olive brown with sparse grayish aerial mycelium. F-AaN1A, 11 weeks. 1x.

Fig. 2.2. The same culture of *L. orchidicola* (as in Fig. 2.1), viewed from the bottom.

Fig. 2.3. Terminal and lateral, pyriform, clavate, obovate, and subglobose conidia of a prolifically-sporulating isolate. Arrowheads point to two secondary conidia. UAMH 5422. Scale bar = 6 µm.

Fig. 2.4. Lateral and clusters of terminal, mostly globose, conidia on a conidiogenous hypha and short lateral branch. F-CxN1A. Scale bar = 6 µm.

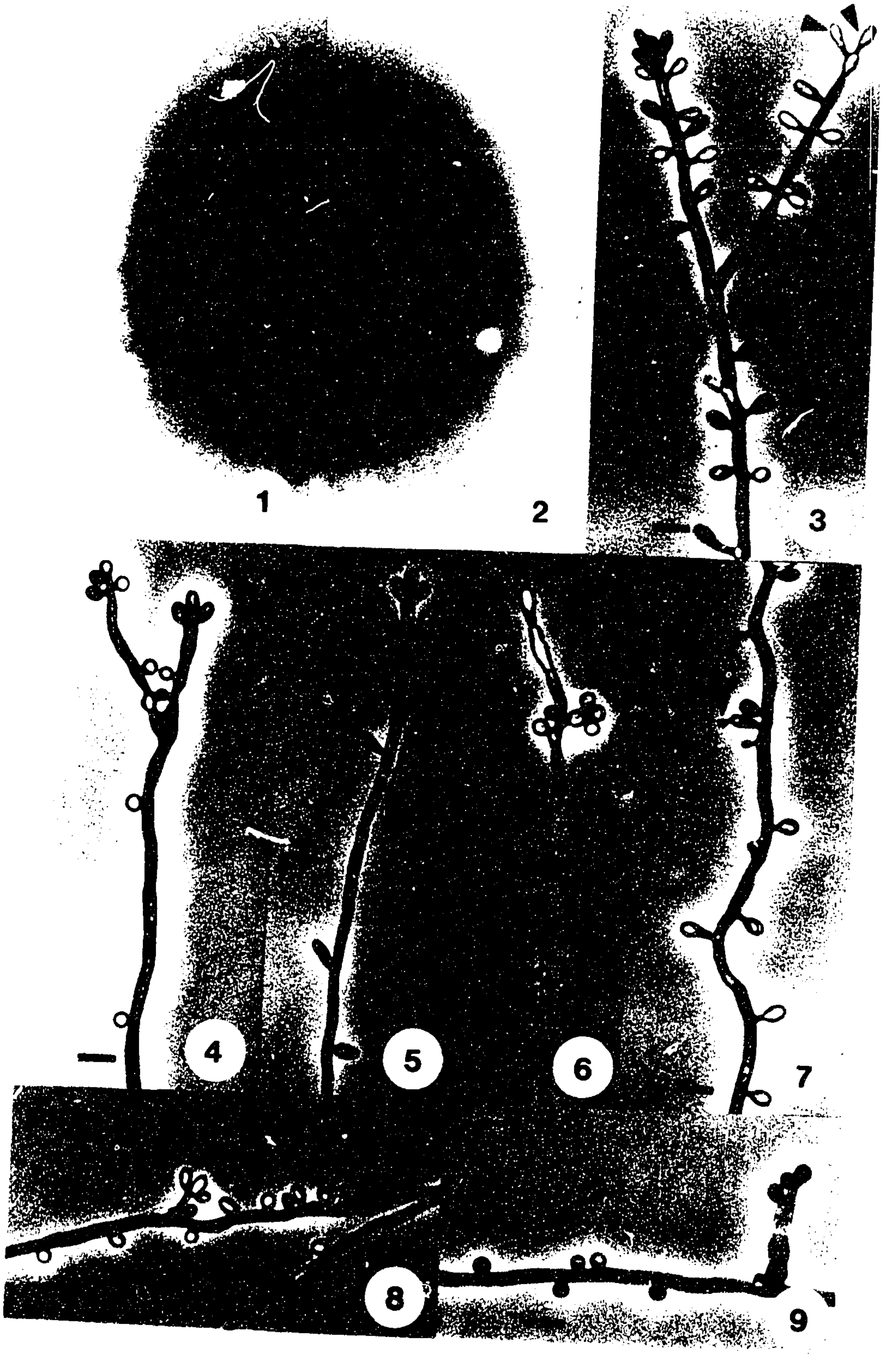
Fig. 2.5. Conidiogenous hypha with lateral solitary conidia and a terminal cluster of conidia arising sympodially. Note the different shapes of conidia, i.e. oval, subglobose, clavate, ellipsoidal, and ovate. UAMH 5422. Scale bar = 6 µm.

Fig. 2.6. Conidiogenous hypha showing three secondary conidia (arrowhead). F-CxN1A. Scale bar = 6 µm.

Fig. 2.7. Lateral solitary conidia with one young secondary conidium (arrowhead). UAMH 5422. Scale bar = 6 µm.

Fig. 2.8. Conidiogenous hyphae with lateral conidia and a short lateral branch bearing terminal conidia. Z63.NR.2a. Scale bar = 6 µm.

Fig. 2.9. Conidiogenous hypha with globose and subglobose lateral conidia and a short lateral branch composed of swollen cells with apical cluster of conidia. F-CxN1A. Scale bar = 6 µm.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Figures 2.10-2.15. SEM micrographs showing lateral and terminal conidia of *Leptodontidium orchidicola* and photographs of its morphology on root systems in axenic culture.

Fig. 2.10. Solitary, oval lateral conidium with short stalk. F-AaN1A. Scale bar = 2 μm.

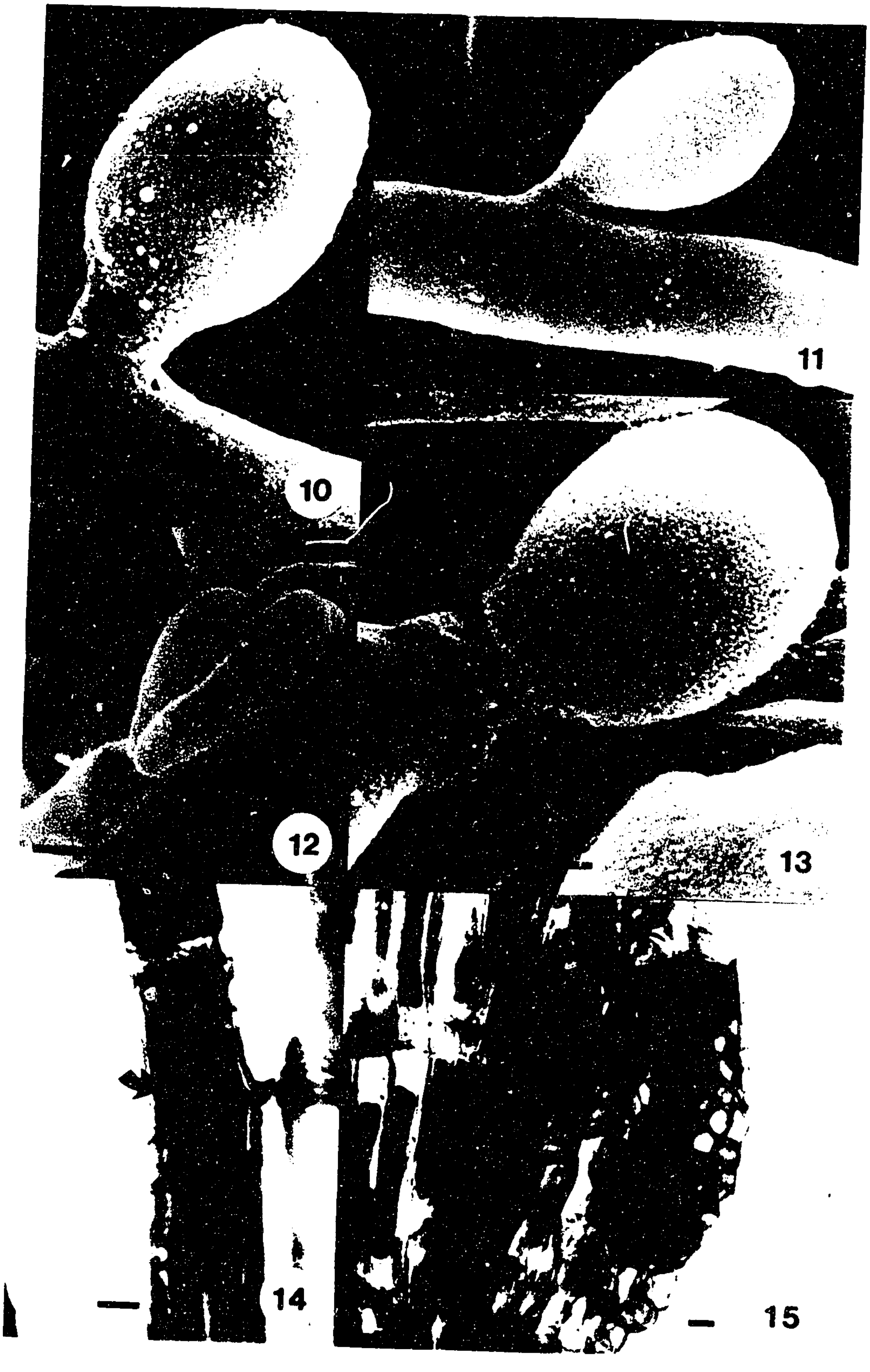
Fig. 2.11. Solitary, sessile, pyriform lateral conidium. F-AaN1A. Scale bar = 2 μm.

Fig. 2.12. Terminal conidia produced sympodially arising in an apical cluster of three conidia. UAMH 5422. Scale bar = 2 μm.

Fig. 2.13. Solitary terminal conidium with its subtending structure starting to disintegrate. F-AaN1A. Scale bar = 2 μm.

Fig. 2.14. Root cells of *Potentilla* showing intracortical branched, swollen and lobed fungal cells (arrow). Scale bar = 6 μm.

Fig. 2.15. Intracellular sclerotium formed by *L. orchidicola* in root of *Betula*. Scale bar = 6 μm.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# Chapter 3

# A COMPARATIVE STUDY OF THE EFFECTS OF THE ROOT ENDOPHYTES *LEPTODONTIDIUM ORCHIDICOLA* AND *PHIALOCEPHALA FORTINII* (FUNGI IMPERFECTI) ON THE GROWTH OF SOME SUBALPINE PLANTS IN CULTURE[1]

## INTRODUCTION

*Leptodontidium orchidicola* Sigler and Currah is one of at least four taxa known among the fungi comprising the *Mycelium radicis atrovirens* (MRA; Melin 1921) complex. It was described by Currah et al. (1987) and detailed studies of its conidiogenesis were made by Fernando and Currah (1995). This dematiaceous hyphomycete has been isolated from roots of terrestrial orchids (Currah et al. 1987; 1990; C. Zelmer, personal communication) and other plants growing in alpine-subalpine habitats with humus-rich soils (Fernando and Currah 1995). The cultural and vegetative characteristics of *L. orchidicola* are similar to those of *Phialocephala fortinii* Wang and Wilcox, another commonly isolated MRA species (Currah et al. 1987; 1988; Stoyke and Currah 1991; 1993). Identification of these fungi is difficult because they rarely sporulate.

The ecological role of MRA is unclear, with some taxa, such as *P. fortinii*, causing a range of effects on host plants from pathogenic to asymptomatic depending on environmental conditions, host, and strain of

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[1] A version of this chapter has been submitted for publication as: Fernando, A.A. and Currah, R.S. A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (Fungi Imperfecti) on the growth of some subalpine plants in culture. Can. J. Bot.

fungus (Wang and Wilcox 1985; Wilcox and Wang 1987; Stoyke and Currah 1991; 1993; Currah et al. 1993; O'Dell et al. 1993). In axenic culture, *L. orchidicola* formed associations with roots of common alpine-subalpine plants without causing any inhibition of root elongation or having apparent effect on seedling growth and survival (Fernando and Currah 1995). With orchids in nature, no indication of pathogenicity has been observed (Currah et al. 1990).

The effects of *L. orchidicola* on axenically-grown *Salix glauca* L. seedlings were observed and compared with those of *P. fortinii.* Resyntheses in pot cultures which simulated natural conditions as closely as possible were also done. Monocultures were established to: 1) determine the effects of four *L. orchidicola* strains on shoot dry weight accumulation of four common alpine-subalpine plant species native to Alberta, i.e. *Potentilla fruticosa* L., *Dryas octopetala* L., *Salix glauca*, and *Picea glauca* (Moench) Voss; 2) determine if the effects of *L. orchidicola* are strain- and/or host-specific; and 3) compare the effects of *L. orchidicola* with those of *P. fortinii.*

Currah et al. (1993) suggested that besides nutrient exchange, the fungus-plant symbiosis may benefit the host plant by reducing the frequency or intensity of grazing by soil microfauna. It may even provide a competitive advantage by parasitizing invasive seedlings or the roots of competing plant species. To determine if any of the strains of fungi under study provides a competitive edge to some plants by adversely affecting others, a pot culture resynthesis experiment involving a combination of the four previously mentioned plant species was done.

# MATERIALS AND METHODS

## Isolation and culture of fungi

Dematiaceous fungi were isolated from roots of plants collected in the vicinity of the treeline in Cardinal River Divide (CRD) near Cadomin, Alberta and from Prince Albert National Park (PANP), Saskatchewan. Fungi were isolated and cultured following the methods of Stoyke and Currah (1991). Four sporulating *L. orchidicola* isolates were also grown on tap water agar (TWA), corn meal agar (CMA), and potato dextrose agar (PDA) plates with cellophane membranes (British cellophane, Bridgewater, U.K.) to test for production of cellulolytic enzymes.

## Axenic resynthesis

Seeds of *Salix* collected from CRD were surface-sterilized and germinated axenically as described by Fernando and Currah (1995). Four sets of thirty-six seedlings with an average root length of 2 mm were transferred onto 2% TWA plates. The plates were either uninoculated or previously inoculated with one of two strains of *L. orchidicola* (UAMH 8151 and UAMH 5422) or *P. fortinii* (UAMH 8148). The ex-type strain (UAMH 5422) was obtained from the University of Alberta Microfungus Collection and Herbarium (UAMH). New isolates were deposited at the UAMH.

All seedlings were grown in the greenhouse at 18°C with 18 h light (2400 lx) and 6 h dark periods for eight weeks. Percent mortality rate and root length of surviving seedlings were determined and analyzed statistically. One-way analysis of variance (ANOVA) procedures were performed and differences detected with Duncan and Least Significant Difference (LSD) multiple range tests (SPSS Inc. 1993). Roots were cleared with 10% potassium hydroxide (KOH), stained with 0.1% chlorazol black in equal volumes of 80% lactic acid, glycerin, and distilled water, and examined under a light microscope.

Roots from a second batch of seedlings were processed for plastic sectioning and light microscopy. Two mm root segments were fixed overnight in 2.5% glutaraldehyde and postfixed for 1.5 h in 2% osmium tetroxide, dehydrated in graded ethanol series followed by propylene oxide, infiltrated gradually over a five-day period and embedded in Spurr's resin (Spurr 1969). Semithin sections (1.5-2 µm) were cut on a Reichert OM U2 ultramicrotome, stained with 0.5% toluidine blue O in 0.1% sodium carbonate (Trump et al. 1961), and examined under a light microscope.

### Resynthesis in pot cultures

Axenically-reared seedlings of *Potentilla*, *Dryas*, and *Salix* (seeds collected from CRD), and *Picea* (seeds from the Alberta Forest Service, Pine Ridge Forest Nursery, Smoky Lake, Alberta) were used as hosts. Fungal strains used as inoculants are presented in Table 3.1.

For the monocultures, each of the four plant species had untreated controls and five fungal treatments, all of which were replicated forty times. For the combination cultures, one seedling of each of the four species were grown together. These combinations also had five fungal treatments plus the untreated controls, replicated forty times.

Inoculum was prepared by thoroughly blending one five-week old fungal colony grown on CMA in a 3.5-inch diameter Petri plate with 1.4 L autoclaved loam, peat moss, and perlite (1:1:1). Controls were prepared with uninoculated CMA. Resulting pH was 5.2.

Seedlings were transplanted into plastic pots (2.5-inch-diameter for monocultures and 4-inch for combination cultures) three to four weeks after germination. All plants were grown in the greenhouse with no special precautions taken to keep contaminants from establishing in the mix. Pots were watered as required with sterile distilled water and rearranged every two weeks to ensure that growth conditions were as uniform as possible.

Plants were harvested after 14 weeks. The shoots of thirty plants from each treatment from both mono- and combination cultures and roots of eighteen plants from each treatment in monocultures were oven-dried and weighed. Inoculants were reisolated from randomly chosen roots of five plants of each series to confirm their presence as root endophytes. Some of the remaining roots were cleared, stained, and examined under a light microscope.

Shoot and root dry weights were analyzed statistically. One-way ANOVA procedures were performed and differences detected with Tukey's honestly significant difference test (Tukey-HSD) using SPSS (SPSS Inc. 1993). When at least one of the assumptions of a parametric test were not met by the data, the analysis used was Kruskal-Wallis one-way ANOVA (SPSS Inc. 1993) and the differences detected with a non-parametric Tukey-type multiple comparisons procedure (Zar 1984).

# RESULTS

## Isolation and culture of fungi

Sporulating *L. orchidicola* isolates were recovered from ten of thirty plants collected at CRD and PANP (Table 3.2). Isolates from at least five other plant species also have cultural and vegetative characteristics of *L. orchidicola* but have not sporulated. All strains tested decomposed the cellophane membranes after about three weeks on CMA, and four weeks on PDA. At five weeks on TWA, there was growth beneath the cellophane membrane but no decomposition was observed.

## Axenic resynthesis

Seedling mortality rate was not significantly different between the treatments and control (Table 3.3). The roots of *Salix* seedlings inoculated with *L. orchidicola* were longer than those of the control and those inoculated with *P. fortinii* (Fig. 3.1; Table 3.3). Considerable breakdown of root cortical cells was observed in all treatments and the control (Figs. 3.2, 3.3, and 3.6).

Both strains of *L. orchidicola* invaded all root tissues including the stele (Figs. 3.3-3.6). Colonization was both inter- and intracellular. Necrosis of the endodermis, pericycle, and vascular tissues was observed. In the stele, sometimes only the thick-walled primary xylem cells appeared intact (Figs. 3.3-3.5) and in extreme cases the entire stele became obliterated (Fig. 3.6). However, the seedlings did not appear outwardly to have any negative effects.

The *P. fortinii*-inoculated seedlings showed no colonization of vascular tissues and in some root segments, ectomycorrhizal characteristics were observed (Figs. 3.7 and 3.8). The Hartig net generally did not go deeper than the outer tangential walls of the cells immediately beneath the epidermal layer (Fig. 3.7). The fungal mantle was thin and irregularly-formed or patchy (Fig. 3.8).

*Phialocephala fortinii* formed microsclerotia more abundantly than either of the strains of *L. orchidicola* (Figs. 3.3, 3.9, and 3.10). Root hairs persisted in inoculated seedlings. These were also sometimes colonized by the fungal inoculant (Fig. 3.11). *Leptodontidium orchidicola* hyphae were hyaline on TWA and in association with the roots making them difficult to see in squash mounts of roots. On the other hand, *P. fortinii* was consistently dematiaceous and therefore easy to locate on or inside the roots.

## Resynthesis in pot cultures

All seedlings in the pot cultures survived. Examination of cleared and stained roots did not show any signs of tissue degradation or colonization of the stelar region. Shoots were also unaffected.

Inoculants were recovered readily from the roots of pot-cultured plants after 14 weeks. Some common conidial and saprophytic fungi (e.g. *Trichoderma* sp.) were also found. Fruiting bodies of *Sphaerosporella brunnea* (Alb. & Schw.: Fr.) Svrcek & Kubicka, an ectomycorrhizal ascomycete, developed in all pots of combination cultures and a few pots of *Dryas*, *Salix*, and *Picea* in monocultures.

The effects of the different treatments on shoot dry weights (SDW) of four alpine-subalpine plants in the monoculture resynthesis experiment are shown in Tables 3.4 and 3.5. As compared with the control, a significant increase in SDW accumulation was observed in the *Potentilla*-Lo1 symbiosis and also in *Picea* when symbiotic with Lo3 and Lo4. A significant SDW reduction was observed in *Potentilla* in symbiosis with

Lo3, Lo4, and Pf, and also in *Dryas* with Lo1, Lo2, and Lo4. All other symbioses showed no significant effect. A comparison of the SDW of Lo- and Pf-treated plants showed that *Potentilla* and *Salix* treated with Lo1 and Lo2, *Salix* with Lo3, and *Picea* with Lo4 resulted in significantly higher SDW accumulation. Only Lo1, Lo2, and Lo4 treatments of *Dryas* resulted in significantly lower SDW, and the rest showed no significant difference from the Pf treatment. The root dry weights (RDW) of plants from the monocultures were measured (Table 3.6) and were generally consistent with the SDW measurements (Table 3.4).

In the combination cultures, only the *P. fortinii-Potentilla* symbiosis resulted in a significantly positive effect when compared with the control (Table 3.7). Fungal treatments of *Picea* showed no significant difference from the control but there were differences in the effects of the *L. orchidicola* treatments, i.e. Lo3 and Lo4 resulted in a significantly higher SDW compared to Lo2 (Table 3.7). Differences among all other symbioses were not significant.

# DISCUSSION

*Leptodontidium orchidicola* has a wide range of hosts and is, therefore, not host-specific. The ease of isolation and culture of this dematiaceous root endophyte indicates that it is not fastidious with respect to carbon source. *Leptodontidium orchidicola* is able to break down cellulose as evidenced by the deterioration of cellophane membranes on CMA and PDA. On TWA, it was observed to grow beneath the cellophane membranes, a manifestation of imminent decomposition. It also produces polyphenol oxidases which suggests that it can break down lignin (Fernando and Currah 1995). These enzymatic abilities, broad host range, and the production of asexual spores are more indicative of saprophytic trophic strategy than a mycorrhizal one (Giltrap 1982; Hutchison 1989).

The collapse of root cortical cells of *Salix* in axenic Petri dish cultures could not be attributed to the colonizing fungi since it was also common in the control roots. This feature was also observed in *Rhododendron* (Peterson et al. 1980) and, to a lesser degree, in lupin and pine roots (O'Dell et al. 1993). It has been suggested that this could be a part of normal root differentiation or an artifact of the growing conditions or fixation procedures (Peterson et al. 1980; O'Dell et al. 1993).

Colonization of *Salix* root tissues by *L. orchidicola* is both inter- and intracellular. It presumably has a capacity to produce cell wall-degrading enzymes enabling it to penetrate into the cells. Certain enzymes produced, e.g. polyphenol oxidases, may have a role in detoxicating the protective compounds produced by the host and aid in fungal invasion of host tissues (Mayer and Harel 1979). *Leptodontidium orchidicola* invaded the stele and destroyed the integrity of *Salix* root tissues. These characteristics indicate that the symbiosis is incompatible and that the infecting fungus is pathogenic (Molina and Trappe 1982; O'Dell et al. 1993). *Leptodontidium orchidicola* exhibited an ability to digest all of the cells in the stele. This is more severe than the effects of *Phialocephala dimorphospora* on red spruce and *P. fortinii* on red pine (Wilcox and Wang 1987). At eight weeks, though, no superficial damage to the seedlings was observed. Moreover, plants infected with *L. orchidicola* appeared normal and had more robust root systems. Apparently, the normal appearance of seedlings does not reflect compatibility in this particular association. It is possible that nutrients are being released from the breakdown of moribund root tissues by *L. orchidicola* and reabsorbed by the roots thereby allowing or promoting root elongation. Alternatively, production of a diffusible growth-promoting compound may be taking place. It is also possible that after a longer incubation time, the pathogenic effects of *L. orchidicola* might still become apparent in *Salix*.

This study is the first to report that *P. fortinii* can form ectomycorrhizal structures with an angiosperm host. The irregular mantle and Hartig net could be an indication of incompatibility in the association. Nevertheless, these results have shown that *P. fortinii* has the ability to form ectomycorrhizas similar to those produced in monoxenic cultures with red spruce and red pine (Wilcox and Wang 1987). In our study, *P. fortinii* seemed to be less aggressive than *L. orchidicola* because the former did not invade the vascular tissues. However, in symbiosis with red spruce and red pine, *P. fortinii* invaded the root vascular tissues of their plant hosts causing cellular lysis (Wilcox and Wang 1987). Apparently, the boundary between a mycorrhizal symbiont and pathogen is only a matter of degree with the fungus being able to change from one type of association to the other even within a single host species or the same root branch (Wilcox and Wang 1987).

Since microsclerotia are formed consistently by *P. fortinii* and *L. orchidicola*, even by strains that exhibited only adverse effects on their hosts, we believe that these are not mycorrhizal structures nor a fungus-host interface for exchange of nutrients. In this case, the microsclerotia are not comparable to the pelotons in orchid mycorrhizas or to the intracellular hyphal coils in ericoid mycorrhizas. Currah and Tsuneda (1993) suggested that microsclerotia are formed by *P. fortinii*, at least partly, for perennation through periods of low nutrient availability.

Both *L. orchidicola* and *P. fortinii* were reisolated easily from the roots of hosts in pot cultures and other fungi were rare. This not only confirms the presence of *L. orchidicola* and *P. fortinii* as root endophytes but also suggests that they were the major colonizers of roots in the pot cultures. We suspect that *Sphaerosporella brunnea* was introduced to the soil mixture with the peat moss (see Danielson 1984). To determine the effect of this fungus, a pot culture resynthesis of *Salix glauca* and *S. brunnea* association was done. Statistical analysis of shoot and root dry weights of 8.5-week-old seedlings indicated no significant difference between uninfected controls and those colonized by *S. brunnea* (Table 3.8). Danielson (1984) also reported that *S. brunnea* formed mycorrhizas with jack pine without affecting shoot weight.

In monocultures, the effects of *L. orchidicola* varied depending on the strain of fungus and the species of plant host. The different strains caused positive, negative, or had no significant effects on their hosts, in which case they could be considered mycorrhizal at the most and weakly parasitic at the least. Similarly, *Alpova diplophloeus* was ectomycorrhizal with five *Alnus* spp. but disrupted root cortical tissues of seven conifers in identical cultural conditions (Molina and Trappe 1982). In pot cultures, the dry weights of *Salix* seedlings inoculated with *L. orchidicola* did not differ significantly from the controls. Cleared and stained roots had neither tissue damage nor hyphal invasion into the stele. This suggests that the pathogenic tendencies of *L. orchidicola* as observed in axenic resynthesis may only be specific for that particular condition because of the artificiality of the system. Our results support the notion that there are fungi which can be mutualistic or antagonistic depending on fungal strain and host (Marx and Bryan 1970; Molina and Trappe 1982; Wilcox 1983) and that the type of fungus-host interaction produced also depends on environmental factors (Marx and Bryan 1970; Giltrap 1982).

The effects of *P. fortinii* on host dry weight accumulation in pot monocultures were either negative or not significant. These results are similar to previous resynthesis studies with *P. fortinii* (Wilcox and Wang 1987; Stoyke and Currah 1991; 1993; Currah et al. 1993; O'Dell et al. 1993) and suggest amensal, parasitic, or neutral associations. It appears that the effects of *P. fortinii* are host-specific like those of *L. orchidicola.* A previous study with *Rhododendron brachycarpum* as the plant host (Currah et al. 1993) also pointed out the strain-specific effects of *P. fortinii.*

*Leptodontidium orchidicola* and *P. fortinii* may yet be shown to be involved in a pathogen-mediated competition between plants but the results of this work are inconclusive. The results obtained from the pot combination resynthesis are markedly different from those of the monoculture resynthesis. Most notable is the significantly positive effect of *P. fortinii* on the SDW of *Potentilla* in the combination set-up when the same symbiosis had a significantly negative result in monocultures. Moreover, all previous resynthesis studies which used identified isolates of *P. fortinii* had only either negative or not significant results. This reiterates the fact that the conditions employed in the synthesis affect the nature of the fungus-host association produced (Giltrap 1982). The combination resynthesis may have emphasized the tendency of the fungi to have a predilection for certain hosts along with the ability to colonize a wide range of plants.

Table 3.1. Strains of *Leptodontidium orchidicola* (Lo) and *Phialocephala fortinii* (Pf) used in the resynthesis studies.

| Fungal strain | Plant source | Family | Location |
|---|---|---|---|
| UAMH 8151 (Lo1) | *Artemisia norvegica* Fries | Asteraceae | Cardinal River Divide, AB |
| UAMH 8152 (Lo2) | *Pedicularis bracteosa* Benth. | Scrophulariaceae | Cardinal River Divide, AB |
| UAMH 8149 (Lo3) | *Carex* sp. | Cyperaceae | Cardinal River Divide, AB |
| UAMH 5422 (Lo4) (ex-type) | *Platanthera hyperborea* (L.) Lindl. | Orchidaceae | Devonian Botanic Garden, AB |
| UAMH 8148 (Pf) | *Salix glauca* L. | Salicaceae | Cardinal River Divide, AB |

Table 3.2. *Leptodontidium orchidicola* strains isolated from Cardinal River Divide, Alberta and Prince Albert National Park, Saskatchewan.

| Strain | Plant source | Family |
|---|---|---|
| UAMH 8151 | *Artemisia norvegica* Fries | Asteraceae |
| UAMH 8152 | *Pedicularis bracteosa* Benth. | Scrophulariaceae |
| UAMH 8149 | *Carex* sp. | Cyperaceae |
| F-TsN6B | *Trollius albiflorus* (A. Gray) Rydb. | Ranunculaceae |
| F-CaM2B | *Castilleja* sp. | Scrophulariaceae |
| F-RsN5B | *Rubus* sp. | Rosaceae |
| F-EnN3B | *Erigeron* sp. | Asteraceae |
| F-AcN7B | *Achillea* sp. | Asteraceae |
| F-HmN2B | *Heracleum lanatum* Michx. | Apiaceae |
| F-AsM1C | *Abies balsamea* (L.) Mill. | Pinaceae |

Table 3.3. Mean mortality rate (%) and root length (mm) of *Salix glauca* in axenic Petri dish resynthesis.

| Fungal treatments | Mortality rate (n=36) | Root length (n=7) |
|---|---|---|
| Lo1 | 54.40 a | 54.43 b |
| Lo4 | 73.60 a | 57.14 b |
| Pf | 54.40 a | 20.71 a |
| Control | 73.40 a | 22.00 a |

Note: Data were analyzed by one-way ANOVA and differences detected with Duncan and Least Significant Difference (LSD) multiple range tests. Values followed by the same letter in each column do not differ significantly ($\alpha=0.05$).

Table 3.4. Effects of fungal treatments on shoot dry weights (g) of four alpine-subalpine plants in pot monoculture resynthesis.

| Fungal treatments | *Potentilla* (mean) | *Dryas* (mean) | *Salix* (rank sum) | *Picea* (rank sum) |
|---|---|---|---|---|
| Lo1 | 0.1620 c | 0.1127 a | 3538.5 b | 2936.5 abc |
| Lo2 | 0.1337 b | 0.1173 a | 3063.0 b | 1917.0 a |
| Lo3 | 0.1067 a | 0.1620 b | 3152.5 b | 3478.5 bc |
| Lo4 | 0.1073 a | 0.0947 a | 2571.0 ab | 3693.5 c |
| Pf | 0.0980 a | 0.1663 b | 1449.0 a | 2424.0 ab |
| Control | 0.1343 b | 0.1927 b | 2516.0 ab | 1840.5 a |

Note: *Potentilla* and *Dryas* data were analyzed by one-way ANOVA and differences detected with Tukey-HSD. *Salix* and *Picea* data were analyzed by Kruskal-Wallis one-way ANOVA and differences detected with a non-parametric Tukey-type multiple comparisons procedure. Values followed by the same letter in each column do not differ significantly ($\alpha$=0.05). n=30.

Table 3.5. Summary of effects of fungal treatments on shoot dry weight (g) of four alpine-subalpine plants in comparison with the control in pot monoculture resynthesis ($\alpha$=0.05).

| Treatments | *Potentilla* | *Dryas* | *Salix* | *Picea* |
|---|---|---|---|---|
| Lo1 | + | - | o | o |
| Lo2 | o | - | o | o |
| Lo3 | - | o | o | + |
| Lo4 | - | - | o | + |
| Pf | - | o | o | o |

Note: (+) Indicates significant increase in shoot dry weight; (-) indicates significant decrease in shoot dry weight; (o) shoot dry weight not significantly different from control.

Table 3.6. Effects of fungal treatments on root dry weights (g) of four alpine-subalpine plants in pot monoculture resynthesis.

| Fungal treatments | *Potentilla* (mean) | *Dryas* (mean) | *Salix* (rank sum) | *Picea* (mean) |
|---|---|---|---|---|
| Lo1 | 0.3141 c | 0.0352 a | 1402.5 b | 0.0289 b |
| Lo2 | 0.2181 ab | 0.0363 a | 1027.5 b | 0.0207 a |
| Lo3 | 0.1919 a | 0.0564 b | 1161.0 b | 0.0310 b |
| Lo4 | 0.1832 a | 0.0341 a | 859.5 ab | 0.0294 b |
| Pf | 0.1975 a | 0.0718 c | 479.0 a | 0.0264 ab |
| Control | 0.2733 bc | 0.0606 bc | 956.5 ab | 0.0215 a |

Note: *Potentilla*, *Dryas*, and *Picea* data were analyzed by one- way ANOVA and differences detected with Tukey-HSD. *Salix* data were analyzed by Kruskal-Wallis one-way ANOVA and differences detected with a non-parametric Tukey-type multiple comparisons procedure. Values followed by the same letter in each column do not differ significantly ($\alpha$=0.05). n=18.

Table 3.7. Effects of fungal treatments on shoot dry weights (g) of four alpine-subalpine plants in pot combination cultures.

| Fungal treatments | *Potentilla* (mean) | *Dryas* (mean) | *Salix* (mean) | *Picea* (rank sum) |
|---|---|---|---|---|
| Lo1 | 1.0457 a | 0.1130 a | 3.4027 a | 2929.0 ab |
| Lo2 | 1.0415 a | 0.0980 a | 2.8253 a | 1932.0 a |
| Lo3 | 1.0453 a | 0.1193 a | 2.5797 a | 3194.5 b |
| Lo4 | 1.0514 ab | 0.0977 a | 2.7574 a | 3296.5 b |
| Pf | 1.0597 b | 0.1013 a | 3.1616 a | 2469.0 ab |
| Control | 1.0475 a | 0.0860 a | 2.6646 a | 2469.0 ab |

Note: No two treatments of *Dryas* have shown significant difference at the .05 level using Kruskal-Wallis one-way ANOVA. *Potentilla* and *Salix* data were transformed using $Y^{-0.024}$ and $Y^{-0.5}$, respectively, analyzed by one-way ANOVA and differences detected with Tukey-HSD. *Picea* data were analyzed by Kruskal-Wallis one-way ANOVA and differences detected with a non-parametric Tukey-type multiple comparisons procedure. Values followed by the same letter in each column do not differ significantly ($\alpha$=0.05). n=30.

Table 3.8. Statistical analysis (t-test) of dry weight accumulation of *Salix glauca* seedlings grown in the presence and absence of *Sphaerosporella brunnea*. n=21.

| | Mean weight (g) | | |
|---|---|---|---|
| | Uncolonized (Control) | Colonized with *S. brunnea* | 2-Tail Significance |
| Roots | 0.0333 | 0.0276 | 0.559 |
| Shoots | 0.0363 | 0.0363 | 0.997 |

Figures 3.1-3.6. Morphological and anatomical effects of *Leptodontidium orchidicola* and *Phialocephala fortinii* on *Salix glauca* seedlings.

Fig. 3.1. Eight week old *Salix* seedlings grown axenically on TWA in Petri plates: control (C); *P. fortinii*-inoculated (Pf); UAMH 8151-inoculated (Lo1); and UAMH 5422-inoculated (Lo4). 1x.

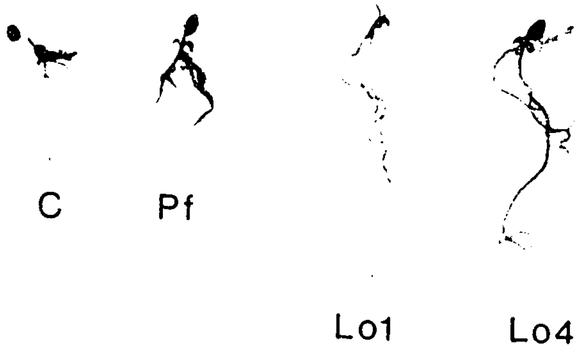
Fig. 3.2. Transverse section of *Salix* root (uninoculated control) from the axenic cultures showing fungus-free tissues and breakdown of cortical cells (cc). Scale bar =10 µm.

Fig. 3.3. Transverse section showing *L. orchidicola* (Lo4) hyphal invasion, collapsed cortical (cc) and vascular cells (vc), and a microsclerotium (arrow) filling a cortical cell. Scale bar = 10 µm.

Fig. 3.4. Inter- and intracellular hyphal penetration (arrowheads) and degraded vascular cells (vc). Lo4-inoculated. Scale bar = 10 µm.

Fig. 3.5. Vascular region showing intact xylem cells (xc) and extensive invasion by *L. orchidicola* (Lo1). Scale bar = 10 µm.

Fig. 3.6. Transverse section showing an obliterated vascular region (vc): other tissues are in various stages of collapse. Lo1-inoculated. Scale bar = 10 µm.



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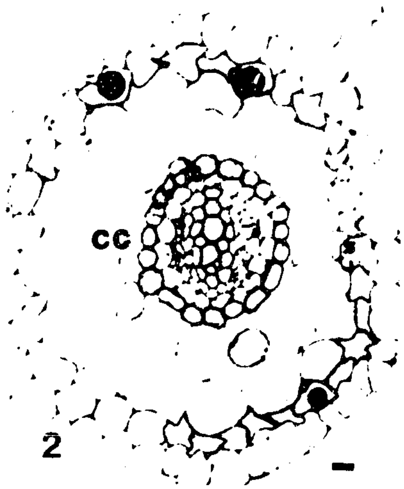

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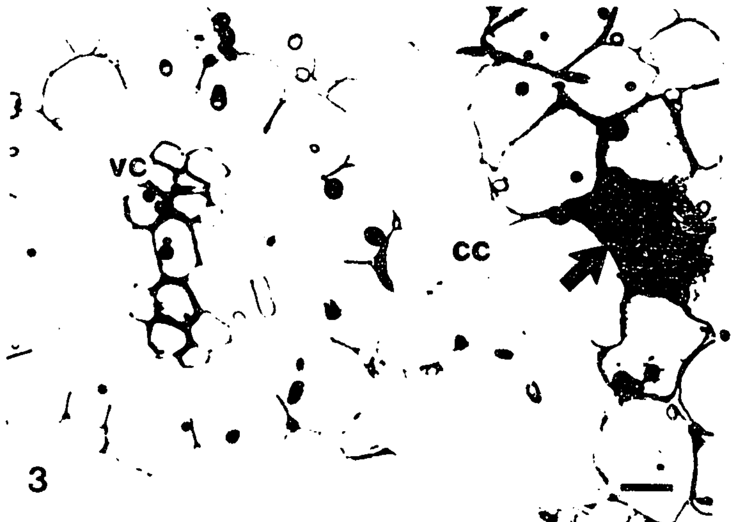

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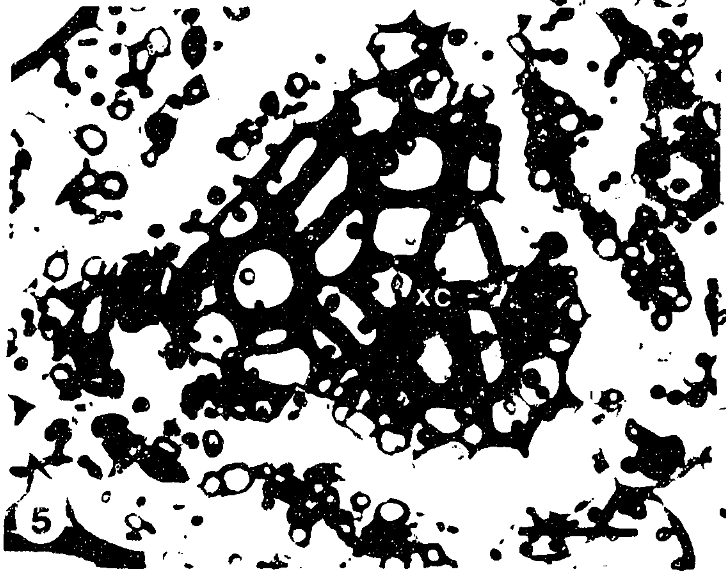

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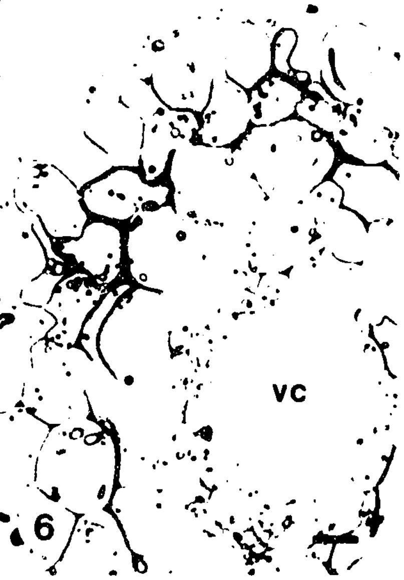

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Figures 3.7-3.11. Sections of roots of axenically-grown *Salix glauca* inoculated with *Phialocephala fortinii*. Scale bar = 10 µm.

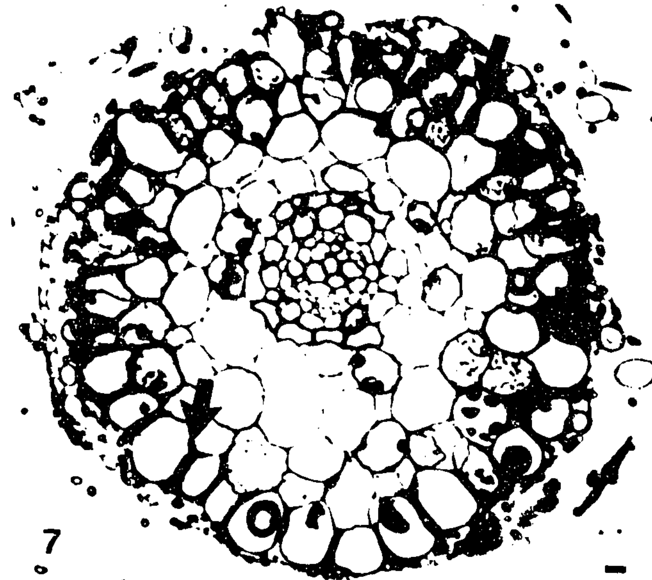
Fig. 3.7. Transverse section showing Hartig net (arrows) associated with the epidermal layer.

Fig. 3.8. Transverse section of a lateral root with a patchy fungal mantle (arrows) and some intercellular penetration (arrowheads) in the epidermal region.

Fig. 3.9. Root transverse section showing microsclerotia (arrows).

Fig. 3.10. Longitudinal section through the epidermis showing microsclerotia (arrows).

Fig. 3.11. Root transverse section showing root hairs colonized by *P. fortinii* (arrows).



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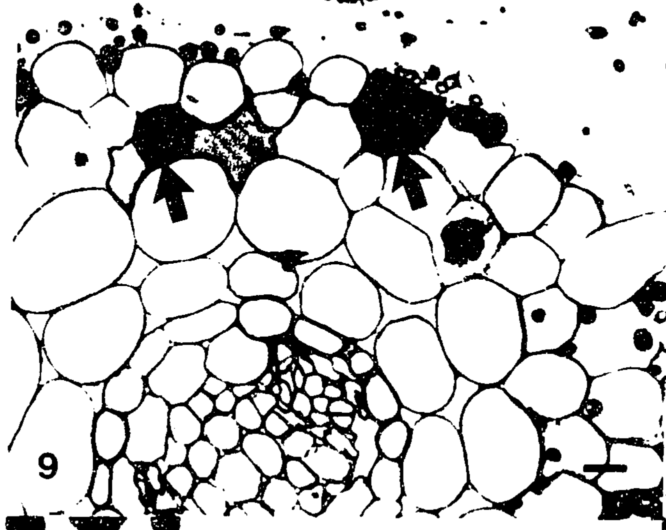

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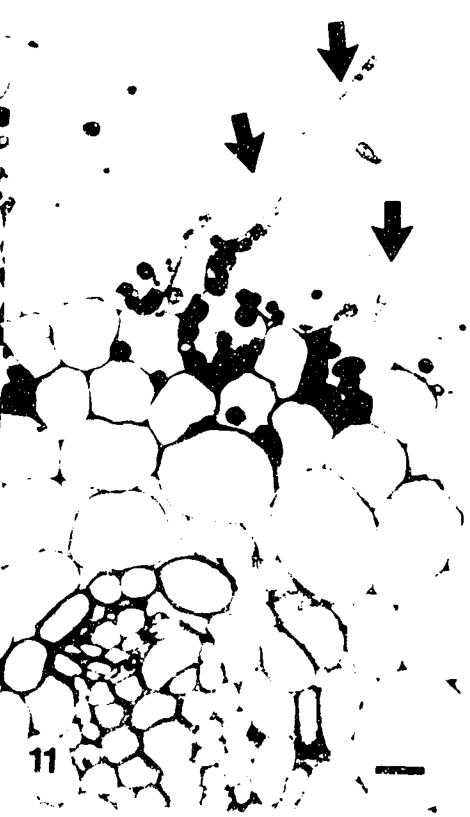

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# Chapter 4

# GENERAL DISCUSSION AND CONCLUSION

Proper identification of the fungus involved in any plant-fungus association is necessary for understanding symbioses and for ensuring reproducibility of experimental work. Currah and Tsuneda (1993) have recognized that difficulties in the taxonomy within the MRA complex have caused problems in elucidating the ecological role of members of this group, hence their study on the vegetative and reproductive morphology of *P. fortinii*.

A summary of the cultural [illegible], and reproductive characteristics of *L. orchidicola* an[illegible] *fortinii* are given in Table 4.1. The most valuable characteristics in differentiating between these two fungi are the type of conidia produced and their mode of conidiogenesis. *Phialocephala fortinii*, after prolonged incubation at cold temperatures, forms conidia that are released from phialides (Wang and Wilcox 1985; Currah et al. 1987; 1988; Currah and Tsuneda 1993), while *L. orchidicola* forms blastic conidia without special manipulation (Fernando and Currah 1995).

This study is the first to try to examine the ecological role of *L. orchidicola. Leptodontidium orchidicola* can live in symbiosis with a wide range of plant species. Its relative ease of isolation and culture, production of polyphenol oxidases and cellulases, and production of conidia are more indicative of saprophytic and/or parasitic trophic strategies than a mycorrhizal one.

*Leptodontidium orchidicola* exhibited a range of symbiotic associations from pathogenic to mycorrhizal, while *P. fortinii* was weakly parasitic to mycorrhizal. Resynthesis experiments have demonstrated that the effects of *L. orchidicola* on its plant hosts could vary depending on the strain of fungus, host taxa, and conditions of synthesis. Similarly, the effects of *P. fortinii* also vary according to its host and cultural conditions. A previous study with *Rhododendron brachycarpum* as the host has also demonstrated the strain-specific effects of *P. fortinii* (Currah et al. 1993). The results of the resynthesis studies support the notion that the types of symbioses produced depend on several factors, i.e. strain of fungus, host, and environment (Marx and Bryan 1970; Giltrap 1982; Molina and Trappe 1982; Wilcox 1983).

*Salix* root sections revealed that *L. orchidicola* has the ability to invade the stele and cause cellular lysis. These characteristics are consistent with the concepts of pathogenicity and incompatibility in the fungus-plant association (Molina and Trappe 1982; O'Dell et al. 1993; Dix and Webster 1995). *Phialocephala fortinii*, on the other hand, exhibited an ability to form an ectomycorrhizal relationship with *Salix* by forming a Hartig net and fungal mantle. Wilcox and Wang (1987) reported that *P. fortinii* formed these ectomycorrhizal features with red spruce and red pine in monoxenic cultures but the axenic resynthesis with *Salix* is the first to show that *P. fortinii* can also form this type of association with an angiosperm host.

The interesting results obtained from the study of root sections warrant a further investigation, with a developmental approach, of the anatomy of the association formed between *L. orchidicola* or *P. fortinii* and alpine-subalpine plants. The use of sections in studying symbiotic organs is worthwhile since there are aspects which are more evident in sections than when using squashed specimens only, e.g. the specific tissues invaded by the fungi can be clearly delimited when observed in sectioned specimens. The importance of close examination of the interface formed by a fungus-root association has been emphasized by Massicotte et al. (1987). Apparently, striking changes can occur over very short distances in the root and misinterpretations are possible if only random portions of roots are studied. The developmental approach should also include longer incubation time. According to Bracker and Littlefield (1973), the interface not only covers space but also "a region in time" and differences may occur from one developmental stage to another.

Another interesting aspect to investigate is the exchange of nutrients that may be occurring between *L. orchidicola* or *P. fortinii* and their plant hosts. This could be studied by using labelled elements, e.g. C, P, and N, to trace their translocation. This is important because nutrient exchange is a key consideration in a mycorrhizal association.

In a fungus-plant symbiosis, the fungus may benefit the host plant not only by giving it nutrients but also by reducing the frequency or intensity of grazing, or by aiding the plant in a pathogen-mediated competition (Currah et al. 1993). The latter involves, on the part of the fungus, parasitizing invasive seedlings or the roots of competing plant species. The aim of the pot combination resynthesis was to establish whether or not *L. orchidicola* and *P. fortinii* are involved in a pathogen-mediated competition. The results obtained were markedly different from those of the monoculture resynthesis. This supports the notion that the type of the fungus-host association produced is affected by the conditions of the synthesis employed (Giltrap 1982). Until procedures for the study of pathogen-mediated competition are improved, the roles of *L. orchidicola* and *P. fortinii* in plant communities are still only a matter for speculation.

## Summary

1) Although the cultural and vegetative characteristics of *L. orchidicola* are similar to *P. fortinii*, another commonly isolated MRA species, its mode of conidiogenesis and the type of conidia it produces are distinctly different. *Leptodontidium orchidicola* forms single-celled conidia which develop blastically at non-specific loci along the lateral or terminal walls of conidiogenous hyphae. Mature conidia are variable in shape and may be sessile or short-stalked. Secondary conidia often develop blastically from the distal end of undehisced primary conidia.

2) *Leptodontidium orchidicola* is relatively common in roots of plants from alpine-subalpine and boreal habitats and it can be easily isolated and cultured.

3) *Leptodontidium orchidicola* produces polyphenol oxidases and is able to degrade cellulose.

4) When grown in axenic culture with native host plants in the genera *Picea, Betula*, and *Potentilla*, *L. orchidicola* exhibited inter- and intracellular penetration of epidermal and cortical cells but did not cause any discernible pathogenic effects.

5) In axenic culture with *Salix glauca* seedlings, *L. orchidicola* caused a marked increase in host root length while at the same time invaded the stele and caused extensive cellular lysis which are manifestations of pathogenicity. *Phialocephala fortinii* formed a Hartig net and thin, patchy mantle and did not invade the stele.

6) In pot monocultures with *Potentilla fruticosa, Dryas octopetala, Salix glauca*, and *Picea glauca* seedlings, the effects of four *L. orchidicola* strains on host dry weight were strain- and host-specific; those of *P. fortinii* were also host-specific.

7) In pot monocultures, *L. orchidicola* formed a range of symbiotic associations that could be considered mycorrhizal to parasitic whereas the effects of *P. fortinii* suggest only amensal, parasitic, or neutral association.

8) In pot combination cultures, the *P. fortinii-Potentilla* symbiosis resulted in a significantly positive effect in the growth of the host plant in contrast with the results from the same symbiosis in monoculture resynthesis. The hypothesis that *L. orchidicola* and *P. fortinii* are involved in a pathogen-mediated competition between plants does not have a conclusive result. Further studies are needed before any valid conclusions can be drawn.

9) The resynthesis experiments demonstrated that the culture or synthesis conditions have an effect on the way *L. orchidicola* and *P. fortinii* behave and the types of association that they form with their plant hosts.

Table 4.1. Cultural, vegetative, and reproductive characteristics of *Leptodontidium orchidicola* and *Phialocephala fortinii* and their behaviour in roots.

| | *Leptodontidium orchidicola* | *Phialocephala fortinii* |
|---|---|---|
| Growth rate on CMA | 66-84 mm diam. in 22 days | 40-60 mm diam. in 21 days |
| Cultural characteristics on CMA | hyaline to olivaceous brown; mostly submerged with sparse grayish aerial mycelium; greenish black below; margins submerged, entire,whitish or very pale | mouse gray to dark gray; felt-like and plane or sulcate from above; greenish black to black below; margins submerged, entire or fibrillose, glabrous or whitish |
| Cultural characteristics on PDA | whitish first becoming olivaceous or brownish gray to dark gray, felt-like and plane or sulcate from above; pale to dark olivaceous gray or grayish brown to black below | mouse gray to dark gray; felt-like and plane or sulcate from above; greenish black to black below |
| Aerial hyphae | walls smooth or with fine broad and flat asperulations; hyaline to olivaceous brown; 2-3 µm diam.; may form toruloid cells, hyphal loops and strands | walls smooth or with dome-shaped bumps or balloon-like blisters; hyaline to olivaceous brown or greenish; 2-5 µm diam.; may form toruloid cells, hyphal loops and strands |
| Submerged hyphae | composed of toruloid cells that may form sclerotic masses; sclerotia rare; may form chlamydospores | composed of toruloid cells; sclerotia common |
| Conidiogenous apparatus | undifferentiated hyphae | complex, branched, fan-shaped; phialidic |
| Conidia | 1-3 x 3-6 µm; globose, subglobose, ellipsoidal, oval, ovate, obovate, pyriform, clavate, or spathulate; lateral solitary or terminal in small clusters; forms secondary conidia | primary conidium bullet-shaped, 1-1.5 x 2.5-3 µm; secondary conidia more or less spherical; released from phialides |
| Behaviour in roots | penetrates roots inter- and intracellularly; forms microsclerotia; may invade stele and cause cellular necrosis | penetrates roots inter- and intracellularly; forms microsclerotia; may form Hartig net and mantle |

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# Appendix 1. Glossary of mycological terms used

**anastomosis** - fusion of somatic hyphae.

**arbuscule** - a finely branched organ produced by endomycorrhizal fungi inside host root cells.

**Ascomycotina** - Subdivision of Dikaryomycota; forms endogenous meiospores in asci and has a restricted dikaryon; generally called ascomycetes

**ascus** (pl.=**asci**) - the meiosporangium of the ascomycetes; originally as a spore-gun.

**asperulate** - delicately rough with projections or points.

**Basidiomycotina** - Subdivision of Dikaryomycota; forms exogenous meiospores on basidia and has an extended dikaryon; generally called basidiomycetes.

**basidium** (pl.=**basidia**) - the meiosporangium of the basidiomycetes; produces exogenous meiospores (usually 4) on special projections called sterigmata.

**blastic** - one of the two basic sorts of conidiogenesis, characterized by a marked enlargement of a recognizable conidial initial before the initial is delimited by a septum.

**chlamydospore** - an often thick-walled, resistant mitospore, formed by many fungi.

**colony** - a discrete mycelium of a fungus.

**conidiogenesis** - the process of conidium formation.

**conidiogenous** - giving rise to conidia.

**conidium** (pl.=**conidia**) - a specialized, non-motile asexual spore, usually falling off readily, not developed by cytoplasmic cleavage.

**culture** - a growth of one organism or of a group of organisms for the purpose of experiment.

**dematiaceous** - pigmented, more or less darkly.

**denticle** - a small tooth-like projection especially one on which a spore is borne.

**Dikaryomycota** - a division of true fungi comprising the Subdivisions Ascomycotina and Basidiomycotina and their anamorphs; characterized by hyphae with chitinous walls and perforate septa, and (usually) the occurrence of a dikaryotic phase.

**ectomycorrhiza** - ectotrophic mycorrhiza; mycorrhiza in which a dikaryomycotan mycelium ramifies through the soil, forms a mantle around individual rootlets, and grows between cells of the root cortex (and/or epidermis) forming a Hartig net.

**endomycorrhiza** - endotrophic mycorrhiza; hyphae grow between and within root cells.

## Appendix 1 (Continued)

**endophyte** - any organism living within the tissues of a plant.

**Fungi Imperfecti** - fungi which are, or are suspected to be, the anamorphs of ascomycetes or basidiomycetes; conidial fungi.

**fungus** (pl.=**fungi**) - non-photosynthesizing eukaryotes that absorb their food; usually producing a network of apically extending, branched tubes called hyphae.

**glabrous** - smooth, not hairy.

**hyaline** - transparent or nearly so; colourless.

**Hartig net** - the intercellular hyphal network formed by a mycorrhizal fungus upon the surface of a root.

**hypha** (pl.=**hyphae**) - the tubular architectural module of almost all fungi.

**Hyphomycetes** - conidial anamorphs (mostly ascomycetous, some basidiomycetous) producing exposed conidiophores.

**inoculate** - to put a microorganism into an organism or a substratum.

**isolate** - the first 1-spore or pure isolation of a fungus from any place.

**mantle** - a compact layer of hyphae enclosing short feeder roots of ectomycorrhizal plants.

**mycelium** (pl.=**mycelia**) - collective term for hyphae; the vegetative thallus of a fungus excluding organs of sporulation or sclerotia.

**mycorrhiza** (pl.=**mycorrhizae** or **mycorrhizas**) - a symbiotic relationship between a filamentous fungus and the roots of a higher plant.

**peloton** - the coiled mass of hyphae which occurs inside the cortical cells of an endomycorrhiza.

**phialide** - a cell which develops one or more open ends from which a basipetal succession of conidia develops without an increase in length of the phialide itself.

**phialidic** - describes a process of conidiogenesis with the conidia produced by a phialide.

**schizolytic** - secession of conidia involving a splitting of the delimiting septum so that one half of the crosswall becomes the base of the seceding conidium and the other half covers the apex of the conidiogenous cell.

**sclerotium** (pl.=**sclerotia**) - a firm, usually rounded mass of hyphae with or without the addition of host tissue, normally lacking spores; a resting or overwintering structure.

**septum** (pl.=**septa**) - a cell wall or partition.

## Appendix 1 (Continued)

**symbiosis** - the intimate living together of unlike organisms.

**sympodial** - characterized by continued growth, after the main axis has produced a terminal spore, by the development of a succession of apices each of which originates below and to one side of the previous apex.

**vesicle** - swollen, lipid-filled cells produced inside plant roots by most VAM fungi.

**vesicular-arbuscular mycorrhiza** - VAM; endomycorrhiza; plant roots colonized by mutualistic fungi of the Glomales; forms vesicles and arbuscules.

**Woronin bodies** - two small spherical objects that sit, one on each side, near the pore of the ascomycete septum.

**Zygomycota** - a division of true fungi; fast-growing, terrestrial fungi with no motile cells; produce zygosporangia (usually thick-walled, often ornamented, multinucleate resting sporangia formed following anastomosis of gametangia arising from compatible mycelia or from the same mycelium); generally called zygomycetes.

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# Appendix 2. Percent mortality rate and root length* of *Salix glauca* seedlings in Petri dish resynthesis

| Mortality rate (%) | | | | Root length (mm) | | | |
|---|---|---|---|---|---|---|---|
| Control | Pf-inoculated | Lo1-inoculated | Lo4-inoculated | Control | Pf-inoculated | Lo1-inoculated | Lo4-inoculated |
| 100 | 100 | 60 | 60 | 12 | 21 | 114 | 133 |
| 100 | 60 | 67 | 100 | 64 | 27 | 60 | 53 |
| 50 | 20 | 20 | 80 | 17 | 42 | 74 | 39 |
| 100 | 75 | 75 | 78 | 11 | 15 | 25 | 68 |
| 17 | 17 | 50 | 50 | 17 | 12 | 24 | 21 |
| | | | | 17 | 7 | 30 | 16 |
| | | | | 16 | 21 | 54 | 70 |

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*Seven seedlings were randomly chosen from the total seedlings that survived and scored for root length.

# Appendix 3. Shoot dry weights (g) of four alpine-subalpine plants in pot monoculture resynthesis: uninoculated control and inoculated with *Phialocephala fortinii* (Pf) or *Leptodontidium orchidicola* (Lo)

| Sample number | Control | | | | Pf-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.09 | 0.17 | 0.14 | 0.05 | 0.09 | 0.15 | 0.10 | 0.02 |
| 2 | 0.18 | 0.17 | 0.10 | 0.04 | 0.11 | 0.23 | 0.13 | 0.02 |
| 3 | 0.13 | 0.20 | 0.13 | 0.01 | 0.09 | 0.13 | 0.11 | 0.02 |
| 4 | 0.18 | 0.19 | 0.06 | 0.01 | 0.11 | 0.18 | 0.05 | 0.01 |
| 5 | 0.11 | 0.27 | 0.04 | 0.01 | 0.11 | 0.17 | 0.05 | 0.01 |
| 6 | 0.21 | 0.27 | 0.08 | 0.02 | 0.12 | 0.17 | 0.06 | 0.02 |
| 7 | 0.16 | 0.18 | 0.13 | 0.02 | 0.15 | 0.20 | 0.05 | 0.01 |
| 8 | 0.17 | 0.14 | 0.10 | 0.01 | 0.17 | 0.18 | 0.03 | 0.02 |
| 9 | 0.17 | 0.19 | 0.05 | 0.01 | 0.07 | 0.18 | 0.06 | 0.01 |
| 10 | 0.19 | 0.18 | 0.04 | 0.01 | 0.13 | 0.23 | 0.04 | 0.02 |
| 11 | 0.15 | 0.18 | 0.06 | 0.01 | 0.15 | 0.16 | 0.05 | 0.01 |
| 12 | 0.14 | 0.23 | 0.05 | 0.01 | 0.12 | 0.17 | 0.03 | 0.02 |
| 13 | 0.13 | 0.19 | 0.06 | 0.01 | 0.10 | 0.18 | 0.03 | 0.02 |
| 14 | 0.17 | 0.26 | 0.07 | 0.01 | 0.11 | 0.23 | 0.04 | 0.01 |
| 15 | 0.16 | 0.33 | 0.09 | 0.01 | 0.10 | 0.17 | 0.02 | 0.01 |
| 16 | 0.14 | 0.22 | 0.03 | 0.01 | 0.10 | 0.15 | 0.04 | 0.01 |
| 17 | 0.10 | 0.16 | 0.04 | 0.01 | 0.06 | 0.14 | 0.02 | 0.01 |
| 18 | 0.14 | 0.20 | 0.06 | 0.01 | 0.07 | 0.17 | 0.02 | 0.01 |
| 19 | 0.16 | 0.28 | 0.04 | 0.01 | 0.08 | 0.18 | 0.03 | 0.01 |
| 20 | 0.13 | 0.20 | 0.07 | 0.01 | 0.08 | 0.19 | 0.03 | 0.01 |
| 21 | 0.13 | 0.21 | 0.05 | 0.01 | 0.08 | 0.19 | 0.03 | 0.02 |
| 22 | 0.14 | 0.18 | 0.05 | 0.01 | 0.09 | 0.20 | 0.02 | 0.02 |
| 23 | 0.12 | 0.16 | 0.04 | 0.01 | 0.08 | 0.22 | 0.01 | 0.02 |
| 24 | 0.09 | 0.12 | 0.05 | 0.01 | 0.08 | 0.12 | 0.03 | 0.01 |
| 25 | 0.10 | 0.16 | 0.05 | 0.01 | 0.06 | 0.10 | 0.03 | 0.01 |
| 26 | 0.10 | 0.12 | 0.04 | 0.01 | 0.07 | 0.20 | 0.03 | 0.01 |
| 27 | 0.07 | 0.19 | 0.03 | 0.01 | 0.09 | 0.11 | 0.03 | 0.01 |
| 28 | 0.07 | 0.16 | 0.02 | 0.01 | 0.11 | 0.08 | 0.03 | 0.01 |
| 29 | 0.12 | 0.16 | 0.02 | 0.01 | 0.08 | 0.10 | 0.02 | 0.01 |
| 30 | 0.08 | 0.11 | 0.02 | 0.01 | 0.08 | 0.11 | 0.02 | 0.02 |

# Appendix 3 (Continued)

| Sample number | Lo1-inoculated | | | | Lo2-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.15 | 0.16 | 0.15 | 0.04 | 0.16 | 0.20 | 0.24 | 0.02 |
| 2 | 0.18 | 0.12 | 0.10 | 0.04 | 0.11 | 0.18 | 0.16 | 0.01 |
| 3 | 0.16 | 0.11 | 0.10 | 0.02 | 0.14 | 0.15 | 0.06 | 0.01 |
| 4 | 0.20 | 0.15 | 0.08 | 0.01 | 0.18 | 0.12 | 0.33 | 0.02 |
| 5 | 0.22 | 0.19 | 0.07 | 0.02 | 0.12 | 0.09 | 0.20 | 0.01 |
| 6 | 0.18 | 0.12 | 0.10 | 0.02 | 0.12 | 0.13 | 0.07 | 0.01 |
| 7 | 0.19 | 0.10 | 0.07 | 0.02 | 0.12 | 0.10 | 0.22 | 0.01 |
| 8 | 0.14 | 0.09 | 0.08 | 0.02 | 0.15 | 0.10 | 0.03 | 0.02 |
| 9 | 0.18 | 0.12 | 0.12 | 0.02 | 0.18 | 0.08 | 0.04 | 0.02 |
| 10 | 0.15 | 0.13 | 0.09 | 0.02 | 0.10 | 0.14 | 0.15 | 0.01 |
| 11 | 0.19 | 0.13 | 0.09 | 0.01 | 0.13 | 0.20 | 0.15 | 0.01 |
| 12 | 0.17 | 0.12 | 0.06 | 0.01 | 0.13 | 0.16 | 0.11 | 0.01 |
| 13 | 0.16 | 0.18 | 0.08 | 0.01 | 0.13 | 0.13 | 0.07 | 0.01 |
| 14 | 0.16 | 0.14 | 0.14 | 0.01 | 0.15 | 0.20 | 0.07 | 0.01 |
| 15 | 0.18 | 0.13 | 0.10 | 0.02 | 0.12 | 0.10 | 0.04 | 0.01 |
| 16 | 0.13 | 0.15 | 0.07 | 0.02 | 0.14 | 0.10 | 0.05 | 0.01 |
| 17 | 0.12 | 0.08 | 0.08 | 0.02 | 0.11 | 0.13 | 0.02 | 0.01 |
| 18 | 0.10 | 0.11 | 0.08 | 0.02 | 0.19 | 0.14 | 0.12 | 0.01 |
| 19 | 0.15 | 0.10 | 0.06 | 0.02 | 0.17 | 0.08 | 0.02 | 0.01 |
| 20 | 0.14 | 0.20 | 0.09 | 0.01 | 0.19 | 0.10 | 0.07 | 0.01 |
| 21 | 0.17 | 0.10 | 0.03 | 0.01 | 0.12 | 0.11 | 0.06 | 0.01 |
| 22 | 0.17 | 0.10 | 0.05 | 0.01 | 0.09 | 0.10 | 0.06 | 0.01 |
| 23 | 0.11 | 0.11 | 0.04 | 0.01 | 0.12 | 0.09 | 0.09 | 0.01 |
| 24 | 0.16 | 0.09 | 0.03 | 0.02 | 0.12 | 0.10 | 0.07 | 0.02 |
| 25 | 0.20 | 0.06 | 0.06 | 0.01 | 0.16 | 0.09 | 0.04 | 0.02 |
| 26 | 0.20 | 0.06 | 0.08 | 0.02 | 12 | 0.10 | 0.02 | 0.01 |
| 27 | 0.17 | 0.05 | 0.05 | 0.01 | 12 | 0.07 | 0.03 | 0.01 |
| 28 | 0.19 | 0.06 | 0.06 | 0.01 | 0.16 | 0.07 | 0.04 | 0.01 |
| 29 | 0.10 | 0.07 | 0.06 | 0.02 | 0.10 | 0.07 | 0.05 | 0.01 |
| 30 | 0.14 | 0.05 | 0.09 | 0.01 | 0.06 | 0.09 | 0.05 | 0.01 |

# Appendix 3 (Continued)

| Sample number | Lo3-inoculated | | | | Lo4-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.14 | 0.18 | 0.15 | 0.02 | 0.08 | 0.21 | 0.11 | 0.01 |
| 2 | 0.11 | 0.24 | 0.20 | 0.03 | 0.11 | 0.17 | 0.08 | 0.01 |
| 3 | 0.13 | 0.19 | 0.19 | 0.01 | 0.16 | 0.16 | 0.05 | 0.01 |
| 4 | 0.17 | 0.28 | 0.07 | 0.02 | 0.08 | 0.12 | 0.13 | 0.01 |
| 5 | 0.12 | 0.13 | 0.12 | 0.02 | 0.14 | 0.15 | 0.08 | 0.02 |
| 6 | 0.16 | 0.22 | 0.08 | 0.02 | 0.10 | 0.10 | 0.05 | 0.02 |
| 7 | 0.15 | 0.12 | 0.09 | 0.02 | 0.11 | 0.08 | 0.06 | 0.02 |
| 8 | 0.11 | 0.30 | 0.08 | 0.02 | 0.11 | 0.14 | 0.10 | 0.02 |
| 9 | 0.16 | 0.12 | 0.12 | 0.02 | 0.10 | 0.12 | 0.08 | 0.02 |
| 10 | 0.10 | 0.16 | 0.16 | 0.02 | 0.11 | 0.21 | 0.11 | 0.01 |
| 11 | 0.12 | 0.21 | 0.16 | 0.02 | 0.14 | 0.12 | 0.08 | 0.02 |
| 12 | 0.13 | 0.17 | 0.08 | 0.02 | 0.14 | 0.11 | 0.05 | 0.02 |
| 13 | 0.12 | 0.18 | 0.05 | 0.02 | 0.09 | 0.11 | 0.06 | 0.02 |
| 14 | 0.13 | 0.16 | 0.08 | 0.02 | 0.18 | 0.09 | 0.04 | 0.02 |
| 15 | 0.10 | 0.21 | 0.05 | 0.02 | 0.13 | 0.1 | 0.06 | 0.02 |
| 16 | 0.11 | 0.23 | 0.09 | 0.01 | 0.12 | 0.06 | 0.06 | 0.01 |
| 17 | 0.07 | 0.09 | 0.03 | 0.02 | 0.09 | 0.06 | 0.03 | 0.03 |
| 18 | 0.08 | 0.15 | 0.04 | 0.01 | 0.13 | 0.05 | 0.04 | 0.02 |
| 19 | 0.10 | 0.17 | 0.05 | 0.02 | 0.13 | 0.05 | 0.03 | 0.02 |
| 20 | 0.09 | 0.15 | 0.06 | 0.02 | 0.08 | 0.06 | 0.04 | 0.02 |
| 21 | 0.07 | 0.22 | 0.06 | 0.02 | 0.08 | 0.07 | 0.05 | 0.02 |
| 22 | 0.08 | 0.20 | 0.05 | 0.02 | 0.10 | 0.07 | 0.04 | 0.02 |
| 23 | 0.08 | 0.14 | 0.06 | 0.01 | 0.10 | 0.08 | 0.05 | 0.02 |
| 24 | 0.08 | 0.16 | 0.06 | 0.02 | 0.14 | 0.08 | 0.04 | 0.02 |
| 25 | 0.06 | 0.06 | 0.03 | 0.02 | 0.11 | 0.04 | 0.05 | 0.06 |
| 26 | 0.08 | 0.07 | 0.04 | 0.01 | 0.06 | 0.06 | 0.04 | 0.03 |
| 27 | 0.11 | 0.11 | 0.04 | 0.02 | 0.09 | 0.04 | 0.03 | 0.04 |
| 28 | 0.11 | 0.06 | 0.05 | 0.02 | 0.08 | 0.05 | 0.06 | 0.03 |
| 29 | 0.07 | 0.07 | 0.04 | 0.01 | 0.08 | 0.04 | 0.06 | 0.03 |
| 30 | 0.06 | 0.11 | 0.03 | 0.02 | 0.05 | 0.04 | 0.02 | 0.02 |

Appendix 4. Root dry weights (g) of four alpine-subalpine plants in pot monoculture resynthesis: uninoculated control and inoculated with *Phialocephala fortinii* (Pf) or *Leptodontidium orchidicola* (Lo)

| Sample number | Control | | | | Pf-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.476 | 0.050 | 0.11 | 0.034 | 0.232 | 0.113 | 0.10 | 0.038 |
| 2 | 0.342 | 0.051 | 0.12 | 0.034 | 0.216 | 0.058 | 0.10 | 0.029 |
| 3 | 0.262 | 0.119 | 0.14 | 0.019 | 0.295 | 0.070 | 0.04 | 0.021 |
| 4 | 0.358 | 0.081 | 0.25 | 0.029 | 0.244 | 0.078 | 0.09 | 0.035 |
| 5 | 0.245 | 0.049 | 0.16 | 0.026 | 0.327 | 0.096 | 0.12 | 0.022 |
| 6 | 0.321 | 0.052 | 0.18 | 0.023 | 0.249 | 0.074 | 0.02 | 0.026 |
| 7 | 0.376 | 0.047 | 0.15 | 0.020 | 0.202 | 0.073 | 0.08 | 0.019 |
| 8 | 0.226 | 0.038 | 0.10 | 0.019 | 0.209 | 0.078 | 0.08 | 0.035 |
| 9 | 0.285 | 0.051 | 0.11 | 0.019 | 0.204 | 0.063 | [illegible] | 0.018 |
| 10 | 0.207 | 0.081 | 0.13 | 0.010 | 0.194 | 0.055 | 0.10 | 0.025 |
| 11 | 0.212 | 0.044 | 0.11 | 0.017 | 0.201 | 0.067 | 0.06 | 0.029 |
| 12 | 0.250 | 0.063 | 0.11 | 0.013 | 0.128 | 0.076 | 0.07 | 0.026 |
| 13 | 0.231 | 0.058 | 0.09 | 0.022 | 0.173 | 0.062 | 0.05 | 0.028 |
| 14 | 0.244 | 0.069 | 0.10 | 0.023 | 0.176 | 0.065 | 0.05 | 0.028 |
| 15 | 0.150 | 0.056 | 0.05 | 0.020 | 0.098 | 0.047 | 0.03 | 0.031 |
| 16 | 0.244 | 0.041 | 0.11 | 0.017 | 0.113 | 0.075 | 0.11 | 0.025 |
| 17 | 0.255 | 0.093 | 0.23 | 0.021 | 0.138 | 0.063 | 0.27 | 0.024 |
| 18 | 0.236 | 0.048 | 0.36 | 0.021 | 0.156 | 0.080 | 0.25 | 0.016 |

## Appendix 4 (Continued)

| Sample number | Lo1-inoculated | | | | Lo2-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.335 | 0.050 | 0.22 | 0.039 | 0.194 | 0.048 | 0.22 | 0.025 |
| 2 | 0.347 | 0.033 | 0.17 | 0.029 | 0.299 | 0.051 | 0.17 | 0.019 |
| 3 | 0.418 | 0.050 | 0.20 | 0.025 | 0.213 | 0.029 | 0.20 | 0.023 |
| 4 | 0.447 | 0.040 | 0.15 | 0.037 | 0.208 | 0.028 | 0.[illegible] | 0.020 |
| 5 | 0.404 | 0.023 | 0.19 | 0.037 | 0.191 | 0.033 | 0.19 | 0.023 |
| 6 | 0.335 | 0.029 | 0.15 | 0.036 | 0.249 | 0.042 | 0.15 | 0.022 |
| 7 | 0.323 | 0.042 | 0.26 | 0.029 | 0.321 | 0.028 | 0.26 | 0.024 |
| 8 | 0.377 | 0.035 | 0.18 | 0.036 | 0.236 | 0.029 | 0.18 | 0.015 |
| 9 | 0.286 | 0.034 | 0.15 | 0.021 | 0.171 | 0.049 | 0.15 | 0.013 |
| 10 | 0.392 | 0.033 | 0.22 | 0.023 | 0.212 | 0.034 | 0.22 | 0.013 |
| 11 | 0.267 | 0.041 | 0.14 | 0.021 | 0.215 | 0.030 | 0.14 | 0.021 |
| 12 | 0.324 | 0.045 | 0.15 | 0.022 | 0.238 | 0.058 | 0.15 | 0.017 |
| 13 | 0.303 | 0.029 | 0.16 | 0.022 | 0.096 | 0.025 | 0.16 | 0.024 |
| 14 | 0.189 | 0.035 | 0.13 | 0.032 | 0.225 | 0.033 | 0.13 | 0.023 |
| 15 | 0.198 | 0.021 | 0.14 | 0.025 | 0.151 | 0.043 | 0.14 | 0.020 |
| 16 | 0.214 | 0.018 | 0.18 | 0.039 | 0.183 | 0.032 | 0.18 | 0.038 |
| 17 | 0.311 | 0.024 | 0.22 | 0.026 | 0.284 | 0.029 | 0.22 | 0.012 |
| 18 | 0.184 | 0.052 | 0.23 | 0.021 | 0.240 | 0.032 | 0.23 | 0.020 |

## Appendix 4 (Continued)

| Sample number | Lo3-inoculated |  |  |  | Lo4-inoculated |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.209 | 0.096 | 0.15 | 0.053 | 0.227 | 0.051 | 0.15 | 0.016 |
| 2 | 0.193 | 0.054 | 0.19 | 0.030 | 0.198 | 0.036 | 0.06 | 0.037 |
| 3 | 0.214 | 0.059 | 0.16 | 0.031 | 0.229 | 0.058 | 0.23 | 0.021 |
| 4 | 0.272 | 0.072 | 0.15 | 0.037 | 0.198 | 0.042 | 0.12 | 0.042 |
| 5 | 0.228 | 0.050 | 0.16 | 0.030 | 0.195 | 0.027 | 0.11 | 0.031 |
| 6 | 0.218 | 0.075 | 0.11 | 0.033 | 0.195 | 0.035 | 0.13 | 0.028 |
| 7 | 0.272 | 0.045 | 0.14 | 0.036 | 0.175 | 0.036 | 0.09 | 0.031 |
| 8 | 0.121 | 0.043 | 0.14 | 0.026 | 0.175 | 0.038 | 0.11 | 0.022 |
| 9 | 0.215 | 0.061 | 0.13 | 0.020 | 0.133 | 0.038 | 0.13 | 0.030 |
| 10 | 0.168 | 0.053 | 0.17 | 0.029 | 0.161 | 0.045 | 0.13 | 0.025 |
| 11 | 0.171 | 0.056 | 0.11 | 0.029 | 0.125 | 0.027 | 0.11 | 0.044 |
| 12 | 0.202 | 0.053 | 0.13 | 0.027 | 0.250 | 0.040 | 0.08 | 0.028 |
| 13 | 0.100 | 0.054 | 0.08 | 0.035 | 0.206 | 0.032 | 0.10 | 0.023 |
| 14 | 0.217 | 0.091 | 0.07 | 0.024 | 0.260 | 0.019 | 0.09 | 0.024 |
| 15 | 0.121 | 0.027 | 0.21 | 0.031 | 0.153 | 0.023 | 0.11 | 0.047 |
| 16 | 0.155 | 0.042 | 0.22 | 0.026 | 0.106 | 0.019 | 0.19 | 0.032 |
| 17 | 0.180 | 0.043 | 0.25 | 0.023 | 0.214 | 0.021 | 0.17 | 0.027 |
| 18 | 0.198 | 0.041 | 0.34 | 0.038 | 0.097 | 0.026 | 0.18 | 0.022 |

Appendix 5. Shoot dry weights (g) of four alpine-subalpine plants in pot combination cultures: uninoculated control and inoculated with *Phialocephala fortinii* (Pf) or *Leptodontidium orchidicola* (Lo)

| Sample number | Control | | | | Pf-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.03 | 0.02 | 1.08 | 0.01 | 0.07 | 0.02 | 1.25 | 0.01 |
| 2 | 0.34 | 0.03 | 0.33 | 0.01 | 0.03 | 0.11 | 0.17 | 0.01 |
| 3 | 0.13 | 0.04 | 0.10 | 0.01 | 0.09 | 0.03 | 0.23 | 0.01 |
| 4 | 0.10 | 0.05 | 0.38 | 0.01 | 0.08 | 0.08 | 0.07 | [illegible] |
| 5 | 0.09 | 0.04 | 0.60 | 0.01 | 0.01 | 0.12 | 0.71 | 0.01 |
| 6 | 0.07 | 0.02 | 0.44 | 0.03 | 0.05 | 0.01 | 0.44 | 0.01 |
| 7 | 0.17 | 0.06 | 0.23 | 0.01 | 0.12 | 0.23 | 0.15 | 0.02 |
| 8 | 0.09 | 0.09 | 0.40 | 0.01 | 0.06 | 0.10 | 0.15 | 0.02 |
| 9 | 0.14 | 0.05 | 0.31 | 0.01 | 0.17 | 0.06 | 0.21 | 0.01 |
| 10 | 0.04 | 0.13 | 0.28 | 0.02 | 0.05 | 0.09 | 0.13 | 0.02 |
| 11 | 0.17 | 0.04 | 0.17 | 0.01 | 0.13 | 0.18 | 0.16 | 0.02 |
| 12 | 0.12 | 0.12 | 0.24 | 0.01 | 0.07 | 0.10 | 0.14 | [illegible] |
| 13 | 0.15 | 0.12 | 0.23 | 0.01 | 0.10 | 0.11 | 0.14 | [illegible] |
| 14 | 0.03 | 0.09 | 0.26 | 0.01 | 0.09 | 0.07 | 0.12 | [illegible] |
| 15 | 0.15 | 0.05 | 0.12 | 0.01 | 0.20 | 0.17 | 0.08 | 0.01 |
| 16 | 0.17 | 0.06 | 0.12 | 0.01 | 0.27 | 0.11 | 0.09 | 0.01 |
| 17 | 0.20 | 0.08 | 0.17 | 0.01 | 0.09 | 0.18 | 0.09 | 0.01 |
| 18 | 0.30 | 0.15 | 0.10 | 0.01 | 0.10 | 0.02 | 0.12 | 0.02 |
| 19 | 0.19 | 0.05 | 0.18 | 0.02 | 0.14 | 0.10 | 0.13 | 0.02 |
| 20 | 0.31 | 0.16 | 0.05 | 0.01 | 0.14 | 0.05 | 0.04 | 0.03 |
| 21 | 0.16 | 0.14 | 0.13 | 0.01 | 0.15 | 0.16 | 0.08 | 0.01 |
| 22 | 0.09 | 0.27 | 0.08 | 0.02 | 0.06 | 0.04 | 0.14 | 0.01 |
| 23 | 0.16 | 0.07 | 0.08 | 0.01 | 0.07 | 0.08 | 0.11 | 0.01 |
| 24 | 0.24 | 0.17 | 0.06 | 0.02 | 0.08 | 0.12 | 0.04 | 0.01 |
| 25 | 0.16 | 0.10 | 0.29 | 0.01 | 0.11 | 0.20 | 0.03 | 0.01 |
| 26 | 0.14 | 0.06 | 0.10 | 0.02 | 0.10 | 0.10 | 0.05 | 0.01 |
| 27 | 0.28 | 0.07 | 0.06 | 0.01 | 0.19 | 0.10 | 0.05 | 0.01 |
| 28 | 0.46 | 0.03 | 0.07 | 0.02 | 0.04 | 0.13 | 0.07 | 0.01 |
| 29 | 0.17 | 0.11 | 0.11 | 0.02 | 0.19 | 0.09 | 0.04 | 0.02 |
| 30 | 0.47 | 0.11 | 0.03 | 0.02 | 0.14 | 0.08 | 0.08 | 0.01 |

# Appendix 5 (Continued)

| Sample number | Lo1-inoculated | | | | Lo2-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | [illegible] | 0.12 | [illegible] | 0.02 | 0.19 | 0.10 | 0.84 | 0.01 |
| 2 | 0.17 | 0.07 | [illegible] | 0.03 | 0.35 | 0.11 | 0.16 | 0.01 |
| 3 | 0.14 | 0.03 | [illegible] | 0.05 | 0.21 | 0.12 | 0.18 | 0.01 |
| 4 | 0.19 | 0.16 | [illegible] | 0.01 | 0.16 | 0.07 | 0.73 | 0.01 |
| 5 | 0.12 | 0.07 | 0.26 | 0.01 | 0.05 | 0.07 | 0.72 | 0.01 |
| 6 | 0.11 | 0.10 | 0.15 | 0.02 | 0.22 | 0.10 | 0.25 | 0.01 |
| 7 | 0.14 | 0.12 | 0.11 | 0.01 | 0.13 | 0.02 | 0.31 | 0.01 |
| 8 | 0.36 | 0.08 | 0.12 | 0.01 | 0.18 | 0.12 | 0.45 | 0.01 |
| 9 | 0.08 | 0.12 | 0.18 | 0.01 | 0.07 | 0.05 | 0.40 | 0.01 |
| 10 | 0.04 | 0.24 | 0.14 | 0.02 | 0.07 | 0.07 | 0.41 | 0.02 |
| 11 | 0.14 | 0.08 | 0.16 | 0.02 | 0.14 | 0.01 | 0.26 | 0.01 |
| 12 | 0.14 | 0.12 | 0.13 | 0.01 | 0.28 | 0.02 | 0.27 | 0.01 |
| 13 | 0.20 | 0.12 | 0.09 | 0.01 | 0.13 | 0.05 | 0.32 | 0.01 |
| 14 | 0.20 | 0.16 | 0.10 | 0.02 | 0.25 | 0.09 | 0.30 | 0.02 |
| 15 | 0.36 | 0.06 | 0.09 | 0.02 | 0.08 | 0.07 | 0.23 | 0.01 |
| 16 | 0.11 | 0.23 | 0.09 | 0.01 | 0.12 | 0.09 | 0.16 | 0.01 |
| 17 | 0.10 | 0.12 | 0.14 | 0.01 | 0.08 | 0.12 | 0.22 | 0.01 |
| 18 | 0.12 | 0.06 | 0.17 | 0.02 | 0.22 | 0.11 | 0.13 | 0.01 |
| 19 | 0.33 | 0.03 | 0.07 | 0.02 | 0.36 | 0.12 | 0.08 | 0.01 |
| 20 | 0.25 | 0.23 | 0.06 | 0.02 | 0.36 | 0.11 | 0.04 | 0.01 |
| 21 | 0.12 | 0.07 | 0.06 | 0.01 | 0.19 | 0.18 | 0.09 | 0.01 |
| 22 | 0.10 | 0.09 | 0.07 | 0.01 | 0.40 | 0.09 | 0.04 | 0.01 |
| 23 | 0.19 | 0.13 | 0.05 | 0.01 | 0.21 | 0.11 | 0.06 | 0.01 |
| 24 | 0.03 | 0.32 | 0.05 | 0.01 | 0.14 | 0.19 | 0.08 | 0.01 |
| 25 | 0.32 | 0.18 | 0.04 | 0.02 | 0.34 | 0.03 | 0.09 | 0.01 |
| 26 | 0.21 | 0.04 | 0.04 | 0.01 | 0.21 | 0.32 | 0.05 | 0.02 |
| 27 | 0.33 | 0.02 | 0.06 | 0.01 | 0.21 | 0.18 | 0.06 | 0.01 |
| 28 | 0.35 | 0.07 | 0.04 | 0.02 | 0.35 | 0.07 | 0.08 | 0.01 |
| 29 | 0.20 | 0.10 | 0.03 | 0.02 | 0.44 | 0.07 | 0.02 | 0.01 |
| 30 | 0.11 | 0.08 | 0.04 | 0.01 | 0.22 | 0.08 | 0.06 | 0.01 |

# Appendix 5 (Continued)

| Sample number | Lo3-inoculated | | | | Lo4-inoculated | | | |
|---|---|---|---|---|---|---|---|---|
| | *Potentilla* | *Dryas* | *Salix* | *Picea* | *Potentilla* | *Dryas* | *Salix* | *Picea* |
| 1 | 0.08 | 0.06 | 0.30 | 0.01 | 0.02 | 0.02 | 0.99 | 0.01 |
| 2 | 0.09 | 0.03 | 0.72 | 0.01 | 0.16 | 0.07 | 0.16 | 0.02 |
| 3 | 0.23 | 0.12 | 0.09 | 0.03 | 0.26 | 0.06 | 0.28 | 0.01 |
| 4 | 0.15 | 0.11 | 0.07 | 0.01 | 0.11 | 0.06 | 0.12 | 0.01 |
| 5 | 0.14 | 0.04 | 0.48 | 0.02 | 0.04 | 0.03 | 0.56 | 0.01 |
| 6 | 0.09 | 0.10 | 0.62 | 0.01 | 0.02 | 0.02 | 0.96 | 0.02 |
| 7 | 0.28 | 0.07 | 0.14 | 0.01 | 0.11 | 0.05 | 0.34 | 0.02 |
| 8 | 0.19 | 0.10 | 0.09 | 0.02 | 0.17 | 0.09 | 0.33 | 0.03 |
| 9 | 0.21 | 0.10 | 0.18 | 0.02 | 0.09 | 0.10 | 0.24 | 0.02 |
| 10 | 0.08 | 0.15 | 0.27 | 0.03 | 0.07 | 0.12 | 0.42 | 0.02 |
| 11 | 0.12 | 0.14 | 0.20 | 0.02 | 0.21 | 0.08 | 0.25 | 0.01 |
| 12 | 0.14 | 0.12 | 0.50 | 0.01 | 0.09 | 0.15 | 0.17 | 0.02 |
| 13 | 0.03 | 0.09 | 0.51 | 0.01 | 0.14 | 0.10 | 0.11 | 0.02 |
| 14 | 0.12 | 0.21 | 0.34 | 0.02 | 0.07 | 0.25 | 0.11 | 0.02 |
| 15 | 0.07 | 0.22 | 0.28 | 0.02 | 0.22 | 0.07 | 0.15 | 0.02 |
| 16 | 0.18 | 0.11 | 0.25 | 0.02 | 0.10 | 0.25 | 0.11 | 0.02 |
| 17 | 0.13 | 0.12 | 0.32 | 0.01 | 0.12 | 0.04 | 0.14 | 0.01 |
| 18 | 0.26 | 0.03 | 0.15 | 0.01 | 0.23 | 0.12 | 0.08 | 0.01 |
| 19 | 0.17 | 0.15 | 0.14 | 0.03 | 0.16 | 0.24 | 0.09 | 0.02 |
| 20 | 0.30 | 0.04 | 0.13 | 0.02 | 0.19 | 0.18 | 0.05 | 0.01 |
| 21 | 0.20 | 0.10 | 0.12 | 0.02 | 0.09 | 0.04 | 0.08 | 0.02 |
| 22 | 0.13 | 0.26 | 0.06 | 0.02 | 0.14 | 0.08 | 0.08 | 0.02 |
| 23 | 0.40 | 0.08 | 0.09 | 0.01 | 0.16 | 0.08 | 0.10 | 0.03 |
| 24 | 0.28 | 0.10 | 0.19 | 0.01 | 0.14 | [illegible] | 0.12 | 0.01 |
| 25 | 0.44 | 0.09 | 0.13 | 0.03 | 0.07 | 0.06 | 0.17 | 0.02 |
| 26 | 0.09 | 0.11 | [illegible] | 0.02 | 0.32 | 0.09 | 0.06 | 0.01 |
| 27 | 0.33 | 0.18 | 0.06 | 0.01 | 0.44 | 0.06 | 0.05 | 0.04 |
| 28 | 0.12 | 0.17 | 0.07 | 0.01 | 0.24 | 0.06 | 0.11 | [illegible] |
| 29 | 0.19 | 0.25 | 0.08 | 0.04 | 0.22 | 0.03 | 0.07 | 0.01 |
| 30 | 0.30 | 0.13 | 0.05 | 0.01 | 0.17 | 0.22 | 0.06 | 0.01 |

Appendix 6. Shoot and root dry weights (g) of *Salix glauca* seedlings grown in the presence and absence of *Sphaerosporella brunnea*

| Sample number | Uncolonized (Control) Shoots | Uncolonized (Control) Roots | Colonized with *S. brunnea* Shoots | Colonized with *S. brunnea* Roots |
|---|---|---|---|---|
| 1 | 0.017 | 0.014 | 0.006 | 0.003 |
| 2 | 0.008 | 0.001 | 0.010 | 0.003 |
| 3 | 0.011 | 0.005 | 0.010 | 0.007 |
| 4 | 0.004 | 0.001 | 0.016 | 0.006 |
| 5 | 0.020 | 0.007 | 0.008 | 0.005 |
| 6 | 0.024 | 0.014 | 0.006 | 0.004 |
| 7 | 0.028 | 0.011 | 0.020 | 0.010 |
| 8 | 0.028 | 0.017 | 0.043 | 0.018 |
| 9 | 0.010 | 0.005 | 0.024 | 0.012 |
| 10 | 0.055 | 0.037 | 0.009 | 0.007 |
| 11 | 0.062 | 0.046 | 0.009 | 0.006 |
| 12 | 0.103 | 0.093 | 0.025 | 0.028 |
| 13 | 0.026 | 0.037 | 0.055 | 0.057 |
| 14 | 0.035 | 0.029 | 0.041 | 0.032 |
| 15 | 0.085 | 0.076 | 0.049 | 0.031 |
| 16 | 0.052 | 0.033 | 0.063 | 0.030 |
| 17 | 0.028 | 0.016 | 0.026 | 0.022 |
| 18 | 0.076 | 0.101 | 0.030 | 0.037 |
| 19 | 0.028 | 0.051 | 0.033 | 0.044 |
| 20 | 0.044 | 0.081 | 0.207 | 0.134 |
| 21 | 0.018 | 0.025 | 0.073 | 0.084 |