AN ABSTRACT OF THE DISSERTATION OF

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Signature redacted for privacy.

Abstract approved: _____
James M. Trappe

Plant succession is among the fundamental concepts in ecology. Studies addressing plant recruitment, successional mechanisms, and the role of root-colonizing fungi with focus on dark-septate endophytes (DSE) were conducted in the laboratory and on the forefront of receding Lyman Glacier (North Cascade Range, Washington, U.S.A.).

Primary successional studies primarily focus on biotic interactions although initial recruitment is controlled abiotically. In a field study, we found that plant distribution is not random but determined by sites that allow seed trapping and protect germinating seeds from desiccation. An additional hypothesis that early successional plant individuals form centers of establishment for subsequent colonizers was tested by assaying the effect of willow shrubs on invading plants. Results suggested that while the canopy had either neutral or inhibitory effects on new seedling establishment, the soil beneath the canopies favors establishment.

The role of fungi in succession is poorly known. We present results of eight years of floristic surveys of the ectomycorrhizal macrofungi at the site. The data show that the communities of ectomycorrhizal fungi differ between primary and secondary successional sites.

Plants on the glacier forefront were frequently colonized by nonmycorrhizal DSE. We reviewed the literature on DSE and listed reports of about 600 colonized plant species from the tropics to arctic and alpine habitats. Although they appear to be ubiquitous, the ecological role of DSE is poorly understood: results of past experiments are inconsistent and do not explain the role of DSE in their natural habitats. We further investigated the host-DSE association and its relevance to plant fitness on the glacier forefront in laboratory and field studies. We conclude that some DSE may function as typical mycorrhizal associates but that the research tools for studying these associations are inadequate and biased. A field study on the distribution of discrete RAPD-phenotypes (individuals) showed that DSE colonize several plant species under natural conditions. A single fungal individual can colonize adjacent plants, potentially providing a mycelial pathway for resource flow as hypothesized for ectomycorrhizal systems.

Primary Succession on a Receding Glacier Forefront – with Special Emphasis on Root-Colonizing Fungi

by

Ari Jumpponen

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CONTRIBUTION OF AUTHORS

Several co-authors contributed to the chapters. Dr. Efren Cázares collected most of the data for chapter 4 and assisted in field work. Drs Rauni Ohtonen and Henry Väre assisted in experimental design, field work, data entry, data analyses, and faithfully edited the manuscripts for chapters 2 and 3. Dr. Kim Mattson and Dr. James Trappe participated all stages of the process, including the initiation, brain-stroming, money-gathering, design, analysis, and editing.

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DEDICATION

This dissertation is dedicated to all loving cats and people!

PREFACE

From northern forest to glacial plain
Dwell Deuteromycotina strains
Deep in soil, happily entwined
Among the roots of fir and pine

Until that peaceful symbiosis
Is spoiled in manner quite atrocious
By intrepid mycology gang
Of stalwart scientists, again.

On tundra vast, in woods humongous,
They delve to find the lowly fungus,
Rejoicing as hyphae come to light,
"Darn, another dark-septate root endophyte!"

What drives their odd quest, you may wonder,
Why tear the landscape thus asunder with
Disturbance incongruous and profane?
What knowledge do they hope to gain?

Simply this: Are DSE promiscuous
or anamorphs globally ubiquitous?
So now you know. They're quite perplexed
By the secret world of fungal sex!

Yes, indeed, for mycology buddies,
That's the focus of their studies, which
Begs the question that ends this song,
Have they been in the field just a little too long?!!

Caryn Davis, 1997

PRIMARY SUCCESSION ON A RECEDING GLACIER FOREFRONT – WITH SPECIAL EMPHASIS ON ROOT-COLONIZING FUNGI

CHAPTER 1

INTRODUCTION

Succession is regarded by many ecologists as the most important ecological concept after that of the ecosystem itself (Cherrett 1989). Modern forest and range production depends on our ability to predict the consequences of management activities. This is especially true during this era of potentially drastic climate changes. The preservation of plant, fungus, and animal species, biotic communities, and productive as well as aesthetically pleasing landscapes, will be aided by knowledge of the patterns and processes of vegetation change.

Fundamental concepts of succession are best based on natural, primary successional communities. Where to find a site where primary succession over time can be studied efficiently? As Ellenberg (1988) phrases it, "Nowhere can succession be studied more profitably than in the valley below the front of a large glacier." Among the greatest advantages of conducting successional research at a glacier forefront is the ease of interpreting spatial distribution of phenomena on a temporal scale (Matthews 1992). The approach of substituting space for time, commonly employed at glacier forefronts, has been criticized due to an implicit assumption of "nonchanging", continuous environment (Austin 1981). Despite the criticism, Pickett (1989) concludes that while unreliable for deeper understanding of successional change, space-for-time substitution is useful for qualitative analyses and hypothesis generation. The relatively severe climate usually encountered at arctic

and alpine glacier forefronts, combined with the infertile substrate, limits the number of organisms able to survive, thereby providing a relatively simple community for studies in field ecology. Finally, glacier forefronts are sharply defined and restricted in size, allowing a thorough and comprehensive investigation of a finite habitat. In summary, glacier forefronts, as the one used in this dissertation, supply field ecologists with simple experimental field laboratories for studying primary successional changes (Matthews 1992, Walker and Chapin 1987).

In this dissertation I utilize the well-documented glacier retreat at Lyman to infer successional patterns and mechanisms in true primary successional ecosystem, emphasizing the importance of root-colonizing fungi. A poorly-known group of root-inhabiting fungi, the dark, septate endophytes (DSE), were studied in detail both under field and laboratory conditions and their potential position in the mutualism-parasitism continuum (*sensu* Johnson *et al.*, 1997) addressed. All the chapters are self-supporting manuscripts with individual introductions; only a brief overview is provided here.

In Chapters 2 and 3 of this dissertation, the Lyman Glacier study site is introduced and the observed successional change in plant communities is described and interpreted in a temporal scale constructed from the historical records. Subjective descriptions of the successional change are an invaluable observational tool to understand the dynamics of early successional plant communities. As subjective studies pose serious problems, Usher (1992) calls for more objective approaches for studying successional dynamics. These approaches, unfortunately, may fail if long-term studies with permanent plots and annual records cannot be established.

Although the plant community development and space-for-time framework is of fundamental importance for succeeding chapters, the main focus of these chapters lies in the successional mechanisms. While biotic interactions, facilitation and competition in particular (see below), are essential in the dynamic nature of any community, the early dynamics in

purely primary successional habitats are under strict abiotic control (Houle, 1997). Only as plants gain more control over their physical environment do interactions between plants become more significant (Houle, 1997). As plants on the Lyman Glacier forefront established on nonvegetated terrain, we sought general rules or factors that govern plant establishment under these conditions. Essentially, the goal in Chapter 2 was to define safe sites (*sensu* Harper, 1961) for the first establishing plant individuals. Instead of separately surveying seed banks or transplant survival, we characterized the microenvironment appropriate for plant establishment ,where the seed bank accumulates, recruited seed germinate, and recent germinants survive.

Biotic interactions and their relevance to succession have generally received more attention than abiotic factors. A recent issue of *Ecology* (1997; Volume 7) had a special feature dedicated to competition and facilitation in plant communities. Facilitation is among the primary mechanisms by which early succession is suggested to occur in the plant community (see Callaway, 1995; Connell and Slatyer, 1977; Pickett *et al.*, 1987; Matthews, 1992) although it is frequently acknowledged that several successional mechanisms occur simultaneously (Holmgren *et al.*, 1997). Proposed factors in facilitation include vegetation-induced changes in microclimate and the physical and chemical properties of soil (Campbell *et al.*, 1990; Manders and Richardson, 1992). Although in no case has it been demonstrated that the presence of a particular species is prerequisite for establishment of others (Matthews, 1992), nucleation and facilitation have been suggested to be significant mechanisms (Campbell *et al.*, 1990).

Opportunities to experimentally address questions of successional mechanisms in primary successional ecosystems are rare. Because nitrogen is generally limiting during early stages of community development (Vitousek and Walker, 1989; Walker, 1993; Chapin *et al.*, 1994), studies of facilitation in primary successional systems have concentrated on taxa with symbiotic nitrogen fixation ability and their effect on plant community development (Walker and Chapin, 1986; Morris and Wood, 1989; Vitousek and Walker,

1989; Blundon *et al.*, 1993; Chapin *et al.*, 1994). Vascular plants with symbiotic nitrogen-fixation, however, are rare on the Lyman Glacier forefront and thus of minor importance. Hence, addressing the importance of the dominating nonnitrogen-fixing shrubs was fundamental. Chapter 3 describes a study designed to determine if the most prominent established woody shrubs, *Salix* ssp., enhance the subsequent plant establishment and survival, *i.e.*, do they form facilitative centers ("nuclei" in Yarranton and Morrison, 1974; "nurse plants" in Niering et al., 1963) for plant establishment?

Coincidental to other research activity, we accumulated a large number of fungal collections from the glacier forefront and surrounding secondary successional plant communities. Studies of arctic and alpine fungal communities are infrequent (reviewed in Graf and Horak 1993). The fungal flora is thus poorly known, and discovery of new or rare and endangered species is not uncommon (Cázares and Trappe 1990, 1991a, 1991b, Graf and Horak 1993, Jumpponen *et al.*, 1997). Again, glacier forefronts have a special importance for several reasons. They represent a new, primary successional parent material distinctively different from the immediate surroundings. As a result, forefronts provide a habitat for ruderal organisms absent from adjacent secondary successional sites. Mycologists, however, have rarely utilized opportunities to study the recently exposed, primary successional substrates provided by glacier forefronts.

Both pathogenic (Van der Putten and Peters, 1997) and mutualistic (reviewed in Watkinson and Freckleton, 1997) root inhabiting fungi can influence plant community change by altering the respective competitive capabilities of their hosts during the succession. Furthermore, mycorrhizal associations are essential to establishment of mycorrhiza-dependent plants (Clay 1990, Allen 1991, Trappe and Luoma 1992). However, mycorrhizal associations of primary successional ecosystems are rarely studied (but see Cázares 1992 and Helm *et al.* 1996). Helm *et al.* (1996) were the first to describe the below-ground community and its change over time since glacier retreat (*i.e.* substrate age) and changing plant community composition. They found

that mycorrhizal colonization occurs soon after seed germination, different mycorrhizal types dominate at different successional stages, and ectomycorrhizal diversity increases from early stages to later stages.

The focus of our sporocarp collections was on ectomycorrhizal fungi. Data on their occurrence in primary vs. adjacent secondary successional ecosystems were used in Chapter 4 to test the current successional model for ectomycorrhizal fungi. That model is based on a series of studies conducted by the Institute of Terrestrial Ecology (ITE) on agricultural land planted with birch in Scotland (see Last *et al.*, 1987). The concepts of ectomycorrhizal succession and temporal change in the fungal community derived from these studies state that a narrow selection of agarics colonize root systems in young forests and is finally replaced by species more persistent and stress tolerant. This 'early-' and 'late-state' model has found support in some studies; but it is unclear how the model relates to primary successional sites, as stand characteristics change with the age of the primary host species. Keizer and Arnolds (1994) propose an alternative model based on dividing stand rotation into six phases that relate to both canopy closure and stand age. However, different factors, including host species composition, host age and stand characteristics, are likely to contribute to the diversity of ectomycorrhizal fungi present in a stand at any given time. The separate effects of the factors are difficult to identify and isolate. An additional problem with the successional studies is the lack of appropriate replication.

Cázares (1992) extensively studied the mycorrhizal status of the plants invading the retreating forefront of Lyman Glacier. He concluded that first invading plants were mainly facultative mycorrhizal. However, even the species usually considered nonmycorrhizal were frequently colonized by root-associated fungi called "dark, septate endophytes" (DSE) by Haselwandter and Read (1982). In Chapter 5, the history, ecology and host interactions of these little known and poorly understood root symbionts is reviewed.

Several approaches to understand the function and importance of DSE have been tried. In Chapters 5, 6, and 7 the controversy between the different

approaches and the problems that are involved in working with sterile or asexual symbiotic (*sensu* de Bary, 1887; "living together, sharing homes") fungi are clearly outlined. So far, there is no consensus whether the miscellaneous root endophytes are casual rhizosphere organisms, weak plant pathogens, or actually mutualisic organisms whose interactions with their hosts slide along the parasitism-mutualism continuum (Chapters 5, 6, and 7). The problems of defining a life strategy of a given DSE are pointed out in Chapters 5 and 7 where different techniques were employed to test the effects of *Phialocephala fortinii* Wang and Wilcox on *Pinus contorta* Doug.

Finally, in Chapter 8, the spatial distribution of *Phialocephala fortinii* individuals was studied under natural conditions to collect information on their potential role in facilitation and plant community change during primary succession. The term "individual" in mycology tends to be problematic: mycelial, vegetative units that are usually (in case of *P. fortinii* always) invisible, sets limits to how to study individuals and populations comprised of them. The terms "genet" and "ramet", common for vegetatively extending plants (Harper, 1977), have been adopted in describing respectively genetically different mycelia and identical units resulting from asexual or vegetative propagation (Brasier and Rayner, 1987). Defining a genet or a ramet is an important first step towards understanding fungal individuals.

As outlined in Chapter 8, a variety of tools ranging from somatic compatibility to advanced molecular techniques have been applied to identify fungal individuals. *Phialocephala fortinii*, our target organism, lacks the ability to recognize a genetically identical soma, *i.e.*, as vegetative haploid states are confronted, the somatic compatibility is overlaid by mating compatibility. As a result, only molecular tools can successfully be used for sorting out different phenotypes. In absence of any macroscopic structures, one is forced to collect material randomly (roots potentially colonized by the target fungus) in order to obtain tissue to isolate the inhabiting fungi. As pointed out in several occasions in this dissertation, it is fortunate that we

possess methods to culture some of the fungi and turn them into 'work horses' for experimental purposes.

Understanding the distribution of fungal individuals and their ability to simultaneously colonize several plant individuals even of different genera or families, is an essential initial test for potential "fungal guilds" that can connect plant individuals to each other and serve as potential pathways for nutrients (see Read *et al.*, 1985; Simard *et al.*, 1997).

In summary, the dissertation consisting of the eight chapters below combines different approaches to study a successional ecosystem and its dynamics. This dissertation has a strong emphasis on root colonizing fungi: ectomycorrhizal and endophytic. In the course of these studies facilitative mechanisms attributable to the root colonizing fungi were addressed.

CHAPTER 2

CHARACTERIZATION OF "SAFE SITES" FOR PLANT RECRUITMENT ON A PRIMARY SUCCESSIONAL GLACIER FOREFRONT

Ari Jumpponen, Henry Väre, Kim G. Mattson,
Rauni Ohtonen, and James M. Trappe

ABSTRACT

We characterized safe sites (*sensu* Harper *et al.*, 1961) for five early colonizers (*Abies lasiocarpa, Juncus drummondii, J. mertensianus, Saxifraga ferruginea, S. tolmiei*) that had survived at least one growing season on the recently deglaciated Lyman Glacier forefront in the North Cascade Mountains, Washington, USA. Sites with concave surfaces, coarse substrate, and in the vicinity of large rocks were associated with higher plant occurrence. The distribution of plants is determined by the presence of sites that facilitate seed trapping and provide protection from desiccation. Additionally, large rocks provide a microsite with extended growing season due to earlier snow melt. By presenting these data, we hope to draw attention to the abiotic factors that control spatial distribution of plants and precede the biotic interactions in this primary successional sere.

INTRODUCTION

Most studies on succession and successional mechanisms have concentrated on biotic interactions (see reviews by Connell and Slatyer, 1983; Pickett *et al.*, 1987; Chapin *et al.*, 1994; Callaway, 1995; Holmgren *et al.*, 1997 and several others). While biotic interactions, facilitation and competition in particular, are essential in plant community development, the early dynamics in primary successional[1] habitats are under strict abiotic control (Houle, 1997). As plants establish and modify their physical environment, intra- and interspecific interactions between plant individuals become more significant (Houle, 1997). Also, in primary successional environments, distribution of

1 Primary succession is defined as vegetation development on newly formed or exposed substrate. It proceeds on raw parent material rather than a developed or modified soil, and is usually characterized by low nitrogen and organic matter (see Glenn-Lewin *et al.*, 1992; Matthews, 1992).

wind-dispersed propagules is controlled by abiotic, environmental factors after the seeds have been released from the seed source (Bigwood and Inouye, 1988).

Although it has been demonstrated that subtle differences in seed distribution and soil surface properties have dramatic effects on successful plant establishment and survival (Sheldon, 1974; Harper *et al.*, 1965; Hamrick and Lee, 1987; Huenneke and Sharitz, 1990), the initial establishment and plant recruitment in primary successional systems has been largely ignored. Harper *et al.* (1961) proposed the concept of "safe sites" to describe a microsite suitable for germination and establishment. Safe sites under natural conditions must not only provide appropriate microhabitats for germination but must also allow establishment and survival. The importance of these safe sites is even more pronounced in primary successional habitats as they are frequently characterized by harsh environmental conditions due to drought and/or exposure (Matthews, 1992; Chapin, 1993) that may induce early mortality of germinants (Chapin and Bliss, 1989). Furthermore, mechanisms of primary and secondary seed dispersal govern the availability of seeds in the proper safe sites.

The objective of this study was to define and characterize the microenvironments (safe sites) for initial plant colonization on the recently deglaciated Lyman Glacier forefront in the North Cascade Mountains, Washington, USA. A combination of various factors governing plant dispersal and establishment was evaluated by characterizing the microenvironment around newly-established, existing plants. Successful establishment requires a site promoting trapping of seeds and vegetative propagules from primary or secondary dispersal. Although the existing seed bank is an essential component in plant recruitment and the resulting community, it may be a poor predictor of the community able to survive and reproduce in that site (van der Valk, 1992). Also, seed accumulation is only the first step towards successful seedling recruitment; the site must also provide conditions that support both germination and survival of those

species present in the soil seed bank. Furthermore, one substrate or site may support high germination, while another supports high survivorship (Reader and Buck, 1986; Huenneke and Sharitz, 1990). To avoid the confusion resulting from different effects of particular life stages, and instead search for generalities, we located the plants that had survived through at least one growing season on otherwise nonvegetated primary successional terrain close to the glacier terminus and described those sites.

MATERIALS AND METHODS

Study Site

Retreating Lyman Glacier (48° 10′ N, 120° 53′ W; elevation 1800 m) has a recently exposed forefront 1100 m long with an elevation drop of only about 60 m and no distinctive recessional moraines. The glacier and its forefront occupy a cirque and a north-south oriented, U-shaped valley bounded by cliffs that rise up to 600 m above the valley floor. The forefront parent material is a heterogeneous glacial till ranging from clay-sized particles to boulders intermingled with deposits of glacial-fluvial sediments (for a detailed description see Cázares, 1992 and Jumpponen *et al.* 1998b).

The vegetation on ridges and benches adjacent to the forefront are in the ecotone between the upper parkland subzones of the *Abies lasiocarpa* zone and *Tsuga mertensiana* zone of the North Cascade Range (Franklin and Dyrness, 1973). The glacier forefront was divided into the following vegetational phases in Jumpponen *et al.* (1998b): (1) A barren phase less than 20 yr old closest to the glacier; (2) a 20–30-yr-old phase characterized by scattered individuals or small patches of the early seral species, *Juncus drummondii, J. mertensianus, Luzula piperi, Saxifraga ferruginea* and *S. tolmiei,* the "rawmark" community of Franklin and Dyrness (1973); (3) a

recruitment such as a bare rock or in standing water, an alternative site was selected by 90° rotation clockwise. Within the area used in this study, it was always possible to locate a control site within 50 cm of the safe site.

Table 2.1. List of plant species and the observed numbers recorded within the first 200 m from the Lyman Glacier terminus during the description of the safe sites. Nomenclature follows Hitchcock and Cronquist (1973).

Family	Species	Observations
Pinaceae	*Abies lasiocarpa* (Hook.) Nutt.	43
	Pinus albicaulis Engelm.	<20[a]
	Pinus contorta Dougl.	<20[a]
Compositae	*Antennaria lanata* (Hook) Greene	<20[a]
	Hieracium gracile Hook.	<20[a]
Ericaceae	*Phyllodoce empetriformis* (Sw.) D. Don	<20[a]
Onagraceae	*Epilobium alpinum* L.	<20[a]
	Epilobium latifolium L.	<20[a]
Juncaceae	*Juncus drummondii* E. Meyer	102
	Juncus mertensianus Bong.	59
	Luzula piperi (Cov.) Jones	<20[a]
Rosaceae	*Luetkia pectinata* (Pursh) Kuntze.	<20[a]
Caryophyllaceae	*Sagina saginoides* (L.) Britt.	<20[a]
Saxifragaceae	*Saxifraga ferruginea* Grah.	295
	Saxifraga tolmiei T. and G.	41

a – excluded from the analyses due to too few observations

Statistical Analyses

The five plant species with more than 20 records occurrence (Table 2.1) were analyzed separately by logistic regression: sites with a plant represented an event while the nonvegetated ones represented a non-event. After preliminary analyses, autocorrelated variables (among those listed above) were omitted and four continuous or categorical variables (Table 2.2) selected for final analyses. A full model with two continuous variables (distance to a rock and proportion of coarse substrate), two categorical variables (plot surface, with plateau as the reference level, and topology, with position on a flat surface as the reference level), and all two-way interactions was fit on the Bernoulli-distributed, binary, presence/absence data. Only the main effects

Table 2.2. Description of the variables used in the logistic regression analyses to characterize the safe sites for the early establishing plants in the primary successional habitat on the Lyman Glacier forefront.

Variable	Type	Description
1 Distance from a rock	Continuous	Distance from the edge of a rock ≥ 20cm in diameter to the site center.
2 Topology	Categorical	Relative topology of site compared to local topogaphy within 1m: elevated, depressed, or flat[a].
3 Contour	Categorical	Surface shape of the site: concave, convex or plateau[a].
4 Substrate particle size	Continuous	Percent of site covered with particles ≥ 2mm but ≤ 2cm in diameter[b].

a – reference level of the categorical variable in the analyses
b – rocks ≥ 2cm in diam were not considered as substrate

and two-way interactions were included in the saturated model because of the vast number of potential interaction terms and the difficulties of interpretation of multi-level interactions. Saturated models were first compared to additive models with no interaction terms by drop-in-deviance tests (Ramsey and Shafer, 1996). If the interactions were not found significant at $\alpha = 0.05$, only additive models were studied further and an additive model with lowest Q^2 (McCullagh and Nelder, 1989) was selected. If drop-in-deviance tests showed saturated models significantly more informative at $\alpha = 0.05$ than the additive models, the 'best model' with interaction terms was again selected by minimizing Q. Data were analyzed in SAS using the GENMOD procedure (SAS, 1997).

RESULTS

In the present study we used logistic regression on binary data to describe the safe sites for plant recruitment in primary successional system on a retreating glacier forefront. The selected models for the included five plant species are displayed in Table 2.3.

Occurrence of *Abies lasiocarpa, Juncus mertensianus,* and *Saxifraga ferruginea* was more likely close to large (>20 cm) rocks. An increase of 1 cm in the distance from a rock decreased recruitment probability of *A. lasiocarpa, J. mertensianus,* and *S. ferruginea,* by 8.57, 5.02, and 1.72%, respectively (Table 2.3). For *Juncus drummondii,* the immediate vicinity of large rocks was particularly important in otherwise unlikely locations for recruitment (elevated positions): a centimeter increase in the distance from a rock decreased odds for recruitment in elevated positions by 23,16 %, *i.e.,* 5 cm

2 $\mathbf{Q = D + 2\,q\,d}$

where D = model deviance
q = number of explanatory parameters in the model
d = scaling parameter (due to Bernoulli distribution, here always 1)

increase reduced odds to less than a half, while no such effect was visible in depressed positions. On the other hand, the rock effect was minimal or nonexistent in presence of other recruitment-facilitating parameters such as coarse substrate for *J. drummondii* or concave surfaces for *Saxifraga tolmiei*. For example, *J. drummondii* data indicated that coarse substrate interacted with distance from rocks: coarse substrate precluded the effects of substantial obstructions or *vice versa*.

Although included in the models, topology had no clearly significant effect on *Abies lasiocarpa* or *Saxifraga tolmiei* recruitment (Type 3 analysis P=0.0901 and 0.1960, respectively). *J. mertensianus* data suggests that recruitment may be more likely in the depressions rather than in flat positions or elevated positions. *Saxifraga ferruginea* recruitment was substantially (100-39.67=60.33%; Type 1 analysis P=0.0009) less likely in the elevated positions than on flats or depresions which did not differ.

Surface contour, as described in Table 2.2, were the most influential parameter explaining presence of plants. While convex and plateau surfaces did not differ, concave surfaces had 4.0 (*Juncus mertensianus*) and up 6.2 (*Juncus drummondii*) times higher odds for plant recruitment than the plateau surfaces (Table 2.3). For *Saxifraga tolmiei*, surface contour interacted with distance from large rocks and allowed no clear conclusions. It appears that when concave surfaces were located near the substantial physical obstructions they had a lesser effect. However, this *S. tolmiei* data set contained fewest observations and might be less reliable than those with less limited data.

Increase in coarse substrate also usually enhanced plant recruitment. A percent unit increase in surface coverage by coarse substrate (> 2 mm in diam) resulted in over 2 % increase in *Abies lasiocarpa, Juncus mertensianus, Saxifraga ferruginea,* and *S. tolmiei* recruitment probability. The increase translates to doubling the recruitment probability by increasing the

Table 2.3. Logistic regression models characterizing the safe sites for the five plant species recorded in the primary successional site on the Lyman Glacier forefront. Odds ratio indicates the proportion of events compared to the non-events (ratio between vegetated sites and nonvegetated sites under the condition defined by the parameter.

Parameter	Odds-ratio	95% confidence intervals		P (χ^2, DF=1)[a]
		Lower	Upper	
Saxifraga ferruginea				
Intercept	0.0735	0.0358	0.1456	0.0001
Distrock (P=0.0084, χ^2, DF=1)[b]	0.9828	0.9693	0.9956	0.0108
Topology (P=0.0001, χ^2, DF=2)[b]	–	–	–	–
depressed	1.0321	0.6163	1.7286	0.9041
elevated	0.3967	0.2288	0.6861	0.0009
Surface (P=0.0001, χ^2, DF=2)[b]	–	–	–	–
concave	4.8423	3.0851	7.7014	0.0001
convex	1.1181	0.6792	1.8415	0.6605
Coarse (P=0.0001, χ^2, DF=1)[b]	1.0262	1.0192	1.0338	0.0001
Deviance=828.1043				
Q=842.1043				

Table 2.3. (continued)

Parameter	Odds-ratio	95% confidence intervals		P (χ^2, DF=1)[a]
		Lower	Upper	
Juncus drummondii				
Intercept	0.1002	0.022	0.4119	0.0020
Distrock (P=0.0001, χ^2, DF=1)[b]	0.7783	0.4119	0.9337	0.0203
Topology (P=0.9708, χ^2, DF=2)[b]	–	–	–	–
depressed	1.1164	0.6127	2.8982	0.8186
elevated	1.1275	0.3583	3.61	0.8380
Surface (P=0.0001, χ^2, DF=2)[b]	–	–	–	–
concave	6.2227	3.2511	12.4547	0.0001
convex	1.1443	0.4934	2.5777	0.7476
Coarse (P=0.2035, χ^2, DF=2)[b]	1.009	0.995	1.0253	0.2335
Distrock*Topology (P=0.0153, χ^2, DF=2)[b]	–	–		–
distrock*depressed	1.013	0.9456	1.1196	0.7543
distrock*elevated	0.7686	0.5655	0.9602	0.0479
Distrock*Coarse (P=0.0187, χ^2, DF=1)[b]	1.0022	1.0003	1.0047	0.0454
Deviance=378.9326				
Q=398.9326				

Table 2.3. (continued)

Parameter	Odds-ratio	95% confidence intervals		P (χ^2, DF=1)[a]
		Lower	Upper	
Abies lasiocarpa				
Intercept	0.0092	0.0013	0.0494	0.0001
Distrock (P=0.0001, χ^2, DF=1)[b]	0.9143	0.8553	0.9614	0.0024
Topology (P=0.0901, χ^2, DF=2)[b]	–	–	–	–
depressed	1.8791	0.5399	7.5754	0.3376
elevated	0.7255	0.1802	3.2043	0.6553
Surface (P=0.0001, χ^2, DF=2)[b]	–	–	–	–
concave	5.9417	2.4269	16.4299	0.0002
convex	0.5532	0.1131	2.1276	0.4138
Coarse (P=0.0007, χ^2, DF=1)[b]	1.0263	1.0104	1.0450	0.0023
Deviance=207.7984				
Q=221.7984				
Juncus mertensianus				
Intercept	0.0082	0.0015	0.0369	0.0001
Distrock (P=0.0011, χ^2, DF=1)[b]	0.9498	0.9125	0.9816	0.0054
Topology (P=0.0028, χ^2, DF=2)[b]	–	–	–	–
depressed	2.8994	1.0341	9.483	0.0550
elevated	0.8251	0.2428	3.0389	0.7615
Surface (P=0.0001, χ^2, DF=2)[b]	–	–	–	–
concave	4.0177	1.9568	8.6755	0.0002
convex	0.4454	0.1184	1.3679	0.1847
Coarse (P=0.0001, χ^2, DF=1)[b]	1.0288	1.0145	1.0453	0.0002
Deviance=269.9394				
Q=283.9394				

Table 2.3. (continued)

Parameter	Odds-ratio	95% confidence intervals		p (χ^2, DF=1)[a]
		Lower	Upper	
Saxifraga tolmiei				
Intercept	0.0147	0.0018	0.0916	0.0001
Distrock (P=0.0002, χ^2, DF=1)[b]	0.8146	0.0916	0.9973	0.1748
Topology (P=0.1960, χ^2, DF=2)[b]	–	–	–	–
depressed	2.8705	0.5109	14.5647	0.1702
elevated	1.2678	0.2488	7.1642	0.7770
Surface (P=0.8089, χ^2, DF=2)[b]	–	–	–	–
concave	1.4154	0.4659	4.4044	0.5426
convex	1.0219	0.1457	8.1507	0.9824
Coarse (P=0.0010, χ^2, DF=1)[b]	1.0253	1.0095	1.0440	0.0033
Distrock*Surface (P=0.0123, χ^2, DF=2)[b]	–	–		–
concave	1.2119	1.0227	1.5863	0.0797
convex	0.6924	0.1692	1.2834	0.4175
Distrock*Topology (P=0.8543, χ^2, DF=2)[b]	–	–		–
distrock*depressed	0.9991	0.7964	1.601	0.9950
distrock*elevated	0.9628	0.7179	1.5571	0.8119
Deviance=206.9117				
Q=228.9117				

a type 1 analysis; significance of the parameter as sole explainer of variation; set of previous parameters does not affect the parameter significance

b type 3 analysis; significance of the parameter after accounting for all the previous parameters in the model

proportion of coarse substrate by 50 %. *Juncus drummondii* data were an exceptions (Table 2.3); substrate interacted with distance from substantial rocks in a manner similar to surface contour described above (with *Saxifraga tolmiei*). Coarse substrate precluded the effects of substantial objects or *vice versa*.

DISCUSSION

Concave surfaces had substantially greater overall plant recruitment than the convex and plateau surfaces. The five species are primarily dependent on wind dispersal. Spatial variation in seed rain and non-uniform distribution of sites where seeds are trapped result in strongly clustered spatial distribution of the soil seed bank (Ryvarden, 1971; Rabinowitz and Rapp, 1980; Reichman, 1984; Price and Reichman, 1987; Bigwood and Inouye, 1988). Small depressions and concave surfaces which provide a 'wind shadow' where surface wind velocities are lower and surface water flow stops, may gather substantial quantities seeds from horizontal seed movement (Reichman, 1984). Depressions appear to effectively collect seeds from primary and secondary dispersal (Reichman, 1984; Matlack, 1989). Harper *et al.* (1965) concluded that the majority of seeds of two *Plantago* ssp. were in depressions or near obstructions the researchers used to create heterogeneity in a controlled study of microsites.

Besides trapping seeds from transient populations, shallow depressions may also provide greater moisture than surrounding soils and enhance seed germination in environments where surface desiccation may be a major factor (Watt, 1919). Early successional sites are characterized by intense radiation at the soil surface resulting in extreme fluctuations in soil temperature and rapid drying of surface soils (Chapin, 1993). The importance of protection against water loss and desiccation has been emphasized for successful germination and establishment (Harper *et al.*, 1965; Sheldon, 1974;

Hamrick and Lee, 1987): drought may, indeed, be the major cause of seedling mortality during the first year after germination in primary succession (Chapin and Bliss, 1989). Clustering of the seeds, as result of seed trapping, in small depressions or other locations likely to enhance germination and survival would result in a strong effect similar to that observed here. The conditions for plant survival and growth, however, are generally less restrictive than those for germination; provided that the emerging radicle is able to penetrate the soil surface, the likelihood of establishment is high (Sheldon, 1974).

Plant occurrence generally was higher near large rocks, especially in otherwise unlikely locations for occurrence. Livingston (1972) pointed out that *Juniperus communis* establishment was more frequent near rocks in New England pastures. The mechanism described by Livingston (1972) was fecal deposition of seeds by robins resting on rocks after feeding on juniper berries. Livingston (1972) also described greater moisture levels due to a "micro-watershed" created by the rocks. Fowler (1986; 1988), similarly, observed increased survival and growth near rocks. She interpreted this to result mainly from reduced evaporation, but did not acknowledge the "micro-watershed" effect. We assume both to be important as extended periods of drought may occur annually in the glacier forefront where water retention is poor due to low organic matter content. Furthermore, rocks may create points for mountain dew to accumulate and improve moisture conditions in absence of precipitation. We have also observed in several occasions that elevated rocks result in earlier snow melt in their immediate vicinity thus prolonging the short growing season at Lyman Glacier forefront. The rock effect, however, was minimal or nonexistent in presence of other recruitment-facilitating parameters (coarse substrate, *J. drummondii*; concave surfaces, *Saxifraga tolmiei*) suggesting potentially similar mechanisms of seed trapping and burial as discussed above.

There are several likely factors involved in the greater occurrence of plants with greater amounts of coarse substrate. Like the small scale

depressions of the concave surfaces, coarse substrate contains seed trapping cavities between the particles. Also coarse substrate allows partial burial between the particles, improving the moisture retention around the seed and increasing the likelihood of successful germination. Surface water flow would move small seeds into the minute cavities and cracks in the surface and further percolate through the surface substrate with vertical flow (Bigwood and Inouye, 1988). The protection offered by the cavities and the associated seed burial are essential for seeds to remain viable (Enright and Lamont, 1989). Hamrick and Lee (1987) concluded that optimal conditions for successful establishment and survival were provided by small irregularities in the soil surface particularly in areas prone to desiccation. Such burial may ensure that seeds remain viable but dormant until conditions are favourable for successful germination (Stamp,1989). Although seed predation was not directly measured, seed burial further reduces likelihood of seed discovery by predators (Enright and Lamont, 1989). Finally, coarse substrate may increase the likelihood of radicle penetration and seed anchorage. Smooth and compacted surfaces provide few possibilites for the emerging radicle to successfully penetrate the surface (Sheldon, 1974). The substrate at the glacier forefront is mainly glaciofluvial silt intermingled with some coarser fragments. Silt frequently forms cement-like, crusty surfaces when desiccated precluding seed trapping and radicle penetration.

Although included in all models, the topology had no significant effect on *Abies lasiocarpa, Juncus mertensianus,* or *Saxifraga tolmiei* recruitment. Plant occurrence is less dependent on microtopography than on parameters describing the microsite. *Saxifraga ferruginea* recruitment, however, was less likely in the elevated positions than on depressed or flat surfaces.

It is surprising that no obvious differences appeared in the safe site characteristics between the five plant species studied here. Establishing vegetation is not only an outcome of differential seed dispersal and spatial distribution of the seed bank but results also from species specific preferences in germination and survival (Reader and Buck, 1986; Price and Reichman,

1987). Seed size and seed morphology have been described as important factors in dispersal and distribution of plants (Rabinowitz and Rapp, 1981; Reichman, 1984; Peart and Clifford, 1987) and partly responsible for floristic diversity and spatial distribution of plants (Sheldon, 1974; Chapin, 1993). In this study, we encountered mainly wind dispersed species but not necessarily ones with small seeds (see Table 2.1). Our safe site characterizations were based on species with fairly large seeds with wings (*Abies lasiocarpa*) as well as species with fairly small seeds with no such devices (*Juncus drummondii, J. mertensianus, Saxifraga ferruginea,* and *S. tolmiei).* Yet, the safe site requirements were highly similar despite different seed characteristics. Based on both field and laboratory experiments with four sympatric *Erodium* species, Stamp (1989) concluded that no differences existed in microhabitat requirements between plants with different seed sizes. Instead, he suggested that the seed size may mainly account for the ability to take advantage of different seasonal conditions if not different microsites.

The early stages in the life cycle (seed dispersal, seed distribution, germination, *etc.*) seem more important than subsequent stages in determining the distribution and abundance of plants (Reader and Buck, 1986). Hamrick and Lee (1987) concluded that greatest mortality occurs between dispersal and establishment. As this stage of the plant life cycle has the highest mortality, the distribution of plants and their safe sites in primary successional systems are determined by the abiotic microenvironment that governs the likelihoods for seed trapping, successful germination, and establishment. In light of the potential importance of drought-induced mortality (Chapin and Bliss, 1989), safe sites have received surprisingly little attention in studies of primary succession. In this paper, our intention is to draw attention to the patterns of initial plant colonization as an essential mechanism in defining the plant distribution and spatial community structure in a primary successional habitat. We hope to initiate further interest in the abiotic factors preceding the biotic interactions and mechanisms in primary succession.

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CHAPTER 3

EFFECTS OF ESTABLISHED WILLOWS ON PRIMARY SUCCESSION ON LYMAN GLACIER FOREFRONT, NORTH CASCADE RANGE, WASHINGTON, U.S.A.: EVIDENCE FOR SIMULTANEOUS CANOPY INHIBITION AND SOIL FACILITATION

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ABSTRACT

The effect of established shrub willows (*Salix commutata* and *S. phylicifolia*) was tested in a primary successional ecosystem at Lyman Glacier forefront in the North Cascade Range (Washington, U.S.A.). To examine the hypothesis that early successional plant individuals form centers of establishment for subsequent vascular plant colonizers, two experiments were conducted to assay the effect of shrub willows on the establishment and survival of indigenous plants. First, the occurrence of indigenous plant species under willow canopies was compared with their occurrence beyond the canopies (experiment 1). Second, the separate effects of willow canopies and associated soils on germinant emergence and survival of an indigenous taxon, *Pinus contorta*, were evaluated (experiment 2). Both experiments indicated that the shrub willows do not serve as nuclei that facilitate the establishment of new, emerging plant individuals. In experiment 1, the willow canopy had no effect on the observed frequency of most indigenous taxa. Five species, however, were negatively associated with the willow canopies. In experiment 2, willow canopies inhibited the germinant emergence of *P. contorta*. The greatest emergence occurred in soils transferred from beneath willow canopies to areas beyond the canopies. Results from the two experiments suggest that while the willow canopy is either neutral or inhibitory in its effect on establishment of indigenous plants, the soil developing beneath the willow can actually be a positive factor towards plant establishment.

INTRODUCTION

Primary establishment and survival of new plants may take years of unsuccessful attempts before plant communities develop. Facilitation is one mechanism by which succession occurs in the plant community (see Connell

and Slatyer, 1977; Pickett et al., 1987; Matthews, 1992, Callaway, 1995). During facilitation, established individual plants modify their environment (Morris and Wood, 1989; Blundon et al., 1993; Pugnaire and Haase, 1996) and serve as "nurse plants" (Niering et al., 1963; Franco and Nobel, 1988). These established plants enhance both the establishment and survival of new plants and ultimately may place themselves at a competitive disadvantage. Proposed mechanisms for such facilitation include changes in microclimate as well as changes in the physical and chemical properties of soil (Franco and Nobel, 1988; Campbell et al., 1990; Manders and Richardson, 1992; Callaway, 1994; Callaway, 1995; Jacquez and Patten, 1996; Pugnaire and Haase, 1996). A special case of facilitation is a close positive interaction known as nucleation, which refers to early successional plant individuals forming centers (nuclei) of establishment for subsequent colonizers (Yarranton and Morrison, 1974).

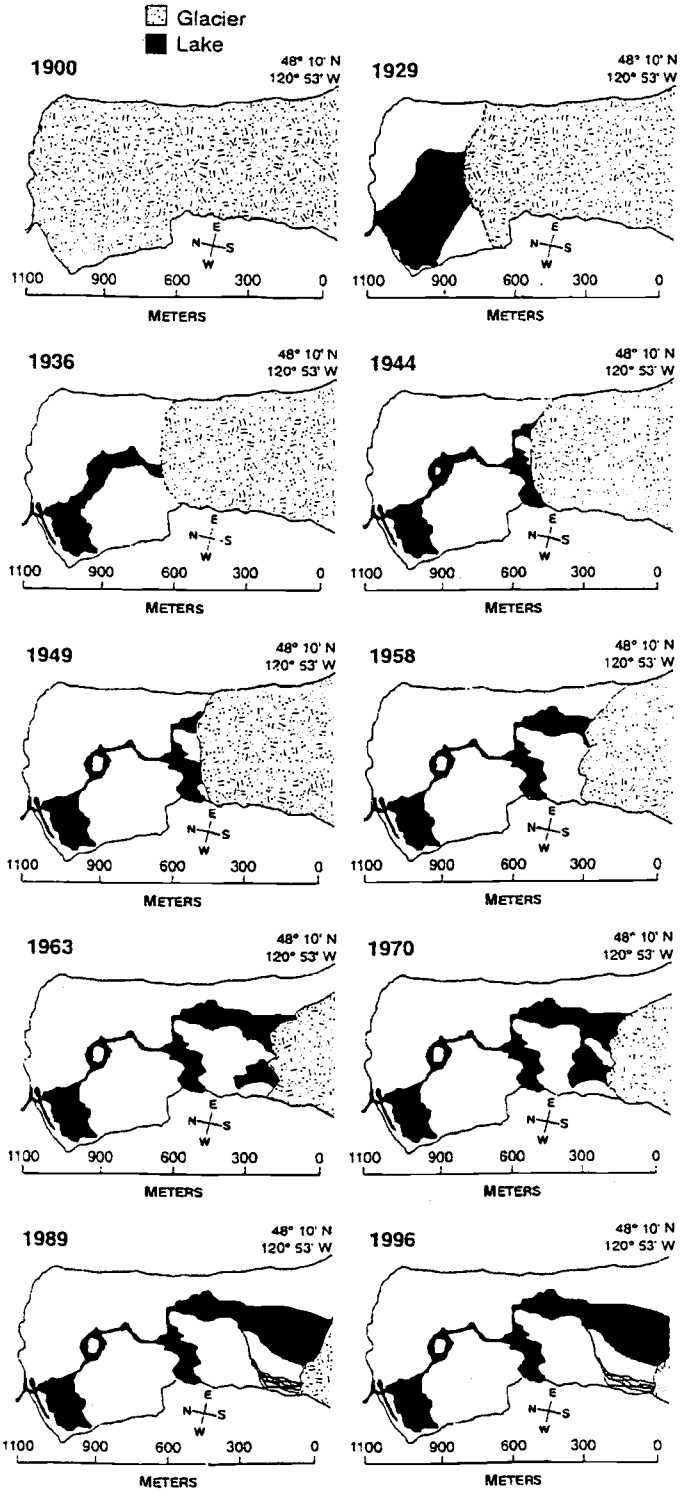
Nucleation has been suggested to be a significant mechanism in secondary succession (Campbell et al., 1990). Opportunities to conduct studies addressing questions of successional mechanisms in primary successional ecosystems are rare. Studies of primary successional systems have mainly concentrated on taxa with symbiotic nitrogen fixation ability and their effect on the plant community development (Walker and Chapin, 1986; Morris and Wood, 1989; Vitousek and Walker, 1989; Blundon et al., 1993; Chapin et al., 1994). Nitrogen-fixing taxa are important during primary succession because nitrogen is limiting during the early stages of community development (Vitousek and Walker, 1989; Walker, 1993; Chapin et al., 1994).

The main objective of this study was to determine the response of indigenous taxa to the physical environment created by a canopy of non-nitrogen-fixing taxa in an ecosystem undergoing primary succession. The forefront of Lyman Glacier in the North Cascade Range, Wenatchee National Forest, Glacier Peak Wilderness Area, Washington, U.S.A. was selected as the study site. An undisturbed deposit of Mount Mazama ash outside the late 1890s terminal moraine shows that the glacier has not advanced beyond that position in the last ca. 6000 yr. The glacier has been receding steadily since the

1890s. The site represents natural primary successional communities: no exotic weeds are present. The retreat of the glacier has been frequently recorded over the past century: photos are available for 1900 to 1944 (Lindsley and Harrison photographs at Special Collections Division, University of Washington Libraries, Seattle), for 1940 to 1970 (U.S. Geological Survey), and every year since 1986 (the authors); annual snow survey data of the terminus retreat are available for 1929 to 1943 (Chelan County, Public Utility District No. 1). Consequently, the chronosequence of plant establishment as new substrate emerged from under the melting ice and subsequent community development can be documented with unusual precision (Fig. 3.1). Nitrogen-fixing *Lupinus latifolius* were present on less than 1 % of over 1000 0.25-m^2 diameter plots on the successional sere (Jumpponen et al., unpublished data), and only one individual of *Alnus sinuata* has been located on the sere. The frequency of these individuals is sufficiently low that any significant effects of biological nitrogen fixation on successional patterns at the glacier forefront would be extremely localized and have little overall impact.

Two separate experiments were conducted to assay the effect of the nuclei formed by shrub willows on the vascular plant establishment at the Lyman Glacier forefront. First, the occurrence of indigeneous plant species under willow canopies was compared with their occurrence in the open. Second, the separate effects of willow canopies and associated soils on germinant emergence and survival of an indigenous species, *Pinus contorta*, were evaluated. Because willows affect soil properties, especially organic matter and nitrogen contents, in primary successional substrates, these properties were analyzed from samples along the chronosequence under and away from the willow canopies.

Figure 3.1. Schematic map of the retreat of Lyman Glacier. Terminus location of 1929 and 1936 (dashed line) estimated based on snow survey data, others on photographs by Lindsley (1900), Harrison (1944), U.S. Geological Survey (1949, 1958, 1968, 1970), and the authors (1989, 1996). Scale bar: distance from the current (1996) glacier terminus.



MATERIALS AND METHODS

Study Area

Lyman Glacier is at 48° 10′ N, 120° 53′ W; the elevation of the present terminus is about 1800 m. The deglaciated forefront is 1100 m long with an elevational drop of only about 60 m and no distinctive recessional moraines. The forefront parent material is a heterogeneous glacial till ranging from clay-sized particles to boulders intermingled with deposits of glacial-fluvial sediments (for a description of the chemical characteristics see Cázares, 1992). The glacier and its forefront occupy a cirque and a north-south oriented, U-shaped valley bounded by cliffs that rise up to 600 m above the valley floor and culminate at Chiwawa Mountain (2430 m) at the head of the cirque.

The vegetation on ridges and benches adjacent to the forefront are in the ecotone between the upper parkland subzones of the *Abies lasiocarpa* zone and *Tsuga mertensiana* zone of the North Cascade Range (Franklin and Dyrness, 1973). The primary tree species on and near the forefront are *Abies lasiocarpa, Larix lyallii,* and *Tsuga mertensiana. Picea engelmannii* and *Pinus contorta* occur on the forefront in small numbers, and *Pinus albicaulis* is common on the higher surrounding ridges but has not been found so far on the forefront itself. Plant communities adjacent to the forefront include heath shrub, lush herb, and dwarf sedge (Franklin and Dyrness, 1973).

The forefront can be divided into the following vegetational phases: (1) A barren phase less than 20 yr old closest to the glacier; (2) a 20–30-yr-old phase characterized by scattered individuals or small patches of the early seral species, *Juncus drummondii, J. mertensianus, Luzula piperi, Saxifraga ferruginea* and *S. tolmiei,* the "rawmark" community of Franklin and Dyrness (1973); (3) a phase roughly 30 to 70 yr old characterized by scattered willow shrubs, principally *Salix phylicifolia* and *S. commutata,* and occasional Pinaceae mixed with the rawmark/low herbaceous communities of Franklin

and Dyrness (1973) dominated by species of Cyperaceae, Juncaceae, Onagraceae, Saxifragaceae, and Scrophulariaceae, but including scattered individuals of other families; and (4) a phase roughly 70–100 yr old, characterized by transition from low herbaceous to early stages of the heath shrub and lush meadow parkland communities containing individuals of *Abies lasiocarpa, Larix lyallii,* and *Tsuga mertensiana* among patches dominated by several members of Ericaceae. The boundary between the barren Phase 1 and rawmark community Phase 2 is well defined. The rawmark Phase 2 is also relatively well demarcated from the willow dominated Phase 3. Phase 3, however, merges in a highly variable ecotone with the early stages of heath shrub/lush meadow parklands of Phase 4. Douglas (1971) and Douglas and Bliss (1977) describe plant communities of the North Cascade Range in detail. The vegetation on the forefront has not reached full coverage on any of the phases described above; the communities are still open and have a substantial proportion of exposed mineral soil. Willow shrubs were chosen for the two experiments described here for the following reasons: (1) they are the first larger plants providing enough physical cover to serve as nuclei for subsequent colonizers and (2) their distribution at the site ranges from the 30-yr-old region to the terminal moraine; no willows are present at regions younger than 30 yr old.

Experiment 1: Effect of Willow Shrubs on Frequencies of Indigenous Species

Sampling

Presence or absence of indigenous plant taxa were tallied within and beyond willow canopies to study effects of the physical environment created by those canopies on the establishment of new plant individuals. A design modified from Blundon et al. (1993) was employed and the conclusions were

based on presence and absence rather than on growth or coverage of a given species.

Six zones representing 10-yr intervals as approximated from the photographic record (Fig. 3.1) were selected in the area where willows occurred on the forefront: 30–39, 40–49, 50–59, and 60–69 yr old of the Phase 3 willow/rawmark/low herbaceous communities, and 70–79 and 80–89 yr old of the Phase 4 early transitional stage towards heath shrub/lush meadow parkland. Twenty to fifty willow canopies were randomly selected within each of the six zones. The diameter of the vertical projection of each willow canopy was recorded, and it was used as an outer boundary of a plot in which presence or absence of all species were scored. An equal number of plots of similar size were located beyond the willow canopies within the same zone. The diameter of the plots under and beyond canopies ranged from 0.25 to 3.0 m. The plots were never placed closer than 3 m to any willow or conifer canopy to minimize any possible effects of the canopy. This limited the number of possible plots in the oldest zones where canopies were larger and more numerous. As a result, the average size of a plot and the number of plots differed among zones. This sampling scheme resulted in 172 plots within willow canopies and 172 beyond any canopies.

Statistical Analysis

The data were analyzed by use of contingency tables and logistic regression. Contingency tables tested for the overall effect of the canopies while logistic regression tested whether the effect of willow canopies varied among zones. Two-by-two contingency tables were used to assess presence and absence data collected for each species (Blundon et al., 1993) and Fisher's exact test was applied to test the effect of canopies. Because of potential problems in tables with low cell counts (SYSTAT, 1992), the tests of species with counts of fewer than five in any of the cells were excluded. The null

hypothesis of no canopy effect was tested at alpha of 0.05. Greater frequency of a given species growing under a canopy compared to in the open was interpreted as a positive effect of the canopies, whereas lower frequency was interpreted as a negative effect.

Logistic regression (Hosmer and Lemeshow, 1982) was applied on the presence and absence data of the 12 most prominent species. No logistic regression model was fit with fewer than 40 observations of presence in the 344 plots. A model was used with zone age as a continuous variable, canopy as a categorical variable and interaction of zone age and canopy. No model selection was performed, because the main interest was to see whether the effect of canopy changed over time.

All Fisher's exact tests were performed with SYSTAT (SYSTAT, 1992) and all logistic regression analyses were performed with SAS in CATMOD procedure (SAS, 1989a).

Experiment 2: effects of willow canopies and willow soil on emergence of Pinus contorta germinants

Establishment of Seeding Treatments

Germinant emergence and survival of *Pinus contorta* were assayed for seed sown in treatments where the presence of willow canopies and soils from beneath willow canopies were controlled. *P. contorta* was chosen as a test plant because (1) it occurs naturally at the site but is present at only low frequency, (2) its seeds germinate reliably and are easy to manipulate under laboratory conditions, and (3) *P. contorta* seeds more readily available than seeds of other indigenous conifers (*Abies lasiocarpa, Larix lyallii, Pinus albicaulis,* and *Tsuga mertensiana*) at the study site.

Seed of *P. contorta* was obtained from high-altitude populations (1540–

2430 m) growing in a zone between 4 km west and 172 km east of the crest of the Cascade Range and between 42° and 45° N. The seeds were stratified by soaking in deionized water for 24 h followed by incubation at 4° C for 5 d. They were next treated with 30% H_2O_2 for 55 min and packed in Ziploc plastic bags with moistened paper towels for transportation to the site. A subsample of the seeds was separated to determine the viability and germination rate in the laboratory. After 3 wk of incubation, 71.5 % (± 1.0 %) of the seeds germinated under these conditions.

Seeds were sown within and beyond willow canopies in four zones representing approximately 30, 45, and 60 yr old of the Phase 3 willow/rawmark/low herbaceous community and 85 yr old of the Phase 4 early transitional stage towards heath shrub/lush meadow parkland. The 30-yr-old zone was at the edge of the boundary between the Phase 2 rawmark community and the Phase 3 community. Within each of these zones, five representative canopies of *Salix* spp. were chosen.

Four soil treatments were applied under each canopy as well as on an open site 3 m from the center of the canopy. Each treatment was applied on a 7x13-cm plot, a size defined by the bags used in Soil Trenching and Soil Transfer treatments (see below). The treatments were: (1) Control—gentle raking of the top 2 cm of soil to create a seedbed; (2) Soil Mixing—complete mixing of the soil in place to a depth of 10 cm; (3) Soil Trenching—removing the soil to a depth of 10 cm, mixing and placing it into a plastic-lined paper bag (7 x 13 x 10 cm, the bottom perforated for drainage), then replacing the bagged soil back into its pit; (4) Soil Transfer—a Soil Trenching treatment, but instead of replacing the bagged soil into its pit, placing the bag of under-canopy soil in the open-site pit and the bag of open-pit soil in the under-canopy pit.

After the soil treatments were applied, ten *P. contorta* seeds were sown on each. A "mulch" of either gravel or willow litter was placed on top of the treated soils to represent the conditions at the treatment area. In the case of the transferred soils, the litter was kept with soils taken from under the

canopies and placed in the open-sites. Likewise, the surface gravel of the open sites was transferred with those soils to the under-canopy positions.

The soils were thoroughly watered at sowing time and two days later; two days after watering, heavy rain fell on the study area. The plots received no other treatment prior to recording of surviving germinants 8 wk later. Germinant emergence and survival for the first 8 wk (from here on referred to as emergence) was scored for all plots. Data were treated as proportion of successful events (emergence) in the total number of trials (number of seedlings/number of seed sown in a single plot).

Statistical Analysis

A logistic regression model with zone age (treated as a continuous variable), presence of the canopy (categorical with two levels) and the treatments (three categorical variables with two levels) was fit on the binomial seedling emergence data. Only the main effects were fit because of the large number of empty cells in the experimental design, so interactions could not be tested. To identify the most influential main effects, a model selection based on the Akaike Information Criterion (AIC) (SAS, 1989b) was performed. All analyses were performed in SAS LOGISTIC procedure (SAS, 1989b).

Characterization of soil nitrogen and organic matter contents

Sampling and Chemical Analysis

The top 10 cm of soil was sampled at the four zones used in experiment 2. All exposed and visible litter and organic debris were removed. One

additional sample was collected from the barren zone at <30 yr exposure from under the ice. Two willow canopies were selected in three zones representing ca. 30, 45, and 60 yr old of the Phase 3 willow/rawmark/low herbaceous community, and one was similarly chosen in the 85 yr old of the Phase 4 early transitional stage towards heath shrub/lush meadow parkland. The 30-yr-old zone was at the edge of the boundary between the Phase 2 rawmark community and the Phase 3 community. In each sampled willow, two samples were collected from beneath the willow canopy and two at 3 m from the edge of the canopy.

Soils were sieved through 2-mm mesh and organic matter was determined by loss on ignition (Davies, 1974) with a subsample checked against analyses by Leco Carbon-Nitrogen analyzer. Nitrogen was determined by Kjeldahl method (Thomas et al., 1967) and colorimetrically analyzed for total nitrogen concentration (% by dry weight) with an Alpkem Rapid Flow Analysis system Model 300.

Statistical Analysis

A multiple linear regression model with zone age (treated as a continuous variable) and presence of the canopy (categorical variable with two levels) was fit on the soil organic matter and nitrogen concentration data. Both main effects and interaction were fit in the full model. Model selection was performed according to suggestions in Ramsey and Shafer (1996). All analyses were performed in SYSTAT using General Linear Models procedure (SYSTAT, 1992).

RESULTS

Experiment 1: Effect of willow shrubs on frequencies of indigenous species

Willows did not affect the presence or absence of most indigenous plants tested (Table 3.1). No species showed a positive association with willow canopies. Negative associations with willows were significant for five species (*Epilobium alpinum, E. latifolium, Juncus mertensianus, Saxifraga ferruginea,* and *S. tolmiei*) of the 18 tested. Logistic regression analysis confirmed the negative effect of willow canopies on *Saxifraga ferruginea* and *S. tolmiei,* but not on *J. mertensianus* or *E. latifolium* (data not shown); observations of *E. alpinum* were too few to complete an analysis. No significant interaction occurred between the zone age and willow canopy, indicating that the effect of willow canopies did not change over the chronosequence (Table 3.1). Logistic regression analysis of a few species resulted in a model with a lack of fit, indicating that these patterns of frequency distribution cannot be explained with a logistic regression model (*Cassiope mertensiana, Epilobium latifolium, Pedicularis groenlandica* and *Phyllodoce empetriformis* in Table 3.1).

Experiment 2: Effect of Willow Shrubs on Emergence of Pinus contorta Germinants.

Only 64 germinants from the total of 1600 *Pinus contorta* seed survived at the study site. This equals an emergence rate of 4% as opposed to the germination rate of about 70% determined in the laboratory. No mortality among the shoots nor signs of herbivory were detected following the 8-wk period between sowing and data collection.

Table 3.1. Test of effect of willow canopies on presence/absence of indigenous plant taxa at Lyman Glacier forefront (experiment 1). Canopy effect was tested with Fisher's exact test. Interaction between zone age and canopy was tested with logistic regression.

Taxon[a]	Number of plots with taxon present		Canopy effect	Zone age x canopy
	Within canopy	Beyond canopy		
Cyperaceae				
Carex scopulorum Holm	10	8	None	c
Ericaceae				
Cassiope mertensiana (Bong) G.Don	44	34	None	b
Phyllodoce empetriformis (Sw.) D. Don	77	75	None	b
Phyllodoce glanduliflora (Hook.) Cov.	13	14	None	c
Vaccinium deliciosum Piper	38	34	None	p=0.4867
Juncaceae				
Juncus drummondii E. Meyer	141	145	None	p=0.3801
Juncus mertensianus Bong.	48	75	Negative**	p=0.3277
Luzula piperi (Cov.) Jones	96	103	None	p=0.1696
Pinaceae				
Abies lasiocarpa (Hook.) Nutt.	50	48	None	p=0.4806

Table 3.1. (continued)

Taxon[a]	Number of plots with taxon present Within canopy	Beyond canopy	Canopy effect	Zone age x canopy
Onagraceae				
Epilobium alpinum L.	10	25	Negative*	c
Epilobium latifolium L.	21	40	Negative*	b
Salicaceae				
Salix commutata Bebb	7	11	None	c
Salix phylicifolia L.	21	25	None	p=0.1551
Saxifragaceae				
Saxifraga ferruginea Grah.	90	123	Negative***	p=0.3231
Saxifraga tolmiei T. & G.	21	36	Negative*	p=0.0994
Scrophulariaceae				
Pedicularis groenlandica Retz.	59	46	None	b
Veronica wormskjoldii Roem. & Schult.	6	12	None	c

a = Nomenclature follows Hitchcock and Cronquist (1973).
b = Model with lack of fit; residual X^2 of the model significant at 0.05 level.
c = Too few observations to fit logistic regression model
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

Neither the soil mixing nor soil trenching treatments significantly affected emergence of *P. contorta,* so these variables were excluded from the final reduced model as inferred from AIC-values. The residual X^2 was highly nonsignificant ($p = 0.6389$) indicating that the selected reduced model was well fit.

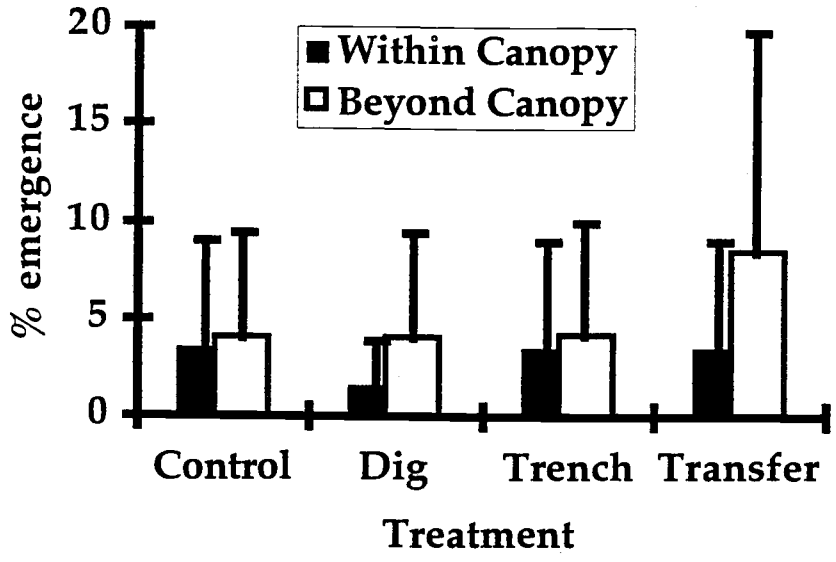
Zone age negatively affected emergence resulting in a mean decrease of 1.6% per year (95% confidence interval: 1.0 – 2.3%) over the 30 to 85-yr old portion of the chronosequence (Table 3.2). Willow canopies also had a strong negative effect on emergence. Estimated germinant emergence within willow canopies was 57% of that beyond willow canopies (95% confidence interval: 43.7–74.2%). Transfer of soil produced a significant 1.8-fold higher emergence than soil left in place (95% confidence interval: 1.4–2.3; Fig. 3.2). The highest emergence thus occurred in those treatments where soil from beneath the canopies was transferred to the open.

Table 3.2. Logistic regression model describing *Pinus contorta* germinant emergence in experiment 2. Only the final reduced model is displayed.

Variable	DF	Odds ratio[c]	95% confidence intervals		Probability
			Lower	Upper	
Intercept	1	0.108	0.074	0.158	0.0001
Zone age[a]	1	0.984	0.977	0.990	0.0149
Canopy[b]	1	0.570	0.437	0.742	0.0332
Soil transfer[b]	1	1.768	1.352	2.313	0.0338
		Model likelihood ratio (DF = 2)[d] = 0.6389			

a = continuous variable
b = indicator (dummy) variable
c = ratio of the proportion of events (emergence) to the proportion of nonevents (see results for interpretation)
d = test for goodness of fit

Figure 3.2. Proportion (mean ± standard deviation) of Pinus contorta seed resulting in germinant emergence in main treatments in experiment 2.



Characterization of soil nitrogen and organic matter contents

Both the nitrogen and organic matter contents of the soil tended to increase with time over the chronosequence through the 60-yr-old zone, although variation was high in samples from some locations (Table 3.4). The decrease in nitrogen and organic matter at the 85-yr-old sampling site was unexpected, but may be due to its location on the south slope of the rather tall terminal moraine, where leaching and wind or water erosion may be more pronounced than at the other, relatively flat sites. As a result, the zone age did not contribute to the explanatory power of our models, nor did the regression coefficients significantly differ from zero (Table 3.3). However, zone age was

Table 3.3. Reduced, additive, linear regression model describing total soil nitrogen (N = 15) and soil organic matter (N = 33) beyond and under the willow canopies in the Lyman Glacier forefront.

Variable	Total soil N % by dry weight	Soil organic matter % by dry weight
Intercept	- 0.003 ± 0.011 ^{ns}	0.620 ± 0.816 ^{ns}
Zone age (yr)[a]	0.000 ± 0.000 ^{ns}	0.007 ± 0.014 ^{ns}
Canopy[b]	0.043 ± 0.015 **	1.144 ± 0.518 *
Model (df = 2)	R^2 = 0.251 Model P = 0.027	R^2 = 0.169 Model P = 0.098

a = continuous variable.
b = indicator (dummy) variable.
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$
ns = $P > 0.05$.

included in the final models for both soil organic matter and nitrogen concentrations as a confounding factor resulting in a reduced, additive model containing no interaction.

Samples under willow canopies consistently produced higher nitrogen and organic matter contents than did those in the nearby open sites (Table 3.4). Based on our linear regression model, presence of a willow canopy resulted in an increase of 0.04 per cent units (95 % confidence interval: 0.01 – 0.07) in total soil nitrogen and 1.144 per cent units (95 % confidence interval: 0.13 – 2.16) in soil organic matter (Table 3.3). Most of the organic matter in the open sites was probably in the form of fine roots, including mycorrhizae, which passed the sieve mesh. Even several meters from the nearest willow and in soil supporting no other plants, an impressive amount of fine roots were evident when samples were extracted.

Table 3.4. Total soil nitrogen and soil organic matter beyond and under the willow canopies at Lyman Glacier forefront. Values are means±standard errors. Sample sizes are 2 for soil nitrogen and 4 for soil organic matter except where otherwise indicated.

	Total soil N % by dry weight		Soil organic matter % by dry weight	
Age zone	Under canopy	Beyond canopy	Under canopy	Beyond canopy
< 30	—[b]	0.001 ± —[a]	—[b]	0.001 ± —[a]
30	0.022 ± 0.003	0.007 ± 0.002	1.20 ± 0.099	0.67 ± 0.219
45	0.053 ± 0.038	0.016 ± 0.002	2.33 ± 0.739	1.37 ± 0.114
60	0.097 ± 0.074	0.006 ± 0.005	3.03 ± 1.574	1.04 ± 0.334
85	0.025 ± —[a]	0.010 ± —[a]	1.62 ± 0.185	0.57 ± 0.015

a = Single observation used.
b = No willows present in this zone.

DISCUSSION

Both experiments showed that the shrub willows (*Salix commutata* and *S. phylicifolia*) do not serve as "nurse plants" or nuclei that facilitate the establishment of vascular plants from 30 yr onward. In experiment 1, the willow canopy did not affect the observed frequency of most indigenous taxa. The five species negatively associated with the willow canopies suggest that willows inhibit their establishment. In experiment 2, willow canopies inhibited the germinant emergence of *P. contorta*. However, the greatest emergence occurred in soils transferred from beneath willow canopies to areas beyond the canopies (Fig. 3.2). These findings indicate that although the willow canopy is either neutral or inhibitory in its effect on establishment of indigenous plants, the soil developing beneath the willow can contribute towards successful plant establishment.

Facilitation has been demonstrated in numerous other studies (see Callaway, 1995), although interference may be the major determinant of plant community structure and dynamics (Aarssen and Epp, 1990; Goldberg and Barton, 1992). The existing plants that aid the establishment of new plant individuals have been referred to as "nuclei" (Yarranton and Morrison, 1974; Blundon et al., 1993) or "nurse plants" (Niering et al., 1963; Franco and Nobel, 1988). Given that plants compete directly with one another for resources, one would expect that nucleation is not a typical phenomenon. In fact, where facilitation has been documented, some specific indirect mechanisms existed that resulted in the favored establishment of invading plants within the nucleus. These mechanisms have been described as altered microclimate (Yarranton and Morrison, 1974; Allen and Allen, 1988; Franco and Nobel, 1988; Callaway, 1994; Berkowitz et al., 1995; Pugnaire and Haase, 1996), nutrient accumulation (Yarranton and Morrison, 1974; Campbell et al., 1990; Franco and Nobel, 1988; Pugnaire and Haase, 1996), increased seed input via perching birds (Campbell et al., 1990), alteration of soil conditions by the accumulation of soil organic matter (Morris and Wood, 1989; Blundon et al.,

1993; Pugnaire and Haase, 1996), and protection from herbivores (Niering et al., 1963; McAuliffe, 1988; Callaway, 1992). Whether an invading plant successfully establishes and survives within a nucleus is the net outcome of the opposite effects of inhibition and facilitation (Connell et al., 1987; Walker and Chapin, 1987; Franco and Nobel, 1988; Callaway, 1994; Berkowitz et al., 1995; Callaway and King, 1996).

Lower frequency of successful establishment of the five plant taxa and *Pinus contorta* under willow canopies is thought to be due to shading. As indicated by transect data (Jumpponen et al., unpublished), these five species were dominant at the earliest successional stage of glacier forefront, either before or at the point where willows began to occur. In contrast, those taxa not affected by willow canopies had the highest frequencies later in the successional sere. The early seral species are likely adapted to high levels of light and therefore are inhibited by the canopy shade. The same case can be made for *P. contorta,* a shade-intolerant species (Fowells, 1965). Competition for light has been suggested as important in controlling the establishment and performance of neighboring plants under previously established plant individuals (Franco and Nobel, 1988; Callaway, 1992; Berkowitz et al., 1995; Callaway and King, 1996).

Herbivory and seed predation have been hypothesized as mechanisms of decreased emergence or establishment within nuclei (Morris and Wood, 1989; Callaway, 1992). No signs of herbivory on *P. contorta* were observed within or beyond the canopies in experiment 2 at the 8-wk observation time. Seed and seedling predation, however, cannot be ruled out; small mammals are frequently encountered on the glacier forefront (Cázares and Trappe, 1994) and newly emergent *Abies* seedlings growing in the open are consumed, probably by birds, at the site (Trappe, unpublished data). If the latter were true for the emergent *Pinus contorta* seedlings, it would mean that even more seedlings had emerged in the open than we recorded as emergent/survivors making the suggested canopy inhibition even more obvious.

Morris and Wood (1989) suggested potential effects of allelopathic substances produced by established plants to inhibit plant establishment. This possibility cannot be excluded in experiment 1; however, no such allelopathic mechanism was observed in experiment 2. On the contrary, the logistic regression model suggested that *P. contorta* shoot emergence was almost two-fold higher in transferred soils. If allelopathy that was associated with the soil characteristics were operating, one would expect equally low emergence in the transferred soil.

Enhanced emergence in soils from beneath the willow canopies is hypothesized to result from: (1) higher soil nitrogen or (2) organic matter contents, and (3) higher mycorrhizal inoculum potential in the transferred soil. Primary successional ecosystems are generally low in nitrogen (Vitousek and Walker, 1989; Matthews, 1992). Higher nitrogen concentrations have been observed under willows than in the open at Lyman Glacier forefront (Tables 3 and 4) and under non-nitrogen-fixing poplars at Glacier Bay in Alaska (Crocker and Major, 1955). Furthermore, the addition of nitrogen resulted in significant, almost two-fold, increase in growth of *Pinus contorta* seedlings grown in the greenhouse in soil from the Lyman Glacier forefront (Jumpponen, Mattson and Trappe, unpublished data), showing that plant growth is strongly nitrogen limited and that even small additions may enhance performance. This supports the importance of willow shrubs (vegetation in general [see Chapin et al., 1994]) in storing nutrients in the organic pool. Rapid accumulation of nitrogen during the earlier stages of succession, followed by a plateau, seems to be a general rule in primary successional seres regardless of the type of vegetation (Matthews, 1992; Walker, 1993).

Young soils are naturally low in organic matter; glacial soils begin with extremely low or no organic matter (Jenny, 1980; Matthews, 1992). At Lyman Glacier forefront, the soils are still low in organic matter almost 100 yr after the glacier retreat. However, there is nearly a two-fold increase in soil organic matter beneath the willow canopies compared to beyond them (Table 3.3).

Increased soil organic matter enhances plant performance through a variety of mechanisms, such as increased water-holding capacity, increased nutrient availability, and more favorable rhizosphere environment. Our observations at Lyman Glacier suggest that soil moisture stress can become severe in mid to late summer and strongly limit establishing plants. Soil organic matter may serve as a moisture reservoir during drought.

Cázares (1992) and Trappe and Luoma (1992) hypothesized that the patterns of plant establishment and community development depend on availability of propagules of mycorrhizal fungi. Similarly, the successional change in plant communities is governed by interactions between plant individuals (Bazzaz, 1990; Connell and Slatyer, 1977) as well as the interactions between plants and soil microbiota (Allen and Allen, 1984, 1988, 1990). Root-associated fungi are of special importance because of their ability to access resources not available to plant roots and create below-ground connections between plant individuals (Allen and Allen, 1990; Harley and Smith, 1983; Newman, 1988; Read et al., 1985). Soil transferred from under the willow canopies may have contained more diverse and viable microflora which could have stimulated seed germination and facilitated survival through the first growing season when the canopy inhibition was excluded.

The logistic regression model indicated that the germinant emergence of *Pinus contorta* decreased with zone age. For example, emergence at the 85-yr-old zone was 54% of that at the 30-yr-old zone. This result should be viewed with caution. The change in emergence over the zones was not linear but rather appeared to display a stepped function where emergence dropped dramatically between the 45-yr-old and 60-yr-old zones. The reduced emergence at the oldest zones may result from factors independent of zone age.

Our data suggest that willow shrubs may be either neutral or inhibitory to other vascular plants and that this interaction is species specific. Callaway (1994) provides a prime example for species-specific interaction: while two winter annuals were clearly positively associated with the perennial shrub,

Arthrocnemum subterminale, survival of the third was substantially increased by the removal of the *Arthrocnemum* canopies. Our two experiments showed no net positive effect was created by the physical microenvironment under the canopies. The role of the willow shrubs, however, may be essential in controlling the transition of the plant community from dominance by the early colonizers. The 100 yr of exposure to the environment at the Lyman Glacier forefront may not be enough to unveil possible positive effects of shrub willows on the late seral species. As shown in experiment 2, the willows may modify the substrate favorably for establishment of the later seral species.

In his comprehensive treatise on the ecology of recently deglaciated terrain, Matthews (1992, p. 271) states: "Many workers have proposed that facilitation is an important process on glacier forelands. In no case, however, has it been demonstrated that the prior occurrence of a particular species is necessary and sufficient for the later occurrence of a different species." Our experiments also show the potential difficulties in pinpointing mechanisms, which may have opposite effects, working simultaneously on any successional sere (Walker and Chapin, 1987). Detailed experimental manipulations are necessary to separate these mechanisms in primary succession. This should be the prerequisite to the introduction of a general theoretical framework and mechanistic models of community development in successional seres.

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CHAPTER 4

ECTOMYCORRHIZAL FUNGI IN LYMAN LAKE BASIN: A COMPARISON BETWEEN PRIMARY AND SECONDARY SUCCESSIONAL SITES.

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ABSTRACT

The results of eight years of floristic field surveys of the ectomycorrhizal macrofungi at the subalpine Lyman Lake Basin (Glacier Peak Wilderness area in the North Cascade Mountains, Washington, USA) are reported. The basin was divided into three sites based on their ecological and geological history (primary successional glacier forefront vs. secondary successional habitat), the range of host species (presence or absence *Larix lyallii, Pinus albicaulis,* and *Polygonum bistortoides*), and the habitat type (alpine parkland vs. subalpine *Tsuga mertensiana – Abies amabilis* community). A total of 145 collections of ectomycorrhizal fungi were obtained. The collections represented 68 taxa, 25 genera, 14 families and 7 orders. Cortinariaceae was the most species rich family with a total of 25 taxa, some of which remained unidentified. *Cortinarius* was the most species-rich genus with a total of 17 species. The two secondary successional sites shared 12.1% of the occurring species; while the primary successional site shared only 2 and 5.1% with the two secondary successional sites. No ectomycorrhizal species occurred on all three sites. The secondary successional sites shared seven species (*Boletus edulis, Elaphomyces granulatus, Hydnotrya variiformis, Rhizopogon subsalmoneus, R. vulgaris, Russula emetica* and *Thaxterogaster pingue*) while the primary successional site shared two species with the parkland (*Fuscoboletinus aeruginascens* and *Suillus cavipes*) and only one with subalpine site (*Inocybe lacera*). Sixty-one species occurred at only one of the three sites. These data show that the communities of ectomycorrhizal fungi differ between the primary and secondary successional sites. The role of the ecological and geological history vs. the role of differences in the host plant composition and age remains unclear. Similarly, the life history strategies of the ectomycorrhizal fungi are difficult to construct due to the sparse data available and possible differences between different habitat types. The results are discussed in the context of succession, fungal life strategies, and ecology of alpine fungi.

INTRODUCTION

Communities of macrofungi have been only infrequently studied in arctic and alpine environments (but see e.g. Favre, 1955; Graf, 1994; Lange, 1946, 1949; Lange and Skifte, 1967; Laursen and Ammirati, 1982; Laursen *et al.*, 1987; Petersen, 1977; Petrini and Laursen, 1993; Senn-Irlet, 1987; Trappe, 1988; Watling, 1988). As a result, the fungal flora in these environments is poorly known, and discovery of new or rare and endangered species is not uncommon (Cázares and Trappe 1990, 1991a, 1991b, Graf and Horak 1993, Jumpponen *et al.*, 1997).

Alpine glacier forefronts have a special importance for several reasons. They represent a new, primary[3] successional parent material distinctively different from the immediate surroundings. Forefronts provide a unique habitat for a variety of ruderal or pioneering organisms absent from adjacent secondary[2] successional sites. The combination of primary and secondary successional ecosystems adjacent to each other supplies field ecologists with experimental field laboratories for studying similarities and differences between the two, and ultimately allowing inferences of the successional patterns in these environments.

Mycologists have rarely utilized the research opportunities provided by primary successional ecosystems created by glacier forefronts (see Alfredsen, 1997; Horak, 1960; Matthews, 1992 and references therein). Mycorrhizal associations and mycorrhizal flora of these primary successional ecosystems

3 Primary succession is defined as vegetation development on newly formed or exposed substrate. It proceeds on raw parent material rather than a developed or modified soil, and is usually characterized by low nitrogen and organic matter (Glenn-Lewin *et al.*, 1992; Matthews, 1992).

4 Secondary succession is defined as replacement of pre-existing vegetation following disturbance (Glenn-Lewin *et al.*, 1992). Developed soil with an organic component and a biological legacy as at least a propagule bank is present.

are even more rarely studied (Cázares, 1992; Helm *et al.*, 1996; Rossow *et al.*, 1996). One successional model of ectomycorrhizal fungus community development is based on the series of studies conducted by the Institute of Terrestrial Ecology (ITE) in Scotland (Deacon *et al.*, 1983; Dighton *et al.*, 1986; Fleming, 1983; Fleming *et al.*, 1984;Last *et al.*, 1987; Mason *et al.* 1982; Mason *et al.*, 1983). This 'early-' and 'late-stage' model has found support (Helm *et al.*, 1996; Jansen, 1991; Visser, 1995) as well as criticism (Keizer and Arnolds, 1994; Termorshuizen, 1991) in several studies but has not been addressed by a comparison between true primary and secondary successional systems. Such studies are timely to understand to what degree the fungal flora at the primary successional sites differ from those in the surrounding secondary successional communities.

This paper has three primary objectives: (1) to report the results of eight years of floristic field surveys of the ectomycorrhizal macrofungi in the transition zone between subalpine coniferous forest and alpine meadow habitat at the Lyman Lake Basin in the North Cascade Mountains, Washington, USA, (2) to compare the ectomycorrhizal fungus flora observed in the primary successional ecosystem at the forefront of Lyman Glacier with those observed in adjacent secondary successional habitats beyond the terminal moraine of Lyman Glacier, and (3) to comment on the ecology of the fungi collected in the course of this study.

MATERIALS AND METHODS

Lyman Lake Basin, located between Spider Pass (48° 10′ 52″ N, 120° 53′ 87″ W) and Cloudy Pass (48° 12′ 13″ N, 120° 55′ 56″ W) in the Glacier Peak Wilderness Area, Wenatchee National Forest, in Washington, USA, contains distinctively different, primary and secondary successional habitats. The primary successional habitat, Lyman Glacier Forefront, has deglaciated during the past century. The glacier has not exceeded its terminal moraine of the late

1890's for the past ca 6,900 years, as beyond that moraine is an undisturbed deposit of volcanic ash from the Mount Mazama eruption (W. Long, pers. comm.). The glacier has been receding steadily since the 1890s vacating an 1100 m forefront for establishment and invasion by pioneering colonizers. The terminal moraine distinctly separates the primary successional glacier forefront from the secondary successional stands which have been exposed to fire and recreational disturbances within the past century. Periodic photographs between 1898 and 1915 (Lindsley photographs at Special Collections Division, University of Washington, Seattle) evidence that most of the eastern Lyman Lake Basin was burned at the turn of the century, most likely to facilitate the intensive prospecting activity at the Central North Cascades region. However, some old growth patches by Lyman Lake survived the fires. Growing recreational activity results in frequent small scale disturbances on trails and at camp sites in the popular Lyman Lake Basin.

To facilitate comparison of the ectomycorrhizal fungus flora we divided the Lyman Lake Basin into three different sites based mainly on their different ecological (primary vs. secondary successional ecosystem) and geological history (young soils of exposed parent material vs. developed soils with a strong litter and humus accumulation), flora of ectomycorrhizal hosts (presence or absence of *Larix lyallii, Pinus albicaulis,* and *Polygonum bistortoides,* which are common at both Lyman Glacier Forefront and Glacier View Ridge, but absent at Lyman Lake), and the habitat type (alpine parkland vs. subalpine *Tsuga mertensiana – Abies amabilis* community). The site descriptions are summarized in Table 4.1.

Lyman Glacier Forefront supports a naturally evolved primary successional community: no exotic weeds have been detected (Jumpponen *et al.,* 1998b). Vegetation is developing from earliest colonization of barren parent material to a plant community resembling that at Glacier View Ridge beyond the terminal moraine. The course of the vegetation change on Lyman Glacier Forefront is described more in detail in Cázares (1992) and Jumpponen

Table 4.1. Description of the three sites in the Lyman Lake Basin.

Site	Elevation	Ectomycorrhizal Hosts	Site Description
Lyman Glacier Forefront	ca 1800 m	*Abies lasiocarpa* *Alnus sinuata* *Larix lyallii* *Picea engelmannii* *Pinus contorta* *Polygonum bistortoides* *Populus trichocarpa* *Salix commutata* *S. nivalis* *S. phylicifolia* *Tsuga mertensiana*	A primary successional[1] community on a receding glacier forefront. Vegetation is undergoing a change from a community dominated by Saxifragaceae towards a community that closely resembles an alpine parkland with patches of coniferous stands and Ericaceous shrubs including *Vaccinium* ssp. *Phyllodoce* ssp. and *Cassiope mertensiana* and *Luetkia pectinata* (Jumpponen et al., 1998b).
Glacier View Ridge	1800 – 2100 m	*Abies amabilis* *A. lasiocarpa* *Larix lyallii* *Pinus albicaulis* *Polygonum bistortoides* *Salix* spp. *Sorbus sitchensis* *Tsuga mertensiana*	A secondary successional[2] community with recent disturbance history by fire. Site is located beyond Lyman Glacier and clearly demarcated by the terminal moraine. Vegetation ranges from alpine parkland with coniferous stands and Ericaceous heathlands (with *Vaccinium* sp. *Phyllodoce* sp. and *Cassiope mertensiana* and *Luetkia pectinata*) to a krumholz with low growing conifers.

Table 4.1. (continued)

Site	Elevation	Ectomycorrhizal Hosts	Site Description
Lyman Lake	1650 – 1800 m	*Abies amabilis* *A. lasiocarpa* *Alnus sinuata* *Picea engelmannii* *Pinus monticola* *Salix* spp. *Sorbus sitchensis* *Tsuga mertensiana*	A secondary successional[2] community with recent disturbance history by fire and recreational activities. Vegetation consists of closed stands of mixed conifers.

et al. (1998b). Vegetation at the secondary successional, Glacier View Ridge resembles the ecotone between the upper parkland subzones of the *Abies lasiocarpa* Zone and *Tsuga mertensiana* Zone of the North Cascade Range (Franklin and Dyrness 1973) extending above timberline to *A. lasiocarpa* and *Pinus albicaulis* krumholz (timberline and krumholz as defined in Art, 1993). Plant communities adjacent to the forefront include heath shrub, lush herb, and dwarf sedge (Franklin and Dyrness 1973) in a conifer parkland consisting of trees ranging from seedlings to 100 + years old. The vegetation at the other secondary successional site, Lyman Lake, includes components of old growth stands with uneven stand age dominated by *Abies lasiocarpa*. Several other conifers are also present (Table 4.1). *Larix lyallii*, however, has not been encountered at the Lyman Lake site. The plant community represents a transition between the mature phase of *Tsuga mertensiana – Abies amabilis* community (Douglas, 1972) and the *Abies lasiocarpa* dominated krumholz stands (Douglas, 1971; Douglas and Bliss, 1977) both characteristic to Western North Cascades region (Douglas, 1971; Douglas, 1972; Douglas and Bliss, 1977).

Ectomycorrhizal macromycetes have been collected during 16 expeditions to Lyman Lake Basin between 1988 and 1996, except for 1991 when the sites were not visited (Table 4.2). Sporocarps were collected as encountered during the course of the visits. Due to other research activity in the primary successional community of Lyman Glacier Forefront site, it received more attention than the surrounding secondary successional communities. As a result, it is safe to conclude that fungi of the secondary successional sites are absent at the primary successional site if not encountered there. The opposite may be incorrect although we attempted to cover the fungal flora in the secondary successional communities as thoroughly as possible. Some years witnessed poor fruiting because of warm, dry summers and autumns followed by early frost. For example, a thorough search revealed no sporocarps of any mycorrhizal species at the glacier forefront in 1996. No epigeous sporocarps were observed at the secondary successional sites either, although several hypogeous species were collected.

Table 4.2. Dates of expeditions to the sites at Lyman Lake Basin.

Year	Expedition Dates	Year	Expedition Dates
1988	05.VII – 11.VII 12.VIII – 17.VIII 31.VIII – 6.IX	1992	15.VIII – 17.VIII 07.IX – 13.IX
1989	04.VIII – 08.VIII 14.VIII – 17.VIII 29.VIII – 02.IX 19.IX – 22.IX	1993	12.IX –17.IX
1990	31.VIII – 03.IX 14.IX – 26.IX	1994	22.VIII – 28.VIII
1991	–	1995	20.VII – 26.VII 06.IX – 11.IX
		1996	24.VIII – 28.VIII

Macroscopic features of the fresh specimens were recorded at the sites. The specimens were then dried in the field with a propane field drier. Microscopic features were later described from dried material mounted in 5% KOH, cotton blue in lactic acid and/or Melzer's reagent. Nomenclature follows Hansen and Knudsen (1992), Hansen and Knudsen (1997) and Moser (1983) for Agaricales, Boletales, Gautieriales, Russulales, and Thelephorales with a few exceptions: the genera *Boletinus* and *Chalciporus*, for example, are not recognized here. Species of Cortinariaceae were identified in part with assistance by Joe Ammirati. The collections are, or will be, deposited in the Cryptogamic Herbarium at Oregon State University (OSC).

Similarities and differences in the ectomycorrhizal fungal flora between the three sites were estimated as follows. A pairwise Jaccardian similarity matrix using the presence/absence data describing the flora at the three sites was calculated using CORR procedure (SYSTAT, 1992). The pairwise matrix of similarities indicates the proportion of shared taxa when

only the taxa present on either or both of two compared sites are considered. This approach should not be considered as a statistical analysis. Rather, the correlation matrix (Table 4.3) provides an efficient tool for visualizing and summarizing similarities and differences between the sites.

RESULTS

During the eight years of field survey 145 collections of ectomycorrhizal fungi were obtained. These consisted of 68 species representing 25 genera, 14 families and 7 orders (Table 4.4). Thirty-seven species were recorded at the Lyman Lake site. Lyman Glacier Forefront and Glacier View Ridge sites had 13 and 28 species, respectively. Ten species were common to two sites, and 58 were unique to one of the three sites: 10 species were unique to Lyman Glacier

Table 4.3. The Jaccardian pairwise correlation matrix calculated for the three sites (lower left matrix). The values represent the proportion of shared taxa of those present at one or both of the compared sites (actual values presented in the upper right matrix). LGF = Lyman Glacier Forefront, GVR = Glacier View Ridge, LL = Lyman Lake.

	Jaccardian correlation matrix		
	LGF	GVR	LL
LGF	–	2/39	1/49
GVR	0.051	–	7/58
LL	0.020	0.121	–

Forefront, 19 to Glacier View Ridge and 29 to Lyman Lake. Cortinariaceae was the most species-rich family in all the sites with 25 taxa, some of which remained unidentified due to inadequate collections. *Cortinarius* was the most species-rich genus with 17 species recorded. A total of 14 species of hypogeous fungi were collected: 13 in the secondary successional sites and one in the primary successional site (*Hymenogaster glacialis*). *H. glacialis* is endemic to this area and known only from two collections from the glacier forefront site. Five of the hypogeous taxa were found on both secondary successional sites while two were unique to Glacier View Ridge and six to Lyman Lake.

The Jaccardian similarity coefficients calculated with the binary (presence/absence) data indicate the species composition at Glacier View Ridge and Lyman Lake were most alike (Table 4.3). They shared 7 of the total of 58 species observed at the two sites (12.1%). The primary successional site at the glacier forefront shared only 5,1% and 2% of the species with Glacier View Ridge and Lyman Lake, respectively. This represents 2 shared species of the 39 encountered at Glacier View Ridge and glacier forefront and 1 of 49 encountered at Lyman Lake and glacier forefront.

Table 4.4. Ectomycorrhizal species representing the 145 collections obtained at the Lyman Lake Basin during eight years of field survey. Presence of a taxon is indicated by +-signs. LGF = Primary successional Lyman Glacier Forefront site, GVR = Secondary successional Glacier View Ridge site, LL = Secondary successional Lyman Lake site. For more information about the sites please see Table 4.1. Number in parenthesis indicates the number of recorded species within each order and family.

Ectomycorrhizal taxa	LGF	GVR	LL
ASCOMYCOTA (4)			
Elaphomycetales (2)			
Elaphomyceteceae (2)			
Elaphomyces granulatus Fries		+	+
E. muricatus Fries			+
Pezizales (2)			
Discinaceae (2)			
Hydnotrya cerebriformis Harkness			+
H. variiformis Gilkey		+	+
BASIDIOMYCOTA (64)			
Agaricales (40)			
Amanitaceae (4)			
Amanita muscaria (L. : Fr.) Hooker			+
A. pantherina (D.C. : Fr.) Schumacher			+
A. vaginata (Bull. : Fr.) Vittadini			+
Amanita sp.1		+	
Cortinariaceae (25)			
Cortinarius cinnamomeus (L. : Fr.) Fr.			+
C. croceus (Schaeff.) Bigeard & Guillemin		+	
C. decipiens (Pers. : Fr.) Fr.	+		
C. duracinus Fr.			+
C. fulvoochrascens Henry		+	
C. idahoensis (Ammirati & Smith) Ammirati			+
C. montanus Kauffm.			+
C. mutabilis Smith	+		
C. semisanguineus (Fr.) Gill.		+	
C. tenebricus Favre	+		
C. violaceus (Fr.) Fr.			+
Cortinarius sp. 1	+		
Cortinarius sp. 2		+	
Cortinarius sp. 3		+	
Cortinarius sp. 4		+	
Cortinarius sp. 5			+
Cortinarius sp. 6	+		

Table 4.4. (continued)

Ectomycorrhizal taxa	LGF	GVR	LL
Hebeloma strophosum (Fr.) Sacc.			+
Hebeloma sp. 1		+	
Hymenogaster glacialis Cázares & Trappe	+		
Inocybe lacera (Fr. : Fr.) Kumm.	+		+
I. lanuginosa (Bull. : Fr.) Kumm.		+	
Inocybe sp. 1		+	
Rozites caperata (Pers. : Fr.) Karst.			+
Thaxterogaster pingue (Zeller) Singer & Smith		+	+
Hygrophoraceae (5)			
Hygrocybe miniata (Fr.) Kumm.			+
Hygrophorus parvulus Peck		+	
H. pudorinus (Fr.) Fr.			+
H. purpurascens (Fr.) Fr.		+	
H. subalpinus Smith			+
Tricholomataceae (6)			
Catathelasma ventricosum (Peck) Singer			+
Laccaria bicolor (Maire) Orton			+
L. laccata (Scop. : Fries) Berkeley & Broome			+
L. montana Singer	+		
L. nobilis Smith		+	
Tricholoma zelleri (Stuntz & Smith) Onr. & Tylr.			+
Boletales (13)			
Boletaceae (5)			
Boletus calopus Fr.			+
B. edulis Bull. : Fr.		+	+
B. piperatus Fr.		+	
B. rubripes Thiers			+
B. smithii Thiers			+
Gomphidiaceae (3)			
Fuscoboletinus aeruginascens (Secr.) Pom. & Smith	+	+	
F. ochraceoroseus (Snell) Pom. & Smith		+	
Suillus cavipes (Opat.) Smith & Thiers	+	+	
Rhizopogonaceae (3)			
Rhizopogon subcaerulescens Smith		+	
R. subsalmoneus Smith		+	+
R. vulgaris (Vitt.) M. Lange		+	+
Xerocomaceae (2)			
Gastroboletus ruber (Zeller) Cázares & Trappe			+
G. turbinatus (Snell) Smith & Singer			+

Table 4.4. (continued)

Ectomycorrhizal taxa	LGF	GVR	LL
Gautieriales (1)			
Gauteriaceae (1)			
Gautieria monticola Harkness		+	
Russulales (9)			
Russulaceae (7)			
Lactarius deliciosus (Fr.) Gray		+	
L. pseudomucidus Smith		+	
L. uvidus (Fr. : Fr.) Fr.	+		
Lactarius sp. 1	+		
Russula emetica Fr.		+	+
Russula fragilis var. fragilis (Pers : Fr.) Fr.	+		
Russula sp. 1			+
Elasmomycetaceae (2)			
Macowanites lymanensis Cázares & Trappe			+
Martellia cf. parksii			+
Thelephorales (1)			
Thelephoraceae (1)			
Sarcodon subfelleus (Harr.) Harr.			+

DISCUSSION

Ectomycorrhizal Species Shared Between Sites

No ectomycorrhizal fungi were common to all three sites. Glacier View Ridge and Lyman Lake shared seven species (*Boletus edulis, Elaphomyces granulatus, Hydnotrya variiformis, Rhizopogon subsalmoneus, R. vulgaris, Russula emetica* and *Thaxterogaster pingue*) while Lyman Glacier Forefront shared two species with Glacier View Ridge (*Fuscoboletinus aeruginascens* and *Suillus cavipes*) and only one with Lyman Lake (*Inocybe lacera*).

The only taxon (*Inocybe lacera*) common to Lyman Glacier Forefront and Lyman Lake sites is a small, morphologically variable, widespread species

fruiting prolifically at nutrient-poor sites. According to Kuyper (1986) some *I. lacera* varieties occur at disturbed sites, such as old fireplaces. It is also common in forest nurseries (Trappe and Strand, 1969). We collected *I. lacera* frequently at the Lyman Glacier Forefront site in association with *Salix* spp., but less frequently at Lyman Lake. The specimens at Lyman Lake were collected at rather heavily used camp sites, on trails, or along the lake shore suggesting a ruderal habit and an ability to form mycorrhizae in wet habitats. Keizer and Arnolds (1994) concluded that *I. lacera* prefers young stands, although they collected it in *Quercus*-stands of various ages, including stands less than 20-yr-old and over 50-yr-old. Jansen (1991) reports *I. lacera* from both old and young stands of *Pseudotsuga menziesii*. Our data do not allow accurate inferences of the host range of *I. lacera* as our collections were obtained in mixed communities of conifers and *Salix* spp. at the glacier forefront and in mixed communities of conifers at Lyman Lake. Our data, however, suggest that *I. lacera* has a wider host range than suggested in Graf (1994). In his studies of snowbed communities Graf (1994) found *I. lacera* at sites with *Salix herbacea* as the main ectomycorrhizal host although he collected it under *S. retusa*. *S. herbacea* does not occur at Lyman Lake Basin. Kuyper (1986) suggests a wider host range that includes *Betula* spp., *Quercus* spp., *Castanea* spp., *Pinus* spp., *Picea* spp., and *Salix* spp. Indeed, *I. lacera* has been reported from Northern Europe in association with *Picea abies*, *Pinus sylvestris* (Ohenoja and Väre, 1993; Väre *et al.*, 1996) and *Betula* sp. (Lange and Skifte, 1967) and in North America with *Pseudotsuga menzisii* (Trappe and Strand, 1969). Despite the evidence for wider host range for *I. lacera*, there may be some varieties with a strong host preference for *Salix* spp.

Two ectomycorrhizal taxa (*Fuscoboletinus aeruginascens* and *Suillus cavipes*) were common to Lyman Glacier Forefront and Glacier View Ridge. Both are specific to *Larix* in the Pacific Northwestern USA (Arora, 1986; Smith and Thiers, 1971). They have also been reported in the transitional zones between the subalpine forest and alpine tundra in the European Alps in association with *Larix decidua* (Moser, 1982, as *Suillus aeruginascens* and

Boletinus cavipes). No *Larix* occurs at the Lyman Lake. *F. aeruginascens* and *S. cavipes* were found only once at Lyman Glacier Forefront. *S. cavipes* on Lyman Glacier Forefront was collected under an *Alnus sinuata* shrub near *Larix lyallii*. The single collection of a large *Cortinarius mutabilis* from the glacier forefront was obtained under the same shrub at the same time. That shrub appears to be the only representative of *Alnus* at the glacier forefront. Jumpponen *et al.* (1998a) found nitrogen to be low in the glacial soil. Nitrogen limitation likely affects mycorrhizal formation and fungal fruiting.

Five of the seven taxa common to the Lyman Lake and the Glacier View Ridge sites but absent at the forefront site were hypogeous: *Elaphomyces granulatus, Hydnotrya variiformis, Rhizopogon subsalmoneus, R. vulgaris,* and *Thaxterogaster pingue*. Hypogeous fungi depend on animal vectors for their spore dispersal (Trappe and Maser, 1977; Cázares and Trappe, 1994). Several species of potentially mycophagous mammals frequently visit the primary successional site as indicated by tracks, sightings or fecal deposits of mule deer (*Odocoileus hemionus*), mountain goats (*Oreamnos americanus*), hoary marmots (*Marmota caligata*), and pikas (*Ochotona princeps*). Cázares and Trappe (1994) studied samples of mammal feces from the Lyman Glacier Forefront site and identified spores of *Elaphomyces* sp., *Rhizopogon* sp., and *Thaxterogaster pingue*. Hence, the propagules are distributed even to the primary successional site. We hypothesize that the main reason for the absence of these five species on the forefront is the lack of legacies of mature forests, especially soil development (organic matter content, well developed litter and humus layers, etc.). Amaranthus *et al.* (1994) showed that most hypogeous fungi in their study were found in mature Douglas fir stands but not in adjacent young reforested stands. More than a third of those were present only in or under coarse woody debris at various stages of decay. This indicates the importance of mature stand structure and those legacies associated with such structure in modifying the fungal communities. Some species require the decayed wood habitat (e.g., *Hydnotrya variiformis* and *Inocybe lanuginosa*; see Arora, 1986).

The additional two species common to the secondary successional sites but absent at the primary successional site, *Boletus edulis* and *Russula emetica*, may similarly depend on these legacies present mature forest stands and soils. *Boletus edulis* has been proposed to be associated with older rather than younger forest stands (Hintikka, 1988; Keizer and Arnolds, 1994). *B. edulis* is a common cosmopolitan through Europe and North America in deciduous and coniferous forests including subalpine and arctic tundra (Arora, 1986; Hansen and Knudsen, 1992; Heikkilä, 1982; Miller, 1987; Smith and Thiers, 1971; Thiers, 1975). Horak (1960) reported *R. emetica* associated with *Salix herbacea* and *S. retusa* at glacier forefront sites in the European Alps indicating that mature forest legacies are not necessary for this species. Keizer and Arnolds (1994) concluded, similarly, that *R. emetica* is likely to have no preference for the successional stage of the forest stands or age of the hosts. *R. emetica* occurs commonly in both deciduous and coniferous forests in northern Europe (Hansen and Knudsen, 1992; Ohenoja and Väre, 1993) and has also been reported from arctic and alpine tundra in North America in association with *Salix arctica* and *S. rotundifolia* (Miller *et al.*, 1973).

Ectomycorrhizal Species Unique to the Lyman Glacier Forefront Site

The fewest ectomycorrhizal species were encountered at the primary successional site at the glacier forefront (13 in total). Disturbance generally decreases numbers of species and individuals of ectomycorrhizal fungi (Zak, 1992). Primary successional ecosystems are extreme examples of disturbance where the biotic component is initially absent (Glenn-Lewin *et al.*, 1992; Matthews, 1992). Plant and fungal propagule banks, organic matter, and organically bound nutrients are set at zero. The harsh environment selects for ruderal species able to colonize newly exposed niches. In general, most of the taxa observed on the glacier forefront produced small fruit bodies, with the exception of those specimens obtained in a single fruiting event under the

Alnus sinuata shrub, suggesting small investment on the reproductive structures and ruderal nature of these organisms. Most species unique to this site are common cosmopolitans that occupy a variety of habitats and have often been reported in association with *Salix* spp.

Of the 13 hypogeous taxa encountered in our survey, only one, *Hymenogaster glacialis,* was on the primary successional site. Indeed, this species is known only from the two collections at Lyman Glacier forefront in association with the willows (species described by Cázares and Trappe, 1990). A closely related species *H. saliciphilus* was recently described in association with *Salix herbacea* in a snow-bed habitat in the Swiss Alps (Graf and Horak, 1993). To our knowledge this species has not been recorded beyond its type locality either. Are these two rarely-encountered species results of local adaptation from their potentially epigeous, Cortinariaceous ancestor to harsh, true alpine habitats, as hypothesized in Graf and Horak (1993)? Or, do they have means to migrate between alpine habitats that can be far away? Given the dependency on the limited dispersal by the mycophagous fauna the former seems more likely. Some hypogeous species occur commonly at elevations near timberline (Trappe, 1988): *Elaphomyces granulatus, Hydnotrya variiformis, Rhizopogon subsalmoneous* and *R. vulgaris*. These species occurred in both of our secondary successional subalpine sites but never in the primary successional site. They occur at lower elevations as well and are thus able to migrate to the high-elevation habitats despite their dependence on the animal dispersal.

Five *Cortinarius* spp. were restricted to the forefront: *C. tenebricus, C. decipiens, C. mutabilis* and two unidentified species. *C. tenebricus* is the third most frequently recorded taxon at the glacier forefront after *Inocybe lacera* and *Laccaria montana.* It has been observed in the alpine areas in both Europe and North America. Moser and McKnight (1987) reported it in association with *Salix* sp. from Yellowstone National Park and Graf (1994) with *Salix herbacea* from the Swiss Alps. Our specimens, like theirs, were collected among willows (*Salix commutata* and *S. phylicifolia*) in areas deglaciated

approximately 60 to 100 yr ago. *C. decipiens* is associated with both deciduous and coniferous trees (Moser, 1983) or *Salix* thickets (Hansen and Knudsen, 1992) in Europe. Ohenoja and Väre (1993) reported it in pure stand of *Picea abies* as well as in mixed stands of conifers and birch in Northern Finland. *C. decipiens* is also common in coniferous stands in North America but may also occur in association with deciduous trees (Arora, 1986). We collected *C. decipiens* under *Salix commutata*.

Laccaria montana, the only recorded *Laccaria* sp. on Lyman Glacier Forefront, fruits prolifically; almost annually. It occurs among the first established *Salix commutata* and *S. phylicifolia* individuals on substrate vacated by the glacier approximately 30 yr ago. *L. montana* appears to be the first ectomycorrhizal colonizer at the primary successional site. It persists through the whole forefront area, including the almost 100-yr-old region by the terminal moraine. We have never recorded *L. montana* in the secondary successional sites. It fruits prolifically in the Swiss Alps and is the most frequently encountered species in *Salix herbacea*-dominated communities (Graf, 1994). Senn-Irlet (1993) found that *L. montana* was among the most frequent and voluminous fungal species fruiting in her *Salix* spp. dominated alpine mires. The data so far indicate that *L. montana* may colonize species of Betulaceae, Pinaceae, and Salicaceae (Mueller, 1992). We collected it mostly among *Salix* spp. although sometimes under *Abies lasiocarpa*. Graf (1994) pointed out that *L. montana* has only been recorded from the Alps in Europe. Alfredsen (1997), however, reported it also from alpine Norway. It may be primarily restricted to alpine environments, although Mueller (1992) describes its distribution as arctic, boreal and montane in North America.

Three species of Russulaceae, *Lactarius uvidus*, an unidentified *Lactarius*, and *Russula fragilis*, occurred among willows on the glacier forefront. Horak (1960) reported *L. uvidus* from a glacier forefront in eastern Alps. It has also been reported from arctic and alpine sites in association with *Salix* spp. (Petersen, 1977; Ohenoja, 1971; Gulden *et al.*, 1985) as well as with *Picea abies* and *Betula* spp. (Ohenoja and Väre, 1993). Only a single collection

of *R. fragilis* was collected at the Lyman Glacier Forefront site in association with willows. *R. fragilis* has been previosly reported in association with various deciduous and coniferous hosts, most commonly with *Quercus* spp. or *Betula* spp. (Hansen and Knudsen, 1992). Arora (1986), however, describes *R. fragilis* to be common in variety of habitats, but especially under conifers.

Ectomycorrhizal Species Unique to the Glacier View Ridge Site

The Glacier View Ridge site is a close secondary successional counterpart for Lyman Glacier Forefront. The two are separated by less than 100 m, but they are demarcated by the outermost terminal moraine. Glacier View Ridge has well developed litter and humus layers that are absent at Lyman Glacier Forefront. Despite the immediate proximity and the similarity in ectomycorrhizal host species of these two sites (Table 4.1), they shared only two ectomycorrhizal fungal species.

A single *Amanita* collection was obtained at Glacier View Ridge in an *Abies lasiocarpa* stand. We were not able to identify this collection. The specimen resembles closely *A. vaginata* (Bull.:Fr.) Vittadini but differs by having remnant patches of partial veil attached to the stipe. As a result, we felt reluctant to call it *A. vaginata*. Knudsen and Borgen (1987) point out that several white *Amanitas* in section *Vaginatae* may be albino forms of other species and further studies are required to sort out the taxonomy.

Two of the five *Boletus* spp., *B. edulis* and *B. piperatus*, were recorded at Glacier View Ridge. *B. piperatus*, unique to this site, was under *Larix lyallii* with a dominant ground cover of *Luetkia pectinata*. *B. piperatus* has been suggested to prefer older rather than younger stands (Keizer and Arnolds, 1994; as *Chalciporus piperatus*). Its presence only in the secondary successional habitat just across the terminal moraine of Lyman Glacier supports this. *B. piperatus* has been reported from arctic and alpine regions in

Europe (Heikkilä, 1982; Moser, 1982; Ohenoja and Väre, 1993), even above the tree line in alpine Norway (Gulden and Lange, 1971).

The Cortinariaceae was the most species-rich family with 10 of the total of 29 species collected at the Glacier View Ridge site. Six of the total of seventeen *Cortinarius* spp. (*C. croceus, C. pellstoniana, C. semisanguineus,* and three unidentified species) and one of the two *Hebeloma* spp. were unique to Glacier View Ridge. *C. semisanguineus* has been reported in dry *Pinus sylvestris* forests in Northern Finland (Ohenoja and Väre, 1993; Väre *et al.* 1996). Our collection was found under a *Pinus albicaulis* with dominant understory of *Phyllodoce empetriformis.*

Two of the three Inocybe spp., *I. lanuginosa* and an unidentified specimen, were unique to Glacier View Ridge. *I. lanuginosa* was collected on well-decayed wood under *Abies lasiocarpa.* It is widespread in the western United States and frequently occurs on "very rotten" wood (Arora, 1986). Hansen and Knudsen (1992) describe the habitat similarly: "very rotten stumps of deciduous trees". Ohenoja and Väre (1993) reported *I. lanuginosa* from Northern Finland in association with *Betula* sp, but it more commonly associates with conifers (Moser, 1983).

Two of the four species of *Hygrophorus, H. parvulus* and *H. purpurascens,* were unique to Glacier View Ridge. *H. parvulus* occurred in the krumholz community near shrubby *Abies lasiocarpa* at an elevation of over 2000 m. *H. parvulus* occurs in deciduous and mixed woods in North America (Hesler and Smith, 1963) and in Europe (Hansen and Knudsen, 1992). We are unaware of any data of its presence at alpine or arctic regions. The single collection of *H. purpurascens* was obtained in a stand of *Larix lyallii* and *Abies lasiocarpa* just outside the terminal moraine of Lyman Glacier. It is common in the Cascade Mountains and fruits prolifically under conifers (Arora, 1986).

One of the four *Laccaria* spp., *L. nobilis,* was collected only at Glacier View Ridge. A closely related species, *L. bicolor,* was collected at Lyman Lake, but not at Glacier View Ridge (see below). *L. nobilis* is known only from

North America where it is found in association with members of Pinaceae (Mueller, 1992).

Two of the three *Lactarius* spp., *L. deliciosus* and *L. pseudomucidus*, were only recorded at Glacier View Ridge. *L. deliciosus* was recorded only once in association with a few individuals of *Pinus alicaulis*. *L. deliciosus* is an abundant cosmopolitan and has been been reported from the European subarctic in, for example, northern Finland (Ulvinen, 1963). *L. pseudomucidus* appears to be endemic to California and Pacific Northwest where it commonly occurs with a variety of conifers (Arora, 1986). Hesler and Smith (1979) report it from Alaska as well.

Two hypogeous species, *Gautieria monticola* and *Rhizopogon subcaerulescens*, were unique to Glacier View Ridge. *G. monticola*, among the most common hypogeous species in Pacific Northwest, was collected under *Abies lasiocarpa*. We have also collected it at lower elevation close to Hart Lake below Lyman Basin. It is likely frequent in this area but has probably escaped detection at Lyman Lake. A single collection of *R. subcaerulescens* was obtained under *Abies lasiocarpa* where the sporocarps lay partially exposed. *R. subcaerulescens* is widely distributed and occurs commonly throughout Pacific Northwest (Arora, 1986; Smith and Zeller, 1966). All of the several varieties of *R. subcaerulescens* are found in elevations above 1200 m in association with conifers including *Abies amabilis, A. lasiocarpa, Pinus albicaulis, P. contorta,* and *Tsuga mertensiana* (Hosford, 1972), all of which are present in the Lyman Lake Basin.

Glacier View Ridge harbored substantially lower numbers of ectomycorrhizal fungi than the Lyman Lake site but twice as many as Lyman Glacier Forefront. The obligate *Larix* associates, with the exception of *Fuscoboletinus ochraceoroseus*, were recorded at the glacier forefront indicating adaptation to a wide range of environmental conditions and efficient dispersal and reproduction. Most of the species unique to Glacier View Ridge have a wide host range (e.g. *Boletus piperatus, Inocybe* spp., and *Laccaria nobilis*) and have no obvious reason to be restricted to this site.

Glacier View Ridge and Lyman Lake, however, have some obvious differences: Glacier View Ridge is an open parkland community with only few shaded, moist habitats, and less woody debris on the ground than Lyman Lake. Additionally, it seems that the burn in the turn of the century was more intense in the drier ridge site leaving a fairly homogenous habitat as opposed to a variety of habitats at Lyman Lake that maintained their characteristics after the fire. Some of the species probably have escaped detection at Lyman Lake. It has been suggested that at least five years of observation are needed to approach full inventory of the ectomycorrhizal fungi of a given site, as many fruit infrequently (O'Dell *et al.*, 1996). Visiting the sites only once or twice a year (see Table 4.2) is inadequate to cover the fungal flora completely even if done over many years. Despite the 8-year duration of our studies many species were recorded only once.

Ectomycorrhizal Species Unique to Lyman Lake Site

The largest number of ectomycorrhizal species was collected at the Lyman Lake site, 39 in all. Over 50% of recorded species were present at this site, most of which were unique to this site. Several factors may account for the larger ectomycorrhizal fungus diversity at the Lyman Lake site. Closed canopy structure and the old-growth legacy missed by the fires at the turn of the century provide a wide variety of habitats. Additionally, its uneven host ages and sizes likely increase the diversity of mycorrhizal fungi. The Lyman Lake site is at a slightly lower elevation in a more protected setting than the other two sites and thus has a less harsh environment.

Three out of four species of *Amanita, A. muscaria, A. pantherina,* and *A. vaginata,* were found only at Lyman Lake. All these three species are common in both deciduous and coniferous stands and widely distributed (Arora, 1986; Hansen and Knudsen, 1992; Jenkins, 1986). *A. vaginata* has been reported from alpine regions in Europe (Bas, 1982) and Greenland (Kobayasi,

et al., 1971; Petersen, 1977). It can occur both in association with coniferous and deciduous trees (Arora, 1986; Hansen and Knudsen, 1992), including *Salix* spp. (Kobayasi, *et al.*, 1971; Petersen, 1977). The absence of mature trees or stands possibly explains the absence of *Amanita* sp. on the Lyman Glacier Forefront. Fleming *et al.* (1984) postulated that *A. muscaria* typically colonizes only older trees. Similarly, Keizer and Arnolds (1994) concluded that *A. muscaria* preferred "medium aged" rather than young stands. Fleming *et al.* (1986) point out that even when no mature trees are present *A. muscaria* can develop resident ectomycorrhizae and mycelia but only rarely produces sporocarps. Moser (1982), for example, observed *A. muscaria* at the timberline zone, but not in the krumholz (kampfzone) indicating presence in the high-elevation habitats. We collected all of the reported *Amanita* spp. only once at the secondary successional sites. The fruiting may be infrequent and resident mycelium may be present even if no fruiting is observed.

Four of the five species of *Boletus* were present in the Lyman Lake site. Three of these, *B. calopus, B. rubripes,* and *B. smithii,* were found only at this site. We collected the bitter-tasting *B. calopus* under *Abies lasiocarpa* and *Tsuga mertensiana*. *B. calopus* is common at higher elevation in the Western United States (Arora, 1986; Smith and Thiers, 1971; Thiers, 1975). *B. rubripes* was obtained at a camp site at Lyman Lake in association with *Abies amabilis* and *Tsuga mertensiana*. It is thought to be endemic to western North America and Mexico (Arora, 1986; Thiers, 1975). *B. smithii,* similarly, has been collected only in western United States, where it can be quite common in association with conifers (Arora, 1986). We collected *B. smithii* in a mixed stand of *Tsuga mertensiana* and *Abies* spp.

Eight species of Cortinariaceae were collected only at Lyman Lake including six *Cortinarius* spp. (*C. cinnamomeus, C. duracinus, C. idahoensis, C. montanus, C. violaceus,* and an unidentified species), *Hebeloma strophosum,* and *Rozites caperata*. The genus *Cortinarius* frequently counts for most fungal taxa in surveys like ours (*e.g.* Väre *et al.*, 1996). We collected *C. montanus* under *Abies lasiocarpa*. According to Arora (1986) it is often

common under old-growth conifers in Cascade Mountains. Our collection of *C. violaceus* was obtained in a stand mixed with *Abies* spp. and *Tsuga mertensiana*. *H. strophosum* can be found in coniferous woods in Europe (Moser, 1983) and is also widely distributed in North America (Arora, 1986). Our collection was obtained under *Abies* spp. and *Tsuga mertensiana*. We collected *R. caperata* in association with *Abies lasiocarpa* and *Salix* sp. at the Lyman Lake site. Hintikka (1988) recorded *R. caperata* in older rather than younger stands. Taken together these findings suggest that *R. caperata* may be restricted to more mature forest stands. *R. caperata* has been encountered with woody shrubs in alpine (Hansen and Knudsen, 1992) and arctic habitats (Lange, 1949).

Three species of Hygrophoraceae, *Hygrocybe miniata, Hygrophorus pudorinus,* and *H. subalpinus,* were found only at Lyman Lake. *H. miniata* was collected in a moist shaded by the outlet of Lyman Lake. *H. miniata* is most frequent on rotting logs or moss in mixed woods (Arora, 1986). It is common and widely distributed in North America (Arora, 1986). It is unclear whether *H. miniata* is mycorrhizal; Väre *et al.* (1996), for example, treat it as a bryophilic taxon. Our collection of *Hygrophorus pudorinus* was under *Tsuga mertensiana* and *Abies* spp. We are not aware of any other *H. pudorinus* reports from alpine habitats. *H. subalpinus* is endemic to the mountainous western North America where it associates with various conifers (Arora, 1986). We collected it under *Abies lasiocarpa.*

Two of the four *Laccaria* spp., *L. bicolor* and *L. laccata,* were found only at the Lyman Lake site. *L. bicolor* is among the most common *Laccaria* spp. of coniferous forests in North America (Mueller, 1992). It is frequent in northern Europe (*e.g.* Väre *et al.*, 1996; Ohenoja and Väre, 1993) occurring in lichen heaths in alpine areas (Hansen and Knudsen, 1992) and *Salix herbacea* dominated montane heaths in Scotland (Watling, 1987). Keizer and Arnolds (1994) concluded that *L. bicolor* prefers younger stands. We collected it at a heavily used camp site with compacted soil under mature *Abies amabilis,* suggesting that it occurs in disturbed sites rather than in association with

young host plants. Jansen's (1991) data on *L. bicolor* in *Pseudotsuga menziesii* stands show no obvious preference for either young or older stands. *L. laccata* was collected at the same camp site as *L. bicolor* but also in nearby conifer stands. *L. laccata* is common and widely distributed throughout North America and "can be found almost anywhere anytime" (Arora, 1986). Horak (1960) and Alfredsen (1997) reported *L. laccata* on a glacier forefront sites in Europe. Hintikka (1988) observed *L. laccata* in association with younger *Pinus sylvestris* stands. These reports suggest that *L. laccata* occupies a variety of habitats independent of substrate or host age. It may also occupy primary successional sites given the right conditions.

Two additional species of Tricholomataceae, *Catathelasma ventricosum* and *Tricholoma zelleri* (likely to be conspecific with European *T. focale*), were unique to the Lyman Lake site. *C. ventricosum* is usually associated with conifers (Arora, 1986). We encountered a single, gregarious fruiting under *Abies* spp. with 15 large basidiocarps in an area of about 5 x 10 m. *T. zelleri*, similarly, was collected only once in our area. *T. zelleri* is "extremely abundant under conifers in Pacific Northwest" (Arora, 1986). Our collection was associated with *Abies lasiocarpa*.

The two species of *Gastroboletus*, *G. ruber* and *G. turbinatus*, were only found at Lyman Lake. *G. ruber* fruits only with *Tsuga mertensiana* and seems to occur exclusively in the Cascade Mountains of Wasington and Northern Oregon (Cázares and Trappe, 1991b). We have collected *G. ruber* at several occasions at a single location in compacted soil on a campground, where it is often partially erumpant and once on the riparian area below the Lyman Lake outlet. *G. turbinatus*, on the other hand, has been encountered only once, in association with *Tsuga mertensiana* and *Abies* spp. It is widely distributed throughout the temperate conifer forests of North America (Thiers, 1975) and common in Pacific Northwest under conifers and occasionally oaks (Arora, 1986).

One of the two *Hydnotrya* spp., *H. cerebriformis*, was collected only at the Lyman Lake site. This collection was obtained on a compacted, bare

campground surrounded by a stand of *Abies amabilis* and *A. lasiocarpa*. No detectable decayed woody debris present. *H. cerebriformis*, similarly to *H. variiformis* (see above), is more usually associated with woody debris in coniferous forests (Arora, 1986).

The only hypogeous Russulales, *Macowanites lymanensis* and *Martellia* cf. *parksii*, were collected only at Lyman Lake. *M. lymanensis* is known only from two collections from this type locality (Cázares and Trappe, 1991a). This species, like several other hypogeous species, was collected on the compacted campground surrounded by *Abies amabilis* and *A. lasiocarpa*. The only *Martellia* collection was obtained at the same campground. The only epigeous *Russula* from Lyman Lake remains unidentified.

One of the two *Elaphomyces* spp., *E. muricatus*, was found only at the Lyman Lake site. We have collected it at several occasions and different locations at this site in association with *Abies* spp., *Picea engelmannii*, and *Tsuga mertensiana*. *E. granulatus* is widely distributed in the northern hemisphere and is associated with a great diversity of hosts.

The only collection of Hydnaceae, *Sarcodon subfelleus*, was unique to this site in a stand of mixed conifers. Little has been published or known about the ecology of *S. subfelleus*. Agerer (1991) considers *Sarcodon imbricatus* (L.:Fr.) Karst. to be mycorrhizal. We believe *S. subfelleus* and several other members of the genus similarly mycorrhizal.

The greatest number of species, especially unique species, at Lyman Lake was most likely facilitated by its largest variety of microhabitats. These are mainly created by the multilayered canopy structure and uneven age and size distribution of ectomycorrhizal hosts. Most of the fungal species we encountered are likely to be adapted to maintaining their niche in relatively stable environments, but disturbance is a frequent event even at this site. The small-scale disturbances at campgrounds and on trail sides open niches to species with ruderal life strategies.

CONCLUSIONS

Most of the species (58 out of the total of 68) collected during our survey were unique to one site. Some of this is due to the different host species composition in the three sites, e.g., the *Larix*-associated taxa. Moreover, the fungal communities clearly differ between the secondary and primary successional sites despite similar host plant composition and close physical proximity. Organic matter, decaying woody debris in particular, is essential to developing the characteristics of a mature stand in Pacific Northwestern United States. Lack of such characteristics is among the more likely explanations for the differences observed between the primary and secondary successional systems. Species such as *Hydnotrya cerebriformis, H. variiformis, Hygrocybe miniata* and *Inocybe lanuginosa* reported to fruit in microhabitats rich in such debris, were present only at the secondary successional sites. The role of the ecological and geological history vs. the age distribution of the hosts still remains unclear.

The ectomycorrhizal fungal communities also differ substantially between the two secondary successional sites. Glacier View Ridge has less coarse woody debris as it probably burned more intensely at the turn of the century than did Lyman Lake area. This may result in lower frequency of fungal species associated with decaying wood. Additionally, large scale disturbances, such as intensive fire, homogenize otherwise patchy distribution of different microhabitats (see Zak, 1992 and references therein). This may have additionally limited the species diversity observed at the Glacier View Ridge. Alternatively, shade and complete canopy cover may be essential to the fruiting of some species. The alpine parkland habitat that dominates Glacier View Ridge as well as the open primary successional habitat rarely provide such conditions.

The 'early'- and 'late-stage' model introduces the succession of ectomycorrhizal fungi in terms of age of the plant community or age of the

dominant overstory vegetation. Furthermore, the model is interpreted to propose that the community structure of fruiting mycorrhizal species is regulated by changes in host physiology, photosynthate allocation in particular, as a result of aging (Danielson and Visser, 1989). Some of the species we recorded only at the secondary successional sites are considered late successional (e.g. *Amanita muscaria, Cortinarius semisanguineus, Hygrophorus purpurascens,* and *Rozites caperata;* Fleming *et al.,* 1984; Hintikka, 1988; Jansen, 1991; Visser, 1995). It remains unclear whether the presence of these species at secondary successional sites in our study is a result of changes in host physiology due to aging or differences in other characteristics between primary and secondary successional sites.

The 'early-' and 'late-stage' model further states that a narrow selection of ectomycorrhizal fungi with a wide host range colonize the root systems in young trees and is joined and replaced by other species (see Last *et al.,* 1987). Similarly, the species richness and diversity increase at least until canopy closure (Dighton *et al.,* 1986; Last *et al.,* 1987). The few ectomycorrhizal fungi dominating our primary successional site (3 species collected frequently, 13 in total) were mostly absent at the older, secondary successional sites. These data, however, merely indicate that differences exist. It is unclear to what degree the differences can be attributed to the different life history strategies of the ectomycorrhizal fungi, age of the ectomycorrhizal hosts, successional stage of the habitat, or greater diversity of habitats available at the secondary successional sites.

There is a vast body of evidence leaving no doubt concerning the presence of different life strategies of ectomycorrhizal fungi (see references in Frankland, 1992; Last *et al.,* 1987; Visser, 1995). Some patterns revealed by our data support adaptation of some species of ectomycorrhizal fungi to early successional habitats. *Cortinarius tenebricus* and *Laccaria montana,* both almost annually observed at the glacier forefront, were absent at both adjacent secondary successional sites. Several species were observed only at the disturbed areas within the secondary successional sites, *e.g.,* campsites and

trails, which may allow establisment of some potentially ruderal species. Some species at the secondary successional sites were only collected within these disturbed habitats (*e.g., Boletus rubripes, Laccaria bicolor, Gastroboletus ruber, Hydnotrya cerebriformis,* and *Macowanites lymanensis*). These species may be ruderal or merely able to tolerate frequent disturbances and persist for long periods. Nonetheless, their absence at the primary successional site suggests they may depend on legacies of late successional communities, such as abundance of soil organic matter. Other disturbance-adapted species, on the other hand, may depend on efficient dispersal, and colonization of vacated niches, but not on the legacies associated with mature plant commuties. *Inocybe lacera,* for example, was collected both at a campsite in the secondary successional site and at the glacier forefront.

Studies addressing the different life strategies of ectomycorrhizal fungi are rare and based on the sparse evidence of unpredictable fruiting. Our conclusions about the life history strategies of some species disagreed with those published elsewhere (e.g., *Laccaria bicolor, L. laccata,* and *Russula fragilis;* see discussion above). Keizer and Arnolds (1994) point out that such disagreements in interpretation of fungal life history strategies may result from differencies in habitats. They found that their conclusions about the relation of fungal species to the age of oak stands differed substantially from those based on Hintikka's (1988) observations in pine stands. Our subalpine habitats dominated by *Abies* spp., and *Tsuga mertensiana* likely lead to yet different interpretations about the life histories of the ectomycorrhizal fungi and their relation to succession of their primary hosts.

Relevance of the 'early'- and 'late-stage' model is questionable in primary successional sites as it was derived from secondary successional systems. The glacier forefront is a relatively homogeneous open habitat lacking the moist microsites rich in organic matter and coarse woody debris which are abundant at Lyman Lake. Keizer and Arnolds (1994) concluded that the 'early-' and 'late-stage' model fails to acknowledge changes in the physical and chemical characteristics of soil as well as stand architecture.

They propose an alternative model based on dividing stand rotation into six phases that relate to both canopy closure and stand age. However, their alternative model does not address other successional changes (*e.g.*, in species composition) taking place in natural ecosystems. As Keizer and Arnolds (1994) concluded in their study, the succession of ectomycorrhizal fungi appears much more complicated than the relatively simple model of 'early-' and 'late-stage' fungi. The different factors, including host species composition, host age and stand characteristics, are all likely contributing to the ectomycorrhizal fungal flora present in a stand at a given time. The separate effects of the factors are difficult to isolate. More data are needed to gain better understanding if the successional changes are visible in below-ground systems, as the interpretation of the below-ground mycoflora from fruiting is inaccurate at best (see e.g., Gardes and Bruns, 1996).

Fungal succession in a natural setting requires further study, preferably with true replication. Appropriate sites for conducting such studies are rare. As a result, lack of true replication is a common problem of successional studies in natural environments. Glacier forelands and their immediate surrounding communities may prove invaluable as field laboratories for studying these successional phenomena and searching for generalities in dynamics of the fungal communities on landscape and ecosystem level.

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CHAPTER 5

DARK-SEPTATE ENDOPHYTES: A REVIEW WITH SPECIAL REFERENCE TO FACULTATIVE BIOTROPHIC SYMBIOSIS

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ABSTRACT

Dark-septate root endophytes (DSE) have been reported for about 600 plant species representing about 330 genera and 100 families. DSE fungi occur from the tropics to arctic and alpine habitats and comprise a heterogeneous group that overlaps with *Mycelium radicis atrovirens* (MRA). Numerous species of undescribed sterile and anamorphic taxa may also await discovery. DSE are likely to overlap functionally and ecologically with soil fungi, saprotrophic rhizoplane-inhabiting fungi, obligately and facultatively pathogenic fungi, and mycorrhizal fungi. Although DSE are abundant in washed root and soil samples from various habitats, and are easily isolated from roots of ecto-, ectendo-, endo-, and nonmycorrhizal host species, their ecological functions are little understood. Studies of DSE thus far have yielded inconsistent results and only poorly illustrate the role of DSE in their natural habitats. These inconsistencies are largely due to the uncertain taxonomic affinities of the strains of DSE used. In addition, because different strains of a single anamorph taxon seem to vary greatly in function, no clear generalizations on their ecological role have been drawn. This paper reviews the current literature on DSE and their ecology and discusses the need for and direction of future research.

INTRODUCTION

The concept of mycorrhizae, the fungus-root (Greek: myco = fungus, rhizae = root), was introduced by A. B Frank (1885). Symbiosis was initially described by A. de Bary (1887) as the long-term coexistence and interaction of two or more dissimilar organisms (Greek: syn-/sym- = with, together; bios = life). Fungi have several kinds of symbiotic interactions with plant roots, ranging from antagonism and mutualism to frequently observed but still little understood functions. Fungal colonizations that do not fit the identified

categories of mutualistic or pathogenic symbiosis have been referred to in a variety of ways: "casual mycorrhizal" (Burges, 1936), "endophytic" (Currah *et al.*, 1987; Stoyke and Currah, 1991; Stoyke *et al.*, 1992; Väre *et al.*, 1992), "pseudomycorrhizal" (Melin, 1923; Thomas, 1943; Robertson, 1954; Kowalski, 1970; 1973; Wang and Wilcox, 1985; Wilcox and Wang, 1987*a*), "weakly pathogenic" (Wang and Wilcox 1985; Egger and Paden, 1986*a*; Wilcox and Wang, 1987*a*), "dark-septate" (Haselwandter and Read, 1982; Haselwandter, 1987; Cázares, 1992; Väre *et al.*, 1992), "*Rhizoctonia*-like" (Peyronel, 1924; Haselwandter and Read, 1980), or "septate endophytes" (O'Dell *et al.*, 1993). Some researchers (Lewis, 1973; Smith and Smith, 1990) have approached the problem from a more functional point of view by characterizing root-fungus interactions on the basis of whether flow of resources (nutrients, carbohydrates, *etc.*) is uni- or bidirectional.

However the association between a plant individual and a fungus colonizing its roots may be described, the host-fungus interaction may not always be accurately understood. This is predictable when fungi of unknown identity produce structures of unknown function when colonizing host roots. Nevertheless, it is essential to acknowledge the potentially important ecological role of these associations, however poorly known and understood they may currently be.

The objective of this paper is to review the literature of miscellaneous, root-associated, dark-septate endophytic (DSE) fungi. Despite several studies addressing various aspects of DSE and their relationships with colonized host plants, very little is known of their taxonomic affinity, host range, and ecology. In this context, we will refer to endophytic fungi in a broad sense: they colonize living plant tissue without causing any apparent, overt negative effects (Hirsch and Braun, 1992). We include *Mycelium radicis atrovirens* (MRA; Melin, 1922; 1923) and similar types of root colonization, all hereafter to be termed DSE. We recognize that an unknown number of fungal taxa are involved, and that a considerable functional and ecological overlap may exist between soil fungi, saprotrophic rhizoplane-inhabiting

fungi, strictly pathogenic fungi, mycorrhizal fungi, and fungal endophytes. Because Richard and Fortin (1974) reviewed the literature on MRA, we will only briefly cover the earlier work and emphasize what has been published since.

EARLY OBSERVATIONS ON DSE

In the initiating work of Frank (1885), it was strictly referred to what were later termed 'ectomycorrhizae' of trees. It was Gallaud (1905) who initially reported another type of root colonization by septate endophytes on *Allium spaerocephalum* L. and *Ruscus aculeatus* L. Peyronel (1922) observed similar fungal structures on the roots of *Triticum aestivum* L. He subsequently noted other type of root colonization, later termed 'endomycorrhizae' (Peyronel 1922, 1923). While isolating ectomycorrhizal fungi and aseptically resynthesizing mycorrhizae, Melin (1922, 1923) isolated a brown, or blackish, 'pseudomycorrhizal' fungi. He called these sterile, root-associated fungi *Mycelium radicis atrovirens* and *Rhizoctonia sylvestris*. Neither formed ectomycorrhizae, *R. sylvestris* produced sclerotia on the root surfaces, whereas MRA produced only intracellular sclerotia. No taxonomic affinity for these 'pseudomycorrhizal' fungi was suggested at that time. MRA has since been applied to any sterile, dark, and septate fungi isolated from roots or soil. From Melin's descriptions of culture characteristics and root colonization, it is clear that the MRA group partially overlaps with what we now call DSE.

Peyronel also documented colonization by pigmented root endophytes on 135 species of angiosperms (Peyronel, 1924, Appendix 1). He recognized the close resemblance of his observations to roots colonized by *Rhizoctonia*. Accordingly, Peyronel referred to the observed structures as "*Rhizoctonia*-like", but he was convinced that more than one fungal taxon – not even necessarily *Rhizoctonia* spp. – was involved in these root colonizations

(Peyronel, 1924). He described the root colonization as having simple, branched hyphae that occasionally produced short, branched, clavate, barrel-shaped segments similar to chlamydospores of *Oidium* or *Monilia*. Within the cortical cells of the host root, these swollen hyphae occasionally aggregated into groups of thick-walled cells that he termed "nodulo stromatico" or stromatic nodules.

Melin (1925 and references therein) reported similar structures in several members of Pinaceae. He called his observations 'pseudomycorrhizas' (false mycorrhizae) to emphasize what he interpreted to be an atypical expression of mycorrhizal colonization. He had also concluded that the 'pseudomycorrhizas' expressed a parasitic rather than mutualistic pattern of behaviour (Melin, 1924). He insisted on the term 'pseudomycorrhiza' because this type of root colonization could be observed under conditions where ectomycorrhizae did not thrive or develop at all.

Melin may not have realized, however, that it is possible for pseudomycorrhizal fungi to coexist with mycorrhizal fungi. Endophytic and ectomycorrhizal fungi also can colonize roots concurrently (Hatch, 1934; Manka and Truszkowska, 1958; Trappe, 1962; Sengupta *et al.*, 1989; Dhillion, 1994), as can arbuscular and ectomycorrhizal fungi (Blaschke, 1991*b*; Cázares and Trappe, 1993; Dhillon, 1994). Recording the presence of the endophyte may be difficult when the roots are colonized and covered by mantle of ectomycorrhizal fungi. Melin (1922, 1923, 1925) reported that fungal colonization of host roots was characterized by persistent intracellular hyphae and that neither Hartig net nor mantle was present. Instead, single, thick-walled, dark brown hyphae radiated from the root surface into the soil. Detailed morphological studies of colonized roots have since revealed, however, that DSE occasionally develop a partial Hartig net and a thin fungal mantle only a few cell layers thick (O'Dell *et al.*, 1993; Fernando and Currah, 1996).

HOSTS AND GEOGRAPHICAL RANGES

Richard and Fortin (1974) pointed out that DSE fungi are widely distributed in coniferous boreal forests in many parts of the northern hemisphere. Most of the early studies referred to tree species and DSE. Similar morphologies of colonized roots had also been described elsewhere, *e.g.*, from Australian Liliaceae (Burges, 1936). DSE-like root colonizations have since been reported in a variety of habitats other than coniferous boreal forests. Colonization resembling DSE has been noted in approximately 600 plant species representing about 330 genera and 100 families (Appendix 1). These include species and genera usually considered arbuscular, ericoid, orchid, and ecto-, and nonmycorrhizal. There seems to be no particular rule governing the species DSE colonize. They have been observed in many plant families of quite different life strategies, suggesting biotrophic interaction with little or no host specificity. Appendix 1 includes observations from habitats ranging from South African coastal plains and lowlands (Allsopp and Stock, 1993) to tropical (*e.g.*, Thomazini, 1974; Sengupta *et al.*, 1989), temperate (Ahlich and Sieber, 1996), subalpine (Cazarés, 1992; Stoyke *et al.*, 1992; Jumpponen and Trappe, 1996), alpine (Bisset and Parkinson, 1979*a*, *b*, *c*; Haselwandter and Read, 1980; Read and Haselwandter, 1981; Allen *et al.*, 1987; Haselwandter, 1987; Blaschke, 1991*a*, *b*), maritime Antarctic (Christie and Nicolson, 1983), and arctic (Bisset and Parkinson 1979*c*; Bledsoe *et al.*, 1990; Kohn and Stasovski, 1990; Väre *et al.*, 1992; Ahlich and Sieber, 1996) zones.

Most authors cited above and in Appendix 1 observed root endophytes with darkly pigmented, septate hyphae that, in most cases, formed intracellular structures similar to those termed micro-sclerotia by Read and Haselwandter (1981). Various hyaline fungi have also been reported to have colonized the root systems (see Kohn and Stasovski, 1990; Stoyke *et al.*, 1992; Ahlich and Sieber, 1996). The sterile DSE-like mycelium seems to be frequent in soil as well as root systems, however. DSE-like mycelium dominated plate counts in studies of cultured fungi from washed soil or root samples from the

subantarctic (Heal *et al.*, 1967), boreal coniferous forests in Canada (Summerbell, 1988, 1989), temperate and boreal forests in Northern and Central Europe (Holdenrieder and Sieber, 1992; Ahlich and Sieber, 1996), and exotic pine plantations in New Zealand (Chu-Chou, 1979; Chu-Chou and Grace, 1982). Courtois (1990) reported *Phialophora dimorphospora* and another unidentified *Phialophora* sp. in addition to several sterile isolates from spruce-roots and 'rootfree' soil collected in the Black Forest region in Germany. It is currently unclear whether the isolates from soil indicate omnipresence of saprotrophic DSE or extramatrical mycelium of facultative biotrophic DSE extending into the soil from host roots.

Unfortunately, isolating and particularly identifying these imperfect fungi is laborious. Thus, few attempts to identify the root endophytes from field samples have been reported (Richard and Fortin, 1973, 1974; Wang and Wilcox, 1985; Currah *et al.*, 1987; Currah *et al.*, 1988; Currah and Sherburne, 1992; Currah and Tsuneda, 1993; O'Dell *et al.*, 1993; Ahlich and Sieber, 1996; Fernando and Currah, 1996). However, these and additional reports from inoculation bioassays indicated wide host ranges for some of the known anamorphic species (Table 5.1). None of the known fungal endophytes appear to express any host specificity. For example, *P. fortinii* can colonize at least 20 plant species in either natural or experimental conditions (Table 5.1).

The numerous reports of DSE colonization indicate a global distribution in a variety of ecosystems. In effect, DSE appear to be found wherever they are sought. Their abundance in different habitats and on different kinds of hosts is still largely unknown, however. It is important to bear in mind that these observations of colonized host roots have been incidental to other work. No systematic survey focusing on DSE has been conducted, despite their probable world-wide distribution and high frequency. The wide distribution of DSE and their profusion in soil and roots of host species that belong to a great variety of plant families suggest not only a

Table 5.1. Host species reported to be colonized by the five described anamorphic taxa of DSE. Reports include notations of DSE isolated from the root systems (natural) or inoculated and shown to colonize a host plant (aseptic/openpot cultures). References displayed in Appendix 1. Nomenclature follows Database of North American Plants (USDA-NRCS) (available at http://www.ars-grin.gov/npgs/tax/index.html) and new provisional Global Plant Checklist (available at http://bgbm3.bgbm.fu-berlin.de/IOPI/GPC/query.html) where applicable.

Species of endophyte	Host species	Location	Conditions	Reference
Chloridium paucisporum	*Betula alleghansis*	–	Aseptic	Wilcox & Wang, 1987*b*
	Picea rubens	–	Aseptic	Wilcox & Wang, 1987*b*
	Pinus resinosa	NY, U.S.A.	Natural	Wang & Wilcox, 1985
Leptodontidium orchidicola	*Abies balsamea*	Alberta, Canada	Natural	Fernando & Currah, 1996
	Achillea sp.	Alberta, Canada	Natural	Fernando & Currah, 1996
	Artemisia norvegica	Alberta, Canada	Natural	Fernando & Currah, 1995
	Betula pumila	–	Aseptic	Fernando & Currah, 1995
	Calypso bulbosa	Alberta, Canada	Natural	Currah *et al.*, 1987, 1988; Currah & Sherburne, 1992
	Carex sp.	Alberta, Canada	Natural	Fernando & Currah, 1995
	Castilleja sp.	Alberta, Canada	Natural	Fernando & Currah, 1996
	Coeloglossum viride	Alberta, Canada	Natural	Currah *et al.*, 1987; Currah & Sherburne, 1992; Fernando & Currah, 1995
	Corallorhiza maculata	Alberta, Canada	Natural	Currah *et al.*, 1987; Currah & Sherburne, 1992
	C. trifida	Alberta, Canada	Natural	Currah *et al.*, 1990; Fernando & Currah, 1995
	Dryas octopetala	-	Open pot culture	Fernando & Currah, 1996
	Erigeron sp.	Alberta, Canada	Natural	Fernando & Currah, 1996
	Heracleum lanatum	Alberta, Canada	Natural	Fernando & Currah, 1996

Table 5.1. (continued)

Species of endophyte	Host species	Location	Conditions	Reference
L. orchidicola	*Listera borealis*	Alberta, Canada	Natural	Currah *et al.*, 1990
	Pedicularis bracteosa	Alberta, Canada	Natural	Fernando & Currah, 1995
	Picea glauca	–	Aseptic	Fernando & Currah, 1995
	Piperia unalascensis	Alberta, Canada	Natural	Fernando & Currah, 1995
	Platanthera hyperborea	Alberta, Canada	Natural	Currah *et al.*, 1987; Currah & Sherburne, 1992; Fernando & Currah, 1995
	Potentilla fruticosa	–	Aseptic/Open pot culture	Fernando & Currah, 1995, 1996
	Rubus sp.	Alberta, Canada	Natural	Fernando & Currah, 1996
	Salix glauca	-	Aseptic/Open pot culture	Fernando & Currah, 1996
	Spiranthes lacera	Alberta, Canada	Natural	Fernando & Currah, 1995
	Trollius albiflorus	Alberta, Canada	Natural	Fernando & Currah, 1996
Phialocephala dimorphospora	*Picea* sp.	Schwarzwald, Germany	Natural	Courtois, 1990
	Picea mariana	?	Natural	Richard & Fortin, 1973, 1974
	Picea rubens	–	Aseptic	Wilcox & Wang, 1987*b*
	Pinus resinosa	–	Aseptic	Wang & Wilcox, 1985; Wilcox & Wang, 1987*b*
P. fortinii	*Abies alba*	Switzerland	Natural	Ahlich & Sieber, 1995
	Alnus rubra	Canada	Natural	Ahlich & Sieber, 1995
	Amerorchis rotundifolia	Alberta, Canada	Natural	Currah *et al.*, 1987
	Andromeda polifolia	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Calluna vulgaris	Switzerland	Natural	Ahlich & Sieber, 1995

Table 5.1. (continued)

Species of endophyte	Host species	Location	Conditions	Reference
P. fortinii	*Calypso bulbosa*	Alberta, Canada	Natural	Currah *et al.*, 1987; Currah *et al.*, 1988
	Cassiope mertensiana	Alberta, Canada	Natural	Currah & Tsuneda, 1993; Hambleton & Currah, 1997
	C. tetragona	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Chamaedaphne calyculata	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Deschampsia caespitosa	-	Open pot culture	A. Jumpponen, unpubl.
	Dryas octopetala	-	Open pot culture	Fernando & Currah, 1996
	Empetrum nigrum	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Fagus sylvatica	Switzer land	Natural	Ahlich & Sieber, 1995
	Gaultheria humifusa	Canada	Natural	Hambleton & Currah, 1997
	G. shallon	Canada	Natural	Ahlich & Sieber, 1995
	Kalmia microphylla	British Columbia, Canada	Natural	Currah & Tsuneda1993
	K. polifolia	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Loiseleuria procumbers	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Luetkea pectinata	Alberta, Canada	Natural	Currah & Tsuneda1993
	Lupinus latifolius	Washington, U.S.A.	Natural	O'Dell *et al.*, 1993
	Menziesia ferruginea	Alberta, Canada	Aseptic, Natural	Stoyke & Currah, 1993; Hambleton & Currah, 1997
	Picea abies	Czechoslovakia; Germany; Switzerland; Sweden	Natural	Cerny & Cudlin, 1989; Ahlich & Sieber, 1995; Dahlberg, *et al*, 1997

Table 5.1. (continued)

Species of endophyte	Host species	Location	Conditions	Reference
P. fortinii	*Pinus contorta*	–	Aseptic/Growth Pouch/Open pot culture	O'Dell *et al.*, 1993; Jumpponen *et al.*, 1998
	P. resinosa	–	Aseptic	Wilcox & Wang, 1987a
	P. sylvestris	Finland; Germany; Switzerland	Natural	Wang & Wilcox, 1985; Ahlich & Sieber, 1995
	Potentilla fruticosa	–	Open pot culture	Fernando & Currah, 1996
	Phyllodoce empetriformis	Alberta, Canada	Natural	Hambleton & Currah, 1997
	P. glanduliflora	Alberta, Canada	Natural	Hambleton & Currah, 1997
	Rhododendron albiflorum	Alberta, Canada	Natural	Hambleton & Currah, 1997
	R. brachy-carpum	-	Aseptic	Currah *et al.*, 1993
	R. obtusum	Tottori, Japan	Natural	Currah & Tsuneda, 1993
	Salix glauca	-	Aseptic/Open pot culture	Fernando & Currah, 1996
	Vaccinium membranaceum	Alberta, Canada	Natural	Hambleton & Currah, 1997
	V. myrtilloides	Alberta, Canada	Natural	Hambleton & Currah, 1997
	V. myrtillus	Switzerland	Natural	Ahlich & Sieber, 1995
	V. scoparium	Alberta, Canada	Natural	Hambleton & Currah, 1997
	V. uliginosum	Alberta, Canada	Natural	Hambleton & Currah, 1997
	V. vitis-idaea	Alberta, Canada	Natural	Hambleton & Currah, 1997
Phialophora finlandia	*Betula alleghansis*	–	Aseptic	Wilcox & Wang, 1987*a, b*
	Picea rubens	–	Aseptic	Wilcox & Wang, 1987*a, b*
	Pinus resinosa	–	Aseptic	Wilcox & Wang, 1987*a, b*
	P. sylvestris	Suonenjoki, Finland	Natural	Wang & Wilcox, 1985

globally ubiquitous presence and lack of host specificity, but also a role of importance in natural ecosystems. The function of DSE – when present in soil or colonizing host roots – is still poorly known and conclusions from the previous research are somewhat contradictory, as will be discussed below.

Since Melin's (1922, 1923, 1924, 1925) characterization of 'pseudomycorrhizal' colonization, studies on conifer roots have yielded additional reports of structures inferring 'pseudomycorrhizal' colonization (Rayner and Levisohn, 1941; Levisohn 1954; Laiho, 1965; Mikola, 1965; Kowalski, 1973). In most of those studies, unfortunately, the fungi colonizing the roots remained sterile and thus unidentified. Consequently, it has been difficult to discern their potential functions and ecological roles. Mikola (1965) addressed the problem of the unknown ecological role and the uncertain taxonomic affinity of root-colonizing – yet not necessarily mycorrhizal – fungi, by suggesting the use of "nonmycorrhizal roots" as a category that would include feeder rootlets of conifers either colonized solely intracellularly or completely free of any fungal colonization. This approach, however, leaves frequently observed fungal colonization of plant roots beyond recognition and discussion. Also, it deprives the unknown symbiotic (*sensu* de Bary, 1887) fungi, that do not form any "typical" mycorrhizal structures (see Harley and Smith, 1983), from appropriate terminology for describing their manifestations in natural ecosystems.

Asexual reproductive structures of fungi colonizing roots in this manner were ultimately found and described by Wang and Wilcox (1985), Currah *et al.* (1987) and Fernando and Currah (1996). Thus enabling identification of some of the root endophytic fungi as well as experimental inoculations for studies on the comparative morphology of roots colonized by known form taxa. The morphology of DSE-colonized roots had been reported to resemble ectomycorrhizal (Wilcox and Wang, 1987*b*; O'Dell *et al.*, 1993), ectendomycorrhizal (Wilcox and Ganmore-Neumann, 1974; Wang and Wilcox, 1985; Wilcox and Wang, 1987*a*), and pseudomycorrhizal colonization (Wang and Wilcox, 1985). In some cases the morphological structures of the

colonized root even have suggested a pathogenic relationship between the host plant and fungi (Wang and Wilcox, 1985; Wilcox and Wang, 1987*a*). The variety of root morphologies observed has made it difficult to sort out the fungal endophytes and their effects on hosts. Morphological structures of DSE in colonized host roots do not fall clearly into any previously described category of mycorrhizal, parasitic, or pathogenic associations. Thus, the terms were introduced to describe the patterns of this root-fungus interaction: 'dark septate' (Read and Haselwandter, 1981) or 'septate' endophytes (O'Dell *et al.*, 1993).

MORPHOLOGY OF ROOTS COLONIZED BY DSE

The pattern of root colonization by DSE is similar in roots of different plant species that are otherwise mainly considered as ericoid, orchid or ectomycorrhizal (see Väre *et al.*, 1992; Currah *et al.*, 1993; O'Dell *et al.*, 1993; Stoyke and Currah, 1993). Plants that do not form mycorrhizae also have similar fungal structures when colonized by DSE (Haselwandter and Read, 1982; Cázares, 1992; Väre *et al.*, 1992). Root colonization by *Phialocephala fortinii* best exemplifies DSE colonization and has been described in detail by several authors. Consequently, the general pattern of *P. fortinii* colonization is just briefly summarized here. Initial colonization is usually characterized by superficial hyphae (Currah and Van Dyk, 1986) that have also been called 'runner hyphae' (McKeen, 1952; Deacon, 1973). The individual hyphae usually grow along the depressions between adjacent epidermal cells (Currah *et al.*, 1993). "A loose hyphal network on the root surface" (Stoyke and Currah, 1993) or "loose wefts of hyphae" (O'Dell *et al.*, 1993) may develop during the superficial colonization. After colonizing the root surfaces, the hyphae penetrate into the outer cortical cells (Stoyke and Currah, 1991; O'Dell *et* al., 1993; Stoyke and Currah, 1993). The hyphal strands can also occupy the space between cortical cells along the main axis of the root. Penetration into

the root hairs has also been observed and may be a potential mode of entry into the root cortical layer (O'Dell *et al.*, 1993). Alternatively, the hyphae can accumulate in the cracks created by developing lateral roots and penetrate into the cortex via this route, (T.E. O'Dell, personal communication). Once into the epidermal layer, the hyphae can grow parallel to the main axis of the host root and from cell to cell within the epidermis (Currah *et al.*, 1993; O'Dell *et al.*, 1993). The hyphae pass through adjoining epidermal cell walls by narrow penetration tubes (Currah *et al.*, 1993), a pattern very different from that of colonization by arbuscular or ericoid mycorrhizae. The penetration tube occasionally arises from an appressorium-like structure formed prior to penetration through the cell walls (A. Jumpponen, unpublished).

During intracellular colonization endophytes occasionally form clusters of inflated, rounded, thick-walled cells within the cortical cells (O'Dell *et al.*, 1993). These frequently encountered structures have been referred to as 'thick pseudoparenchymatic mass (Melin, 1923; Robertson, 1954), 'sclerotia' (Melin, 1923; Hatch, 1934; Stoyke and Currah, 1991; O'Dell *et al.*, 1993; Stoyke and Currah, 1993; Fernando and Currah, 1995), 'microsclerotia' (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Haselwandter, 1987), or 'sclerotial bodies' (Levisohn, 1954; Wilcox and Wang, 1987*b*). The clusters of fungal cells within root cells have been described as filled with 'closely packed, thick-walled cells' (McKeen, 1952), 'groups of swollen cells' (Deacon, 1973), 'intracellular sclerotia of compact, darkly pigmented and irregularly lobed, thick-walled hyphae' (Stoyke and Currah, 1991), or 'thick-walled, irregularly lobed and compacted cells which sometimes formed sheets several cells thick' (O'Dell *et al.*, 1993).

Occasionally structures resembling ectomycorrhizae may also occur with ectomycorrhizal host plants. O'Dell *et al.* (1993) reported 'labyrinthine tissue (similar to Hartig net tissue)' in roots of *Pinus contorta* when inoculated with *Phialocephala fortinii*. Similarly, Fernando and Currah (1996) reported a Hartig net and a thin, patchy mantle when *Salix glauca* was inoculated with *P. fortinii*. In addition, occasional hyphal coils (Haselwandter

and Read, 1980) or peloton-like structures of coiled or looped, branched hyphae within root cells have been reported when DSE colonized ericaceous hosts (Currah *et al.*, 1993). Stoyke and Currah (1991) pointed out that none of their DSE isolates from alpine ericaceous plants displayed dense coiling similar to that frequently observed in ericoid mycorrhizae. A. Jumpponen (unpublished) inoculated *Vaccinium membraneum* seedlings with strains of *Phialocephala fortinii* and observed no coiling, but frequent production of thick-walled, inflated microsclerotia.

Wilcox and Wang (1987*a*) suggest that the morphology of the colonized root is mainly controlled by the host plant. Morphology may also change with time (Wilcox and Ganmore-Neumann, 1974). Wilcox and Ganmore-Neumann (1974) described the morphology of *Pinus sylvestris* roots inoculated with "a black imperfect fungus". While observing inoculated seedlings at 2-mo intervals, they reported the structures changing from a combination of intra- and intercellular invasion to those more typical of ectendomycorrhizae. A. Jumpponen and J.M. Trappe (ubpublished) conducted a similar assay with *Pinus contorta* and *Phialocephala fortinii* and observed chronological development similar to that described above. They found that a similar, discontinuous, patchy mantle developed within the 6-mo incubation, just as O'Dell *et al.* (1993) earlier observed for this host-fungus association.

When describing the fungal structures within roots colonized by DSE, it is necessary to allow adequate time for the structures to develop as well as for differences due to the host plants before making conclusions. For example, A. Jumpponen (unpublished) never observed a patchy, pseudoparenchymatous, mantle when *Phialocephala fortinii* was inoculated on nonectomycorhizal hosts (*Deschampsia caespitosa* or *Vaccinium membraneum*) while such structures frequently appeared with an ectomycorrhizal host (*Pinus contorta*). Some basic structures, however, seem constant for colonization by DSE regardless of the host species. The presence of sparse superficial mycelium, penetration into the cortical layer, and

subsequent occasional formation of chlamydospore-like, thick-walled, inflated, rounded cells within the cortical cells of the host root are all common to known form-taxa DSE. *Phialocephala fortinii, Phialophora finlandia, Leptodontidium orchidicola,* and isolates of Pezizales, as well as several unidentified isolates, all develop structures similar to those described above when colonizing a variety of host plants (Wilcox and Ganmore-Neumann, 1974; Read and Haselwandter, 1981; Egger and Paden, 1986*b*; Wilcox and Wang, 1987*a, b*; Currah *et al.*, 1993; O'Dell *et al.*, 1993; Stoyke and Currah, 1993; Fernando and Currah, 1995, 1996). More data are needed, however, to validate whether DSE are morphologically or taxonomically uniform. The current evidence seems not to favour such uniformity, as will be discussed below. A standardized vocabulary, however, is necessary to describe these various structures of DSE colonization. For this purpose, we propose the following terms: 'runner hyphae' for the individual, superficial fungal strands following the depressions between epidermal cells; 'superficial net' for the superficial colonization; 'appressorium' for the swollen structure preceeding penetration through a host cell wall; 'penetration tube' for the thin structure penetrating through the cell wall; 'microsclerotia' for the intracellular rounded, thick walled cells.

TAXONOMIC AFFINITIES OF DSE

Only few taxonomic affinities of DSE have been recognized. Most are currently placed within deuteromycetes and their relation to teleomorphic (meiotic) taxa is unknown. Fortunately, at least some DSE are fairly easy to bring into pure culture. Conidiogenesis and sporulation of the cultures is necessary for identification. Production of conidiospores is infrequent and some strains sporulate only after culture manipulation, such as extended incubation in low temperatures (Richard and Fortin, 1973; Wang and Wilcox, 1985; Ahlich and Sieber, 1996; Fernando and Currah, 1995). Conidiogenesis

may require incubation for over a year (Ahlich and Sieber, 1996), making the identification both difficult and time consuming. Even after culture manipulation, conidiogenesis can only be induced in a few isolates; most remain sterile and unidentifiable (see Stoyke *et al.*, 1992; Ahlich and Sieber, 1996).

In the absence of conidial structures, anamorphic species of DSE are similar species of *Rhizoctonia*, such as *Rhizoctonia endophytica* (see Saksena and Vaartaja, 1960). This is mainly due to the paucity of other distinctive characteristics in culture. Some species of *Rhizoctonia* and DSE also share a number of morphological characteristics. They may have septate, darkly pigmented, smooth to verrucose hyphae, lipid compounds within the hyphae, abundant inflated, barrel-shaped (monilioid) cells or chains, and occasionally produce sclerotia (compare Saksena and Vaartaja, 1960; 1961; Kendrick 1961; Wang and Wilcox, 1985; Currah and Tsuneda, 1993). As suggested by Currah *et al.* (1987), fungi with these characteristics have been frequently, but not necessarily correctly, referred to as *Rhizoctonia* (*e.g.* Peyronel, 1924; Burges, 1936; Saksena and Vaartaja, 1960, 1961; Haselwandter and Read, 1980; Currah *et al.*, 1988). It may, however, be that some *Rhizoctonia* share an ecological niché in addition to apparent morphological similarities.

The paucity of taxonomic characteristics, the difficulty of inducing conidiogenesis, and the range of variation in culture makes study of these groups of root-colonizing fungi difficult and tedious. To further complicate matters, morphology of the fungi in culture may depend on media and growth conditions (see Tu and Kimborough, 1975). Asexual structures can also be similar between different anamorph taxa, which are mainly separated by characteristics of conidia and conidiophores (Wingfield *et al.*, 1987). Even separating ascomycetes from basidiomycetes on the basis of asexual characteristics can be difficult (see Parmeter and Whitney, 1970). Classification and grouping based on conidial structures does not represent natural (phylogenetic) relationships among these diverse anamorphs.

Similar morphological characteristics do not necessarily represent homologous traits or can be results from several separate evolutionary events. Consequently, the deuteromycetes, comprised of fungi known by their asexual rather than sexual characteristics, is an artificial taxonomic and nomenclatural entity (see Gams, 1995; Seifert *et al.*, 1995; Taylor 1995). We may be better able to address problems of taxonomic identities of DSE as well as their relationships to other fungal taxa by sequencing and cladistic analyses.

Determining the identity of the root-colonizing fungus by its root morphology is also difficult. Some characteristics of root colonization are shared by DSE and *Rhizoctonia* and by DSE and ectendomycorrhizal fungi (*e.g.*, E-strain). Colonization by DSE or *Rhizoctonia* may include inter- and intracellular fungal growth and production of sclerotial bodies – or chlamydospores – within or among the cortical cells (Saksena and Vaartaja, 1960, 1961). Confusion between DSE and E-strain is also possible. E-strain, similarly to some DSE, occasionally forms a thin, discontinuous mantle, and the hyphae can penetrate regularly into the cortical cells of the host plants (Mikola, 1965; Wilcox *et al.*, 1974). Danielson (1982), however, pointed out characteristics that easily differentiate E-strain from DSE: E-strain fungi have a tendency to anastomose and produce chlamydospores, even on the root surfaces.

The identity and number of fungal (anamorphic or teleomorphic) species included in the group DSE are still uncertain. The isolates typically do not sporulate or, when they do, produce only scanty conidia. The difficulty of identifying the root endophytes led researchers to use various names to describe similar root-fungus associations. As previously discussed, Peyronel (1924) called such colonization that he observed on 135 taxa of angiosperms '*Rhizoctonia*-like'. Possibly some of those species actually were colonized by *Rhizoctonia* species. Several researchers have referred to isolates of sterile non-Rhizoctonia fungi from orchids as '*Rhacodium* spp.' (Harvais and Hadley, 1967; Harvais, 1974) or given them names within *Rhizoctonia* (*e.g.* Curtis, 1939). The true taxonomic affinity of these fungi is obviously

uncertain. Some of the reported *Rhizoctonia* or *Rhacodium* spp. may be synanamorphs belonging to the MRA complex (Currah *et al.*, 1987) or DSE.

Despite Melin's (1923) accurate description of his sterile isolates, it wasn't until the 1960s before any taxonomic identities of root or soil associated fungi in the MRA complex were suggested. Gams (1963) identified two cultures of MRA isolated from soil as *Phialocephala dimorphospora* Kendrick. Richard and Fortin (1973) were able to identify 15 of the 41 strains of MRA they isolated from roots of various woody plants in central and northern Europe as *P. dimorphospora*. *P. dimorphospora* commonly appears to be associated with decaying wood, soil, and pseudo- or ectomycorrhizae (Kendrick, 1961; Gams, 1963; Richard and Fortin, 1973). Richard and Fortin (1973), however, were somewhat uncertain of the accuracy of their identification: "the conidiophores of *P. dimorphospora* isolates were generally darker and the collarette more conspicuous." Still, they felt that their sporulating isolates were, indeed, *P. dimorphospora*. When studying slides of one of Richard and Fortin's cultures, Wang and Wilcox (1985) pointed out a possibility of misidentification in the previous work and concluded that at least some of the *P. dimorphospora* isolates may actually have been one of the later described anamorphic species, *Phialocephala fortinii*.

Additional anamorphic species from the MRA complex were described on the basis of the conidial structure: *Phialocephala fortinii, Phialophora finlandia, Chloridium paucisporum* (Wang and Wilcox, 1985), and *Leptodontidium orchidicola* (Currah *et al.*, 1987). We decided to exclude *P. radicicola* Cain (Cain, 1952) from this discussion. It is thought to be a nonpathogenic (or parasitic) anamorph that colonizes roots of grasses and cereals and shares characteristics typical of the roots colonized by DSE (see Deacon, 1973), but reports of its presence are sparse and usually from agricultural fields. The above four taxa have all been isolated, identified, and reported from various hosts and habitats (Table 5.1).

Establishing connections between DSE and sexually reproducing taxa, *i.e.*, anamorph-teleomorph relationships, would be helpful for inferring the possible systematic and functional relationships between genera, species and strains of DSE. Currently the terms MRA and DSE, as employed by many investigators, represents a heterogeneous mix of strains of deuteromycetes. Many DSE likely have an ascomycetous origin. Lobuglio *et al.*, (1996) analysed the small subunit of the ribosomal RNA gene (18S). Their analysis showed an ascomycetous affinity for *Phialocephala fortinii* and *Phialophora finlandia*. Our preliminary phylogenetic analysis based on the same region also clearly placed all the included DSE within ascomycetes (A. Jumpponen, unpublished). Similarly, *Leptodontidium orchidicola* has a likely ascomycetous affinity as judged by the septal ultrastructure (Currah and Sherburne, 1992). The placement of DSE within ascomycetes is still an open question. In our opinion two likely options exist. Currah *et al.*, (1993) reported small aggregations of apothecium-like structures on the surface of the substrate in cultures in which *Rhododendron brachycarpum* was inoculated with *Phialocephala fortinii*. Even though the ascomata remained sterile and never matured, the observed characteristics suggest an affiliation with inoperculate Discomycetes (Leotiales). Although not designed to address the placement of DSE, the 18S data by Lobuglio *et al.* (1996) also positioned *P. fortinii* close to inoperculate Discomycetes. *Phialophora finlandia*, however, was placed either with inoperculate (Leotiales) or operculate (Pezizales) Discomycetes indicating the insufficiencies of the sampling with respect to DSE. Another line of evidence also suggests that some of the DSE are related to the Pezizales. Several species within the Pezizales have been shown to colonize roots of woody plants (Danielson, 1984; Egger and Paden, 1986*a*, *b*). Some of these, such as *Sphaerosporella brunnea* (A. and S.) Svrcek and Kubicka, may be ecto- or ectendomycorrhizal (Danielson, 1984; Egger and Paden, 1986*b*). Others, *e.g.*, *Geopyxis carbonaria* (A. and E.) Sacc. and *Trichophaea hemisphaerioides* Mont., formed only patchy, discontinuous mantles and extensively colonized epidermal and cortical cells (Egger and

Paden, 1986*b*), resembling structures described by O'Dell *et al.*, (1993) in the roots of *Pinus contorta* colonized by *Phialocephala fortinii*. Most other species studied by Egger and Paden (1986*a, b*), however, were clearly pathogens on their test plant, *Pinus contorta*.

Sequencing and cladistic analyses will doubtless be powerful in identifying DSE. Genetic markers and their applications have been shown useful in identifying taxa and strains of DSE (Stoyke *et al.*, 1992; Yan *et al.*, 1995; Jumpponen and Trappe, 1996). They can in part replace the time-consuming culture manipulation. Moreover, they can be used for studying the phylogenetic relationships among the form taxa (*e.g.* Yan *et al.*, 1995; A. Jumpponen, unpublished). Molecular techniques can also be applied to generate new hypotheses and discover new directions where future effort is needed. For example, RFLP data of the ribosomal RNA gene indicates that *P. fortinii* and similar isolates express substantial variation (Stoyke *et al.*, 1992; Harney *et al.*, 1996), suggesting possible heterogeneity in taxa previously identified on the basis of morphological characteristics. Similarly, Yan *et al.* (1995) found disagreement between morphological and molecular identification of *Phialophora* sp.

In addition to addressing questions of DSE identification, sequence analyses can address questions of monophyly, *i.e.*, shared origin of DSE, systematic relatedness, and anamorph-teleomorph relationships. Unfortunately, molecular systematics with ascomycetes, and fungi in general, is fairly recent. As a result, the information available in international databanks is limited. However, more ascomycetous species are being sampled and new sequences made available for further phylogenetic analyses. Analyses of several known and unidentified isolates of DSE based on the small subunit of the ribosomal RNA gene strongly suggest a polyphyletic origin of these fungi (Harney *et al.*, 1996; Lobuglio *et al.*, 1996; A. Jumpponen, unpublished data). Some seem to be related only distantly. *Phialocephala fortinii, Phialophora finlandia, Chloridium paucisporum,* and *Leptodontidium orchidicola* all fall fairly close to each other and possibly

within Leotiales, while some of the unidentified isolates group within Pleosporales. These preliminary analyses, however, are based on only a short, 450 base-pair, partial sequence of the gene and should be interpreted with caution. It seems, however, that a combination of traditional and more recent methods in taxonomy have the potential to produce new anamorph-teleomorph relationships, if only at generic or familial levels. These inferences are necessary to facilitate the categorization of DSE as well as determining their function in natural ecosystems based on the function of known close relatives.

It is not surprising that the ecology of DSE is poorly understood given that several taxa of ascomycetes from different families and even orders are involved. As Allen and Allen (1992, p. 465) explained: "Unfortunately, few field data exist which allow definitive statements regarding the importance of different fungi on plant communities. In part this is due to frequent inability to recognize the vegetative state of fungi associated with plants in the field." Description and identification of new taxa of DSE establishes a starting point for a better understanding of their interactions with hosts (see Kendrick, 1961; Wang and Wilcox, 1985; Currah *et al.*, 1987, Fernando and Currah, 1995). Molecular and morphological systematic studies of the heterogeneous groups of root-inhabiting fungi – DSE (Kendrick, 1961; Wingfield *et al.*, 1987; Currah and Tsuneda, 1993; Fernando and Currah, 1995) and *Rhizoctonia* (*e.g.* Saksena and Vaartaja, 1960; 1961; Harvais and Hadley, 1967; Currah and Zelmer, 1992) – and reevaluation of the taxa will open avenues for separating fungi with different ecological functions by accommodating morphologically or genetically distinct groups.

EFFECTS OF DSE ON THEIR HOSTS

When Melin (1922) first described 'pseudomycorrhizae' and MRA, he wanted to differentiate this structure that he perceived as "harmful to seedlings and trees" from "ectotrophic mycorrhiza which is a necessary condition for their normal development". He concluded that MRA are fungal parasites that may kill the host plant. He also reported that, after forming a thin mycorrhizae, the fungus overgrew and killed the seedlings (Melin, 1923). Robertson (1954) and Hatch and Hatch (1933) confirmed these results in their pure culture syntheses. Robertson (1954) further stated: "About 3 weeks later after inoculation the pine seedlings began to die and in a month they were completely dead and overgrown by the fungus". However, because of the presence of dark mycelium on the surfaces of "healthy elongating" roots of pines in the field samples, Robertson (1954) concluded that these fungi attack roots only under special physiological conditions, such as during the senescence, of the roots and that they are not pathogenic "to healthy roots in natural soils".

The early results indicating pathogenic behaviour of strains belonging to MRA resulted in further tests on the relationship between root endophytic fungi and their host plants. Pathogenicity tests were conducted with unidentified cultures of dark-pigmented, sterile fungi and the host plants *Picea abies* (Schönhar, 1984), *Pinus sylvestris* (Schönhar, 1984), *Chamaecyparis nootkanensis* (Hennon *et al.*, 1990), and *Fragaria vesca* (Wilhelm *et al.*, 1969). Results from these studies with unknown fungal associates were, not surprisingly, inconsistent: the cultures varied from strongly pathogenic (Wilhelm *et al.*, 1969) to weakly pathogenic (Schönhar, 1984) or nonpathogenic (Hennon *et al.*, 1990). Haselwandter and Read (1982) inoculated two *Carex* species with indigenous dark and septate strains from the European Alps. They reported increased dry matter production and shoot phosphorus levels after inoculation and concluded that "the root-fungus association appeared to be of a mutualistic rather than a parasitic nature".

The fungal endophytes of those studies remained unidentified. A. Jumpponen (unpublished) included one of their isolates in a preliminary molecular phylogenetic analysis. It appeared closely related to the type culture of *Phialocephala fortinii* and some other strains identified as *P. fortinii*. It is essential to note that the interaction between a fungus and its host depends strongly on the species of both the fungus and the host as well as the experimental conditions. Any meaningful conclusions about host-fungus relationships must therefore be based on correctly identified fungi and hosts.

With the description of a few species of DSE (Kendrick, 1961; Wang and Wilcox 1985; Currah *et al.*, 1987), interest increased in determining the nature of the interaction between the host and fungal endophyte in specific host-endophyte combinations. Wilcox and Wang (1987*a*) inoculated four identified anamorphic taxa on *Pinus resinosa, Picea rubens,* and *Betula alleghaniensis*. The results were variable and the response of a given host species depended on the host-fungus combination. *Phialocephala dimorphospora* was concluded to be a pathogen, *P. fortinii* pseudomycorrhizal or a pathogen, *Chloridium paucisporum* ectendo- or pseudomycorrhizal, and *Phialophora finlandia* ecto- or ectendomycorrhizal, depending on the host species. Their data on host response included no biomass accumulation, nutrient acquisition, or fitness measurements and were not statistically analyzed. The conclusions were based on the visual appearance of the seedlings and their root systems. When they further studied the positive association between the above mentioned host taxa and *P. finlandia,* they concluded that the fungal colonization "did not result in the diminution of growth but an increase" (Wilcox and Wang, 1987*b*).

Additional inoculation assays with specific host-fungus combinations were conducted. Stoyke and Currah (1993) inoculated *Menziesia ferruginea* with *Phialocephala fortinii* in cellulose agar petri dish cultures. *P. fortinii* physically overgrew the seedlings in absence of competition by other fungi under aseptic conditions. O'Dell *et al.* (1993), however, saw no adverse

reaction or extensive degradation of *Pinus contorta* tissue in response to colonization by *P. fortinii*. Several possible reasons may account for the inconsistent results of these studies. Fernando and Currah (1996) pointed out obvious strain specific differences in the growth responses in bioassays where *P. fortinii* was inoculated on several hosts in aseptic and open pot cultures.

The resynthesis and culturing system as well as the media used in assays can produce incongruent results. Duddridge and Read (1984) and Duddridge (1986) demonstrated a change in the behaviour of ectomycorrhizal fungi in the presence of exogenous carbohydrate. Jumpponen and Trappe (Chapter 7) conducted an experiment to reveal whether the interactions of *P. fortinii* with *Pinus contorta* would change under different levels of supplied exogenous glucose. They found that increased glucose in an aseptic culture system clearly resulted in a substantial increase in host biomass; furthermore, no negative effects on the host plant were observed. They speculated that either the carbohydrates available in the medium were directly transported to the host plant by the living mycelium or that respiration by the fungus increased the CO_2 concentration in the closed culture system, resulting in increased photosynthesis by the pines. The increases in host biomass, however, appeared too large to be accounted for by the increased CO_2 concentration alone. The nutritional status of the fungal endophyte may therefore be an explanation for the controversial earlier results: Wilcox and Wang (1987*a*) and Stoyke and Currah (1993) used a growing medium with readily available carbohydrates, double strength MMN (Marx and Zak, 1965) and cellulose agar (Warcup, 1973), respectively. In contrast, O'Dell *et al.*, (1993) used a method in which the seedlings were grown in a "growth pouch" to which nutrients but no carbohydrates were added. Whatever the reasons for observed inconsistencies in the host response to inoculation, it is obvious that results from any pure culture synthesis should be viewed with some caution.

Other studies similarly support the hypothesis of DSE involvement in host nutrient acquisition. Jumpponen *et al.* (Chapter 6) grew seedlings of *P.*

contorta in nitrogen-limited soil obtained from a glacier foreland in a growth room in a fully factorial design. The treatments included addition of organic matter, addition of nitrogen, and inoculation with a strain of *Phialocephala fortinii*. The inoculation alone did not affect growth, but significantly increased the foliar phosphorus concentration. The combination of inoculation and nitrogen amendment resulted in a more than 50% larger increase in *Pinus contorta* biomass than did the nitrogen amendment alone. These results allow us to conclude that DSE may be involved in host nutrient acquisition and therefore may indeed have a mutualistic, mycorrhizae-like relationship with their host plants.

ECOLOGY

Even if the effects of these fungi on host plants vary with hosts and growth conditions, their abundance in some natural environments (see Berch *et al.*, 1988; Hennon *et al.*, 1990; Cázares, 1992; Holdenrieder and Sieber, 1992) and ubiquitous global presence (Appendix 1) suggest an ecological role of importance. Inter- and intracellular colonization as well as the ability to colonize a wide variety of host plants has led researchers to propose that root endophytes such as *Rhizoctonia* sp. (Warcup, 1985) and *Phialocephala fortinii* (Jumpponen and Trappe, 1996) may form mycelial connections between plant individuals of the same or even different species. These connections could be involved in photosynthate or nutrient transport as suggested by Read *et al.*, (1985) and Newman (1988) for ectomycorrhizal systems. A. Jumpponen and J.M. Trappe (unpublished) tested this hypothesis in a preliminary study employing the stable, heavy nitrogen isotope (^{15}N) in an experiment using the root-mycocosms described in Rygiewicz *et al.*, (1988). *Pinus contorta* seedlings inoculated with *Phialocephala fortinii* grew in a peat-vermiculite substrate in one section of the mycocosm. ^{15}N was added to the adjacent compartment that could be accessed by the fungal hyphae but not

by host roots. The two compartments were separated by an air-filled gap, bridged by the fungal hyphae. The ^{15}N was thus accessible to the pine only via the hyphal bridges. ^{15}N appeared to move from the fungus into the plant; some of the *Phialocephala fortinii*-inoculated *Pinus contorta* seedlings acquired two to three times more ^{15}N than did controls. However, because of the small sample size in the preliminary study, the results were inconclusive.

The observed increases in foliar phosphorus concentrations in the inoculation studies described suggest that at least some DSE may acquire nutrients directly from organic debris by saprotrophic activity. The frequent appearance of sterile, dark mycelium (MRA as most soil isolation assays refer to darkly pigmented, septate mycelium) in soil (Heal *et al.*, 1967; Summerbell, 1988, 1989; Holdenrieder and Sieber, 1992) gives further evidence of the saprotrophic capabilities of DSE. The type collection for the genus *Phialocephala* – *Phialocephala dimorphospora* – was, indeed, isolated from well decayed deciduous wood, and the habitat is described as "often on decaying wood or bark" (Kendrick, 1961). Yet, *P. dimorphospora* has been shown to readily colonize roots of several host species (Richard and Fortin, 1973, 1974; Wang and Wilcox, 1985; Wilcox and Wang, 1987*b*). DSE also grow through dead cells in root cortices without apparent harm to immediately adjacent live tissue (Robertson, 1954; Livingston and Blaschke, 1984). This, in addition to observations of greater frequency of DSE in long roots (Livingston and Blaschke, 1984) or within the basal rather than tip tissue of fine roots (Robertson, 1954), suggests some degree of saprotrophic behaviour.

Several different enzymatic activities have been detected in DSE. Bååth and Söderström (1980) showed cellulolytic and proteolytic activity in an isolate of MRA. Based on an enzymatic activity assay Caldwell *et al.*, (1996) concluded that *Phialocephala fortinii* and other sterile isolates of DSE macro- and microscopically similar to *P. fortinii* can process common detrital carbon and phosphorus polymers. Polyphenol oxidases are produced by *Leptodontidium orchidicola* (Fernando and Currah, 1995) and *P. fortinii* (Currah and Tsuneda, 1993). These enzymes may be involved in processes

such as lignin degradation. Despite these enzymatic activities, it remains unclear whether DSE fungi actually decompose organic debris in their natural environment. Hutchinson (1990) speculated that besides processes of decomposition, these enzymes may be merely involved in fungal resistance to antifungal compounds. Furthermore, penetration through host cell walls may require lignolytic and cellulolytic enzymes. It is interesting to speculate about the ecological role of root-associated endophytes with saprotrophic activities. A continuous mycelial unit could possibly link host plants to each other (see Jumpponen and Trappe, 1996) as well as directly to newly released organic residues that could be utilized if adequate enzymatic abilities were available.

DSE-like colonization has also been observed to occur simultaneously with arbuscular mycorrhizae (Sengupta *et al.*, 1989) or ectomycorrhizae (Randy Currah, personal communication). Similarly, DSE is frequently isolated from root tips colonized by ectomycorrhizal fungi (Hatch, 1934; Trappe, 1962; Holdenrieder and Sieber, 1992). Concurrent occupation by different root-associated fungi may indicate either ongoing change in the composition of the community of root-colonizing fungi or the presence of similar functions performed by a variety of organisms occupying a single root at the same time. On the one hand, DSE colonization appears more frequent in older parts of the root system (Robertson, 1954; Livingston and Blaschke, 1984; A. Jumpponen, unpublished). This may indicate that DSE prefer aging root tissue and thus are found in greater frequency in the later stages of root development. It is also possible that DSE are recycling nutrients from senescent or dead root cells back into the active part of the root system. On the other hand, the endophytic (DSE-like) fungi may function as mycorrhizal fungi taking part in nutrient and water acquisition, especially in stressed environments (Sengupta *et al.*, 1989). The concurrent colonization by DSE and ecto- or endotrophic mycorrhizae would thus provide a back up system during periods when mycorrhizal fungi are inhibited by the environmental

conditions. These hypotheses need to be tested, preferably in natural environments.

Another interesting question is the role and presence of melanins in the hyphae of DSE. Melanins develop in large quantities in organisms that live in stressed environments (Bell and Wheeler, 1986). They appear to play an important role in discouraging grazing on soil micro-organisms by other soil microfauna and they enable the organisms to withstand dessication and microbial lysis (Kuo and Alexander, 1967; Bell and Wheeler, 1986). Melanins may help protect DSE hyphae during seasons of extreme temperatures and drought; DSE may thus broaden their ecological niche, as is true for the strongly melanized *Cenococcum geophilum* (Trappe, 1962). Cázares (1992) and Jumpponen and Trappe (1996) have isolated several DSE from an alpine glacier foreland in northern Washington, USA. This area is frequently exposed to frost and midsummer droughts. Resistance to cold and dessication may play a significant role for the organisms able to persist at the site from year to year.

Currah *et al.*, (1993) hypothesized that the intracellular sclerotial bodies of *Phialocephala fortinii*, also heavily melanized, can be effective as dispersal propagules. As the colonized roots mature, the epidermal cells frequently loosen, separate and slough off the root. The individual, sloughed-off cells can then disperse with the movement of soil like the spores of arbuscular mycorrhizal fungi (Allen, 1991). If this is true, melanins provide a means for greater durability and extended persistence and survival of the individual DSE propagules in soil. According to Currah *et al.*, (1993), presence of persistent propagules, such as the sloughed-off cortical cells filled with sclerotial bodies, could also explain why DSE fungi are frequently isolated from surface sterilized or washed roots and mycorrhizae (Summerbell, 1989; Stoyke and Currah, 1991; Holdenrieder and Sieber, 1992; Stoyke *et al.*, 1992; Jumpponen and Trappe, 1996).

The strategies of reproduction and dispersal of DSE are also almost completely unknown and only rarely hypothesized. As described above,

mycelial fragmentation is among the suggested means of dispersal (Currah *et al.*, 1993). Reproduction and dispersal by conidiospores is also possible. Despite the fact that to date no anamorph-teleomorph connections have been established, sexual reproduction by ascospores is likely. Currah *et al.* (1993) described developing ascomata in their pot culture synthesis. Those ascomata, unfortunately, never matured. As hypothesized by Jumpponen and Trappe (1996), from the high genetic diversity observed in a population assay, a large number of asexually dispersing individuals or frequent sexual recombination would be required to explain the large number of distinct and highly variable phenotypes found on their small study site on a glacier forefront. Both asexual, by either mycelial fragmentation or conidiospores, and sexual reproduction and dispersal are possible. However, more data are needed to understand how the populations of DSE expand, disperse, and maintain themselves.

DISCUSSION AND CONCLUSIONS

Sterile root endophytes are ubiquitous in various habitats. Harley (1950) pointed out that ". . .one is definitely in a position to state that such sterile septate mycelia are to be expected in the external tissues and on the surface of roots of almost any plant. . . ." These endophytes, DSE fungi, have been reported from subalpine and alpine habitats through the tropics to arctic latitudes. Their abundance in tropical and temporal habitats needs further research. By concentrating on recognized and morphologically identifiable forms of mycorrhizae, we may easily overlook groups such as miscellaneous root-inhabiting endophytes and discount their importance.

DSE seem able to colonize a wide range of different hosts, including species known to be vesicular-arbuscular, ericoid, orchid, or ecto- or nonmycorrhizal. In most studies to date all but the most conventional types of root colonization have been ignored. Including root endophytes in

mycorrhizal studies adds laborious steps to the already time-consuming enumeration of mycorrhizae, but it would yield valuable data about the actual importance and frequency of other types of root colonizers.

The morphology of roots colonized by DSE seems to vary from structures resembling ecto- or ectendomycorrhizae to unique structures such as the thick-walled intracellular clusters of rounded fungal cells. It is difficult to find a common denominator for all host-fungus interactions within DSE. Detailed studies of the nature of the interaction between specific and known host-fungus combinations are needed to resolve these problems. Additionally, a way to describe the root-fungus interaction on the basis of both the morphological appearance and function of the dual organ of the colonized root is needed. This calls for careful histological approaches, including electron microscopy, to understand the development and function of colonization. These data can then be integrated with assays focusing on the function of the colonized root and the role of the fungal partner in particular.

DSE clearly comprise a heterogeneous group of known, and possibly unknown and undescribed, taxa of deuteromycetes. These fungi seem to rarely produce conidia and then only after long manipulation in pure culture. The inconsistency and disagreement between results from various studies of DSE are at least partly due to the uncertain identities of the strains used; it is hardly surprising that confusion remains whether these organisms are parasitic, commensal or mutualistic, given the variety of conidial and sterile fungi that produce similar structures when colonizing host roots. Fortunately, molecular approaches offer a promise to provide means to separate and identify these fungi with increased certainty. Availability of identified type cultures and use of molecular tools can simplify the identification of the fungal endophyte by comparing genetic markers. Phylogenetic analyses of DSE may shed light on questions of their origin and provide further help in solving the functional aspects of the fungi in this group.

Pure culture syntheses of DSE with their hosts indicate a variety of interactions, ranging from mutualistic to pathogenic. Host responses to DSE colonization depend on the species of both the endophyte and host, and likely also the environment. These interactions need to be studied in more detail under various conditions, especially in natural habitats of both fungus and host. Although aseptic syntheses are a convenient and valuable initial step for understanding the interaction between the host and the fungus, they may give a biased or even erroneous view of the association. There is a need to find and develop research tools that allow assessment of the questions about the host-fungus interaction in natural environments.

The ecology of DSE in natural ecosystems is still largely unknown and hypotheses are based on sparse evidence. Root-fungus associations may diverge from easily classifiable, morphologically identifiable mycorrhizal types and yet function physiologically as mycorrhizae under natural conditions (Kope and Warcup, 1986). Thus, a primary research focus should be on the functional aspects of the interaction between the two organisms involved in the association.

With the recent development of molecular tools and the availability of type culture material as well as fungal sequences in international nucleotide databases, more emphasis should be put on identifying the true affinity of the fungi, even if only on generic or familial level. Understanding the relationships within DSE, as well as the relationship of DSE to known teleomorphic genera and families will open ways to generate more general understanding of the true nature and ecological importance of these poorly known root-colonizing fungi. In depth understanding of the systematics and taxonomy of DSE may be an invaluable cornerstone preceeding unearthing the interaction between DSE and their host plants.

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CHAPTER 6

MYCORRHIZAL FUNCTIONING OF *PHIALOCEPHALA FORTINII* WITH *PINUS CONTORTA* ON GLACIER FOREFRONT SOIL: INTERACTIONS WITH SOIL NITROGEN AND ORGANIC MATTER.

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ABSTRACT

Plants growing in an environmentally stressed glacier forefront on soil low in N and organic matter have abundant root colonizations by dark-septate fungi. As the plants appeared fit for this severe habitat, it was hypothesized that the dark-septate endophytes were neutral or beneficial rather than detrimental to the plants. To test this hypothesis, we designed a growth room experiment with *Pinus contorta* grown on forefront soil inoculated with the dark-septate fungus, *Phialocephala fortinii,* in the absence of climatic stress. N and organic matter treatments were included to explore interactions with the fungal inoculation. *P. fortinii* colonized roots inter- and intracellularly and occasionally formed microsclerotia. Inoculated plants absorbed significantly more P than noninoculated plants in all combinations of N and organic matter. Without added N, neither inoculation nor organic matter additions improved plant growth or N uptake, showing that N indeed limits plant growth in this substrate. With added N, however, both organic matter addition and inoculation significantly increased total pine biomass production and N uptake. The enhanced P uptake by the *P. fortinii*-inoculated pine regardless of N limitations and increased pine growth and N uptake in the *P. fortinii* treatment when N was not limiting appear as typical mycorrhizal responses.

INTRODUCTION

N and organic matter buildup play important roles in young ecosystems such as glacier forefronts (Chapin *et al.*, 1994; Matthews, 1992; Vitousek and Walker, 1989). The introduction of mycorrhizal propagules is essential for establishment of mycorrhiza-dependent plants (Trappe and Luoma, 1992). Once mycorrhizal plants and their symbiotic fungi establish on

such poorly developed substrates, nutrient capture and cycling is greatly enhanced.

Dark-septate, root-endophytic fungi are common in arctic-alpine habitats (Cázares, 1992; Haselwandter and Read, 1982; Jumpponen and Trappe, 1996; Väre *et al.*, 1992). The interactions of dark-septate endophytes and their hosts are controversial, having been suggested to be pathogenic, neutral or beneficial (Fernando and Currah, 1996; Haselwandter and Read, 1982; O'Dell *et al.*, 1993; Stoyke and Currah, 1993; Wang and Wilcox, 1985; Wilcox and Wang, 1987). Some may function in different ways along the mutualism-parasitism continuum, depending on conditions (Johnson *et al.*, 1997).

Cázares (1992) reported dark-septate endophytes to be common on many plant species on the forefront of Lyman Glacier in the North Cascade Range of Washington State. The plants showed no symptoms of disease and were established in a habitat notable for its poor soil, short growing season and climatic stress. As these plants had to contend with a severely stressful habitat, we hypothesized that the dark-septate colonizations had no adverse effect to the plants; imposition of disease in addition to the environmental stresses would not likely lead to successful establishment. To test this hypothesis, we explored effects of a dark-septate endophyte on *Pinus contorta* Dougl. grown on forefront soil without climatic stress; *P. contorta* represents an early colonizer at the Lyman Glacier forefront. *Phialocephala fortinii* Wang and Wilcox was selected as the endophyte, because it is frequent at the site (Jumpponen and Trappe, 1996) and colonizes roots of *P. contorta* (O'Dell *et al.*, 1993). N and organic matter supplements were included to examine their interactions with the fungus; nitrogen and organic matter are very low away from established plants on the forefront (Jumpponen *et al.*, 1998).

MATERIALS AND METHODS

A composite soil sample was collected at the Lyman Glacier forefront (48° 10′ 14″ N, 120° 53′ 44″ W; elevation ca 1800 m) within an area that had been deglaciated for less than 20 yr and was devoid of plants (see Cázares, 1992 and Jumpponen *et al.*, 1998 for site details). The sample was sieved through 5 mm screen, air dried, and stored at 4°C. The organic matter and N contents of the soil are low and likely limiting plant growth (ca 0.4 % organic matter and 0.001 % N by soil dry weight; Jumpponen *et al.*, 1998). The soil was pasteurized before use.

Seed of *Pinus contorta* was soaked 24 hours in deionized water at 4°C, stratified 14 days at 4°C, then surface sterilized 55 min in 30% hydrogen peroxide under agitation. Five stratified seeds were sown on 80 g of air-dried soil in 115 cm^3 plastic Cone-tainers (Ray Leach Inc., Corvallis, Oregon). N, organic matter, and *Phialocephala fortinii* were applied to each container in a fully factorial design, each treatment at two levels: present or absent. To account for effects of inoculation, an additional control with killed inoculum was included in the experiment. The result was a 2 x 2 x 2 factorial with an additional inoculum control, nine treatment combinations in total. Ten replicates of each treatment were randomly arranged in the growth room.

An equivalent of 100 kg N/ha of sulfur-coated urea was added to the top of soil in half of all containers; the other half were untreated. The application is approximately 35 % of the total N in the nonvegetated forefront soil and less than 5 % of total N underneath shrub canopies in the area deglaciated approximately 60 yr ago. The sulfur-coated pellets were selected because they dissolve over a 6-mo period. Hence, N 'pulses' were avoided during the experiment.

Approximately 1.5% peat by dry weight of soil was added and thoroughly mixed to half of all treatments as the organic matter treatment; the other half were left untreated. The additions resulted in organic matter contents similar to naturally occurring by the terminal moraine on Lyman

Glacier forefront (Jumpponen *et al.*, 1998). To minimize nutrient additions, the peat was leached with 0.2 N HCl and then thoroughly washed with distilled H_2O. Thus, the focus in this treatment was on effects of organic matter on physical and chemical soil characteristics rather than on nutrient input.

The inoculum of *Phialocephala fortinii* (SE24, strain isolated by O'Dell *et al.* (1993), maintained at USDA-PNW Research Station, Corvallis, Oregon) was mixed with vermiculite and Modified Melin Nordkrans media (Marx and Zak, 1965), then incubated for 8 weeks. The control inoculum was separated from the same batch and autoclaved for 25 minutes. A total of 10 ml of live or killed inoculum was applied to the top of the soil mixture in inoculated treatments and controls.

Conditions in the growth room were 25°C and a 16/8 hour day/night cycle using an irradiance intensity ca 300 μE within the photosynthetically active range. After emergence of the first seedling, subsequent germinants were removed to maintain only one per container. After 12 weeks of growth (the approximate growth season at Lyman Glacier forefront), soil was washed from the roots and the colonized root length was estimated by the gridline intersection method (Giovannetti and Mosse, 1980). Dark and septate, superficial, inter-, and intracellular mycelium was observed. After examination, seedlings were dried at 80°C for 48 hours and the dry weights of shoot and root recorded.

Due to the small accumulation of foliar biomass in some treatments, foliage from three seedlings within a treatment were pooled for foliar nutrient analyses. Foliar tissue N and P were analyzed by the Kjeldahl method (Thomas *et al.*, 1967) with an Alpkem Rapid Flow Analyzer Model 300.

A one-way analysis of variance was first used to compare the killed-inoculum control against the no-inoculum control. Analysis of variance showed no significant differences in growth ($p=0.322$, 0.931, and 0.481 for shoot, root and total biomass, respectively) or nutrient concentrations

(p=0.460 and 0.141 for total N and P, respectively). Because of the strong N effect on growth (ca 50 % increase, p>0.001, in shoot, root and total biomass) and foliar N concentration (ca 100 % increase, p>0.001) and absence of three way interactions (p=0.492, 0.297, 0.357, p=0.832, and 0.199 for shoot, root, total biomass, total N, and total P, respectively), effects of organic matter and fungal inoculation were analyzed separately for the two nitrogen regimes as 2 x 2 factorial design: two treatments (organic matter and fungal inoculation) on two levels (present or absent).

All response variables, except the root/shoot ratios and the nutrient concentrations, were log-transformed prior to analysis to obtain homogeneity of variances between the treatments. Data were subjected to analysis of variance by the General Linear Models procedure in SYSTAT (SYSTAT, 1992) to test for main and interactive effects. Means between the different treatment combinations were compared by Fisher's least significant difference (LSD) test at an alpha level of 0.05.

RESULTS

Root Colonization by Phialocephala fortinii

Root systems of all inoculated seedlings were colonized by the dark-septate mycelium. Intracellular monilioid chains and microsclerotia as well as superficial or inter- and intracellular, papillate mycelium were observed. No root-associated fungi (mycorrhizal or endophytic) were observed in noninoculated treatments. The degree of root colonization by *Phialocephala fortinii* was not significantly affected by application of N or organic matter. Colonized root length ranged from 4 to 20 %.

Growth Response

Addition of N increased both shoot and total biomass > 50% and root biomass > 40 % compared to the control (Fig. 6.1A). Analyzed separately for the two N levels, organic matter, without added N, did not significantly affect shoot biomass, but root biomass decreased by 35 % compared to the control (Table 6.1). Due to the large reduction in root biomass (Fig. 6.1B), total biomass and root/shoot ratio also decreased. With added N, the organic matter amendment resulted in an increase of > 40 % in shoot biomass when compared to the treatment with N application alone. The strong positive effect of N amendment was thus further enhanced in presence of organic matter.

Inoculation with *Phialocephala fortinii* did not significantly affect shoot, root and total biomass when no N was added (Table 6.1, Fig. 6.1A). With added N, fungal inoculation resulted in a 50 % biomass increase over the N treatment alone; the root endophyte hence enhanced growth of the seedling when adequate levels of N were available.

Nutrient Accumulation

The N amendment increased foliar N concentration by 100 % when compared to the control (Fig. 6.1C). Organic matter and fungal inoculum did not affect foliar N concentration when no N was added (Table 6.2). With added N, organic matter and fungal inoculation increased the foliar N concentration by 25 % and 20 %, respectively, in when compared to the N amendment alone. Foliar P concentration significantly increased as a result of inoculation with *Phialocephala fortinii* regardless of N or organic matter treatment (Table 6.2, Fig. 6.1D).

Table 6.1. Shoot, root and total dry weights and Root/Shoot ratios (means ± standard deviation) of *Pinus contorta* grown in Lyman Glacier forefront soil. Results of four treatments applied under two levels of N (no added N and amendment equal to 100 kg-N/ha): OM, organic matter amendment equal to 1.5% soil dry weight; PF, inoculation with *Phialocephala fortinii*. Presence or absence of a factor is indicated by + or –. Numbers in parentheses indicate number of replicates in each treatment. Same letters in a column indicate non-significant differences at alpha = 0.05 based on Fisher's LSD. Source of variation indicates which factors contribute to the observed differences.

Treatment (n)	Shoot (mg)	Root (mg)	Total (mg)	Root/Shoot
		No added N		
OM – PF – (10)	31.30±13.39a	21.60±7.29b	52.90±18.67b	0.72±0.21b
OM + PF – (9)	26.33±5.29a	14.00±2.45a	40.33±7.84a	0.55±0.10a
OM – PF + (10)	29.30±6.22a	19.30±4.27ab	48.80±8.94ab	0.67±0.16ab
OM + PF + (10)	27.60±7.58a	15.50±4.09a	43.10±9.62ab	0.59±0.19ab
Source of variation				
OM	NS	***	*	*
PF	NS	NS	NS	NS
OM x PF	NS	NS	NS	NS
		100 kg-N/ha		
OM – PF – (9)	51.00±14.36a	30.78±10.08a	81.78±21.22a	0.63±0.18b
OM + PF – (10)	72.10±21.94b	32.00±9.14ab	104.10±29.10ab	0.46±0.11a
OM – PF + (10)	82.50±19.80bc	47.40±18.07c	129.90±33.63b	0.57±0.19ab
OM + PF + (10)	103.20±29.81c	43.40±19.79bc	146.60±42.98b	0.43±0.16a
Source of variation				
OM	***	NS	NS	**
PF	**	***	***	NS
OM x PF	NS	NS	NS	NS

NS $p > 0.05$
* $0.05 \geq p > 0.01$
** $0.01 \geq p > 0.001$
*** $0.001 \geq p$

Figure 6.1. Shoot and root dry weights and N and P concentrations (error bar = mean ± standard deviation) of *Pinus contorta* seedlings grown in soil from Lyman Glacier forefront. Four treatment combinations applied under two levels of N are shown (no added N and amendment equal to 100 kg-N/ha): OM, organic matter amendment equal to 1.5% soil dry weight; PF, inoculation with *Phialocephala fortinii*. Presence or absence of a factor is indicated by + or –.

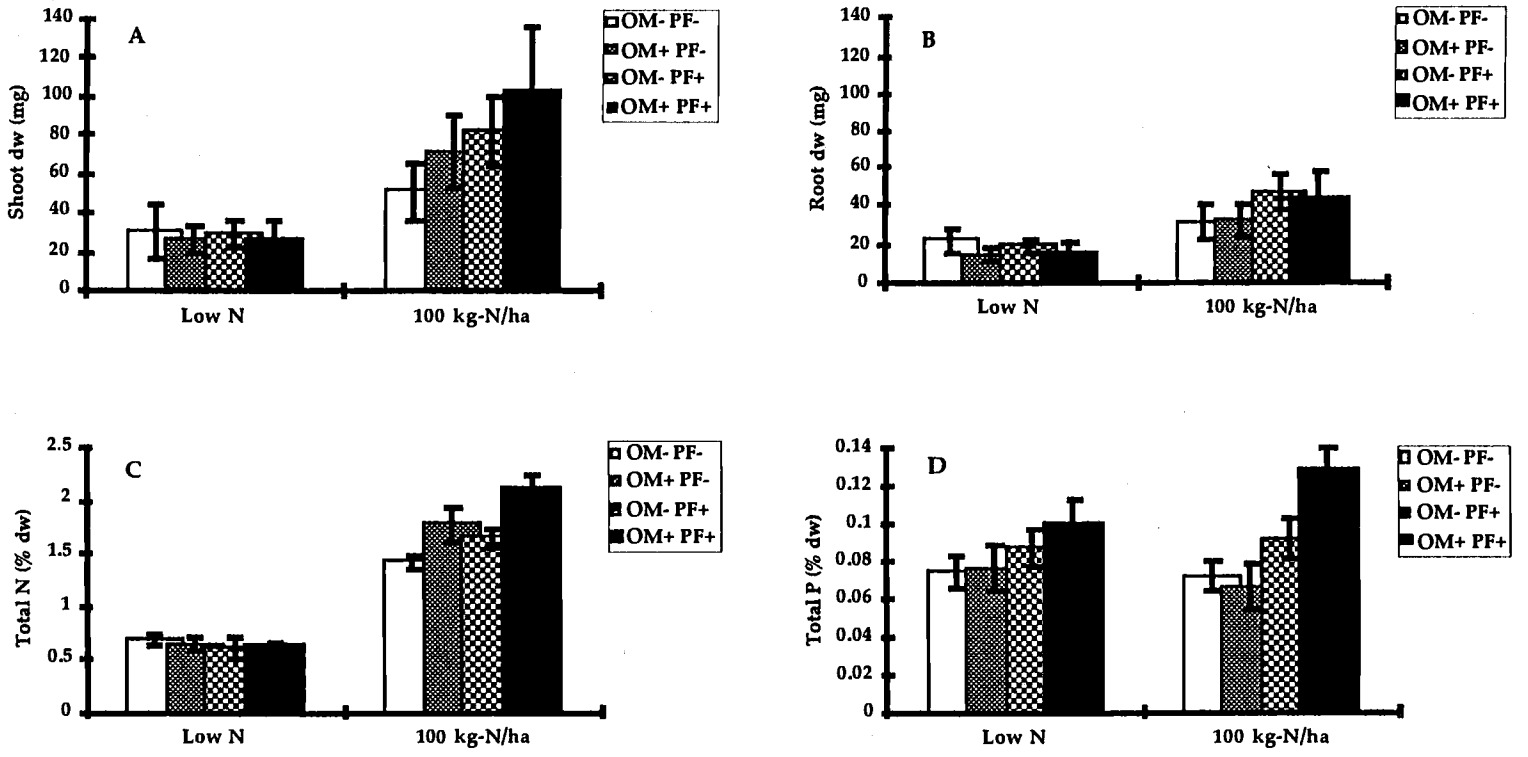

Table 6.2. N and P concentrations (means ± standard deviation) in *Pinus contorta* foliage following four treatment combinations applied under two levels of added N. For legends and symbols, see Table 1.

Treatment (n)	Low N		100 kg-N/ha	
	Total N (% by dw)	Total P (% by dw)	Total N (% by dw)	Total P (% by dw)
OM – PF – (3)	0.690±0.057a	0.074±0.006a	1.414±0.067a	0.072±0.013a
OM + PF – (3)	0.639±0.031a	0.076±0.012a	1.780±0.149c	0.066±0.012a
OM – PF + (3)	0.608±0.114a	0.087±0.007b	1.642±0.024b	0.092±0.011b
OM + PF + (3)	0.623±0.039a	0.100±0.012b	2.110±0.181d	0.128±0.012b
Source of variation				
OM	NS	NS	***	NS
PF	NS	**	**	**
OM x PF	NS	NS	NS	NS

NS $p > 0.05$
* $0.05 \geq p > 0.01$
** $0.01 \geq p > 0.001$
*** $0.001 \geq p$

DISCUSSION

The experiment reaffirmed earlier conclusions that N limits plant growth on the Lyman glacier as on other glacier forefronts (Chambers *et al.*, 1987, 1988, 1990; Jumpponen *et al.*, 1998). Addition of N together with organic matter in the absence of *Phialocephala fortinii* enhanced N uptake by the seedlings, presumably because the organic matter reduced leaching losses of N. With added N, organic matter also increased shoot and total biomass and foliar N concentration as compared to N application alone. These responses likely resulted from altered ion exchange capacity, increased aeration, or decreased bulk density (Cheng, 1977). Addition of organic matter alone in the low N treatment reduced root and total biomass. The reduction was possibly due to immobilization of the already low soil N by soil microorganisms (Paul and Clark, 1989).

Phialocephala fortinii enhanced P uptake by the pine regardless of N or organic matter treatments. Enhanced P uptake is, of course, among the better known mycorrhizal functions (Harley and Smith, 1983; Smith and Read, 1997). When the N limitation was overcome by added N, *P. fortinii* produced two responses classically regarded as mycorrhizal: enhancement of growth and P uptake (Harley and Smith, 1983; Smith and Read, 1997). Haselwandter and Read (1982) reported analogous increases in growth and P uptake by a *Carex* sp. inoculated with a dark-septate endophyte. Their sterile isolate is similar to *P. fortinii* based on preliminary analyses of partial sequences of the small subunit of the nuclear ribosomal DNA (Jumpponen, unpublished data).

Because *P. fortinii* seems to have behaved as a parasite or pathogen under some experimental conditions (Currah *et al.*, 1993; Fernando and Currah, 1996; Stoyke and Currah, 1993; Wang and Wilcox, 1985, Wilcox and Wang, 1987), its status as a mycorrhizal fungus under our experimental conditions might be questioned. That is a matter of how "mycorrhiza" is defined. The definition of Gerdemann (1970) as modified by Harley (1992) and

then Trappe (1996) can be applied: "Dual organs of absorption formed when symbiotic fungi inhabit healthy absorbing organs (roots, rhizomes or thalli) of most terrestrial plants and many aquatics and epiphytes." In our experiment, the roots and fungus formed a dual organ of absorption with no symptoms of disease; living together, hence in symbiosis (Lewis, 1973). As Johnson *et al.* (1997) point out, the accuracy of this definition is "not compromised if the dynamic nature of plant responses to mycorrhizal associations is accepted and they are considered to be generally mutualistic, with occasional commensal and parasitic excursions from this norm." Most established plants in the Lyman Glacier forefront seem fit for that stressful habitat, lack symptoms of disease, and are colonized by dark-septate endophytes such as *P. fortinii* (Cázares, 1992; Jumpponen and Trappe, 1996). The circumstances when that fungus was pathogenic may have been "occasional excursions" from the norm or reflect differences between strains of this anamorphic taxon (Currah *et al.*, 1993; Fernando and Currah, 1996).

From our results and those of Haselwandter and Read (1982), we cannot say unequivocally that the symbiosis was mutualistic: although the pine and sedge benefited, we have no direct evidence of benefit to the fungus. However, as *P. fortinii* produced intracellular structures possibly analogous to arbuscules or the hyphal coils of ericoid mycorrhizae, it is reasonable to assume that the fungus obtained photosynthates from the host.

How does the pine reaction to *P. fortinii* differ from a reaction to any casual soil or rhizosphere fungus? A major difference is that *P. fortinii* forms intracellular colonizations within the root cortical tissue. As with arbuscular and ericoid mycorrhizae, it has direct contact with the root cells. Some soil fungi such as *Penicillium bilaji* solubilize P from rock phosphate without colonizing root tissue, but the solubilized P is not available to mycorrhiza-dependent host plants in the absence of the mycorrhizal fungus (Kucey, 1987). Accordingly, the enhancement of P uptake by hosts colonized by *P. fortinii* strongly supports the mycorrhizal functioning of that system. The increased N and P uptake in the high N - *P. fortinii* combination also indicates

enhanced absorbing capacity for the host when its roots are colonized, probably because the architecture of the extramatrical hyphae is more efficient at nutrient capture than is that of the roots, and the hyphae serve as a pathway for nutrient transport (Read *et al.*, 1985; Newman, 1988). Various combinations of nonmycorrhizal soil organisms may enhance plant growth (Linderman, 1988), either by mobilizing soil nutrients or producing growth regulators (Shivanna *et al.*, 1994). Suggested mechanisms for such growth promotion are similar to those demonstrated for mycorrhizal fungi and, indeed, have often been deduced with no consideration of whether or not mycorrhizal fungi were functioning in the experimental systems (Linderman, 1988).

Results from our study indicate that plant growth is limited by the low levels of N in the soil in primary successional ecosystem at Lyman Glacier forefront. The function and importance of the root endophytes require further examination. These fungi may function as beneficial root symbionts under some circumstances. Further studies, *e.g.*, with radioisotopes or stable heavy isotopes, are needed to determine the directions of net nutrient fluxes as well as to define the nature of the association between dark-septate root endophytes and their host plants.

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CHAPTER 7

PERFORMANCE OF PINUS CONTORTA INOCULATED WITH TWO STRAINS OF ROOT ENDOPHYTIC FUNGUS, PHIALOCEPHALA FORTINII: EFFECTS OF SYNTHESIS SYSTEM AND GLUCOSE CONCENTRATION

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ABSTRACT

Melanized, root-colonising fungi are ubiquitous. Their ecological role, however, is poorly understood, and results of studies of associations between these fungi and their potential host plants are controversial. The culture system under which the association is studied may also affect the host-fungus interaction. Two experiments on the association between *Pinus contorta* Dougl. and a root-inhabiting endophytic fungus, *Phialocephala fortinii* Wang and Wilcox, were conducted to study the host response to inoculation. First, *P. contorta* seedlings were inoculated with two strains of *P. fortinii* and grown under aseptic conditions with five levels of glucose in the medium. Multiple linear regression analysis was employed to study the effects of inoculation and glucose concentration. Second, the same two strains of *P. fortinii* were inoculated on *P. contorta* seedlings in open pot cultures. Inoculation resulted in substantial increase in all biomass components of the host plant in the aseptic culture system. Total biomass, for example, was increased approximately 60 and 90 % when seedlings were inoculated with strain 1 and strain 2, respectively. No seedling mortality was observed following fungal inoculation after six months of incubation. Inoculation increased host biomass with increasing glucose concentration, while glucose concentration did not significantly affect host biomass when no inoculum was added. Inoculation lowered foliar nitrogen and phosphorus concentrations. The effect of glucose concentration on the foliar nutrient concentrations varied between the two strains. In the open pot cultures, inoculation did not affect biomass or foliar nutrient concentration. We hypothesise that the observed increases in host growth in the aseptic culture system are due to fungal respiration in a closed culture system, the carbohydrates made available to the host plant by the fungus, or, most likely, combination of both. The ecological role of *P. fortinii* and validity of aseptic culture assays are discussed.

INTRODUCTION

Phialocephala fortinii Wang and Wilcox is among the few known anamorphic taxa of root-colonising hyphomycetes belonging to the *Mycelium radicis atrovirens* (MRA; Melin, 1921; 1923) complex of fungi with melanised hyphae. Also referred to as 'dark-septate endophytes' (Haselwandter and Read, 1982), they appear ubiquitous with a circumglobal distribution (Richard and Fortin, 1974) including Antarctica (Heal *et al.*, 1967; Christie and Nicholson, 1983). Even though fungi belonging to MRA frequently inhabit roots of many plant species (Haselwandter and Read, 1982; Currah *et al.*, 1988, Stoyke and Currah, 1991; Cázares, 1992; Stoyke and Currah, 1993; Ahlich and Sieber, 1996; Jumpponen and Trappe, 1996), their ecological role remains poorly understood.

Symbioses between *P. fortinii* and several of its potential host plants can vary from pathogenic to mutualistic depending on host plant and strain of fungus (Wang and Wilcox, 1985; Wilcox and Wang, 1987; Stoyke and Currah, 1991; 1993; O'Dell *et al.*, 1993; Fernando and Currah, 1996; Jumpponen *et al.* unpublished). The experimental culture system often affects the observed host-fungus association (Giltrap, 1982). Exogenous supply of carbohydrates may strengthen the fungal colonisation of host roots (Duddridge and Read, 1984; Gibson and Deacon, 1990; Molina and Trappe, 1992) or produce abnormal behaviour of the host plant or the fungus (Duddridge and Read, 1984). Usually these abnormalities shift the balance of the symbiosis in favour of the fungal partner resulting in, for example, intracellular penetration by the fungus in ectomycorrhizal systems (Duddridge, 1986), death of host cells (Duddridge and Read, 1984) or enhanced ability to colonise normally nonsusceptible host species (Molina and Trappe, 1992).

Here we report two separate experiments on the effects of a root-inhabiting endophytic fungus, *P. fortinii*, on growth of *Pinus contorta* Dougl. Seedlings of *P. contorta* were grown aseptically with five levels of glucose in

the medium and in open pot cultures where no exogenous carbohydrates were supplied. The objectives were to study effects of glucose gradient on mortality and biomass of *P. contorta* inoculated with two strains of *P. fortinii*. The results obtained in the aseptic and open pot cultures were compared to evaluate the significance of the different culture systems as well as to infer the possible ecological importance of *P. fortinii*.

MATERIALS AND METHODS

Aseptic Synthesis

Pinus contorta was selected as a test species because it is colonised by *P. fortinii* (O'Dell *et al.*, 1993; Jumpponen *et al.*, 1998). Seeds were stratified by 24 h incubation in water followed by cold treatment of 14 d at 4°C. Seeds were then surface sterilised with 30% H_2O_2 for 55 min under agitation and aseptically planted onto tap water agar on Petri plates to germinate and screen for possible contamination. Germinating, contamination-free seeds were aseptically transplanted into the culture system described by Molina (1979): glass test tubes 300 x 38 mm filled with *ca* 110 cm^3 of vermiculite-sphagnum peat moss mixture (10 : 1) and 70 ml of modified Melin-Norkrans medium (MMN) (Marx, 1969), capped with 50-ml glass beakers and autoclaved for 30 min. Five concentrations of glucose in the medium, 0, 2.5, 5, 10 and 20 g l^{-1}, were selected to study effects of glucose gradient on plant-fungal interactions.

Seedlings were grown alone for four weeks prior to fungal inoculation (Molina, 1979). Inoculum of *P. fortinii* was added as 0.25 cm^2 plug excised from an edge of an actively growing colony on potato dextrose agar (PDA). Two strains of *P. fortinii* were selected: the type culture isolated from Europe and used by Wang and Wilcox (1985) in describing the form-taxon (strain 1), and a North-American strain (strain 2) isolated by O'Dell *et al.* (1993).

Noninoculated controls received a PDA plug of equal size from an empty plate. Ten replicates of each level of glucose in combination with each inoculum treatment were prepared for a total of 150 individual culture tubes. Due to contamination or failure in seed coat release from the germinants, six synthesis tubes were discarded and excluded from the analyses. This resulted in a total N=144.

Seedlings were grown for six months under a mixture of red-end spectrum fluorescent grow-lights and fluorescent cool white lights at irradiance intensity of 300 μmol m^{-2} s^{-1} within the photosynthetically active range (PAR) with a 16 h photoperiod. To avoid substrate and root overheating, the bottom third of the synthesis tubes was submerged in a 10°C water bath while the ambient temperature ranged from 18–22°C. At harvest the plants and substrate contained in the synthesis tubes were carefully removed and roots were gently cleaned of substrate with running tap water. Root colonisation was observed under a dissecting microscope and confirmed when necessary under a compound microscope. No staining was necessary to confirm the colonization by melanized endophytes; even during the intracellular colonization the fungal hyphae was clearly visible. All root systems of inoculated seedlings were colonised by dark, septate mycelium. No fungi were observed in noninoculated treatments. A small, random subsample was cleared with 10 % KOH and stained with trypan blue to confirm absence of hyaline root colonizing fungi. Colonised root length was estimated by the gridline intersection method (Giovannetti and Mosse, 1980). After root examination, whole plants were dried at 80°C for 48 h and separated into roots, stems and foliage. Total, root, shoot, stem and foliar dry weights were recorded.

Two separate composite samples of the foliage of three randomly selected individual plants in each treatment were collected for foliar nutrient analyses. Foliar tissue was digested by Kjeldahl method (Thomas *et al.*, 1967) and digested solutions were colorimetrically analysed for total nitrogen and phosphorus concentration (% by dry weight) with an Alpkem Rapid Flow

Analysis system Model 300 series. The concentrations of nitrogen and phosphorus in the foliage were also converted to absolute quantities (mg by dry weight).

The data were analysed by multiple linear regression. The glucose gradient was assigned as a continuous variable while the fungal strains were assigned as two 'dummy' variables receiving values of either 0 or 1 indicating absence or presence of a particular strain, respectively (for details in dummy-coding see Jongman *et al.*, 1995; Ramsey and Shafer, 1996). The following multiple linear regression model was fit to the data to estimate the effects of inoculation with the two strains of *P. fortinii* and glucose concentration on biomass components, R : S-ratio, and the foliar nutrient concentrations:

$$\text{Mean}[Y] = b_0 + b_1X_{s1} + b_2X_{s2} + b_3X_{gl} + b_4X_{s1}X_{gl} + b_5X_{s2}X_{gl}$$

where

Y = response variable (biomass component, R : S-ratio, nutrient concentration)

X_{s1} = 'dummy' variable for strain 1, receives a value of 1 if inoculated with strain 1 otherwise 0

X_{s2} = 'dummy' variable for strain 2, receives a value of 1 if inoculated with strain 2 otherwise 0

X_{gl} = glucose concentration (g l^{-1}) in the medium

b_0 = coefficient for the control intercept (reference intercept)

b_1 = coefficient for the difference in intercepts between control and strain 1

b_2 = coefficient for the difference in intercepts between control and strain 2

b_3 = coefficient for the control slope (reference slope)

b_4 = coefficient for the difference in slopes between control and strain 1

b_5 = coefficient for the difference in slopes between control and strain 2

This model describes three different regression lines, one for each inoculation treatment, as a linear function of glucose concentration[5]. The multiple linear regression model allows the following set of individual hypotheses to be tested: (1) if the intercept and slope coefficients for the control treatment differ from zero (H_0: b_0, $b_3 = 0$) and (2) if any of the intercept and slope coefficients for the inoculated treatments differ from those of the control (H_0: b_1, b_2, b_4, $b_5 = 0$). Accordingly, Student's t-tests estimate probability for the hypotheses that a coefficient equals zero in the specified regression model ($b_i = 0$; i=0, 1, 2, 3, 4, 5). A significant *P*-value ($P \leq 0.05$) for coefficients b_1, b_2, b_4 and b_5 indicates a significant difference between the intercept or slope coefficient for the control treatment and that coefficient for either inoculation treatment, while a significant *P*-value for b_0 or b_3 indicates that the intercept or slope coefficient for the control treatment is different from zero.

The control treatment was excluded from the fungal colonisation analysis due to absence of any fungal colonisation in this treatment and the following model was fit.

$$\text{Mean}[Y] = b_1 + b_2X_{s2} + b_4X_{gl} + b_5X_{s2}X_{gl}$$

where parameters are as described above.

5 The three linear regression lines described by the model are as follows:
(1) a response of the host in the control, or reference, treatment (Mean[Y] = $b_0 + b_3X_{gl}$; intercept = b_0, slope = b_3) when both X_{s1} and X_{s2} equal zero,
(2) a response of the host in the treatment inoculated with strain 1 (Mean[Y] = $b_0 + b_1X_{s1} + b_3X_{gl} + b_4X_{s1}X_{gl}$; intercept = $b_0 + b_1$, slope = $b_3 + b_4$) when X_{s1} equals one but X_{s2} equals zero,
(3) a response of the host in the treatment inoculated with strain 2 (Mean[Y] = $b_0 + b_2X_{s2} + b_3X_{gl} + b_5X_{s2}X_{gl}$; intercept = $b_0 + b_2$, slope = $b_3 + b_5$) when X_{s1} equals zero but X_{s2} equals one.
There are no data for the interaction between the two strains ($X_{s1} = 1$ and $X_{s2} = 1$ simultaneously); this was omitted from the model. Intercept and slope coefficients for the three regression lines (Fig. 7.1) described above are displayed in Tables 7.1 and 7.2. Intercept coefficient gives the value at the point where glucose concentration is zero, *i.e.*, the regression line described by the model intercepts the y-axis. The slope coefficient gives the unit response to addition of 1 g l^{-1}

In this analysis, strain 1 was selected as the reference level. The model describes only two different linear regression lines[6].

All statistical analyses were performed with the General Linear Models procedure in SYSTAT™ (1992). Model selection was excluded because the purpose of the analyses was to test hypotheses about variables that were included by statistical design.

Open Pot Synthesis

An open pot synthesis was employed to study the association between the two strains of *P. fortinii* and *P. contorta* seedlings in nonsterile conditions. Inocula of *P. fortinii* were prepared in 2000 ml flasks containing 1200 ml vermiculite and 700 ml MMN and incubated for six weeks. The vermiculite inocula were enclosed in cheesecloth and thoroughly rinsed with running deionised water to remove excess medium and minimise the amount of nutrients not bound in the fungal tissue. The control inoculum (strain 1) was separated and devitalised by autoclaving in 125∞C for 20 min. Ten replicates for each of the two strains and the control with killed inoculum were prepared by mixing the respective inocula with vermiculite-spaghnum peat moss mixture (10 : 1). This resulted in a total N=30. Seeds of *P. contorta,* stratified and sterilised as described for the aseptic synthesis were planted directly into 115 cm^3 plastic Pinecells™ (Stueve & Sons Inc., Corvallis, Oregon) containing the vermiculite inoculum substrate.

6 The two linear regression lines described by the model are as follows:
(1) a response of the colonisation in the treatment inoculated with strain 1 (Mean[Y] = $b_1 + b_4X_{gl}$; intercept = b_1, slope = b_4).
(2) a response of the colonisation in the treatment inoculated with strain 2 (Mean[Y] = $b_1 + b_2X_{s2} + b_4X_{gl} + b_5X_{s2}X_{gl}$; intercept = $b_1 + b_2$, slope = $b_4 + b_5$) when X_{s2} equals one.
Coefficients for the two regression lines are displayed in Table 7.1.

Seedlings were grown for six months under a mixture of red-end spectrum fluorescent grow lights and fluorescent cool white lights at irradiance intensity of 250 μmol m^{-2} s^{-1} (PAR) with a 16 h photoperiod in ambient temperature of 18 – 22°C. The seedlings were watered to saturation two to three times a week. No fertilizer was applied during the study. Harvest and foliar nutrient analyses followed the protocol described for the aseptic synthesis.

Analysis of variance (ANOVA) was employed to study differences between the three inoculation treatments applied in the pot culture synthesis. Pairwise comparisons were not necessary since ANOVA indicated no differences between the different inoculation treatments. All analyses were performed using General Linear Models procedure in SYSTAT™ (1992).

RESULTS

Aseptic Synthesis

The multiple regression models were generally highly significant, indicating that one or more of the included variables significantly differed from zero (see *P*-values for model in Tables 1 and 2). The variation explained by the model (see R^2 in Tables 1 and 2) varied between from *ca* 20 to 80 %, but was generally 50 % for most biomass and nutrient variables, typically leaving half of the variation unexplained (stochastic patterns in the data not due to the explanatory variables included by the statistical design) and thus not accounted for by the design.

Table 7.1. Total, shoot, foliar, stem and root dry weights (mg), R : S-ratio of *Pinus contorta* and colonisation by *Phialocephala fortinii* in the aseptic (N=144; df for regression model = 5, and error = 138) and open pot cultures (N=30; df for model = 2; df for error = 27). Aseptic culture data were analysed using multiple linear regression[a, c]; values = coefficient ± standard error. P-values from Student's t-tests indicate if coefficients different from zero. Open pot culture data were analysed by analysis of variance; values = mean ± standard error. (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Aseptic cultures				
Coefficient[a]	Total dw (mg)	Shoot dw (mg)	Needle dw (mg)	Stem dw (mg)
Intercept coefficients[d]				
b_0 (Control)	64.20 ± 9.13 ***	48.67 ± 6.64 ***	38.88 ± 6.06 ***	9.83 ± 1.26 ***
b_1 (Strain 1)	38.28 ± 13.01 **	22.44 ± 9.54 **	20.00 ± 8.64 *	2.41 ± 1.79 ns
b_2 (Strain 2)	58.34 ± 12.76 ***	35.25 ± 9.35 ***	29.29 ± 8.47 ***	5.93 ± 1.76 **
Slope coefficients[e]				
b_3 (Control)	0.46 ± 0.90 ns	0.52 ± 0.66 ns	0.58 ± 0.60 ns	-0.07 ± 0.12 ns
b_4 (Strain 1)	4.36 ± 1.26 ***	2.49 ± 0.93 **	1.77 ± 0.84 *	0.72 ± 0.17 ***
b_5 (Strain 2)	7.04 ± 1.25 ***	3.98 ± 0.92 ***	3.23 ± 0.83 ***	0.76 ± 0.17 ***
Model (df = 5)	R^2 = 0.657 Model $P < 0.001$	R^2 = 0.554 Model $P < 0.001$	R^2 = 0.511 Model $P < 0.001$	R^2 = 0.530 $P < 0.001$

Open pot cultures				
Treatment	Total dw (mg)	Shoot dw (mg)	Needle dw (mg)	Stem dw (mg)
Control	86.15 ± 9.06	44.40 ± 5.20	32.30 ± 4.52	12.10 ± 1.47
Strain 1	95.30 ± 9.00	52.86 ± 4.73	40.97 ± 3.73	11.89 ± 1.29
Strain 2	89.24 ± 14.03	50.78 ± 7.39	39.88 ± 5.76	10.90 ± 1.70
	P = 0.844	P = 0.586	P = 0.392	P = 0.833

Table 7.1. (continued)

Aseptic cultures			
Coefficient[a]	Root dw (mg)	RS-ratio	Colonization (%)
Intercept coefficients[d]			
b_0 (Control)	15.49 ± 3.80 ***	0.350 ± 0.040 ***	– [b, c]
b_1 (Strain 1)	15.87 ± 5.42 **	0.113 ± 0.057 [ns]	32.70 ± 3.77 ***
b_2 (Strain 2)	23.13 ± 5.32 ***	0.142 ± 0.056 *	-21.20 ± 5.21 ***
Slope coefficients[e]			
b_3 (Control)	- 0.05 ± 0.38 [ns]	-0.005 ± 0.004 [ns]	– [b, c]
b_4 (Strain 1)	1.87 ± 0.53 **	0.010 ± 0.006 [ns]	1.64 ± 0.36 ***
b_5 (Strain 2)	3.05 ± 0.52 ***	0.013 ± 0.006 *	–1.03 ± 0.50 *
Model (df = 5)	R^2 = 0.638	R^2 = 0.261	R^2 = 0.804
	Model $P < 0.001$	Model $P < 0.001$	$P < 0.001$

Open pot cultures			
Treatment	Root dw (mg)	RS-ratio	Colonization (%)
Control	41.75 ± 5.69	1.04 ± 0.25	– [c]
Strain 1	42.43 ± 4.56	0.81 ± 0.04	8.50 ± 3.70
Strain 2	38.45 ± 6.91	0.72 ± 0.08	19.40 ± 5.7
	$P = 0.877$	$P = 0.355$	$P = 0.121$

a = Model: Mean[Y] = $b_0 + b_1X_{s1} + b_2X_{s2} + b_3X_{gl} + b_4X_{s1}X_{gl} + b_5X_{s2}X_{gl}$.

b = The control treatment was excluded from the analysis and strain 1 used as a reference level.

c = Model: Mean[Y] = $b_1 X_{s1} + b_2X_{s1}X_{gl} + b_4X_{s2} + b_5X_{s2}X_{gl}$.

d = Intercept coefficient gives the value at the point where glucose concentration is zero, *i.e.*, the regression line defined by the model intercepts the y-axis.

e = Slope coefficient gives the unit response to 1 g l^{-1} addition of glucose.

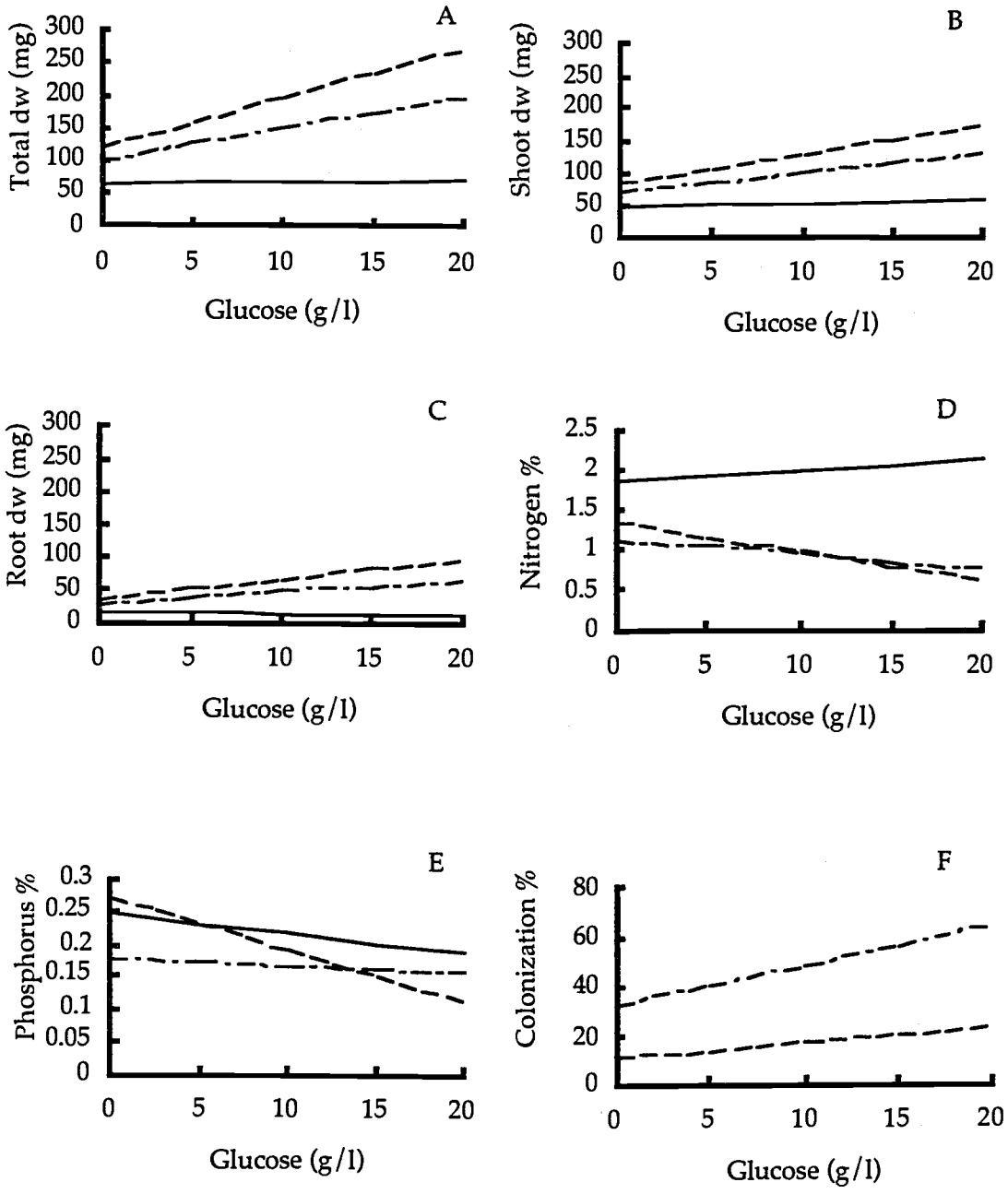
Biomass

All biomass components of *P. contorta* increased in the aseptic culture system as a result of inoculation with two strains of root-colonising fungus, *P. fortinii* (Table 7.1, Fig. 7.1). No seedling mortality was observed after fungal inoculation during the six months of incubation in pure culture synthesis. Increasing glucose concentration did not significantly affect *P. contorta* biomass when no inoculum was added. However, in treatments inoculated with either of the two *P. fortinii* strains, increase in glucose concentration resulted in substantial increase in host biomass.

Total biomass increased by approximately 60 and 90 % when seedlings of *P. contorta* were inoculated with strain 1 and strain 2, respectively, based on the multiple regression analysis (intercepts b_0, $b_0 + b_1$, $b_0 + b_2$ indicate the total biomass in treatment with no added glucose for control, strain 1 and strain 2 treatments in Table 7.1 and Fig. 7.1A, respectively). Addition of glucose significantly increased total biomass of inoculated seedlings (slopes $b_3 + b_4$ and $b_3 + b_5$ in Table 7.1 for treatments inoculated with strains 1 and 2, respectively), while no such significant trend was evident in the noninoculated treatment (coefficient $b_3 = 0.46$; 95% confidence interval from -1.32 to 2.24; $P = 0.361$). Inoculation with strain 1 and strain 2 increased total biomass by approximately 4.82 mg (slope $b_3 + b_4$ in Table 7.1; 95% confidence interval 2.35 – 7.29) and 7.50 mg (slope $b_3 + b_5$ in Table 7.1; 95% confidence interval 5.05 – 9.95),respectively, per g glucose l^{-1} MMN in the substrate. This amounts to more than a 50 % increase in total dry weight, when 12 g or 9 g of glucose is added to the media in cultures inoculated with strains 1 and 2, respectively[7].

7 Values calculated from the coefficients in Table 7.1 as follows: 50 % increase to the biomass in treatments with no glucose (biomass equals intercepts $b_0 + b_1$ and $b_0 + b_2$ for strains 1 and 2, respectively) results from glucose addition of $(b_0 + b_1) \times [2(b_3 + b_4)]^{-1}$ and $(b_0 + b_2) \times [2(b_3 + b_5)]^{-1}$ for strains 1 and 2, respectively.

Figure 7.1. Total (A), shoot (B), and root (C) dry weights, nitrogen (D) and phosphorus (E) concentrations (% by dry weight), and root colonization (F; % colonized root length) of *Pinus contorta* seedlings in controls (—) and treatments inoculated with strain 1 (– – –) and strain 2 (– – –). Values estimated using the multiple linear regression model and the coefficients displayed in Tables 7.1 and 7.2. Note the difference in the scale bars in figures D and E.



The effects of inoculation and glucose gradient on the root biomass were greater than on the total biomass. Inoculation with either strain of *P. fortinii* resulted in more than a two-fold (100 %) increase in root dry weight (compare b_0 to $b_0 + b_1$ and $b_0 + b_2$ Table 7.1, Fig. 7.1C). Again, addition of glucose had no significant effect in absence of the fungus (coefficient $b_0 = -0.05$; 95% confidence interval from – 0.80 to 0.70; $P = 0.623$). The root response to the glucose gradient in the inoculated treatments was proportionally larger than that of the total biomass: an increase of 50 % in root biomass required a glucose addition of 8 or 6g l^{-1} in treatments inoculated with strains 1 and 2, respectively[7].

Shoot, foliar and stem biomass also exhibited a strong positive response to inoculation (Table 7.1, Fig. 7.1B). Again, shoot, foliar, and stem biomass increased significantly with increasing glucose concentration when inoculated with either of the two strains. Due to the proportionally larger increase in the root than in the shoot biomass the R : S-ratio was significantly larger in the treatments inoculated with strain 2 than in controls. The addition of glucose resulted in larger growth response in the roots than in the shoots of the seedlings inoculated with either fungal strain. Accordingly, the R : S-ratio increased significantly (Table 7.1).

Root colonisation varied from 5 to 100 % and 1 to 50 % in seedlings inoculated with strains 1 and 2, respectively. Colonisation was approximately three times higher with strain 1 than strain 2 (Table 7.1, Fig. 7.1F). Colonisation increased by 1.64 (95% confidence interval 0.91 – 2.26) and 0.61 percent units (95% confidence interval – 0.40 – 1.62) per gram of glucose added to the medium for strains 1 and 2, respectively. This interprets to 50 % increase in observed colonisation, when 10 g (95% confidence interval 7.23 – 17.96) or 9 g [95% confidence interval from 3.54 to infinity (*i.e.*, no addition would increase colonisation)] of glucose is added to the media in cultures inoculated with strains 1 and 2, respectively[7].

Nutrient Analyses

Foliar total nitrogen concentration (% by dry weight) was lowered as a result of fungal inoculation (Table 7.2, Fig. 7.1D): strain 1 resulted in almost 40 % and strain 2 in almost 30 % decrease in the total nitrogen in foliage. Glucose concentration did not affect total foliar nitrogen except when seedlings were inoculated with fungal strain 2, which lowered foliar nitrogen concentration with increasing glucose concentration (95 % confidence interval - 0.077 to 0.005 percent units per gram of glucose added). Foliar total phosphorus concentration was not affected by glucose gradient or inoculation with strain 2 but was decreased by almost 30 % as a result of inoculation with strain 1 (Table 7.2, Fig. 7.1E). Inoculation with strain 2 did not affect the foliar phosphorus concentration (% by dry weight) but resulted in over a two-fold increase in the total foliar phosphorus content (mg in foliar tissue). Addition of glucose did not affect the foliar nutrient concentrations in the treatments which received no inoculum.

Open Pot Synthesis

No significant inoculation effects on biomass components or foliar nutrient concentrations were observed in the open pot cultures (Tables 7.1 and 7.2). In the open pot synthesis, root colonisation varied from 0 to 35 % and from 4 to 48 % in seedlings inoculated with strain 1 and strain 2, respectively. Root colonisation by the two strains did not differ significantly.

Table 7.2. Foliar nitrogen and phosphorus concentration (%) and content (mg) of *Pinus contorta* in the aseptic (N=30; df for regression model = 5, and error 24) and open pot cultures (N=6; df for model = 2, and error = 3). Legend as in Table 7.1. (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

	Aseptic cultures			
Coefficient[a]	N concentration (%)	N content (mg)	P concentration (%)	P content (mg)
Intercept coefficients[b]				
b_0 (Control)	1.867 ± 0.142 ***	2.189 ± 0.507 ***	0.248 ± 0.020 ***	0.286 ± 0.091 **
b_1 (Strain 1)	-0.738 ± 0.201 **	-0.180 ± 0.717 ns	-0.069 ± 0.029 *	0.048 ± 0.128 ns
b_2 (Strain 2)	-0.511 ± 0.201 *	-1.037 ± 0.717 ns	0.025 ± 0.029 ns	0.346 ± 0.128 *
Slope coefficients[c]				
b_3 (Control)	0.013 ± 0.013 ns	0.049 ± 0.049 ns	-0.003 ± 0.002 ns	-0.001 ± 0.009 ns
b_4 (Strain 1)	-0.030 ± 0.020 ns	-0.020 ± 0.070 ns	0.002 ± 0.003 ns	0.006 ± 0.012 ns
b_5 (Strain 2)	-0.049 ± 0.020 *	-0.066 ± 0.070 ns	-0.005 ± 0.003 ns	-0.006 ± 0.012 ns
Model (df = 5)	$R^2 = 0.742$	$R^2 = 0.164$	$R^2 = 0.561$	$R^2 = 0.356$
	Model $P < 0.001$	Model $P = 0.473$	Model $P = 0.001$	Model $P = 0.048$

	Open pot cultures			
Treatment	N concentration (%)	N content (mg)	P concentration (%)	P content (mg)
Control	0.660 ± 0.012	0.633 ± 0.146	0.129 ± 0.016	0.120 ± 0.016
Strain 1	0.629 ± 0.145	0.832 ± 0.178	0.109 ± 0.032	0.144 ± 0.040
Strain 2	0.696 ± 0.088	0.795 ± 0.129	0.123 ± 0.013	0.139 ± 0.009
	$P = 0.892$	$P = 0.656$	$P = 0.809$	$P = 0.796$

a = Model: $\text{Mean}[Y] = b_0 + b_1X_{s1} + b_2X_{s2} + b_3X_{gl} + b_4X_{s1}X_{gl} + b_5X_{s2}X_{gl}$.

b = Intercept coefficient gives the value at the point where glucose concentration is zero, *i.e.*, the regression line defined by the model intercepts with the y-axis.

c = Slope coefficient gives the unit response to 1 g l^{-1} addition of glucose.

DISCUSSION

We observed a substantial increase in *P. contorta* biomass when inoculated with two strains of *P. fortinii* under aseptic conditions while no such response could be observed in the open pot cultures. Increase in the glucose concentration resulted in substantial increase in host biomass in the aseptic system when fungus was present but not when no inoculum was added. These results indicate that the inoculation bioassays can lead to different conclusions depending on the conditions under which the syntheses are performed. Moreover, the association between *P. fortinii* and its potential host plants cannot be clearly categorised as either parasitic, pathogenic or mutualistic on the basis of these results.

Growth responses as well as nutrient acquisition have traditionally been considered indicators of the nature of the symbiosis (*sensu* de Bary 1887) between host plant and its root associated fungal symbionts (Harley and Smith, 1983; Francis and Read, 1995; Smith and Read, 1997). In this study, biomass of *P. contorta* was increased under aseptic conditions due to inoculation with *P. fortinii*. Previous results from synthesis studies suggest neutral (O'Dell *et al.*, 1993; Fernando and Currah, 1996), parasitic or pathogenic (Wang and Wilcox, 1985; Wilcox and Wang, 1987; Stoyke and Currah, 1993; Fernando and Currah, 1996) association between *P. fortinii* and its hosts. Positive growth responses to inoculation with *P. fortinii* are rarely reported (but see Stoyke and Currah, 1993; Fernando and Currah, 1996; Jumpponen *et al.*, 1998). Most of these previous studies, however, included no statistical evaluation nor any biomass measurements, but were based on visual appearance or observed mortality during the study (Wang and Wilcox, 1985; Wilcox and Wang, 1987; O'Dell *et al.*, 1993). The strong positive growth response observed in this study conflicts with the earlier reports. However, as opposed to the aseptic culture system, no significant effects on *P. contorta* growth occurred in the open pot cultures when inoculated with the same two strains of *P. fortinii*. This exemplifies the difficulty of assaying host–fungus

associations in culture systems with only the fungal associate and its host plant. Subtle responses in our open pot cultures make interpretation of results difficult. In addition, results vary depending on the conditions under which the association between the host plants and their fungal associates are tested. For example, Jumpponen *et al.* (1998) observed no growth response to inoculation of *Pinus contorta* with a strain of *P. fortinii* under low nitrogen regimen while inoculation increased host biomass by approximately 50% when nitrogen fertilizer was applied. Similarly, in the current study, no host responses were observed in open pot cultures which received no additional nutrients. It is difficult, therefore, to definitely assess the association between *P. fortinii* and its potential host plants as either parasitic, pathogenic or mutualistic based on inoculation studies alone.

Original objective of this study was to determine whether *P. fortinii* would more aggressively colonise and increase host mortality when higher levels of glucose were available in the medium. Results of pure culture synthesis studies suggest that *P. fortinii* can be pathogenic reducing host vigour, and occasionally killing (Wilcox and Wang, 1987), or physically overgrowing and finally suffocating the host plant (Stoyke and Currah, 1993). Surprisingly, we observed no mortality in the experiments employing either aseptic or open pot cultures. On the contrary, biomass of *P. contorta* was substantially increased as a result of inoculation. Furthermore, increase in glucose concentration, *i.e.*, increase in readily available carbohydrates for the fungus, resulted in an additional substantial increase in host biomass in the pure culture systems inoculated with either of the two *P. fortinii* strains. It is essential to note that glucose concentration in the aseptic system had no significant effect on the *P. contorta* biomass when no fungus was present. It is possible that the fungus transferred the carbohydrates in the growing medium to the host plant (McDougal and Dufrenoy, 1946), a situation that may occur in synthesis where easily accessible simple carbohydrates are made available (Duddridge and Read, 1984). It has been shown that carbohydrates can be transferred through mycelial strands of ectomycorrhizal fungi under

natural conditions (Finlay and Read, 1986). Based on significant increase in foliar phosphorus concentration due to inoculation with *P. fortinii*, Jumpponen *et al.* (1998) concluded that *P. fortinii* may function as a mutualistic mycorrhizal symbiont under some circumstances and participate in nutrient uptake and transport as shown in mycorrhizal systems (Read *et al.*, 1985; Newman, 1988; Simard *et al.*, 1997). Alternatively, fungal respiration elevated the CO_2 concentration in the culture system with poor gas exchange. This allowed the host to efficiently continue photosynthesis and incorporation of carbon into its living biomass resulting in the observed strong growth response. Increased growth due to elevated ambient CO_2 concentration has been reported frequently (Wong *et al.*, 1992; Poorter, 1993; Baxter *et al.*, 1994a, Idso and Idso, 1994; Roumet *et al.*, 1996). During the harvest, we observed visually that the mycelial colonisation of the substrate was clearly more extensive in the treatments with higher concentration of glucose. Increased fungal biomass resulting in higher level of maintenance respiration and elevated CO_2 concentration, would not only explain the observed strong growth response to the inoculation and glucose concentration, but also the discrepancy between the results of the aseptic and open pot syntheses. Growth responses observed in our study, however, are very large compared to the growth responses attributed to a two-fold increase in CO_2 concentration reported by Poorter (1993). Although we did not measure the final CO_2 concentration in the culturing systems, it is unlikely that CO_2 concentration alone accounted for the total increase in biomass accumulation. A more probable explanation is that a combination of the carbohydrate transfer by the fungus and the elevated CO_2 concentration due to fungal respiration resulted in the increased host biomass accumulation that was associated with increased glucose concentration seen in our study. The more extensive mycelial colonisation of the substrate in treatments with higher glucose concentration may also have improved the acquisition of other nutrients (*e.g.*, phosphorus).

Foliar concentration of nitrogen was lowered as a result of fungal inoculation in the aseptic culture system. The role of *P. fortinii* in nutrient acquisition and transport remains unclear although Jumpponen and Trappe (1996) and Jumpponen *et al.* (1998) hypothesised that *P. fortinii* facilitate nutrient transport via the mycelial network. Additionally, increased phosphorus acquisition has been shown in plants inoculated with *P. fortinii* (Jumpponen *et al.*, 1998). We hypothesise that in our experiment, enhanced plant growth increased the nitrogen requirement resulting in the observed lower foliar nitrogen concentration in the inoculated treatments. The inoculation increased the foliar biomass by more than 50%. Because no nutrients were added to the aseptic system during the course of this study nor was the experiment conducted in a non-limiting system, the dilution of nutrient concentration due to increased biomass could be assumed. Enrichment of CO_2, here due to fungal respiration, may result in similar decrease in foliar nitrogen concentration (Luo *et al.*, 1994; Roumet *et al.*, 1996), some of which simply attributed to a dilution by larger biomass (Baxter *et al.*, 1994b, Roumet *et al.*, 1996). Even though the glucose gradient resulted in no significant reduction in the foliar nitrogen concentration except when inoculated with strain 2, the regression coefficients suggest a negative association between glucose and foliar nitrogen concentration in both inoculated treatments. This further supports the dilution of nitrogen due to larger carbohydrate accumulation in the treatments inoculated with *P. fortinii*.

No inoculation effects similar to those in the aseptic cultures on biomass components or foliar nutrient concentration were observed in the open pot cultures. Results from these two experiments indicate that the conclusions from synthesis studies may vary depending on the culture conditions. Pure culture syntheses have been criticised in several occasions due to the bias introduced by use of a aseptic culture system (Duddridge and Read, 1984; Molina and Trappe, 1992). Similarly, such systems poorly reflect the natural environment where the host-fungus interaction takes place.

Furthermore, despite their obvious value to well-being of a plant individual, neither nutrient acquisition nor biomass accumulation may reliably judge host-fungus association, whose effects extend over the life of a plant individual. Studies able to account for measures of long term durability and persistency such as fitness seem timely. Fitness of a species or an individual includes not only survival or biomass accumulation but also ability to contribute towards the future community via reproduction. Similarly, holistic studies of plant communities and their association with root-colonising fungal communities, including mycorrhizal and endophytic fungi, are necessary to provide insights to the dynamics and function of below-ground communities and shed light on the poorly understood components of the below ground ecosystems.

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CHAPTER 8

SPATIAL DISTRIBUTION OF DISCRETE RAPD-PHENOTYPES OF A ROOT ENDOPHYTIC FUNGUS, PHIALOCEPHALA FORTINII, AT A PRIMARY SUCCESSIONAL SITE ON A GLACIER FOREFRONT

Ari Jumpponen

ASTRACT

Phialocephala fortinii Wang and Wilcox is among the few known anamorphic taxa of root-colonising hyphomycetes belonging to the *Mycelium radicis atrovirens* complex. This 'dark-septate endophyte' has a global distribution and colonises a wide variety of host plants. In the present study, spatial distribution of discrete genets of *P. fortinii* on the forefront of a receding glacier was assayed by randomly amplified polymorphic DNA (RAPD) technique. A total of 74 isolates of *P. fortinii* were obtained from nine plant species, typically ecto-, ericoid-, or non-mycorrhizal. The isolates expressed a substantial variation, sharing approximately half of the RAPD-markers on the average. Three isolates belonging to a single genet were obtained from two plant individuals separated by a distance of nearly 2 m. The continuity of this genet was assessed by a subsequent sampling the following summer. No isolates similar to any of the genets collected the year before were observed. Consequently, the identical isolates from the previous year were concluded to represent discontinuous ramets. Two additional larger genets were observed between the identical isolates. These inhabited roots of several plant individuals representing three different species. The results are discussed with respect to host specificity, ecological role of the dark-septate endophytes and their population dynamics.

INTRODUCTION

Phialocephala fortinii Wang and Wilcox is among the few known anamorphic taxa of root-colonising hyphomycetes belonging to the *Mycelium radicis atrovirens* (MRA; Melin, 1922, 1923) complex. Also referred to as 'dark-septate endophytes' (Haselwandter and Read, 1982), they appear ubiquitous with a circumboreal (Richard and Fortin, 1974) or global distribution including maritime Antarctic (Christie and Nicolson, 1983) and

extreme arctic habitats (Väre *et al.*, 1992). Although fungi belonging to the MRA complex frequently inhabit roots of many plant species (Haselwandter and Read, 1980; 1982; Currah *et al.*, 1988; Stoyke and Currah, 1991; Cázares, 1992), their ecological role remains poorly understood.

Cázares (1992) discovered that the dark-septate endophytes were common to most plants in primary and early successional communities on the forefront of the rapidly receding Lyman Glacier of the North Cascade Mountains of Washington State, USA. The plants harbouring the dark-septate endophytes included species that are usually considered ecto-, VA-, ericoid-, or non-mycorrhizal. Cázares (1992) suggested that *Salix* spp. characteristically established as individual shrubs; subsequently, a variety of other plant species would become established in the immediate proximity of these shrubs. Furthermore, Cázares (1992) concluded that dark-septate endophytes were common on roots of all species in these willow-patch communities.

The tools and principles of population ecology have been applied to studying fungal communities only recently. This is mainly due to the inability to recognise fungal individualism and, furthermore, the difficulty of identifying individual units of fungal mycelium (Dahlberg, 1995). The terms genet and ramet, commonly used for vegetatively extending higher plants (Harper, 1977), have been adopted in describing genetically different mycelia and identical units resulting from asexual or vegetative propagation (Brasier and Rayner, 1987). Here, a genet is defined as a collection of isolates with identical phenotypes inferred from randomly amplified polymorphic DNAs (RAPDs), while a ramet refers to a collection of such identical phenotypes likely to be physically continuous.

Several approaches have been used to identify fungal genets. Somatic incompatibility, which refers to the fungal system of recognising 'self' and 'nonself' mycelium resulting in either acceptance or rejection, has been applied to fungi with both pathogenic (see Rayner *et al.*, 1984; Rayner 1991) and mutualistic (see Dahlberg, 1995; Dahlberg and Stenlid, 1995) associations

with their host. Similarly, allozyme patterns have in some cases enabled identification of genets in fungi (Sen, 1990; Rodrigues *et al.*, 1995; el Karkouri *et al.*, 1996). The recent, rapid evolution of molecular techniques has facilitated the development of tools that can be applied to fungal populations. Restriction fragment length polymorphism (RFLP; Egger *et al.*, 1991; Bae *et al.*, 1994; Egger, 1992; Smith *et al.*, 1992; Matsumoto and Fukumasa-Nakai, 1995), RAPDs (Jacobson *et al.*, 1993; de la Bastide *et al.*, 1994; Liew and Irwin, 1994; Peever and Milgroom, 1994; Gosselin *et al.*, 1995; Raffle *et al.*, 1995; Perotto *et al.*, 1996;) and amplified fragment length polymorphism (AFLP; Mueller *et al.*, 1996) have been reported useful and efficient in assaying of both spatial and temporal distribution and variation of genets in populations of fungi.

The main objective of this study was to expand current knowledge of dark-septate root endophytes and their potential functions in their natural environment by assaying the spatial distribution of discrete RAPD-phenotypes (genets) of *P. fortinii*. *Phialocephala fortinii* appears to be among the major root-colonising fungal components during the early stages of the primary successional alpine ecosystem at Lyman Glacier, Washington, USA. Studies described here were conducted to detect whether large, single genets of *P. fortinii* simultaneously colonise root systems of several established plant individuals in a developing primary successional plant community, or if *P. fortinii* comprises a fungal population of several small, genetically discrete individuals. It was hypothesised that dark-septate mycelium connecting roots of already established *Salix*-shrubs with roots of their understory plants could serve as a link for nutrient and carbon transfer between the willows and the understory plants.

MATERIALS AND METHODS

Site Description

Lyman Lake basin (48° 10′ 14″ N, 120° 53′ 44″ W) is located in the Glacier Peak wilderness area in the North Cascades Mountain range in Washington, USA. The basin contains distinctly different, primary and secondary successional habitats. The primary successional forefront habitat has rapidly deglaciated during the past century. The glacier has not exceeded its terminal moraine of the late 1890's for the past ca 6,900 years, as beyond that moraine is an undisturbed deposit of volcanic ash from the Mount Mazama eruption. The glacier has been receding steadily since the 1890s, vacating an 1100 m forefront for invasion by pioneering colonisers. The elevation of the present terminus is approximately 1800 m. The forefront parent material is a heterogeneous glacial till ranging from clay-sized particles to boulders intermingled with deposits of glacial-fluvial sediments (for a description of the chemical characteristics see Cázares, 1992). The glacier and its forefront occupy a cirque and a north-south oriented, U-shaped valley bounded by cliffs that rise up to 600 m above the valley floor and culminate at the 2430-m high Chiwawa Mountain at the head of the cirque. The steep cliffs surrounding the glacier and its forefront have facilitated the maintenance of the indigenous flora: no introduced, exotic weeds have been detected thus far (Jumpponen *et al.* 1997). Therefore, the Lyman Glacier forefront site supports a naturally evolved primary successional community. Vegetation is developing from the early colonisation of barren parent material to a plant community resembling that beyond the terminal moraine. The course of the vegetation change on Lyman Glacier forefront is described more in detail in Cázares (1992) and Jumpponen *et al.* (1998*b*).

Sampling

Three representative willows (*Salix commutata* Bebb and *Salix phylicifolia* var. *planifolia* (L.) Sm.) were selected in 1993 on each of two areas deglaciated approximately 30 and 65 yr ago. Ten randomly selected plant individuals were dug up in the immediate surroundings of each of the selected willows, totaling sixty individuals of various indigenous species. Distribution of the sampled plant individuals is shown in Figures 8.1 and 8.2. The soil and attached root systems were kept intact to minimize damage to the root systems or endophytes within the roots. The samples were sealed in plastic bags for transport to the laboratory and kept cold with ice from the glacier until they could be stored in a 4°C refrigerator. All the root samples were processed within two weeks of sampling.

The site was visited again during the next summer (1994). This time, the same three willow shrubs included in the first sampling from the area deglaciated 30 yr ago were resampled. An area where a single genet was isolated in 1993 from the roots of two separated *Phyllodoce empetriformis* (Sw.) D. Don individuals was intensively sampled to test the physical continuity of this genet. In this second sampling, the willow roots were excavated and followed within this area. Ten plants with their roots intermingled with the willow roots were sampled together with the willow roots. This resulted in twenty root samples from each of the three selected willow shrubs, ten from the willow and ten from adjacent indigenous species. Once collected, the samples were treated as during the first sampling.

Isolation of the Root Endophytes

Each root sample was washed free from attached soil. Ten pieces of roots, approximately 1 cm in length, with dark-septate mycelium were collected from each root sample using a stereo microscope. Root pieces were stored in water until surface sterilised in 30 % H_2O_2 for 30-60 s and transferred toPetri plates containing 25 ml of MMN (Modified Melin Norkrans) media (Marx, 1969) without rinsing. The root pieces were incubated at room temperature until fungal colonies were detected, usually in less than two weeks. Fungal colonies possessing the macroscopic characteristics of *P. fortinii* (as described in Wang and Wilcox, 1985 and Currah and Tsuneda, 1993) were selected and transferred onto a new plate. The macroscopic and microscopic characteristics were checked as isolates developed; the colour and texture of the colony, the presence of papillate hyphae, toruloid cells, and hyphal coils and strands were checked (Currah and Tsuneda, 1993). Isolates were stored at 4°C for three to six months to enhance conidiophore production (Currah and Tsuneda, 1993); no conidiophores were detected. The identity of the isolates was confirmed by comparison of the RFLPs of PCR-amplified (Polymerase Chain Reaction; Saiki *et al.*, 1988) ITS-region (Internal Transcribed Spacer) of nuclear ribosomal RNA gene (Gardes *et al.*, 1991; Erland *et al.* 1994; Gardes and Bruns, 1996*a*; 1996*b*) with the type culture of *P. fortinii* (Wang and Wilcox, 1985). The ITS-region was amplified with primers ITS1 and ITS4 with the cycle parameters described in Lee and Taylor (1990) and digested according to the manufacturer's instructions with 5 units of restriction enzymes, MspI, RsaI and TaqI (Promega, Madison, Wisconsin). Only two of the macroscopically prescreened isolates possessed an RFLP pattern different from that of the type culture (data not shown); these were omitted from further analyses. Representative isolates were deposited in the ectomycorrhizal culture collection at the Pacific Northwest Research Station, Forestry Sciences Laboratory, Corvallis, Oregon.

DNA Extraction and RAPD Analysis of Isolates

The fungal tissue for DNA extraction was grown on a permeable cellophane membrane covering MMN Petri plates. The whole colony, with the transfer plug excluded, was scraped from the membrane with a microscope slide within two weeks after transferring and placed into a 1.5 ml Eppendorf tube. The tube and its contents were frozen in liquid nitrogen and stored in -20°C until the time of DNA extraction.

DNA was extracted using the protocol of Lee and Taylor (1990) with these modifications: the tissue was ground with a micropestle attached to an electric drill in 50 µl of the lysis buffer (Lee and Taylor, 1990), followed by an addition of 350 µl of the buffer and incubation of 30 min in 65°C. The (25/24/1) extraction was repeated twice prior to precipitation with -20°C 90% ethanol and vacuum drying. DNA was resuspended in 100 µl of TE buffer and 4 µg of RNAse-A was added. The RNA was digested for 60 minutes, the phenol/chloroform/isoamyalcohol extraction repeated two more times and followed by precipitation and drying as above and, finally, resuspension in 25 µl of TE. The DNA concentration was measured with a fluorometer and each extract diluted to a final concentration of 0.5 ng ul^{-1}.

RAPDs were generated by PCR with four (UBC409, UBC431, UBC438 and UBC450; TAG GCG GCG G, CTG CGG GTC A, AGA CGG CCG G, CGG AGA GCC C, respectively) arbitrary decamers (Williams *et al.* 1990) chosen from the fifty that were included in the preliminary screening (UBC401 – UBC450; University of British Columbia, Vancouver, BC, Canada). Amplification was performed in 25 µl reaction volumes [1x reaction buffer supplied with Taq polymerase, 1.3 µg $µl^{-1}$ BSA, 100 µM dNTPs, 1.8 µM $MgCl_2$, 0.2 µM primer, 1 unit of Taq polymerase (Promega, Madison, Wisconsin) and 2 ng of template DNA]. The cycle consisted of 3 minutes at 93°C (predenaturation) followed by 44 cycles of 1 minute at 93°C (denaturation), 1 minute at 37°C (annealing) and 2 minutes at 72°C (extension). The final

extension step was set to 10 min at 72°C to assure the completion of the reaction. Electrophoresis of the reaction products was performed in 2% agarose gels in 1X TBE buffer. PCR products were visualised in the gel by staining with ethidium bromide. Gels were photographed on a UV transilluminator.

The samples from 1993 and 1994 were analysed separately. The loci from the two separate samplings were not considered comparable due to potential reproducibility problems (see Meunier and Grimont, 1993; Tommerup *et al.*, 1995). Consequently, continuity of the identical isolates detected in 1993 was tested via a side by side comparison with the samples from 1994. All samples were amplified as two replicates within a reaction and all the reactions were performed twice, totalling four repeated reactions per sample. Only the consistently reproduced amplicons were scored for presence or absence, 1 or 0, respectively. Approximately 80 % of the amplicons were scored due to reproducibility and consistency. This resulted in a total of 45 fragments (6-18 fragments per primer) in the analysis of 34 isolates obtained in the 1993 sampling and 44 fragments (4-18 fragments per primer) in the analysis of the 40 isolates from the 1994 sampling.

A pairwise Jaccardian similarity matrix[8] was calculated from the binary (presence or absence) data using the CORR procedure in SYSTAT (1992). This matrix was used to generate a phenetic dendrogram by the simple linkage method (nearest neighbour linkage) using the CLUSTER procedure (SYSTAT, 1992).

8 Jaccardian pairwise similarity for two isolates x and y: $F_{xy}(F_{xy} + F_x + F_y)^{-1}$, where
F_{xy} = total number of amplicons common to both isolates x and y
F_x = total number of amplicons unique to isolate x
F_y = total number of amplicons unique to isolate y

RESULTS

The molecular tools used in this study proved powerful in identifying the isolates similar to *P. fortinii*. Macro- and micromorphological characteristics were usually reliable for identification of the obtained isolates despite the absence of asexual reproductive structures (conidiophores); polymorphisms between isolates morphologically similar to the type culture of *P. fortinii* were observed only infrequently in the ITS-RFLP assay (data not shown). The RAPD assay reproducibly identified a range of discrete phenotypes.

Isolation attempts from 17 of a total of sixty plant individuals sampled in 1993 resulted in one or more *P. fortinii* isolates per plant, 34 isolates in total. *P. fortinii* was isolated at a similar frequency from areas deglaciated 30 and 65 yr ago: 9 *vs.* 8 plant individuals were colonised by *P. fortinii* resulting in 19 *vs.* 15 isolates, respectively (Table 8.1). One to four isolates of *P. fortinii* were obtained from a single root system in the 1993 sampling (Table 8.1). The isolates from the root systems of individual plants represented one or two genets. In the subsequent sampling in 1994, up to seven isolates per root system were obtained from 12 of the twenty plants sampled within the area where the recovery rate was greatest in 1993 (Table 8.1, Fig. 8.1). These isolates represented up to four genets per root system. In the two subsequent samplings in 1993 and 1994 at the Lyman Glacier forefront, isolates of *P. fortinii* were obtained from nine plant species, typically ecto-, ericoid, or non-mycorrhizal.

Table 8.1. *Phialocephala fortinii*-harbouring plant species at Lyman Glacier forefront in 1993 and 1994. Sample number refers to a location where sample was collected (Fig. 1 and 2). Number of isolates indicates total number of isolates obtained from the root system of a given plant individual, number of genets refers to discrete RAPD phenotypes identified among these isolates. The numbers in parentheses indicate which of the three larger genets in Fig. 1 were isolated from that plant individual. Nomenclature follows Hitchcock and Cronquist (1973).

Deglaciated	Year	Host Plant	Sample Number	Mycorrhizal Habit	Isolates	Genets
30 yr ago	1993	*Phyllodoce empetriformis*	1	Ericoid	4	2(1)
–	1993	*P. empetriformis*	2	Ericoid	1	1
–	1993	*P. empetriformis*	3	Ericoid	2	1(1)
–	1993	*P. empetriformis*	6	Ericoid	2	1
–	1994	*P. empetriformis*	12	Ericoid	3	1(3)
–	1994	*P. empetriformis*	14	Ericoid	4	1(3)
–	1994	*P. empetriformis*	16	Ericoid	2	2(3)
–	1994	*P. empetriformis*	18	Ericoid	3	2(2)
–	1994	*P. empetriformis*	20	Ericoid	1	1
–	1994	*P. empetriformis*	21	Ericoid	2	1(2)
–	1993	*Phyllodoce glanduliflora*	9	Ericoid	2	1
–	1993	*Salix commutata*	4	Ecto	2	1
–	1993	*S. commutata*	5	Ecto	2	1
–	1994	*S. phylicifolia*	10	Ecto	7	4(2)
–	1993	*Juncus sp.*	7	Non	1	2
–	1994	*Juncus sp.*	13	Non	4	1(3)
–	1994	*Juncus sp.*	15	Non	5	1(3)
–	1993	*Tsuga mertensiana*	8	Ecto	3	1
–	1994	*Luzula piperi*	11	Non	4	2(2,3)
–	1994	*L. piperi*	17	Non	3	2(2,3)
–	1994	*L. piperi*	19	Non	2	1
65 yr ago	1993	*Juncus sp.*	22	Non	3	2
–	1993	*P. empetriformis*	23	Ericoid	1	1
–	1993	*P. empetriformis*	24	Ericoid	2	1
–	1993	*P. empetriformis*	27	Ericoid	2	2
–	1993	*P. empetriformis*	28	Ericoid	3	2
–	1993	*Cassiope mertensiana*	25	Ericoid	2	2
–	1993	*S. phylicifolia.*	26	Ecto	1	1
–	1993	*Vaccinium deliciosum*	29	Ericoid	1	1

Figure 8.1. Plants sampled (o = sampled 1993, + sampled 1994, no *P. fortinii* isolated) in the area deglaciated 30 yr ago. Table 8.1 lists plants which hosted *Phialocephala fortinii* in 1993 (samples 1-9) and 1994 (10-21). Arrows indicate the two identical RAPD-phenotypes (Genet 1). The plants within the circles hosted *Phialocephala fortinii* Genets 2 and 3. Asterisk indicates plants with more than one genet. Number ten refers to root samples of the *Salix phylicifolia* harboring *Phialocephala fortinii* in 1994; associated species is indicated by the lower right number.

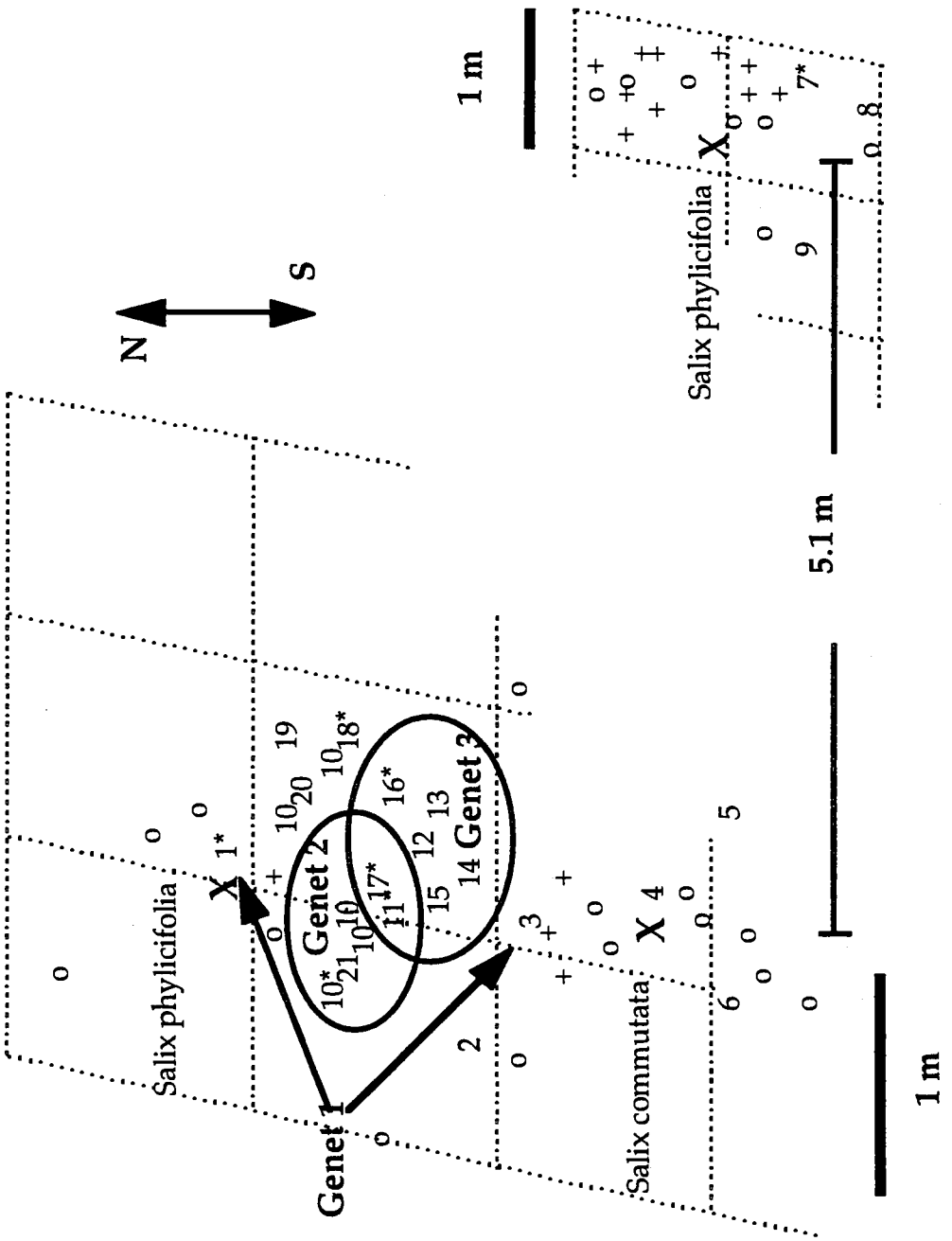
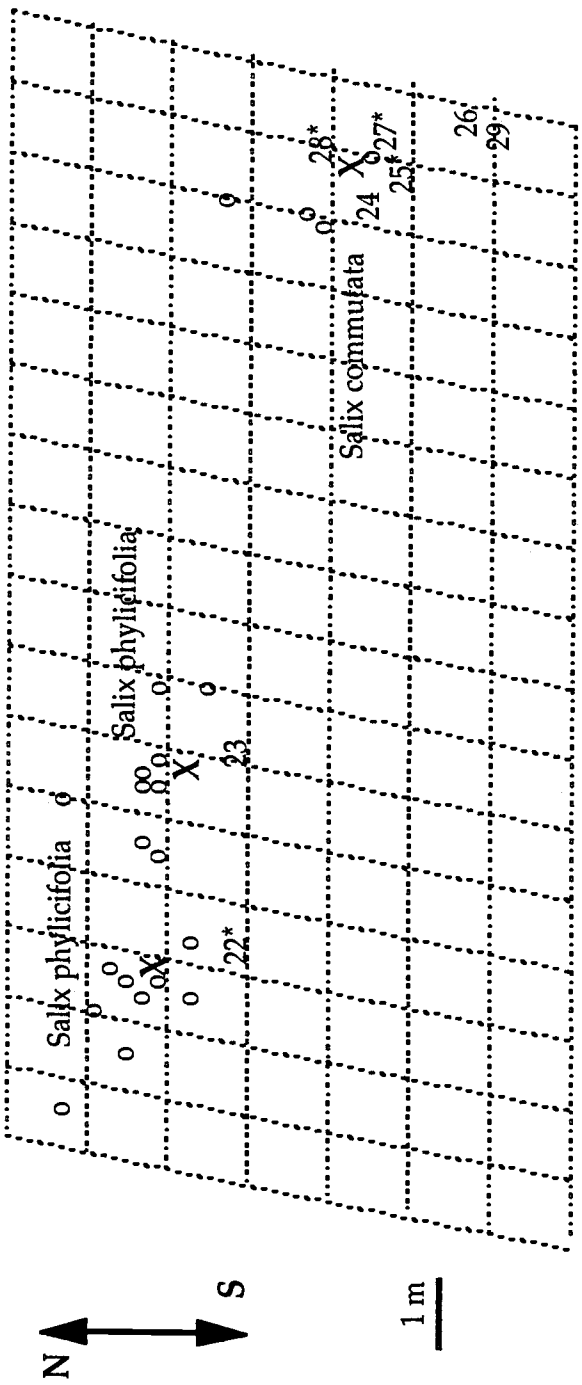


Figure 8.2. Distribution of plant individuals sampled in 1993 (o = isolations yielded no *P. fortinii*) at the Lyman Glacier forefront in the area deglaciated approximately 65 yr ago. Table 8.1 lists the sampled plant individuals from which *Phialocephala fortinii* was isolated (samples 22-29).



Despite the monomorphism in ITS-RFLPs, the genets inferred from RAPD phenotypes were highly variable sharing an average of 52.8 % (± 15.2 % standard deviation) and 85.0 % (± 16.1 % standard deviation) of the fragments scored in the analysis of 1993 and 1994 data, respectively (Figures 8.3 and 8.4). The genet corrected, *i.e.*, with monomorphic RAPD-phenotypes removed, averages were 51.7 % (± 15.5 %) and 78.5 % (± 15.2 %) for the data from 1993 and 1994, respectively. Even the genets obtained from a single root system occasionally shared less than forty percent of the fragments (*e.g.*, isolates 2201, 2202 and 2203 from *Juncus* sp. in Fig. 8.3).

In the 1993 sampling, a single genet was detected from root systems of two *Phyllodoce empetriformis* individuals separated by a distance of almost 1.5 m (Genet 1 in Fig. 8.1). The following year, this area was revisited and intensively sampled to test the continuity of this genet. Forty isolates of *P. fortinii* were obtained from 12 colonised plant individuals. A *Salix phylicifolia* shrub, whose roots were followed in the 1994 sampling, shared a genet (Genet 2 in Fig. 8.1) with two *Phyllodoce empetriformis* and two *Luzula piperi* individuals in addition to three genets unique only to the *S. phylicifolia* roots alone. The shared fungal genet was thus isolated from the roots of five different plant individuals representing three different taxa. The two *L. piperi* plants that shared Genet 2 harboured another common genet (Genet 3 in Fig. 8.1). Genet 3 was found to be shared among three individual *P. empetriformis*, two *L. piperi* and two *Juncus* sp. plants but was not detected in the *S. phylicifolia* roots. This genet was thus isolated from the roots of a total of seven individual plants also representing three different taxa. Neither of these larger genets was identical to the isolates obtained during the earlier sampling. Genet 1 is likely physically discontinuous and consists of two ramets resulting from fragmentation. Or, alternatively, *P. fortinii* remains in the root systems for only a short period of time and recolonises them frequently from new propagules. No *P. fortinii*-isolates were obtained from the roots of the other two adjacent willow shrubs included in the 1994 sampling (Fig. 8.1).

Figure 8.3. Clustering of 34 isolates of *Phialocephala fortinii* isolated from the roots of 17 individual plants at Lyman Glacier forefront in 1993. Branch lengths indicate Jaccardian similarities (%). Simple linkage method (nearest neighbour) was employed. First two digits refer to plant individual from which the isolates were obtained (Table 8.1), the following two to individual isolate numbers from that plant individual.

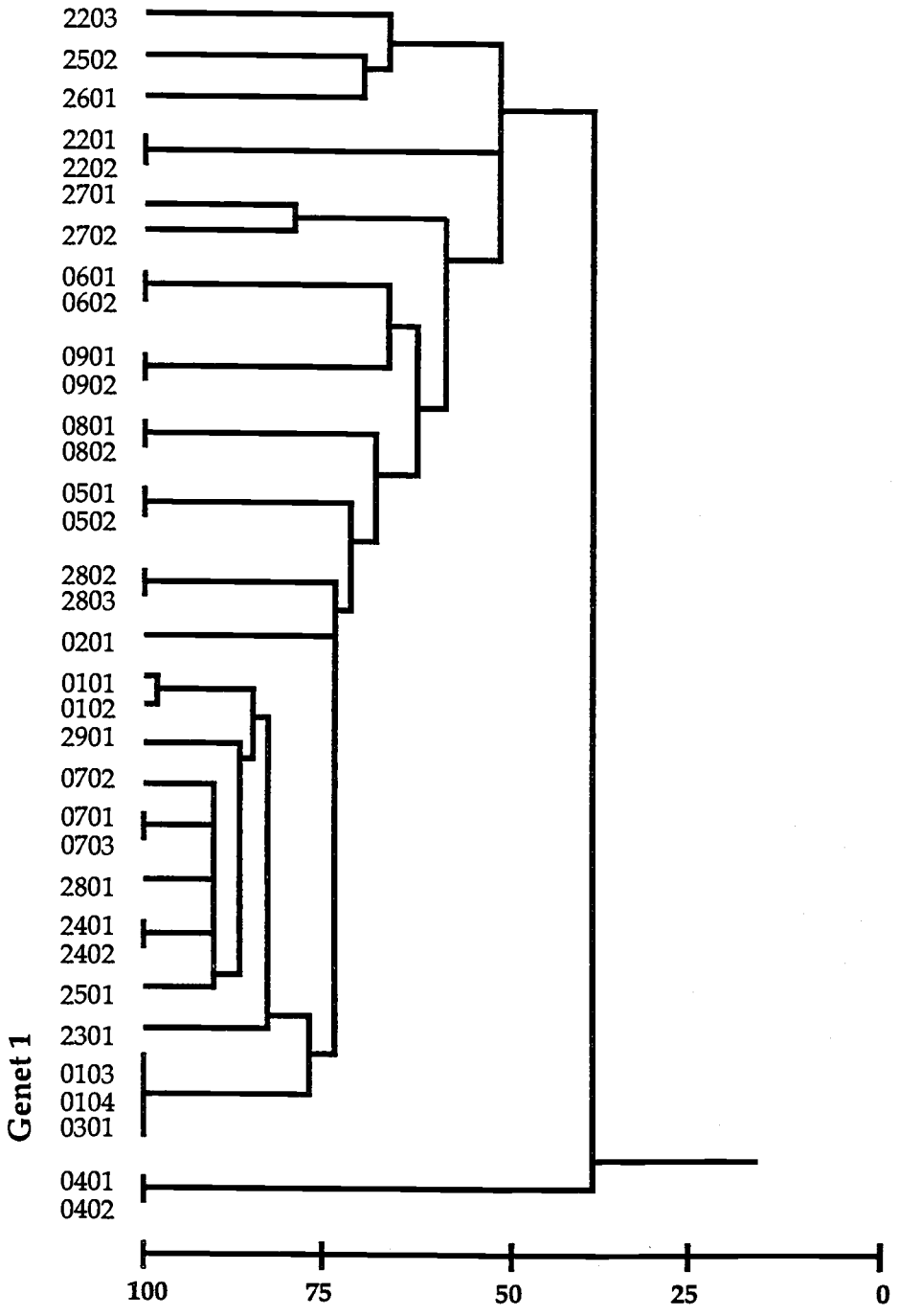
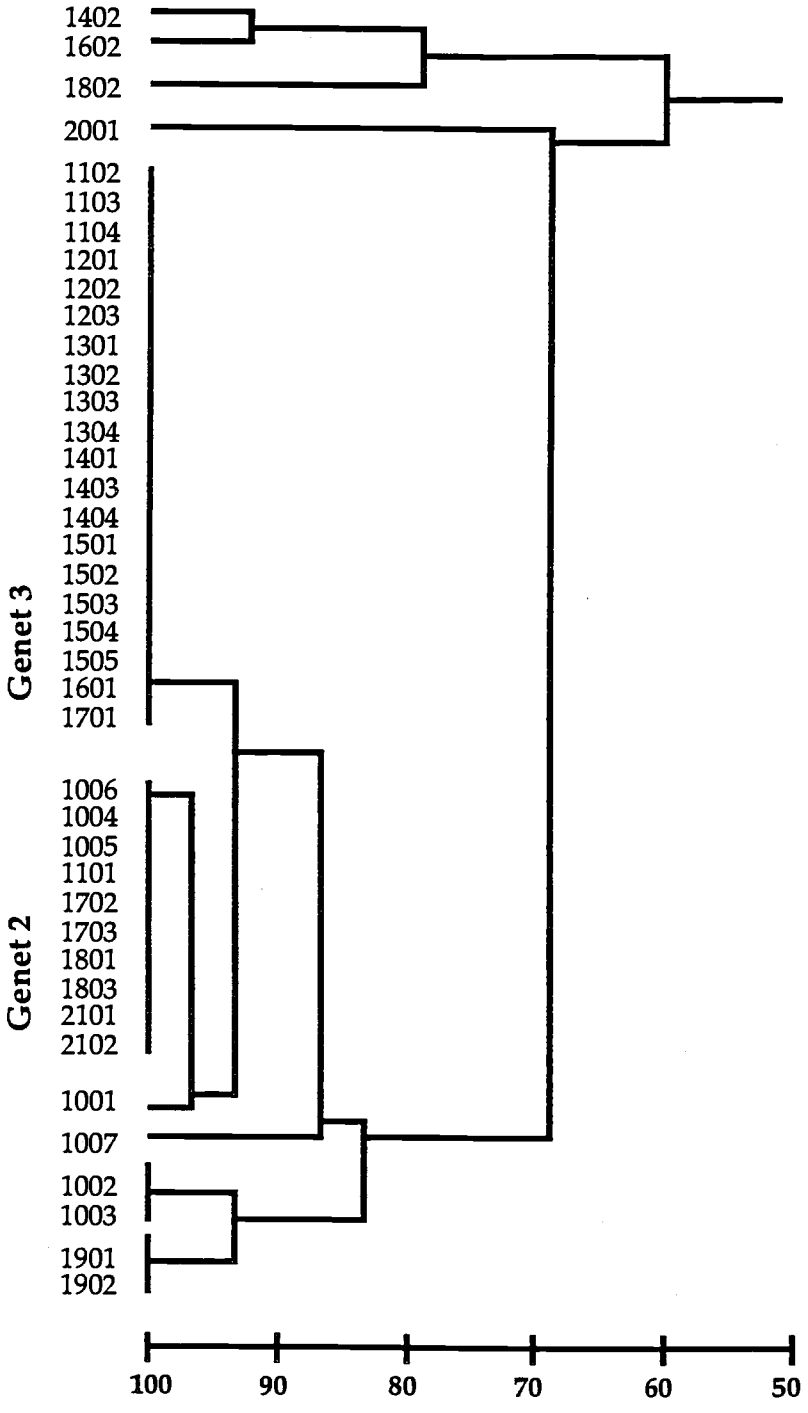


Figure 8.4. Clustering of 40 isolates of *Phialocephala fortinii* isolated from the roots of 17 individual plants at Lyman Glacier forefront in 1994. Branch lengths indicate Jaccardian similarities (%). Simple linkage method (nearest neighbour) was employed. First two digits refer to plant individual from which the isolates were obtained (Table 8.1), the following two to individual isolate numbers from that plant individual.



DISCUSSION

ITS–RFLPs (Gardes and Bruns, 1996*a*) employed in this study have been shown to bear little variation within a species but typically reliably vary between even closely related species (Gardes *et al.*, 1991). In this study, isolates grouped by their macro- and micromorphological characteristics were further tested by restriction digestion of the PCR-amplified ITS-region. The preliminary screening by the visual macro- and microscopic characteristics seemed to exclude isolates deviating from the type culture of *P. fortinii*. Several unidentified macroscopically distinct morphotypes were observed emerging from the surface sterilised roots. *Phialocephala fortinii*-like colonies, however, appeared to be the most frequent individual type. The areas where the isolates were obtained were vacated by the glacier approximately 30 and 65 yr ago. At the former, more recently deglaciated area, the fungal flora appears to consist of only a few fungal species. During eight years of field collecting, Trappe, Jumpponen and Cázares (see Chapter 4) observed only a single fruiting species, *Laccaria montana* Singer, in this general area. It is in the regions deglaciated more than ten years earlier, vacated approximately 45 yr ago, that other fruiting species (*Inocybe lacera* (Fr. : Fr.) Kumm., *Cortinarius tenebricus* Favre) have been observed. Given the possible low diversity in the fungal flora, *P. fortinii* may be an essential root coloniser in the early successional community.

Willow patches in the area deglaciated 65 yr ago were also sampled for root colonising endophytic fungi. *Phialocephala fortinii* was isolated at a similar frequency as compared to the areas deglaciated more recently. This observation suggests that *P. fortinii* is present throughout the glacier forefront. It has also been frequently isolated from material collected from mature forest soils (see Currah *et al.*, 1987; Currah *et al.*, 1988; Ahlich and Sieber, 1995). Root colonisation by dark-septate endophytes similar to *P. fortinii* have been observed in habitats ranging from South African coastal plains and lowlands (Allsopp and Stock, 1993) to tropical (*e.g.* Sengupta *et al.*,

1989; Thomazini, 1974), temperate (Ahlich and Sieber, 1995), subalpine (Cázares, 1992; Stoyke *et al.*, 1992), alpine (Allen *et al.*, 1987; Bisset and Parkinson 1979a, 1979b, 1979c; Blaschke 1991a, 1991b; Haselwandter, 1987; Haselwandter and Read, 1980; Read and Haselwandter, 1981; Stoyke and Currah, 1991), maritime Antarctic (Christie and Nicholson, 1983), and arctic (Ahlich and Sieber, 1995; Bisset and Parkinson 1979c; Väre *et al.*, 1992) zones. These numerous reports indicate a global distribution and adaptation to a variety of different ecosystems by the dark-septate endophytes.

Nine different plant species were found to be colonised by *P. fortinii* on the glacier forefront. These species are known as ecto (*Salix commutata, S. phylicifolia* and *Tsuga mertensiana*), ericoid (*Phyllodoce empetriformis, P. glanduliflora, Cassiope mertensiana* and *Vaccinium deliciosum*), or non- (*Juncus* sp. and *Luzula piperi*). Jumpponen and Trappe (unpublished) tabulated over 600 plant species representing about 330 genera and 100 families reported to be colonised by dark septate endophytes (Appendix 1). *Phialocephala fortinii* alone has been isolated from field-collected root samples or shown to be able to colonise a variety of species in controlled bioassays (see Table 5.1). Taken together, our own and the above observations indicate that *P. fortinii* is likely to have no or very little host specificity. Furthermore, it does not seem to be restricted to any of the known host-fungus associations, *i.e.* it seems able to colonise plant species with different habits.

The areas studied here had been deglaciated fairly recently and therefore have allowed establishment of fungal individuals only for a limited time. The population of *P. fortinii*, however, expressed a substantial variation as inferred from the RAPD-markers used in this study. Even the fungal individuals obtained from a single root system seemed distant based on the proportion of shared RAPD-fragments. Several possible reasons are suggested for the observed high diversity.

First, the majority of variable RAPD-fragments are likely to represent noncoding DNA and, as a result, not be affected by natural selection. These

neutral loci may bear more variation within and among populations than functional gene products such as allozymes. Jacobson *et al.* (1993) showed that somatically compatible isolates, which are frequently interpreted as representatives of a single genet, of *Suillus granulatus* (L. : Fr.) Kuntze may have distinct RAPD phenotypes. Similarly, Rizzo *et al.*, (1995) found that somatically compatible isolates of *Armillaria ostoyae* (Romagn.) Henrik from a mixed *Pinus*-stand in Minnesota were distinguishable by nuclear DNA fingerprints. These results and ours indicate larger variation in RAPD or fingerprint phenotypes than in the expression of the genes coding for somatic compatibility systems.

Second, the mitochondrial genome may contribute to the variation observed in RAPD-phenotypes (*e.g.*, Aagard *et al.*, 1995; Aagard, 1997). A colony with uniform nuclear genotype may be a mosaic of mitochondrial genomes due to different patterns of nuclear and mitochondrial migration after anastomosis of hyphae of compatible mating types (Hintz *et al.*, 1988; May and Taylor, 1988; Specht *et al.*, 1992; Anderson and Kohn, 1995). Although nuclearly uniform, mitochondrial mosaics may be identified as distinct individuals based on the RAPD-phenotypes resulting in overestimation of both numbers of individuals and genetic variation in the studied population. One needs, however, to bear in mind the differences in the basidiomycetous and ascomycetous lifecycles: while the vegetative state is dikaryotic in most basidiomycetes, it is haploid in most ascomycetes. The lifecycle of anamorphic, ascomycetous *P. fortinii* is currently unknown, but its vegetative state is most likely haploid. Mitochondrial mosaics without a meiotic event, therefore, are considered unlikely.

Third, the studied population at the glacier forefront may result from asexual propagation from a large and diverse population surrounding the forefront. The high degree of observed variation in the forefront suggests a large number of vegetatively dispersing individuals able to invade and establish at the study site. Mycelial expansion or asexual (clonal) dispersal was evidenced in this study by isolation of identical genets from root systems

of several plant individuals. At least in one case, these genets were likely discontinuous and resulted from asexual propagation (isolates representing Genet 1 in Fig. 8.1). Alternatively, based on estimated growth of 0.3 – 1.6 m yr^{-1} for various species of *Armillaria* under natural conditions (Kile, 1983; Risbeth 1991; Shaw and Roth, 1976; Smith *et al.*, 1992), it is possible that a single genet may have vegetatively expanded over this distance. However, given the short period of plant colonisation in the glacier forefront, short snow-free growing season and relatively harsh site conditions, vegetative expansion seems an unlikely explanation.

Fourth, the high variation and large number of individuals obtained in this very limited sampling could result from frequent sexual recombination and propagation. Isolates obtained during the 1994 sampling were more similar than the diverse isolates from 1993, suggesting a sibling relationship. Spores may disperse over long distances but most likely land near the sporocarp, resulting potentially in clusters of closely related individuals. Although only asexual reproduction is known for *P. fortinii*, sexual propagation cannot be ruled out. No connection between the anamorphic *P. fortinii* and any teleomorphic taxon is known. However, Currah *et al.*, (1993) observed small, dark inconspicuous apothecia in pots of *Rhododendron brachycarpum* inoculated with *P. fortinii*. The apothecia never matured, but based on this observation they suggested *P. fortinii* as an anamorph of an apothecial member of Leotiales. Unpublished (Jumpponen) sequence data from the nuclear small subunit of the ribosomal RNA gene placed Pezizales basal to several strains of *P. fortinii* while several other sterile, root-colonising, melanised fungi grouped within Pleosporales or Dothidiales. Members of these families typically produce small, darkly coloured ascocarps which may easily escape detection in the field (see discussion in Jumpponen *et al.*, 1997). Therefore, sexual reproduction may be frequent and explain the observed diversity despite the lack of a known teleomorph. Furthermore, smaller variation among the forty isolates obtained in the 1994 sampling than those in the 1993 sampling indicates a

close relatedness between the 1994 isolates from a limited area (see Fig. 8.3 and 8.4): they may be siblings from a single or few mating events that resulted in short-distance dispersal and colonisation of adjacent vacant root systems.

Several plant individuals of different species were observed to be colonised by the same fungal genets. For adjacent plant individuals with intermingled root systems, these genets are likely to be physically continuous, *i.e.* ramets as defined earlier. It is unlikely that plant individuals separated by a considerable distance would share a continuous, single ramet. Rather, as shown by the one example in the presented data, identical genets can be expressions of two or more independent ramets of a genet. Fragmentation is a common feature in clonal plants (Harper, 1977). Several examples from filamentous fungi suggest that fragmentation may also be a frequent phenomenon in fungi in their natural environments (Thompson and Rayner, 1982; Holmer and Stenlid, 1991; Dahlberg and Stenlid, 1994; Rizzo *et al.*, 1995). The likelihood of fragmentation increases with age of a fungal genet. Division of a single, continuous genet into genetically identical, discontinuous ramets may result from physical division of the vegetative mycelium (Dickman and Cook, 1989) or from asexual propagation (Anderson and Kohn, 1995). Given the considerable distance between the observed ramets at the harsh alpine glacier forefront site suffering from an extended dry period during the summer, division by dispersal of asexual propagules (conidiospores, hyphal fragments, or sclerotia) seems more likely. Currah *et al.*, (1993) hypothesised that the intracellular sclerotial bodies, frequently produced by *P. fortinii* while colonising host root tissue, can be effective propagules when the root cells filled with mycelium are loosened and sloughed off the root. The heavily melanised mycelium and sclerotial bodies of *P. fortinii* may play an essential role in increasing the longevity of such propagules by discouraging grazing and protecting against dessication (Kuo and Alexander, 1967; Bell and Wheeler, 1986). However, studies on the feeding of some common soil fungivores show preferences for darkly

pigmented to non-pigmented fungi (Mitchell and Parkinson, 1976; Visser and Whittaker, 1977; Parkinson, 1988).

It is likely that adjacent plant individuals are connected via a shared mycelial ramet. The significance and ecological role of dark-septate endophytes, including *P. fortinii,* is presently unclear. Various studies of the interaction between the endophytes and their hosts have yielded conflicting results. Fernando and Currah (1996) concluded that inoculation of a given host species with *P. fortinii* may result in increased or decreased growth depending on the strain of the fungus used in the study. Jumpponen *et al.,* (1998*a*) observed substantial increase in biomass and foliar concentrations of nitrogen and phosphorus as a result of inoculation of *Pinus contorta* with a strain of *P. fortinii.* The increased foliar nutrient concentrations suggest involvement of the fungal endophyte in nutrient acquisition. It is possible that under some circumstances colonisation by a root endophyte may result in a positive (mutualistic), mycorrhiza-like host response. Extramatrical mycelium penetrating into soil matrices inaccessible to the host plant and transporting nutrients to the host seems possible. Similarly, connection between plant individuals via shared mycelium may allow flow of photosynthates as suggested for ectomycorrhizal systems (Read *et al.,* 1985; Simard *et al.,* 1997). Such transport of carbohydrates may be significant for the development of early successional plant communities.

The role of root-associated microorganisms in plant succession has been poorly studied. Allen and Allen (1984; 1988) have hypothesised that VA-mycorrhizae may partially regulate the successional change of plant communities. Similarly, root endophytes, *P. fortinii* included, may affect the successional change in plant communities. They may serve as a fungal network providing means of facilitation via the shared mycelium or change the relative competitive fitness as a result of host response to colonisation. Alternatively, they may be involved in the nutrient acquisition from organic debris. *P. fortinii* and *Leptodontidium orchidicola* have been shown to

possess enzymatic activities allowing utilisation of detrital organic compounds (Caldwell *et al.*, 1996; Currah and Tsuneda, 1993; Fernando and Currah; 1995). Such abilities may be crucial in early successional communities where supply of nutrients, nitrogen in particular, may be sparse (Matthews, 1992; Jumpponen *et al.*, 1998*b*) and nutrients bound to recently deposited litter can be elementary for successful establishment and survival of new plant individuals. The data so far are too few to allow any definite answers about the potential role of the dark-septate endophytes, especially in the successional context. However, whatever their precise role, the sharing of genets among plant species suggest that *P. fortinii* may play a fundamental role in adaptation and interaction among the entire plant community during primary succession.

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CHAPTER 9

CONCLUSIONS

SUMMARY

In this thesis, plant community changes over time on the well-documented glacier retreat at Lyman Glacier are described. This information is then used to infer successional patterns and mechanisms in a true primary successional ecosystem with special emphasis on the role of fungi, in particular the dark, septate endophytes.

The microenvironments (safe sites) most favorable for plant invasion are defined and characterized. Successful establishment does not appear random but depends on a site that traps seeds and vegetative propagules from primary or secondary dispersal. This site must also support both germination and survival after the seeds are trapped. Minor wind shadows collect the seeds; the ability of a safe site to protect seeds and establishing seedlings from desiccation on the exposed glacier forefront is then the primary mechanism determining which sites are favourable. A substrate that allows seed to anchor and protects seed from predation may be important.

Although abiotic interactions are crucial during initial plant establishment, biotic interactions dominate as soon as some plant cover has established. The response of indigenous taxa to the physical environment created by willow canopies was studied on the primary successional glacier forefront. First, the occurrence of indigeneous plant species under willow canopies was recorded and then compared with that in the open. Second, the separate effects of willow canopies and associated soils on germinant emergence and survival of *Pinus contorta* were studied. The data show that willow shrubs may be either neutral or inhibitory to other vascular plants. The two experiments indicated that no net positive effect was created by the

physical microenvironment under the canopies. The role of established willow shrubs, however, may control the transition of the plant community from dominance by the early colonizers. The willows may modify the substrate favorably for establishment of the later seral species. In summary, the data confirmed previous postulation by Matthews (1992): the prior occurrence of a particular species (in our case willow) was not necessary or sufficient to account for the later occurrence of a different species. The experiments also show the potential difficulties in identifying successional mechanisms: detailed experimental manipulations are necessary to separate these mechanisms, yet the studies are difficult carry out in primary successional habitats.

Although not directly addressed, the potential importance of root associated fungi was hypothesized in Chapter 3. The observations of ectomycorrhizal fruiting spanning eight years of floristic studies in the Lyman Lake basin were presented. The data seem to support the prevailing hypothesis ('early-' and 'late-stage' model) on succession of mycorrhizal fungi. This, however, may be an artifact of the environment. The glacier forefront is a relatively homogeneous open habitat lacking the moist microsites rich in organic matter and coarse woody debris which abound at adjacent secondary successional sites. It was concluded that the "early-" and "late-stage" model fails to acknowledge changes in the physical and chemical characteristics of soil as well as stand architecture. The different factors, including host species composition, host age and stand characteristics, all likely contribute to diversification of the ectomycorrhizal fungal flora.

Dark, septate root endophytes (DSE) have been frequently encountered during previous studies in arctic and alpine environments. The current literature on this poorly known, heterogeneous group is reviewed in Chapter 5. Colonization by DSE has been reported for about 600 plant species representing about 330 genera and 100 families. DSE fungi occur in habitats ranging from the tropics to arctic and alpine. Studies on DSE thus far have yielded inconsistent results and only poorly illustrate the role of DSE in their

natural habitats. These inconsistencies are largely due to the uncertain taxonomic affinities of the strains of DSE. In addition, because different strains of a single anamorph taxon seem to vary greatly in function, no clear generalizations on their ecological role have been drawn. Furthermore, the experimental tools used to study host fungus relationships may result in biased or even erroneous conclusions.

The plants colonized by DSE in the Lyman Glacier habitat showed no symptoms of disease and had survived in its poor soil, short growing season and climatic stress. We hypothesized that the DSE had no adverse effect on the plants. To test this hypothesis, we studied effects of one strain of *Phialocephala fortinii* on *Pinus contorta* grown on forefront soil. In this experiment, *P. fortinii* enhanced P uptake by the pine regardless of N or organic matter treatments and increased host growth when N was available: it produced classical mycorrhizal responses. It was concluded that *P. fortinii*-colonized plant roots fit the definition of mycorrhiza (Trappe, 1996): "Dual organs of absorption formed when symbiotic fungi inhabit healthy absorbing organs (roots, rhizomes or thalli) of most terrestrial plants and many aquatics and epiphytes."

Two additional experiments on the effects of two *P. fortinii*-strains on growth of *P. contorta* were carried out. Seedlings of *P. contorta* were grown aseptically with five levels of glucose in the medium and in open pot cultures where no exogenous carbohydrates were supplied. A substantial increase in *P. contorta* biomass was observed when inoculated with either strain of *P. fortinii* under aseptic conditions, whereas no such response could be observed in the open pot cultures. Increase in the glucose concentration resulted in substantial increase in host biomass in the aseptic system when fungus was present but not when no inoculum was added. These clearly show that the inoculation bioassays can lead to different conclusions depending on the conditions under which the syntheses are performed. Moreover, the association between *P. fortinii* and its potential host plants cannot be clearly

categorised as either antagonistic, pathogenic or mutualistic on the basis of experiments like this.

Finally, in a field study, using randomly amplified polymorphic DNA (RAPD) technique, spatial distribution of discrete genets of *P. fortinii* on the forefront of a receding glacier was assayed. The 74 isolates of *P. fortinii,* obtained from ecto-, ericoid-, or non-mycorrhizal hosts expressed substantial variation. The isolated genets simultaneously inhabited roots of several plant individuals representing three different species.

We conclude that *P. fortinii* is likely to have no or very little host specificity. Furhermore, it is possible that adjacent plant individuals are connected via shared mycelial units. As a result, *P. fortinii* may partially regulate the successional change of plant communities: (1) they may serve as a fungal network providing means of facilitation via the shared mycelium, (2) they may be involved in nutrient acquisition from organic debris and thereby improve the nutritional status of the host plants, or (3) may alter the interspecific competitive capabilities via differential growth promotion or inhibition.

RECOMMENDATIONS FOR FUTURE RESEARCH

Several aspects of successional communities and their dynamics were touched during this thesis research. The abiotic aspects controlling early plant colonization in primary successional communties have received little attention. The early stages in the life cycle seem more important than subsequent stages in determining the distribution and abundance of plants. Our intention was to draw attention to the patterns of plant colonization as an essential mechanism in defining the plant distribution and spatial community structure in a primary successional habitat. Potential ways to continue this work include repeating field studies such as ours in other habitats, designing *in situ* experiments addressing the relative importance of,

e.g., desiccation or granivory, or conducting designed laboratory experiments following examples set by, *e.g.*, Harper *et al.* (1965) or Sheldon (1974).

Successional mechanisms as well as the theoretical framework have recently received substantial attention. Detailed experimental manipulations are necessary to separate these mechanisms in primary succession. This should be prerequisite to the formulation of a general theoretical framework and mechanistic models of community development in successional seres.

At the moment, little is known about non-nitrogen fixing plants and their relative importance compared to nitrogen fixers in primary succession. Nitrogen fixers in primary succession have received more attention for obvious reasons: primary successional habitats are low in the nitrogen and organic matter required for capturing deposited nitrogen. Sites where the effects of nitrogen fixers and non-nitrogen fixers can be compared would provide an invaluable opportunity for tests of specific effects.

The importance of soil microorganisms in successional change of plant communities has been overlooked. It is essential to see if, for example, mycorrhizal fungi do allow facilitation via established mycelial guilds. A first and important step towards understanding the role of soil microbiota is a comprehensive understanding of their community structure and its change along changing environmental gradients, such as ones provided by glacier forefronts. Another approach is to assay the communities of root colonizing fungi of different hosts along a successional sere and infer which are the organisms shared by the hosts dominating different stages of a successional sere. This would allow constructing and testing specific hypotheses on the effects of particular species or groups of organisms at different successional stages.

As pointed out in the review (Chapter 5), the data so far are too few to allow definitive answers about functions of DSE, especially in the successional context. To understand the role of strictly heterotrophic organisms (such as fungi), one must understand their effects on potential hosts. In addition to continuing the work in simple experimental systems

mainly designed to address growth and nutrient acquisition effects, further studies, *e.g.*, with radioisotopes or stable heavy isotopes, are needed to determine if net nutrient fluxes are uni- or bidirectional. The most important task on the way towards improved understanding of DSE and their host associations is, however, to assure correct taxonomic affinities, either morphological or molecular, of the organisms studied.

The availability of molecular tools to virtually any researcher allows some estimation of taxonomic affinity. This may be the most crucial way of bringing the communities of root-associated fungi within our reach. I'll elaborate with a quote from Allen and Allen (1992, p. 465): "Unfortunately, few field data exist which allow definitive statements regarding the importance of different fungi on plant communities. In part this is due to frequent inability to recognize the vegetative state of fungi associated with plants in the field." I believe it is timely to attempt more holistic ways to identify the components in below-ground communities and their relative importance.

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APPENDICES

APPENDIX 1: PLANT SPECIES REPORTED TO BE COLONIZED BY DSE.

Reports include any notation of dark, septate hyphae observed in the root systems. Current taxon name given first and the one used by the original author(s) in parentheses. References displayed in short format in Appendix 2. Nomenclature follows Database of North American Plants (USDA-NRCS) (available at http://www.ars-grin.gov/npgs/tax/index.html) and new provisional Global Plant Checklist (available at http://bgbm3.bgbm.fu-berlin.de/IOPI/GPC/query.html) where applicable.

Plant Taxon	Climate type	References
ANGIOSPERMAE		
DICOTYLEDONEAE		
Aceraceae		
Acer circinatum	temperate	J.M. Trappe, unpubl.
Acer pseudoplatanus	temperate	Dominik & Pachlewski, 1955
Aizoaceae		
Carpobrotus edulis (C. acinaciformis)	temperate	Allsopp & Stock, 1993
Dorotheanthus bellidiformis	temperate	Allsopp & Stock, 1993
Ruschia macowanii	temperate	Allsopp & Stock, 1993
Sesuvium portulacastrum	tropical	Sengupta *et al.*, 1989
Anacardiaceae		
Rhus rosmarinifolia	temperate	Allsopp & Stock, 1993
Apocynaceae		
Nerium oleander	temperate	Peyronel, 1924
Vinca minor	temperate	Demeter, 1923; Peyronel, 1924
Araliaceae		
Oplopanax horridus	temperate	J.M. Trappe, unpubl.
Asclepiadaceae		
Cynanchum vincetoxicum	temperate	Peyronel, 1924
Avicenniaceae		
Avicennia marina	tropical	Sengupta *et al.*, 1989
A. officinalis	tropical	Sengupta *et al.*, 1989
Stachytarpheta cayennensis	tropical	Thomazini, 1974
Betulaceae		
Alnus rubra	temperate	Ahlich & Sieber, 1995
Betula alleghaniensis	temperate	Wilcox & Wang, 1987*b*
B. pendula (*B. alba*)	temperate	Melin, 1923; Abuzinadah & Read, 1989
B. pubescens	temperate	Ahlich & Sieber, 1995
B. pumila	temperate	Fernando & Currah, 1995
Bignoniaceae		

Jacaranda decurrens	tropical	Thomazini, 1974
Pyrostegia venusta	tropical	Thomazini, 1974
Tabebuia ochracea	tropical	Thomazini, 1974
Boraginaceae		
Mertensia ciliata	temperate	J.M. Trappe, unpubl.
M. maritima	arctic	Väre *et al.*, 1992
Myosotis alpestris	alpine	Stoyke & Currah, 1991
Pulmonaria officinalis	temperate	Peyronel, 1924
Symphytum tuberosum	temperate	Peyronel, 1924
Buddlejaceae		
Buddleja davidii	temperate	Stevenson, 1964
Cactaceae		
Coryphantha sp.	temperate	Johansen, 1931
Gymnocalycium sp.	temperate	Johansen, 1931
Mammillaria parkinsonii	temperate	Johansen, 1931
Campanulaceae		
Campanula glomerata	alpine	Demin, 1971
C. lasiocarpa	temperate	Currah & Van Dyk, 1986
C. linifolia (*C. scheuchzeri*)	alpine	Read & Haselwandter, 1981
C. uniflora	arctic	Väre *et al.*, 1992
Phyteuma hemisphaericum	alpine	Read & Haselwandter, 1981
P. michelii	temperate	Peyronel, 1924
P. ovatum (*P. halleri*)	alpine	Peyronel, 1924
Caprifoliaceae		
Linnaea borealis	temperate	Currah & Van Dyk, 1986
Lonicera involucrata	temperate	J.M. Trappe, unpubl.
Sambucus racemosa	temperate	Mejstrik, 1969, 1971
Caryocaraceae		
Caryocar brasiliense	tropical	Thomazini, 1974
Caryophyllaceae		
Arenaria ciliata	alpine	Haselwandter & Read, 1980
Cerastium alpinum	arctic	Bledsoe *et al.*, 1990; Kohn & Stasovski, 1990
C. arcticum	arctic	Väre *et al.*, 1992
C. regelii	arctic	Väre *et al.*, 1992
C. uniflorum	alpine	Haselwandter & Read, 1980
Colobanthus quitensis	subantarctic	Christie & Nicolson, 1983
C. subulatus	subantarctic	Christie & Nicolson, 1983
Gypsophila repens	temperate	Peyronel, 1937
Melandrium apetalum	arctic	Bledsoe *et al.*, 1990
Minuartia biflora	alpine, arctic	Stoyke & Currah, 1991; Väre *et al.*, 1992
M. rubella (*Arenaria rubella*)	alpine, arctic	Cázares, 1992; Väre *et al.*, 1992
Sagina intermedia	arctic	Väre *et al.*, 1992
Silene acaulis	alpine, arctic	Haselwandter & Read, 1980; Currah & Van Dyk, 1986; Bledsoe *et al.*, 1990; Kohn & Stasovski, 1990; Väre *et al.*, 1992
S. undulata	temperate	Allsopp & Stock, 1993

S. vulgaris	alpine	Blaschke, 1991a, 1991b
Stellaria holostea	temperate	Dominik, 1957
S. humifusa	arctic	Bledsoe *et al.*, 1990
S. longipes	arctic	Bledsoe *et al.*, 1990
Celastraceae		
Paxistimaa myrsinites	temperate	J.M. Trappe, unpubl.
Chenopodiaceae		
Beta vulgaris	temperate	Peyronel, 1923; Peyronel, 1924
Suaeda maritima	tropical	Sengupta *et al.*, 1989
Cistaceae		
Cistus incanus (C. villosus)	temperate	Litav, 1965
Helianthemum nummularium	alpine	Stelz, 1968
Compositae		
Achillea clavennae	alpine	Read & Haselwandter, 1981
A. millefolium	alpine	Peyronel, 1924
A. ptarmica	temperate	Harley & Harley, 1987
Achyrocline satureioides	temperate	Thomazini, 1974
Anaphalis margaritacea	alpine, temperate	Cázares, 1992; J.M. Trappe, unpubl.
Antennaria lanata	alpine	Stoyke & Currah, 1991; J.M. Trappe, unpubl.
Arctotheca calendula	temperate	Allsopp & Stock, 1993
Arnica cordifolia	alpine	Currah & Van Dyk, 1986
Artemisia genipi	alpine	Read & Haselwandter, 1981
A. laxa	alpine	Read & Haselwandter, 1981
A. norvegica	alpine	Fernando & Currah, 1995;, 1996
Aster alpigenus	subalpine	Cázares, 1992
Calendula officinalis	temperate	Peyronel, 1924
Centaurea montana	alpine	Peyronel, 1924
C. uniflora	alpine	Peyronel, 1924
Chaptalia nutans	tropical	Thomazini, 1974
Chrysanthemum alpinum (*Leucanthemopsis alpina*)	alpine	Haselwandter & Read, 1980; Read & Haselwandter, 1981
C. leucanthemum (*Aster leucanthemum*)	temperate	Peyronel, 1924
Coreopsis tinctoria	temperate	Yousef, 1946
Crepis aurea	alpine	Read & Haselwandter, 1981
C. nana	alpine	Cázares, 1992
Erigeron sp.	temperate	Fernando & Currah, 1996
E. perigrinus	alpine	Currah & Van Dyk, 1986
Helianthus annuus	temperate	Shterenberg, 1951
Hieracium villosum	alpine	Read & Haselwandter, 1981
Homogyne alpina	alpine	Peyronel, 1924
Leontodon hispidus	temperate	Peyronel, 1924
Leontopodium alpinum	alpine	Read & Haselwandter, 1981
Luina stricta	alpine	J.M. Trappe, unpubl.
Nothocalais alpestris	alpine	J.M. Trappe, unpubl.
Petasites frigidus (*P. nivalis*)	alpine	Currah & Van Dyk, 1986
Senecio fremontii	alpine	Cázares, 1992; J.M. Trappe, unpubl.

S. cymbalarioides	alpine	Cázares, 1992
S. incanus	alpine	Read & Haselwandter, 1981
S. squalidus	temperate	Stevenson, 1964
S. triangularis	alpine	Currah & Van Dyk, 1986
Sonchus tenerrimus	temperate	Peyronel, 1924
Taraxacum arcticum	arctic	Väre *et al.*, 1992
T. officinale	temperate	Peyronel, 1924
Connaraaceae		
Rourea sp.	tropical	Thomazini, 1974
Convolvulaceae		
Convolvulus arvensis	tropical	Iqbal *et al.*, 1982
Cornaceae		
Cornus canadensis	temperate	Currah & Van Dyk, 1986
Crassulaceae		
Crassula filiformis	temperate	Allsopp & Stock, 1993
Jovibarba globifera (S. hirtum)	temperate	Zach, 1909
Sempervivum angustifolium	temperate	Zach, 1909
S. arachnoideum	alpine	Zach, 1909; Haselwandter & Read, 1980; Read & Haselwandter, 1981
S. carpaticum	temperate	Zach, 1909
S. ciliosum	temperate	Zach, 1909
S. funkii	temperate	Zach, 1909
S. grandiflorum (S. gaudinii)	temperate	Zach, 1909
S. heuffelii (S. patens)	temperate	Zach, 1909
S. leucanthum	temperate	Zach, 1909
S. marmoreum (S. assimile)	temperate	Zach, 1909
S. montanum (S. braunii)	temperate	Zach, 1909
S. soboliferum	temperate	Zach, 1909
S. tatari	temperate	Zach, 1909
S. tectorum (S. acuminatum)	temperate	Zach, 1909
Cruciferae		
Capsella bursa-pastoris	alpine	Peyronel, 1924
Cardamine nymanii	arctic	Väre *et al.*, 1992
Cochlearia officinalis	arctic	Väre *et al.*, 1992
Draba adamsii	arctic	Väre *et al.*, 1992
D. alpina	arctic	Väre *et al.*, 1992
D. corymbosa	arctic	Väre *et al.*, 1992
D. fladnizensis	arctic	Väre *et al.*, 1992
D. nivalis	arctic	Väre *et al.*, 1992
D. oligosperma	alpine	Cázares, 1992
D. subcapitata	arctic	Bledsoe *et al.*, 1990; Väre *et al.*, 1992
Erysimum pallasii	arctic	Kohn & Stasovski, 1990
Iberis sempervirens	temperate	Peyronel, 1924
Sisymbrium officinale	temperate	Harley & Harley, 1987
Thlaspi rotundifolium	alpine	Peyronel, 1924
Empetraceae		
Empetrum nigrum	alpine, boreal	Hambleton & Currah, 1997; A. Jumpponen, unpubl.

Ericaceae		
Arctostaphylos rubra	alpine	Currah & Van Dyk, 1986
A. uva-ursi	alpine	Stoyke & Curah, 1991
Calluna vulgaris	temperate; alpine	Ahlich & Sieber, 1995; A. Jumpponen, unpubl.
Cassiope mertensiana	alpine	Stoyke & Currah, 1991; Cázares, 1992; Currah & Tsuneda, 1993; Hambleton & Currah, 1997; A. Jumpponen, unpubl.
C. tetragona	arctic	Kohn & Stasovski, 1990; Stoyke & Currah, 1991; Hambleton & Currah, 1997
Gaultheria humifusa	alpine	Hambleton & Currah, 1997
G. shallon	temperate	Ahlich & Sieber, 1995
Kalmia microphylla	temperate	Currah & Van Dyk, 1986
K. polifolia	alpine	Hambleton & Currah, 1997
Ledum glandulosum	alpine	Cázares, 1992
Loiseleuria procumbens	alpine	Stoyke & Currah, 1991; Hambleton & Currah, 1997
Menziesia ferruginea	alpine	Stoyke & Currah, 1991; Stoyke & Currah, 1993; Hambleton & Currah, 1997
Phyllodoce empetriformis	alpine	Cázares, 1992; Stoyke & Currah, 1991; Hambleton & Currah, 1997
P. glanduliflora	alpine	Stoyke & Currah, 1991; Cázares, 1992; Hambleton & Currah, 1997
Rhododendron albiflorum	alpine	Hambleton & Currah, 1997
R. brachycarpum	temperate	Currah *et al.*, 1993
R. kaempferi (R. obtusum)	temperate	Currah & Van Dyk, 1986
Vaccinium cespitosum Michx.	subalpine	Cázares, 1992
V. deliciosum	alpine	Cázares, 1992; A. Jumpponen, unpubl.
V. membranaceum	alpine	Stoyke & Currah, 1991; Hambleton & Currah, 1997
V. myrtilloides	alpine	Hambleton & Currah, 1997
V. myrtillus	boreal, temperate	Ahlich & Sieber, 1995; A. Jumpponen, unpubl.
V. scoparium	alpine	Stoyke & Currah, 1991; Hambleton & Currah, 1997
V. uliginosum	alpine	Hambleton & Currah, 1997
V. vitis-idaea	alpine	Hambleton & Currah, 1997
Euphorbiaceae		
Excoecaria agallocha	tropical	Sengupta *et al.*, 1989
Fabaceae		
Acacia saligna	temperate	Allsopp & Stock, 1993
Fagaceae		
Fagus sylvatica	temperate	Ahlich & Sieber, 1995
Geraniaceae		
Pelargonium triste	temperate	Allsopp & Stock, 1993

Gentianaceae		
Cotylanthera tenuis	temperate	Figdor, 1897
Gentiana calycosa	alpine	J.M. Trappe, unpubl.
G. glauca	alpine	Currah & Van Dyk, 1986
G. prostrata	alpine	Currah & Van Dyk, 1986
Gentianella propinqua		
Grossulariaceae		
Ribes bracteosum	temperate	J.M. Trappe, unpubl.
R. cereum	temperate	J.M. Trappe, unpubl.
R. divaricatum	temperate	J.M. Trappe, unpubl.
Tiarella trifoliata	temperate	J.M. Trappe, unpubl.
Guttiferaceae		
Hypericum maculatum (*H. quadrangulum*)	alpine	Peyronel, 1924
Juglandaceae		
Juglans regia	temperate	Peyronel, 1924
Labiatae		
Ajuga reptans	temperate	Peyronel, 1924
Lamium album	temperate	Harley & Harley, 1987
Prunella vulgaris (*Brunella vulgaris*)	temperate	Peyronel, 1924
Salvia pratensis	temperate	Peyronel, 1924
S. verbenaca	temperate	Peyronel, 1924
Teucrium chamaedrys	temperate	Peyronel, 1924
T. polium	temperate	Peyronel, 1924
Lauraceae		
Laurus nobilis	temperate	Peyronel, 1924
Leguminosae–Caesalpiniaceae		
Chamaecrista calycioides (*Cassia calycioides*)	tropical	Thomazini, 1974
C. catharctica (*Cassia cathartica*)	tropical	Thomazini, 1974
C. fasciculata (*Cassia chamaecrista*)	tropical	Thomazini, 1974
C. chrysocarpa (*Cassia chrysocarpa*)	tropical	Thomazini, 1974
C. rotundifolia (*Cassia rotundifolia*)	tropical	Thomazini, 1974
Gleditsia triacanthos	temperate	Thomas, 1943
Senna multijuga (*Cassia multijuga*)	tropical	Müller & Frémont, 1935
S. occidentalis (*Cassia occidentalis*)	tropical	Thomazini, 1974
S. siamea (*Cassia siamea*)	tropical	Müller & Frémont, 1935
S. tora (*Cassia tora*)	tropical	Thomazini, 1974
S. uniflora (*Cassia uniflora*)	tropical	Thomazini, 1974
Leguminosae–Mimosoideae		
Mimosa pudica	tropical	Thomazini, 1974
Anadenanthera colubrina	tropical	Thomazini, 1974
Styphnodendron adstringens	tropical	Thomazini, 1974
Leguminosae–Papilionoideae		

Aspalathus spinescens	temperate	Allsopp & Stock, 1993
Astragalus alpinus	alpine	Currah & Van Dyk, 1986
A. vexilliflexus	alpine	Currah & Van Dyk, 1986
Cicer arietinum	tropical	Shterenberg, 1951
Indigofera suffruticosa	tropical	Thomazini, 1974
Lens culinaris (*L. esculenta*)	temperate	Shterenberg, 1951
Lupinus caudatus	temperate	O'Dell & Trappe, 1992
L. latifolius	alpine, temperate	O'Dell & Trappe, 1992; O'Dell *et al.*, 1993
L. laxifloris	alpine, temperate	O'Dell & Trappe, 1992
L. lepidus	alpine, temperate	O'Dell & Trappe, 1992
L. leucophyllus	temperate	O'Dell & Trappe, 1992
L. wyethii	temperate	O'Dell & Trappe, 1992; J.M. Trappe, unpubl.
Medicago falcata	temperate	Demin, 1971
M. sativa	temperate	Peyronel, 1924
Melilotus indica (*M. parviflora*)	tropical	Iqbal *et al.*, 1982
M. officinalis Pall.	temperate	Peyronel, 1924
Ornithopus compressus	temperate	Peyronel, 1924
Oxytropis foetida	temperate	Peyronel, 1937
O. campestris (*O. jordalii*)	alpine	Currah & Van Dyk, 1986; O'Dell & Trappe, 1992
Phaseolus vulgaris	temperate	Shterenberg, 1951
Stylosanthes humilis	temperate	Williams, 1985
Trifolium alpestre	alpine	Malan, 1938
T. alpinum	alpine	Malan, 1938
T. medium	temperate	Malan, 1938
T. pratense (*T. nivale*)	temperate	Peyronel, 1924; Malan, 1938
T. repens	temperate	Peyronel, 1924; Malan, 1938; Powell, 1980; J.M. Trappe, unpubl.
T. subterraneum	temperate	Williams, 1985
T. thalii	temperate	Malan, 1938
Trigonella foenum-graecum	temperate	Peyronel, 1924
Vicia cracca	temperate	Malan, 1938
V. sepium	temperate	Malan, 1938
V. tetrasperma	tropical	Iqbal *et al.*, 1982
Vigna radiata	tropical	Venkatamaran *et al.*, 1989
Zornia gemella (*Z. diphylla*)	tropical	Thomazini, 1974
Malpighiaceae		
Byrsonima intermedia	tropical	Thomazini, 1974
Melastomataceae		
Miconia sp.	tropical	Thomazini, 1974
Moraceae		
Morus alba	temperate	Peyronel, 1924
M. nigra	temperate	Peyronel, 1924
Myrtaceae		
Eucalyptus delegatensis	temperate	Chu-Chou & Grace, 1982
E. fastigata	temperate	Chu-Chou & Grace, 1982

E. nitens	temperate	Chu-Chou & Grace, 1982
E. regnans	temperate	Chu-Chou & Grace, 1982
E. saligna	temperate	Chu-Chou & Grace, 1982
Psidium sp.	tropical	Thomazini, 1974
Ochnaceae		
Ouratea spectabilis	tropical	Thomazini, 1974
Onagraceae		
Epilobium anagallidifolium (*E. alpinum*)	alpine, subalpine	Peyronel, 1924
E. angustifolium	subalpine	Currah & Van Dyk, 1986; Cázares, 1992
E. latifolium	subalpine	Currah & Van Dyk, 1986; Cázares, 1992
Circaea alpina	alpine	Peyronel, 1923;, 1924
Oleaceae		
Ligustrum vulgare	temperate	Boullard *et al.*, 1963
Olea europaea	temperate	Peyronel, 1924
Oxalidaceae		
Oxalis montana (*O. acetosella*)	temperate	Peyronel, 1924
O. corniculata	temperate	J.M. Trappe, unpubl.
O. obtusa.	temperate	Allsopp & Stock, 1993
O. oregana	temperate	Peyronel, 1924
O. pes-caprae (*O. cernua*)		
Papaveraceae		
Papaver lapponicum (*P. radicatum*)	arctic	Bledsoe *et al.*, 1990; Kohn & Stasovski, 1990
P. rhoeas	temperate	Peyronel, 1924; Harley & Harley, 1987
Pedaliaceae		
Craniolaria integrifolia	tropical	Thomazini, 1974
Piperaceae		
Piper plantagineum	tropical	Stanczak-Boratynska, 1954
Plantaginaceae		
Plantago lanceolata	temperate	Peyronel, 1924
P. major	temperate	Peyronel, 1924
P. media	temperate	Peyronel, 1924
Plumbaginaceae		
Aegialitis rotundifolia	tropical	Sengupta *et al.*, 1989
Polemoniaceae		
Phlox diffusa	alpine	J.M. Trappe, unpubl.
Polemonium acutifolium	alpine	Currah & Van Dyk, 1986
P. boreale	arctic	Väre *et al.*, 1992
P. pulcherrimum	temperate	J.M. Trappe, unpubl.
Polygonaceae		
Eriogonum compositum	temperate	J.M. Trappe, unpubl.
Oxyria digyna	alpine, arctic	Bledsoe *et al.*, 1990; Cázares, 1992; A. Jumpponen, unpubl.
Polygonum bistorta	alpine	Peyronel, 1924
P. bistortoides	alpine	J.M. Trappe, unpubl.
P. newberryi	temperate	J.M. Trappe, unpubl.

P. persicaria	temperate	Harley & Harley, 1987
P. viviparum	alpine, arctic	Currah & Van Dyk, 1986; Bledsoe *et al.*, 1990
Rumex alpestris (*R. arifolius*)	alpine	Peyronel, 1924
R. alpinus	alpine	Peyronel, 1924
R. cordatus	temperate	Allsopp & Stock, 1993
Portulacaceae		
Portulaca oleracea	temperate	Peyronel, 1924; Thomazini, 1974
Primulaceae		
Anagallis arvensis	temperate	Peyronel, 1924
Androsace chamaejasme	alpine	Currah & Van Dyk, 1986; Stoyke & Currah, 1991
Cyclamen hederifolium (*C. neapolitanum*)	temperate	Peyronel, 1924
C. persicum (*C. vernale*)	temperate	Peyronel, 1924
Primula glutinosa	alpine	Haselwandter & Read, 1980
P. minima	alpine	Read & Haselwandter, 1981
P. vulgaris (*P. acaulis*)	temperate	Peyronel, 1924
Soldanella alpina	alpine	Peyronel, 1924
Ranunculaceae		
Aconitum delphinifolium	alpine	Currah & Van Dyk, 1986
A. napellus (*A. firmum*)	alpine	Peyronel, 1924
Anemone deltoidea	temperate	J.M. Trappe, unpubl.
A. drummondii	alpine	J.M. Trappe, unpubl.
A. multifida	alpine	Currah & Van Dyk, 1986
A. narcissifolia	alpine	Peyronel, 1924
Aquilegia flavescens	alpine	Currah & Van Dyk, 1986
A. vulgaris	temperate	Peyronel, 1924
Caltha palustris (*C. arctica*)	alpine	Peyronel, 1924
Coptis occidentalis	temperate	Trappe unpubl.
Ranunculus biternatus	subantarctic	Christie & Nicolson, 1983
R. bulbosus	temperate	Peyronel, 1924
R. ficaria	temperate	Peyronel, 1924
R. glacialis	alpine	Haselwandter & Read, 1980; Read & Haselwandter, 1981
R. montanus	alpine	Peyronel, 1924
R. nivalis	arctic	Väre *et al.*, 1992
R. paludosus (*R. flabellatus*)	temperate	Peyronel, 1924
R. pygmaeus	arctic	Miller, 1981
R. sulphureus	arctic	Bledsoe *et al.*, 1990; Väre *et al.*, 1992
Trollius europaeus	alpine	Peyronel, 1924
T. laxus (*T. albiflorus*)	alpine	Currah & Van Dyk, 1986
Rhizophoraceae		
Bruguiera gymnorrhiza	tropical	Sengupta *et al.*, 1989
Ceriops decandra	tropical	Sengupta *et al.*, 1989
Rosaceae		
Alchemilla xanthochlora (*A. vulgaris*)	alpine	Peyronel, 1924
Dryas integrifolia	arctic	Kohn & Stasovski, 1990

D. octopetala	alpine, arctic	Fernando & Currah, 1996
Eriobotrya japonica	temperate	Peyronel, 1924
Fragaria virginiana	subalpine	Currah & Van Dyk, 1986
Luetkea pectinata	alpine	Stoyke & Currah, 1991; Cázares, 1992; Currah & Tsuneda, 1993; J.M. Trappe, unpubl.
Potentilla erecta (*P. tormentilla*)	alpine	Peyronel, 1924; Dominik & Pachlewski, 1955
P. fruticosa	alpine	J.M. Trappe, unpubl.
P. hyparctica	arctic	Väre *et al.*, 1992
P. nivea	arctic	Kohn & Stasovski, 1990
P. plattensis	alpine	Currah & Van Dyk, 1986
Prunus americana	temperate	Thomas, 1943
P. dulcis (*P. amygdalis*)	temperate	Peyronel, 1924
P. pensylvanica	temperate	Thomas, 1943
P. persica Batsch	temperate	Peyronel, 1924
Rubus sp.	temperate	Fernando & Currah, 1996
R. acaulis (*R. arcticus*)	alpine	Currah & Van Dyk, 1986
R. idaeus	temperate	Berkeley, 1936
R. lasiococcus	temperate	J.M. Trappe, unpubl.
R. pedatus	alpine	Stoyke & Currah, 1991
R. pubescens	temperate	Currah & Van Dyk, 1986
Sorbus aucuparia	temperate	Dominik & Pachlewski, 1955
Rubiaceae		
Cinchona pubescens (*C. succirubra*)	tropical	Steinmann, 1929
Coffea arabica	tropical	Ciferri, 1929
Galium oreganum	temperate	J.M. Trappe, unpubl.
G. triflorum	temperate	J.M. Trappe, unpubl.
Palicourea rigida	tropical	Thomazini, 1974
Tocoyena formosa	tropical	Thomazini, 1974
Rutaceae		
Citrus aurantium	subtropical	Peyronel, 1924
C. limon	subtropical	Peyronel, 1924
Salicaceae		
Populus sp.	temperate	Harris & Jurgenson, 1977
P. nigra	temperate	Dominik, 1956
Populus tremula	temperate	Melin, 1923
Salix sp.	temperate	Harris & Jurgenson, 1977
S. commutata	alpine	Cázares, 1992; A. Jumpponen, unpubl.
S. glauca	alpine	Dhillion, 1994
S. hastata	alpine	Dhillion, 1994
S. herbacea	alpine	Dhillion, 1994
S. lanata	alpine	Dhillion, 1994
S. myrsinites	alpine	Dhillion, 1994
S. nigricans	alpine	Dhillion, 1994
S. phylicifolia	alpine	Cázares, 1992; Dhillion, 1994; Jumpponen; unpubl.
S. polaris	arctic	Väre *et al.*, 1992

S. repens	boreal	A. Jumpponen, unpubl.
S. reticulata	alpine	Dhillion, 1994; A. Jumpponen, unpubl.
S. reticulata (S. nivalis)	alpine	Cázares, 1992
S. retusa	alpine	Ahlich & Sieber, 1995
Santalaceae		
Thesium sp.	temperate	Allsopp & Stock, 1993
Saxifragaceae		
Parnassia fimbriata	alpine	Currah & Van Dyk, 1986
P. palustris	alpine	Peyronel, 1924
Saxifraga bronchialis	alpine	Currah & Van Dyk, 1986
S. bryoides	alpine	Haselwandter & Read, 1980; Read & Haselwandter, 1981
S. caesia	alpine	Haselwandter & Read, 1980
S. caespitosa	arctic	Väre *et al.*, 1992
S. ferruginea	alpine	Cázares, 1992
S. flagellaris	arctic	Väre *et al.*, 1992
S. hyperborea	arctic	Väre *et al.*, 1992
S. lyallii	alpine	Currah & Van Dyk, 1986
S. nivalis	arctic	Väre *et al.*, 1992
S. oppositifolia	alpine, arctic	Currah & Van Dyk, 1986; Bledsoe *et al.*, 1990; Väre *et al.*, 1992
S. punctata	alpine	Cázares, 1992
S. rotundifolia	temperate	Peyronel, 1924
S. tolmiei	alpine	Cázares, 1992
S. tricuspidata	alpine	Currah & Van Dyk, 1986
Scrophulariaceae		
Castilleja miniata	temperate	J.M. Trappe, unpubl.
Dischisma capitatum	temperate	Allsopp & Stock, 1993
Linaria vulgaris	temperate	Peyronel, 1924
Melampyrum pratense (*M. vulgatum*)	alpine	Dominik & Pachlewski, 1955
Mimulus lewisii	alpine	Cázares, 1992
M. tilingii	alpine	Cázares, 1992
Pedicularis bracteosa	alpine	Currah & Van Dyk, 1986; J.M. Trappe, unpubl.
P. capitata	alpine, arctic	Currah & Van Dyk, 1986; Bledsoe *et al.*, 1990; Kohn & Stasovski, 1990
P. contorta	alpine	J.M. Trappe, unpubl.
P. dasyantha	arctic	Väre *et al.*, 1992
P. flammea	alpine	Currah & Van Dyk, 1986
P. groenlandica	alpine	Currah & Van Dyk, 1986; Cázares, 1992; A. Jumpponen, unpubl.; J.M. Trappe, unpubl.
P. hirsuta	arctic	Kohn & Stasovski, 1990; Väre *et al.*, 1992
P. howellii	temperate	J.M. Trappe, unpubl.
P. kanei (*P. lanata*)	alpine, arctic	Currah & Van Dyk, 1986; Bledsoe *et al.*, 1990
P. ornithorhyncha	alpine	J.M. Trappe, unpubl.

P. racemosa	temperate	J.M. Trappe, unpubl.
P. sudetica	arctic	Bledsoe *et al.*, 1990
Penstemon davidsonii	alpine	Cázares, 1992; J.M. Trappe, unpubl.
Verbascum thapsus	temperate	Peyronel, 1924
Veronica agrestis	temperate	Iqbal *et al.*, 1982
V. cusickii	alpine	J.M. Trappe, unpubl.
V. filiformis	temperate	Harley & Harley, 1987
V. wormskjoldii (*V. alpina*)	alpine	Currah & Van Dyk, 1986; Cázares, 1992
Solanaceae		
Nicotiana tabacum	temperate	Peyronel, 1923, 1924; Koch, 1935; Hildebrand & Koch, 1936
Solanum grandiflorum	tropical	Thomazini, 1974
S. lycocarpum	tropical	Thomazini, 1974
S. nigrum	temperate	Peyronel, 1924
S. palinacanthum	tropical	Thomazini, 1974
S. tuberosum	temperate	Peyronel, 1922, 1923, 1924; Shternberg, 1951; Gelcerova, 1961
Sterculiaceae		
Waltheria communis	tropical	Thomazini, 1974
Theaceae		
Camellia japonica (*Thea japonica*)	temperate	Steinmann, 1929
C. sinensis	temperate	Gadd, 1929; Tunstall, 1940
Tiliaceae		
Tilia tomentosa	temperate	Mejstrik, 1969, 1971
Ulmaceae		
Ulmus americana	temperate	Thomas, 1943
Umbelliferae		
Aegopodium podagraria	temperate	Peyronel, 1924
Apium graveolens	temperate	Peyronel, 1924
Chaerophyllum hirsutum. (*C. cicutaria*)	temperate	Peyronel, 1924
Heracleum maximum (*H. lanatum*)	temperate	Fernando & Currah, 1996
Lomatium martindalei	temperate	J.M. Trappe, unpubl.
Peucedanum ostruthium	temperate	Peyronel, 1924
P. verticillare	temperate	Peyronel, 1924
Pimpinella saxifraga	temperate	Demin, 1971
Urticaceae		
Urtica membranacea	temperate	Peyronel, 1924
Valerianaceae		
Valeriana sitchensis	temperate	J.M. Trappe, unpubl.
Violaceae		
Viola biflora	temperate	Peyronel, 1924
V. calcarata	alpine	Peyronel, 1924
V. canina	temperate	Peyronel, 1924
V. hirta	temperate	Peyronel, 1924

V. nuttallii	temperate	J.M. Trappe, unpubl.
V. odorata	temperate	Peyronel, 1924
V. palustris	alpine	Peyronel, 1924
Vitaceae		
Vitis vinifera	temperate	Peyronel, 1923, 1924; Shterenberg & Kostyuk, 1955; Kostyuk & Shterenberg, 1959; Schubert, 1979; Schubert *et al.*, 1981
Zygophyllaceae		
Zygophyllum spinosum	temperate	Allsopp & Stock, 1993
MONOCOTYLEDONAE		
Alliaceae		
Allium cepa	temperate	Peyronel, 1924
A. sativum L	temperate	Peyronel, 1924
A. sphaerocephalum	temperate	Gallaud, 1905
Aloeaceae		
Aloe arborescens.	temperate	Gelcerova, 1961
Amaryllidaceae		
Galanthus nivalis	temperate	Peyronel, 1924
Araceae		
Arum italicum	temperate	Peyronel, 1923, 1924
Asparagaceae		
Asparagus densiflorus	temperate	Yousef, 1946
Asphodelaceae		
Asphodelus ramosus	temperate	Peyronel, 1924
Colchicaceae		
Colchicum autumnale	temperate	Peyronel, 1924
Colvallariaceae		
Colvallaria majalis	temperate	Peyronel, 1924
Disporum trachycarpum	temperate	Currah & Van Dyk, 1986
Maianthemum bifolium	temperate	Peyronel, 1924
M. racemosum (Smilacina racemosa)	temperate	Currah & Van Dyk, 1986
Schellhammera undulata	tropical	Burges, 1936
Cyperaceae		
Carex sp.	alpine	Fernando & Currah, 1995, 1996
C. arenaria	temperate	Dominik & Pachlewski, 1955
C. curvula	alpine	Haselwandter & Read, 1980; Read & Haselwandter, 1981
C. firma	alpine	Haselwandter & Read, 1980, 1982; Read & Haselwandter, 1981; Blaschke, 1991*b*
C. membranacea	arctic	Bledsoe *et al.*, 1990
C. misandra	arctic	Bledsoe *et al.*, 1990
C. nardina	arctic	Kohn & Stasovski, 1990
C. nigricans	alpine	Cázares, 1992
C. scirpoidea	arctic	Kohn & Stasovski, 1990
C. scopulorum	alpine	Cázares, 1992
C. sempervirens	alpine	Read & Haselwandter, 1981; Haselwandter & Read, 1982;

		Blaschke, 1991*b*
Cyperus rotundus	temperate	Shvartsman, 1955
Eriophorum scheuchzeri	arctic	Bledsoe *et al.*, 1990; Väre *et al.*, 1992
Kobresia myosuroides	arctic	Kohn & Stasovski, 1990
Uncinia meridensis	subantarctic	Christie & Nicolson, 1983
Graminaceae		
Agropyron cristatum	temperate	Shterenberg, 1951
A. fragile (A. sibiricum)	temperate	Shterenberg, 1951
Agrostis alpina	alpine	Read & Haselwandter, 1981
A. canina	temperate	Dominik & Pachlewski, 1955
A. idahoensis	temperate	J.M. Trappe, unpubl.
Alopecurus alpinus	arctic	Bledsoe *et al.*, 1990; Väre *et al.*, 1992
Ammophila arenaria (*A. arundinaceae*)	temperate	Dominik & Pachlewski, 1955
Anthoxanthum odoratum	alpine	Read & Haselwandter, 1981
Avena sativa	temperate	Peyronel, 1924; Shterenberg, 1951; Balis, 1970; Deacon, 1973
Bromus inermis	temperate	Shterenberg, 1951
Corynephorus canescens	temperate	Dominik & Pachlewski, 1955
Cynodon dactylon	temperate	Peyronel, 1924
Dactylis glomerata	temperate	Nicolson, 1959; Balis, 1970
Deschampsia alpina	arctic	Väre *et al.*, 1992
D. antarctica	subantarctic	Christie & Nicolson, 1983
D. caespitosa	alpine	Blaschke, 1991*a*, *b*; Cázares, 1992; A. Jumpponen, unpubl.
D. flexuosa	alpine	Read & Haselwandter, 1981
Dupontia fisheri	arctic	Bledsoe *et al.*, 1990
Echinolaena inflexa	tropical	Thomazini, 1974
Festuca brachyphylla	arctic	Kohn & Stasovski, 1990; Väre *et al.*, 1992
F. contracta	subantarctic	Christie & Nicolson, 1983
F. halleri	alpine	Read & Haselwandter, 1981
F. hyperborea	arctic	Väre *et al.*, 1992
F. ovina (*F. vivipara*)	arctic	Väre *et al.*, 1992
F. quadriflora (F. pumila)	alpine	Blaschke, 1991*a*, *b*
F. rubra (*F. cryophila*)	alpine, arctic	Balis, 1970; Väre *et al.*, 1992
F. violacea	alpine	Read & Haselwandter, 1981
Hierochloe alpina	arctic	Väre *et al.*, 1992
Holcus mollis	temperate	Peyronel, 1924
Hordeum vulgare	temperate	Peyronel, 1922, 1923, 1924; Shterenberg, 1951; Balis, 1970
Lolium multiflorum (*L. italicum*)	temperate	Balis, 1970; Deacon, 1973
L. perenne	temperate	Williams, 1985
Lolium pratensis (*Festuca arundinacea*)	temperate	Shterenberg, 1951
Melinis minutiflora	tropical	Thomazini, 1974
Nardus stricta	alpine	Peyronel, 1924
Oreochloa disticha	alpine	Read & Haselwandter, 1981

Paspalum virgatum	tropical	Johnston, 1949
Phippsia algida	arctic	Väre *et al.*, 1992
P. concinna	arctic	Väre *et al.*, 1992
Phleum alpinum	alpine	J.M. Trappe, unpubl.
P. pratense	temperate	Balis, 1970; Deacon, 1973
Poa alpigena	arctic	Väre *et al.*, 1992
P. alpina	alpine, arctic	Read & Haselwandter, 1981; Blaschke, 1991*b*; Väre *et al.*, 1992
P. annua	alpine	Peyronel, 1924
P. arctica	arctic	Kohn & Stasovski, 1990; Väre *et al.*, 1992
P. fendleriana (P. cusickii)	alpine	Cázares, 1992
P. laxa	alpine	Haselwandter & Read, 1980; Read & Haselwandter, 1981
P. nevadensis	alpine	Cázares, 1992
Porteresia coarctata	tropical	Sengupta *et al.*, 1989
Saccharum officinarum	tropical	Johnston, 1949
Secale cereale	temperate	Peyronel, 1924; Shterenberg, 1951
Sesleria albicans	alpine	Blaschke, 1991*b*
S. albicans (S. varia)	alpine	Blaschke, 1991*a*
S. disticha	alpine	Haselwandter & Read, 1980; Read & Haselwandter, 1981
Setaria verticillata	temperate	Peyronel, 1924
Stenotaphrum secundatum	tropical	Thomazini, 1974
Tribolium uniolae	temperate	Alsopp & Stock, 1993
Trisetum spicatum	arctic	Väre *et al.*, 1992
Triticum aestivum	temperate	Peyronel, 1922, 1923, 1924; Shterenberg, 1951; Strzemska, 1953; Bilai, 1955; Khrushcheva, 1955, 1960; Gelt'ser, 1962;Balis, 1970; Scott, 1970; Deacon, 1973; Deacon, 1981; Iqbal *et al.*, 1982
T. turgidum (*T. polonicum*)	temperate	Strzemska, 1953
Zea mays	temperate	Peyronel, 1922, 1923l, 1924; Shterenberg, 1951; McKeen, 1952
Haemodoraceae		
Wachendorfia parviflora	temperate	Allsopp & Stock, 1993
Hyacinthaceae		
Ornithogalum umbellatum	temperate	Peyronel, 1924
Iridaceae		
Antholyza ringens	temperate	Allsopp & Stock, 1993
Babiana cf. *nana*	temperate	Allsopp & Stock, 1993
Crocus imperati	temperate	Peyronel, 1924
C. vernus	alpine	Peyronel, 1924
Iris albicans	tropics	Yousef, 1946
I. germanica	temperate	Peyronel, 1924
Romulea schlechteri	temperate	Allsopp & Stock, 1993
Juncaceae		

Juncus drummondii	alpine	Cázares, 1992; A. Jumpponen, unpubl.
J. biglumis	arctic	Bledsoe *et al.*, 1990
J. inflexus	temperate	Harley & Harley, 1987
Luzula arctica	arctic	Väre *et al.*, 1992
L. campestris	temperate	J.M. Trappe, unpubl.
L. confusa	arctic	Kohn & Stasovski, 1990
L. piperi	alpine	Cázares, 1992; A. Jumpponen, unpubl.; J.M. Trappe, unpubl.
L. nivalis	arctic	Bledsoe *et al.*, 1990
L. spicata	alpine	Read & Haselwandter, 1981
Liliaceae		
Calochortus apiculatus	temperate	Currah & Van Dyk, 1986
C. subalpinus	alpine	J.M. Trappe, unpubl.
Erythronium grandiflorum	alpine	Currah & Van Dyk, 1986
Lilium martagon	temperate	Peyronel, 1924
Melanthiaceae		
Tofieldia glutinosa	alpine	Cázares, 1992
T. pusilla	alpine	Currah & Van Dyk, 1986
Veratrum lobelianum	alpine	Peyronel, 1924
V. viride	temperate	J.M. Trappe, unpubl.
Xerophyllum tenax	temperate	J.M. Trappe, unpubl.
Orchidaceae		
Amerorchis rotundifolia	temperate	Currah *et al.*, 1987
Calypso bulbosa	temperate	Currah *et al.*, 1987; Currah *et al.*, 1988
Coeloglossum viride	temperate	Currah *et al.*, 1990; Fernando & Currah, 1995
Corallorhiza maculata	temperate	Currah *et al.*, 1987
C. trifida	alpine	Currah *et al.*, 1990; Fernando & Currah, 1995
Listera borealis	temperate	Currah *et al.*, 1990;
Piperia unalascensis	temperate	Fernando & Currah, 1995
Platanthera hyperborea		
Spiranthes lacera	temperate	Fernando & Currah, 1995
Palmae		
Phoenix canariensis	tropics	Stanczak-Boratynska, 1954
P. paludosa	tropics	Sengupta *et al.*, 1989
Ruscaceae		
Ruscus aculeatus	alpine	Gallaud, 1905
Smilacaceae		
Smilax syringoides	tropical	Thomazini, 1974
Trilliaceae		
Paris quadrifolia	temperate	Schlicht 1889
GYMNOSPERMAE		
Cupressaceae		
Chamaecyparis nootkatensis	temperate	Hennon *et al.*, 1990
Juniperus communis	temperate	Dominik, 1961
J. rigida	temperate	Tominaga, 1971
J. scopulorum	temperate	Thomas, 1943

J. virginiana	temperate	Thomas, 1943
Thuja plicata	temperate	Ahlich & Sieber, 1995
Pinaceae		
Abies alba	temperate	Blaschke, 1981; Ahlich & Sieber, 1995
A. balsamea	temperate	Fernando & Currah, 1996
A. lasiocarpa	alpine	Cázares, 1992; Cázares & Trappe, 1993
A. spectabilis	temperate	Ahlich & Sieber, 1995
Picea abies	boreal, temperate	Melin, 1922; Livingston & Blaschke, 1984; Haug *et al.*, 1988; Cerny & Cudlin, 1989; Holdenrieder & Sieber, 1992; Ahlich & Sieber, 1995; Dahlberg, Jonsson & Nylund, 1997
P. engelmannii	temperate	Thomas, 1943
P. glauca	temperate	Danielson & Visser, 1990; Danielson, 1991
P. mariana	temperate	Richard & Fortin, 1973, 1974
P. pungens	temperate	Thomas, 1943
P. rubens	temperate	Wilcox & Wang, 1987b
P. sitchensis	temperate	Levisohn, 1954; Schild *et al.*, 1988
Pinus banksiana	temperate	Danielson & Visser, 1989; Danielson, 1991
P. contorta	temperate	Thomas, 1943; Levisohn, 1954; Egger & Paden, 1986; Danielson & Visser, 1990; O'Dell *et al.*, 1993
P. flexilis	temperate	Thomas, 1943
P. muricata	temperate	Horton, Cázares & Bruns, 1998
P. mugo	temperate	Ahlich & Sieber, 1995
P. nigra	temperate	Levisohn, 1954
P. ponderosa	temperate	Thomas, 1943
P. radiata	temperate	Levisohn, 1954; Chu-Chou, 1979
P. resinosa	temperate	Wang & Wilcox, 1985; Wilcox & Wang1987*b*
P. sylvestris	boreal, temperate	Melin, 1922; Levisohn, 1954; Robertson, 1954; Kowalski, 1973; Wang & Wilcox, 1985; Ahlich & Sieber, 1995
Pseudotsuga menziesii	temperate	Thomas, 1943
Tsuga dumosa	temperate	Ahlich & Sieber, 1995
T. heterophylla	temperate	Ahlich & Sieber, 1995
T. mertensiana	alpine	Cázares, 1992; Cázares & Trappe, 1993; A. Jumpponen, unpubl.
Podocarpaceae		
Podocarpus spinulosus	temperate	McLuckie, 1923
EQUISETOPSIDA		
Equisetaceae		
Equisetum arvense	temperate	Boullard, 1957

E. hyemale	temperate	Boullard, 1957
E. scirpoides	alpine	Currah & Van Dyk, 1986
E. sylvaticum	temperate	Boullard, 1957
LYCOPSIDA		
Lycopodiaceae		
Diphasiastrum alpinum (Lycopodium alpinum)	alpine	Freeburg, 1962
Hyperenzia phlegmaria (L. phlegmarium)	tropical	Burgeff, 1938; Boullard, 1957, 1979;
H. selago (L. appressum; L. lucidulum)	alpine	Burgeff, 1938; Freeburg, 1962
Lycopodium cernua (L. cernuum)	temperate	Freeburg, 1962
Selaginellaceae		
Selaginella selaginoides	alpine	Bruchmann 1897
S. selaginoides (S. spinulosa)	temperate	Bruchmann 1897
POLYPODIOPSIDA		
Adiantaceae		
Adiantum venustum	temperate	Iqbal *et al.*, 1981
Onychium japonicum	tropical	Iqbal *et al.*, 1981
Aspleniaceae		
Asplenium dalhousiae (Ceterachopsis dalhousiae)	temperate	Iqbal *et al.*, 1981
A. pseudofontanum	temperate	Iqbal *et al.*, 1981
A. trichomanes	temperate	Iqbal *et al.*, 1981
Phyllitis scolopendrium	temperate	Fontana, 1959
Blechenaceae		
Blechnum spicant	alpine	Fontana, 1959
Cyatheaceae		
Alsophila extensa	tropical	Boullard, 1957
Dennstaedtiaceae		
Pteridium aquilinum	temperate	Dominik & Pachlewski, 1955; J.M. Trappe, unpubl.
Dryopteridaceae		
Dryopteris filix-mas	temperate	Boullard, 1957
D. fragrans	arctic	Kohn & Stasovski, 1990
D. odontoloma	temperate	Iqbal *et al.*, 1981
Polystichum longchitis	temperate	J.M. Trappe, unpubl.
Hymenophyllaceae		
Hymenophyllum membranaceum	tropical	Boullard, 1957
H. tunbrigense	tropical	Boullard, 1957
Pleuromanes pallidum (Trichomanes pallidum)	tropical	Boullard, 1957
Marattiaceae		
Danaea elliptica	temperate	Boullard, 1957
D. nodosa	temperate	West, 1917
Marattia alata	tropical	West, 1917
Matoniaceae		
Matonia foxworthyi	temperate	Boullard, 1957
Ophioglossaceae		

Botrychium virginianum	temperate	Jeffrey 1897
B. pumicola	temperate	F. Camacho, unpubl.
Helminthostachys zeylanica	tropical	Nozu, 1961
Polypodiaceae		
Leptochilus auriculatus	tropical	Boullard, 1957
Pteridiceae		
Pteris cretica	temperate	Iqbal *et al.*, 1981
Schizaeaceae		
Schizaea pusilla	temperate	Britton & Taylor, 1901
Woodsiaceae		
Athyrium filix-femina	temperate	Iqbal *et al.*, 1981
Diplazium polypodioides	tropical	Iqbal *et al.*, 1981
Gymnocarpium dryopteris	temperate	Berch & Kendrick, 1982
Matteuccia struthiopteris	temperate	Berch & Kendrick, 1982
Onoclea sensibilis	temperate	Berch & Kendrick, 1982
PSILOTOPSIDA		
Psilotaceae		
Psilotum nudum		Bernatsky 1899; Bierhorst, 1953

APPENDIX 2: SHORT REFERENCES FOR APPENDIX 1.

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