Native Ectomycorrhizal Fungi of Limber and Whitebark Pine: Necessary for Forest Sustainability?

Paper

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Abstract—Ectomycorrhizal fungi are an important component of northern coniferous forests, including those of *Pinus flexilis* (limber pine) and *P. albicaulis* (whitebark pine) which are being decimated by white pine blister rust and mountain pine beetles. Ectomycorrhizal fungi are known to promote seedling establishment, tree health, and may play a role in forest sustainability. The goal of this research is to discover the native ectomycorrhizal fungi associated with these two pines in the Rocky Mountain region. Here we report 32 species of ectomycorrhizal fungi associated with whitebark pine, 26 with limber pine, with an overlap of 14 species (primarily suilloids). The ectomycorrhizal fungi can be grouped into 1. generalists, 2. western conifer associates, 3. calcareous species (limber pine) and 4. specialists for five-needle pine or stone pines (primarily suilloids). Some of the *Suillus* species occur with stone pines globally, suggesting a long co-evolutionary history and important ecological roles. Their association with limber pines is newly reported. These five-needle pine specialists could confer a competitive advantage over spruce and fir when present. A preliminary study of the physiology of the suilloid fungi reveals intra- and inter-specific variation in pH preference/tolerance *in vitro*. Strains with limber pines from calcareous sites exhibit a broader pH tolerance than those found with whitebark pine which is restricted to high elevations. It is hoped that these efforts contribute to an understanding of the native ectomycorrhizal fungi with whitebark and limber pine and provide information useful towards sustaining these tree species, including strain selection for inoculation of nursery seedlings.

Introduction

Ectomycorrhizal fungi are an important component of northern coniferous forests (Smith and Read 1997), including those of limber (*Pinus flexilis*) and whitebark pine (*P. albicaulis*) (Mohatt and others 2008). These two pine species are currently being seriously impacted by white pine blister rust and mountain pine beetles in much of their range (Schwandt 2006). Pines are highly dependent on ectomycorrhizal fungi for establishment (Hasselquist and others 2005) and growth (Smith and Read 1997). Ectomycorrhizal fungi are assumed to play a strong role in forest sustainability. It is acknowledged that a successful reforestation strategy for whitebark pine will need to incorporate information on links between seed/seedling performance and various biotic and abiotic factors (Bower and Aitken 2008). One factor that has not been addressed previous to our research is the beneficial association between ectomycorrhizal fungi and whitebark and limber pines (Mohatt and others 2008).

There are 7,000-10,000 species of ectomycorrhizal fungi (Taylor and Alexander 2005) associated with trees and woody shrubs. Each tree species hosts a particular subset of these fungi. Some, such as Douglas fir are capable of hosting over 2,000 species of ectomycorrhizal fungi (Trappe 1962) while others such as alder host only a few (Brunner and others 1992; Hirose and others 2010). Fungal associations are further restricted by soil type, climate, tree age and other factors (Cripps 2003). As five-needle pines, both whitebark and limber pine are likely to host a fairly limited set of ectomycorrhizal fungi further restricted by the harsh nature of growing sites (Mohatt and others 2008).

Ectomycorrhizal fungi (as species or strains) vary in host specificity, soil preference, host age requirements, dispersal strategies, ability to enhance nitrogen (N) or phosphorus (P) uptake, types of N and P accessed and in protective abilities against pathogens, drought, heavy metals, and soil grazers (Smith and Read 1997). The more we learn, the less functional redundancy appears to be the rule with each species/ecotype filling a unique niche (Tedersoo and others 2009). Therefore, the set of benefits provided to hosts depends on the specific community of fungi present on a root system or in a forest. Diversity of ectomycorrhizal fungi in a community can range from a few species on stressed or disturbed sites to hundreds in mature forests (Tedersoo and others 2009).

The goal of this research is to discover the native ectomycorrhizal fungi associated with limber and whitebark pine. Prompt attention is necessary as host-specific fungi and certain ecotypes could disappear along with specific pine populations. Here we report the ecological (not applied) aspects of this research focused on the Rocky Mountain region. We compare the ectomycorrhizal fungi found with whitebark pine (Mohatt and others 2008) to our new findings on the ectomycorrhizal fungi associated with limber pine. This is of interest for restoration purposes and because evolutionary histories of the two pine species differ, but they can co-occur. We do not know if they host the same ectomycorrhizal species. Also, in order to begin to understand the physiological diversity of ectomycorrhizal fungi associated with these pines, we examined pH preferences of various strains of suilloid fungi (of high importance to pines) to see if this links with host (limber/whitebark) or original soil type (calcareous/not). Here we report preliminary results. It is hoped that these efforts contribute to a greater understanding of the ectomycorrhizal fungi with whitebark and

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limber pine and provide information useful towards sustaining these tree species.

Methods

Fruiting bodies of ectomycorrhizal fungi were collected from various sites in the north-central Rocky Mountains, primarily in the Greater Yellowstone Area (GYA), mountain ranges in southwest Montana, and Waterton Lakes National Park from 2001 to 2010. Sporocarps were collected, described, photographed, dried and tissue-cultured when possible. Voucher specimens are in the MONT Herbarium at Montana State University. The description of sites A-K is as follows:

- *Site A. Greater Yellowstone Area* (largest site) includes *a*) New World district: approximately 2590- 3105 m a.s.l. (8497-10187 ft), Fisher and Miller Creek drainages, Gallatin National Forest, Beartooth/Absaroka Mountains, Park County, Montana b) Dunraven Pass, approximately 2682 m a.s.l. (8800 ft), northeast side of Yellowstone National Park and c) Beartooth Pass, approximately 2890 m a.s.l. (9480 ft), east side of pass, Beartooth/Absaroka Mountains, Custer National Forest, Carbon County, Montana. Predominately whitebark pine with some scattered spruce and *Vaccinium* understory present. Mineral soil has a pH of 5.8 on Beartooth Pass area.
- *Site B. Sacajawea Saddle,* approximately 2700 m a.s.l (8860 ft), Bridger Mountains, Gallatin National Forest, Gallatin County, Montana. Predominately limber pine with occasional whitebark pine at lower elevations and a pure stand across the saddle. Mineral soil has a pH of 6.3 at lower elevations.
- *Site C. Golden Trout Lake,* approximately 2590 m a.s.l. (8497 ft), across Gallatin Canyon from Big Sky, Gallatin County, Montana. Predominantly in whitebark pine with a *Vaccinium* understory. Soil pH was not tested.
- *Site D. Gravelly Mountain Range,* approximately 2500-2630 m a.s.l. (8202-8628 ft), Gravelly Mountains, Madison County, Montana. Limber pine at lower elevations and whitebark pine at higher elevations. Soil pH is 5.5 in whitebark pine area.
- *Site E. Waterton Lakes-Glacier International Peace Park, and Crow's Nest pass,* approximately 1524-1890 m a.s.l. (5000-6200 ft), southern Alberta, Canada. Limber pine at Horseshoe Basin, whitebark pine at Cameron Trail, mixed at Lineham Trail, and limber pine on Crow's Nest Pass. Soil pH was not tested.
- *Site F. Lewis and Clark State Park,* approximately 1675 m a.s.l. (5500 ft), above trail from Visitor Center, Lewis and Clark State Park, Jefferson County, Montana. Pure stand of limber pine with grass and forbs understory. Soil has a pH of 6.9.
- *Site G. Red Lodge,* approximately 2300 m a.s.l. (7600 ft), on ridge opposite Red Lodge Ski area, Custer National Forest, Carbon County, Montana. Predominately limber pine of mixed ages with Douglas fir at lower elevations. Mineral soil has a pH of 7.2.
- *Site H. Crown Mountain*, approximately 2011 m a.s.l. (6600 ft), upper Whitewater Creek drainage, Front Range/Lewis Mountains, Lewis and Clark National Forest, Lewis and Clark County, Montana. Predominately limber pine with occasional Douglas fir at lower elevations. Mineral soil has a pH of 7.6.
- *Site I. Storm Lake,* approximately 2511 m a.s.l. (8238 ft), west of Anaconda, Deer Lodge Forest, Deer Lodge County, Montana. Primarily in whitebark pine. Soil pH not tested.
- *Site J. Red Mountain*, approximately 2347 m a.s.l. (7770 ft), upper Alice Creek drainage, Helena National Forest, Lewis and Clark County, Montana. Predominately limber pine with mixed Douglas fir and lodgepole pine at lower elevations. Soil pH is 6.31.
- *Site K. Avalanche Lake*, approximately 2473 m a.s.l. (9000 ft), Madison Range, Lee Metcalf Wilderness, Gallatin National Forest, Madison County, Montana. Predominately whitebark pine with some scattered spruce and *Vaccinium* understory present. Soil pH was not tested.

Tissue Culture of Fungi and Method for pH Study

Isolations of fungi into pure culture were generally attempted within 24 hours of fruiting body collection. Tissue was removed aseptically from the context of mushroom caps and placed on sterile Modified Melin Norkran's agar (Molina and Palmer 1982) supplemented with 50 mg l^{-1} each of ampicillin and tetracycline. Duplicate tissue samples were placed in vials of 2 percent CTAB (buffer) and frozen for subsequent DNA analysis. Parafilm-sealed petri dishes were incubated at room temperature (22-25°C) until visible signs of growth appeared at which point they were subcultured on a modified MMN medium that lacked malt extract and contained biotin at 1.0 g per liter and a trace element solution. Stock cultures were maintained on slants of the latter medium at 4°C.

Three hypogeous (subterranean) suilloid fungi were examined including isolates of *Rhizopogon roseolus* (Corda) Th. Fr. from Avalanche Lake (CLC 2489), Beartooth site (CLC 2469) and Crown Mountain (CLC 2475). The first two isolates were associated with whitebark pine whereas the last was associated with limber pine. Four isolates of epigeous suilloid fungi were examined including: *Suillus sibericus* Singer from the Beartooth site (CLC 2472) and New World site (CLC 2345); both with whitebark pine. Two limber pine associates were studied including *Suillus tomentosus* var. *discolor* Smith, Thiers, Miller (CLC 2422) from Sacajawea and *Suillus cf placidus* (Bon.) Singer (CLC 2473) from Crown Mountain. It should be noted that associates from sites with limber pine generally have higher pH soils.

For pH work, the base medium was MMN salts (Molina and Palmer 1982) lacking malt extract and with glucose

reduced to 5 g l^{-1} giving a final C:N ratio of approximately 30:1. As malt extract was removed the base medium was supplemented with biotin 1.0 g l^{-1} and a trace elements solution (Dickie 1998). Media were buffered with MES buffer according to Hilger et al. (1986) and others adjusted to pHs of 4.0, 5.5 and 7.0 with either HCl or NaOH prior to autoclaving (20 min at 121°C). Gellan (Phytagel, Sigma Life Sciences, St Louis, USA) was used at 6.5 g l^{-1} as a solidifying agent following (Dickie and others 1998). The pH of each treatment was verified after autoclaving.

Fungi to be used as inoculum were grown on plates with 20 ml MNN agar medium lacking malt extract at 22°C for at least 21 days. Plates were aseptically center-inoculated with one disk obtained from the active colony margin with a sterile 6 mm cork borer and sealed with parafilm. Three replicate plates were established for each treatment. Twenty disks were placed on pre-dried and weighed filter paper and dried overnight at 60°C. The average dry weights of these disks were used to remove the contribution of agar plugs from final mycelial dry weights. Plates were subsequently incubated at 22-25°C. Plates were examined weekly and radial growth measurements were obtained in two directions at right angles. Data were recorded on colony morphology and pigment production as described by (Keller 1996). At the end of the growing period plates were photographed under ambient natural light with lids removed on a Dreadnaught gray (# 6099 Daler-Rowney, Berkshire, UK) background for subsequent area and color analysis.

Dry weights for use in density calculations were collected when the fastest growing treatment had covered approximately two-thirds of the plate surface (90 X 15 mm dishes), for our isolates this ranged from 21 to 42 days. Colonies were removed by cutting around the margins with a scalpel and transferring this to a weighing dish to obtain a fresh weight. Colonies were then cut into pie-shaped wedges and placed in plastic drink cups. Citrate buffer pH 6.0 was then added to give a solution volume to mycelium-gel weight ratio of 10:1; the covered cups then stood 24 to 72 h prior to filtration and rinsing (Dickie and others 1998). Mycelium on pre-dried filter paper was weighed after 24 h at 60°C.

Results

Forty-five species of ectomycorrhizal fungi were collected from whitebark and limber pine forests (Table 1) and are considered putative associates of these tree species. Most species in the important suilloid group have been confirmed molecularly on roots and by mycorrhizal synthesis with seedlings in the greenhouse (Cripps and others 2008; Mohatt and others 2008). Other species await molecular or synthesis confirmation. Of these, 32 species of ectomycorrhizal fungi are reported for whitebark pine (Table 1, column 2), 26 species for limber pine (Table 1, column 3) with an overlap of approximately 14 species at this point in time. Examples of ectomycorrhizal species are shown in Figure 1.

The small pH study tested the growth of ectomycorrhizal fungi *in vitro* at pH 4, 5.5 and 7 (Figure 2). The two strains of *Suillus* from whitebark pine forests (CLC 2422, CLC 2345) grew similarly at pH 4 and pH 5.5, but growth was significantly reduced at pH 7. The two *Suillus* strains from limber pine forests (CLC 2472, CLC 2473) grew comparably at all three pH levels, thus spanning a pH range of 1000x. Similarly, the two *Rhizopogon s*trains from whitebark pine forests grew at pH 4 and pH 5.5, but growth was significantly reduced at pH 7. The *Rhizopogon* strain from the limber pine forest grew at all three pH levels, but there was a lag phase at pH 7 (data not shown). In general, limber pine occurs on higher pH soils than whitebark pine.

Discussion

Overall, the diversity of species of ectomycorrhizal fungi in whitebark and limber pine forests in the north-central Rocky Mountain region appears relatively limited in comparison to other tree species (Mohatt and others 2008). This could reflect dry, cold conditions at these high elevations which are not conducive to fungal fruiting. Alternatively, results might mean that these pines are dependent on a rather limited set of ectomycorrhizal fungi. For the studied area, the ectomycorrhizal fungi in whitebark pine forests consist of 33 percent in Boletales (primarily suilloids), 21 percent in Cortinariales, 12 percent in Russulales, with 33 percent as other types (mostly *Hygrophorus* and *Tricholoma* species). For limber pine the numbers are 20 percent in Boletales (suilloids), 16 percent in Cortinariales, 28 percent in Inocybaceae (mostly *Inocybe* from one site), 16 percent in Russulales and 16 percent as miscellaneous types. This is in line with results from a study of ectomycorrhizal fungi with the European stone pine (*Pinus cembra*) done by Keller (1997) who found 44 percent in Boletales, 32 percent in Cortinariales, 6 percent in Inocybaceae, 11 percent in Russulales and 7 percent from other groups. This suggests that these limber and whitebark pine forests host a Boletales-Cortinariales type of ectomycorrhizal community as reflected by aboveground sporocarps. Fungi not, or rarely, represented by sporocarps such as thelephoroids, *Cenococcum, Amphinema,* and *Piloderma* have been confirmed on roots of whitebark pine seedlings (Mohatt and others 2008), but roots of limber pine have not yet been analyzed and so these fungi are not included in Table 1. However, whitebark pine seedlings from a belowground ectomycorrhizal perspective were found to be dominated by *Cenococcum,* suilloids and Cortinariales (Mohatt and others 2008). However, proportions of these species are known to change on seedling roots after severe fire (Trusty and Cripps, these proceedings).

Ecologically, the ectomycorrhizal fungi reported can be grouped into a) generalists known to associate with many tree species under a variety of conditions (*Amphinema, Cenococcum, Piloderma, thelephoroid fungi*), b) high-elevation western conifer associates including species of *Cortinarius, Russula, Lactarius, Tricholoma* and *Hygrophorus,* c) fungi with a preference for calcareous and/or sandy soil (i.e. *Inocybe* species found on one limber pine site), and d) those specific for pines, five-needle pines or stone pines (*Suillus, Rhizopogon,*

Table 1. Fruiting ectomycorrhizal species collected in whitebark (*Pinus albicaulis,* PA) and limber pine (*P. flexilis,* PF) forests in the Greater Yellowstone Area and north to Waterton Lakes National Park. Sites are: A= New World Mine district and YNP (PA), B= Sacagawea Saddle (PA, PF), C= Golden Trout Lake (PA), D= Gravelly Range (PA, PF), E= Waterton Lakes and Glacier Parks (PA, PF), F= Lewis and Clark State Park (PF), G= Red Lodge (PF), H= Crown Mt. (PF), I= Storm Mt (PA), J= Red Mt. (PF), and K= Avalanche Lake (PA). na = not checked.

Species	*P. albicaulis*	*P. flexilis*	Both PA & PF
BASIDIOMYCOTA - AGARICALES			
AMANITACEAE			
Amanita "alpina" A.H. Smith nom. prov.	A, B, C		
Amanita muscaria sl (Fr.) Gray	A		
HYGROPHORACEAE			
Hygrophorus gliocyclus Fr.	A, B, D, E	G, E	yes
Hygrophorus marzuolus (Fr.) Bres.	A, C		
Hygrophorus olivaceoalbus (Fr.:Fr.) Fr.	A		
Hygrophorus subalpinus A. H. Smith	A,C		
Hygrophorus sp. (aff. *H. piceae*)	B		
TRICHOLOMATACEAE			
Leucopaxillus paradoxis (Cost. & Durfour) Boursier	D		
Leucopaxillus gentianus (Quél.) Kotl.		G	
Tricholoma argyraceum (Bull.) Sacc.		F, D	
Tricholoma moseri Singer	A ,B, C, D	D, F	yes
CORTINARIACEAE			
Cortinarius cf *clandestinus* A.H. Smith	A, D		
Cortinarius duracinus Fr.	A, B, E	E?	
Cortinarius "flavobasalis" McKnight & Moser	A, D		
Cortinarius flavoroseus nom. prov.	A, D		
Cortinarius aff. *fulminoides* (Moser) Moser	B, D		
Cortinarius cf *subolivescens* A.H. Smith	A, D	C? B?	yes?
Cortinarius cf *evernius* Fr.		D	
Hebeloma sp.		G, J	
INOCYBACEAE			
Inocybe aff. *arenicola* (Heim) Bon		F	
Inocybe dulcamara (Alb. & Schw.) Kummer		F	
Inocybe cf. *fraudens* (Britz.) Sacc.		F	
Inocybe nitidiuscula (Britz.) Sacc.		F	
Inocybe aff. *rufuloides* Bon		F	
Inocybe sororia group Stuntz	A	B, D, F	yes
Inocybe splendens group Heim		F	
RUSSULALES			
Lactarius cf. *deterrimus* Gröger	A, B, D	D	yes
Russula albonigra (Krombh.) Fr.	E?	E?	yes?
Russula brevipes Peck	A	E?	yes?
Russula torulosa /queletii group	A, D, E	G, E?	yes
BOLETALES			
SUILLOIDS			
Chroogomphus sp. nov. (secotioid)	A		
Rhizopogon evadens A.H. Smith	A, D, I?	B, D	yes
Rhizopogon milleri A.H. Smith	A, D	D	yes
Rhizopogon molligleba	A		
Rhizopogon roseolus group	A	B, H, J	yes
Rhizopogon olivaceofuscus	A		
Suillus cf *placidus/plorans group*		H	
Suillus sibiricus (Singer) Singer	A, C, D, E	B, D, E, G	yes
Suillus subalpinus M.M. Moser	A, C, I?		
Suillus tomentosus var. *discolor* (bluing)	A, C, D, I	B, D, E?	yes
Suillus sp. (veil)		B, E, H, J	yes
THELEPHORALES			
Pseudotomentella sp.	A	na	
Tomentellopsis sp.	A	na	
Thelephora caryophylla		E	
ASCOMYCOTA			
Cenococcum geophilum Fr.	A, B, C, D, E	na	yes?
Total Species	**32**	**26**	**14**

Figure 1. Ectomycorrhizal fruiting bodies of fungi associated with whitebark pine. a. *Rhizopogon* species ("pogies") and b. *Suillus sibiricus*. *Photos by C. Cripps*.

Chroogomphus). The latter group is termed suilloid fungi (Bruns et al., 2002).

Limber pine is thought to have a southwestern history with possible Mexican pine relatives while whitebark pine appears historically related to Eurasian stone pines (Richardson 1998). However, relationships may be rearranged as suggested by new molecular data (Gernandt and others 2005; Tomback and Linhart 1990; Tsutsui and others 2009). These different histories combined with preferences for different soil types (more calcareous in general for limber pine) might lead to the conclusion that this should be reflected in differences in ectomycorrhizal communities. However, on some study sites, the pines co-occur, and have the potential to host the same ectomycorrhizal fungi. There appears to be only a 25 percent overlap in ectomycorrhizal fungi species for the two pines, although examination of roots is likely to increase the overlap of generalist fungi. Species that separate the two are limber pine associates with a strong preference for calcareous or sandy soil. Interestingly, many of the shared species are suilloid fungi i.e. *Suillus* and *Rhizopogon* species. These fungi are specialists and some occur only with pines in the Section *Strobus* (Grubisha and others 2002; Hirose and others 2010; Kretzer and others 1996). Other suilloid species are further restricted to five-needle or stone pines (Moser 2004).

Suillus sibiricus is known to be restricted to five-needle pines and occurs with stone pines world wide in Europe and Asia (Moser 2004) and it is now recorded with both whitebark and limber pine in the Rocky Mountains. *Suillus tomentosus* var. *discolor* also appears restricted to five-needle pines, but more information is needed to determine its molecular relationship to *S. tomentosus*, a three-needle associate. *Suillus subalpinus* is known only with whitebark pine in the north-central Rocky Mountains (Moser 2004). Other suilloids known to occur with stone pines worldwide are *Suillus plorans* and *S. placidus*, neither of which has been recorded on our sites. Keller (1997) reports *Suillus placidus, S. plorans* and *S. sibiricus* with the stone pines in Europe. Only a few of the hundreds of *Rhizopogon* species have been recorded with limber and whitebark pines on our sites, primarily *Rhizopogon evadens, R. milleri*, and a species in the *roseolus* group also found with three-needle lodgepole pine (Dowie and others, in prep.). A few other species have been reported with *P. albicaulis* but are not confirmed (Molina and Trappe 1992). A new secotioid species of *Chroogomphus* species is recorded with whitebark pine from our study sites. The *Suillus* species appear to be those restricted to five-needle pines or stone pines (Grubisha and others 2002). This host restriction is less clear for the *Rhizopogon* species however, *R. evadens* is both common and widespread with whitebark pine on many of our sites.

Rhizopogon and *Suillus* species (suilloids) are multi-age fungi that occur on both young seedlings and mature trees (Tedersoo and others 2009). They grow well in culture and have been used successfully for restoration purposes. While selected *Rhizopogon* species are used commercially as an inoculum for pine seedlings in North America, it is primarily the *Suillus* species that have been used to regenerate stone pines in Europe for 50 years (Weisleitner, personal communication). The use of ECM fungi restricted to five-needle pines in restoration practices is more likely to give these pines a competitive advantage; typical commercial inoculum generally contains fungi that favor other tree species. *Rhizopogon* is more dependent on mammals (squirrels, deer, etc.) for their spore dispersal, while *Suillus* spores are also dispersed by wind (Ashkannejhad and Horton 2006).

Soil conditions are known to further restrict the set of ECM fungi that can occur with a single host. Cripps (2003) found acidic conditions to restrict the ECM soil community for aspen (*Populus tremuloides*). Our early results demonstrate both strong intraspecific (ecotypes) and interspecific differences in pH growth responses for isolates of five-needle pine suilloid fungi; these results appear linked to the original soil pH and host plant. A wider pH tolerance was exhibited (in terms of growth) in isolates obtained from sites with higher soil pH that were generally associated with limber pine. Earlier work has shown strain and species variation in tolerance to soil acidification *in vitro* (Willenberg and others 1990), but little has been done to try to link original soil properties with pH responses (Smith and Read 1997). Earlier work on pH effects with pure cultures has been summarized by Hung and Trappe (1983). Much more work will

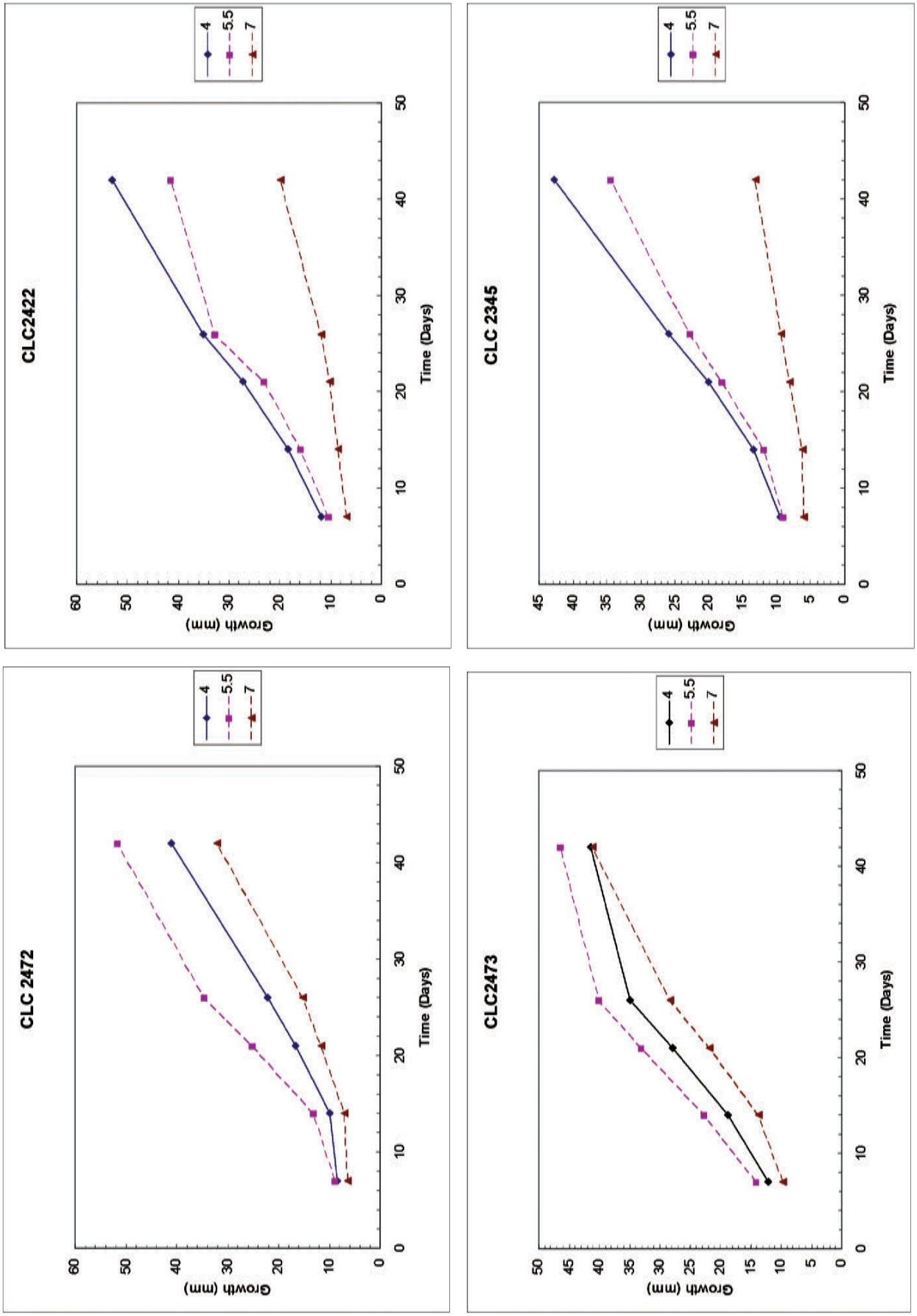

Figure 2. Growth of ectomycorrhizal fungi *in vitro* at pH 4, 5.5 and 7. The two strains of *Suillus* from whitebark pine forests (CLC 2345, CLC 2422) grew comparably at pH 4 and pH 5.5, but growth was reduced at pH 7. The two strains of *Suillus* from limber pine forests (CLC 2472, CLC 2473) grew comparably at all three pH levels, with a slight lag at pH 7. In general, limber pine occurs on higher pH soils than whitebark pine.

be needed to determine if our *in vitro* results translate to differences in mycorrhizal formation and function under greenhouse or field conditions (Dunabitia and others 2004; Ek and others 1994). It should be noted that these are only preliminary results, but limber pine occurs from the plains to timberline, and isolates from this pine, had a wider pH tolerance that those from whitebark pine forests more restricted to high elevations.

It has been suggested that ectomycorrhizal fungi have the ability to alter the course of plant establishment and succession through control of tree nitrogen uptake (Kronzucker and others 1997). Generally these fungi absorb a range of nitrogen sources not necessarily available to non-mycorrhizal pine roots; an important consideration given the potential effects of soil pH (as observed herein) on the nitrogen cycle. These fungi are also prolific producers of protease and phosphatase enzymes that mineralize soil organic compounds; again little is known about the pH response of these enzymes and whether site adaptation occurs (Antibus and others 1986). The current study also reveals differences in pigment production by different fungi in response to pH (results not shown). The production of pigments and low molecular weight organic compounds potentially add carbon sources to soil that could influence the size and structure of the rhizosphere community and be important in the release of essential elements from soil minerals (Rosling 2009). Our plans for future work include an examination of a wider range of ectomycorrhizal fungal species for their ability to use a variety of inorganic and organic nitrogen sources *in vitro*. In addition we are working with greenhouse inoculations and ^{15}N stable isotope analyses to better understand the range of physiological variation found in fungi associated with whitebark and limber pine. Applications from this type of research might include: assessment for the presence of appropriate native fungi in restoration, selection of species/ecotypes for nursery inoculation of seedlings, and monitoring out-planted seedlings for mycorrhizal colonization on various pH soils. Applied research on this topic is reported in Cripps and Grimme (these proceedings).

Whitebark and limber pines are involved in complex plant-fungus coevolutionary interactions under different abiotic conditions across the landscape (Hoeksema 2010). The suilloid fungi are an important group of mycorrhizal symbionts with pines and five-needle pines globally and should be considered as an important aspect of their ecology, distribution and sustainability (Wu and others 2000). It should also be considered that host-specific fungi and particular ecotypes could disappear along with specific pine forests.

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