FINAL REPORT

Project Title

Whitebark Pine Restoration, Dunraven Pass
(Monitoring mycorrhizal status of seedlings)

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Project Location: Yellowstone National Park, Dunraven Pass area

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This report details findings to date and proposed and accomplished deliverables.

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PART 1: SUMMARY, EVALUTION, OPTIONS

Introduction: *Pinus albicaulis* (whitebark pine) is a species in decline from white pine blister rust, mountain pine beetles and fire suppression. The planting of nursery grown rust-resistant seedlings is fast becoming a widely implemented restoration strategy for this tree species. This study was generated by the need for more information on factors which affect the survival of planted whitebark pine seedlings. All pines require the appropriate mycorrhizal fungi on their roots to survive in nature; lack of appropriate fungi can adversely affect the restoration process. The removal of mature whitebark pines along Dunraven Pass for road construction in 2004 and subsequent planting of 4,000 of seedlings along the pass provided an opportunity to determine if appropriate mycorrhizal fungi for whitebark pine seedlings were present and if nursery seedlings were colonized in a timely fashion. Secondly, many of these seedlings were planted in "replaced soil" that included wood mulch; this replaced soil was assessed for the presence of appropriate mycorrhizal fungi in a greenhouse study. Third, a concern over the possibility of nursery seedlings harboring and bringing exotic nursery fungi into the Park was addressed.

Goal: of this project was to determine if whitebark pine seedlings planted for the purpose of restoration along Dunraven pass have access to, and are effectively colonized by the native ectomycorrhizal fungi necessary for their establishment, health and sustainability, and if not, to consider the remedial steps that might be taken in the future.

Objectives:

1. To determine if appropriate mycorrhizal fungi are present in replaced and native soil along Dunraven Pass where whitebark pine seedlings are to be planted [GREENHOUSE SOIL BIOASSAY].

2. To determine if exotic "nursery fungi" are present on seedling roots before planting, and if they persist after planting or are replaced by native mycorrhizal fungi [NURSERY TREES].

3. To assess the mycorrhizal colonization of roots of whitebark pine seedlings nine months after planting along Dunraven Pass and to correlate results with abiotic/biotic factors [ON- SITE MONITORING].

4. To assess future options if seedlings are not effectively colonized.

Results from this project contribute to results from other researchers to help optimize restoration strategies for whitebark pine, and in particular when the planting of rust resistant seedlings is deemed necessary within the Greater Yellowstone Ecosystem.

1. GREENHOUSE SOIL BIOASSAY determined that native mycorrhizal fungi are available in the replaced and native soil along Dunraven Pass, at least at low levels.

A. The Soil Bioassay technique revealed that native mycorrhizal fungi appropriate for whitebark pine seedling colonization were available in the two soils (replaced soil & soil from whitebark pine forests). These fungi were found on roots of 50-60% of seedlings grown in these soils in the greenhouse.
B. The soil bioassay revealed Suilloid fungi to be present in both soils. These fungi are important pine seedling establishment)
C. The number of viable mycorrhizae on seedling roots was low after 1 year in the greenhouse. Soil Bioassay technique could be further optimized or this is true reflection of low levels of fungi.
D. Nursery E-strain fungus persisted on the roots, however native fungi were still able to colonize the seedlings.

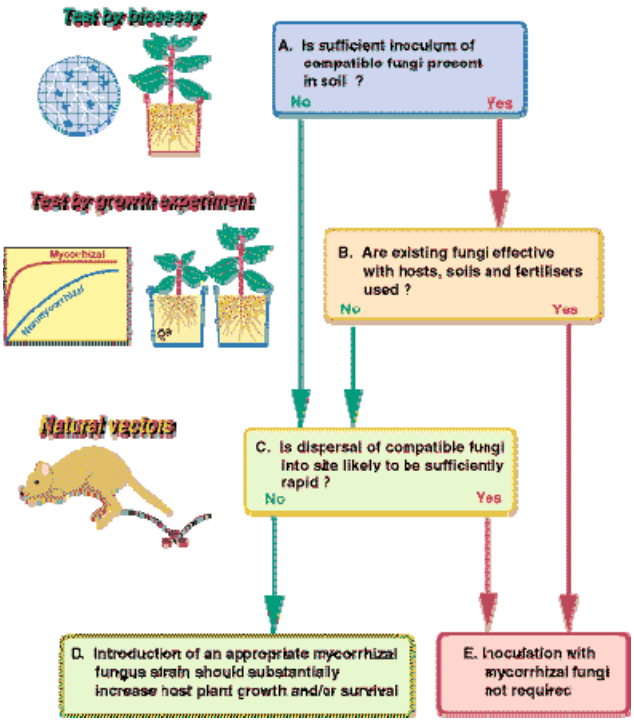
2. NURSERY SEEDLINGS hosted non-native mycorrhizal fungi, including *Thelephora* and E-strain, prior to outplanting, but these were present at low levels.

These are likely different strains than those native to Yellowstone Park, although at the species level, the species do exist within the Park. E-strain was the most common on the nursery seedlings and does not appear to be problematic on sites examined at this point, but should be monitored especially for other soil types. *Thelephora* was present in low levels on nursery seedlings; this fungus could be more problematic, but we did not find it on outplantings. Nursery seedlings with copious white mycorrhizae (likely *Thelephora*) should be monitored on out-planting to ensure this fungus does not spread in natural systems, or is replaced by native fungi.

3. ON SITE MONITORING: Mycorrhizal colonization of out-planted whitebark pine seedlings on 10 sites 9 months after planting on Dunraven Pass revealed native fungi were present on 50% of sites but colonization levels of roots were low (< 4% on average).

A. Native mycorrhizal fungi were present on planted pine seedling roots on 50% of sites.
B. 70% of healthy whitebark pine seedlings examined hosted some type of mycorrhizal fungus and only 10% of the compromised seedlings were colonized. The compromised condition of the B seedlings could be due to abiotic factors which precluded mycorrhization or due to a priori lack of mycorrhizae.
C. Mycorrhizal colonization rates were mostly low < 1% of the whole root system, with an overall average of 4.4%. One seedling was well-colonized at 30% showing significant mycorrhization can occur in 9 months under the right conditions.
D. The presence/abundance of mycorrhizae did not correlate directly with % survival by site in this study, however it did correlate somewhat with the diversity of fungi on roots.
E. Most of the mycorrhizae occurred from 4-12 cm in the soil, but occurred deeper/shallower on the burned sites. This could have implications for planting.
F. Both native and exotic nursery mycorrhizal fungi were found on out-planted whitebark pine seedlings after 9 months. Thus nursery fungi persisted, but native fungi were able to colonize the seedlings.
G. A diversity of native mycorrhizal fungi was found on planted whitebark pine seedlings after 9 months, including the important Suilloids. This shows Suilloid fungi are in the soil and colonized the whitebark pine seedlings on some sites. These fungi are important for pine regeneration.
H. Most of the diversity (number of species) of native mycorrhizal fungi was found on the North side of the pass, and this area supports extensive mature whitebark pine forests.
I. While appropriate mycorrhizal fungi are present, the low colonization rates are a concern.

4. Recommendations and future options should include 1) long term monitoring of seedlings: seedlings should be assessed again in 3-5 years to see if mycorrhizal colonization of planted seedlings has occurred and how this correlates with survival. This could include continued monitoring to determine the persistence of nursery fungi. It would also be important see if root systems were still in a cone-tainer shape and still lacked roots growing into the soil. 2) Individual soils on sites could be used in a Greenhouse bioassay to determine if fungi are still present on each site and if this correlates with subsequent survival by site. 3) The Greenhouse Bioassay method should be evaluated with different aged trees to see if younger seedlings are more amenable to colonization by mycorrhizal fungi. This could have implications for out-planting age. 4) We do not believe that retroactive inoculation with mycorrhizal fungi should be implemented on these sites. The sites are more valuable as controls and many are near roadways and parking lots. 5) We are currently working to develop an inoculation technique for whitebark pine seedlings. Eventually survival inoculated and uninoculated seedlings could be compared in selected areas of YNP, particularly in areas a distance from mature whitebark pine forests or where there is poor seedling survival. 6) A commercial mycorrhizal inoculum should NOT be used for seedlings in YNP. Commercial fungi do not occur with whitebark pine and could upset delicate natural systems.7) Most of the fungi we have discovered are pine associates and do not occur with spruce and fir; if used as inoculum these native fungi could promote a mycorrhizal system not conducive to other tree species. 8) The low colonization rates of seedlings on sites suggest that inoculation of native fungi in the greenhouse might be a benefit on some sites. Smith & Cordell found that inoculation of 5 million seedlings (pine and oak) increased survival from 50 to 90% and ultimately reduced reforestation costs because replanting was not necessary. 9) If subsequent plantings of whitebark pine are planned, the flow chart below should be used to determine if inoculation is necessary. 10) We are also gaining information on the mycorrhizal fungi found with whitebark pine in mature forests and information is not included at this time.



Brundrett et al. 1996. Working with Mycorrhizas in Agriculture. CSIRO.

Fig. 6. Whitebark pine seedling planted on Dunraven Pass with mature whitebark pine tree in the Background.

PART 2: IN DEPTH REPORT WITH DETAILS OF FINDINGS

Accomplishments

1. GREENHOUSE SOIL BIOASSAY *to determine if fungi are available in the replaced soil*. In spring 2006 (June 1), soil was collected by the PI and a student from 10 areas along Dunraven Pass where previously stockpiled soil had been replaced (and contained wood mulch). For a positive control, soil was collected from 10 areas within mature whitebark pine forests. The soil was taken to the MSU Plant Growth Center on the Bozeman Campus. Half of each soil type was steam-sterilized for negative controls. Two year old whitebark pine seedlings obtained directly from the Coeur d'Alene nursery were planted in the treatment soils mixed 1:1 with course vermiculate. Four treatments of 10 seedlings each (1. replaced soil, 2. pasteurized replaced soil, 3. native soil and 4. pasteurized native soil) were placed under typical greenhouse conditions and watered minimally. Seedlings were not fertilized (this prevents mycorrhizal colonization) and were grown for approximately 1 year in the MSU Plant Growth Center. At this time seedlings were assayed for a) colonization by mycorrhizal fungi b) diversity of fungi, and c) species composition (native or not). Root systems were soaked and washed, mycorrhizal root tips inspected for colonization. Diversity was determined by sorting the ectomycorrhizae into morphotypes and counting them. Fungal identity was determined by both morphotyping (dissecting/microscope) and molecular identification. Molecular work consisted of DNA extraction, PCR with fungal specific primers, and sequencing of the ITS region (by Berkeley and Davis Sequencing Labs). Results were subjected to comparison with those in the Genbank database for identification. This was also done by a graduate student.

The goal was to determine if seedlings were colonized by any native mycorrhizal fungi that might be present in the two soils. Use of pasteurized soils as a control also allowed detection of non-native fungi from the nursery seedlings. The Bioassay was set up in late Sept/early October, 2006 in the MSU Plant Growth Center.

2. SITE SET-UP & ASSESSMENT OF NURSERY SEEDLINGS (PRE-PLANTING) *to determine if non-native nursery fungi are present on roots.* In Fall 2006 (Sept 11-13) approximately 4,000 whitebark pine seedlings were planted along Dunraven pass by Mike Angermeyer and crew under the supervision of Dan Reinhart. Kay Izlar (graduate student at U of MT) set up 10 plots on the pass where whitebark pine seedlings were planted with data (Tab. 1).

Table 1. Location of field sites in Yellowstone National Park, along Dunraven Pass, Zone 12, NAD 83. Data from Whitebark Pine Mapping Project (K. Izlar 2007).

	UMT E	*UMT N*	*elevation*	*aspect*	*slope*	*vegetation*	*burned*
1	543253	4959160	8900 ft	W	30^0	50%	-
2	543225	4959232	8885 ft	flat	flat	5%, disturbed, bare	-
3	543239	4959241	8880 ft	W	40^0	20%, rocky, duff, bare mix	-
4	543130	4957295	8583 ft	SE	25^0	50%, grasses, rocky, stable	-
5	543172	4957556	8600 ft	ESE	15^0	30%, disturbed, pioneer	-
6	543884	4961573	8897 ft	NNW	20^0	30%, brome, lupine, rocky	+
7	543940	4961640	8844 ft	WNW	30^0	10%, unstable, bare rocks	+
8	543467	4962733	8667 ft	S	$5\text{-}25^0$	25%, bare soil, small rocks	-
9	544060	4961780	8880 ft	W+E?	$0\text{-}10^0$	10-90%, native grass/bare	-
11	543322	4960009	8873 ft	NW	5-20%	10%, some overstory	-

On Sept. 14th the MSU crew picked up 10 seedlings from this batch which had been grown at the Coeur D'Alene Nursery to assess roots for the presence of nursery fungi. These seedlings were stored in a refrigerator for a few days before they were picked up and were transported to the MSU Mycology Lab in a cooler. Roots were washed and the presence of nursery fungi determined by visual observation (morphotyping) and species were confirmed in some cases by molecular analysis (ITS sequencing) by a graduate student.

3. ON-SITE MONITORING ***to determine if whitebark pine seedlings planted along Dunraven Pass are colonized by mycorrhizal fungi*****.** Nine months after planting, on June 6, 2007, Kay Izlar and Mary Hektner measured % survival of seedlings on the 10 plots and the MSU crew took this opportunity to learn the location and extent of each plot along the Pass. On June 28 2007 an MSU crew revisited each plot to sample seedlings for evaluation of mycorrhizal colonization. Two seedlings in the vicinity of each plot (but not on the plot) were removed in their entirety: a compromised seedling (a majority of needles were brown) and a healthy seedling (a majority of needles were green). In some cases, seedlings were of necessity located a distance from the plot or on the margin of the roadway. Each seedling was placed in an individual ziplock bag, moistened with non-chlorinated water, labeled by plot and placed in a cooler for transport to the MSU Mycology Lab.

At the MSU lab, roots were immediately cleaned of soil in non-chlorinated water over screens, and the various mycorrhizal types were sorted into morphotypes for each seedling. A general estimate of % mycorrhization was done visually with a dissecting scope and grid for each root system. The frequency of each fungal species and diversity of morphotypes/species was recorded for native and putatively non-native fungi. Some types were recognized visually and some were confirmed molecularly. In this case, DNA was extracted from the mycorrhizae, and fungal specific primers used with PCR to amplify the ITS region to confirm identifications.

Findings regarding the presence of mycorrhizal fungi 1) in soil on Dunraven Pass 2) on whitebark pine nursery seedlings before planting and 3) on roots of seedlings 9 months after planting on Dunraven Pass.

1. GREENHOUSE SOIL BIOASSAY determined native fungi are available in the replaced and native soil, at least in low levels.

A. The Soil Bioassay technique was reasonably successful, and revealed native mycorrhizal fungi appropriate for whitebark pine on 50-60% of seedlings grown in the non-sterilized soils (Fig. 1). Both native soil and replaced soil hosted native fungi at nearly the same frequency, although diversity was somewhat higher in the native soil (Table 2, columns T1 and T2). It was not possible to correlate these finding with individual sites. Results for the replaced soil could have originated from only one sample, since soil samples were mixed.

B. Nursery E-strain fungus persisted in all four treatments however native fungi were still able to colonize the seedlings. E-strain appears to persist more under greenhouse conditions than in the field (Table 2 & 7). This nursery fungus does not appear to be a problem for out-plantings of nursery seedlings in YNP. However other results suggest that this fungus may persist in other types of soil type or under other conditions so this should always be a consideration (Trusty & Cripps, unpublished). The presence of nursery E-strain was confirmed molecularly in most cases.

Fig.1. Greenhouse bioassay. Whitebark pine seedlings planted in 1) soil from native whitebark pine forest 2) replaced mulched soil, and 3) two controls (sterilized soils).

Table 2. Colonization of 2-year-old nursery seedlings by mycorrhizal fungi after one year in various soils from Dunraven Pass (YNP). Seedlings were planted in cone-tainers in greenhouse conditions.

	T1. Replaced soil N=10	***T2. native soil N=10***	*T3. Sterilized replaced soil (control) N=10*	*T4. sterilized native soil (control) N=10*
% seedling survival	**100%**	**100%**	80%	90%
% seedlings with nursery fungi	**60%**	**80%?***	40%?	50%?*
% of seedlings with native fungi	**60%**	**50%**	10%†	0%
Diversity of Fungi	**3-6 species of native fungi , also E-strain**	**2-5 species of native fungi, also E-strain**	E-strain	E-strain

* confirmed molecularly † possible incomplete sterilization

C. The soil bioassay revealed Suilloid fungi to be present in native and replaced soil **(**Table 3). A variety of native fungi (found in the soil) colonized seedling roots in both non-sterilized treatments. Most were Suilloid fungi of 3-6 types, plus *Cenococcum* and *Amphenima*. Each seedling was typically colonized by one species of fungus. The nursery fungus *Wilcoxina* (E-strain) occurred on all treatments but was difficult to indentify in sterile soils. Suilloid fungi are important in early pine establishment, so this is an important finding.

Table 3. The diversity of mycorrhizal fungi that colonized root tips of whitebark pine seedlings grown in unsterilized native and replaced soils.

Diversity of native mycorrhizal fungi on root tips from native and replaced soil			
Tree	T2. Native soil	T1. Replaced soil	Species
1.	1	1	Both Suilloid types, possible *Rhizopogon*
2.	0	1	*Suillus cf pseudobrevipes*
3.	1	0	*Rhizopogon subbadius*
4.	0	0	none
5.	1	0	Suilloid type
6.	1	0	*Rhizopogon vulgaris*
7.	2	0	Suilloid type, *Amphenima sp.*
8.	0	1	Suilloid type
9.	0	1?	*Suillus cf luteus* type in replaced
10.	0	1?	?
Total	3-6 native species	2-5 spp.	
mean	0.6	0.5	

D. The actual number of viable mycorrhizae on seedling roots <u>was low</u> after 1 year in the greenhouse and the Soil Bioassay technique could be optimized further (Table 4). Numbers of mycorrhizae are shown below, and while the fungi were present, they did not colonize the seedlings rapidly under our nursery conditions. We have two considerations to test in the future: the soil mixture used may not have been optimal. Secondly, the advanced age of the seedlings could be prohibitive to mycorrhizal colonization and the younger seedlings may be more amenable to inoculation. This might also be applicable to out-planted seedlings. A small side project revealed that when young (few month old) seedlings were placed in the soil, they were rapidly colonized by Rhizopogon in a few weeks (data not shown).

Table 4. Actual numbers of viable mycorrhizae on roots of whitebark pine seedlings in greenhouse after 1 year in native and replaced soil.

Tree no.	*A1. native soil N=10*		*A2. sterilized native soil (control) N=9*		*B1. replaced soil N=10*		*B2. sterilized replaced soil (control) N=8*	
	Suilloids	E-strain	Suilloids	E-strain	Suilloids	E-strain	Suilloids	E-strain
1	72**	46[b]	-	107	164*	-	-	-
2	-	289*	-	48	98*	-	-	-
3	125*	68	-	D	-	390[b]	-	-
4	-	-	-	36	-	33?	D	D
5	75**	97[b]	-	123[b]	-	91*	D	D
6	195*	100[b]	-	D	149	52?	-	-
7	51**	4[a]	-	-	-	-	-	235
8	-	165[b]	-	173[b]	254**	16[b]	-	88[b]
9	-	72[b]	-	-	118	-	154*[†]	43
10	-	77[b]	D	D	182	23?	-	8
Freq	50%	80%	0%	50%	60%	60%	10%	40%

*molecular confirmation, ** = morphotype identification, b = bifurcate, potential E-strain, a = *Amphenima*
D = dead roots, [†] incomplete sterilization.

2. Examination of nursery seedling roots prior to outplanting, revealed that non-native mycorrhizal fungi, including *Thelephora* and E-strain, were present at low levels.

Three mycorrhizal fungi were discovered on the whitebark pine seedlings (Fig. 2) directly from the nursery that are potentially non-native species/strains: *Wilcoxina* (E-strain), *Thelephora terrestris* and *Cenococcum geophilum*. For the ten seedlings examined half (50%) appeared to host the E-strain fungus, 20% the fungus *Thelephora terrestris* confirmed by molecular analysis, and one had 5 root tips of *Cenococcum geophilum.* The first two are common nursery fungi, and the latter is common in many natural systems. All of these are found in natural systems as well however strains vary for different regions. These are likely different strains than those native to Yellowstone Park, although at the species level, the species do exist in the Park. E-strain was the most common and does not appear to be problematic at this point, but should be monitored. *Thelephora* was present in low levels, this fungus could be more problematic, but we did not find it again on outplantings. Nursery seedlings with copious white mycorrhizae (likely *Thelephora*) should be monitored on out-planting to ensure this fungus does not spread to natural systems.

Fig. 2. Mycorrhizal types on seedlings directly from the nursery before planting. A & B. Young E-strain fungus initiating bifurcation of short roots. C. Young *Thelephora terrestris* mycorrhizae. D. *Cenococcum geophilum* mycorrhizae. Most nursery fungi were E-strain type (*Wilcoxina*).

3. Mycorrhizal colonization of out-planted whitebark pine seedlings on 10 sites 9 months after planting on Dunraven Pass revealed native fungi were present on 50% of sites but colonization levels were low.

A. Native mycorrhizal fungi were present on whitebark pine seedling roots on 50% of sites (Table 5). Seedlings on most sites north of the pass hosted native mycorrhizal fungi except for site 8 which is windy and exposed (it also had a low survival rate). No mycorrhizal fungi were found with seedlings on the two sites south of the pass. For the top of the pass, Site 1 hosted native fungi, and sites 2 and 3 appeared to host only nursery E-strain fungus.

B. ***A recorded 70% of healthy whitebark pine seedlings examined hosted some type of mycorrhizal fungus*** **and** ***only 10% of the compromised seedlings were colonized*** (Table 5). An average of 99 mycorrhizae/root system occurred on healthy seedlings and 6.9 on compromised seedlings, but standard deviations were high so statistical significance could not be confirmed at a 95% confidence level using a paired T-test (P=0.19). Sample size was minimal due to the impracticality of removing additional seedlings from monitored sites. While there was a strong

correlation between compromised seedling condition and lack of mycorrhizal colonization, it cannot be concluded that the lack of mycorrhizae was the cause health decline. Other abiotic factors, could also have compromised the seedling prior to mycorrhization. This should be tested further.

***C. Mycorrhizal colonization rates were low and most were < 1% of the whole root system,* with an overall average of 4.4%** (Table 2). One possible explanation for the low colonization rates might be that 9 months was insufficient time for this to occur. However, the seedling on site 6 which was almost one third colonized (Fig. 3), shows that under the correct conditions mycorrhization of these whitebark pine seedlings can occur rapidly in the field. This means that likely some other factor was preventing timely colonization. This is possibly lag time until fungi arrive or the age of the seedling could bes prohibitive to mycorrhization.

Table 5. Number of viable mycorrhizal root tips on nursery whitebark pine seedlings planted in 10 sites along Dunraven Pass in 9/11-13 and roots samples taken 6/28 (after nine months).

sites	*Healthy seedlings*	*Compromised seedlings*	*% seedling survival**	*Habitat notes*
1. Top of Pass	15	0	87%	slope behind visitor area to north
2. Top of Pass	32	0	96%	disturbed area adjacent to parking lot
3. Top of Pass	8	0	81%	slope behind visitor area to south
4. S of Pass	0	0	84%	disturbed area adjacent to parking lot
5. S of Pass	0	0	59%	open slope adjacent to distant conifer forest
6. N of Pass	670**	0	42%	burned in 1988, previously mixed conifer
7. N of Pass	136	0	100%	burned in 1988, previously mixed conifer
8. N of Pass	0	0	30%	windy, exposed, no trees
9. N of Pass	117	69	94%	unburned below mature whitebark pine
11. N of Pass	14	0	44%	unburned mature whitebark pine, spruce-fir
Total	992	69		
mean per tree	99.2±206	6.9±22	68%	*p = 0.192 due to high st. dev.*
frequency	70%	10%		

* Kay Izlars seedling survival data for site taken in same time period as mycorrhizal monitoring.
** Seedling samples taken from near/not on plot and from directly below mature pine along road.

D. The presence of mycorrhizae did not correlate directly with % survival in this study. Three of the sites with low survival rates (5, 8 and 11), also had no or few mycorrhizae. However, a low survival rate was also recorded for site 6 which had the highest actual number of mycorrhizae. The high number of mycorrhizae recorded for site 6 results primarily from one well-colonized seedling (Fig. 3) taken a distance from the site (of necessity) and under a mature whitebark pine. Also, a high number of mycorrhizae were found on healthy seedlings from the two burn sites resulting from the 1988 fires, one site had high survival and the other low so it would be of interest to investigate the differences between these two sites (Table 5).

E. Most of the mycorrhizae occurred from 4-12 cm in the soil, but did occur deeper/shallower on the burned sites (Table 6). Root systems of the planted whitebark pine seedlings all retained their cone-tainer shape, and roots had not spread into the surrounding native soil after 9 months. It was therefore possible to divide the root systems into 4 cm sections for assessment of average

depth of the mycorrhizae which was assessed only for the healthy seedlings. Results suggest that the top few cm of soil is not conducive to mycorrhizal fungi (too dry or cold). This may have implications for planting depth in various soil types and for spreading the roots into native soil.

Table 6. Number of viable mycorrhizae in healthy whitebark pine seedlings by soil depth for 10 sites (0-4 cm = surface layer).

Number of viable mycorrhizal root tips for healthy seedlings by depth					
sites	0-4 cm	4-8 cm	8-12 cm	12-16 cm	% mycorrhizal
1. Top of Pass	0	0	15	0	< 1%
2. Top of Pass	0	0	5	27	< 1%
3. Top of Pass	0	8	0	0	< 1%
4. S of Pass	0	0	0	0	0%
5. S of Pass	0	0	0	0	0%
6. N of Pass	65	312	245	48	< 30%
7. N of Pass	76	12	28	20	< 5%
8. N of Pass	0	0	0	0	0%
9. N of Pass	0	82	15	10	< 5%
11. N of Pass	0	14	0	0	< 1%
Total	141	428	308	105	44
Average	14.1	42.8	30.8	10.5	4.4%

Descriptive statistics by soil depth

Variable	N	Mean	StDev
0-4 cm	10	14.1	29.8
4-8 cm	10	42.8	97.8
8-12 cm	10	30.8	75.9
12-16 cm	10	10.5	16.4

Fig. 3. A. cone-tainer shaped roots of whitebark pine seedlings planted along Dunraven pass in Yellowstone National Park after 9 months. B. Bifurcate (branched) root tips indicate mycorrhizal activity on short roots. C. Heavy mycorrhizal activity on root system of seedling from Site 6 (1988 burn area). Arrows indicate mycorrhizae. This seedling was heavily colonized by four types of native species of mycorrhizal fungi.

F. ***Both native and exotic nursery mycorrhizal fungi were found on out-planted whitebark pine seedlings after 9 months.*** Healthy seedlings on 50% of the sites hosted native fungi and seedlings on 30% of the sites were colonized by nursery fungi (Table 7). These two groups of fungi did not overlap except on one site. It appears that when native fungi are present, they replace nursery fungi for seedlings establishment. However, it is not clear why complete colonization by the native fungi is not accomplished in 9 months. Compromised seedlings did not host any fungi except in one case. Again the presence of native fungi did not correlate with survival rates after 9 months.

Native species were primarily Suilloid types; *Cenococcum* occurred on three sites as well. This is likely a native strain, since in all cases sclerotia were present and prolific. Only one of the compromised seedlings hosted a mycorrhizal fungus, potentially a species of *Piloderma*. The presence of E-strain (nursery fungus) could not be verified on the compromised trees, but it did occur on 30% of the healthy trees, overlapping with native species in one case.

Table 7. Presence or absence of native and nursery fungi on whitebark pine seedlings assessed nine months after planting in 10 sites along Duraven Pass, Yellowstone National Park. Seedlings were planted in Sept. 2006 and assessed in June 2007.

	Number of viable mycorrhizal root tips					
sites	A. Healthy seedlings		B. Compromised seedlings		Habitat notes	seedling survival**
	native*	nursery†	native*	nursery†		
1. Top of Pass	+[c]	-	-	-	visitor area to north	86%
2. Top of Pass	-	+	-	-	visitor area parking lot	96%
3. Top of Pass	-	+	-	-	visitor area to south	81%
4. S of Pass	-	-	-	-	disturbed, near parking lot	84%
5. S of Pass	-	-	-	-	open slope near conifer forest	58%
6. N of Pass	+[c]	+	-	-	burn, previously mixed conifer	42%
7. N of Pass	+	-	-	-	burn, previously mixed conifer	100%
8. N of Pass	-	-	-	-	windy, exposed, no trees	30%
9. N of Pass	+[c]	-	p	-	unburned, whitebark pine area	93%
11. N of Pass	+	-	-	-	Unburned, whitebark pine area	44%
	50%	30%	10%	0%		

* primarily native Suilloid-types (1-5 species) †*Wilcoxina* (E-strain) nursery fungus ** Izlar data

[c] native *Cenococcum* in addition to native *Rhizopogon* p = *Piloderma*, a native fungus

G. A diversity of native mycorrhizal fungi were found on planted whitebark pine seedlings 9 months after planting, including the important Suilloids. <u>This shows that Suilloid fungi are in the soil and available to whitebark pine seedlings on some sites.</u> At least five species of native mycorrhizal fungi were found colonizing the planted whitebark pine seedlings (Table 8). Molecular results revealed most to be Suilloids (species of *Suillus* and *Rhizopogon*). These are important fungi for the regeneration of whitebark pine seedlings. The closest match to a species in Genbank are shown below, and genus names are confirmed but further phylogenetic study is needed for actual species determination. Most of these important fungi are specific for pines (not spruce and fir) and some may be specific for 5-needle pines. The finding of a diversity of native Suilloid fungi on the whitebark pine seedlings shows that these fungi are present in the soil. These were found on five of the sites (1, 6, 7, 9, 11) on roots of healthy seedlings.

Table 8. Genera and tentative species of ectomycorrhizal fungi found with whitebark pine along Dunraven Pass identified by molecular techniques. Species names are not yet definitive.

ID	*Taxa*	*Accession #*	*BP/% match*
6A1	Suillus neoalbidipes,	L54112	572/600 (95%)
6A1B1	Suillus neoalbidipes,	AY880940	598/616 (97%)
6A1B2	Suillus neoalbidipes,	AY880940	563/595 (94%)
6AC	Suillus cf variegatus	DQ179130	409/429 (95%)
7A1	Rhizopogon subbadius	AF377152	772/785 (98%)
9A	Rhizopogon subbadius,	AF377152	550/586 (93%)
9B1	Amphinema sp.	AJ893289	585/598 (97%)
YNP1	Rhizopogon subcaerulescens,	M91613	72/74 (97%)
RZP1	Suillus sp., unusable	NA	NA
N7B	Amphinema byssoides,	AJ893289	560/578 (96%)
RHIZ 1	Rhizopogon luteorubescens	AJ810038	689/692 (99%)
CEN	Cenococcum geophilum	NA	NA

Fig. 4. Proximity of sampled whitebark pine seedling to mature trees on site 6 helps explains existence of *Cenococcum* (it usually exists in the canopy) and other Suilloids on a burn site.

H. Most of the diversity (number of species) of native mycorrhizal fungi was found on the rth side of the pass, and this area supports extensive mature whitebark pine forests (Table 9). Mature whitebark pine forests are found north of the pass and some on top, and some are extensive pure stands. Sites on the south side of the pass (which lacked mycorrhizae) were in subalpine areas and forests were primarily comprised of subalpine fir with some spruce. It is not known if some sites support higher mammal populations which are vectors for spreading mycorrhizal fungi, and particularly squirrels, voles, and deer.

Table 9. Diversity of native mycorrhizal or E-strain nursery fungi on root tips by site. B indicates a bifurcate condition of roots and potential E-strain fungus not confirmed by molecular or morphological data. Naïve Suilloid types include *Rhizopogon* and *Suillus*, both important Suilloids.

Diversity (number of species) of mycorrhizal native or E-strain nursery fungi on root tips by site*				
sites	Healthy seedlings	Compromised seedlings	% survival	Habitat notes
1. Top of Pass	2	0	86%	Suilloid type, *Cenococcum geophilum*
2. Top of Pass	1*	0	96%	b = bifurcate roots, possible E-strain
3. Top of Pass	1*	0	81%	b = possible E-strain
4. S of Pass	0	0	84%	NA
5. S of Pass	0	0	58%	NA
6. N of Pass	3+	0	42%	*S. neoalbidipes, S. variagatus, C. geophilum,* b = possibly E-strain, others
7. N of Pass	2	0	100%	*R. subbadius, Amphenima sp*
8. N of Pass	0	0	30%	NA
9. N of Pass	2	1	93%	9A = *R. subbadius, C. geophilum* 9B = *Amphenima* type + dead root tips
11. N of Pass	1	0	44%	Suilloid type
Total	992	69	86%	
mean per tree	99.2±206	6.9±22	80%	*p = 0.192 due to high st. dev.*
frequency	70%	10%		

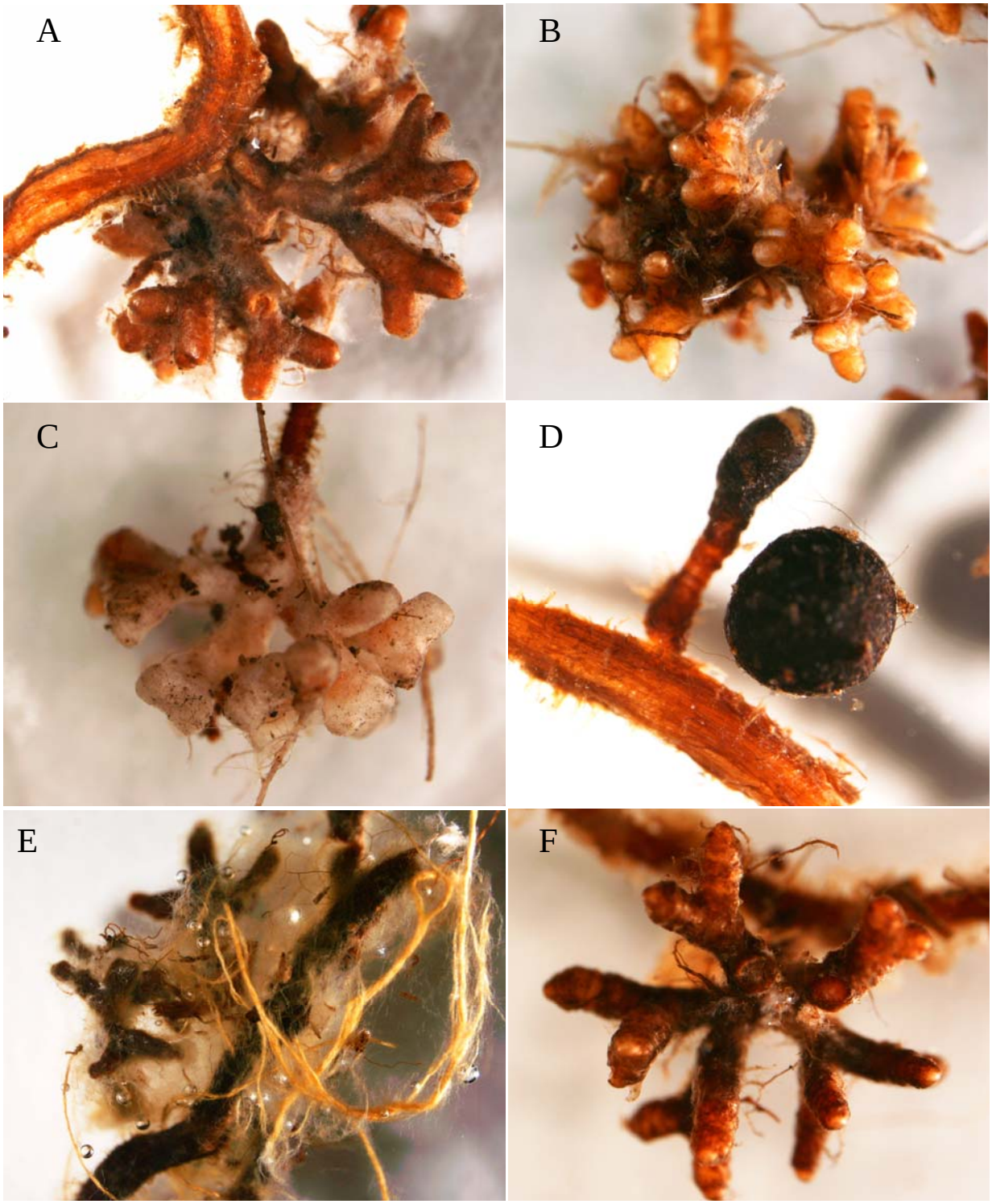


Fig. 5. Diversity of native mycorrhizal fungi occurring on whitebark pine seedlings planted on Dunraven Pass. A. *Suillus 1*. B. Suillus 2 neoalbidipes/variagatus. C. *Rhizopogon cf subbadius*. D. *Cenococcum geophilum*. E. *Amphenima* type. F. Copper type (older).

Recommendations

See pg 4.

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