2009 WHITEBARK PINE
RESTORATION PROJECT PROGRESS REPORT

Submit the following information electronically to John Schwandt (jschwandt@fs.fed.us) by 11/30/2009

Project Title: Inoculation of Whitebark Pine with Native Mycorrhizal Fungi in the Nursery: Phase 2 (continuing project).

Project Contact: Dr. Cathy L. Cripps, Plant Sciences & Plant Pathology Dept., Montana State University, Bozeman, MT 59717, 406-994-5226, ccripps@montana.edu

Location: Montana State University Plant Growth Center.

Size of Treated Area: Continued & additional Greenhouse trials at the MSU Plant Growth Center, MSU. Native mycorrhizal fungi collected from Rocky Mountain Region. Possible transfer to outdoor nursery sites.

Reported in FACTS (if applicable)? (Y/N/na): NA

Objective(s) (from original request)**:**
All pines, including whitebark pine, need ectomycorrhizal fungi to survive in nature. Non- mycorrhizal seedlings are at risk when planted in soil lacking appropriate mycorrhizal fungi in natural habitats. The overall survival of whitebark pine out-plantings is poor on many restoration sites (Izlar 2007) and the availability of appropriate mycorrhizal fungi needs to be considered in addition to other biotic and abiotic conditions. This is particularly a concern for whitebark pine where nursery seedlings are planted on burns, in ghost forests and in areas where live whitebark pine has not existed for years (Wiensczyk et al. 2002). Mycorrhizal fungi decline in the soil over time and whitebark pine depends on many specialist fungi (suilloids) restricted to 5-needle or stone pines (Mohatt, Cripps, Lavin 2008).

The ultimate goal of our research program is to develop methods for the inoculation of whitebark pine seedlings with native fungi on a large scale for use in nurseries and to ultimately assess survival of inoculated seedlings in the field under various conditions.

Phase 1 (2008) is completed (see final report). The main goal of phase I was to expand information on the ectomycorrhizal fungi with whitebark pine and develop methods for inoculation of whitebark pine seedlings with native ectomycorrhizal fungi under nursery conditions. Over 26 strains of native ectomycorrhizal fungi were tested, using various kinds of inoculum, and methods of inoculation. In brief, we had success in synthesizing the first mycorrhizae between native fungi and whitebark pine. We identified *Suillus sibiricus*, various other *Suillus* species, and *Rhizopogon* species to be the most effective in forming mycorrhizae under greenhouse conditions. Secondly, spore slurries produced more mycorrhizae in a shorter time than soil inoculum, but spore sources are not always available.

Phase 2 (2009, year 1 of 2) is an ongoing project. The overarching goal is to continue to work towards a 'reliable' method for consistent colonization of whitebark pine in the greenhouse with native fungi. The primary objectives are to continue towards development of a mycorrhizal inoculum for whitebark pine by assessing a) inoculation procedures in the nursery, b) effects of various soil substrates on mycorrhizal formation c) effects of fertilizer on mycorrhizal formation and d) survival of inoculated seedlings in out-planting trials. In addition, we are to work on a method for addition of mycorrhizal fungi "at planting".

Item	Requested 09 WBKP Funding $	Other-Source 09 Funding $	Description /Source/in-kind
Salary	12,000	3,000	Cripps donation of 1 month salary time
Travel	400		
Other (specify) Greenhouse rental	2,400		
Contracting		600	Coeur D'Alene seedlings already donated
Equipment			
Supplies	1,000	300	Greenhouse material donated from MSU
Other (specify)			
Totals	**15,800**		

Did FHP funding get used or obligated; if not briefly explain. NA

Project Status: (Is the project complete? If not, what remains to be accomplished? – provide time line)

Phase 1 is complete. Phase 2 is a continuing two year project and is not complete. We gained additional isolates of native mycorrhizal fungi to test this summer. Isolates were selected on past performance of the species in Phase 1 trials. Experiments to examine fertilizer and soil effects are set up, seedlings are inoculated with selected native fungi, and are being maintained in the MSU Plant Growth Center (see Appendix 1 for experimental design). We will subject seedlings to cold treatment over the winter and re-inoculate them; we expect to assess data in the spring. This winter we are working on developing an inoculum to be "added at planting". We will also inoculate seedlings (provided by Melissa Jenkins) to be grown in the Coeur D'Alene Nursery—inoculation will take place at the nursery itself as a move towards testing FS nursery conditions as amenable to inoculation. We hope that inoculation can take place before seedlings go into cold treatment, and possibly again afterwards. These seedlings will be planted by the Forest Service (Melissa Jenkins, as per her other seedlings) in various treatments (including burns) and later assessed for survival. We do not expect results from this part of the project for 1 or 2 years. This summer we will continue to collect sporocarps for spore slurries and to test shelf life of current spore slurries, and do additional green house experiments. We are amenable to additional studies as fits the needs of J. Schwandt.

Results: (what did you accomplish and what have you learned)

Collection of Sporocarps of Native ECM Fungi

We collected sporocarps of native fungi from whitebark pine forests to develop into spore slurries. We made several field trips for this purpose and were highly successful due to high precipitation this field season. A surprise was to see the widespread distribution of *Suillus sibiricus* in many whitebark pine forests. Several isolates of native fungi were selected (from Phase 1) for further testing in the greenhouse as an inoculum for whitebark pine seedlings. Our current list of isolates of native ectomycorrhizal fungi collected from whitebark pine forests is shown in Table 1. Many isolates were grown out on MMN (some with antibiotics) for isolation and are maintained in the lab on MMN media in petri dishes and are now in tubes on agar. Spore slurries were developed from sporocarps when possible.

Table 1. Initial screening of native ectomycorrhizal fungi for potential use as inoculum for whitebark pine seedlings as assessed by growth characteristics on various substrates. Mycorrhizae denotes if mycorrhizae formed in any of the experiments (whether spore slurry or soil inoculum). CLC 2400 isolates are new from 2009.

No.	Mycorrhizal species	Location	Source	Host	Plate[a]	Liquid[b]	Soil[c]	Seedling[d]	Mycorrhiza
CLC 2035	*Rhizopogon subpurp.*	New World	sporocarp	*P. albicaulis*	M+	-	-	-	+
CLC 2036	*Rhizopogon* sp.	New World	sporocarp	*P. albicaulis*	M+	-	-	-	na
WO 81.1	*Tricholoma moseri*	New World	sporocarp	*P. albicaulis*	M -	-	-	-	na
Rhiz 1w	*R.* cf *ochraceorubens*	Waterton Park	sporocarp	*P. contorta*	M+	-	-	-	na
Hyp 1	*R.* cf *salebrosus*	Waterton Park	sporocarp	*P. flexilis*	M+	-	-	-	na
GDP 1	*Rhizopogon.* sp. 1	Glacier Park	roots	*P. flexilis*	M+	-	-	-	na
UB 7	*Rhizopogon* sp. 2	Fridley Burn	native soil	*P. albicaulis*	M+		-	-	na
CLC 2199	*Suillus* sp. (veil)	Dunraven	sporocarp	*P. albicaulis*	M++	+	+	+	na
CLC 2294	*R. subbadius*	Dunraven	sporocarp	*P. flexilis*	M++	+	+	+	+
CLC 2341	*S. subalpinus*	New World	sporocarp	*P. albicaulis*	M++	+	+	+	+
CLC 2344	*S. variegatus*	New World	sporocarp	*P. albicaulis*	M++	+	+	+	+
CLC 2345a	*S. sibiricus (thick)*	Dunraven	sporocarp	*P. albicaulis*	M++	+	+	+	-
CLC 2345b	*S. sibiricus (thin)*	New World	sporocarp	*P. albicaulis*	M+	-	-	-	++
CLC 2346	*S.* cf *brevipes*	Dunraven	sporocarp	conifers	M -	-	-	-	+
CLC 2347c	*S. subalpinus*	Dunraven	sporocarp	*P. albicaulis*	M+	-	-	-	na
VT 1009	*Cenococcum geophil.*	Eastern US	roots	Conifers	M ++	+	+	+	-
CLC 2375	*S. sibiricus*	Beartooth	sporocarp	*P. albicaulis*	S	N/A	N/A	+	+/-
CLC 2377	*R. subpurpurascens*	Beartooth	sporocarp	*P. albicaulis*	S	N/A	N/A	+	+
CLC 2379	*R.* cf *evadens* R 1	Dunraven	sporocarp	*P. albicaulis*	S	N/A	N/A	+	+
CLC 2380a	*R.* cf *molligleba* R2	Dunraven	sporocarp	*P. albicaulis*	S	N/A	N/A	+	+
CLC 2380b	*R.* sp. (yellow) R3	Dunraven	sporocarps	*P. albicaulis*	S	N/A	N/A	+	na
CLC 2381a	*R. olivaceofuscus* 4,5	New World	sporocarp	*P. albicaulis*	S	N/A	N/A	+	+
CLC 2382	*Thaxterogaster* sp.	New World	sporocarp	*P. albicaulis*	S	N/A	N/A	+	-
NW Hyp 1	*Hypogeous* 1	New World	sporocarp	*P. albicaulis*	S?	N/A	N/A	-	na
NW Hyp 2	*Hypogeous* 2	New World	sporocarp	*P. albicaulis*	S?	N/A	N/A	-	na
CLC 2432	*S. sibiricus*	Fox Meadow	sporocarp	Mixed PA/L		N/A	N/A	-	na
CLC 2433	*S. variegatus*	Fox Meadow	sporocarp	Mixed PA/L	M+	N/A	N/A	-	na
CLC 2440	*S. sibiricus*	Gravelly Mt	sporocarp	*P. albicaulis*	M+/S	N/A	N/A	+	+
CLC 2445	*S. sibiricus*	New World	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2441	*Suillus sp.*	Gravelly Mt	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2447	*S. subalpinus*	New World	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2449	*S. sibiricus*	New World	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2450	*S. sibiricus*	New World	sporocarp	*P. albicaulis*	M+/S	N/A	N/A	-	na
CLC 2451	*Rhizopogon*	New World	sporocarp	*P. albicaulis*	M+/S	N/A	N/A	-	na
CLC 2466	*Suillus sp*	Beartooth	sporocarp	*P. albicaulis*	M+/S	N/A	N/A	-	na
CLC 2467	*Suillus sp*	Beartooth	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2469	*Rhizopogon sp*	Beartooth	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2470	*Rhizopogon sp*	Beartooth	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2472	*Suillus sp*	Beartooth	sporocarp	*P. albicaulis*	M+/S	N/A	N/A	-	na
CLC 2487	*S. subalpinus*	Avalanche Lk	sporocarp	*P. albicaulis*	M+/S	N/A	N/A	-	na
CLC 2489	*Rhizopogon sp*	Avalanche Lk	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2500	*S. variegatus*	Storm Lake	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2503	*Suillus sp*	Storm Lake	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2505	*Suillus sp*	Storm Lake	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2508	*Suillus sp*	Storm Lake	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2509	*Suillus subalpinus*	YNP	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2510	*Suillus variegatus*	YNP	sporocarp	*P. albicaulis*	M+	N/A	N/A	-	na
CLC 2511	*S. sibiricus*	YNP	sporocarp	*P. albicaulis*	M+	N/A	N/A	+	na
CLC 2544	*Rhizopogon sp*	Beartooth	Sporocarp	*P. albicaulis*	S	N/A	N/A	+	na
XX07	*Rhizopogon* sp.	Yellowstone	grizzly scat	*P. albicaulis*	S	N/A	N/A	+	na

[a] growth on Petri ‘plates’ of MMN (M+ = growth, M++ = vigorous growth, M- = poor growth).
[b] growth in ‘liquid’ MMN media (+ = growth, - = no growth).
[c] growth in peat:vermiculite (1:9 v/v) ‘soil’ mix (+ = growth, - = no growth).
[d] fungi used to inoculate whitebark pine seedlings.
S = spores from fruiting bodies used for direct inoculation of seedlings.

Development of spore slurries

The 2009 field season had high precipitation and produced lots of sporocarps in whitebark pine forests. We took advantage of this and gathered sporocarps from several sites (especially suilloids) and processed them into spore slurries either directly or dried them for later use (Table 2).

For inoculum development, sporocarps were stripped of mature hymenium or gleba, and these portions were then either a) developed directly into spore slurries b) dehydrated or c) frozen. For spore slurries, the tissue was blended and then some were filtered; all are kept refrigerated until ready to be used. The shelf life of slurries is currently being assessed.

Several native ectomycorrhizal fungi identified as good root colonizers in Phase 1 were used to inoculate one and two year old seedlings for Phase 2. Extra seedlings from Phase 1 plus additional new germinants from the Coeur D'Alene nursery were used in Phase 2.

Table 2. Native ectomycorrhizal fungi from whitebark pine forests developed into inoculum or for future inoculum. Numbers are spore concentrations in the slurries.

Native ectomycorrhizal fungus	CLC #	Host	Source	Inoculum Type	Treatment	Spore Count (spores/ml)	Inoculation date
Suillus sp (veil)	2199	WBP	Dunraven	spore slurry	fresh, not filtered		5.28.09
Rhizopogon sp.	2251	WBP	New World	spore slurry	Fresh filtered	6.1×10^6	NA
Suillus subalpinus	2341	WBP	New World	soil?	mycelium?	NA	5.28.09
Suillus cf variegatus	2344	WBP	New World	Soil?	Mycelium?	NA	5.28.09
Suillus sibiricus	2345	WBP	New World	soil?	mycelium?	NA	5.28.09
Suillus sibiricus	2375	WBP	New World	spore slurry	Fresh filtered	2.0×10^6	10.16.09
Rhizopogon. subpurpurascens	2377	WBP	New World	spore slurry	1 yr, not filtered	6.4×10^7	5.28.09
Rhizopogon evadens	2379	WBP	New World	spore slurry	1 yr, not filtered	1.2×10^7	5.28.09
Rhizopogon molligleba	2380	WBP	New World	spore slurry	1 yr, not filtered	3.1×10^7	5.28.09
Rhizopogon olivaceofusca	2381	WBP	New World	spore slurry	1 yr, not filtered	6.1×10^6	5.28.09
Thaxterogaster pinque	2382	Mixed	New World	spore slurry	1 yr, not filtered	2.8×10^6	NA
Suillus sibiricus	2440	WBP	Gravelly Mts	spore slurry	Fresh filtered	3.6×10^6	10.16.09
Suillus sibiricus	2440	WBP	Gravelly Mts	spore slurry	fresh filtered1:10	8.5×10^5	10.16.09
Suillus sibiricus	2440	WBP	Gravelly Mts	spore slurry	frozen filtered	2.3×10^6	10.16.09
Suillus sibiricus	2440	WBP	Gravelly Mts	spore slurry	dry filtered	2.9×10^6	10.16.09
Rhizopogon sp	2451	WBP	New World	dried	gleba	NA	10.16.09
Rhizopogon sp	2452	WBP	New World	dried	gleba	NA	NA
Suillus cf variegatus	2466	WBP	Beartooth	dried	hymenium	NA	NA
Suillus sibiricus	2472	WBP	Beartooth	dried, lots	hymenium	NA	NA
Suillus subalpinus	2487	WBP	Avalanche Lk	dried	hymenium	NA	NA
Suillus subalpinus	2509	WBP	Dunraven	dried	hymenium	NA	NA
Rhizopogon sp.	2544	WBP	Beartooth Mts	spore slurry	fresh filtered	7.2×10^7	NA
Rhizopogon sp.	2544	WBP	Beartooth Mts.	spore slurry	fresh filtered 1:5	8.8×10^6	10.16.09

Experiments currently underway in MSU Plant Growth Center

Experiment 1: Examination of the effects of fertilizer on mycorrhizal colonization of whitebark pine seedlings with native fungi (CLC 2440, *Suillus sibiricus*). Several fertilizer regimes including one similar to that used in the Coeur D'Alene nursery were selected for assessment to determine if fertilizer deters mycorrhizal colonization (and at what level). See experimental design in Appendix 1, Table 3.

Experiment 2: Examination of the effects of soil type on mycorrhizal colonization of whitebark pine seedlings for various isolates of native fungi. Three soil types (two used by the Coeur D'Alene nursery were selected to be tested. This includes a peat:vermiculite mix, a peat:bark mix and soil mix 2 (peat:MSU mix:vermiculite). This test will help determine if the type of substrate can affect the mycorrhizal colonization process. See experimental design in Appendix 1, Table 4.

We are awaiting results on these experiments and expect to assess them after cold treatment in the spring.

Summary of results to date:

1. Inoculum: spore slurries are developed and ready for use. We are testing shelf-life.
2. Inoculum: to be used 'at planting' is being developed, but studies show it is preferable to inoculate seedlings in the greenhouse.
3. Whitebark pine seedlings are inoculated for various tests are we are awaiting results for
    a) tests for fertilization effects
    b) tests for soil/substrate effects
4. Inoculum: is ready for Melissa Jenkins seedlings and will be applied in spring.

Changes needed or Problems Encountered:

We had no problems except the usual difficulties of obtaining seedlings (released seeds) which are now overcome. If John Schwandt would like some of his seedlings inoculated for a small trial, we are ready for that, and would inoculate them in the greenhouse at the same time we inoculate Melissa's seedlings. An upcoming concern is that when we inoculate seedlings in the Coeur D'Alene nursery, we will request that the fertilizer regime be changed for some of the treatments. Hopefully this can be worked out with Kent Eggleston and the nursery crew.

Sharing Results/Products/Outcomes:

Attached separately please find:
Report from Phase 1 that is being rewritten as a paper for submission to a journal.
Also:
Presentations were previously sent in with Phase I (can be resubmitted on request).
We will present our results at the High-Five Conference in 2010.

Suggestions for how the overall program can be improved to better meet your needs: (suggestions regarding RFP solicitation and evaluation process, etc.)

It is still a bit unclear as to the ending date of our 2-years of funding for Phase 2. Do the funds need to be used up by a certain date (what is it?) or can work be extended with a no cost extension until it is used up (is it open-ended). We are trying to be efficient and effective with the funding available to us.

Appendix 1 is below. Greenhouse Experimental Set-up.
Appendix 2 is below. Photos of inoculation techniques.

Appendix 1: Experimental set-ups in the MSU Plant Growth Center.

Table 3. Test to examine effects of fertilizer on mycorrhizal colonization of whitebark pine seedlings with native fungi (CLC 2440, *Suillus sibiricus*).

Styrofoam blocks

Row	Isolate	origin	host	Type	Fertilizer	Notes
1	None, control	NA	NA	NA	no	
2	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	slurry (full)	no	
3	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	slurry (1:10)	no	
4	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	dried (______)	no	
5	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	frozen (_____)	no	
6	*S. sibiricus* CLC 2421		*P. flexilis*	slurry (full)	no	
7	*Rhizopogon* CLC 2544	Beartooths	*P. albicaulis*	slurry (full)	no	
8	*S. americanus* CLC 2564	Ohio	*P. strobus*	slurry (full)	no	
9	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	slurry (full)	yes	Fert trmt 1
10	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	slurry (full)	yes	Fert trmt 2
11	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	slurry (full)	yes	Fert trmt 3
12	None, control	NA	NA	NA	yes	Fert trmt 1

Table 4. Test to examine effects of soil type on mycorrhizal colonization of whitebark pine seedlings for various isolates of native fungi.

Containers, spore slurries

Row	Isolate	origin	host	Soil Type	Dry/slurry	Seedling age
1	None, control	NA	NA	P:V 50:50	NA	5 mon. 4/28/09
2	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	P:V 50:50	slurry	5 mon. 4/28/09
3	*S. sibiricus* CLC 2275	New World	*P. albicaulis*	P:V 50:50	1yr old- slurry	5 mon. 4/28/09
4	*S. sibiricus* CLC 2421	Sacajawea	*P. flexilis*	P:V 50:50	slurry	5 mon. 4/28/09
5	*Rhizopogon* CLC 2544	Beartooths	*P. albicaulis*	P:V 50:50	slurry	5 mon. 4/28/09
6						
7	None, control	NA	NA	P:B	NA	5 mon. 4/28/09
8	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	P:B	slurry	5 mon. 4/28/09
9	*S. sibiricus* CLC 2275	New World	*P. albicaulis*	P:B	1yr old- slurry	5 mon. 4/28/09
10	*S. sibiricus* CLC 2421	Sacajawea	*P. flexilis*	P:B	slurry	5 mon. 4/28/09
11	*Rhizopogon* CLC 2544	Beartooths	*P. albicaulis*	P:B	slurry	5 mon. 4/28/09
12						
13	None, control	NA	NA	soil mix 2	NA	5 mon. 4/28/09
14	*S. sibiricus* CLC 2440	Gravelly Mts	*P. albicaulis*	soil mix 2	slurry	5 mon. 4/28/09
15	*S. sibiricus* CLC 2275	New World	*P. albicaulis*	soil mix 2	1yr old- slurry	5 mon. 4/28/09
16	*S. sibiricus* CLC 2421	Sacajawea	*P. flexilis*	soil mix 2	slurry	5 mon. 4/28/09
17	*Rhizopogon* CLC 2544	Beartooths	*P. albicaulis*	soil mix 2	slurry	5 mon. 4/28/09
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Appendix 2: Figures of project at the MSU Plant Growth Center.

Figs. 1-4. Inoculatation of whitebark pine seedlings. A. Liquid inoculum being applied to small seedlings, B. Soil inoculum being applied to small seedlings, C. Inoculation gun being used for larger seedlings, D. Synthesized ectomycorrhizae on inoculated seedlings in the greenhouse.