Native mycorrhizal fungi & whitebark pine restoration

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Figure 1.1: Range of whitebark pine (WPEF) 2014.
Whitebark pine (*Pinus albicaulis*)

- High elevations, at treeline
- Broad spreading tops

Whitebark pines
Elevation range: 8000-12,000 ft

Lodgepole pines
Elevation range: 6000-10,000 ft
• Round purple cones
• Needles in bundles of 5
• Whitish, light colored bark
Seeds (pine nuts) are dispersed by birds that often plant them in burns.
Seeds are planted in clusters by birds
Germinate in a group
Grow together, into multi-stemmed trees
Pine nuts are also a food source for bears and squirrels.

Mammals also eat mycorrhizal fungi and spread the spores.
Threats

- White pine blister rust
- Mountain pine beetle
- Fire exclusion
- Climate change

Over 90% decline in many populations
A Range-Wide Restoration Strategy for Whitebark Pine (*Pinus albicaulis*)

- Gather cones
- Extract seeds
- Grow seedlings
- Plant seedlings
Plus trees

Seeds removed

Seeds scarified

Bags of cones

Seeds planted
Coeur D’Alene Forest Nursery, Idaho: whitebark pine seedlings
Whitebark pine ‘rust resistance’ trials
Coeur D’Alene Forest Nursery

Not ‘rust resistant’

‘Rust resistant’
Seedling survival has been generally low.

A major assessment (Izlar 2007) showed around a 50% survival rate overall. This has been improving over the time.
What we have learned:
Seedling survival is higher on **burns** & when planted near **protective objects (microsite)**
Can we use **Native Mycorrhizal Fungi** to improve seedling survival?

**Fungi**
- Enhance nitrogen uptake
- Protect against drought
  - Aggregate soil
  - Hold soil moisture

**Plants**
- Provide sugars (food) to the fungus
In nature:

- trees need mycorrhizae to survive
- many different species of fungi on roots
- each has a unique function
- some are host specific
Native Mycorrhizal Fungi with Whitebark Pine

- Waterton Lakes National Park
- Glacier National Park

3 national parks
7 mountain ranges

Grizzly Bear Range

Yellowstone Park
### BASIDIOMYCOTA

#### AMANITACEAE
- Amanita alpinicola *Cripps*
- Amanita muscaria

#### HYGROPHORACEAE
- Hygrophorus glioocyclus
- Hygrophorus marzuolus
- Hygrophorus olivaceoalbus
- Hygrophorus piceae
- Hygrophorus subalpinus

#### TRICHOLOMATACEAE
- Leucopaxillus paradoxis
- Tricholoma moseri

#### CORTINARIACEAE
- Cortinarius ahsii
- Cortinarius clandestinus
- Cortinarius cf duracinus
- Cortinarius *bridgei* *Cripps*
- Cortinarius flavobasilis
- Cortinarius aff. fulminoides
- Cortinarius subolivescens
- Cortinarius spp.
- Dermocybe crocea

#### RUSSULALES
- Lactarius deterrimus
- Russula albonigra
- Russula brevipes
- Russula cf queletii
- Russula sp. 1
- Russula sp. 2

#### BOLETALES
- Boletus edulis
- Chroogomphus sp. nov.
- Rhizopogon evadens
- Rhizopogon milleri
- Rhizopogon roseolus
- Rhizopogon olivaceofuscus
- Suillus discolor
- Suillus cf placidus
- Suillus sibiricus
- Suillus subalpinus
- Suillus sp.

#### THELEPHORALES
- Thelephora caryophylla
- Pseudotomentella nigra
- Tomentelloid type

#### ATHELIACEAE
- Piloderma byssinum
- Amphinema byssoides

#### HYMENochaetaceae
- Coltricia sp.

#### ASCOMYCOTA
- Cenococcum geophilum (no sporocarps)*
- Wilcoxina mikolae
- Wilcoxina rehmii

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**Suilloids!**
- host specific
- on seedlings and on mature trees
Native Ectomycorrhizal Fungi useful for inoculation

- Spores germinate in culture (spore slurry inoculum)
- Mycelium grows in culture (soil inoculum)
- Associate with seedlings and mature trees
- Host specific!

**Suilloids!**

**Suillus**
- Sticky cap
- Pores (sponge)
- Stem with or without dots

**Rhizopogon** *(pogies)*
- Round or oval
- Brown pores inside
- Grows underground
Collecting native mycorrhizal fungi

Next: How to recognize our star performers
Suillus sibiricus = Suillus americanus

- Yellow cap
- With red patches
- Yellow pores
- Yellow & pink stem
Suillus discolor

- Dull yellow-gold
- Black dots on stem
- Stains blue when cut
Suillus subalpinus

- Brown cap
- Yellow pores
- White stem with brown dots

Not as good for inoculation: also on conservation list
Rhizopogon evadens

pinkish, round
grows underground
Citizen Science Program

- Mushroom clubs
- Park Service employees
- Forest Service employees

This program is now closed
Suilloids!

Keep cool!
**Selecting, Transporting, Storing**

**Selection**
- Need to be mature to produce spores
- Not old, rotten, or full of maggots

Yes!

- Pogies
  - White inside
  - No! too young

No! too old

- Suillus
  - Worm holes

**Collection containers**
- Baskets
- Paper bags
- Boxes
- Plastic boxes

Not plastic bags!

**Transporting & Storage!**

Always keep them cool
- in the cooler or refrigerator
Remove pore surface (contains spores) for Suillus
Save the Pores (contain spores)

Throw out the flesh
From this

Whole mushroom

To this

Pores (spongy layer) only
For Rhizopogon, slice them up!

Or dry them on a food dehydrator.
Making a spore slurry from fresh pores (spongy layer)

Recipe

Materials
- coffee grinder
- glass canning jars
- funnel

Ingredients
1. 50 gms of fungal tissue
2. Water as needed
   - distilled or spring water
   - not chlorinated!

Movie clip
Dilute spore slurry to 1 million spores/ml

Slurry color chart  or  Use a counting chamber
(Haemocytometer & microscope)

Typically this means diluting the spores slurry 10 or 20 times.

Store in refrigerator
How long does it last?

Dark Chocolate  Milk Chocolate  Tea color

Then calculate the number of spores/ml .....
How to inoculate Seedlings with Spore Slurries

We use 3 ml of slurry (@ 1 million spores/ml) = 3 million spores/container

Drip method using a pipette

On soil surface near base of seedling

Inoculation gun

Cattle vaccination gun
Alternative: Make a Soil Inoculum

1. Tissue culture the fungus
   Grow in petri dish

2. Grow in liquid media, blend

3. Add to sterilized jars of peat: vermiculite

4. Add to containers or mix into soil
Suillus mycorrhizae on inoculated whitebark pine seedling roots

Look like ‘little hands’
What we have learned about:

When to inoculate

How to inoculate

Conditions for inoculation

Kinds of inoculum
Optimizing mycorrhizal colonization

**Container size:** short or long

**Soil substrate:** makes a difference!
- Peat: composted bark (7:3)
- Verm: peat: sand: loam (6:5:2:2)
- Not: Sunshine mix
- Not: high pH

**Seedling age:**
- 1 yr to 1.5 years
- Takes weeks/months for mycorrhizae to form

**Watering regime**
- Not: chlorinated water if possible

**Fertilization**
- Minimize
- Stop fertilization 1 month before inoculation
- Or use low nitrogen fertilizer: NPK 4:25:15
  - Or fertilize less often

**Types of inoculation**
- Fresh spore slurry is best
- Spore slurry from dried material works
- Soil inoculum works more slowly

**Inoculation method**
- Cattle vaccination gun
- Drip onto roots

**Strains of fungus**
- Some strains better than others
- Consider ‘seed zones’
Results from one Greenhouse Study
seedlings were then planted in burn soil
Colonized by Suillus mycorrhizae  Not colonized

• The stable isotope composition $N^{15}$ was lower for colonized seedling
  - confirms that the higher N content is a result of mycorrhizal colonization

Biomass of colonized seedlings 1.7 times greater

Foliar nitrogen content of colonized seedlings 1.66 times greater
Inoculation versus Colonization

- Not all inoculated seedlings become colonized (0-100%)

- Some un-inoculated seedlings can become colonized (at least at the Idaho Forest Nursey)

- For experiments, need to assess colonization status of each seedling

Colonized or not!
Field Studies
Improved survival is the ultimate goal

Difficult to assess effects of mycorrhizal colonization for the field studies because:

• Usually not possible to assess colonization at out-planting

• Seedlings are bundled together for planting and during cold storage for spring plantings

• Long term assessment is necessary survival can remain high on all treatments for a few years
Gravelly Mts, Eureka Burn

- Severe burn
- Beetle-killed trees prior
- Sorted trees in storage
- GPS individual seedlings
36,000 seedlings planted each year
Site 1: Seedling GPS points

Seedling Health
3 months post planting

Legend
Health Rating

Health of 1000 seedlings/site after 3 yrs
Highest health rating = 4

Health of 1000 seedlings/site after 2 yrs
Site 2

Colonization Status
When to inoculate

On burns

- **Light burn?** Mycorrhizal fungi not reduced?
- **Severe burn?** Mycorrhizal fungi reduced
- **Beetle-killed area?** Mycorrhizal fungi reduced
- **Rust killed area?** Mycorrhizal fungi reduced
- **Source of inoculum nearby?** mature living trees
- **Animal vectors present?** squirrels, deer, etc

Economically feasible to inoculate greenhouse seedlings?
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References


