Native mycorrhizal fungi & whitebark pine restoration

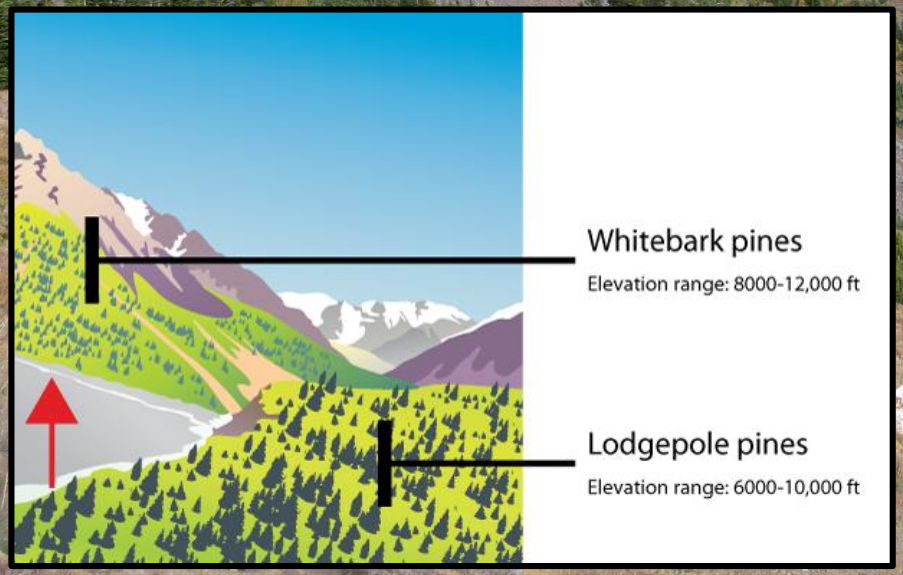
Figure 1.1: Range of whitebark pine (WPEF) 2014).

Dr Cathy Cripps

Montana State University

Whitebark pine (*Pinus albicaulis*)

-High elevations, at treeline
-Broad spreading tops



- Round purple cones
- Needles in bundles of **5**
- Whitish, light colored bark

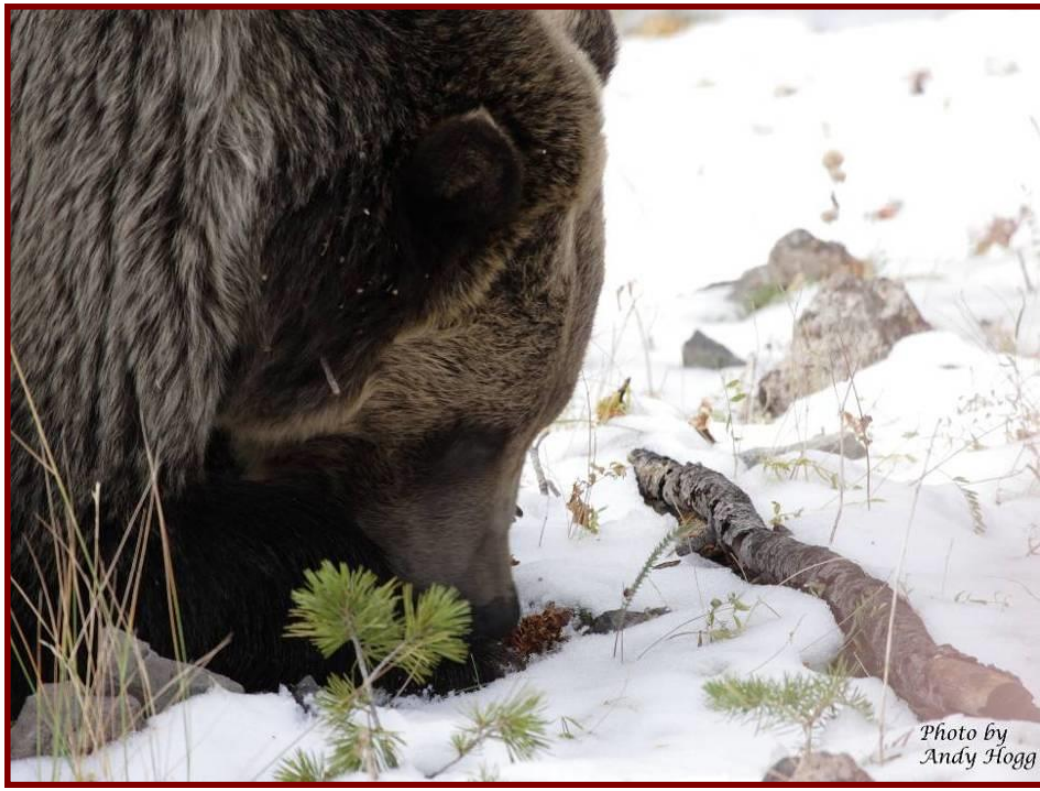
Seeds (pine nuts) are dispersed by birds that often plant them in burns

Clark's nutcracker
Nucifraga columbiana

Seeds are planted in clusters by birds
Germinate in as 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


Ghost Forests

Dead Whitebark Pine, Gravelly Range, Montana

USDA

United States Department of Agriculture

Forest Service

Rocky Mountain Research Station

General Technical Report RMRS-GTR-279

June 2012

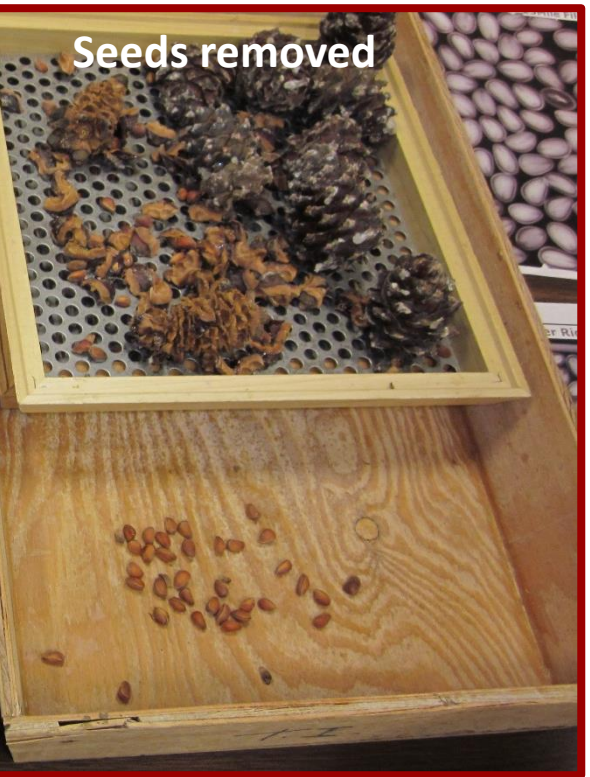
A Range-Wide Restoration Strategy for Whitebark Pine (*Pinus albicaulis*)

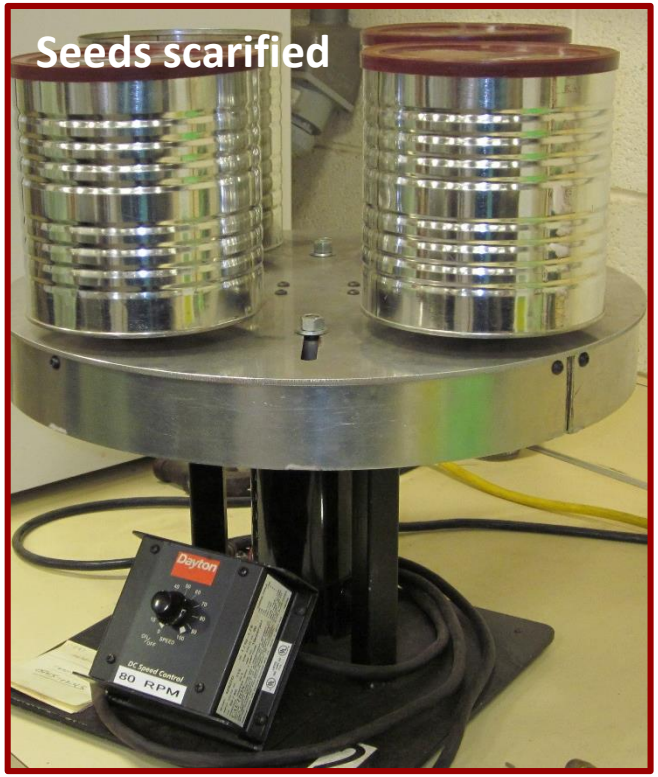
Restoration

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


Plus trees


Bags of cones


Seeds removed


Seeds scarified
Dayton
80 RPM


Seeds planted

Coeur D'Alene Forest Nursery, Idaho: whitebark pine seedlings

Whitebark pine ‘rust resistance’ trialsCoeur D’Alene Forest Nursery

Seedling survival has been generally low

A major assessment (Izlar 2007) showed around a 5o% 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)

WLNP photo

Can we use Native Mycorrhizal Fungi to improve seedling survival?

Fungi

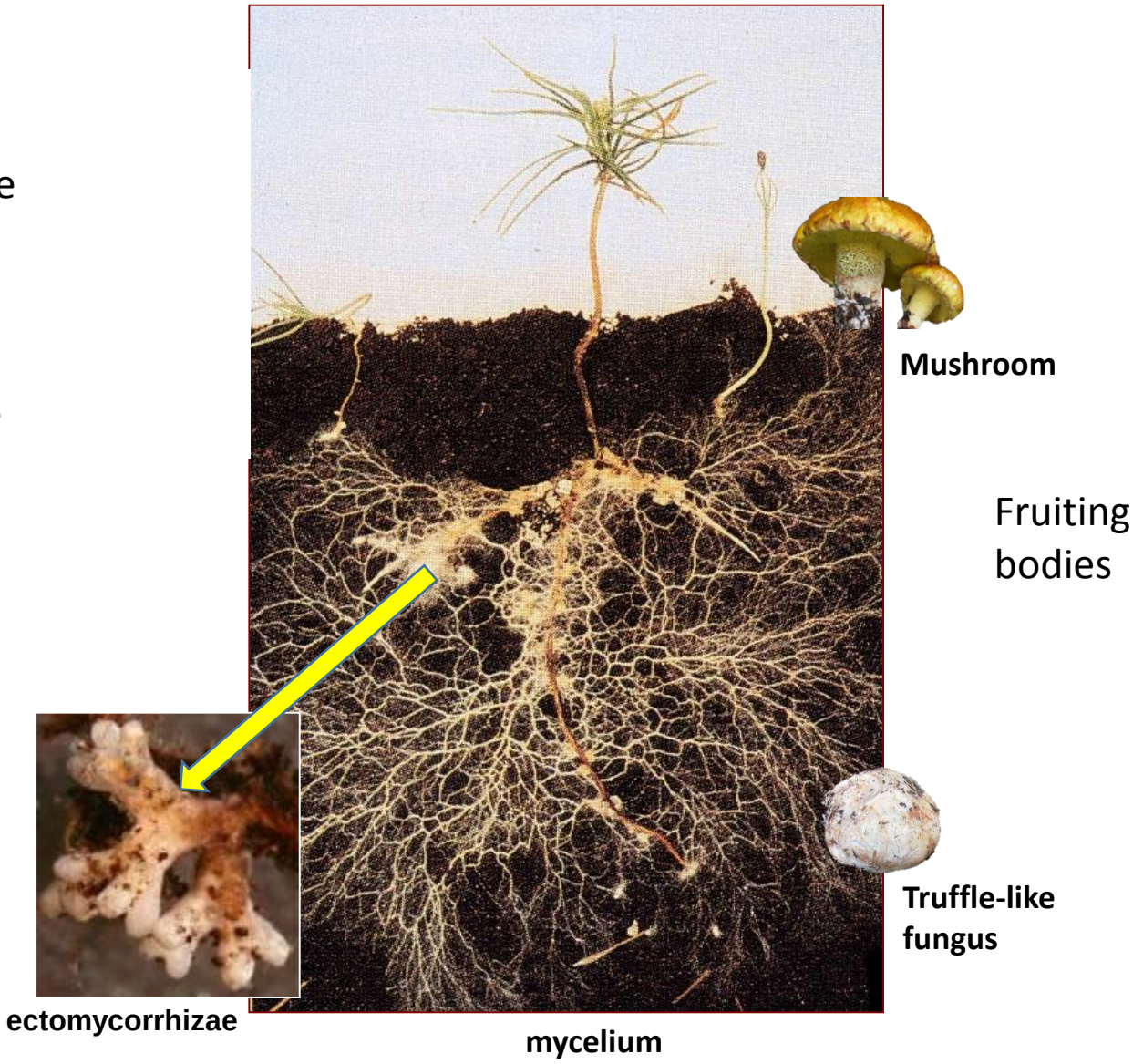
- Enhance nitrogen uptake
- Protect against drought

  Aggregate soil

  Hold soil moisture

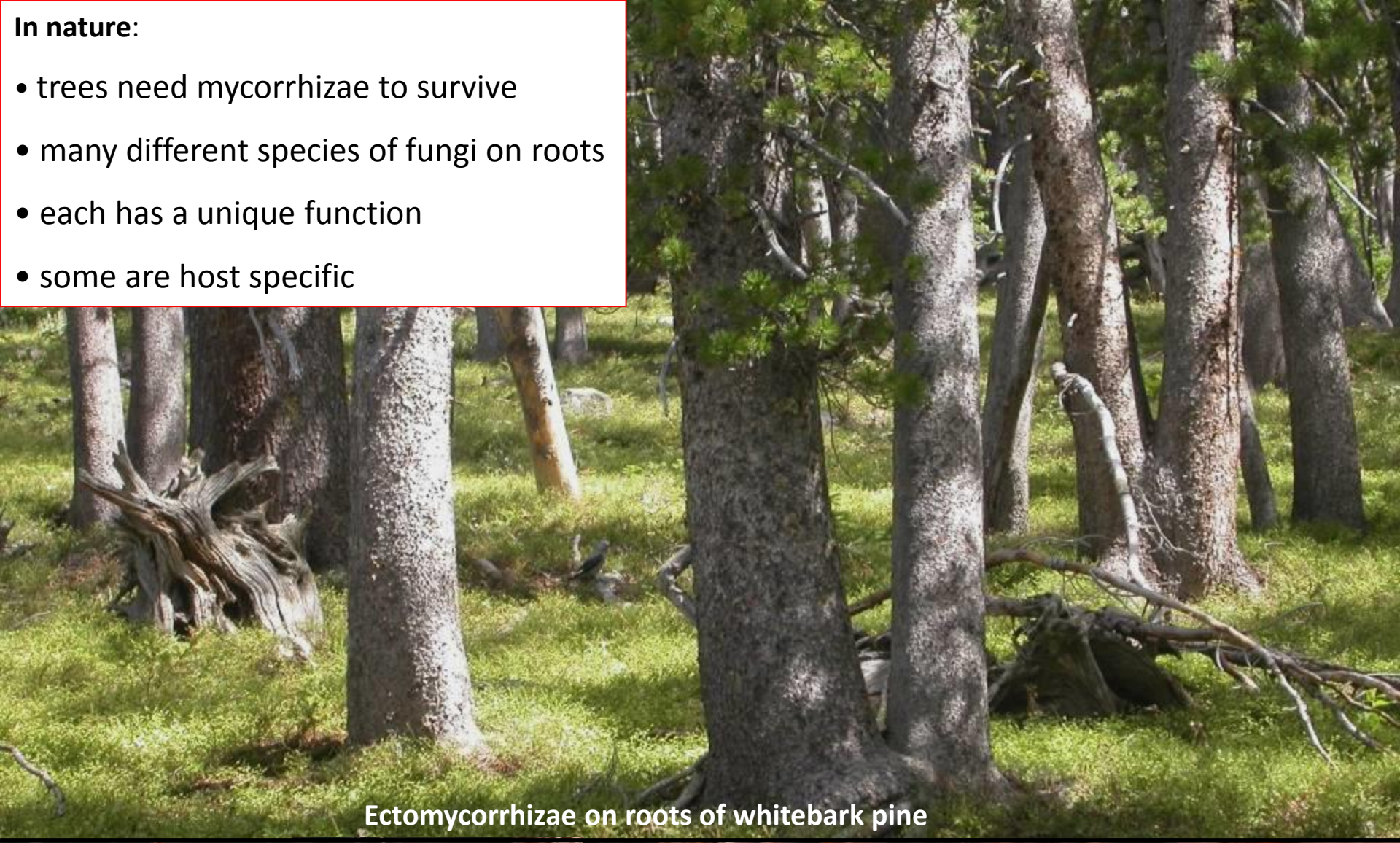
Plants

- Provide sugars (food) to the fungus



In nature:

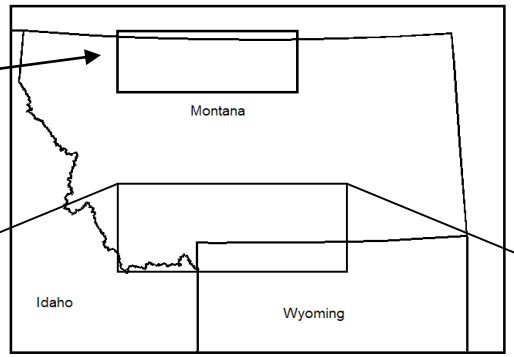
- trees need mycorrhizae to survive
- many different species of fungi on roots
- each has a unique function
- some are host specific



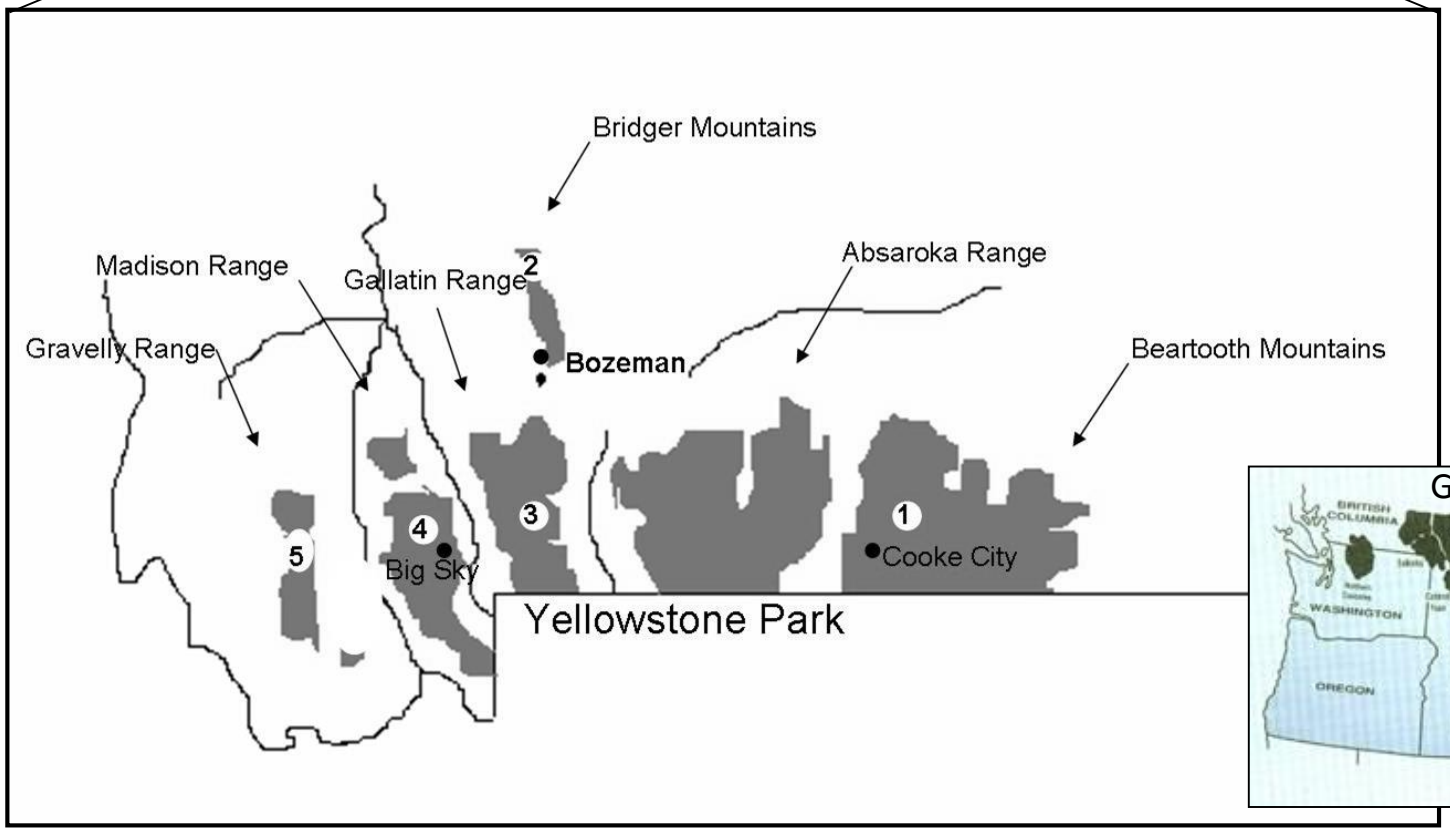
Ectomycorrhizae on roots of whitebark pine

Native Mycorrhizal Fungi with Whitebark Pine

Waterton Lakes National Park
Glacier National Park



3 national parks
7 mountain ranges



50 species of Native Ectomycorrhizal Fungi with Whitebark Pine

BASIDIOMYCOTA

AMANITACEAE
Amanita alpinicola Cripps
Amanita muscaria

HYGROPHORACEAE
Hygrophorus gliocyclus.
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

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

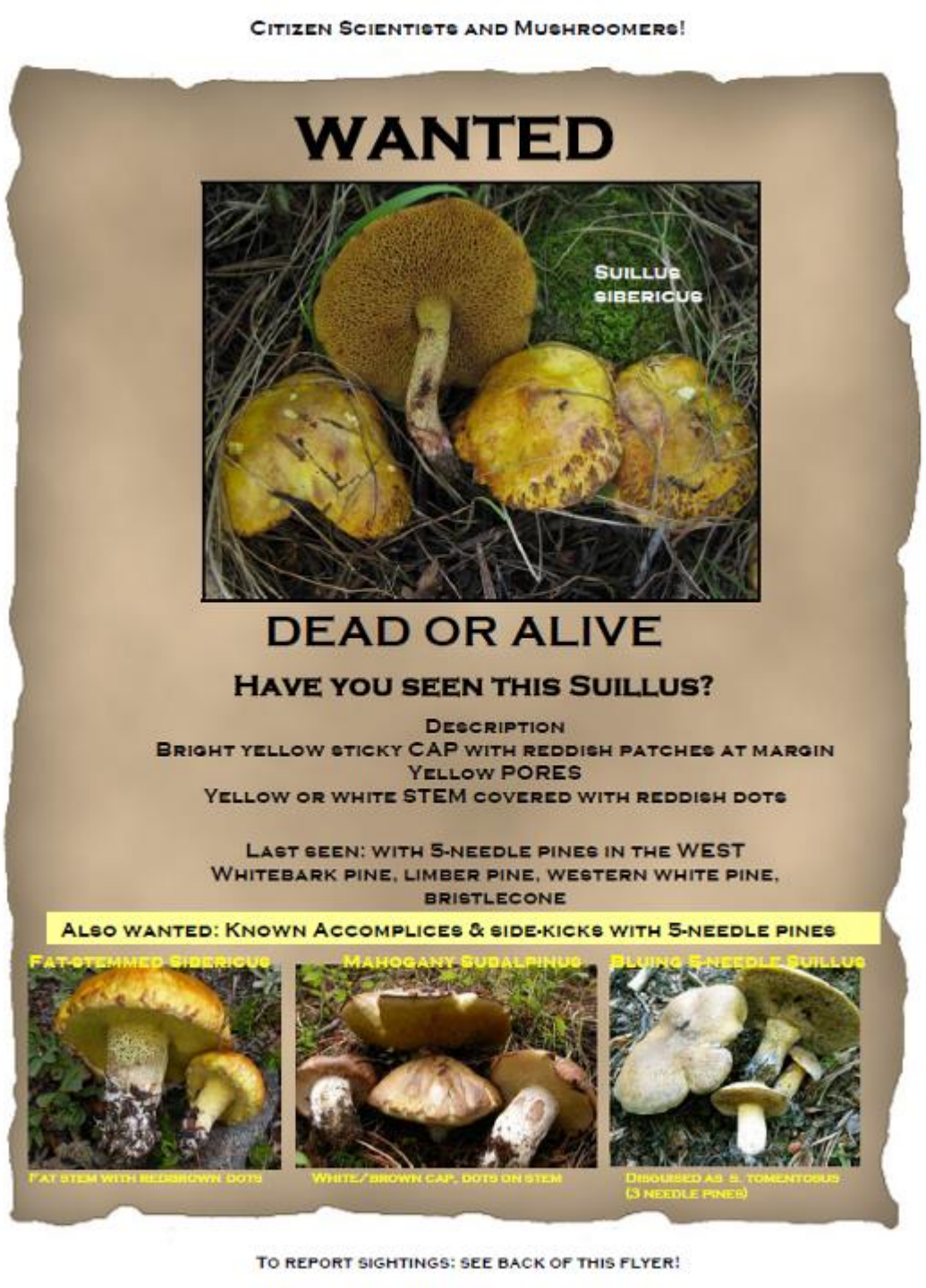
Rhizopogon evadens

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

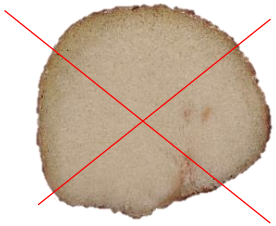
Selection

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

Yes!

Pogies

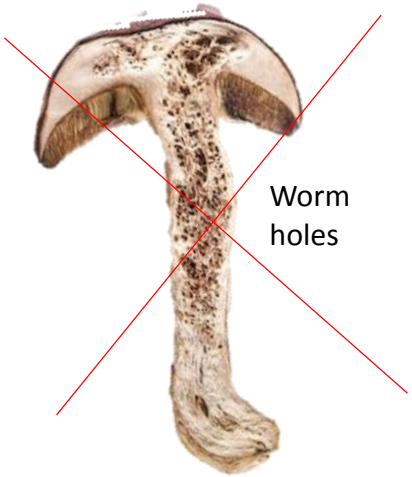
No! too young



White inside

Suillus

No! too old



Worm holes

Collection containers

- Baskets
- Paper bags
- Boxes
- Plastic boxes

Not plastic bags!

Slimy mushrooms

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

<u>Dilute</u> spore slurry to 1 million spores/ml

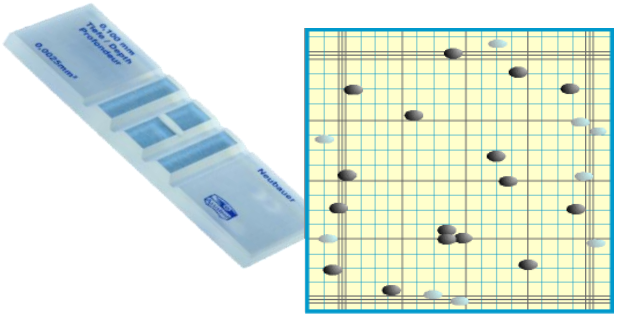
Slurry color chart

or

Use a counting chamber

(Haemocytometer & microscope)

Dark Chocolate **Milk Chocolate** **Tea color**



Then calculate the number of spores/ml …..

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

Store in refrigerator
How long does it last?

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 pippette

On soil surface near base of seedling

Inoculation gun

Cattle vaccination gun

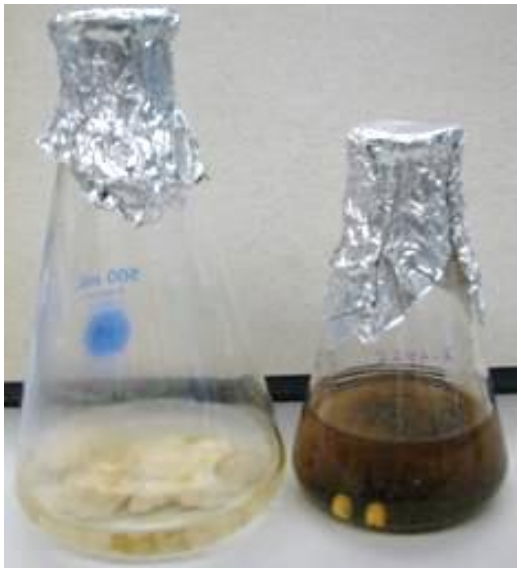
Alternative: Make a Soil Inoculum

Tissue culture the fungus
Grow in petri dish

1

Grow in liquid media, blend

2



Add to sterilized jars of peat: vermiculite

3

4

Add to conetainers or mix into soil

Suillus mycorrhizae on inoculated whitebark pine seedling roots

Look like ‘little hands’

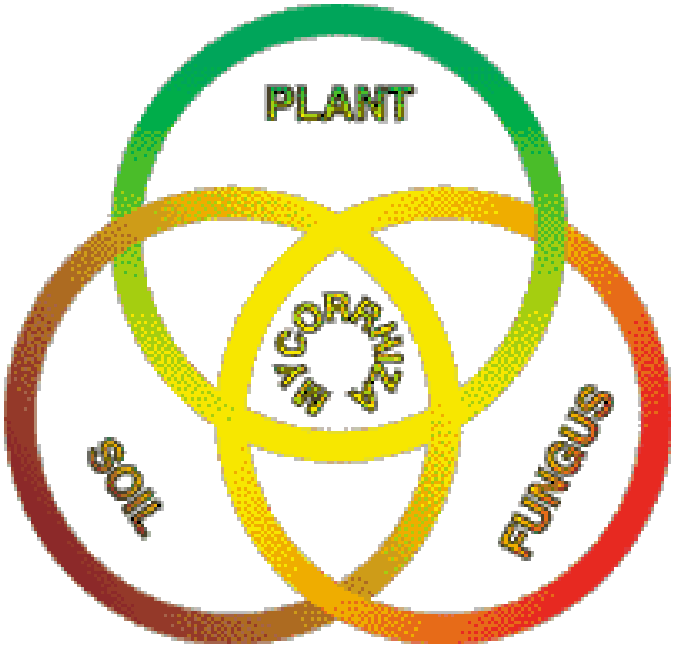
What we have learned about:

When to inoculate

How to inoculate

Conditions for inoculation

Kinds of inoculum



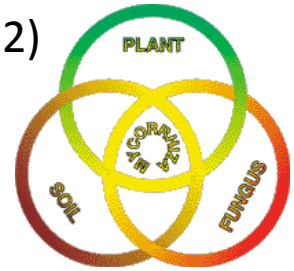
Optimizing mycorrhizal colonization

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

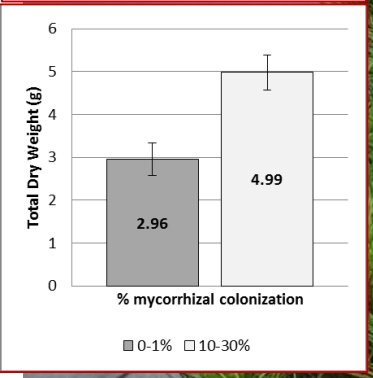
Strains of fungus
-some strains better than others
-consider ‘seed zones’

Results from one Greenhouse Study

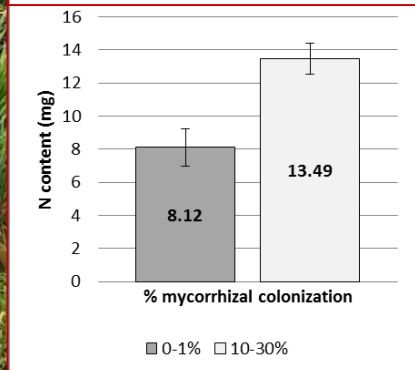
seedlings were then planted in burn soil

Colonized by Suillus mycorrhizae **Not colonized**

<u>Biomass</u> of colonized seedlings 1.7 times greater



<u>Foliar nitrogen content</u> of colonized seedlings 1.66 times greater



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

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

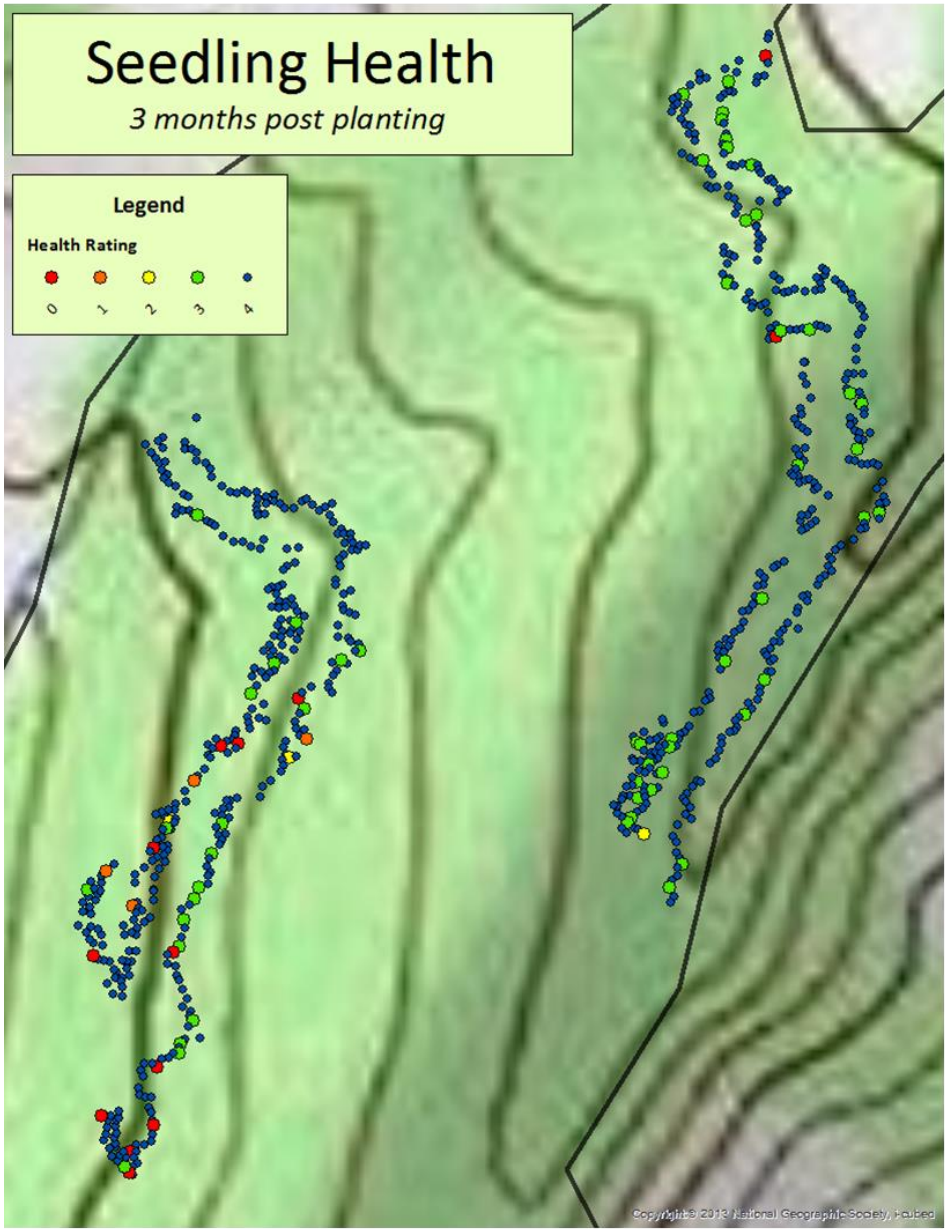
Gravelly Mts, Eureka Burn

- Severe burn
- Beetle-killed trees prior

- Sorted trees in storage
- GPS individual seedlings

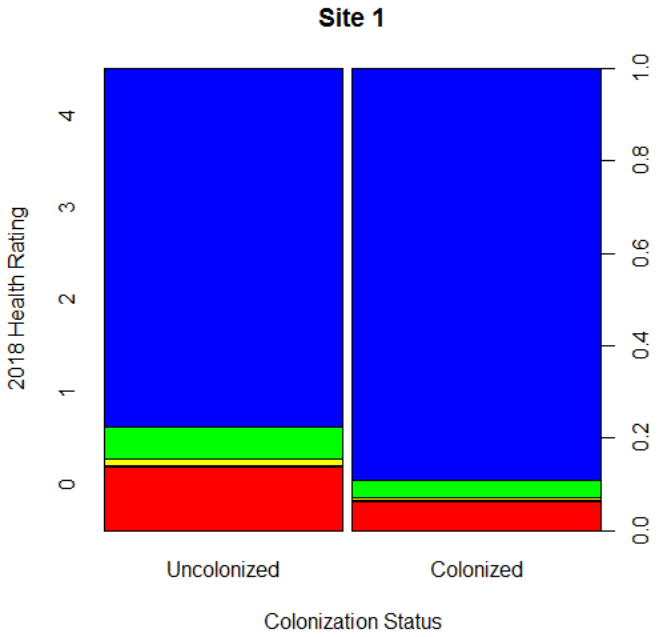
36,000 seedlings
planted each year

Site 1: Seedling GPS points

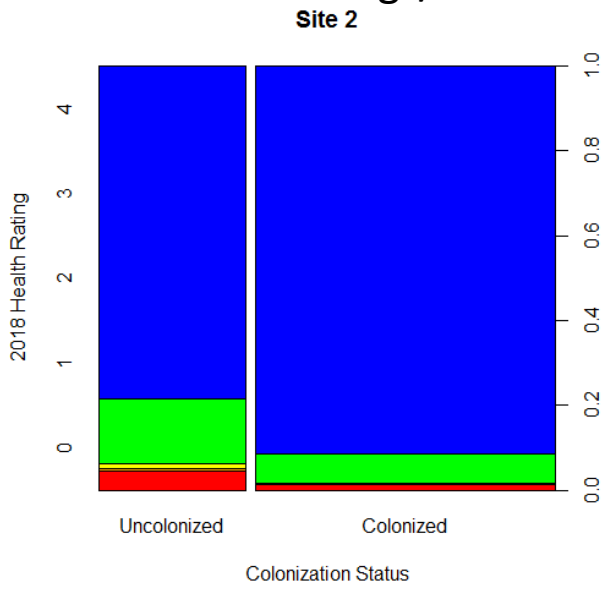


Health of 1000 seedlings/site 1 after 3 yrs

Highest health rating = 4



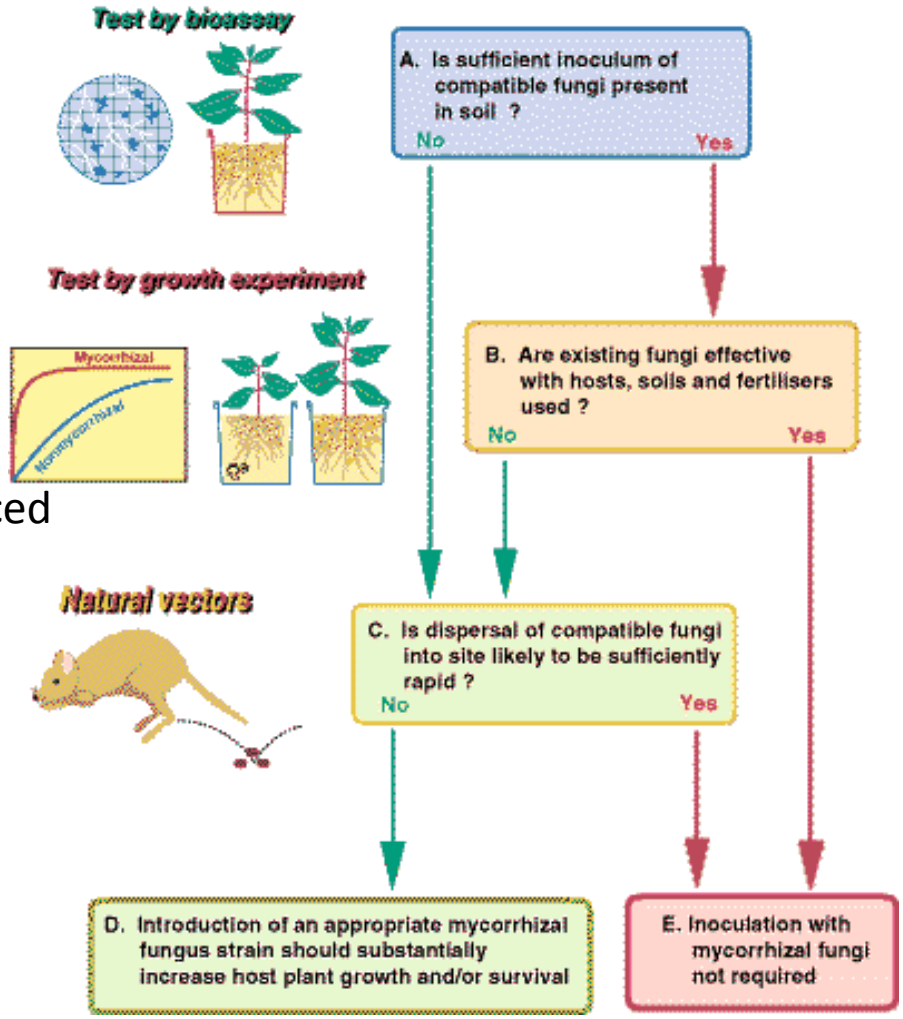
Health of 1000 seedlings/site after 2 yrs



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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Questions?