Inoculation of Whitebark Pine Seedlings with Native Ectomycorrhizal Fungi

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Most ecosystems are predicated on the existence of microscopic fungi on the roots of plants (that form mycorrhizae) E.O. Wilson

What are Ectomycorrhizal fungi?

- mutualistic with tree roots

- promote phosphorus & nitrogen uptake in trees
- protect tree roots (heavy metals, grazers, etc)
- improve drought tolerance
- improve tree health & seedling establishment

Trees: Birch, Oak, Willow, Aspen, Eucalyptus, Alder, Pine, Spruce, Fir, Larch, Douglas fir

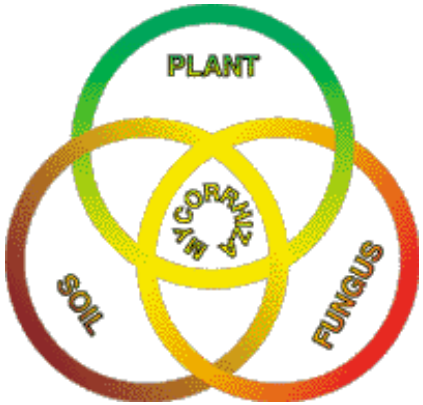
All pines depend on ectomycorrhizal fungi to survive in nature (Read 1998)

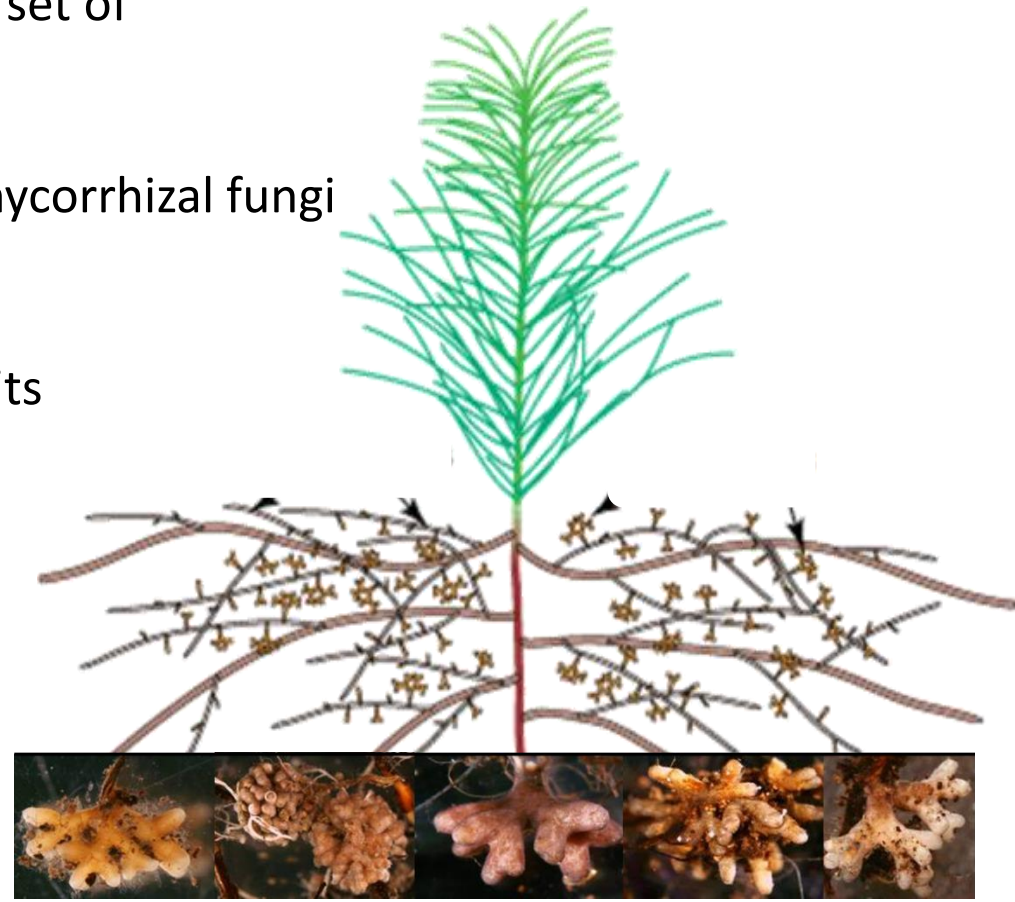
Can these native mycorrhizal FUNGI be used as a tool in WHITEBARK PINE restoration?

There are many kinds of Ectomycorrhizal fungi: which ones could be useful?

5,000-8,000 species of fungi

- Each tree species hosts a particular set of mycorrhizal fungi
- Each tree hosts a 'community" of mycorrhizal fungi (many different species)
- Each fungus provides unique benefits
- Some ECM fungi are host specific





Cripps, CL 2002. Mycorrhiza. In: Pscheidt & Ocamb, Pacfic NW Plant Disease Management Handbook.

Austria has been inoculating European Stone Pine (*Pinus cembra*) seedlings for restoration for 50 years

To stabilize slopes from avalanches after trees were cut down for grazing

Visit to Federal Institute Nursery
Innsbruck, Austria, 2008

Inoculation with ectomycorrhizal fungi: Increased seedling survival from 50% to 90 % at high elevations

Weisleitner Austrian National Nursery, Pers. Comm.

Still on the 50 yr old pines today!

Native **<u>Suillus species</u>**

Ectomycorrhizae

What fungi are ectomycorrhizal with whitebark pine? Which ones are important and/or useful in restoration?

- About 50 species of ECM fungi with whitebark pine
- The **Suilloids** we found are specific for WBP, or 5-needle pines, or pines

***Suillus** sibiricus*

One of the same species used in Austria!

Rhizopogon ("pogies") grow underground

Kate Mohatt, Cripps & Lavin (2006) Ectomycorrhizal fungi of whitebark pine (a tree in peril) revealed by sporocarps and molecular analysis of mycorrhizae from treeline forests in the Greater Yellowstone Ecosystem. Can. J. Bot. 86(1): 14-25.

Cripps & Antibus. 2011. Native Ectomycorrhizal fungi of limber and whitebark pine. Hi-Five Proceedings.

Native Ectomycorrhizal fungi were screened to see which colonized whitebark pine seedling roots in the greenhouse

- Tested different inoculum types, substrates, fertilizers, etc

Results: *Suillus sibiricus* colonized whitebark pine roots most efficiently!

Cripps & Grimme. 2011. Inoculation and successful colonization of whitebark pine seedlings with native ectomycorrhizal fungi under greenhouse conditions. Hi-Five Proceedings

Lonergan & Cripps, C.L. 2013. Use of a low nitrogen fertilizer as a strategy to maintain ectomycorrhizal colonization on whitebark pine seedlings. *Native Plants Journal* 14(3): 213-224.

Field Study: Waterton Lakes National Park

Does inoculation with *Suillus* improve seedling survival?

21 plots, 1000 seedlings planted & monitored

1) Burns with beargrass roots

2) Burns without beargrass roots

3) Unburned area with beargrass

4) Unburned areas without beargrass

- **Inoculated** with native ECM fungi/not
- Planted in **microsites**/not
- Planted in clusters of 3

Terra-torched, and not severe/intense burns

Inoculation with *Suillus sibiricus*, planting in microsites, and on burn sites resulted in higher survival of out-planted whitebark pine seedlings (3 yrs)

Lonergan, Cripps, Smith, C. 2014. Influence of site conditions, shelter objects and ectomycorrhizal inoculation on the early survival of whitebark pine seedlings planted in Waterton Lakes National Park. *Forest Science* 60(3): 603-612.

Cripps, Lonergan, Smith. Survival of whitebark pine seedlings inoculated with ectomycorrhzae. *Nutcracker Notes* 26 15-18.

Marlee Jenkins ongoing graduate work

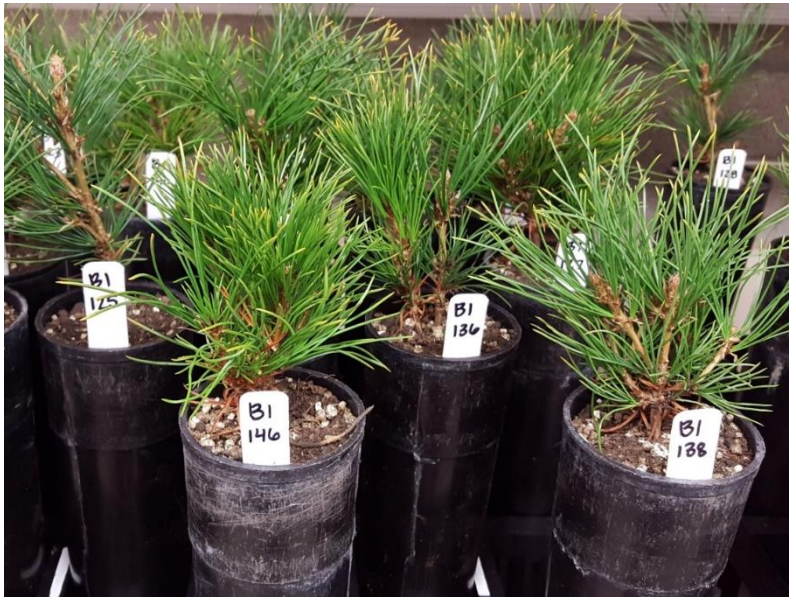
How does colonization with Suilloid fungi affect:

- Whitebark pine seedlings planted on a **severe burn** in the field (survival)
- Whitebark pine seedlings planted in **burn soil** in the greenhouse

What do we know:

- Whitebark pine seedlings have improved survival on burn sites
- Severe burns are known to eliminate ectomycorrhizal fungi in the soil

Seedlings outplanted in burn soil



Seedlings planted in burn soil in greenhouse

Method

Seedlings from the Idaho nursery

are sorted on their arrival at the MSU Plant Growth Center

Roots not colonized with ectomycorrhizal fungi
(use in inoculation experiments)

Roots already colonized with ectomycorrhizal fungi
(usually small amounts of Thelephora, Cenococcum, etc)

50% of seedlings were already colonized; seedlings appeared larger & greener!

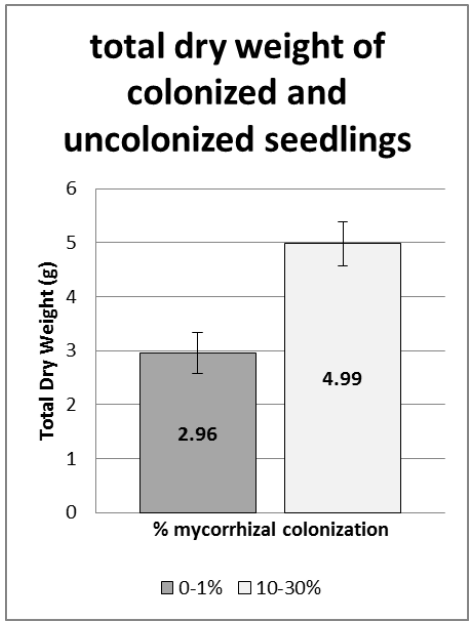
Typically do not expect an early growth response to ectomycorrhizal colonization in slow growing pines?

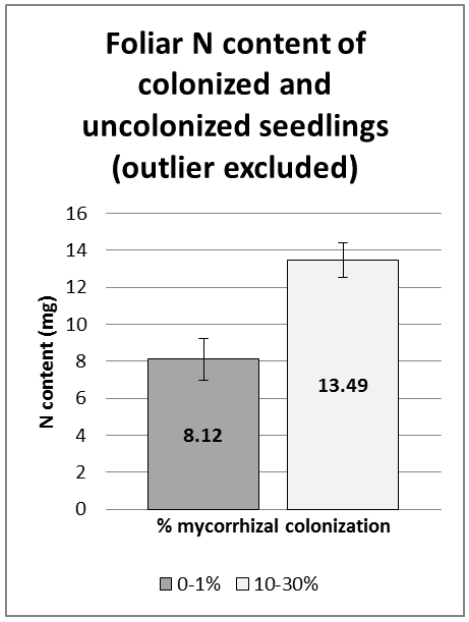
What is this important fungus? DNA analysis of ectomycorrhizae

Results: *Suillus* species! But where did they come from?

Comparison whitebark pine seedling colonized and not colonized by Suilloid fungi in the greenhouse

- Biomass of colonized seedlings was 1.68 times greater
- Total nitrogen content of colonized seedlings was 1.66 times greater





Preliminary data

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

But where did the fungi come from?

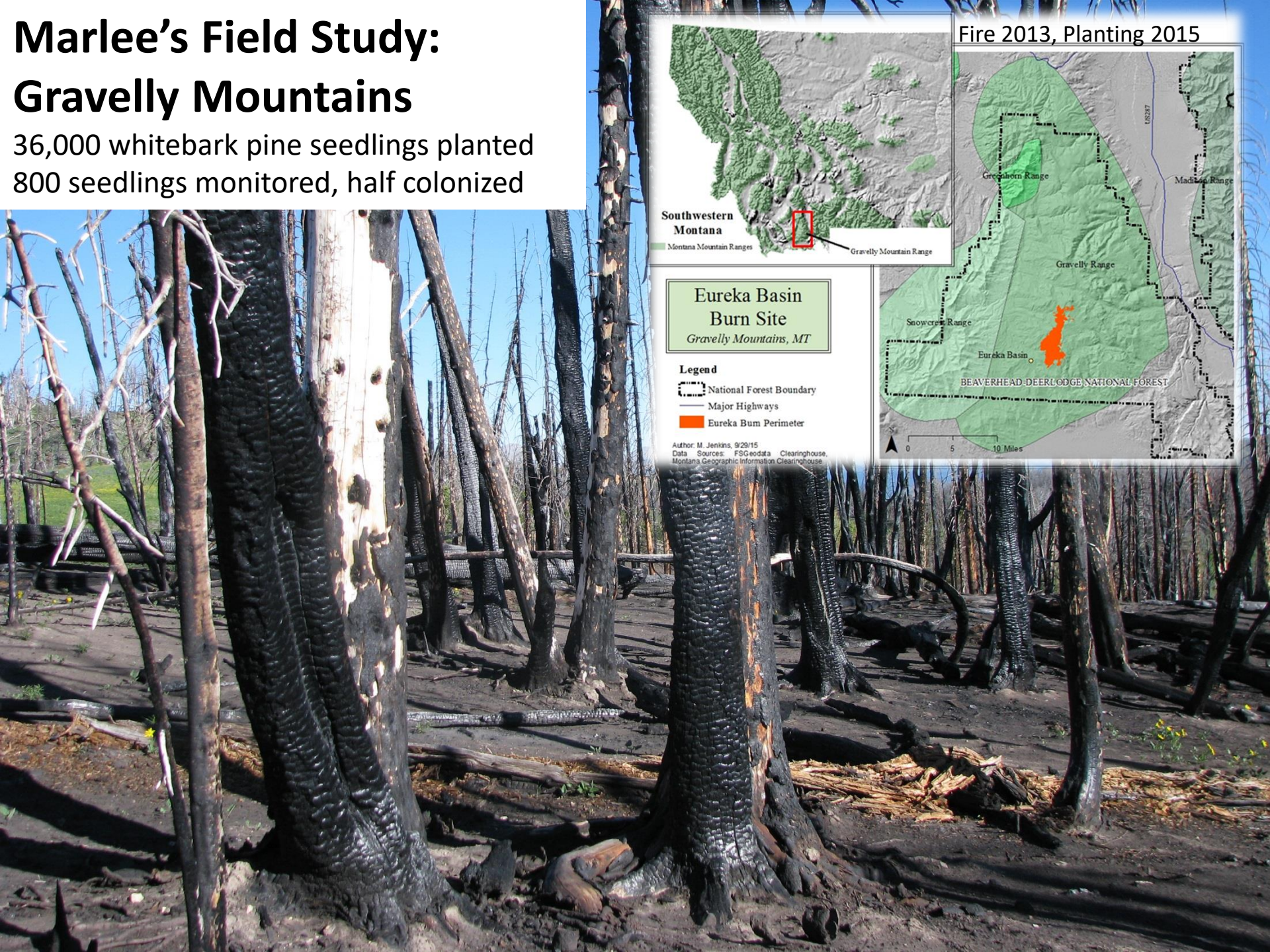
Mystery:
Where did the Suilloid Fungi come from --the Idaho Nursery?

Western white pine planted outside at the Idaho Nursery

Suillus sibiricus **with western white pine at the Idaho Nursery – directly behind the greenhouse where whitebark pine seedlings are grown!**

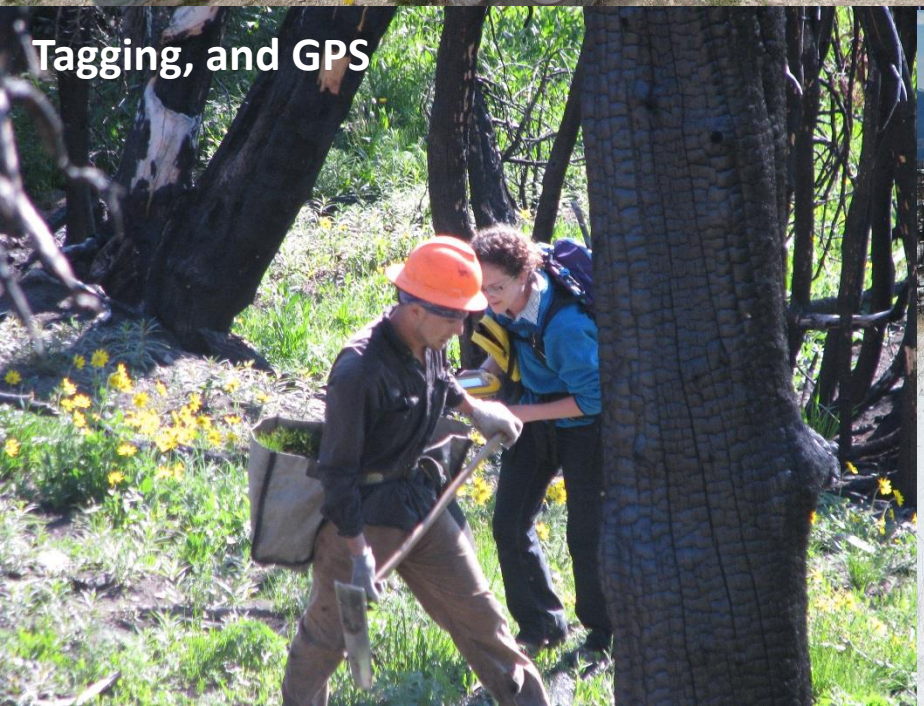
Marlee's Field Study: Gravelly Mountains

36,000 whitebark pine seedlings planted
800 seedlings monitored, half colonized




Seedlings delivered to the site 2015
PERISHABLE
KEEP COOL
FOREMAN
4x4


Planting


Tagging, and GPS


Monitoring in all conditions

Will colonization by suilloid fungi increase seedling survival?

Can we predict the best planting conditions with GIS spatial analysis?

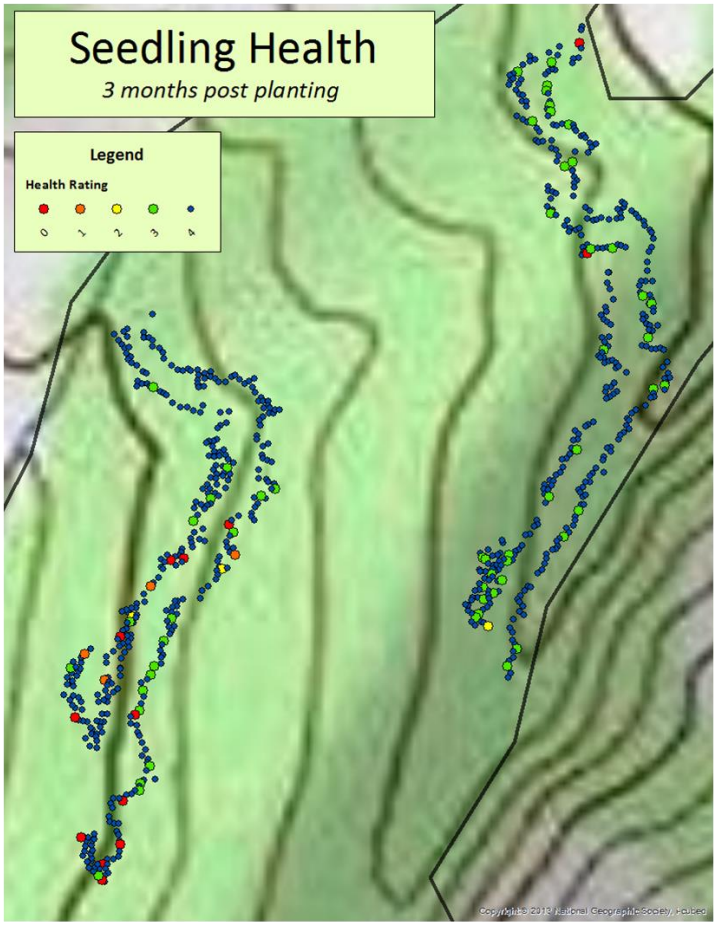


Figure 3. A map of seedling points displaying health ratings 3 months after planting.

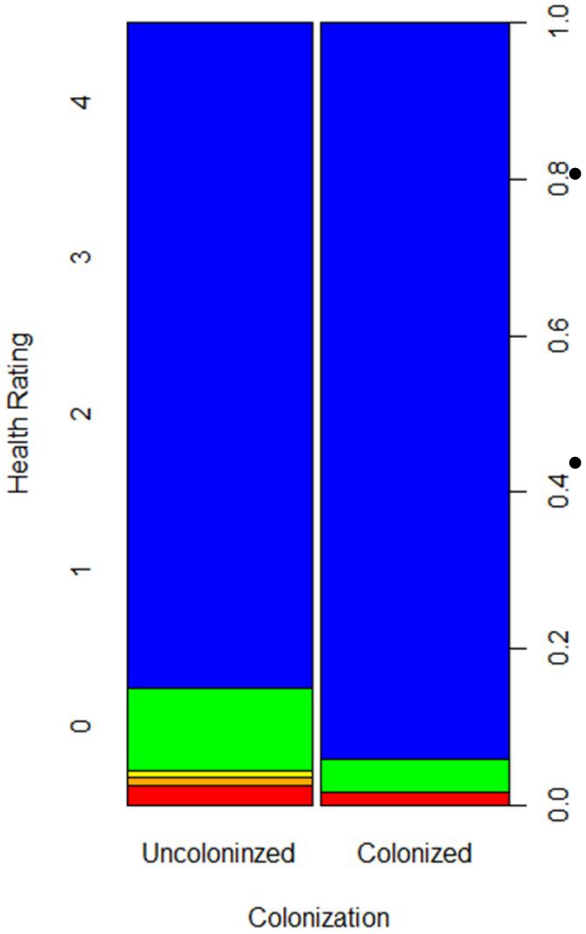


Figure 4. A spineplot of seedling health 3 months after planting.

- Seedling health data attached to GPS points using Esri ArcMap Software
- Relate to site factors including colonization, wetness index, aspect, etc.

2nd study site in the Gravellies is testing: inoculated seedlings versus already colonized seedlings

Suillus NEWS

Suillus subalpinus is proposed for a Global Red List Assessment

It is only known to occur with *Pinus albicaulis.*

Given its host specificity and the declining populations of its host whitebark pine, it is being assessed for the **Global Fungal Red List.**

The new Suilloid study group will sequence 53 complete genomes; we hope the whitebark pine *Suillus* species will be included.

http://iucn.ekoo.se/iucn/species_list/

Technology transfer from MSU to Athabasca University and the Smoky Lake Nursery

Dr. Roland Treu

Robert Sissons, Parks Canada, Waterton Lakes National Prak

THOSE OF YOU IN CANADA: ROLAND NEEDS YOUR SUILLUS WITH WHITEBARK PINE RIGHT NOW!

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Suillus ectomyorrhizae