Influence of site conditions, shelter objects and ectomycorrhizal inoculation on the early survival of whitebark pine seedlings planted in Waterton Lakes National Park

Erin Lonergan & Cathy Cripps, Montana State University
Cyndi Smith, Conservation Biologist, Waterton Lakes National Park
“Working Together to Restore Terrestrial Ecosystems”
--Waterton Lakes National Park--

Parks Canada investing $7 million to restore terrestrial ecosystems. In Waterton Lakes National Park, the restoration of native fescue grasslands and of whitebark and limber pine communities are the main focus of this many-sided project.
Cyndi Smith, Conservation Biologist WLNP

Initiated restoration of whitebark & limber pine in Waterton Lakes National Park

- High WBP mortality (Smith et al. 2008, Smith et al. 2011)
- Restoration began in 2003
- Plus trees identified 2006, cone collection 2006-2011
- Use of Verbenone to protect selected trees
- Seeds sent for rust resistance screening
- Inoculated of seedlings with native ectomycorrhizal fungi


“Use of Native Ectomycorrhizal Fungi in the Restoration of Whitebark Pine”

Plant Sciences & Plant Pathology Dept., Montana State University

- experimental design, planting, inoculation
- monitoring seedlings
- statistical analysis
Our Goal:
was to determine how various factors affect the survival of nursery-grown whitebark pine seedlings planted in the park.

- Planting in **burned** areas (terra-torched)
- Planting in **beargrass**
- Planting with **microsites** (shelter objects)
- Inoculation with **ectomycorrhizal fungi**
Waterton Lakes National Park

• 21 circular plots
• Randomly selected
• 50 meters in diameter
• half of each plot burned

Elevations: 1,500 – 2,000 m

Terra torching

To remove overstory trees, not WBP
4 sets of site conditions on each plot

- Planted in clusters of 3
- Some planted in microsites
- Some inoculated with ectomycorrhizal fungi (in the nursery)

Unburned without beargrass

Unburned with beargrass

Burned with beargrass

Burned without beargrass

Burned, beargrass roots still present

Burned, no beargrass present
How do Ectomycorrhizal Fungi benefit plants?

- enhance nutrient uptake especially nitrogen
- provide protection from drought, pathogens, grazers, heavy metals

In nature:
- trees need mycorrhizae to survive
- many different species of fungi on roots

Method for inoculation with Native Ectomycorrhizal Fungi

Native Suillloid fungi are collected from whitebark pine forests

Fertilization stopped & spores injected onto the soil- 1 to 3 months before planting

Spore slurries are made from mushrooms

Seedlings colonized with ectomycorrhizal fungi

Cripps & Antibus 2011: Hi-Five Proceedings

Cripps & Grimme 2011: Hi-five proceedings
Seedlings were out-planted in clusters

Three ectomycorrhizal treatments

**Inoculated** – seedlings inoculated with **native** ectomycorrhizal fungus in the greenhouse

**Exposed** - seedlings not inoculated but adjacent to inoculated seedlings in a cluster

**Not inoculated** – seedlings were not inoculated or not exposed
Monitoring seedling survival

1000 nursery grown seedlings planted in 2010

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unburned No Beargrass</th>
<th>Unburned Beargrass</th>
<th>Burned No Beargrass</th>
<th>Burned Beargrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-inoculated</td>
<td>27 seedlings</td>
<td>54 seedlings</td>
<td>87 seedlings</td>
<td>92 seedlings</td>
</tr>
<tr>
<td>Exposed</td>
<td>16 seedlings</td>
<td>47 seedlings</td>
<td>99 seedlings</td>
<td>85 seedlings</td>
</tr>
<tr>
<td>Inoculated</td>
<td>41 seedlings</td>
<td>70 seedlings</td>
<td>174 seedlings</td>
<td>191 seedlings</td>
</tr>
</tbody>
</table>

Above normal precipitation in monitoring years one (2011) and two (2012)

Early results – 2 years

Variables

- With/without beargrass
- Unburned/burned
- With/without microsite
- With/without mycorrhizal inoculation

Oct 2011- Aug 2012
Early Seedling Survival 1 and 2 years after planting

Overall 95% survival after 1 year
Overall 69% survival after 2 years

After 2 years, overall survival averaged:
70% on burns
51% unburned areas
Results of **binary logistic regression** of site conditions, shelter object presence, and ectomycorrhizal inoculation treatment on the survival of out-planted whitebark pine seedlings.

### BINARY REGRESSION MODEL

\[
\text{logit(odds of survival)} = \beta_0 + \beta_1 b + \beta_2 bg + \beta_3 so + \beta_4 \text{exposed} + \beta_5 \text{inoculated} + \beta_6 b*bg + \beta_7 b*so + \beta_8 bg*so + \beta_9 b*exposed + \beta_{10} bg*exposed + \beta_{11} b*inoculated + \beta_{12} bg*inoculated
\]

\[\text{Exp}(B) = \text{odds of survival in comparison to outgroup}\]

### OUTGROUP: Unburned without beargrass, Uninoculated, No shelter object

<table>
<thead>
<tr>
<th>Model Terms</th>
<th>Estimate ($\beta$)</th>
<th>SE</th>
<th>Wald $z^2$</th>
<th>df</th>
<th>Prob.</th>
<th>Exp($\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.109</td>
<td>0.387</td>
<td>-2.865</td>
<td>1</td>
<td>0.004</td>
<td>0.330</td>
</tr>
<tr>
<td>Burn</td>
<td>2.083</td>
<td>0.434</td>
<td>4.803</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>8.029</td>
</tr>
<tr>
<td>Beargrass</td>
<td>0.877</td>
<td>0.404</td>
<td>2.168</td>
<td>1</td>
<td>0.030</td>
<td>2.404</td>
</tr>
<tr>
<td>Exposed</td>
<td>0.497</td>
<td>0.468</td>
<td>1.062</td>
<td>1</td>
<td>0.288</td>
<td>1.644</td>
</tr>
<tr>
<td>Inoculated</td>
<td>-0.042</td>
<td>0.399</td>
<td>-0.105</td>
<td>1</td>
<td>0.917</td>
<td>0.959</td>
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<tr>
<td>Shelter object</td>
<td>1.151</td>
<td>0.359</td>
<td>3.211</td>
<td>1</td>
<td>0.001</td>
<td>3.161</td>
</tr>
</tbody>
</table>

### OUTGROUP: Unburned with beargrass, Uninoculated, No shelter object

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<tr>
<td>Intercept</td>
<td>-0.233</td>
<td>0.286</td>
<td>-0.814</td>
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<td>0.792</td>
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<tr>
<td>Burn</td>
<td>0.572</td>
<td>0.347</td>
<td>1.648</td>
<td>1</td>
<td>0.099</td>
<td>1.773</td>
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<tr>
<td>No Beargrass</td>
<td>-0.877</td>
<td>0.404</td>
<td>-2.168</td>
<td>1</td>
<td>0.030</td>
<td>0.416</td>
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<tr>
<td>Exposed</td>
<td>0.798</td>
<td>0.377</td>
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<td>0.035</td>
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<td>Inoculated</td>
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<td>Shelter object</td>
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<td>0.290</td>
<td>1.902</td>
<td>1</td>
<td>0.057</td>
<td>1.735</td>
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</tbody>
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<tr>
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<td>0.384</td>
<td>2.536</td>
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<td>0.011</td>
<td>2.647</td>
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<tr>
<td>Unburned</td>
<td>-2.083</td>
<td>0.434</td>
<td>-4.803</td>
<td>1</td>
<td>&gt; 0.001</td>
<td>0.125</td>
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<tr>
<td>Beargrass</td>
<td>-0.634</td>
<td>0.420</td>
<td>-1.510</td>
<td>1</td>
<td>0.131</td>
<td>0.530</td>
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<tr>
<td>Exposed</td>
<td>-0.583</td>
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<td>-1.608</td>
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<td>0.108</td>
<td>0.558</td>
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<tr>
<td>Inoculated</td>
<td>-0.359</td>
<td>0.332</td>
<td>-1.082</td>
<td>1</td>
<td>0.279</td>
<td>0.698</td>
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<tr>
<td>Shelter object</td>
<td>1.030</td>
<td>0.328</td>
<td>3.141</td>
<td>1</td>
<td>0.002</td>
<td>2.801</td>
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### OUTGROUP: Burned with beargrass, Uninoculated, No shelter object

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<tr>
<td>Intercept</td>
<td>0.340</td>
<td>0.237</td>
<td>1.435</td>
<td>1</td>
<td>0.151</td>
<td>1.404</td>
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<td>Unburned</td>
<td>-0.572</td>
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<td>-1.648</td>
<td>1</td>
<td>0.099</td>
<td>0.564</td>
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<tr>
<td>No Beargrass</td>
<td>0.634</td>
<td>0.420</td>
<td>1.510</td>
<td>1</td>
<td>0.131</td>
<td>1.885</td>
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<tr>
<td>Exposed</td>
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<tr>
<td>Inoculated</td>
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<td>1.331</td>
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<tr>
<td>Shelter object</td>
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<td>0.215</td>
<td>1.996</td>
<td>1</td>
<td>0.046</td>
<td>1.536</td>
</tr>
</tbody>
</table>

### Interactions

<table>
<thead>
<tr>
<th>Model Terms</th>
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<th>Prob.</th>
<th>Exp($\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn x Beargrass</td>
<td>-1.511</td>
<td>0.357</td>
<td>-4.229</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>0.221</td>
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<tr>
<td>Burn x Exposed</td>
<td>-1.080</td>
<td>0.435</td>
<td>-2.483</td>
<td>1</td>
<td>0.013</td>
<td>0.340</td>
</tr>
<tr>
<td>Burn x Inoculated</td>
<td>-0.318</td>
<td>0.377</td>
<td>-0.843</td>
<td>1</td>
<td>0.399</td>
<td>0.728</td>
</tr>
<tr>
<td>Burn x Shelter object</td>
<td>-0.121</td>
<td>0.331</td>
<td>-0.366</td>
<td>1</td>
<td>0.714</td>
<td>0.886</td>
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<tr>
<td>Beargrass x Exposed</td>
<td>0.301</td>
<td>0.426</td>
<td>0.706</td>
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<td>0.480</td>
<td>1.351</td>
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<tr>
<td>Beargrass x Inoculated</td>
<td>0.645</td>
<td>0.377</td>
<td>1.711</td>
<td>1</td>
<td>0.087</td>
<td>1.906</td>
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<tr>
<td>Beargrass x Shelter object</td>
<td>-0.601</td>
<td>0.353</td>
<td>-1.702</td>
<td>1</td>
<td>0.089</td>
<td>0.548</td>
</tr>
</tbody>
</table>

1. Coefficient
2. Wald chi-square value = (Wald Z value)$^2$
3. Odds ratio of survival for the predictors

Lonergan, Cripps, Smith (accepted)
Forest Science
% Survival of Planted Whitebark Pine Seedlings for all Variables

- Highest on burned areas without beargrass, with microsite
- Inoculation effect
- Lowest on unburned sites without beargrass, no microsite
Effect of Planting near shelter objects (microsite) on survival of planted whitebark pine seedlings

In general, planting near microsites improved survival 10-12.5% on burns and 31% on unburned areas.
Effect of ectomycorrhizal inoculation on survival of planted whitebark pine seedlings

Inoculation or exposure to ectomycorrhizal inoculum increased survival 17-24% on unburned sites with beargrass.

Inoculation of 1, 2, or 3 in cluster improved seedling survival.

Site specific inoculation effect

Inoculated (65.6%) exposed (71.5%) controls (48.3%).
Conclusions from early monitoring after 2 years

• This study had some of the **highest early survival rates** of any reports (overall 95% in year 1 and 69% year 2)

What role did exceptional **moisture conditions** have on early survival? What role did treatments have on early survival?

• **Burned (terra-torched) areas without beargrass** roots supported some of the highest seedling survival rates (82%)

• Planting near **shelter objects (microsite)** increased survival 10-12.5% on burns and 31% on unburned areas without beargrass (poor planting sites)

• **Inoculation with native ectomycorrhizal fungi** (or exposure to inoculated seedlings) increased survival 17-24% on unburned sites with beargrass

• **Long-term monitoring** necessary to assess the ultimate effectiveness of the restoration techniques tested.

Izlar 2007:
100,000 seedlings
year 1 = 74%
year 2 3-15l = 38%
Ongoing Efforts

Out-plantings of seedlings inoculated with native ectomycorrhizal fungi

- 500 Whitebark- Glacier 2009
- 1000 Whitebark- Waterton 2010
- 1000– Whitebark Waterton 2011
- 1000– Limber Pine Waterton 2012
- 1000—Whitebark– Waterton 2012
- 1000—Whitebark—Waterton 2013

Can it help increase the survival of expensive WBP seedlings?
Need carefully planned studies for inoculation:

- usual seedling production to 1.5 yrs
- stop fertilization 1-2 months
- inoculate 2-3 months before planting
- monitor, monitor, monitor

Have gun, will travel!

Further information online
2 papers coming soon
We thank Parks Canada for funding this research and the Whitebark Pine Ecosystem Foundation for additional resources, Joyce Lapp, Tara Carolin, and the Glacier Park Revegetation Crew and the volunteers.

And our field assistants
• Ed Barge
• Rosemary Keating
• John Mason
• Don Bachman

Regeneration of whitebark pine is tedious business...thanks to all who have dedicated themselves to saving this important tree species
Erin Longergan is currently searching for employment in the USDA Forest Service, Parks Service, in restoration, or private industry

Particularly in the PNW area (OR, WA)  elonergan3@gmail.com