

Ectomycorrhizal Species Beneficial for Plant Establishment on Abandoned Mine Lands

Shiv Hiremath, Kirsten Lehtoma, and
Jenise M. Bauman

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Ectomycorrhizal (ECM) Fungi

ECM Allows Host Plant:

- Greater access to water and nutrients
- Tolerance of heavy metals
- Protection from disease
- Provides networks to established trees
- Mutualistic to both partners

Marx 1972; Cairney and Chambers 1997; Brundrett 2002; van der Heijden et al. 2003; Walker et al. 2004; Nara 2005



ECM in Plant Establishment:



- Native ECM not adapted to disturbances
- To compensate for this loss, ECM inoculation is commonly used
- ECM fungi may differ in attributes and mutualisms may vary per site



Pisolithus tinctorius

American Chestnut in Restoration



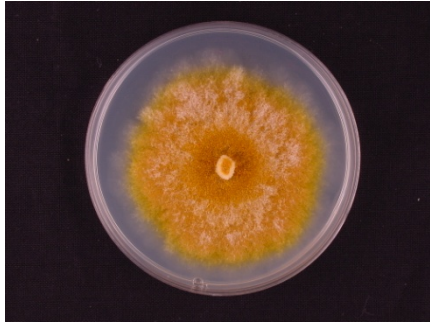
The American Chestnut: Historical Perspective

- Valued in the U.S for its economical and ecological importance
- Occupied 25 percent of the eastern forest
- Encompassed 200 million acres of forest land



<http://www.frw.ca/images/americanchestnut3.gif>

Causal Agent: *Cryphonectria parasitica*

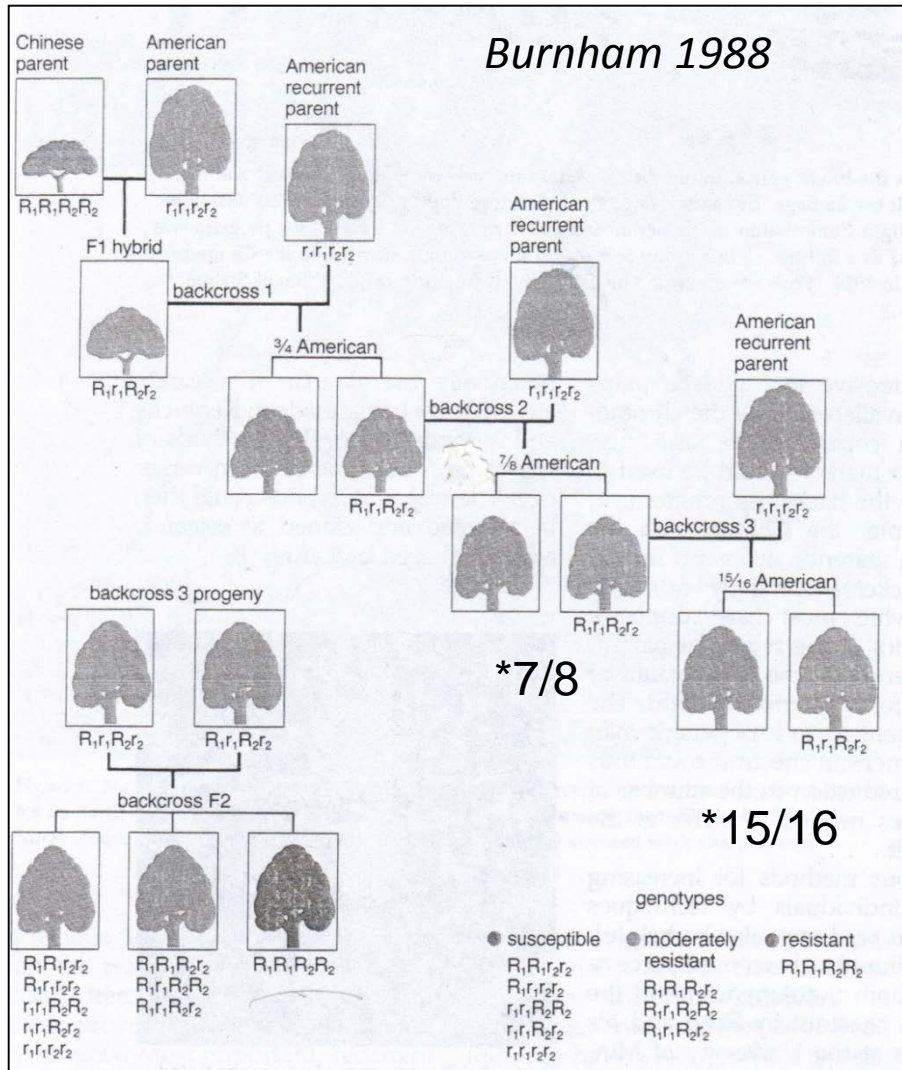


<http://www.appalachianwoods.com/appalachianwoods/images/parkway.jpg>



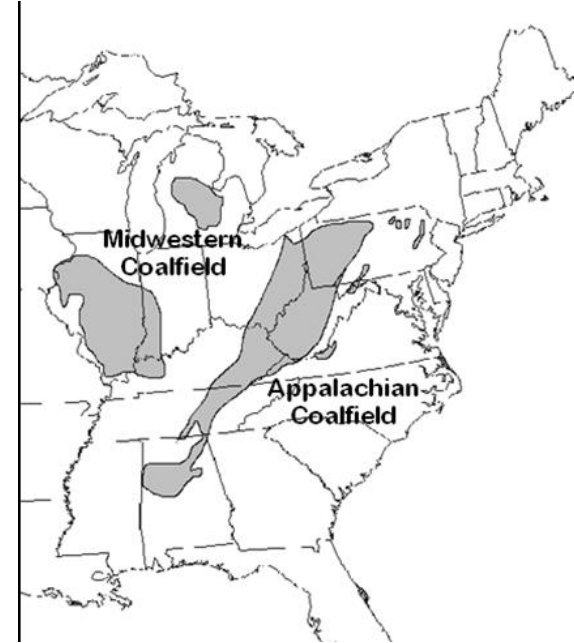
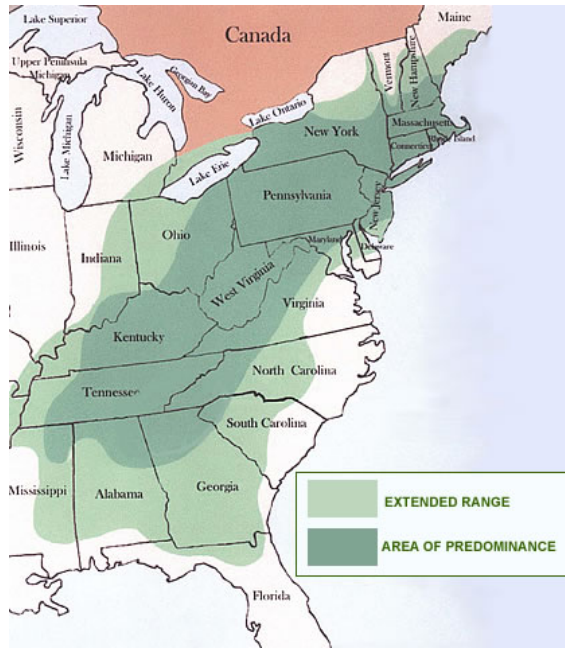
Identified in 1904 and by the 1950s, the American chestnut was devastated throughout native range

Backcrossed Breeding Program



15/16th backcrossed chestnut is ~94% American chestnut genes and only 6% Chinese genes

Chestnut in Coal Mine Reclamation:



- Fast growth rate and tolerance to adverse soil conditions
- Has attributes of both pioneer and late-successional species
- Native range of chestnut compliments active coal mine sites

Mine Sites in Central Ohio:



1. Perry Forest: Abandoned Coal Mine Land (AML) 1950s



2. Tri Valley Wildlife Area: Coal Mine Reclaimed under Surface Mining Control Reclamation Act (SMCRA) 1980s

Abandoned Mine Lands

- Soil Compaction
- No organic matter
- Decreased water holding capacity
- Poor drainage
- Metal Toxicity
- Extreme pH
- Disturbed microbial community



Previous Studies:



- Field inoculation did not work on seeds
- Existing pines provided ECM inoculum for establishing chestnuts on these mine sites



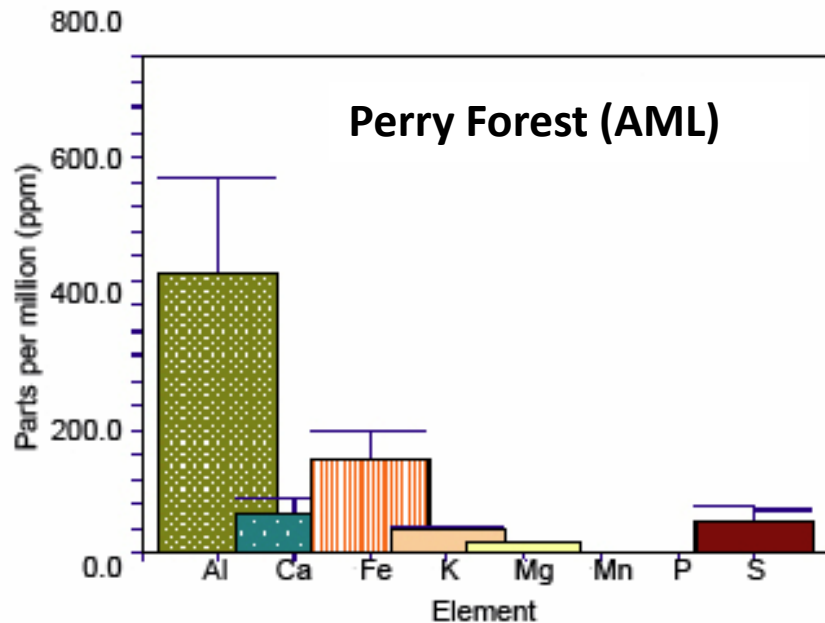
= 1 mm

Current Study: Perry Forest (AML)



The Perry Forest site mined in the 1950s - abandoned gob piles.

No topsoil, very little competition and very little organic matter.



High in aluminum

Low in macro and micro nutrients

High soil temperature

Very low pH - 3.6

Methods:



Photo credit – B. Herendeen



Planted Spring of 2005:

100 Chestnuts inoculated with the ECM *Pisolithus tinctorius* as bare root seedlings

600 Virginia Pines were inter-planted and consisted of the following ECM inoculations:

- *Amanita rubscens*
- *Laccaria laccata*
- *Pisolithus tinctorius*

Seedling volume cm^3 (height x basal diameter²) measured in 2012

ECM Survey Methods:

30 pines and 30 chestnuts were selected for non-destructive sampling



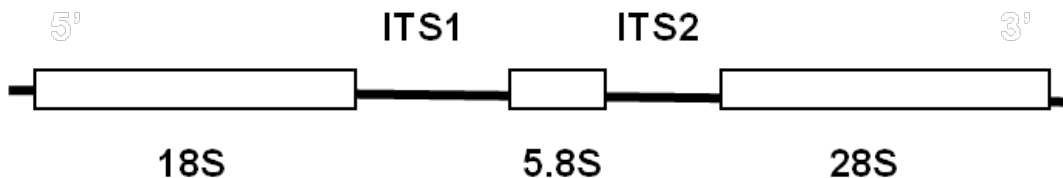
Field Sample



Quantify



Describe and Voucher



DNA sequencing of fungal genes for ECM identity to genera

ECM Fungi Sampled:

Total Species	Total Percent	ECM on Pine	ECM on Chestnut
<i>Russula</i>	30%	33%	24%
<i>Oidiiodendron</i>	26%	15%	45%
<i>Cantharellales</i>	11%	0%	29%
<i>Helotiales</i>	9%	14%	0%
<i>Amanita</i>	6%	10%	0%
<i>Scleroderma</i>	5%	8%	1%
<i>Suillus</i>	4%	7%	0%
<i>Hygrocybe</i>	3%	5%	0%
<i>Unknown sp.</i>	2%	4%	0%
<i>Rhizopogon</i>	2%	3%	0%
<i>Laccaria</i>	1%	1%	0%

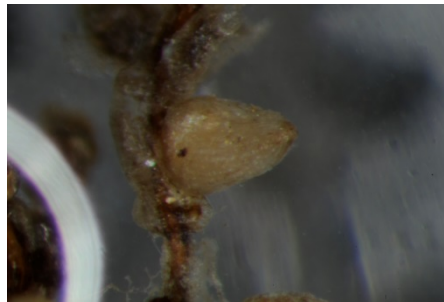
- ECM species *Amanita* and *Laccaria* were part of original inoculum but did not move to chestnut.
- Both pine and chestnut did not maintain *Pisolithus tinctorius* and were replaced by other fungi.

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Russula sp.



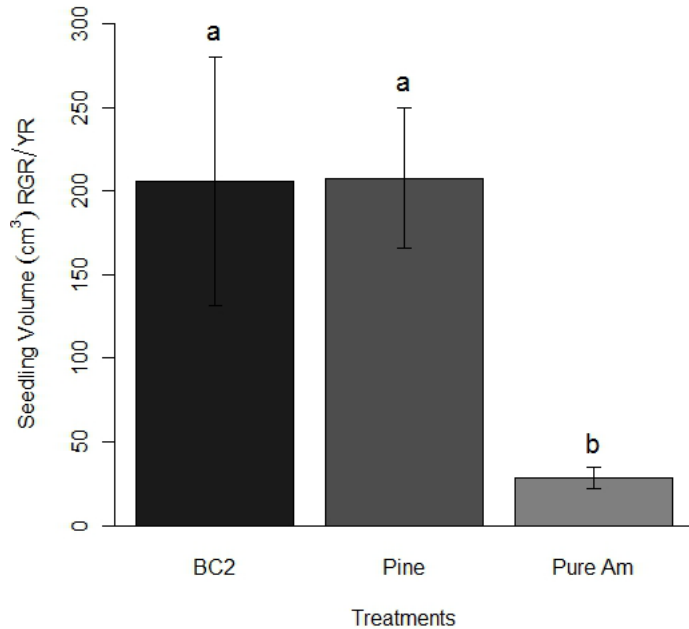
Oidiodendron sp.



Scleroderma sp.

Three most common ECM species shared by both pine and chestnut

Tree Growth



When growth was compared, there were no differences between 7/8 chestnut hybrids and Virginia pine.

Pure American chestnuts were significantly smaller

This difference is attributed to chestnut blight





Planted 2005, photographed October 2012



Planted 2005, photographed October 2012



Planted 2005, photographed October 2012

Perry Forest (AML) Summary



Inoculum replaced after 8 field seasons – no movement to chestnut

8 year old sites decline due to chestnut blight significantly decrease chestnut biomass

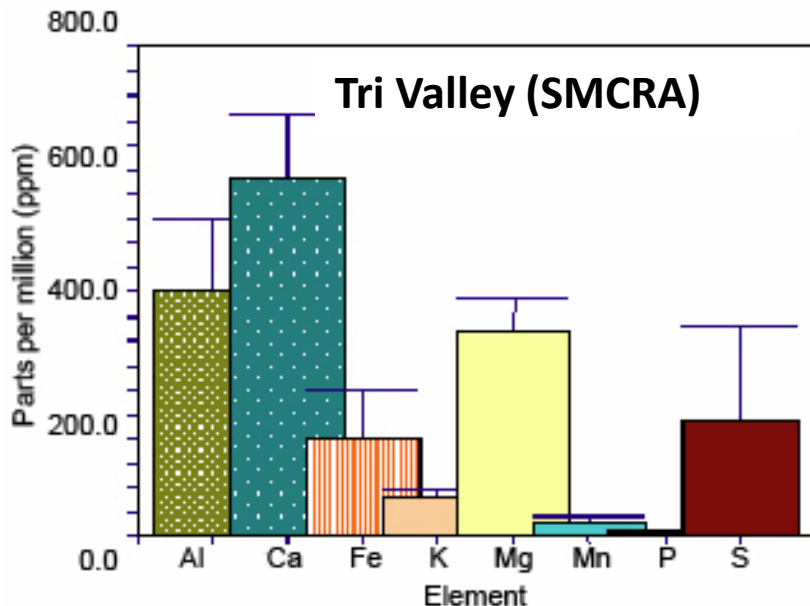
Blight resistant seed lines are surviving well in the field, growing comparable to pine

SMCRA Reclaimed: Tri Valley



Tri Valley site mined in 1978 and reclaimed under SMCRA

Soil is compacted and seeded with aggressive herbaceous species



High in calcium

Low in macro and micro nutrients

pH – 5.5

Methods:

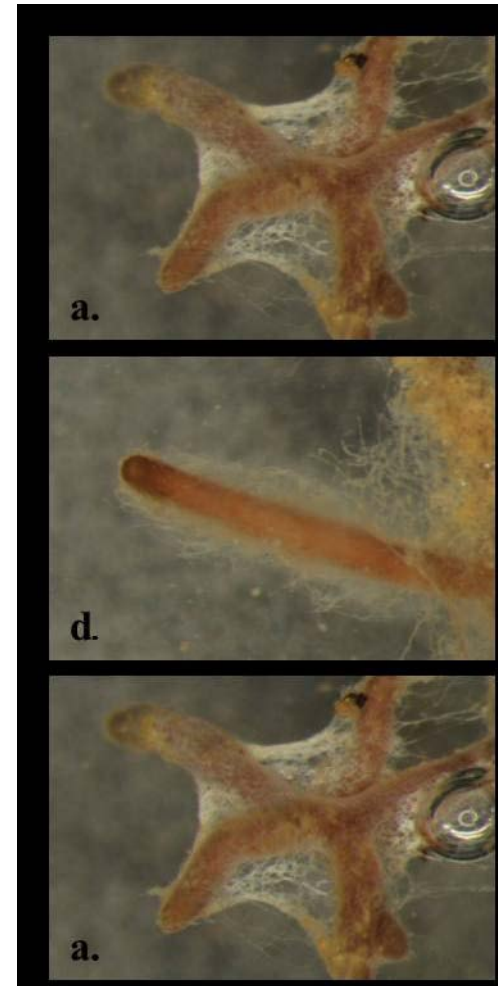


Using the Forestry Reclamation Approach, plots were ripped using 1 m steel shank

- One year-old seedlings used (300):
 - Pure American
 - Hybrid BC3 (15/16)
 - Hybrid BC2 (7/8)
- Inoculated with ECM *Pisolithus tinctorius*
- Sampled 60 chestnuts in 2008 and another 60 in 2012

ECM Species – 2 Years

ECM species	% Total	Accession
<i>Hebeloma</i> sp.1	30.8	GU246983
<i>Hebeloma</i> sp. 2	20.3	GU246984
<i>Cortinarius</i> sp. 1	15.7	GU246986
<i>Scleroderma</i> sp. 1	8.5	GU246989
<i>Thelephora</i> sp.	7.2	GU246993
Unknown ECM 2	3.7	GU246997
<i>Hebeloma</i> sp. 3	3.5	GU246985
<i>Laccaria</i> sp.	3.5	GU246994
Unknown ECM 1	1.5	GU246996
<i>Scleroderma</i> sp. 2	1.3	GU246990
<i>Cortinarius</i> sp. 2	1.2	GU246987
<i>Pisolithus</i> sp.	1.2	GU246988
<i>Tomentella</i> sp.	1.0	GU246992
<i>Cenococcum</i> sp.	0.7	GU246995
Thelephoraceae	0	GU553376



— = 1 mm

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<i>Cenococcum</i> sp.	0.7	GU246995
Thelephoraceae	0	GU553376

Pisolithus inoculum is not maintained

Hebeloma and *Cortinarius* unintentionally introduced

Root colonization of *Hebeloma* and *Cortinarius* are better competitors and their abundance facilitated by site conditions

ECM Species – 5 Years

ECM species	% Total	Accession
<i>Hebeloma</i> sp.1	30.8	GU246983
<i>Hebeloma</i> sp. 2	20.3	GU246984
<i>Cortinarius</i> sp. 1	15.7	GU246986
<i>Scleroderma</i> sp. 1	8.5	GU246989
<i>Thelephora</i> sp.	7.2	GU246993
Unknown ECM 2	3.7	GU246997
<i>Hebeloma</i> sp. 3	3.5	GU246985
<i>Laccaria</i> sp.	3.5	GU246994
Unknown ECM 1	1.5	GU246996
<i>Scleroderma</i> sp. 2	1.3	GU246990
<i>Cortinarius</i> sp. 2	1.2	GU246987
<i>Pisolithus</i> sp.	1.2	GU246988
<i>Tomentella</i> sp.	1.0	GU246992
<i>Cenococcum</i> sp.	0.7	GU246995
Thelephoraceae	0	GU553376

Hebeloma and *Pisolithus* inoculum has disappeared

Cortinarius is becoming the most abundant fungus

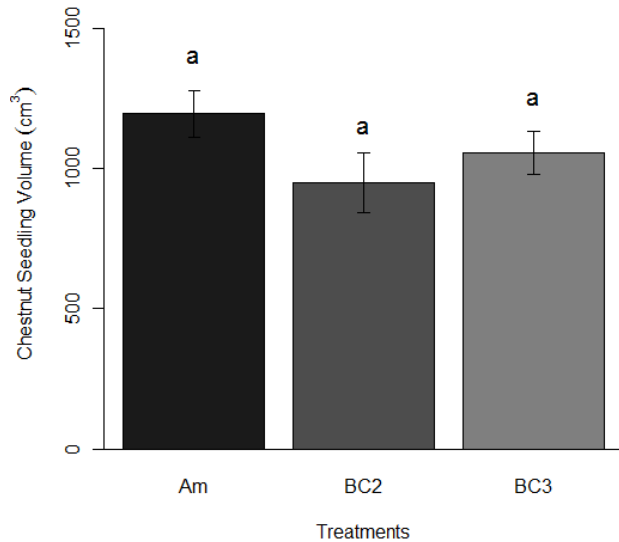
There is a significant correlation between *Cortinarius* and chestnut growth ($P= 0.001$)



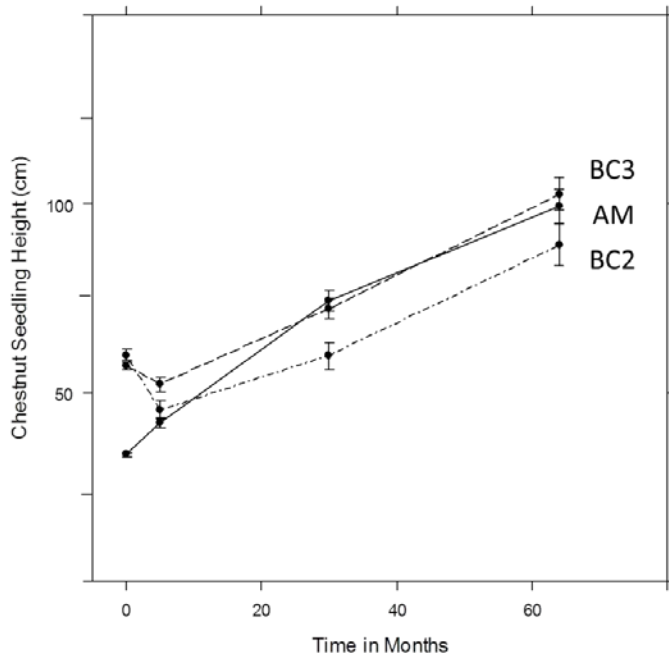


Chestnut Growth

No differences in seedling volume among seed types after five field seasons



Similarities in height ranging between 102.6 cm – 89.1 cm after five years



Chestnut Blight: Canker Data



Pure American Chestnut



Backcrossed Chestnut (BC₃F₁)

SMCRA Site – Tri Valley Summary

ECM inoculum *Pisolithus* quickly replaced by better ECM competitors

Of these, *Hebeloma* is disappearing and being replaced by *Cortinarius*

ECM root colonization and Chestnut growth were increased in mechanically treated plots

Field sites where plants are 5 years old, blight is appearing on pure seedlings

Current Studies: Tracking spread of blight and what is replacing dying pure American chestnuts – how will existing ECM facilitate this?



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