American chestnut (*Castanea dentata* (Marsh.) Borkh.) Response to *Phytophthora cinnamomi* Rands Inoculation Under Varying Substrate pH and Potential Methods to Enhance Resistance Upon Outplanting

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Abstract
The pending reintroduction of American chestnut poses numerous challenges due to limitations in the knowledge base regarding the ecology and silvics of this species. With mine reclamation projects being targeted as potential sites for deployment of blight-resistant American chestnut hybrids, specific knowledge is required to ensure outplanting success. One area of particular concern is how American chestnut hybrid seedlings will respond to infection by the oomycete pathogen, *Phytophthora cinnamomi*, which thrives in soil conditions frequently encountered on mine reclamation sites. The overall purpose of this study is to determine differences between lineages of American chestnut backcross hybrids in physiological and morphological responses to *P. cinnamomi* infestation, as well as the potential for both the inoculation of the ectomycorrhizal fungus, *Pisolithus tinctorius* and the application of phosphite fungicides to mitigate the deleterious effects of *P. cinnamomi*, thus enhancing chestnut resistance upon outplanting. The effects of *P. cinnamomi* will be assessed through morphological (height, root collar diameter, biomass allocation, and root parameters) and physiological (leaf water potential, stomatal conductance, transpiration, and leaf nutrient content) measurements to elucidate significant differences between treatments. We hypothesize that genetic variation between families will yield different levels of resistance to *P. cinnamomi*. We also expect that both *P. tinctorius* inoculation and phosphite treatments will mitigate some of the effects of *P. cinnamomi* infestation, and this effect will vary with soil pH.

Introduction
Prior to the introduction of the chestnut blight, American chestnut (*Castanea dentata* (Marsh.) Borkh.) was a dominant tree species throughout the eastern deciduous forests of the United States, comprising up to 40-50% of the forest canopy (Braun 1950), with a range representing over 800,000 km^2 (Little 1977). American chestnut was a highly valued species in the late 19th century, both economically and ecologically, providing a source of timber, as well as tannic acid and nuts (Youngs 2000). Currently, little modern information is available regarding the ecology and silvics of this species. Restoration efforts may heavily target afforestation of mine reclamation sites due to the abundance of these sites available within the original range of American chestnut (USDA Office of Surface Mining 2006). Mine reclamation sites are often extremely harsh, with poorly drained, compacted soils in which *Phytophthora cinnamomi* may thrive, as well as extreme (high or low) pH conditions, in which many tree species struggle to survive. Ectomycorrhizal associations could potentially mitigate the harmful effects of *P. cinnamomi* infestation, while simultaneously affording more favorable nutrient and moisture relations to American chestnut. The application of phosphites has been shown to mitigate the deleterious effects of *P. cinnamomi* infection (Gentile et al. 2010), and may also prove to be effective in increasing the probability of success of American chestnut outplantings on reclaimed mine sites.

Experimental Design

Experiment 1: Genetic variation in ecophysiological response to P. cinnamomi in hybrid American chestnut seedlings

- 6 × 2 completely randomized factorial design
- 6 Families
- 2 *P. cinnamomi* treatments

Experiment 2: Effects of P. cinnamomi on growth, nutrient uptake, and water relations of American chestnut seedlings under varying pH and ectomycorrhizal treatment

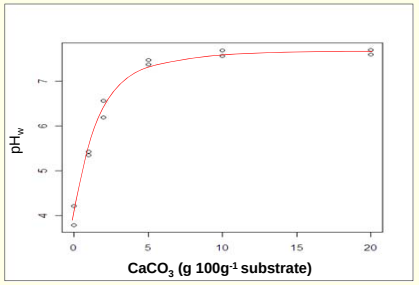
- 3 × 2 × 2 completely randomized factorial design
- 3 pH treatments (4.0, 5.5, 7.0)
- 2 *P. cinnamomi* treatments (inoculated, control)
- 2 *P. tinctorius* treatments (inoculated, control)

Experiment 3: Ecophysiological response of American chestnut seedlings to P. cinnamomi as influenced by varying substrate pH and foliar application of phosphite

- 3 × 2 × 2 completely randomized factorial design
- 3 pH treatments (4.0, 5.5, 7.0)
- 2 *P. cinnamomi* treatments (inoculated, control)
- 2 Phosphite treatments (treated, control)

Materials and Methods

Determination of Substrate pH Amendment



- Three pH levels
- 4.0- 5.5- 7.0
- $CaCO_3$ to raise pH

Figure 1. Standard curve for determination of $CaCO_3$ addition.

Phytophthora cinnamomi Inoculation

- Cultures of *P. cinnamomi* will be obtained from the American Type Culture Collection
- Inoculation treatments carried out following the methodology of Burns and Benson (2000) as adapted by Jeffers et al. (2009)
- Seedlings inoculated early July 2011
- Pots will be flooded to encourage disease development 3 weeks post-inoculation

Pisolithus tinctorius Inoculation

- Basidiospores of *Pisolithus tinctorius* will be applied to the rooting zone at the time of planting
- Rate of approximately 2.75 × 10^7 spores plant^{-1}.
- Seedlings will then be well watered to encourage mycelia growth
- Basidiospores will be provided by Mycorrhizal Applications Inc.

Foliar Application of Phosphite

- Agri-Fos® Systemic Fungicide
- Applied as a foliar spray, at 2.6 mL of phosphite per liter of water, in accordance with the label instructions for *Phytophthora* treatment of tree nuts
- Initial spraying mid-May 2011
- Subsequent application just prior to *P. cinnamomi* inoculation

Expected Outcomes
- Decreased physiological function in response to *P. cinnamomi* inoculation, with regard to all physiological parameters measured
- Foliar nutrient concentrations are expected to be lower in those seedlings experiencing the inoculation treatment
- Seedlings inoculated with *P. cinnamomi* should exhibit retarded height and diameter growth, as well as decreased root volumes
- Families may exhibit varying levels of resistance (i.e., proportion of necrotic root tissue) and survivorship to *Phytophthora* infection, though the extent of this remains unclear
- Survivorship as assessed through mixed effects logistic regression will provide insight as to expected afforestation success in the presence of *P. cinnamomi*
- Both ectomycorrhizal inoculation and phosphite application should mitigate some of the negative effects of *P. cinnamomi* infection. We expect that this effect will vary with different levels of soil pH

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