

Mapping of beech bark disease resistance in Michigan and associated fungal endophyte antagonism of *Neonectria faginata*.

Rachel E. Griesmer, Andrew J. Storer and Dana L. Richter

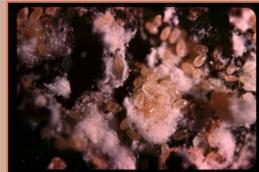
Michigan Technological University

Introduction

Beech bark disease (BBD)

A widespread disease of American beech (*Fagus grandifolia*) originating in Nova Scotia in the late 19th century, discovered in 2001 in Ludington, MI

- A non-native scale insect (*Cryptococcus fagisuga*)
- The invasive beech scale is disseminated predominantly by wind and is the primary factor in BBD spread
- The scale originated in southern Europe (Bulgaria) on *Fagus sylvatica orientalis*
- Two canker-causing fungal species: *Neonectria faginata* (syn=*Nectria coccinea* var. *faginata*), non-native, and highly virulent. The primary species involved in BBD.
- *N. ditissima* (syn=*Nectria galligena*), native.
- Fungus enters through wounds created by *C. fagisuga*, creating a network of coalesced cankers leading to tree mortality



The disease is comprised of three distinct stages:

- Advancing front: Pioneer populations of *C. fagisuga*
- Killing front: Most intense beech mortality; dense populations of *Neonectria* and *C. fagisuga*
- Aftermath zone: Supports lingering populations of beech scale and *Neonectria*
- Resistance and finding mechanisms of resistance is key in overcoming beech bark disease since *C. fagisuga* dispersal cannot be realistically controlled

Endophytes

- Fungi (or bacteria) that live asymptotically within plant tissues as symbionts, latent pathogens or latent saprophytes.
- Recent research has shown them to be antagonistic to insects or pathogens
- Endophyte communities are not well studied in forest trees and may present a potential tool for management

Hypotheses

- Trees resistant to beech bark disease will have different endophyte assemblages than susceptible trees and contribute to an additional resistance phenotype.
- Endophytes in resistant beech will be antagonistic toward *Neonectria*.

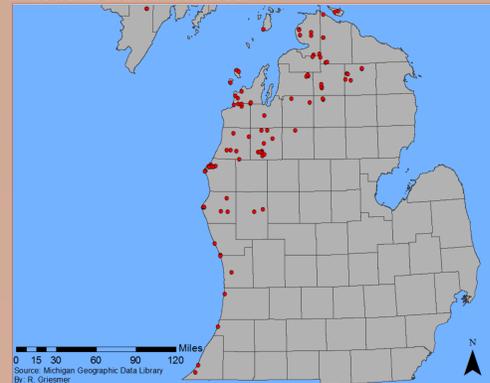
Objectives

- Map the extent of beech bark disease and its spread in Michigan using previously established protocols from BBDMIAS.
- Identify resistant beech in Michigan, focusing on those areas with heavy beech scale (*C. fagisuga*).
- Establish the relative diversity of endophytes in beech cambium tissue in both resistant and susceptible specimens.
- Isolate and identify *N. faginata* in pure culture.
- Grow individually isolated beech endophytes with *Neonectria* to test for antagonism.

Methods

Beech Bark Disease Progression Assessment

- Beech Bark Disease Monitoring and Impact Assessment (BBDMIAS) measurements
- 101 plots (pictured) throughout the lower peninsula of Michigan
- 30 total trees (5x6 or 3x10 arrangement)
- Survey for resistant trees in high density scale areas



Canopy Assessment

Currently being conducted for each plot at each sample point (beech tree). Data provides insight about an individual tree's health:

- Foliage transparency
- Crown density
- Uncompacted live crown ratio (UCLR)
- Crown light exposure
- Crown class
- Percent dieback
- Vigor rating
- Damages
- Percent cover of *C. fagisuga*



Endophyte Isolation

- Extracted from beech cambium using a 1 inch arch punch
- Cambium plugs were further cut into 2-3 mm² cubes
- 3 cubes were plated on one 2% malt agar plate with 3 plates per tree



Endophyte Classification and Antagonism

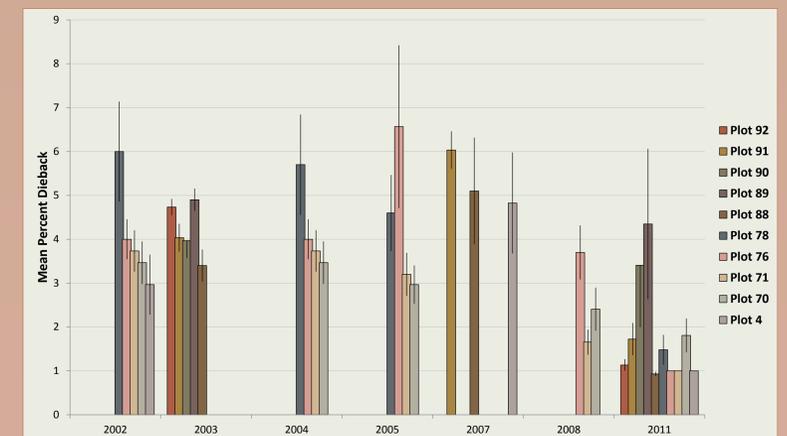
- Fungal isolates were catalogued according to culture morphology
- Microscopic characters were also observed
- 5mm agar plugs from each of the most commonly isolated endophytes and *N. faginata* were placed 2cm apart on a 100mm plate on 2% malt agar with 3 replicates per isolate
- Control plates of each endophyte and *N. faginata* were prepared individually using a 5mm agar plug placed in a center of a petri plate
- Radial growth measurements are ongoing at 1, 2 and 3 weeks

Preliminary Results

Forest Health

- Percent dieback is an indicator of tree decline
- Current year's data compared with previously collected data

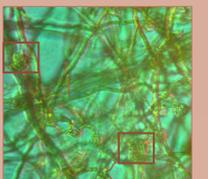
Mean Percent Dieback 2002-2011



Within a small subset of plots, percent dieback (means \pm SE) hasn't significantly changed from 2002 to 2011, though data within plots are highly variable. Data collection from plots where BBD has been established is ongoing.

Fungal Endophyte Identification

- 35 morphotypes were established based on cultural characters after approximately 25 days grown at room temperature
- 2 were given tentative IDs: Photo shows probable *Phialophora* spp. with conidiophores
- The remaining fungi were sterile forms, therefore funding has been requested to sequence the fungal isolates to determine identities based on ITS sequence



Neonectria Antagonism

- After 1 week, one isolate showed evidence of antibiotic activity as a distinct interaction line between colonies (pictured)
- Antagonism plates will continue to be monitored for several weeks



Acknowledgments

Funding: USDA Forest Service, Forest Health Monitoring: Evaluation Monitoring program.

Cooperators: Manfred Mielke (USDA Forest Service), Roger Mech (Michigan Department of Natural Resources), Robert Heyd (Michigan Department of Natural Resources), Paul Bloese (Michigan State University), Richard Mergener (Michigan Department of Natural Resources).

Images: http://treediseases.cfans.umn.edu/labphotos/Beech_Bark_Disease.htm, <http://www.ipmimages.org/images/768x512/1301056.jpg> and Kristina Denison.