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

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**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 cocinea 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 asymptomatically 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 plated 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.

**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.

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Images: [Beach Bark Disease](http://treediseases.cfans.umn.edu/labphotos/Beech_Bark_Disease.htm), [Beach Bark Disease](http://www.ipmimages.org/images/768x512/1301056.jpg) and Kristina Denison.

**Mean Percent Dieback 2002-2011**
Within a small subset of plots, percent dieback (means ± 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.

**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.