# Preliminary assessment of the fungi associated with butternut (*Juglans cinerea*), Japanese walnut (*J. ailantifolia*) and their hybrid (*J. × bixbyi*)

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## Abstract

Endophytic and epiphytic fungi associated with woody plants have been shown to have an effect on interactions between host species and abiotic or biotic stressors. The aim of this study was to isolate and identify a sample of the endophytic and epiphytic fungal populations associated with *J. cinerea*, *J. ailantifolia* and a hybrid between the two species *J. × bixbyi* planted on a common site near Rosemount, MN. Fungi were isolated from a single, medium-sized branch of each species on two types of growth media. Branch segments from each species were either rinsed briefly with sterile DI water before samples were collected or samples were removed and surface sterilized before plating. Plates were monitored for the emergence of fungi until samples were completely overgrown. Isolates were taken from unique colonies and identification of taxa was conducted by sorting isolates into groups based on morphological characteristics and subsequent sequencing of the Internal Transcribed Spacer (ITS) region of the ribosomal DNA. Preliminary results indicate that greater than 20 taxa were represented by 173 isolates. The most common fungi isolated were from the genera *Phoma*, *Epicoccum*, *Sordaria* and *Paraconiothyrium*. Many isolates failed to sporulate in culture and were identified based on ITS sequence only. Data indicate that these tree species and their hybrid, when growing on the same site, harbor similar assemblages of associated fungi. We are currently collecting and identifying fungi associated with these three species on other sites for comparison.

## Additional Materials and Methods

- Samples excised from branches using a sterile 5mm cork borer in August 2011
- Branch segments rinsed with sterile deionized water and samples directly plated (EPI) or surface sterilized in 0.55% sodium hypochlorite solution (ENDO)
- Samples plated on either potato dextrose agar amended with 500µl lactic acid (APDA) or malt extract agar amended with 0.1g streptomycin sulfate (MEA+)
- All isolates maintained for future analysis on 1.5% MEA
- ITS sequence used for ID of a subsample from groups having same morphological features and for all isolates failing to produce features easily recognized. Best BLAST used for genera assignment

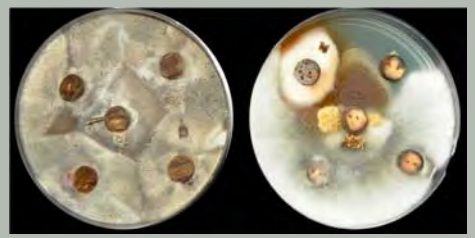

Fig 1. Differences in patterns of emergence and presence of common isolates from excised stem samples

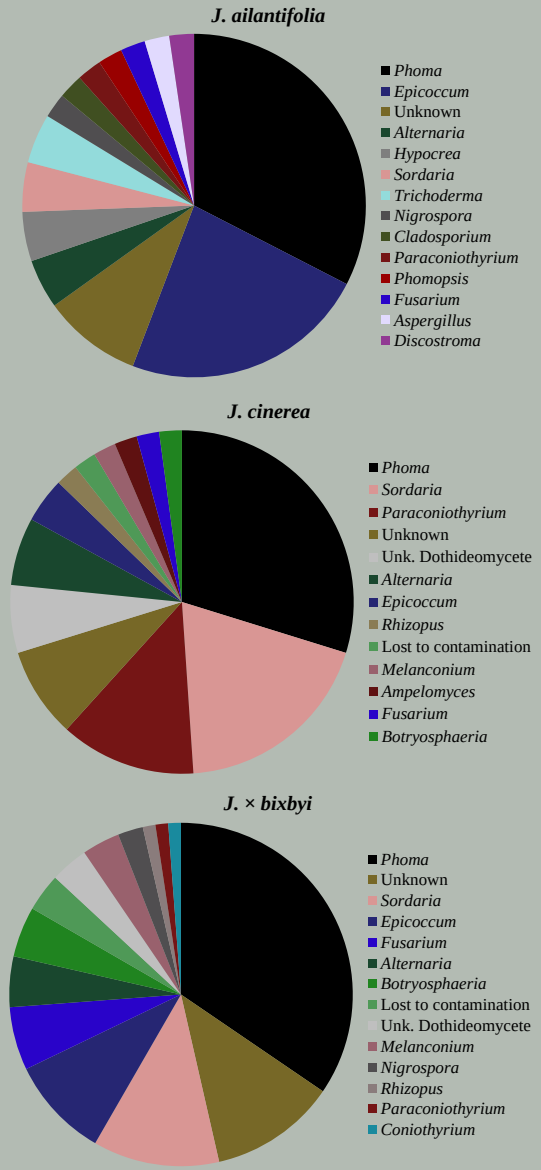


Fig 2. Isolates collected from each tree species and their relative isolation frequencies on that particular host. Tissue type and surface treatment combined. Column lists in order of abundance. "Unknown" – no sequence data to date.

## Results

- 173 isolates collected from *J. cinerea*, *J. ailantifolia* and *J. × bixbyi* bud and stem tissues using two surface treatments and two types of growth media
- *J. cinerea*, *J. ailantifolia* and *J. × bixbyi* colonized by similar assemblages of filamentous fungi in Central Minnesota
- *Phoma spp.* is a common colonizer of the three species studied
- Many genera contain species previously reported to be antagonistic to other fungi collected including *Phoma*, *Epicoccum*, *Paraconiothyrium*, *Ampelomyces*, and *Fusarium*
- *Melanconium oblongum*, the imperfect state of a common weak parasite (*Melanconis juglandis*) on *Juglans spp.* isolated as an endophyte from healthy tissue of both *J. cinerea* and *J. × bixbyi*.

## Conclusions and Future Directions

- Similar assemblages found in all three tree species at this site
- Possibly incomplete census of culturable fungi
- Sampling season, growth rates of fungi, and non-systemic distributions may have influenced results (1)
- Further research will utilize collections from other sites
- An in-vitro fungal antagonism assay will be utilized to characterize interactions between these fungi and the virulent plant pathogen *Ophiognomonia clavigignenti-juglandacearum*, formerly known as *Sirococcus clavigignenti-juglandacearum*.

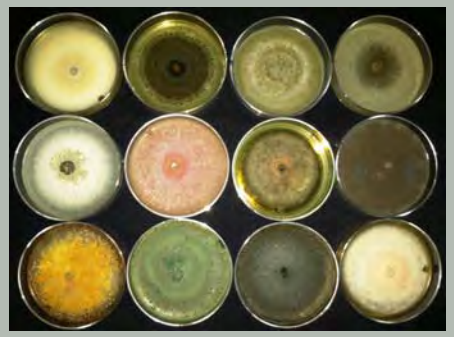

Fig 3. Sample of fungal isolates collected showing morphological differences

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## References

1 - Moricca, S., and Ragazzi, A. 2008. Fungal endophytes in Mediterranean oak forests: A lesson from *Discula quercina*. *Phytopathology 98:380-386.*

**For More Information Visit: http://www.agriculture.purdue.edu/fnr/HTIRC/woeste.html**