

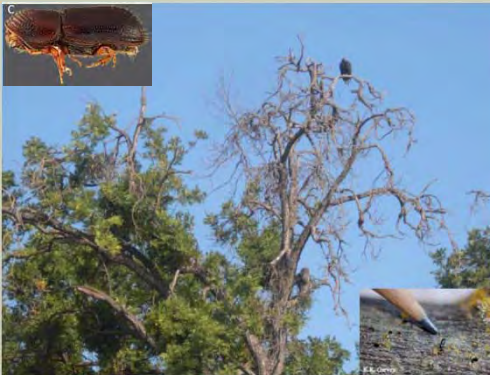
Genetic variation in *Geosmithia moribida*, the fungal associate of thousand cankers disease of walnut

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Abstract

Thousand cankers disease (TCD) has emerged as a major threat to members of Juglandaceae. TCD is caused by a bark beetle/fungal complex comprised of the walnut twig beetle (*Pityophthorus juglandis*) and a newly discovered fungal plant pathogen *Geosmithia moribida*. The goal of this project is to evaluate the spatial genetic structure of *Geosmithia moribida* to establish correlations between genetic diversity and movement out of its native range. Microsatellite alleles were detected using a three-primer method in which the forward primer included a tail on the 5' end that was complementary to an M13 primer that was labeled with a HEX fluorescent probe. Seventeen loci were identified, but 9 were selected for analysis based on consistent amplification and polymorphism. Significant polymorphism was observed, with loci producing as many as 15 different alleles. Preliminary results suggest that genetic variation in *G. moribida* is not largely related to geographic range. Only 2% of genetic variation can be attributed to isolate state of origin, indicating that human activities have played a role in the spread of this fungal/beetle complex. Additionally, host species only accounted for 1% of genetic variation; *G. moribida* is not likely evolving different "races" on different hosts. The large amount of genetic variation indicates a sexual stage is part of the life cycle of this fungus. Sexual recombination of genetic material will mean *G. moribida* will be able to quickly adapt to changes in host and environment outside of its native range.

Level: Undergraduate Division: Research



Above: *Juglans hindsii* affected with Thousand Canker Disease
Corners: *Pityophthorus juglandis* (Tisserat et al., 2009)

Introduction

- Thousand cankers disease (TCD) has emerged as a major threat to members of Juglandaceae. TCD is caused by a bark beetle/fungal complex comprised of the walnut twig beetle (*Pityophthorus juglandis*) and a newly discovered fungal plant pathogen *Geosmithia moribida*.
- TCD occurs in nine western states, and has been recently confirmed in Tennessee, Virginia, and Pennsylvania. Black walnut (*Juglans nigra*), a valuable hardwood species native to the eastern United States, is highly susceptible to TCD.

Goals

Evaluate the spatial genetic structure of *Geosmithia moribida* to establish correlations between genetic diversity and movement out of its native range through:

- Identifying polymorphic microsatellite regions from fungal isolates
- Designing primers to amplify polymorphic regions of fungal DNA using PCR
- Using identified alleles to establish relationships among fungal isolates.



Figure 1. Representation of the range of *Geosmithia moribida* and the locations of recently confirmed cases of thousand cankers disease (shown in red) and the native range of black walnut (*Juglans nigra*) in green.

Methods and Materials

Primers Developed

- DNA from fungal isolates was enriched for microsatellites.
- Polymorphic microsatellites were identified by the use of programs: Tablet and Mega 5. The sequence data was then used to design primers that would amplify polymorphic regions in the DNA



PCR used to amplify desired regions

- Used designed primers in a three primer method that used forward, reverse, and fluorescently tagged M13 simultaneously.
- This method produced a fluorescently tagged product.
- Product was submitted to Purdue Agricultural Genomics center for genotyping.



Genotype results analyzed

- The genotyped results were analyzed using GENEMAPPER to identify allele sizes.
- GENALEX was used to analyze genetic diversity among and within populations.

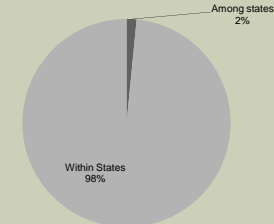


Figure 2. Representation of the 6 states (Oregon, California, New Mexico, Arizona, Colorado, and Tennessee) from where isolates were obtained.

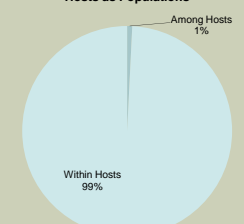
Results

- We genotyped 108 fungal isolates at 9 different loci. On average, each locus produced 8 different alleles. The most polymorphic loci were 194 and 6823, producing 14 and 15 different alleles respectively. A total of 78 different alleles were identified during the course of this study.
- An analysis of genetic variation where populations were defined as states (Arizona, California, Colorado, New Mexico, Oregon, and Tennessee) revealed the majority of genetic variation was contained within each population. Only 2% of molecular variance was among populations.
- When populations were defined as host species (*Juglans californica*, *J. hindsii*, *J. major*, *J. nigra*, *J. regia*), only 1% of molecular variance occurred among populations.

Percentages of Molecular Variance Using States as Populations



Percentages of Molecular Variance Using Hosts as Populations



Discussion

- Collaborators sequenced three genes, internal transcribed spacer region, beta tubulin, and methionine aminopeptidase (MAP). Variation in these genes was used to characterize 48 different haplotypes in *Geosmithia moribida*. An individual tree can host multiple haplotype, and a single haplotype can also be found on multiple host species. Similarly, host species accounts for almost no genetic variation in microsatellite alleles. *G. moribida* does not show signs of evolving to have different "races" on different hosts.
- Because little genetic variability can be attributed to geographic range (state), human activities likely contributed to the spread of the fungus.
- G. moribida* exhibits a high amount of genetic diversity. Though it has not been observed, the fungus likely has a diploid (sexual) stage. Sexual recombination of DNA will allow *G. moribida* to more quickly adapt to changes to host and environment. The recent confirmation of TCD in the eastern United States poses a large threat to *J. nigra* if *G. moribida* is able to adapt to these conditions.

Acknowledgements

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References

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Tisserat, Ned, et al. *Black Walnut Mortality in Colorado Caused by the Walnut Twig Beetle and Thousand Cankers Disease*. Plant Management Network, 11 August 2009