Setting the Mood for *Raffaelea lauricola*

Determination of the Reproductive Capability of the Laurel Wilt Pathogen, *Raffaelea lauricola*

Cassondra Newman[1], Tyler Dreaden[2], Andrew Loyd[1], and Jason Smith[1]; [1]**School of Forest Resources and Conservation**, University of Florida, Gainesville, FL;
[2]USDA Forest Service, Southern Research Station, Forest Health Research and Education Center, Lexington, KY

Introduction

Laurel wilt, a disease caused by the fungus *Raffaelea lauricola,* was first reported in Georgia in 2002. Since its introduction, this disease has caused the death of nearly 500 million trees throughout the southeastern U.S. *Raffaelea lauricola* causes typical wilt symptoms of members in the Lauraceae family, which includes species such as *Persea borbonia* (redbay) and *Persea americana* (avocado) **(Figure 1).** Surveys of *R. lauricola* isolates and its vector, the redbay ambrosia beetle *(Xyleborus glabratus*), from the southeastern U.S. have shown that this pathogen and its vector are propagating clonally, which suggests that this epidemic could have been the result of a single introduction (2). The beetle and fungus were most likely introduced from Taiwan, where there are genetically distinct populations of *R. lauricola*. Although sexual reproduction of the fungus is unknown, two distinct mating types (MAT1-1 and MAT1-2) have been characterized from collections from Asia, which suggests the potential for sexual recombination **(Figure 2).**

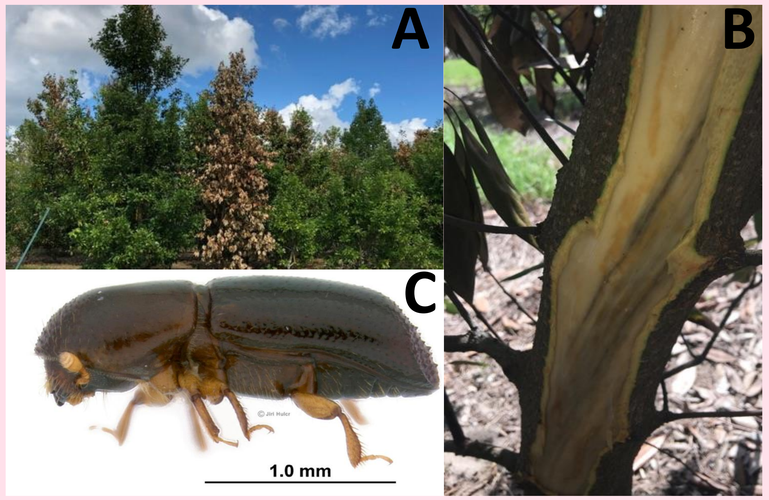

Figure 1. Laurel wilt symptoms and vector. A) wilt symptoms *Persea borbonia* (redbay), B) vascular streaking symptom, and C) *Xyleborus glabratus* (the redbay ambrosia beetle), vector of *R. lauricola (*Photo credit: Jiri Hulcr)

Aim

The major objective of this research was to determine whether artificial pairings of isolates representing opposite mating types would result in sexual recombination and perithecial development.

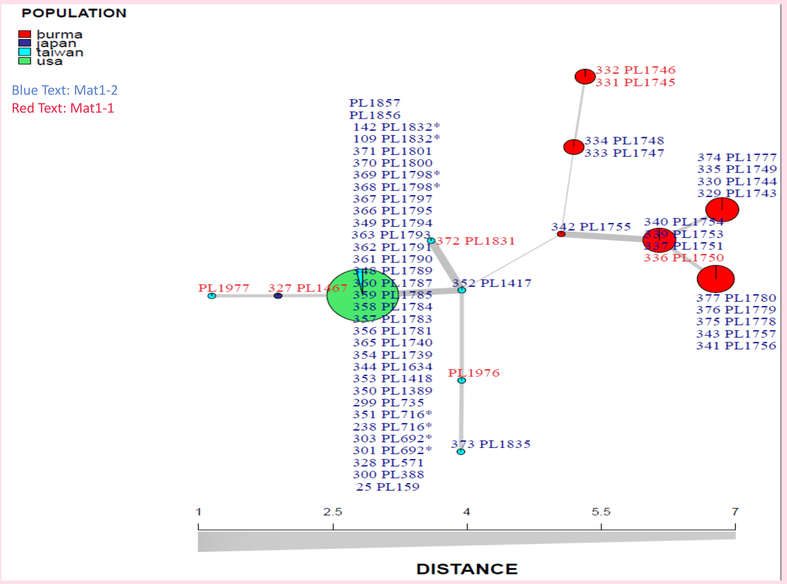

Figure 2. Minimum spanning network tree showing genetic distance of *Raffaelea lauricola* isolates from a global collection. *Raffaelea lauricola* isolate collection from four distinct populations indicated by different colored circles, as well as differentiated mating types indicated by different colored text.

Methodology

Experiment No.	Isolate Type	Orientation	Media Type
1	MAT1 and MAT2	Opposite each other on the plate	water agar + pine wood
2	MAT1 and MAT2	Opposite each other on the plate	¼ malt extract agar + pine wood
3	MAT1 and MAT2	Opposite each other in gallery	artificial beetle gallery in camphor branch
4	MAT1 and MAT2	One mating type established then the opposite mating type suspended on top	¼ malt extract agar + pine wood

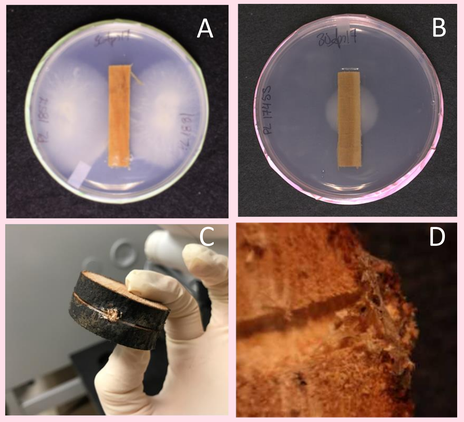

Figure 3. Plating techniques. A) Opposite each other on the plate, B) one mating type followed by conidial suspension of opposite mating type, C) camphor branch dissection for pairing of opposite mating type isolates, and D) a close-up photo of artificial gallery where isolates were inoculated

Results

Of the 120 different pairings of isolates representing opposite mating types, no combination yielded successful mating (i.e. no perethecial development). However, there was no inhibition when isolates grew together, which means they were vegetatively compatible.

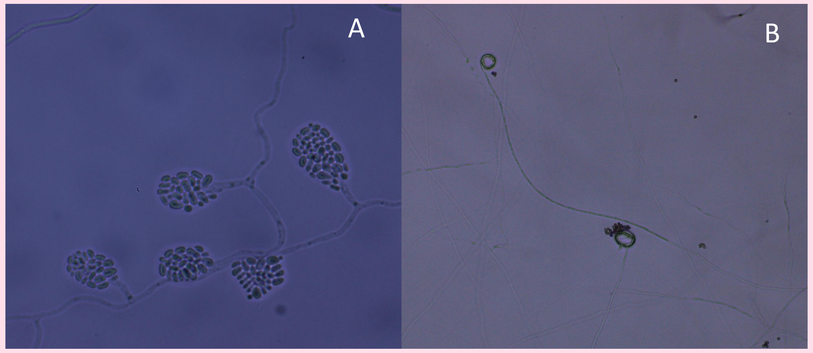

Figure 4. Microscopic analysis of pairing tests. A) large conidia formed in mass along hyphae and B) coiled hyphae that developed in pairing plates after three months

Conclusions

These results suggest that the mating system in *R. lauricola* is complex. The lack of sexual compatibility and development of perithecia (fruiting body) may be contributed to an unknown environmental condition or stimulus that was not present in the mating experiments (1). Likewise, the negative results of these pairing experiments may be due to cryptic gene interactions. It may be extrapolated that sexual reproduction is rare if present at all in this fungus and that it is unlikely to occur in nature. Future work in elucidating the undescribed sexual reproductive stage of *R. lauricola* should be pursued to help determine what genes contribute to its ability to cause disease. It may be useful to sequence all genes that comprise the two mating idiomorphs (only MAT1-2-1 and MAT1-1-3 were used previously to determine the presence or absence of each mating type). More tests in artificial galleries in optimal conditions may be useful in further testing the compatibility of *R. lauricola* isolates.

References

1. Van den Berg, Marco A, and Karunakaran Maruthachalam. "Agar Media Inducing Sexual Reproduction." Genetic Transformation Systems in Fungi, vol. 2, Springer International Pu, 2016.
2. Wuest, Caroline E, et al. "Genetic Variation in Native Populations of the Laurel Wilt Pathogen, Raffaelea Lauricola, in Taiwan and Japan and the Introduced Population in the United States." Plant Disease, vol. 101, Apr. 2017, pp. 619–628., apsjournals.apsnet.org.

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