

# Development of a real-time PCR assay for detection of *Raffaelea lauricola*, causal agent of laurel wilt disease

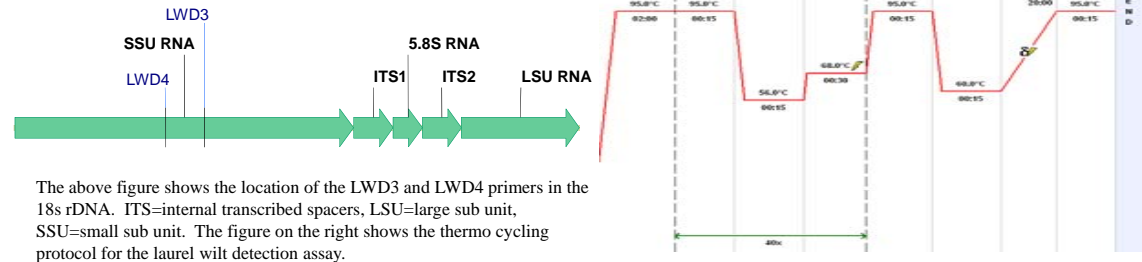
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## Abstract

Laurel wilt disease (LWD), caused by *Raffaelea lauricola* sp. nov. (1) and vectored by the non-native redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff), is a highly destructive disease that affects redbay (*Persea borbonia* (L.) Spreng) and other plant species in the Lauraceae family (2). Due to the need for rapid, sensitive and accurate pathogen detection methods, a real-time PCR detection assay was developed by comparing 18s rDNA sequences from *Raffaelea lauricola* (GenBank accession # EU249466) with host and related fungal sequences present in GenBank. The assay allows successful amplification of a 232 bp fragment that is not likely to be amplified in other fungi known from LWD hosts. Preliminary results from diseased red bay and avocado (*Persea americana*) samples show that the assay results in positive pathogen identification from diseased tissue but does not result in amplification from non-infected host material or other fungal species. This assay is currently being used to study pathogen colonization of host tissue and could be used for multiple applications such as quantitative studies of inoculum density and host resistance.

## Real time PCR assay

Primer sites for the real-time PCR assay were identified by comparing 18s rDNA sequences from *Raffaelea lauricola* (GenBank accession # EU249466) with host and fungal sequences present in GenBank (www.ncbi.nlm.nih.gov). The primers LWD3 and LWD4 amplify a 232 bp product. A melting curve analysis was performed at the end of the PCR protocol. SYBR green was used as the detection method. An Eppendorf Mastercycler ep realplex was used for this analysis.



## Laurel Wilt Disease

*Xyleborus glabratus* (redbay ambrosia beetle) the vector of pathogenic fungus (*Raffaelea lauricola*) was first detected in 2002 near Port Wentworth, Georgia (2). In 2004 the beetle and an unidentified wilt fungus was identified as the causal agent of redbay (*Persea borbonia*) mortality on Hilton Head Island, South Carolina. Currently the disease has spread to over 30 counties in Florida, Georgia, and South Carolina where it has caused extremely high levels of redbay mortality (2). Other members of the Lauraceae family are susceptible to laurel wilt disease to varying degrees. For more information on Laurel Wilt Disease visit [www.fs.fed.us/r8/foresthealth/laurelwilt](http://www.fs.fed.us/r8/foresthealth/laurelwilt)



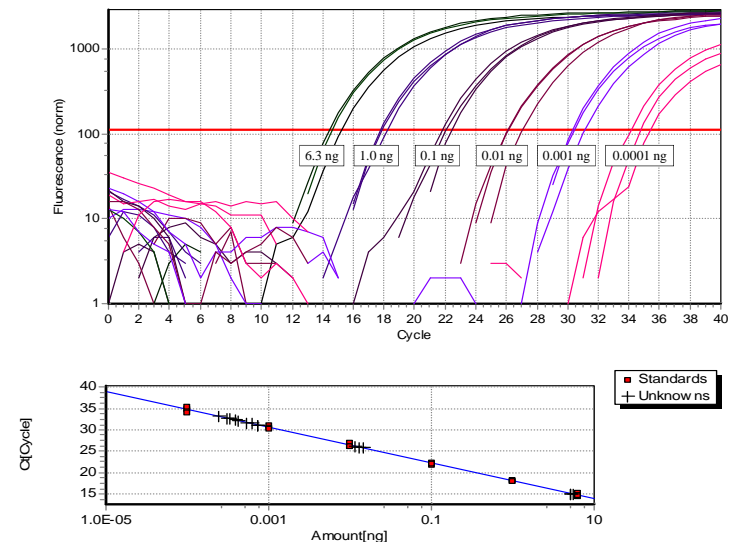
The above left photo shows a wilted redbay infected with laurel wilt disease. The above right photo shows the vascular staining caused by *Raffaelea lauricola* and the entrance holes caused by *Xyleborus glabratus*, the redbay ambrosia beetle.

## Results

The pathogen, *Raffaelea lauricola*, was detected using DNA extracted from wood samples of redbay (*Persea borbonia*), avocado (*Persea americana*), and camphor (*Cinnamomum camphora*); no amplification occurred using DNA extracted from wood samples of uninfected redbay trees. A serial dilution of *Raffaelea lauricola* DNA was used to produce a standard curve, the concentration ranged from 6.3 ng to 0.0001ng. The  $C_t$ , the first cycle that amplification has increased over background levels or threshold cycle, is used to determine the amount of pathogen DNA in each sample.

In addition to the diagnostic value of this procedure, the technique could be used to study pathogen colonization of host tissue and quantification of inoculum density and host resistance.

The figure on the upper right shows the amplification plot, log scale, for the serial dilution used to make the standard curve. The dilutions used were 6.3 ng, 1ng, 0.1ng, 0.01ng, 0.001ng and 0.0001ng of template DNA. The figure to the right shows the standard curve used to determine the amount of *Raffaelea lauricola* DNA in infected wood samples, slope -4.193,  $r^2$  0.997.



## Works Cited

- (1) Harrington, T. C., Fraedrich, S.W., and Aghayeva, D.N. 2008. *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauraceae. Mycotaxon. 104: 399.
- (2) Fraedrich, S. W., Harrington, T. C., Rabaglia, R. J., Ulyshen, M. D., Mayfield, A. E., III, Hanula, J. L., Eickwort, J. M., and Miller, D. R. 2008. A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Dis. 92:215-224.

## Acknowledgments

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