# Development of a Sensitive and Rapid Detection Tool for the Detection of the Laurel Wilt Pathogen

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Laurel wilt (LW), caused by the ambrosia fungus *Harringtonia lauricola* (HL), is a destructive vascular wilt disease affecting many Lauraceae hosts, including important forest species in the southeast United States and commercial avocado trees in Florida. In the past several years, Laurel wilt has expanded its range rapidly through numerous host jumps, which has warranted the development of early detection and monitoring protocols. Current molecular-based detection methods lack the sensitivity to detect HL DNA from complex samples (i.e., host and vector tissues), especially when the titer is low in host tissue in the early and asymptomatic post-infection stages. Our goal is to develop a cost-effective highly sensitive detection method with a simple visualization and interpretation of results for end-users with limited training. A published species-specific IFW SSR region was used to design a TaqMan probe to perform qPCR and traditional PCR amplification. Our cost-effective visualization tool comprises of an inexpensive blue flashlight and barrier filter glass. Using this system, fluorescence can be visualized when positive PCR amplicons are present and thus bypassing costly qPCR protocols. The sensitivity using pure HL DNA of the primer and probe pair with qPCR was 0.32 pg/uL (compared to the lowest sensitivity of 1.6 ng/uL in conventional PCR). Using conventional PCR with blue flashlight, we can detect 0.04 ng/uL of pure HL DNA. Screening susceptible hosts and potential vectors for the detection of the LW pathogen will be an effective tool to control the spread and establishment of this lethal disease in new areas.