

Validation of Molecular Oak Wilt Detection Using Comparative DNA Extraction and Amplification Assays

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Introduction

Oak wilt, caused by the fungal pathogen *Bretziella fagacearum*, is a rapidly spreading vascular disease that poses a major threat to oak populations across the United States. Traditional diagnostic methods rely on culture-based fungal isolation, which is time-intensive, prone to contamination, and often requires up to two weeks for confirmation. To address the need for faster and more reliable diagnostics, molecular techniques such as quantitative PCR (qPCR) and loop-mediated isothermal amplification (LAMP) have been increasingly adopted. This study aimed to validate and compare molecular diagnostic workflows currently used at the Texas Plant Disease Diagnostic Lab (TPDDL) by evaluating multiple DNA extraction methods and downstream amplification assays for oak wilt detection.

Methods

Oak tissue samples submitted to TPDDL were assessed based on size and condition and routed through either conventional culturing or molecular diagnostic workflows. Molecular samples were processed using three DNA extraction methods: the Qiagen DNeasy Plant Mini Kit, the KingFisher Citrus protocol, and the KingFisher Grape protocol. Extracted DNA was analyzed using qPCR targeting a *B. fagacearum*-specific region with TaqMan probes. Cycle threshold (Ct) values were used to compare DNA purity and amplification efficiency across extraction methods. A subset of samples was also tested using a colorimetric LAMP assay to evaluate its suitability for rapid, visual diagnosis. Conventional culturing was conducted in parallel as a reference method.

Results

The Qiagen extraction method consistently produced the lowest and most uniform Ct values, indicating high DNA purity and reliable amplification. The KingFisher Citrus protocol initially showed greater variability but demonstrated improved consistency over time, ultimately yielding Ct values comparable to Qiagen after operator optimization. In contrast, the KingFisher Grape protocol produced higher and more variable Ct values, suggesting reduced DNA quality for oak tissue. When targeting *B. fagacearum* specifically, the Citrus protocol outperformed the Grape protocol with lower average Ct values and tighter distributions. LAMP assays successfully detected positive samples rapidly, confirming their utility for time-sensitive diagnostics, although without quantitative resolution.

Summary

This study demonstrates that molecular diagnostics provide a faster and more scalable alternative to traditional culturing for oak wilt detection. The Qiagen DNeasy Plant Mini Kit remains a gold standard for DNA purity and consistency, while the KingFisher Citrus protocol

offers a strong balance between automation, throughput, and reliability when optimized. The KingFisher Grape protocol was less suitable for oak tissue under the conditions tested. LAMP assays serve as a valuable supplementary tool for rapid screening when speed is prioritized. Overall, validated molecular workflows enhance diagnostic efficiency and support improved disease management strategies for oak wilt.

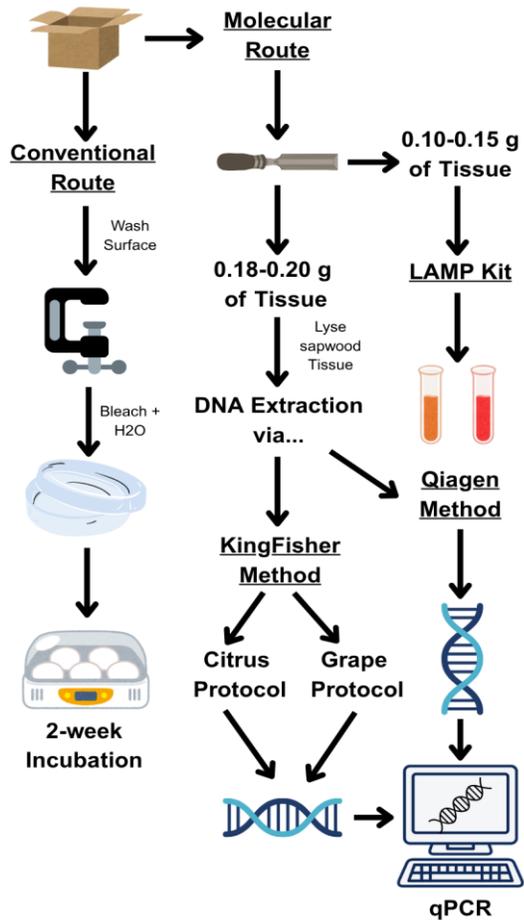


Figure 1. Workflow of oak wilt diagnosis at the Texas Plant Disease Diagnostic Lab. Larger samples proceed to conventional culturing, while smaller or time-sensitive samples follow molecular testing protocols including LAMP and qPCR using Qiagen and KingFisher methods (image created by author).

References

Abbas et al., 2024; Chahal et al., 2024; Koch et al., 2010; Yang & Juzwik, 2017