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

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Abstract

Oak wilt, caused by Bretziella fagacearum, is a destructive vascular disease threatening oak populations across the U.S. Traditional culturing methods used for diagnosis are time-consuming and prone to contamination. Molecular diagnostic approaches, such as qPCR and LAMP, offer faster, more specific detection (Yang & Juzwik, 2017; Abbas et al., 2024).

This project aimed to validate and compare the performance of different DNA extraction protocols (Qiagen Kit, KingFisher Citrus, and KingFisher Grape) in detecting oak wilt via qPCR, and evaluate the utility of LAMP as a rapid diagnostic alternative. Findings support optimized molecular workflows that enhance diagnostic efficiency at the Texas Plant Disease Diagnostic Lab (TPDDL).

Introduction and Objectives

This project was conducted to evaluate molecular assays used in oak wilt detection and determine which DNA extraction protocol yields the most reliable results.

Hypothesis:

- The Qiagen and KingFisher Citrus DNA extraction methods yield higher quality DNA for qPCR detection of B. fagacearum compared to the KingFisher Grape protocol.

Objectives:

- Validate the accuracy and consistency of qPCR across three extraction protocols.
- Determine whether LAMP assays can be used for rapid field-based oak wilt diagnosis
- Support diagnostic workflow optimization at TPDDL.

Methods

Oak tissue samples were assessed and routed based on size and condition as shown in Figure 1 (right side):

- **Larger samples underwent conventional culturing for a14-day incubation**
- **Smaller samples were processed for molecular testing using:**
  - Qiagen DNeasy Plant Mini Kit (silica-column).
  - KingFisher Citrus or Grape protocols utilizing bead-based automation(Ghosh & El-Gebali, 2023).
- DNA was amplified via qPCR targeting B. fagacearum using hydrolysis probes (Chahal et al., 2024).
- Subset was tested with LAMP, a colorimetric assay for rapid endpoint detection (Abbas et al., 2024).

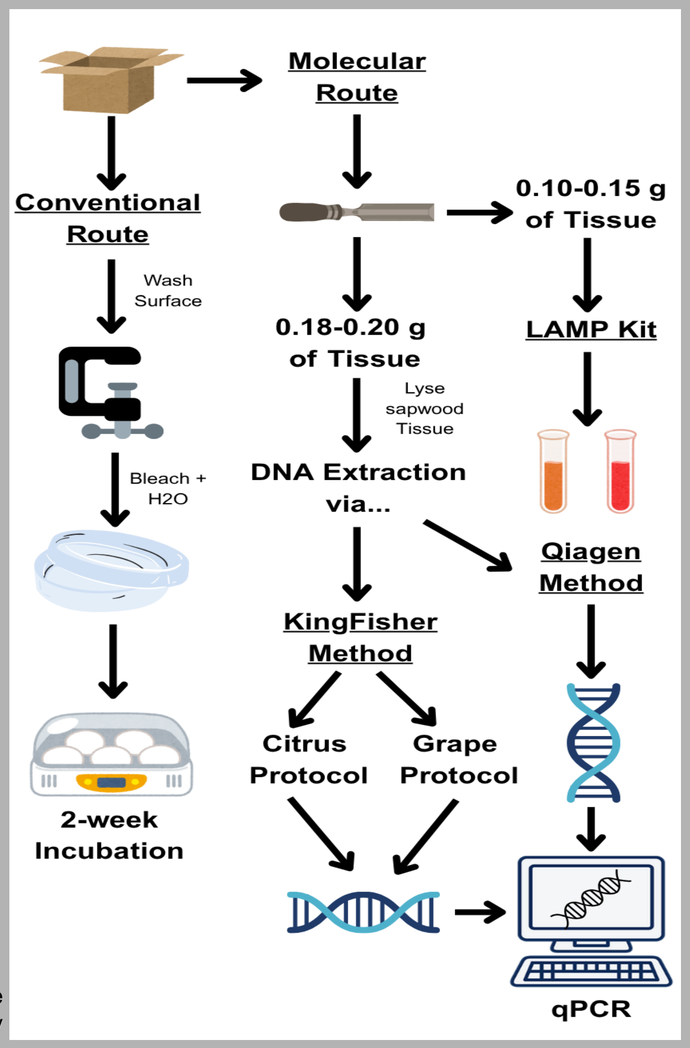

Figure 1. Experimental workflow of the study

Results

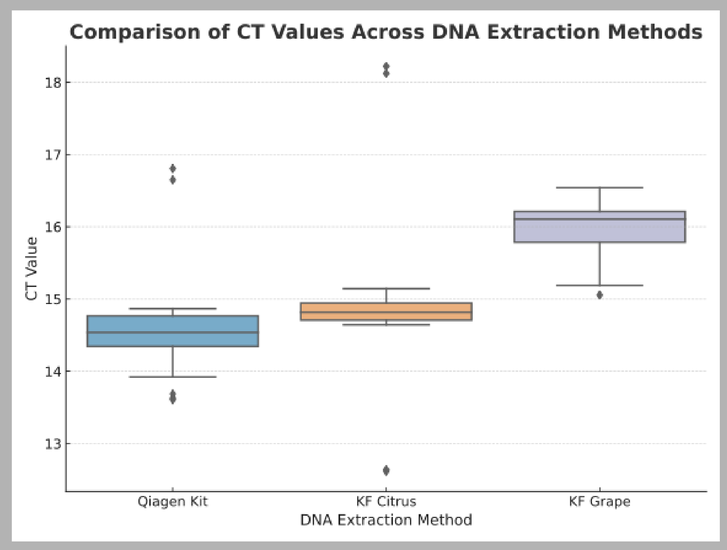

Figure 2: Boxplot comparing Ct value distributions across all three methods.

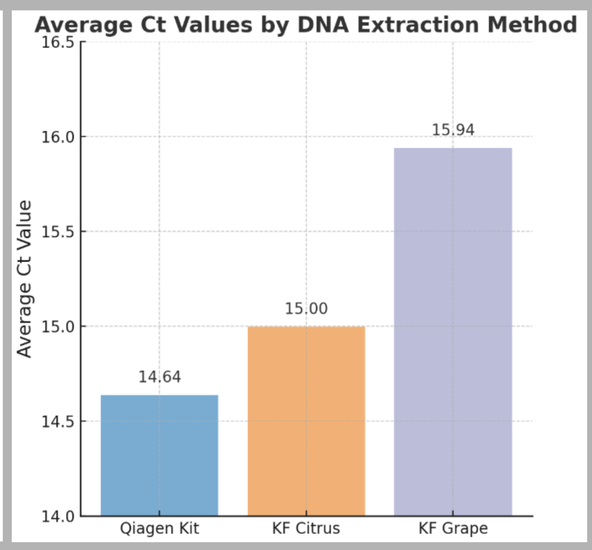

Figure 3: Bar chart summarizing average Ct values for each method.

- Qiagen Kit showed the lowest and most consistent Ct values (~14.6) indicating the highest DNA purity
- KingFisher Citrus produces slightly higher Ct values (~15.0) but demonstrated improved consistency after technician optimization
- KingFisher Grape has the highest and most variable Ct values (~15.9), suggesting lower extraction efficiency.
- Results support the Qiagen and Citrus protocols as the most reliable for qPCR-based oak wilt detection, however **the KingFisher Citrus method offers a promising, automated alternative with higher throughput potential, making it suitable for diagnostic labs with large sample volumes.**

Conclusion

- Qiagen and KingFisher Citrus methods produced high-quality DNA for molecular oak wilt detection.
- Citrus protocol offers a strong balance of throughput and accuracy,
- LAMP assay is effective for rapid visual diagnosis. Reliability testing will be required in the future.
- All testing methods are viable, but the KingFisher Citrus method stands out for its precision and reproducibility

References

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Acknowledgements and Future Work

This project was made possible through the mentorship of Dr. Kevin Ong and support from Hannah Ayala and Jake Ueckert at the Texas Plant Disease Diagnostic Lab (TPDDL). I am forever thankful for the endless support, encouragement, and environment my colleagues created.
Sponsors for high impact experiences for BESC and the BESC poster symposium include the Department of Plant Pathology and Microbiology, the College of Agriculture and Life Sciences, the Office of the Provost and Executive Vice President for Academic Affairs.