PRESSURE CHAMBER EXTRACTION OF XYLEM FLUID: IMPROVING BACTERIAL DETECTION IN PLANTS AFFECTED BY *XYLELLA FASTIDIOSA*

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ABSTRACT
Xylella fastidiosa is the xylem-limited bacterium that causes Pierce's disease of grapevine and oleander leaf scorch. Detection of this pathogen prior to symptom development is critical for improved management of the pathogen. ELISA and PCR are currently used for routine detection of the pathogen; however, both detection methods are limited by low titer or patchy distribution of the bacterium within a host plant. In the study reported here, we directly compared *X. fastidiosa* detection in whole-tissue samples to xylem fluid samples from grapevine and oleander. Collection of xylem fluid samples improved sensitivity of pathogen detection by ELISA (41.0%) compared to whole-tissue samples (20.5%) in asymptomatic grapevine. Additionally, pathogen detection in asymptomatic grapevine by PCR was also improved when xylem samples were tested (66.7%) compared to whole-tissue samples (23.1%). There were no differences in frequency of detection of *X. fastidiosa* in symptomatic grapevines by ELISA or PCR dependent upon sample collection method. Assays of xylem fluid samples did not improve detection of *X. fastidiosa* in symptomatic or asymptomatic oleander compared to assays of whole tissue. Finally, in a direct comparison of ELISA and PCR, we found no significant differences in frequencies of positive grapevine or oleander samples detected.

INTRODUCTION
The xylem-limited bacterium, *Xylella fastidiosa*, has been identified as the causal organism of several economically important diseases in California (Freitag 1951). Pierce's disease of grapes, the most notable of these diseases, is caused when the pathogen interrupts the translocation of water and nutrients through the xylem of affected plants (Purcell 1997). *X. fastidiosa* also causes leaf scorch and declines in elm, sycamore, oak, maple, oleander, and almond (Purcell and Hopkins 1996).

X. fastidiosa collection methods hamper all detection techniques used for pathogen management and study. The most common method of collection, extraction of the bacterium directly from tissue (leaves, shoots, or stems) has several limitations, such as low cell numbers collected and high amounts of plant DNA and organic matter that can interfere with ELISA or PCR, hindering early detection and often resulting in false negatives (Blua, personal communication). Improving the consistency of detection will enhance the study of the interaction between host plants and the pathogen. A better bacterial collection technique would benefit commonly used sensitive detection techniques. Several techniques have been used to collect xylem sap for identification of bacteria. Vacuum extraction has been used to identify bacteria in xylem sap of grapevine (Bell et al. 1995, Guo and Lu 2001).

Using the pressure bomb technique, we hoped to increase the consistency of *X. fastidiosa* detection in plants. This improved technology would allow pathogen diagnostics to be quantified and will improve the detection techniques already being used by concentrating the titer of bacteria being detected. Our intent was to improve bacterial detection in plants used for transmission tests; however, pressure chamber extraction of xylem fluid is applicable to detection of other endophytic bacteria in other plants. We plan to expand the method to detect other bacteria, those with anti-*Xylella* properties, in the vascular tissues of plants that can be used for paratransgenesis.

OBJECTIVES
The goal of the research proposed was to improve the efficiency and consistency of bacterial detection in plants. The technique developed in this research study will be applicable to other plant/pathogen systems, improving detection of pathogens in a simple and cost effective manner. The specific objectives of this work were to:

1. Improve *Xylella fastidiosa* detection methods in oleander and grapevine by extracting DNA from xylem fluid samples rather than whole tissue samples.
2. Develop assay for extraction of bacteria to improve sensitive molecular techniques currently being used for detection of pathogens and endophytic bacteria.

RESULTS AND CONCLUSIONS

Grapevine xylem was relatively easy to collect under pressure from a Scholander pressure bomb with a pipette. It exuded from the cut stem as a clear fluid. In contrast, the collection of xylem fluid from oleander was relatively more difficult to collect using the Scholander pressure bomb because it exuded as a froth, making collection difficult to keep sterile. The froth indicated cell collapse within the stem; therefore samples collected were most likely not pure xylem fluid.

Use of the Scholander pressure bomb to collect samples was more efficient than whole-tissue samples for detecting *X. fastidiosa* in asymptomatic grapevines. Both whole-tissue and xylem fluid samples were collected from 30 grapevines to be tested by ELISA and PCR on August 27, 2002 (10 visually symptomatic and 20 visually asymptomatic) and an additional 30 grapevines were sampled on October 23, 2002 (11 visually symptomatic and 19 asymptomatic). For data analysis, results from these two collection dates were pooled. Statistical analysis of samples pooled across dates did not reveal differences between collection technique in symptomatic plants detection using ELISA (χ^2=0.099 df=1, *p* value=0.7530) or PCR (χ^2=1.867 df=1, *p* value=0.1718). However, *X. fastidiosa* detection in xylem fluid samples of asymptomatic plants was significantly better than whole-tissue extract by ELISA (χ^2=12.045 df=1, *p* value=0.0005) and PCR (χ^2=14.978 df=1, *p* value=0.0001).

Use of the Scholander pressure bomb to collected samples was not more efficient then whole-tissue samples when analyzed by ELISA or PCR. Ninety-four oleanders were sampled on April 22, 2002, 56 symptomatic and 38 asymptomatic, and 30 oleander samples were taken on November 8, 2002, 15 symptomatic and 15 asymptomatic. As in the grapevine samples, results from these two collection dates were pooled for data analysis. In symptomatic plants detection by ELISA using either collection method was not significantly different (χ^2=0.201 df=1, *p* value=0.6539). In symptomatic plants tested by PCR, whole-tissue extraction was significantly more sensitive than xylem fluid samples (χ^2=10.327 df=1, *p* value=0.0013). In asymptomatic plants there was no significant differences between collection methods by ELISA (χ^2=1.941 df=1, *p* value=0.1630) or PCR (χ^2=2.192 df=1, *p* value=0.1387).

There were no significant differences between the ELISA or PCR method of detection in grapevine (χ^2=1.35 df=1, *p* value=0.245) or oleander (χ^2=0.115 df=1, *p* value=0.734).

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