USE OF A POLYMERASE CHAIN REACTION SYSTEM (BIO-PCR) FOR
DETECTION OF ERWINIA RUBRIFACIENS IN SYMPTOMLESS TISSUES OF
WALNUT TREES

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ABSTRACT

Contaminated graftwood is a suspected means by which deep bark canker disease is spread. To investigate this possibility and ultimately to develop a source of bacteria-free budwood from which to produce healthy trees, a highly sensitive method to detect the pathogen in walnut tissue is needed. A procedure known as polymerase chain reaction (PCR) would meet this requirement. The subject of this project was to complete development of a PCR protocol to detect the deep bark canker bacteria in walnut tissue. Primers derived from the DNA sequence of the regulatory gene for exopolysaccharide synthesis produced successful detection of Erwinia rubrifaciens in symptomatic and asymptomatic walnut tissue. Samples of all age wood on two scaffolds displaying external symptoms were tested for E. rubrifaciens using the PCR. The number of positives were very sparse and several unexpected false positives occurred as well. There appear to be too many technical problems with this procedure to warrant further research at this time.

INTRODUCTION

Deep bark canker of English walnut is caused by the bacterium Erwinia rubrifaciens. Although the disease is most prevalent on cultivar Hartley, most cultivars are susceptible and may develop symptoms when placed under exceptional stress such as inadequate water, hot summers, or poor soil. Chemical protection against deep bark canker is not presently, and most likely will not be, available.

The disease may be spread by mechanical harvesters if contaminated shaker pads inflict deep wounds in the bark. Also, previous research indicates that the pathogen may be transmitted via the graftwood. In the latter case, graftwood taken from infected trees, whether these trees displayed external symptoms or not, would result in symptomless but infected trees being distributed through nursery sales.

A method to detect low populations of the pathogen in symptomless tissues used for propagation could be useful in development of a program to produce bacteria-free trees. The polymerase chain reaction (PCR) procedure, combined with Southern blot analysis, is such a method. PCR is an in vitro enzymatic procedure in which a specific segment of a genome is exponentially amplified. It is possible to amplify a discrete DNA fragment many million-fold. Previous research has shown that PCR has allowed the detection of 10-12 cells per milliliter. A refinement of the PCR method, known as bio-PCR detects living cells instead of dead cells and is the method used here.

Past attempts to apply the bio PCR have not produced expected positive results. Refinements of the system and improved sampling were conducted to test the potential usefulness of this procedure for development of disease-free propagation materials.
OBJECTIVES

1. To test and standardize viable cell bio-PCR protocol for *E. rubrificiens* in vitro and in walnut tissue.

RESULTS AND DISCUSSION

Samples were collected from a Hartley walnut tree that had been inoculated with *E. rubrificiens* in 1994 and which had developed external symptoms of the disease on the trunk, primary, and one secondary scaffolds. All the wood on one of the diseased primary and the secondary scaffolds were harvested over a ten week period from mid September through the end of November. On each harvest date, two or three side shoots were removed and returned to the laboratory. Each age wood was identified and removed at each successive base such that all one, two, three- and so on year-old sections were separated. After surface sterilization, a one inch wide band of bark and outer wood located one inch above the section base was removed from the circumference of each section. The bark and outer wood in this band were scraped into a mortar and pestle and ground in 3 ml of buffer. Subsamples were plated and allowed to grow for 26 hours after which the culture plates were washed with buffer and the liquid collected in tubes. The suspensions were centrifuged, the pellet resuspended in 1.0 ml buffer and frozen at -80C until tested with the PCR.

Several positive readings were found but there also were false positives and false negatives as well. This indicates that there remain sufficient difficulties with this procedure that would have to be resolved in order to justify its use as a detection system. The importance of deep bark canker to the walnut industry at this time does not appear to warrant the time and expense required to resolve the issues surrounding this project. We regretfully suggest the research be abandoned for the time being.