CHARACTERIZATION AND IDENTIFICATION PIERCE'S DISEASE RESISTANCE MECHANISMS: ANALYSIS OF XYLEM ANATOMICAL STRUCTURES AND OF NATURAL PRODUCTS IN XYLEM SAP AMONG *VITIS*

Project Leader:
Hong Lin
Exotic & Invasive Disease & Pest Research Unit
USDA-ARS
Parlier, CA 93648

Cooperator:
Andrew Walker
Department of Viticulture and Enology
University of California
Davis, CA, 95616

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ABSTRACT
Understanding and utilizing natural defense mechanisms is a critical component of crop improvement. The ultimate solution to Pierce's disease (PD) problems likely relies on host resistance. This research proposal focuses on examining PD resistance in several grape species. Although some of resistant species have been tested, the mechanisms involving resistance are not well understood. It appears that PD resistance mechanisms vary. Some resistance mechanisms may be related to anatomical characteristics while others may be due to defense chemicals. The strategy that we are developing for this project will provide research tools to investigate PD resistance mechanisms.

INTRODUCTION
Pierce's disease (PD) caused by the *Xylella fastidiosa* (*Xf*) bacterium is a major disease problem in California. The extent to which PD affects the California grape industry has been dramatically increased by the recent introduction and establishment of a more effective vector, the glassy-winged sharpshooter (*Homalodisca coagulata*). Host plant resistance is a critical component of integrated crop management. Understanding the nature or basis of resistance mechanisms and utilizing resistance resources from native grape species offers a direct approach to combating PD.

Resistance to PD had been tested in many grape species (Mortensen et al., 1967, 1977) but the mechanisms involving in PD resistance have not been well characterized, hence, are not well understood. Given the fact that these species were derived from various genetic backgrounds and different origins, it is expected that the mechanisms of PD resistance may be different among grape species.

The hypothesis of this research proposal is that the mechanisms of PD resistance are due to chemical compounds (e.g. anti-microbial activity) present in xylem sap that suppress *Xf* or due to anatomical features of the xylem (e.g. pit membrane) that restricts *Xf*'s mobility or both. Plants produce a vast array of natural defense compounds. Most of these natural products are derived from secondary metabolic pathways. Compounds such as the isoprenoid, phenylpropanoid, alkaloid or fatty acid/polyketide, are continuously expressed in many plants that have proven effective against microbial attacks and insect/animal predation. Other types of compounds that are synthesized *de novo* in response to pathogen attack are known as phytoalexins. Stilbenes are examples of such compounds with low molecular weight phenolics occurring in a number of plant species including grape (Sparvoli et al., 1994). In grape, they act like phytoalexins, in response to against pathogens such as *Botrytis cinerea* (Jeandet et al., 1995), and *Rhizopus stolonifer* (Sarig et al., 1997).

One of the underlining aspects of this study is to determine if *Xf* resistance is confirmed by antimicrobial substances in the xylem sap, thus providing a new approach to controlling PD. Grafting with resistant rootstocks is commonly used to overcome pest and disease problems and nutrients and growth regulators move from the rootstock to the scion. Therefore, identifying and screening xylem antimicrobial compounds, and using these PD resistant plants as rootstocks may provide a unique opportunity for enhancing the expression of PD resistance without genetically modifying the scion. This last point is critical because it will be very difficult to produce economically competitive wine grape cultivars through classical breeding or genetic engineering, because of the conservative and international wine industry. If a rootstock that can confer PD resistance is produced the integrity of wine grape cultivars will be maintained.

OBJECTIVES
1. Evaluate if any structurally related factors are associated with PD resistance. Develop a novel multiple grafting technique to examine the correlation between pathogen movement and the features of anatomical structures. A quantitative PCR-based assay (*Xf*-specific Taq-Man PCR) will be used to measure the mobility of *Xf* and the size of the pathogen population in tissues. Scanning electron microscopy will be used to determine the comparative the anatomical structure of resistant and susceptible plants.
2. To characterize the chemical composition of xylem and identify the substances that may contribute to antimicrobial effects which prevent / suppress *Xf* colonization / multiplication. *In vitro* bioassay and chemical analysis will be used to determine the roles of these compounds.
3. To evaluate the transmissibility of the antimicrobial compounds from resistant plants and evaluate the performance of susceptible scions grafted on these resistant rootstocks.

RESULTS AND CONCLUSIONS

This project was funded in June, 2003. We selected 10 grape species (*Vitis aestivalis, V. candicans, V. champinii, V. labrusca, Muscadinia munsoniana, V. riparia, M. rotundifolia, V. rufotomentosa, V. shuttleworthii, V. simpsonii, V. smalliana, V. tiliifolia*, and *V. vulpina*) from different genetic backgrounds representing a wide range of resistance to PD. Currently, we are propagating these plants in greenhouse for later experiments.

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