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

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ABSTRACT
This research tests the hypothesis that Pierce's disease (PD) resistance is due to the presence of chemical factors, e.g. anti-microbial compounds expressed in the xylem sap that suppress *Xylella fastidiosa (Xf*) and /or are due to anatomical features of the xylem, e.g. pit membrane that restrict *Xf*'s mobility in xylem. A wide range of PD resistance from various genetic backgrounds of *Vitis* species was selected for this study. To determine if pathogen movement *in xylem* is related to anatomic structure, an inter-grafting method was used to evaluate the movement of *Xf* across between PD susceptible and resistant stems. SEM and quantitative PCR were used for this study. To test the effect of xylem sap, an *in vitro* bioassay method was developed. The preliminary bioassay results suggest that xylem saps from PD resistant grapes may have effect when the test was compared with the sap from *V. vinifera cv.* Chardonnay.

INTRODUCTION
Plants have evolved a variety of resistance and tolerance mechanisms against biotic stress. This rich diversity results in part from an evolutionary process driven by selection for acquisition of defense compounds against microbial attack or insect/animal predation. As pesticide use becomes more restricted, it becomes increasingly important to explore and utilize compounds from plant's natural defense systems. Like many other plants, grape species are very diverse. Many *Vitis* species, *V. aestivalis*, *V. arizonica, V. shuttleworthii, V. simpsonii, V. smalliana*, are highly resistant to PD, as have the muscadine species*, Muscadinia munsoniana and M. rotundifolia*. Understanding and utilizing natural defense mechanisms is a critical component of crop improvement. The ultimate solution to PD problems likely relies on host resistance. This research focuses on understanding PD resistance mechanisms in grape species. Although PD resistant species have been identified (Mortensen, et al, 1977), the mechanisms involving resistance have not been well characterized and identified. It appears that PD resistance mechanisms vary – some resistance mechanisms could be related to anatomical aspects while others may be related to xylem chemistry. This research will examine the physiological and anatomical basis of PD resistance. We selected the following grape species to study PD resistance: *V. arizonica*, *V. aestivalis, V. candicans, V. champinii, V. labrusca, M. munsoniana, V. riparia, M. rotundifolia, V. rufotomentosa, V. shuttleworthii, V. simpsonii, V. smalliana, V. tiliifolia,* and *V. vulpina*. 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. *Xylella fastidiosa* is xylem limited and kills vines by inducing or creating vessel blockage leading to disease (Goodwin et al 1988a, 1988b). The pathogenesis of *Xf* appears to be dependent upon its ability to multiply in the xylem vessels and move systematically across vessels. Therefore, the mechanisms of host resistance may act to physically eliminate *Xf* movement or chemically suppress population development, or both. This proposal attempts to determine whether PD resistance is because: 1) anatomical features of the xylem (e.g. pit membrane) eliminate *Xf*'s mobility; 2) chemical compounds (e.g. anti-microbial activity) present in xylem sap suppress *Xf.*

OBJECTIVES
1. Develop an *in vitro* bioassay to determine the roles of compounds present in PD resistant species. Chemically characterize the composition of xylem and identify compound(s) that may contribute to antimicrobial effects which prevent or suppress *Xf* colonization.
2. Examine xylem structure related PD resistance. Use an inter-graft technique to examine the correlation between pathogen movement and xylem anatomy features.

RESULTS
1. Table 1 presents a list of grape species used for bioassays of xylem sap. A 4 inch diameter x 20 inch pressure chamber (PMS Instrument Co., Corvallis, OR) was used to collect xylem sap from shoots. The chamber pressure was gradually increased to 1,000 – 2000 kPa. On average 0.5 to 2.0 ml xylem sap was collected from each sample. Sap collected from infected and non-infected plants was used for bioassays. The xylem sap was first filtered through a 0.22 micron nylon filter. Two bioassays were conducted. The first bioassay was on PW agar medium on which a piece of filter paper saturated with sap solution was placed onto growing *Xf*. Filter paper saturated with 200 µm Tetracycline or water was used as positive and negative controls, respectively. Another bioassay was carried out by directly culturing *Xf* in xylem sap for 10 days prior to spreading sap on a PW plate to check colony formation. Xylem sap from Chardonnay, a PD-susceptible cultivar was used as a positive reference. Using both methods, we screened xylem saps collected from early spring and summer. No inhibitory

effects were observed from the xylem saps collected from early spring. Currently, we are working on the saps collected from growing season. Our preliminary bioassay results indicate that sap from *M. rotundifolia* appears to have effect on *Xf* growth compared with the sap from Chardonnay. Additional xylem sap has been collected from *M. rotundifolia* to confirm the result.

2. To evaluate xylem structure related to PD resistance, we designed an inter-graft method to compare *Xf* movement between PD resistant and PD susceptible stems. Table 2 presents the results of graft combinations with susceptible stems connected with a resistant interstock. We used dormant cuttings for most of grafts. However, *M. rotundifolia* and several other PD resistant species are only successfully grafted with herbaceous cuttings. Because of difficulty in completing these grafts only a limited number of graft combinations could be made, others are still processing. The successfully grafted plants were used for the movement experiment. In August, these plants were mechanically inoculated with 20 µl of mixture of Stag's Leap and Beringer strains (OD_{600}=0.249) at the bottom part of the susceptible stem. Two months after inoculation, PD symptoms began to appear in both the top and the bottom of halves of "Chardonnay -9621-15 - Chardonnay" but not in resistant stems in the middle of inter-grafted plants (Figure 1). We are harvesting leaves and petioles from the bottom, middle and top parts of the each plant to determine *Xf* levels. Currently, we are working on xylem structure among these PD resistant species using SEM.

CONCLUSION

We have commenced a study of the anatomical and chemical aspects of xylem that distinguishes PD resistant species. Understanding and utilizing natural defense mechanisms is a critical component of crop improvement, and our studies will help breeders fine tune selection indices and determine whether xylem chemistry or anatomy characters are more closely involved in PD resistance.

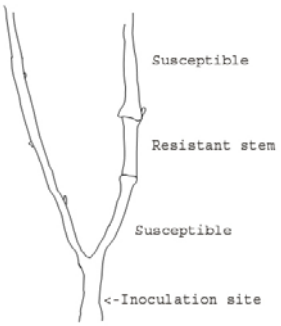
Table 1. List of plants from which the xylem saps were extracted for *in vitro* bioassay.

Resistant species and hybrids

V. arizonica
V. candicans
V. champinii
V. rufotomentosa
V. shuttleworthii Haines City
V. simpsonii
S. smalliana
V. tiliifolia
*M. rotundifoli*a Cowart
V. rupestris Metallique
V. girdiana
V. monticola
V. nesbitiana
8909-15 (*V. rupestris* x *V. arizonica)*
8909-19 *(V. rupestris* x *V. arizonica)*
9621-67 *(V. rupestris* x *V. arizonica)*
9621-94 *(V. rupestris* x *V. arizonica*)

Table 2. Combinations of inter-graft stems used for evaluating *Xf* movement. Plants were mechanically inoculated with *Xf* at the base of the susceptible plants (see picture on the right and the bottom). Petioles and leaves from each part of plants were sampled for *Xf* measurement.

Inter-graft stems		
(Susceptible)	(Resistant)	(Susceptible)
8909-19	8909-15	8909-19
Chardonnay	8909-15	Chardonnay
Chardonnay	Haines City	Chardonnay
Thompson Seedless	8909-05	Thompson Seedless
Fiesta	8909-05	Fiesta
9621-94	9621-67	9621-94



Base of the grafted plants. Yellow arrow points to the first graft union. Red arrow indicates the inoculation point. Symptoms in the lower part of the susceptible Chardonnay plant.	Leaves on the shoot coming from the middle part of the inter-stem of the grafted plant (resistant, 9621- 15). No visible symptoms.	Upper part of the grafted plants. The second graft union is shown in yellow arrow. PD symptoms are present on the upper part of the grafted plants (susceptible Chardonnay).

Figure 1. Inter-grafted plant experiment

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