BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCES DISEASE: XYLEM FLUID CHEMISTRY MEDIATION OF RESISTANCE TO PIERCE'S DISEASE

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INTRODUCTION

The influence of xylem chemistry on the establishment, colonization and movement of *Xylella fastidiosa (Xf)* can be tested at various levels. The chemistry of xylem fluid is relatively simple compared to other plant tissues. Xylem fluid consists of over 98% water, and the major chemical entities monomeric (amino acids, organic acids and sugars) and inorganic ions. Few secondary compounds are present in appreciable quantities in xylem fluid, although peroxidases are often detected in low concentration. We feel the contribution of plant nutrient status is an undervalued component of plant resistance.

Some research areas that we hope to make contributions including: 1) to define the chemical basis of establishment, multiplication and spread of *Xf* in grape genotypes and other plant species; 2) the creation the development of a vastly simplified chemically defined medium; 3) the elucidation of factors that promote *Xf* aggregation and biofilm formation; 4) the determination of the antibacterial properties of lytic peptides; 5) the determination of the presence of peroxidases in xylem fluid and to establish the promotion/inhibition of *Xf in vitro.*

OBJECTIVES

The all-encompassing objective was to establish the role of xylem chemistry on resistance/susceptibility of *Vitis* genotypes to *Xylella fastidiosa* (*Xf*) and Pierce's disease (PD). The objectives are to determine:

1. the resistance of 10 grape genotypes to PD after mechanical inoculation with *Xf* and discern the relationship between chemical profiles of xylem fluid and resistance. (Dr. Andrew Walker, cooperator);
2. the mechanism of resistance to PD of host plants that are common in riparian habitats in California. (Dr. Alexander Purcell, cooperator);
3. validate the influence of specific chemical profiles on the growth and survival of *Xf* by tests *in-vitro* culture;
4. the naturally occurring antimicrobial peptides in *in vitro* experiments for efficacy against *Xf* and study the stability of these peptide compounds in buffer and in xylem fluid, and;
5. the concentrations of peroxidases in xylem fluid of 10 grape genotypes.

RESULTS AND CONCLUSIONS

In 2002, we have analyzed the xylem chemistry of 10 *Vitis* genotypes that expressed differential rates of *Xf* susceptibility. The primary organic compounds (amino acids, organic acids and sugars) and inorganic ions were genotype dependent. Xylem chemistry was influenced by geographic location (California and Florida) and season of the year (dormant and growing season). Very unbalanced chemical profiles occurred. For example, in one study the concentration of glutamine varied between 46% (Chardonnay) and 70% (Dogridge) of the total amino acids in xylem fluid. Chemical profiles among *Vitis* genotypes varied greatly when xylem fluid was collected during the dormant season. Biofilm formation is considered an important component of colony formation of *Xf* and is likely involved in pathogenesis. The relationships of chemical profiles and specific chemical entities to *Xf* colonization, spread and biofilm formation are being investigated. (Biofilm was quantified *in vitro* by the crystal violet/ethanol elution method).

A short-term exposure to xylem fluid from grapevine genotypes caused the development of differential colony numbers and colony size of *Xf* UCLA PD strain when grown on agar culture. In most cases the effect of xylem fluid on colony number was not greatly altered by increasing incubation time from 1 to 24 hours suggesting that the effects on *Xf* are rapid. Anomalous results were obtained showing that colony number decreased with exposure to xylem fluid from PD-susceptible genotypes of *V. vinifera* (Chenin blanc and Chardonnay); however these genotypes formed significantly larger colonies than PD-resistant genotypes. The formation of large colonies may be critical to expression of *Xf* virulence *in-planta,* in that *Xf* may typically survive and persist in PD-resistant *Vitis* genotypes; colonies simply do not form that adhere to xylem walls and occlude vessels. We investigated this phenomenon again in an effort to quantify biofilm formation using *Xf* UCLA and STL strains in liquid culture. *Xf* was incubated for 96 hours in xylem fluid of *V. rotundifolia* Noble and *V. vinifera* Chardonnay. Xylem fluid was collected from dormant, field-grown and screen house grown vines. *Xf* strain and xylem fluid treatment had a highly significant effect on subsequent colony numbers. Biofilm formation varied greatly with xylem fluid treatment. The highest amount of biofilm and the highest ratio of biofilm to colony numbers in solution occurred for *V. vinifera* Chardonnay. These data taken collectively show that the chemistry of xylem fluid can have a profound effect on *Xf* colony number and biofilm formation, and the highest tendency toward biofilm formation occurred for PD-susceptible species.

We examined xylem chemistry throughout the year on a large variety of alternative (non-*Vitis*) host plants and compared these to rates of *Xf* infection. The best statistical correlation was found for percentage of plants infected and the concentration of total amino acids when sampled during the dormant season. This will further our knowledge of resistance mechanisms are the same for other host species as for *Vitis*, and to further our knowledge of alternative hosts that may be important in the spread of *Xf*.

In 2002, we completed the formulated of new chemically-defined media for *Xf*. Several aspects contributed to the completion of this phase. Several alternative methodologies were implemented to assure the complete evaluation of these new formulated diets. New media were evaluated on the basis of agar culture, liquid culture and biofilm formation. The most simple medium that was successful consisted of 4 organic compounds and 3 inorganic salts. Other chemically defined media were based on the chemistry of Chardonnay (a susceptible grape genotype to *Xf*). The performance of *Xf* in different media was dependent on the strain, the media composition and the strain X media interactions. These results support the contention that xylem chemistry may be critical in determining pathogenesis.

The antimicrobial activity of naturally occurring lytic peptides (cecropin A, cecropin B, magainin I, magainin II, indilocidin, lysozyme) has been investigated. The cecropins were the most lethal to *Xf*. The minimum inhibitory concentration for 100% *Xf* mortality in PW^{+} medium was as follows: cecropin A 1 μM, cecropin B 1 μM, indolicidin 10 μM, magainin II 80 μM, magainin I 80 μM, tetracycline 100 μM, lysozyme > 1000 μM. The persistence of lytic peptides in xylem fluid *V. rotundifolia* was investigated. Xylem fluid plus cecropin A (10 or 20 μM) and cecropin B (2, 10, 20 μM) resulted in 100% *Xf* (UCLA strain) mortality for 5 hours or less of incubation. Xylem fluid of *V. rotundifolia* Noble and *V. vinifera* Chardonnay incubated with *Xf* UCLA strain plus cecropin B (1 μM) resulted in high colony counts and low biofilm production for Noble, but low colony counts and high biofilm production for Chardonnay. A timecourse of cecropin B (1, 10, 50 and 100 μM) activity in xylem fluid of *V. rotundifolia* Noble and *V. vinifera* Chardonnay was followed by SDS Page gel electrophoresis. Cecropin B showed reduced activity from 1 to 96 hours, although at a concentration of 100 μM the cecropin B band did not disappear entirely. The cecropin bands disappeared at lower concentrations of cecropin B indicating a loss of stability probably as a result of proteolytic breakdown. Lytic peptides may eventually be incorporated in control strategies for *Xf* via genetic engineering or direct application of compounds into xylem fluid.

Proteins and specifically peroxidases were detected in low concentrations in xylem fluid of all 10 grape genotypes. Protein content and total peroxidase activities varied with genotype. SDS-PAGE gels of peroxidases from the 10 grape genotypes show a different banding pattern and banding intensities indicating that different isozyme exist in different genotypes and also that concentrations vary with genotype. There is ample justification for continuing this work as we feel that proteins, and specifically peroxidase activity is important to redox reactions at the xylem vessel/bacterium interface, and as such may be involved in some of the earlier responses of plants to PD-infection. In addition, we feel that enzymes are involved in the proteolytic breakdown of lytic peptides.

In conclusion, we have found that xylem fluid chemistry varies greatly with genotype, location (i.e. edaphic conditions), and season of the year. Even a short-term exposure to *Xf* to different growth media altered *Xf* colony number. Exposure of *Xf* to different xylem fluid or to chemically defined media can also induce biofilm formation. The development of a 4 organic compound based chemically defined media will now allow us to delineate the role of specific compounds in *Xf* colony formation and biofilm formation. The biological parameters of naturally occurring lytic peptides have been quantified. We have detected the presence of proteins (in very low concentration) in xylem fluid of all grape genotypes. We have also quantified peroxidase isoenzymes in xylem fluid of all genotypes by gel electrophoresis. The role of peroxidase in early stages of *Xf* colonization *in planta* needs to be evaluated.

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