DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

The glassy-winged sharpshooter (GWSS) (*Homalodisca coagulata*) is a serious pest of grapes and a number of other crops. Its most destructive characteristic is that it is an efficient vector of the bacterium that causes Pierce's disease (*Xylella fastidiosa*). The management of this pest will, in all likelihood, require a multilateral set of approaches. Several of these approaches would be enhanced if the GWSS could be reared on a large-scale basis, especially with an artificial diet. Development of such a diet presents challenging barriers, including formulation of a liquid diet that closely simulates the xylem sap that is the target of GWSS. Understanding the feeding biology of this pest is complicated by the fact that it has cosmopolitan selection of hosts: i.e., it is a promiscuous feeder. However, formulation of a suitable diet is complicated by the fact that this species, which is a strict xylem sap-feeder, must be accommodated with a diet presentation system that simulates the vascular system of plants. Because the development of the diet and the feeding system must be done simultaneously, the probability of success of the entire project is reduced.

The approaches to be taken to increase the chances of complete success include the analysis of xylem sap from several host plants, modeling the diets to be bioassayed after those analyses, attempting to use non-flowing and flowing systems of diet delivery, and microscopic studies of the feeding choices made by GWSS when using their natural hosts. Should all of these approaches work suitably, the need for an oviposition system will be born, and it will become a further aim of this project to develop an in vitro-based system that will allow harvesting of GWSS eggs with maximum efficiency.

OBJECTIVES

1. Development of an artificial diet that will permit complete development of GWSS and reproduction of continuous generations of healthy individuals.

2. Development of a feeding system that will allow efficient delivery of diet to the GWSS.

3. Development of an oviposition system for in vitro egg production and efficient harvesting of the eggs.

RESULTS AND CONCLUSIONS

At the time that this report is being written, the funds have just been put in place, so there has been no research progress. We have set up our greenhouse, gotten the permit to have the GWSS shipped, and we have set up our analytical equipment to do the required amino acid and carbohydrate analyses.

FUNCTIONAL GENOMICS OF THE GRAPE-*XYLELLA* INTERACTION: TOWARDS THE IDENTIFICATION OF HOST RESISTANCE DETERMINANTS

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INTRODUCTION

Pierce's disease (PD), caused by *Xylella fastidiosa (Xf)*, is one of the most important diseases of grapevines (Purcell and Hopkins, 1996). Currently, the development of resistant varieties through classical breeding is limited by the absence of resistant phenotypes in *Vitis vinifera*. On the other hand, several wild grape species, not suitable for wine production, are known to either resist or tolerate infection by *Xf*. Therefore, an alternative approach for the development of resistance in cultivated grapes is to identify transcriptional pathways correlated with susceptible or resistant interactions in *Vitis* species. In principle, comparison of these two distinct interactions will reveal functional elements of the host resistance response, or conversely host functions that confer susceptibility (Cummings and Relman, 2000).

The experimental strategies outlined below will use genomics technology to identify genes in *Vitis* species that may be causal to host susceptibility (in the case of *V. vinifera*) or resistance/tolerance (in the case of native *Vitis* species). Such information will considerably increase our knowledge of the *Xylella*-grape interaction, and potentially provide the basis for developing resistance to the PD pathogen in *V. vinifera*.

OBJECTIVES

1. Construction of cDNA libraries from infected and non-infected grape plants of both susceptible *V. vinifera* and tolerant/resistant *Vitis* species (e.g., *V. shuttleworthii* or *V. aestivalis* complex).

2. A total of 30,000 DNA sequencing reactions will be completed in the first year of the project from cDNA products of the above libraries. The resulting sequence information (i.e., Expressed Sequence Tags (ESTs) [Marra et al., 1998]) will be submitted to the National Center for Biotechnology Information (NCBI) in a simple annotated format.

3. An online relational database will be developed in Oracle 8 to distill relationships within the data, and in particular to estimate a minimum gene set expressed during *Xylella*-grape interactions. A Web-based interface to the project database will make the results of this project available to all Pierce's disease researchers, with the intent of stimulating interaction among scientists and accelerating progress towards control of the *Xylella* pathogen in cultivated grapes.

4. Subsequent to EST sequencing and electronic data mining, we will employ functional genomics strategies to first verify and then dissect host gene expression in both susceptible and tolerant/resistant grape genotypes.

RESULTS AND CONCLUSIONS

In the first phase of the project, a total of 120 individuals from both Chardonnay and Cabernet plants (60 plants for each variety) in the Napa Valley of California were randomly selected. For each grape variety, 30 plants were selected located close to riparian areas with a previous history of PD infection and 30 plants were selected located distally from the riparian areas without previous PD infection. At two-week intervals, plants were analyzed for PD using a PCR-based approach with *Xylella*-specific primers (Kim et al., *In Preparation*). By early July the first symptoms of PD infection were observed, and PCR analysis confirmed that two Chardonnay and three Cabernet plants were infected by *Xylella*. Leaves and petioles from these plants were collected and stored at –80 °C until further use for cDNA library construction. These same plants

gave positive PCR results in subsequent weeks, thus confirming the original diagnosis. By late September the frequency and severity of PD symptoms had increased, and tissue was again sampled for cDNA library construction immediately prior to the grape harvest. cDNA libraries are being constructed from these infected and non-infected plants, at time points corresponding to early and late disease development (i.e., early July and late September). DNA sequencing reactions are being carried out at the UC Davis College of Agricultural and Environmental Sciences Core Genome Facility (http://cgf.ucdavis.edu). By March 2002 a total of 30,000 cDNAs will be sequenced and analyzed. These data, corresponding to differences in the transcriptional profiles between infected and non-infected plants, are expected to include host resistance and susceptibility factors. Thus, they will provide the basis for new lines of experimental inquiry focused on testing the efficacy of specific host genes for PD resistance.

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BIOLOGICAL CONTROL OF PIERCE'S DISEASE WITH NON-PATHOGENIC STRAINS OF *XYLELLA FASTIDIOSA*

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INTRODUCTION

Competitive exclusion of plant pathogens with nonpathogenic or less virulent strains has been demonstrated for a number of bacterial, fungal, and viral pathogens. Many nonpathogenic mutants retain the ability to colonize either external or internal plant tissues, and if established first, can effectively compete for colonization and establishment of pathogenic strains. One advantage of this approach for biological control is that the biocontrol agent and target pathogen occupy the same niche and have similar requirements for growth and survival. In addition, the specificity of the biocontrol interaction reduces the possibility of undesirable non-target effects. We propose to construct several nonpathogenic derivatives of *Xylella fastidiosa (Xf)* and test them for preemptive competitive exclusion of pathogenic strains in grape. In practice, such strains could be established in plants at the nursery level or potentially inoculated to mature vines. To construct nonpathogenic mutants, we are taking advantage of the completed genome sequence of the citrus variegated chlorosis (CVC) strain of *Xf* and the rough genome sequence of the almond leaf scorch strain, which is closely related to the PD strains. We predict that genes that are likely to be required for pathogenicity can be identified through comparison of these sequences with known pathogenicity gene sequences from its nearest relative, *Xanthomonas campestris*, or other plant or animal pathogens. PCR methods are being used to amplify these genes from the Pierce's disease strain of *Xf*. Deletions are being created in the genes, and homologous recombination will be used to introduce each deletion independently into the Pierce's disease strain. Each mutant will be tested for virulence and systemic colonization of grapevines, as well as the ability to competitively reduce populations of a pathogenic strain and reduce expression of symptoms.

OBJECTIVES

1. Construct deletion mutations in putative virulence genes of *Xylella fastidiosa*.
2. Test mutant strains for virulence in grapevines.
3. Test mutant strains for biological control of pathogenic strains in grapevines.

RESULTS AND CONCLUSIONS

In our first year, we have cloned a number of putative virulence genes that were identified from the full genome sequence of the CVC strain of *Xf* and from additional sequence information for an oleander and an almond leaf scorch strain available from the DOE-JGI. The genes we have amplified or cloned (in bold) and others we intend to target are as follows:

Genes encoding potential adhesins:

- ***pil*** genes encoding type IV pili
- Three large genes encoding hemagglutinin-like proteins, most closely related to *pspA* in *Neisseria meningitidis*
- Other afimbrial ahesin genes similar to: *hsf* and *hia* from *Hemophilus influenzae; uspA1* from *Moraxella catarrhalis*.

Xanthan gum biosynthesis genes:

- The entire xanthan gum biosynthetic operon (**9 *gum*** genes)

Regulatory genes known to control virulence in *Xanthomonas* or other pathogens:

- *gacA*
- ***rpfB, rpfF***
- ***rsmA***
- ***sphIM*** (Dam methylase)

Mutations have been constructed in several of the cloned genes, and we are attempting to insert them into a wild-type strain by electroporation and homologous recombination. Our initial attempts at transformation with standard plasmid vector systems were not successful. However, we have recently achieved stable transformation of *Xylella* with the vector pCL1920. We also had success with the transformation and mutagenesis system that Dr. Bruce Kirkpatrick at UC Davis recently published. We are therefore confident that our strategy will yield the desired mutants during this next year. These will then be tested for virulence and colonization of grapevines.