

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

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## ABSTRACT

Susceptible *Vitis vinifera* responds to *Xylella* infection with a massive re-direction of gene transcription involving >800 genes with strong statistical support. This number is increased from previous estimates based on use of a more sensitive and robust statistical method known as linear models for microarray data (LIMMA). The transcriptional response to *Xylella* infection is characterized by increased transcripts for phenylpropanoid and flavonoid biosynthesis, ethylene production, adaptation to oxidative stress, and homologs of pathogenesis related (PR) proteins, and decreased transcripts for genes related to photosynthesis. A survey of 22 transcripts by means of *in situ* hybridization reveals that a majority of transcriptional activity is associated with phloem and cortical tissues, consistent with the presence of the pathogen in adjacent xylem elements. DNA sequence analysis of regions 5' to the transcription site for ~200 differentially expressed genes provides a rich source of new gene promoters and the possibility of *in silico* analysis of regulatory cis-elements.

In addition to highlighting potential metabolic and biochemical changes that are correlated with disease, the results suggest that susceptible genotypes respond to *Xylella* infection by induction of a limited, but apparently inadequate, defense response. We have also tested the hypothesis that Pierce's disease results from pathogen-induced drought stress. We compared the transcriptional and physiological response of plants treated by pathogen infection, low or moderate water deficit, or a combination of pathogen infection and water deficit. Although the transcriptional response of plants to *Xylella* infection was distinct from the response of healthy plants to moderate water stress, we observed synergy between water stress and disease. In particular, water stressed plants exhibit a stronger transcriptional response to the pathogen. This interaction was mirrored at the physiological level for aspects of water relations and photosynthesis, and in terms of the severity of disease symptoms and pathogen colonization, providing a molecular correlate of the classical concept of the disease triangle.

## INTRODUCTION

All organisms adapt to external stressors by activating the expression of genes that confer adaptation to the particular stress. In the case of Pierce's disease, such genes are likely to include those coding for resistance or susceptibility to *Xylella fastidiosa* (Xf).

Genomics technology offers an opportunity to monitor gene expression changes on a massive scale (so-called "transcriptional profiling"), with the parallel analysis of thousands of host genes conducted in a single experiment. In the case of Pierce's disease of grapes, the resulting data can reveal aspects of the host response that are inaccessible by other experimental strategies. In May of 2004, the first Affymetrix gene chip was made available for public use, with ~15,700 *Vitis* genes represented. This gene chip has been developed based primarily on collaboration between the Cook laboratory and researchers at the University of Nevada-Reno (Goes da Silva et al., 2005). With the arrival of the Affymetrix gene chip, we are poised to make a quantum leap in the identification of host gene expression in response to Xf.

In addition to enumerating differences between susceptible and resistant genotypes of *Vitis*, this research is testing a long-standing but largely untested hypothesis that pathogen-induced drought stress is one of the fundamental triggers of PD symptom development. The utility of this type of data will be to inform the PD research community about the genes and corresponding protein products that are produced in susceptible, tolerant and resistant interactions. Differences in the transcriptional profiles between these situations are expected to include host resistance and susceptibility genes, and thus provide the basis for new lines of experimental inquiry focused on testing the efficacy of specific host genes for PD resistance. It should be possible, for example, to determine the extent to which resistance responses in grapes are related to well-characterized defense responses in other plant species (e.g., Maleck et al 2002; Tao et al 2003; de Torres et al 2003).

Three co-lateral benefits from the identification of pathogen-induced genes are: (1) the promoters for such genes are candidates to control the expression of transgenes for resistance to Pierce's disease, (2) the protein products of induced genes may have roles in disease resistance, and (3) knowledge of host gene expression can be used to develop improved diagnostic assays for disease. In a related project, we are currently characterizing pathogen-responsive promoters, which will facilitate testing of candidate genes for resistance phenotypes.

## OBJECTIVES

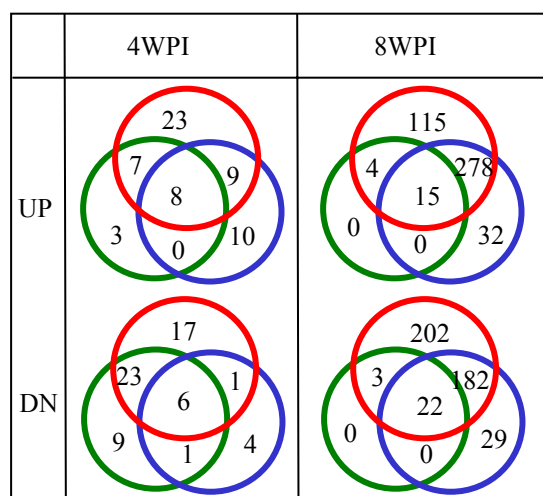
1. Identify genes and gene pathways in susceptible *Vitis vinifera* correlated with *Xylella infection*: (a) identify *Xylella*-responsive genes in *V. vinifera*, (b) distinguish early from late gene expression, and (c) determine the correlation between drought stress and Pierce's disease.
2. Determine host genotype affects on gene expression in response to *Xylella* infection: (a) susceptible *Vitis vinifera* compared to tolerant and/or resistant genotypes of *Vitis* species, and (b) comparison of pathogen-induced gene expression with gene expression triggered by salicylic acid and ethylene.
3. Detailed analysis of candidate genes: (a) Real Time PCR to validate candidate genes identified in objectives 1 and 2, (b) Real Time PCR to study kinetics and specificity of the host response in susceptible and resistant genotypes, and (c) in situ hybridization to establish precise location of plant gene expression relative to bacterial infection.

## RESULTS

### Objective 1 Activities

Microarray experiments under Objective 1 were completed in the prior year of this project. In the current project period we have reanalyzed the Affymetrix data set by a more robust and sensitive statistical approach known as LIMMA (Smyth 2005). Figures 1 and 2 present the overall data in the context of a Boolean diagram and 2-dimensional hierarchical heat map, respectively. Briefly, we have identified 883 differentially expressed genes, of which 448 are up-regulated and 435 are down-regulated. We are currently annotating this expanded gene set by means of MapMan and AraCyc tools, so that genetic and biochemical pathways are more readily inferred from the data.

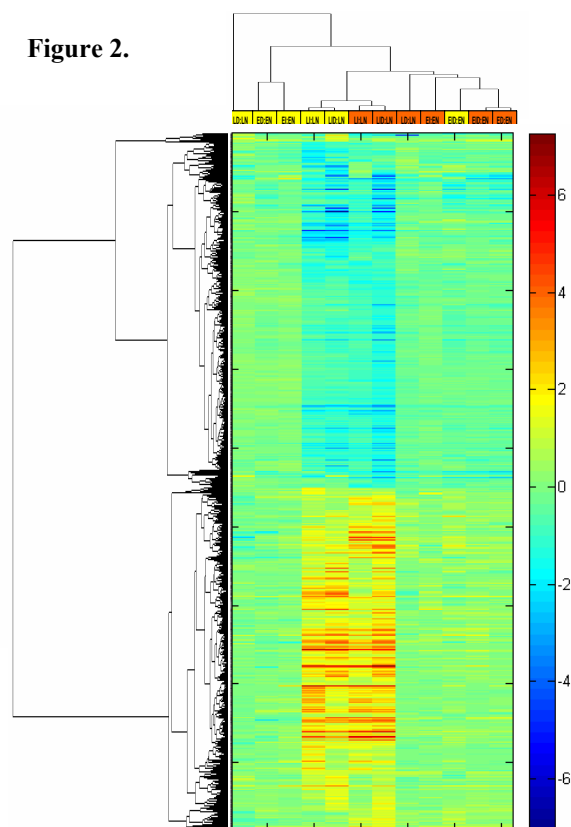
**Figure 1.**



**Figure 1.** Boolean diagram of 883 differentially regulated genes. WPI, weeks post inoculation; UP, up regulation; DN, down regulation; ●, drought; ●, infection; ●, drought + infection.

**Figure 2.** 2-D cluster and heat map of LIMMA pairwise comparisons for 883 genes. The horizontal axis presents experiments by treatments (drought, infection, infection + drought, healthy well watered). The vertical axis presents hierarchical clustering of 883 differentially expressed genes. Colors in the heat map correspond to levels of gene induction/repression, with red-yellow representing induced transcripts and blue representing repressed transcripts. Lanes 4-7 correspond to genes induced or repressed in infected symptomatic plants, with or without water deficit.

**Figure 2.**



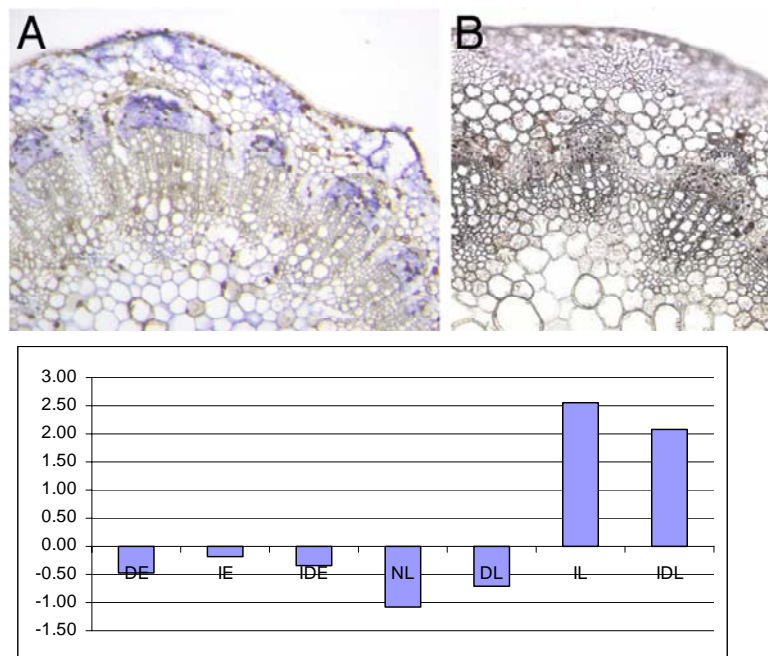
Within the context of the recent LIMMA analysis, we have determined that less than 4% of the genes influenced by *Xf* infection are also influenced by water deprivation. This limited overlap suggests that the host response to *Xylella* is substantially dissimilar to a response to water stress. Among the *Xylella*-responsive genes, 50 genes exhibited significant transcriptional induction or repression within four weeks of infection, considerably in advance of symptom development. We are particularly interested in using real time RT-PCR to determine whether any of these 50 genes may represent rapid responses to the presence of the pathogen. Major categories of early genes include cell wall modifying enzymes/proteins, proteins involved in production of the stress hormone jasmonic acid, pathogenesis-related (PR) proteins, enzymes of the flavonoid biosynthesis pathway, and regulatory proteins involved in gene transcription and protein turnover. The largest category of down-regulated early genes is implicated in photosynthesis.

### Objective 2 Activities

To test the effect of host genotype on gene expression, we conducted a replicated greenhouse experiment involving susceptible plants of *Vitis vinifera* Cabernet Sauvignon and reportedly tolerant hybrids (Blanc du Bois, Champenel, and Black Spanish). Four replicates of each genotype were inoculated with the Fetzner strain of *Xf* and petiole and leaf samples were collected at 2, 4, 8 and 12 weeks post inoculation. Contrary to expectations, severe symptoms developed in Champenel and Black Spanish vines, while only limited symptoms were evident in Blanc du Bois. Petioles are currently being analyzed to determine (1) the extent of pathogen movement in each variety over time, and (2) host gene expression using real time RT-PCR. Depending on the results of these ongoing analyses, we will determine whether to use the collected leaf samples for Affymetrix profiling experiments.

### Objective 3 Activities

24 candidate genes were selected from the Affy chip analysis and used to develop a RealTime PCR assay; this significantly extends our previous RealTime data involving 4 genes. The assay was used to validate results from the Affy chip, especially with respect to quantitative estimates of gene expression. The specificity of host gene expression was addressed by RealTime PCR experiments on field grown plants. *In situ* hybridization experiments were used to evaluate the spatial distribution of transcripts in petiole and leaf samples, with the majority of 22 genes tested revealing transcript accumulation in phloem and cortical tissue, as shown by example in Figure 3.



**Figure 3.** *In situ* hybridization analysis and Affymetrix expression data for a *Xylella*-induced thaumatin transcript.

Panel A, transcript is evident as blue coloration in phloem and cortical tissue of an infected petiole.

Panel B, limited transcript is evident in the cortical tissue of a non-infected petiole.

Panel C, transcript abundance in leaves determined by means of Affymetrix microarray analyses. Scale represents log2 values for gene expression. DE, early drought; IE, early infection; IDE, early drought + infection; NL, non-infected late; DL, late drought; IL, late infection; IDL, infection + drought late. The early time point corresponds for 4 weeks post inoculation (prior to symptom development), while the late time point corresponds to 8 weeks post inoculation (subsequent to symptom development).

### Promoter analysis of *Xylella*-induced genes

Promoter sequences for 240 *Xylella*-induced transcripts are being isolated by means of BLASTN analysis against the *Vitis vinifera* whole genome shotgun dataset. In an initial analysis, transcripts for 32 genes were used to query the dataset, yielding promoter fragments for 30 genes of which 25 promoters contained 1-15kbp of sequence 5' of the translation start site. BLAST analysis with a larger set of the 240 most induced/repressed transcripts is currently underway. Ultimately we intend to query all ~800 *Xylella*-induced transcripts for 5' promoter sequences, producing a sizeable dataset for mining putative cis-regulatory elements and promoter selection.

### CONCLUSIONS

We have identified numerous genes where expression is induced strongly in diseased tissue. The largest fraction of the *Xylella*-responsive transcriptome is synergistically modified in plants that are doubly-treated by pathogen infection and moderate drought stress. One important class of synergistically up-regulated genes encode enzymes of the flavonoid biosynthesis pathway, while the predominant class of synergistically down regulated are from the photosynthesis pathway. A smaller fraction of *Xylella*-responsive transcripts are responsive to the pathogen, but apparently not sensitive to water status. Many of these later genes are annotated as PR proteins implicated in host defense. Taken together, the results are consistent with the existence of two distinct classes of transcriptional response in grapes to *Xylella*. One response is sensitive to plant water status and results in redirection of flavonoid synthesis and photosynthesis genes, and one response is independent of plant water status leading to the activation of defense-related transcripts. Although we observed limited overlap in the genes induced in response to moderate drought stress and the genes induced in diseased tissue, we cannot rule out the possibility that a more severe drought stress may lead to an increase in the coincidence of Pierce's disease and drought-associated gene expression.

Real time RT-PCR with 24 candidate genes was used to evaluate gene expression as a check of the Affymetrix microarray data. The RealTime data confirm the major conclusions drawn from the Affymetrix GeneChip, including the correlation between pathogen infection and gene expression, and the synergistic interaction between infection and water deficit as it relates to the "strength" of gene induction or repression. Genes identified in the Affymetrix microarray analysis were also subject to *in situ* hybridization analysis of petiole tissue. The results indicate that the majority of *Xylella*-induced transcripts exhibit up-regulation specifically in phloem and cortical tissues. Current work focuses on localizing gene expression in infected and non-infected leaf tissue.

### REFERENCES

- de Torres, M., et al. 2003. Expression profiling of the host response to bacterial infection: the transition from basal to induced defence responses in RPM1-mediated resistance. *The Plant Journal* 33:665-676.
- Goes da Silva et al. 2005. Characterizing the grape transcriptome: Analysis of ESTs from multiple *Vitis* species and development of a compendium of gene expression during berry development *Plant Physiology* 139: in press.
- Iandolino, et al. 2004. A rapid and efficient protocol for the isolation of high-quality total RNA from grapevine (*Vitis vinifera* L.) tissues containing high concentrations of polyphenols, polysaccharides and proteins. *Plant Molecular Biology Reporter* 22:269-278.
- Maleck, K., et al. 2001. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. *Nature Genetics* 26:403-410.
- Smyth, G. K. (2005). Limma: linear models for microarray data. In: *Bioinformatics and Computational Biology Solutions using R and Bioconductor*, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420.
- Tao, Y., et al. 2003. Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *The Plant Cell* 15:317-330.

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## FUNCTIONAL TESTING OF PIERCE'S DISEASE-SPECIFIC PROMOTERS FROM GRAPE

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### ABSTRACT

Several projects working toward understanding the genetic basis for susceptibility or resistance at the molecular level in grape to Pierce's disease need to test the ability of candidate genes to alter disease progression or activity of *Xylella fastidiosa* (*Xf*) *in planta*. A major limitation to developing assays to test candidate genes is the ability to regulate expression of these genes in time and space relative to the presence of the pathogen. Currently, we are able to express any candidate transgene constitutively and at high levels but cannot modulate gene expression in relation to pathogen presence. The goal of this research is to clone and characterize unique DNA sequences from grape that specifically regulate the expression of grape genes in tissues that are infected with *Xf*. The result will be the delivery of one or more *Xylella*-responsive promoters from grape to drive the bioassay of any candidate gene at locations where the bacteria reside or where bacterial signals extend. The promoters also would be capable of either increasing or suppressing the expression of a gene of interest. Grower acceptance as well as enabling the most subtle level of transgene expression requires that these genes be expressed only when and where they are needed.

### INTRODUCTION

Among the potential solutions to Pierce's disease (PD) in grapes are approaches based on gene transfer technology. One research priority identified in 2004 by the PD/GWSS Program was the need to identify, clone and characterize unique DNA sequences that specifically regulate the expression of grape genes in tissues that are infected with *Xylella fastidiosa* (*Xf*). This means the candidate genes driven by such promoters will be off (not expressed) and only on (expressed) when the bacteria or their secreted signals are present in the vascular system of the grape plant. Emphasis was placed on the urgency and practical utility of isolating promoters of PD responsive genes. Transgenic technology offers the possibility of modifying specific traits (e.g., PD susceptibility) based on the introduction of novel genes. One of the major bottlenecks in the genetic engineering of grape (or any plant) is the absence of suitable promoters - sequences that regulate gene expression in particular tissues (e.g., vascular tissue) or in response to particular situations (e.g., sharpshooter feeding or *Xylella* infection). In the absence of tissue or response-specific promoters, transgenic strategies for control of PD can use only so-called constitutive promoters. By definition, constitutive promoters are expressed in all cells all the time. By contrast, *Xylella*-inducible promoters have the potential to confer transgene expression at the time and location of bacterial infection, thus delivering therapeutic proteins more precisely to their intended site of action. In addition to increasing the specificity of transgene expression, such promoters should reduce the possibility of unintended side effects in non-target tissues.

In addition to their utility for engineering PD resistance in grape, the advent of *Xf*-induced reporter gene expression would provide an extremely powerful tool to examine host responses in their intact cellular and tissue context. With such tools, it should be possible to examine the chemical and/or physical cues from the insect or pathogen that trigger host gene expression and the deleterious effect of the disease. For example, we have recently determined that host gene expression is induced specifically in live cells of the phloem (Cook 2005). It remains uncertain, however, whether gene expression occurs only in phloem tissue that borders infected xylem elements, or whether bacterial infection can induce host gene expression at a distance. Promoter-GFP fusions being developed in this project should help answer such questions.

### OBJECTIVES

1. Produce stable transgenic grape plants containing promoter-GFP fusions that respond to *Xylella* infection. At this point 4 constructs bearing promoters derived from genes induced in *Xf* infected grape but not in healthy grape tissue will be transformed into Thompson Seedless plants by the UC Davis Plant Transformation Facility. There are four genes, G8946, G9353, G7061 and G7172 to initially be expressed transgenically. These genes were shown to be induced in the phloem of infected petioles and leaves, adjacent to sites of probable *Xf* infection in the xylem as indicated in the 2005 symposium report (Gilchrist et al. 2005). Expected delivery of the first set of transgenics is November 2006.
2. Develop a rapid *in planta* assay to characterize promoter-GFP expression in a series of independent grape transformants derived from each promoter fusion via detached leaf/branch bacterial uptake system. The goal is to identify a series of independent lines for each fusion where transgene expression is strong and reproducible. GFP expression will be monitored in both excised leaves and branches (described below), as well as in stem-inoculated whole plants. The progression of induction will be assessed qualitatively in relation to location bacteria monitored by RT-PCR of both the promoter expression and the presence of the bacterium.