CORRELATION BETWEEN RESISTANCE TO PIERCE'S DISEASE AND *XYLELLA* STRAIN VIRULENCE USING PARTIALLY PURIFIED CULTURE FILTRATE

Project Leaders:
Jiang Lu
Center for Viticulture and Small Fruit Research
Florida A & M University
Tallahassee, FL 32317

Kenneth Bloem
USDA, APHIS, PPQ-CPHST
at Center for Biological Control
Florida A & M University
Tallahassee, FL 32307

Researcher:
Oulimathe Paraiso
Center for Biological Control
Florida A & M University
Tallahassee, FL 32307

Cooperators:
Mehboob Sheikh
Center for Vitic. & Small Fruit Research
Florida A & M University
Tallahassee, FL 32317

Haenkun Yun
Center for Vitic. & Small Fruit Research
Florida A & M University
Tallahassee, FL 32317

Donald Hopkins
Mid-Florida Res. & Educ. Ctr.
University of Florida
Apopka, FL 32703

Reporting Period: The results reported here are from work conducted from March 2003 to September 2004.

ABSTRACT
Previous research at the FAMU Center for Viticulture suggested that cells of a virulent strain of *Xylella fastidiosa* (*Xf*) may produce toxic compounds that could be used to determine varietal susceptibility to Pierce's disease (PD) in grapes. I n the experiments reported here, when grape leaves were challenged with partially purified culture filtrate of *Xf* with different levels of virulence, positive correlations between the degree of leaf necrosis and (1) the virulence of the *Xf* strain and (2) the level of PD resistance were observed.

INTRODUCTION
Pierce's disease (PD), a lethal disease of grapevine, is caused by the bacterium *Xylella fastidiosa* (*Xf*) (Proteobacteria: Xanthomonadales) and is spread by leafhoppers known as sharpshooters. *Xylella fastidiosa* is native to the southeastern U.S., where it reproduces in ornamentals such as crape myrtle, eucalyptus, and hibiscus, but also in various crop plants including citrus, avocado and grapes (Blua et al. 1999). In Florida and other southeastern States, the abundance of *Xf* and vectors such as the glassy-winged sharpshooter (*Homalodisca coagulata*) has precluded commercial production of European grape varieties. The first evidence of PD infection usually is a drying or "scorching" of leaves. Typically, the leaves dry progressively over a period of days to weeks, showing a series of concentric zones of discolored and dead tissue. Vines develop symptoms as the bacteria multiply and begin to block the water conducting system and reduce the flow of water to affected leaves. However, Hopkins (1983) reported that only about 40% of the xylem vessels of infected plants have bacterial occlusions and plants with this percentage of non-functioning vessels typically do not show symptoms of water stress. The PD bacterium also has been reported to produce a phytotoxin or phytotoxins that may damage plant tissues and play an important role in disease initiation and development (Lee 1982).

OBJECTIVES
1. Determine whether partially purified culture filtrate from virulent, weakly virulent and avirulent strains of *Xf* would produce different levels of necrosis when applied to leaves of a given variety of grape.
2. Determine whether partially purified culture filtrate from a given strain of *Xf* would produce different levels of necrosis when applied to leaves from susceptible, tolerant and resistant varieties of grape.

RESULTS AND CONCLUSIONS
Cultures of virulent (PD002), weakly virulent (PD91-2) and avirulent (PD F1) strains of *Xf* were centrifuged to remove cells. The supernatant was filtered and then extracted with ethyl acetate, and the eluate was evaporated to dryness. The powder was then reconstituted in distilled water and applied to the surface of detached leaves of different grape varieties that had been wounded with a sharp needle. After 48 h, the leaves were scored based on the percentage of the leaf surface with necrotic lesions (Table 1).

In general, the mean percentage of leaf necrosis was greater when leaves were challenged with partially purified culture filtrate (PPCF) from the more virulent strains of *Xf*. For example, the leaf necrosis rating for 'Chardonnay', a highly PD susceptible variety of *V. vinifera* grape, was 1.5 for the virulent strain of *Xf*, 0.9 for the weakly virulent strain and 0.3 for the

avirulent strain. The leaf necrosis ratings for Black Beauty, a PD tolerant variety of muscadine grape, were 0.7, 0.4 and 0.1 when challenged with PPCF from the virulent, weakly virulent and avirulent strains of *Xf*, respectively.

In addition, leaves from susceptible varieties of grape generally produced greater levels of necrosis than did leaves from tolerant and resistant varieties. For example, the mean percentage of leaf necrosis for 'Chardonnay', 'Blanc du Bois' (a PD tolerant Florida hybrid bunch grape), Alachua and Noble (PD resistant muscadine grapes) were 1.5, 1.0, 0.6 and 0.0, respectively, when challenged with the PPCF from the virulent strain of *Xf*. Similar and consistent trends also were observed when using PPCF from the weakly virulent and avirulent strains of *Xf*, but as mentioned before, the leaf necrosis ratings were lower, which resulted in less overall differences between susceptible and resistant varieties.

These results suggest that *Xf* may produce extra cellular "toxin(s)" that could cause necrotic lesions when applied to grape leaves and that might have potential in screening grape germplasm and hybrids for PD resistance. The "toxins" extracted from the culture filtrate of more virulent strains of *Xf* produced more necrosis than did the "toxins" from less virulent strains. Leaves from susceptible varieties of grape also reacted more strongly to these "toxins" than did the leaves from resistant grape varieties. At this time the nature of the "toxin(s)" is not known, nor is it known whether the different strains of *Xf* produce different quantities or types of these "toxins". Future studies will attempt to answer these questions and expand the number of PD susceptible and resistant grape varieties and *Xf* strains evaluated with this test.

A

B

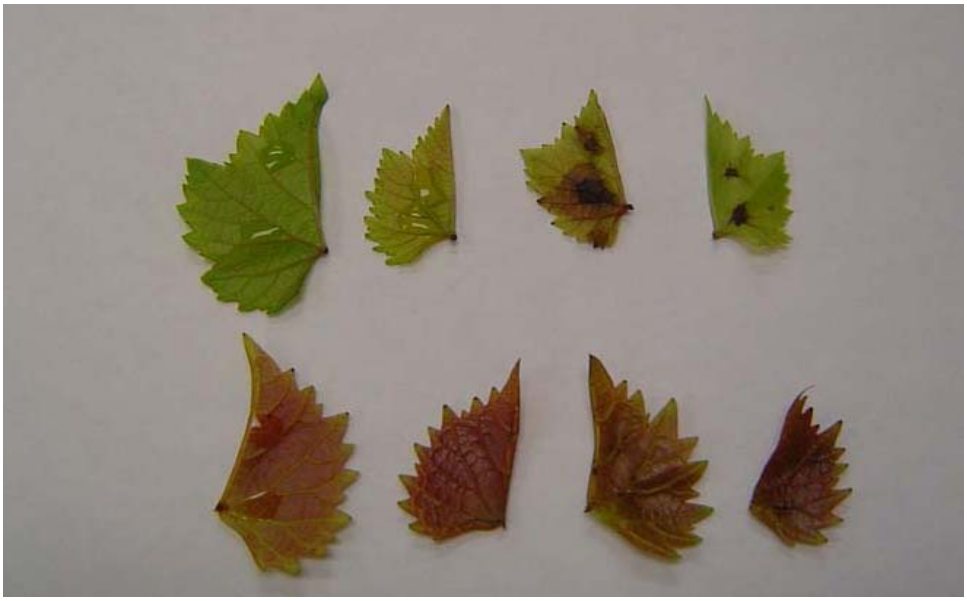


Figure 1. An example of the type of symptoms caused by *Xf* culture filtrate in young 'Chardonnay' (A, PD susceptible) and 'Noble' (B, PD resistant) grape leaves. Lanes 1 and 2 = control leaves treated with distilled water, lane 3 = leaves treated with undiluted culture filtrate from a virulent strain of *Xf* PD002, and 4 = leaves treated with diluted (1:2 vol/vol) culture filtrate of *Xf* PD002. Incubation time was 48 h.

Table 1. Response of grape leaves to partially purified culture filtrate from virulent (PD002), weakly virulent (PD91-2), and avirulent (PD-F1) strains of *Xf* as measured by the amount of necrosis produced. Leaf necrosis ratings were: 0 = no necrotic lesions; 1 = 25% or less of the leaf surface with necrotic lesions; 2 = 26-50% necrosis; 3 = 51-75% necrosis; 4 = 76-100% necrosis. The level of PD resistance: S = Susceptible, T = Tolerant and R = Resistant.

Virulent Strain (PD002)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	1	1	1	1	2	2	1	2	2	2	1.5	0
Blc. Bois (T)	1	1	1	1	1	1	0	1	1	2	1.0	0
Carlos (T)	0	1	0	1	1	1	2	0	1	1	0.8	0
Bl. Beauty(R)	1	1	0	1	1	1	1	1	0	0	0.7	0
Alachua (R)	1	0	1	0	1	0	1	1	0	1	0.6	0
Fry (R)	1	0	0	1	1	0	1	1	0	0	0.5	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Weakly Virulent Strain (PD91-2)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	1	1	1	0	0	1	1	2	1	1	0.9	0
Blc. Bois (T)	1	0	0	0	1	1	0	1	1	0	0.5	0
Carlos (T)	0	0	0	1	0	1	0	0	1	0	0.3	0
Bl. Beauty(R)	0	0	1	1	0	0	1	0	1	0	0.4	0
Alachua (R)	0	1	0	0	0	1	0	0	1	0	0.3	0
Fry (R)	0	0	1	1	0	0	0	0	0	0	0.2	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Avirulent Strain (PD-F1)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	0	1	0	0	0	0	1	0	1	0	0.3	0
Blc. Bois (T)	0	0	0	0	0	1	0	0	0	0	0.1	0
Carlos (T)	0	0	0	0	0	0	0	0	0	0	0.0	0
Bl. Beauty(R)	0	0	0	0	0	0	0	0	1	0	0.1	0
Alachua (R)	0	0	0	0	0	0	0	0	0	0	0.0	0
Fry (R)	0	0	0	0	0	0	0	0	0	0	0.0	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Abbreviations: Chard = Chardonnay, Blc. Bois = Blanc du Bois, Bl. Beauty = Black Beauty.

REFERENCES

Blua, M. J., P. A. Phillips, and R. A. Redak. 1999. A new sharpshooter threatens both crops and ornamentals. Calif. Agric. 53: 22-27.

Hopkins, D. L. 1983. Variability of virulence in grapevine among isolates of the Pierce's disease bacterium. Phytopath. 74: 1395-1398.

Hopkins, D. L. 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopath. 27: 271-290.

Lee, R. F., B. C. Raju, G. Nyland, and A. C. Goheen. 1982. Phytotoxin(s) produced in culture by the Pierce's disease bacterium. Phytopath. 72: 886-888.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service, the USDA Cooperative State Research, Education, and Extension Service, and the Florida Viticulture Advisory Council.

TOWARDS IDENTIFYING PIERCE'S DISEASE RESISTANT GENES FROM A NATIVE AMERICAN GRAPE SPECIES (*VITIS SHUTTLEWORTHII)* – A GENOMICS APPROACH

Project Leaders:
Jiang Lu
Center for Viticulture and Small Fruit Research
Tallahassee, FL 32317

Wayne Hunter
USDA, ARS Horticultural Research Laboratory
Fort Pierce, FL 34945

Cooperators:
Hong Hunag, Xia Xu, and Zhongbo-Ren
Center for Viticulture and Small Fruit Research
Florida A&M University
Tallahassee, FL 32317

Phat Dang
USDA, ARS Horticultural Research Laboratory
Fort Pierce, FL 34945

Reporting Period: The results reported here are from work conducted from January 2003 to September 2004.

ABSTRACT

INTRODUCTION

There are over 160,000 grape ESTs in the public data bases and the vast majority of these ESTs were generated from the European grape varieties (*Vitis vinifera*). However, the European grapes are highly susceptible to the Pierce's disease and they are not necessary possessing all the genes required for providing a full protection against the GWSS and *Xf* attack. On the other hand, PD resistant sources exist in some native North American grape species, particularly those species originated in the southeast United States. For example, *Vitis shuttleworthii*, a species originated from the southeast United States, is considered to be one of the most PD resistant grape, which has long been used for developing PD resistant grape varieties for the deep south - a most severe PD infected area. We therefore propose to search for PD resistant genes from the *Vitis shuttleworthii* grape.

The Viticulture Center at Florida A&M University and the USDA-ARS Horticultural Laboratory at Fort Pierce (Florida) jointly initiated a grape EST project from the native American grape -*Vitis shuttleworthii*, aiming to identify and isolate grape disease resistant genes including the Pierce's disease resistant genes. We have sequenced 30,000 ESTs, and have several on-going experiments for expression analysis and marker development for identifying the PD resistant genes.

OBJECTIVES

The objectives of this research are to identify/isolate PD resistant genes from *Vitis shuttleworthii* grapes and develop EST derived molecular markers for PD resistance. Specifically, the project is gearing towards to: 1) discover genes for PD resistance from *Vitis shuttleworthii* grapes; 2) conduct comparative genomics analysis between *V. shuttleworthii, V. vinifera* grapes and other plant species; 3) develop SSR and SNP markers for PD resistance, which will be used for accelerating the development of PD resistant grape varieties.

RESULTS AND CONCLUSIONS

We have sequenced 30,000 ESTs from a clone of *V. shuttleworthii* grape. Blasting analysis revealed that 13% of the *V. shuttleworthii* ESTs are unique when compared to the existing *Vitis vinifera* NCBI databases, and 3% of the ESTs did not find any homologous sequences among all plant ESTs reported in NCBI. Overall, approximately 7% of ESTs were related to disease / pest defense or stress tolerance genes, and it is obvious that these genes are abundant in the *V. shuttleworthii* grape (Table 1, Table 2).

Table 1. Comparison of transcription factor (TF) families in grape (*V. shuttleworthii, V. vinifera*), *Arabidopsis* and *R*ice

V. vinifera	Arabidopis	Rice
124	190	156
69	144	143
114	105	125
67	82	71
131	72	83
34	36	21
31	28	8
121	81	75
10	6	5

Table 2. Comparison of disease resistant gene (R-gene) families in grape (*V. shuttleworthii, V. vinifera*) and *Arabidopsis*

R-gene Class	Number in V. shuttleworthii	Number in V. vinifera	Number in Arabidopis
TIR-NBS-LRR	11	64	85
CC-NBS-LRR	9	51	41
NBS-LRR	9	64	10
TIR-NBS	3	19	17
CC-NBS	5	30	4
TIR	18	82	36

A series of experiments are being conducted to identify and isolate PD resistant genes through gene expression profiling analysis by using DNA microarrays. Specifically, a comparative analysis of transcriptional profiles of 1) unchallenged *V. shuttleworthii* grapes (control), *Xf* challenged *V. shuttlewothii* grapes (samples will be collected on different timeframes after infection).

For marker development, we are developing SNP and SSR markers from our *V. shuttleworhtii* sequence data set and the *V. vinifera* ESTs in the public domain. Aligned sequences will be mined for Single Nucleotide Polymorphism. A preliminary screening of the SNP and SSR marker from the 12,056 *V. shuttleworthii* ESTs indicated that the SNP and SSR markers are abundant in *V. shuttleworthii* grapes, and around 800 candidate SSR and SNP sites have already been identified. Table 3 shows the distribution of the di-, tri-, and tetra- SSRs from *Vitis shuttleworthii* ESTs, and Table 4 shows the abundant SSRs motifs from *Vitis shuttleworthii* ESTs. We have designed and synthesized the PCR primer pairs using computer software such as Primer3 to flank the SSR loci (partially shown in Table 5). Verification of these primers with PCR amplification on selective grape DNA templates is under way.

Table 3. Distribution of EST derived SSRs from *Vitis shuttleworthii*

Number of ESTs	Number of SSR-ESTs	Motifs		
		di-	tri-	tetra-
10,995	401(3.651[1])	82(20.32[2])	306(76.5)	13(3.2)

[1.] SSR-EST percentage in total EST
[2.] di-nucleotide motif percentage in SSR-EST.

Table 4. Distribution of the abundant (>5) SSR-ESTs among the *V. shuttleworthii* EST data set.

SSR Motif	Number of ESTs
GA/CT	36
AT/TA	13
CAA/GTT	90
ACC/TGG	34
TCT/AGA	19
CAG/GTC	15
AAG/TTC	14
CAC/GTC	14
CTT/GAA	13
CCA/GGT	12
CCT/GGA	12
TGA/ACT	9
TCC/AGG	8
CAT/GTA	7
GAT/CTA	6
TGC/ACG	6
CTC/GAG	5
Total	313

Table 5. A selective set of SSR primer pairs from the *Vitis shuttleworthii* ESTs

Repeat	*Left Sequence*	*Right Sequence*	*Product Size*
GTCGTCGTCGTCGTCGTCGTC	TACAAGAGCCAAGAGGGATT	GGATAACGAAGGAGACAGAGT	245
AGCAGCAGCAGC	AGGGAGATGACAAAGATGAAG	CCAAACACCGTAGGAGAGA	367
AACAACAACAACAAC	AATAATAAGAAGGAGATGCGG	GTTGTGGTGGTCGTGAAG	367
AGCAGCAGCAGC	CAGAGTGTCAGCACAGCA	GCGTTTCTCAAGGTTCTACTT	368
AACAACAACAACAAC	TGACTGGCATACTGATTTACC	CCCAATGAACTACCTTTACCT	368
CGGCGGCGGCGGCGGCGG	ACCCAATGAACTACCTTTACC	AGGAACAAGACAAACAATACACT	113
CCTCCTCCTCCTCCTCCTCCT	TTTATCCCAACAATCAGG	CTTTCACAGCAGAAGAGTT	226
CCTCCTCCTCCTCCTCCTCCT	GCCTTGGACCGAACTATC	CTAAGAAACACCATTCATCAG	226
GAGAGAGAGAGAGAGAGAGAGA	CGACCTAAGAAACACCATTC	CCTTGGACCGAACTATCTG	292
ACCACCACCACCACCACC	CGCATCAGAAGTCATCAAC	ACCCTCACTCTCACACTCAC	238
TCCTCCTCCTCCTCCTCC	ACGGAAGAAGAGAAGAAAGAG	ATCCACCGAAACAAACTTAC	133
AGAAGAAGAAGAAGAAGA	ACAAAGCAGGTAAGTAGCAAA	AAGACGGAAGAAGAGAAGAAA	233
TCTCTCTCTCTC	GTGATTGTTACCGACCTTGA	ATTCCCTTCTTCTCCTTTACC	195
TCTCTCTCTCTC	CCTCGGAAACAAACTTACA	CGAAGAAGAGAAGAAAGAGAAA	195
TGATGATGATGATGATGAGGATGATGA	AAGACCGAAGAAGAGAAGAAA	TAATACCGTGGAAATCACAAA	281
ACCTACCTACCTACCTACCT	TTACCCGACACTGGACAC	ACTTACCACCGAGATGAGG	266

After the potential SNP-EST and SSR-EST are verified, PD segregating populations will be used for marker development. Several populations derived from the hybridization of Native American species/hybrids and *V. vinifera* grapes will be candidates for this purpose. For example, a 183-seedling population of N18-6 x 'Cabernet Sauvignon' has been evaluated for PD resistance for several years in our vineyard. 'N18-6' is a breeding line highly resistant to PD while 'Cabernet Sauvignon' is the best known wine grape variety highly susceptible to PD. Segregating analysis revealed that three dominant genes provide full resistance to the Pierce's disease**.**

FUNDING AGENCIES
Funding for this project was provided by the USDA Cooperative State Research, Education, and Extension Service, and the FAMU-ARS Science Center.

FIELD EVALUATION OF GRAPE ROOTSTOCK RESPONSE TO NATURAL INFECTION BY PIERCE'S DISEASE

Project Leaders:
Jiang Lu and Zhongbo Ren
Center for Viticulture and Small Fruit Research
Florida A&M University
Tallahassee, Florida 32317

Peter Cousins
Plant Genetic Resources Unit, USDA, ARS
New York State Agricultural Experiment Station
Geneva, New York

Reporting Period: The results reported here are for work conducted from November 2, 2003 to October 31, 2004.

ABSTRACT
To understand the adaptation of grape rootstocks commonly used in major grape production areas worldwide to Florida, where Pierce's disease (PD) is the primary limiting factor in grape production, ten important grape rootstocks were cultivated at the experimental vineyard, Florida A&M University, Tallahassee, Florida. Disease resistance and symptoms and growing performance were evaluated. PD symptoms were scored in September and October 2002, 2003, and 2004, with leaf symptoms the basis of scoring. None of the grape rootstocks was completely resistant to PD and the severity of PD varied with rootstock cultivar. St George and Ramsey showed least PD symptoms. Freedom and 44-53 succumbed to PD by the 2004 rating period; of the surviving rootstocks, 3309C had the highest PD score. Overall vine survival, evaluated in 2002, 2003, and 2004, varied among the rootstocks. Based on the performance of ungrafted vines, St George and Ramsey are the most suitable rootstocks in this north Florida environment, where natural infection by PD is very high and vectors and inoculum are abundant.

INTRODUCTION
Rootstocks are used widely in viticulture to provide resistance against soil pests and pathogens and improve scion performance. Choice of rootstock depends on pest populations, soil, and growing conditions. The grape rootstocks in common use world wide are deployed primarily to provide phylloxera and nematode protection (Bouquet 1980, Einset and Pratt 1975, Winkler et al 1974). In contrast, Pierce's disease (PD), caused by gram-negative bacterium *Xylella fastiosa* (*Xf*), is the primary limiting factor of growing *Euvitis* grape in the southeast United States (Lu and Ren 2002, Chen et al 2001). Pierce (1905) reported that rootstock variety affected expression of "California vine disease" (now known as Pierce's disease) in grape. Grape rootstock trials in Mississippi showed a large effect of rootstock trial on vine longevity in a region recognized for high Pierce's disease pressure (Loomis 1965, 1952, Magoon and Magness 1937). In humid and hot regions of the United States, such as Florida, bunch grapes often are highly susceptible to pests and diseases (Olien and Hegwood 1990). When the Florida hybrid bunch grape cultivar Blanc du Bois was grafted on to muscadine, which is relatively tolerant or resistant to the bunch grape pests and diseases common in North America, the scion showed a reduction in both PD and anthracnose symptoms and fruiting improved (Ren and Lu 2002). Growing conditions in Florida are harsh-a successful rootstock for grape industry in that area must be tolerant to PD and adapted to the environment. Evaluation of rootstock performance and survival in Florida would provide useful information on rootstocks performance for humid tropical and subtropical environments, especially where PD is prevalent. Greenhouse screening has been used to investigate the PD resistance, tolerance, and susceptibility of grape cultivars. However, field screening is more applicable, since conditions closely match those in a commercial vineyard. When relying on natural infection in the vineyard, there is no need to inoculate vines or maintain colonies of *Xf* or insect vectors. Field screening is cheap, requires no specialized equipment and can be accomplished quickly, with symptom expression being used as the main criterion. Northern Florida is an ideal test environment due to heavy PD pressure, with abundant vectors, including glassy-winged sharpshooter, and inoculum, in contrast to many other locations, especially California, which demonstrate substantial cycling of PD incidence.

OBJECTIVES
1. Evaluate the response of grape rootstocks to natural field infection by Pierce's disease.

RESULTS AND CONCLUSIONS
Ten grape rootstocks (five replicates of two vines each, ten vines total per rootstock cultivar) were planted in the spring of 2001. Vines were bilaterally cordon trained and spur pruned. Pierce's disease (PD) symptoms were scored in 2002, 2003 and 2004, with symptoms on leaves assessed in a numerical scale from 0 to 5. For PD, 0 represented no symptoms, 1 = minor symptoms up to 15% of leaves with marginal necrosis (MN), 2 = 15-30% of leaves with MN, 3 = 30-50% of leaves with MN, 4 = 50-75% of leaves with MN, 5 = over 75% of leaves with MN or vine dead. Vine vigor was surveyed later fall in 2002. The annual shoot and node growth was recorded from ten randomly sampled shoots per plant, and shoot diameter was taken in the middle of 4th node. Node length was calculated with total node numbers and the length of each shoot. Twenty (4 x 5) random shoots were investigated for shoot death rate from each vine: 5 shoots in each canopy quadrant area divided by the main trunk and trellis wire. A shoot was considered as dead if more than half of the shoot had died. Trunk diameters were measured 50 cm above the ground in fall 2003.

All rootstock vines developed PD symptoms, although the severity varied. The least severe PD scores were seen on Ramsey and St George, with average PD scores of 1.1 and 1.4 in 2002, 1.0 and 1.7 in 2003, and 1.2 and 0.9 in 2004, respectively (Table 1). The consistently low PD scores on these varieties over several years demonstrate that Ramsey and St. George are reliably resistant or tolerant of PD in north Florida.

Freedom (3.7 – 5.0 score in 2002-2003) and 44-53 (2.6 – 2.3 score in 2002-2003) did not survive through the rating period of 2004. That Freedom succumbed to PD is not surprising—this rootstock showed the worst PD symptoms of all the rootstocks in the trial in the previous two years of observations. The 44-53 showed severe PD symptoms in 2002 and 2003, but typically its symptoms were not as severe as those on O39-16 and 3309C, so it was surprising that this rootstock succumbed while O39-16 and 3309C remain in the trial.

Of the surviving rootstocks, 3309C (3.0) and 5BB (2.9) had the most severe PD symptoms in 2004. The 3309C has consistently shown heavy PD symptoms and most of the vines of this rootstock have died (Table 3). The slightly less severe average PD score for 3309C probably reflects the survivorship of this vine (heavier symptoms being related to lower survivorship). Although 5BB showed excellent survivorship in earlier years of the study, it is now beginning to develop PD symptoms. The 5C, 110R, and 101-14 showed moderate PD symptoms over the three year period (Table 1). O39-16 symptoms in 2004 were less severe than in earlier years, when it was among the most symptomatic rootstocks; however, symptom severity overall was lower in 2004.

After four growing seasons in Florida's heavy PD pressure, environment, the survival rate was very different among the rootstocks (Table 2). Only Ramsey shows 100% survival. All Freedom and 44-53 vines have been killed by PD and only one of ten 3309C vines remains alive. Vines greatly deteriorated in the third growing season; from 2002 to 2003, the vine losses of Freedom, 44-53 and 3309C were 87%, 70%, and 50%, respectively. There was less change overall in vine survival from 2003 to 2004. Although Freedom and 44-53 completed their precipitous decline, other varieties may be reaching a "steady state" of vine survival, with diminishing losses to PD. The 110R, 5C, and 101-14, noted for their moderate PD symptoms, have survival rates of at least 80%.

Fishleder (2000) examined the response of grape rootstocks to PD in a greenhouse. In contrast to this study, Fishleder inoculated vines with *Xf*. The results from this study largely coincide with and confirm Fishleder's findings. In particular, both this research and Fishleder's work found St George to show only minor PD symptoms; O39-16, 5C, 5BB, 110R were intermediate in symptom development; and 3309C and Freedom showed severe PD symptoms. However, our results contradict Fishleder's regarding Ramsey. While we observed only low levels of PD symptoms in Ramsey, Fishleder found Ramsey to be one of the most symptomatic of rootstocks tested. What accounts for this disparity in observation? It is possible that the *Xf* strain that Fishleder cultured and used to inoculate the vines growing in the greenhouse was substantially different in pathogenicity or host specificity from the naturally occurring *Xf* prevalent at Tallahassee, Florida. Another possibility is that while the *Xf* populations in the respective studies do not differ in pathogenicity or host specificity, the direct inoculation through pin prick employed by Fishleder is more difficult for the plant to resist than the natural inoculation by insect vectors that is thought to have occurred in the vineyard.

Rootstock performance in north Florida primarily is a factor of PD response. Cultivars differed in their performance and some were markedly superior-these should be further investigated for their influence on scions. Specifically we suggest Ramsey and St George for additional study. These rootstocks survive well under natural inoculation conditions in north Florida. The evaluation of rootstock cultivars in PD limited viticultural regions is important-much PD management research is focusing on augmenting PD resistance and or tolerance in scions, but rootstocks are a critical component of viticulture. As demonstrated here, several rootstocks have substantial levels of PD resistance that should permit their cultivation in PD prone regions, allowing concentration of effort on scion improvement. Additionally, testing the PD response of ungrafted rootstocks indicates the potential for rootstock varieties to be cultivated as nursery mother vines in PD prone regions. Rootstocks identified as resistant or tolerant to PD could be genetic resources for breeding improved PD resistant scion varieties, as in the case of MidSouth and MissBlue, which have PD resistant rootstocks as parents (DeGrasset and Dog Ridge, respectively). PD resistant rootstocks might be necessary for the cultivation of PD tolerant scion varieties if *Xf* spreads to the root system.

Field evaluation of PD resistance in Florida is easy due to high PD pressure resulting from high populations of vectors and bacteria in the area and should be continued as a technique to test PD management strategies and screen plant material.

REFERENCES

Bouquet, A. 1980. *Vitis* x *Muscadina* hybridization: a new way in grape breeding for disease resistance in France. Proc. 3rd Intl. Symp. On Grape Breeding. p 177-197.

Chen, J., Copes, W. E., Walker, R. M., and Lamikanra,, O. 2001 Diseases. p189-239. In: Basiouny, F. M. and Himelrick, D. G., (eds). Muscadine Grapes. ASHS Press, Alexandria, Virginia.

Einset, J. and Pratt. C. 1975. Grapes. p 130-153. In: Janick, J. and Moore, J. N. (eds). Advances in fruit breeding. Purdue University Press, West Lafayette, Indiana.

Fishleder, A. J. 2000. Evaluating grape rootstocks and Vitis and Muscadinia species for resistance and susceptibility to Pierce's Disease. Thesis, University of California, Davis.
Loomis, N. H. 1952. Effect of fourteen rootstocks on yield, vigor, and longevity of twelve varieties of grapes at Meridian, Mississippi. Proc. Amer. Soc. Hort. Sci. 52:125-132.
Loomis, N. H. 1965. Further trials of grape rootstocks in Mississippi. Proc. Amer. Soc. Hort. Sci. 86:326-328.
Lu, J. and Ren, Z. 2002. Evaluation for Pierce's Disease among Muscadine Grapes. XXVIth International Hort. Congr. p 173
Magoon, C. A. and Magness, J.R.. 1937. Investigations on the adaptability of grape root stocks to Gulf Coast conditions. Proc. Amer. Soc. Hort. Sci. 35:466-470.
Olien, W. C. and Hegwood, C. P. 1990. Muscadine—a classic southeastern fruit. HortScience, 25: 726
Pierce, N. B. 1905. The Vineyard: Mr. Pierce and the Lenoir. Pacific Rural Press, 69:79.
Ren, Z. and Lu, J. 2002. Muscadine rootstock increased the resistance of Florida hybrid bunch grape cv. Blanc du Bois to Pierce and Anthracnose diseases. Proc. Fla. State Hort. Soc. 115:108-110.
Winkler, A. J., Cook, J. A., Kliewer, W. M., and Lider, L. A. 1974. General Viticulture. University of California Press, Berkeley.

Table 1. PD symptom scores of the ten grape rootstocks during the second, third, and fourth growing seasons.

Rootstock	PD score		
	2002	2003	2004
O39-16	3.1bc	3.8b	2.3
101-14	2.2d	2.4c	1.9
110R	2.2d	1.8cd	2.3
3309C	3.6b	4.2ab	3.0
44-53	2.6cd	2.3c	---
5BB	2.7cd	1.6cd	2.9
5C	2.2d	1.9cd	2.1
Freedom	3.7b	5.0a	---
Ramsey	1.1e	1.0d	1.2
St. George	1.4e	1.7cd	0.9

Table 2. Vine survival of the ten grape rootstocks after four growing seasons.

Rootstock	Number of living vines				Survival %
	2001	2002	2003	2004	2004
O39-16	9	9	6	6	67
101-14	10	10	10	9	90
110R	10	10	9	8	80
3309C	10	10	5	1	10
44-53	10	10	3	0	0
5BB	10	10	10	7	70
5C	10	10	9	9	90
Freedom	10	8	1	0	0
Ramsey	8	8	8	8	100
St. George	10	9	9	7	70

FUNDING AGENCIES
Funding for this project was provided by the University of California Pierce's Disease Grant Program. Special thanks to California Grapevine Nursery for supplying the grapevines used in this experiment.

MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*. PROGRESS REPORT NUMBER ONE: COMPARISON WITH SYMPTOMS OF WATER DEFICIT AND THE IMPACT OF WATER STRESS

Project Leaders:
Mark A. Matthews
Dept. of Viticulture & Enology
University of California
Davis, CA 95616
mamatthews@ucdavis.edu

Thomas L. Rost
Section of Plant Biology
University of California
Davis, CA 95616
tlrost@ucdavis.edu

Cooperator:
Eleanor T. Thorne
Dept. of Viticulture & Enology
University of California
Davis, CA 95616
etthorne@ucdavis.edu

Reporting period: The results reported here are from work conducted from October 2003 to September 2004.

ABSTRACT
The pathology of diseases such as Pierce's disease (PD) of grapevine (*Vitis vinifera* L.) that are caused by the xylem-limited bacteria *Xylella fastidiosa (Xf)* is widely attributed to vessel occlusion and subsequent water deficits. Grapevines (*Vitis vinifera* L. 'Chardonnay') were exposed to water deficits, stem inoculation with *Xf*, and combinations of both to evaluate whether symptoms of PD were a consequence of water deficits. When vines were inoculated with *Xf* and exposed to water deficits, more extensive PD symptoms developed throughout the plant than when + *Xf* vines were well-watered. However, vines infected with *Xf* exhibited symptoms unique to PD that included inhibited periderm development in stems (green islands), leaf blade separation from the petiole (matchsticks), and irregular leaf scorch. Vines exposed to water deficits and not *Xf*, displayed accelerated periderm development, basal leaf abscission at the stem/petiole junction, and uniform leaf chlorosis. Water deficits induced the development of an abscission zone, but PD did not. Pierce's disease symptoms could not be produced with any of several water deficit treatments, including severing all but one secondary vein near the leaf tip. The results indicate that factors other than water deficits are involved producing the symptoms of PD. We conclude that the widely accepted hypothesis that PD-infected plants develop water deficits that cause green islands, matchsticks, localized leaf scorch, and eventual death of vines should be reevaluated.

INTRODUCTION
The overwhelming consensus among researchers is that the fatal nature of PD is a result of the *Xf* bacteria becoming systemic and blockage occurring in xylem vessels (due to bacterial accumulation, tyloses, gums, and/or emboli), causing water transport to become progressively impaired until the plant is no longer able to function (Goodwin *et al.*1988a, b; McElrone *et al.* 2001, 2003; Newman *et al.* 2003, 2004; California Agricultural Research Priorities 2004). Indeed, Pierce's disease has become nearly synonymous with plant water deficit. This view is largely based on correlative evidence. Hopkins (1988) showed a strong association between reduced water conductance in stems of citrus seedlings and *Xf*-caused disease symptoms. Low leaf water potential and turgor, impaired hydraulic conductance, and higher stomatal resistance were correlated with PD symptoms in grapevines (Goodwin *et al.* 1988a). While reduced leaf water potential, stomatal conductance and stem hydraulic conductivity are characteristic of water deficit, it should be noted that these same features also occur in flooded plants (Kramer & Boyer 1995), so correlations are not necessarily indicative of causality.

From our recent work we observed that, although PD symptoms have been attributed to water deficit, the visual symptoms of PD did not appear to be the same as those resulting from water deficit alone. In grapevine, typical visual symptoms of PD are "green islands," patchy or marginal leaf necrosis (often called leaf scorch), and "matchsticks" (petioles that remain attached to the stem after the laminae have fallen off) (Purcell 1986; Goheen & Hopkins 1988, 1989; Stevenson *et al.* 2004). These symptoms are not characteristic of water deficit symptoms in grapevines (Okamoto *et al.* 2004). In addition, the diagnostic symptoms of PD have never been observed in healthy grapevines exposed to water deficits, nor have they ever been reported to develop as a consequence of water deficits.

Interestingly, citrus trees already infected with *Xf* and subjected to drought displayed accelerated symptom development of citrus variegated chlorosis (Gomes *et al.* 2003). Extended water deficit also increased the severity of Pierce's disease in the woody liana, Virginia creeper (McElrone *et al.* 2001, 2003). Thus, extended water deficit (such as drought) may exacerbate the development of PD symptoms in grapevine as well. However, there are no reports describing the effects of water deficit on *Xf*-infected grapevines, nor has there been a detailed comparison of water deficit and PD symptoms. If the visual symptoms of PD are not, in fact, a result of water deficit, then studies relying on the assumption that water stress is the

ultimate killer of plants suffering from PD may result in misleading information and add years to finding solutions to the PD problem. Therefore, it is important that it be determined which PD symptoms, if any, are a result of water stress, and what role water shortage actually plays in symptom development and vine death.

OBJECTIVES

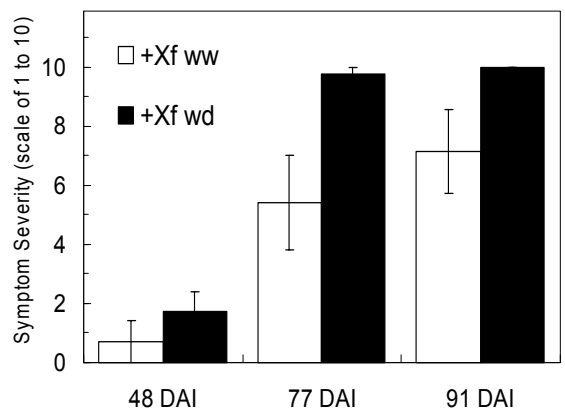
1. Evaluate the impact of vine water status on the development of the visual symptoms of PD.
2. Determine whether visual PD symptoms are a direct result of water deficits.

RESULTS

Objective 1

In the field, extended water deficit exacerbates citrus varigated chlorosis in citrus (Gomes *et al.* 2003) and PD in Virginia creeper (McElrone *et al.* 2001, 2003). Thus, it was not surprising that subjecting potted grapevines to extended water deficit also resulted in a faster and more extensive onset of PD symptoms (barring green islands) than in well-watered *Xf*-infected (+*Xf*) vines. The first clear indications of leaf scorch were seen 48 DAI. Water-stressed +*Xf* vines developed more symptomatic leaves with severe symptoms than well-watered +*Xf* vines (Fig. 1). Interestingly, the leaf scorch and matchstick symptoms in the well-watered +*Xf* plants had the same visual characteristics as in the +*Xf* water-stressed plants. There was no significant difference between well-watered +*Xf* and healthy (-*Xf*) vines in stomatal conductance (0.86 ± 0.09 & 0.69 ± 0.06 cm s^{-1}), transpiration (6.53 ± 0.83 & 5.66 ± 0.83 µg cm^{-2} s^{-1}), and leaf water potentials (-0.60 ± 0.05 & -0.73 ± 0.11 MPa, respectively). Likewise, these parameters were equivalent for water-deficit +*Xf* and –*Xf* vines (0.28 ± 0.04 & 0.34 ± 0.05 cm s^{-1}, 2.41 ± 0.31 & 2.86 ± .39 µg cm^{-2} s^{-1}, -1.07 ± 0.05 & -1.28 ± 0.13 MPa, respectively).

Figure 1. Symptom development during whole plant water deficit and well-watered conditions. Severity of Pierce's disease symptoms in Chardonnay grapevines. Vines infected with *X. fastidiosa* were well-watered (+*Xf* ww; white bars) or subjected to water deficit (+*Xf* wd, black bars). On a scale from 1 to 10, 1 indicates mild symptoms, 10, the most severe PD symptoms. Non-infected values are zero due to the lack of PD symptoms and are not shown.



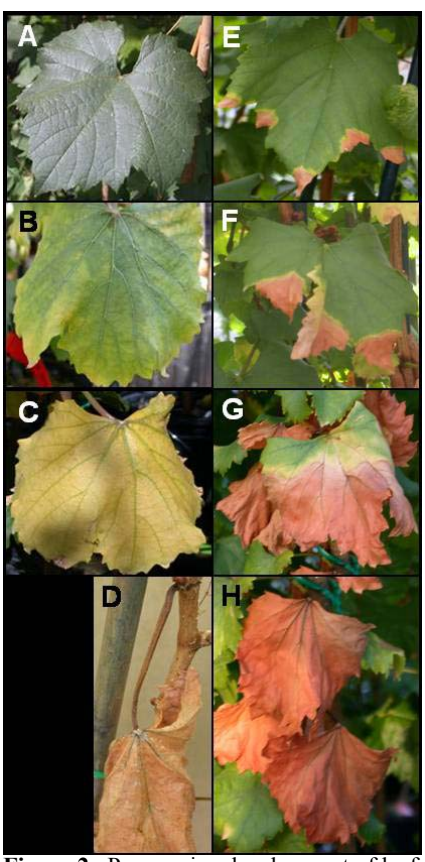


Figure 2. Progressive development of leaf symptoms of non-infected water-stressed Chardonnay leaves (A-D), and Chardonnay with Pierce's disease (E-H).

Objective 2

The results revealed that visual symptoms of Pierce's disease in grapevine are qualitatively and quantitatively different than those of extended water deficit. Regardless of water status, +*Xf* plants displayed symptoms unique to PD. In general, PD symptoms masked water-deficit symptoms. The PD symptoms manifested in laminae, petioles and stems often revealed an interaction between plant and bacteria in which plant responses to *Xf*-infection seemed to be either elicited or suppressed by the bacteria.

Comparison of Visual Symptoms of Water Deficit and PD

To determine whether PD symptoms are a direct result of water deficit, the visual characteristics of well-watered and water-stressed grapevines inoculated with *Xylella* (+*Xf*) or water (–*Xf*) were evaluated. Leaves of well-watered –*Xf* grapevines remained green and healthy throughout the course of the experiments (Fig. 2a). Water-stressed –*Xf* vines gradually developed leaf chlorosis in a fairly uniform pattern over the entire leaf lamina (Fig. 2b-c), with the veins staying green until leaves became necrotic. Leaves remained attached to the stem even after the leaves were apparently dead (Fig. 2d). In contrast, the first PD symptom to appear was leaf scorch. Leaf scorch symptoms started with chlorosis at the margins of the leaves and moved towards the petiole in patches such that sections of necrosis were bordered by slim regions of chlorosis (Fig. 2e-f). As symptoms progressed, laminae of +*Xf* vines became completely necrotic, while the petioles remained green (Fig. 2g-h). Eventually laminae fell from the petioles to form "matchsticks."

In –*Xf* water-stressed plants, two sites of constriction and necrosis developed on petioles, one at the stem/petiole junction (the basal end of the petiole) and the other at the petiole/lamina junction (the distal end of the petiole). At the basal end of the

petiole, a true abscission zone formed. At the distal end of the petiole where the lamina is attached, the tissue constricted and concurrently became necrotic. Observations at the cellular level suggest that the constriction and necrosis at this junction is not an actual abscission zone (Stevenson *et al.* 2004). Neither the abscission zone at the stem/petiole junction nor the fracture zone at the petiole/lamina junction developed until the lamina was severely chlorotic. In +*Xf* vines, a fracture zone also occurred at the petiole/lamina junction. Comparisons of the anatomy of the fracture zone at the petiole/lamina junction of +*Xf* vines and –*Xf* water-stressed vines showed that these fracture zones were identical. However, abscission zones did not develop at the stem/petiole junction of either well-watered or water-stressed +*Xf* plants.

The canes of both +*Xf* and –*Xf* water-stressed plants matured faster, becoming stiffer and more woody than those of the well-watered plants, based on the extent of periderm development up the canes. Stems of water-stressed + *Xf* plants became woody before the well-watered plants. Interestingly, in +*Xf* plants only the well-watered vines developed green islands, having an average of 2.1 ± 0.31 green islands per plant.

Vessel Blockage in Relation to Leaf Scorch Symptoms

Leaf scorch symptoms, in particular, have been considered a direct result of water deficits within the leaf, specifically due to clogged vessels limiting water transport. If leaf scorch is simply a matter of reduced water availability to the leaf margins, then we should be able induce leaf scorch symptoms by selectively severing veins to simulate xylem vessel blockage. To this end, experiments were conducted in which all veins but one were severed such that a single secondary leaf vein connected the two halves of a lamina and was the sole water source for the nearly-severed portion of the leaf. Nearly-severed leaf halves of vines experiencing low transpirational demand in the laboratory appeared turgid and showed no signs of necrosis for up to 36 days. In the greenhouse, under medium to high transpirational conditions, sections of leaves which received water via a single vein remained green and turgid (Fig. 3) for at least 30 days after the veins were severed**.** This was true for leaves of +*Xf* and –*Xf* grapevines alike. Significantly, leaf scorch symptoms of PD did not develop on any of the –*Xf* nearly-severed leaves. Even when these leaf sections did eventually dehydrate after approximately two months, the symptoms were similar to water deficit, not PD.

Figure 3. Turgid leaf of non-infected Chardonnay under moderate to high transpiration 13 days after all but one vein was severed, resulting in a single leaf vein connecting and supplying water to half of the leaf. Black arrow shows secondary vein supplying water to the nearly-severed leaf half.

CONCLUSIONS

In summary, water deficit clearly had an exacerbating effect on the symptom development of PD. Water-stressed +*Xf* vines displayed more extensive PD symptoms throughout the plant than did well-watered vines. Matchstick and leaf scorch symptoms moved up the canes more rapidly than in well-watered vines implying that the bacteria spread more rapidly throughout the plant under water deficit conditions, assuming bacterial proximity is necessary for symptom development. Importantly, with the exception of green islands, extended water deficit did not affect the nature of the PD symptoms. Indeed, in water-stressed +*Xf* plants, PD masked all of the symptoms of water deficit, except green islands, which occurred only in well-watered +*Xf* vines.

Detailed comparisons of the visual symptoms of PD and water deficit revealed that conclusions reached from earlier work, stating that water deficit causes PD symptoms, were not completely correct. The visual characteristics of +*Xf* vines were unique to PD and distinctly different from –*Xf* vines experiencing extended water deficit. The fracture zone at the petiole/lamina junction, common to all treatments, appears to be a plant response to stress and not specifically induced by bacterial infection. In contrast, the lack of an abscission zone in +*Xf* plants implies that the bacteria were in some way suppressing development of an abscission zone. Conversely, water deficit overcame the influence of *Xf* to prevent the occurrence of green islands, possibly by hastening periderm development. Considering that only well-watered +*Xf* vines developed green islands, water deficit could have masked the green island symptom of PD by inducing the periderm of +*Xf* water-stressed canes to develop faster than could the conditions necessary to impair periderm activity leading to green islands. This suggests that the bacteria are in some way inhibiting periderm activity at seemingly random locations.

Finally, based on the dramatic and sudden increase in the number of nonfunctional vessels which was caused by severing leaf veins, it seems clear that xylem vessel blockage, whether due to gums, tyloses or bacterial accumulation, is not responsible for leaf scorch symptoms and that *Xf* bacteria are able to affect plant responses in ways not involving altered vine water status. While occluded xylem vessels may worsen leaf scorch symptoms, several other factors, or combination of factors, may contribute. Ultimately, however, comparison of the leaf scorch symptoms of PD and the chlorosis of extended water-stressed leaves shows that *Xf* bacteria are able to produce, alter or eliminate signals that result in leaf scorch symptoms and that these signals can, to some degree, override signals controlling plant responses to water deficit. (A manuscript containing the completed study will be submitted to a peer-reviewed journal shortly.)

REFERENCES

California Agricultural Research Priorities: Pierce's Disease. (2004) National Research Council, The National Academies Press, Washington, D.C., USA.

Goheen A.C. & Hopkins D.L. (1988) Pierce's disease. In *Compendium of Grape Diseases* (eds R.C. Pearson & A.C. Goheen), pp. 44-45. The American Phytopathological Society Press, St. Paul, MN, USA.

Gomes M.M., Lagôa A.M.M.A., Machado E.C. & Medina C.L. (2003) Abscisic acid and indole-3-acetic acid contents in orange trees infected by *Xylella fastidiosa* and submitted to cycles of water stress. *Plant Growth Regulation* **39**, 263-270.

Goodwin P.H., DeVay J.E. & Meredith C.P. (1988a) Roles of water stress and phytotoxins in the development of Pierce's disease of the grapevine. *Physiological and Molecular Plant Pathology* **32**, 1-16.

Goodwin P.H., DeVay J.E. & Meredith C.P. (1988b) Physiological responses of *Vitis vinifera* cv. "Chardonnay" to infection by the Pierce's disease bacterium. *Physiological and Molecular Plant Pathology* **32**, 17-32.

Hopkins D.L. (1988) Production of diagnostic symptoms of blight in citrus inoculated with *Xylella fastidiosa*. *Plant Disease* **75**, 432-435.

Hopkins D.L. (1989) *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. *Annual Review of Phytopathology* **27**, 271-290.

Kramer P.J. & Boyer J.S. (1995) *Water Relations of Plants and Soils.* Academic Press, San Diego, CA, USA.

McElrone A.J., Sherald J.L. & Forseth I.N. (2001) Effects of water stress on symptomatology and growth of *Parthenocissus quinquefolia* infected by *Xylella fastidiosa*. *Plant Disease* **85**, 1160-1164.

McElrone A.J., Sherald J.L. & Forseth I.N. (2003) Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. *Journal of Experimental Botany* **54**, 419-430.

Newman K.L., Almeida R.P.P., Purcell A.H. & Lindow S.E. (2003) Use of a green fluorescent strain for analysis of Xylella fastidiosa colonization of Vitis vinifera. *Applied and Environmental Microbiology* **69**, 7319-7327.

Newman K.L., Almeida R.P.P., Purcell A.H. & Lindow S.E. (2004) Cell-cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. *Proceedings of the National Academy of Sciences of the USA* **101**, 1737-1742.

Okamoto G., Ueki K., Imai T. & Hirano K. (2004) A comparative study of drought and excess-water tolerance in *Vitis coignetiae* and several table grapes grown in Japan. *Scienitific Reports of the Faculty of Agriculture* (Okayama Univ.) **93**, 39-43.

Purcell A.H. (1986) Pierce's disease. In *Grape Pest Management*, pp.62-69. Cooperative Extension University of California, Division of Agriculture and Natural Resources, pub. 4105.

Stevenson J.F., Matthews M.A. & Rost T.L. (2004) The developmental anatomy of green islands and matchsticks as symptoms of Pierce's disease of grapevines. *Plant Disease* (in press).

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

EPIDEMIOLOGICAL ANALYSES OF GLASSY-WINGED SHARPSHOOTER AND PIERCE'S DISEASE DATA

Project Leader:
Thomas M. Perring
Dept. of Entomology
University of California
Riverside, CA 92521

Cooperators:
Yong-Lak Park
Dept. of Entomology
University of California
Riverside, CA 92521

Charles A. Farrar
Dept. of Entomology
University of California
Riverside, CA 92521

Rayda K. Krell
Dept. of Entomology
University of California
Riverside, CA 92521

Murry P. Pryor
Cooperative Extension
University of California
Bakersfield, CA 93307

Barry Hill
CDFA
Sacramento, CA 95814

Jennifer Hashim
Viticulture Farm Advisor
UC Cooperative Extension
Bakersfield, CA 93307

Maggi Kelly
Environmental Sciences, Policy and
Management Dept.
University of California
Berkeley, CA 94720

Reporting period: The results reported here are from work conducted from July 1, 2004 to October 8, 2004.

ASBTRACT
The progression of PD in vineyards and across a landscape is dependent upon factors related specifically to four components: GWSS, *Xylella fastidiosa* causing PD, grapes, and the environment. When conditions in all four of these areas are optimal, disease spreads with devastating consequence as in Temecula in the late 1990s. Conversely, sub-optimization within any of the four categories can slow or stop disease progress. The aggressive insecticide campaigns against GWSS are prime examples of creating this sub-optimal condition for disease spread. This single approach has been effective, but it may not be sustainable in reduced budget times. The science of epidemiology seeks to determine how the 4 components listed above interact, with the goal of creating long-term, sub-optimal conditions for disease spread. Achieving this goal will enable California producers to continue growing grapes in areas known to have PD and GWSS.

INTRODUCTION
Earlier studies pointed out the importance of the distribution of disease (Weltzien 1972, 1978) and insects (Southwood 1978), but mapping the distribution of disease and insect populations has not been applied to entomological and epidemiological studies until recently. This is mainly because there was a lack of suitable technologies or methods to map the distribution of insects and diseases in the field. Recently, the global positioning system (GPS), the geographic information system (GIS), and geostatistics have been applied to entomological and epidemiological. These technologies combined with advanced statistical methods can facilitate the making of distribution maps and the analyzing and modeling of the spatial phenomena represented on the maps.

OBJECTIVES
The overall goal of this research is to analyze the GWSS and PD data to investigate the relationship between GWSS and PD. The objectives of this research include,

1. Determine the spatial patterns and structures of GWSS and PD distributions, and use these analyses to create statistical distribution maps.
2. Analyze map correlations between GWSS abundance and incidence of PD.
3. Relate the epidemiology of GWSS-transmitted PD to environmental components, and identify characteristics of areas with rapid and slow PD infection rates.

RESULTS AND CONCLUSIONS
This project has just begun, so our report is preliminary at the present time. Prior to analyses, the GWSS and PD data need to be centralized into a geo-referenced database. Fortunately, there has been a tremendous and successful effort to maintain a weekly trapping effort for GWSS in areas of Kern, Tulare, and Ventura Counties. The data have been managed in a geographic information system (GIS) maintained by Rosie Yacoub of CDFA in Sacramento. We are working closely with Rosie to obtain trapping data from Kern County. Secondly, for certain areas there are crop layers that have been entered into the GIS, and we will work closely with the Kern County GIS group to obtain these layers. Within these two data sets we find

information related to two of the four epidemiological components (i.e., GWSS abundance and the agricultural environment). Data from the other two components (i.e., PD and grapes) also have been collected, largely by Barry Hill and Jennifer Hashim (Hill and Hashim 2002, Hashim and Hill 2003). These scientists have directed crews to survey hundreds of vineyards in Kern and Tulare counties over the past four years. Much of the data has been entered and managed in a GIS format at UC Berkeley under the direction of Maggi Kelly. We have begun the process of bringing the PD data together with the GWSS data and crop layers. Once the map databases are constructed and standardized, we will pursue the analyses phases of this project.

REFERENCES

Hashim, J. & Hill, B.L. 2003. Monitoring and control measures for Pierce's disease in Kern County, and epidemiological assessment of Pierce's disease, pp. 95-98. In CDFA (ed.), Proceedings of Pierce's disease research symposium 2003, Coronado, CA.

Hill, B.L. & Hashim, J. 2002. The epidemiology of Pierce's disease, pp. 74-75. In CDFA (ed.), Proceedings of Pierce's disease research symposium 2002, Coronado, CA.

Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect population. Chapman & Hall, London.

Weltzien, H.C. 1972. Geophytopathology. Annu. Rev. Phytopathol. 10:277-298.

Weltzien, H.C. 1978. Geophytopathology, pp 339-360. *In* J.G. Horsfall and E.A. Cowling (eds) Plant disease: An advance treatise, Vol. 2: How disease develops in populations. Academic Press, New York.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

AREA-WIDE EPIDEMIOLOGY OF PIERCE'S DISEASE IN THE COACHELLA VALLEY

Project Leaders:

Thomas M. Perring
Dept. of Entomology
University of California
Riverside, CA 92521

Carmen Gispert
Viticulture Farm Advisor
UC Cooperative Extension Riverside County
Indio, CA 92201

Cooperators:

Charles A. Farrar
Dept. of Entomology
University of California
Riverside, CA 92521

Rayda K. Krell
Dept. of Entomology
University of California
Riverside, CA 92521

Yong-Lak Park
Dept. of Entomology
University of California
Riverside, CA 92521

REPORTING PERIOD: The results reported here are from work conducted from May 1, 2001 to September 30, 2004.

ABSTRACT

This is a continuation of the epidemiology project that was initiated in 2001 in the Coachella Valley. Surveys in 2001 did not detect any Pierce's disease (PD). In 2002, we identified 2 infected vines in one vineyard and 1 infected vine in an adjacent vineyard. These were the first finds of PD in the area since 1983. Intensive surveys in these vineyards over the past 3 years have revealed a total of 16 infected vines. In June 2003, we found PD-infected vines in 2 additional vineyards. Further work in these vineyards has identified a total of 62 vines infected with PD. This past summer (2004), we again surveyed all vineyards in the Valley, finding PD-infected vines at 3 additional sites. Additional searches have identified a total of 19 infected vines in these three vineyards. With the finds this past summer, we now have identified 97 PD-infected vines from 7 vineyards. Except for the two infected vineyards identified in 2002, sharpshooter densities have been low near the sites that have PD.

Since the inception of this project in May 2001, we have used yellow sticky traps to monitor the spatial and temporal abundance of adult glassy-winged sharpshooters (GWSS), *Homoladisca coagulata* (Say) and native smoke tree sharpshooters (STSS), *Homoladisca liturata* Ball in the Valley. In 2001-2003, two peaks were identified in abundance; a broad-peak around a maximum abundance in July and a second smaller peak in winter. Summer densities in 2002 were higher than the same time in 2001 and winter counts in 2003 were higher than winter densities in 2002. This apparent increase in GWSS abundance was altered by the CDFA-sponsored vector control program being implemented through the Riverside County Agricultural Commissioner's Office. This program was initiated in the winter of 2003, and since then, very few GWSS adults have been caught on our traps. Relative densities of the STSS have remained constant throughout the 4-year study period.

INTRODUCTION

The Coachella Valley is home to 11,345 acres of table grapes; in 2003 harvested grapes from this region were valued at $115,939,900 (Riverside County Agricultural Commissioner, 2003). Pierce's disease first was identified in the Valley in 1983 (Goheen 1984), and from that time until recently, it has not been a concern to growers. When the GWSS was identified from the Valley in the early 1990s (Blua et al. 1999), growers became concerned, since this insect had been shown to be instrumental in the devastating spread of PD in the Temecula Valley in the late 1990s. At the request of the table grape growers, we initiated a study in 2001 to determine the spatial and temporal distribution of GWSS, and to identify the distribution of PD in the Valley. From that point in time to the present, we have continued our monitoring efforts, with the intention of describing the epidemiology of GWSS-transmitted PD in this area.

OBJECTIVES

The goal of our studies in the Coachella Valley is to describe the epidemiology of PD in the presence of GWSS, and to use this information to design management strategies to reduce disease spread.
Three objectives are pertinent to this report:

1. Determine the incidence and distribution of PD in the Coachella Valley.
2. Determine the spatial and temporal abundance of sharpshooters in the Coachella Valley.
3. Describe the epidemiology of PD in the Coachella Valley.

RESULTS AND CONCLUSIONS

Determine the incidence and distribution of PD in the Coachella Valley

For the past 4 years, we have searched for PD in the Coachella Valley. In 2001, we visually inspected 300 plants in each of 25 vineyards and all vines in a 60-acre vineyard proximal to an area that had PD in 1983. We collected 233 symptomatic samples and analyzed them with ELISA. None of these plants were positive for *Xylella fastidiosa*, the causal agent of PD. In 2002, we visually sampled 300 plants in each of 25 vineyards, and visually inspected 35,000 vines distributed throughout the Valley. We analyzed (by ELISA) 268 plants from these surveys, and found 2 infected vines in one field and 1 infected vine in an adjacent field. We analyzed (by ELISA) 268 plants from these surveys, and found 2 infected vines in one field and

1 infected vine in an adjacent field. Both fields were in the southeast corner of the Valley (Figure 1). The PD-strain of *X. fastidiosa* was confirmed in these plants with selective-media plating and PCR. These were the first post-GWSS PD finds in the Valley. Intensive sampling in these 2 fields over the past 2 years has found 13 additional vines infected with *X. fastidiosa*. In 2003, we visually inspected an estimated 616,400 vines and samples from 478 vines with suspected PD were subjected to ELISA. Five of these 478 vines were positive for PD. Four of these vines were at one field site and the 5th vine was at another site. Interestingly, neither vineyard was near the infected vineyards identified in 2002, and the fields were not near each other (Figure 1). One of the vineyards was in a fairly isolated location on the west side of the Valley. Further searches of the two infested vineyards found no additional PD infection at one of the sites, however work at the site on the west side of the valley has identified a total of 61 infected vines. We are in the process of characterizing this field to determine the spatial pattern of infection. In the 2004 survey, we observed an estimated 571,861 vines and collected 187 samples to assay for PD. From these assays we identified 5 infected vines, adding 3 vineyards to our list. These vineyards were located in the east-central part of the valley with an additional find in the far southwest corner of the Valley (Figure 1). Further research has identified a total of 19 infected vines from these three vineyards. We are in the process of determining the distribution of PD-infected vines in these vineyards.

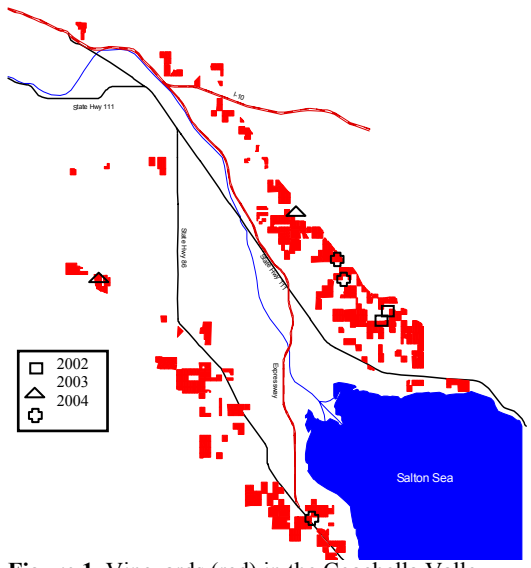


Figure 1. Vineyards (red) in the Coachella Valley, and sites where PD was confirmed in 2002, 2003, and 2004.

Spatial and temporal abundance of sharpshooters

Yellow sticky cards have been used to trap GWSS and STSS adults from May 2001 to the present. These 156 traps are distributed uniformly at one-mile intervals throughout the Coachella Valley. Traps are checked weekly and the total numbers of sharpshooters are recorded.

We discuss the trap data in two distinct time periods. The first, from May 2001 through January 2003, preceded the CDFA treatment program in citrus while the second period from February 2003 to the present has been during the implementation of this areawide program. During the early part of this period, GWSS vastly outnumbered STSS (Figure 2A). While average densities did not exceed 3 GWSS per week, some sites had very high GWSS catches; up to 160 insects per week were trapped (Figure 2B). During the second period of trapping, STSS numbers remained consistent with previous years, and even increased in 2003 (Figure 2B). A few sites reached high densities of STSS, nearly as abundant as the GWSS peaks in 2002. Presently, STSS outnumber GWSS in the Valley. The reason for these seasonal dynamics is that the CDFA treatment program specifically targets citrus, a preferred host of GWSS during certain times of the year. STSS, on the other hand, utilizes a number of desert scrubs and riparian plants, thus its densities have been largely unaffected by the treatment program. STSS is a known vector of PD, but it is not clear how important it is in the epidemiology of the disease.

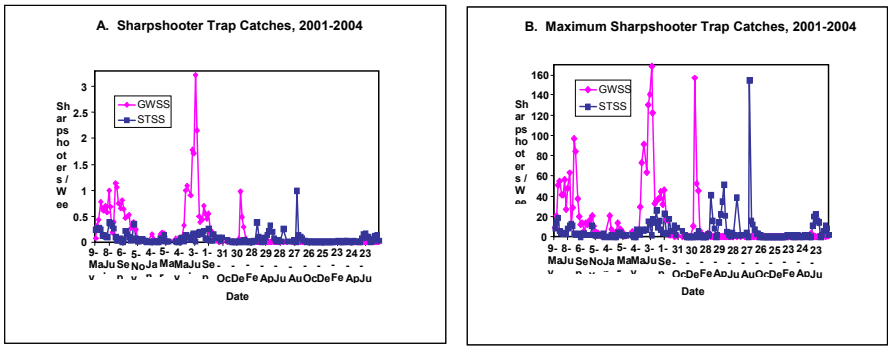


Figure 2. (A) Average number of GWSS (pink) and STSS (blue) trapped per week from 2001 - 2004 in the Coachella Valley. (B) Maximum number of GWSS (pink) and STSS (blue) trapped per week.

GWSS Seasonal Abundance

From 2001-2003, two peaks of adult activity were identified; a broad-peak centered around a maximum abundance in July and a second smaller period of activity in January and February (Figure 3). Summer densities in 2002 were higher than the same time in 2001 and winter counts in 2003 were higher than winter densities in 2002. This apparent general increase in

GWSS abundance was altered by the CDFA-sponsored vector control program being implemented through the Riverside County Agricultural Commissioner's Office. Treatments from this program were initiated in the winter of 2003, and since then, very few GWSS adults have been caught on our traps (Figure 3).

STSS Seasonal Abundance

Generally, trap counts of STSS peaked at about 1/3 the densities of GWSS in 2001 and 2002 (Figure 3). However, in 2003, average densities equaled GWSS, and at certain sites, there were far more STSS than GWSS (Figure 2B). Since STSS have non-citrus hosts throughout the Valley, they have not been affected by the treatments in citrus. It is unclear at this time what role this species may play in the epidemiology of PD in the Coachella Valley, but we will be investigating this as we continue data analysis

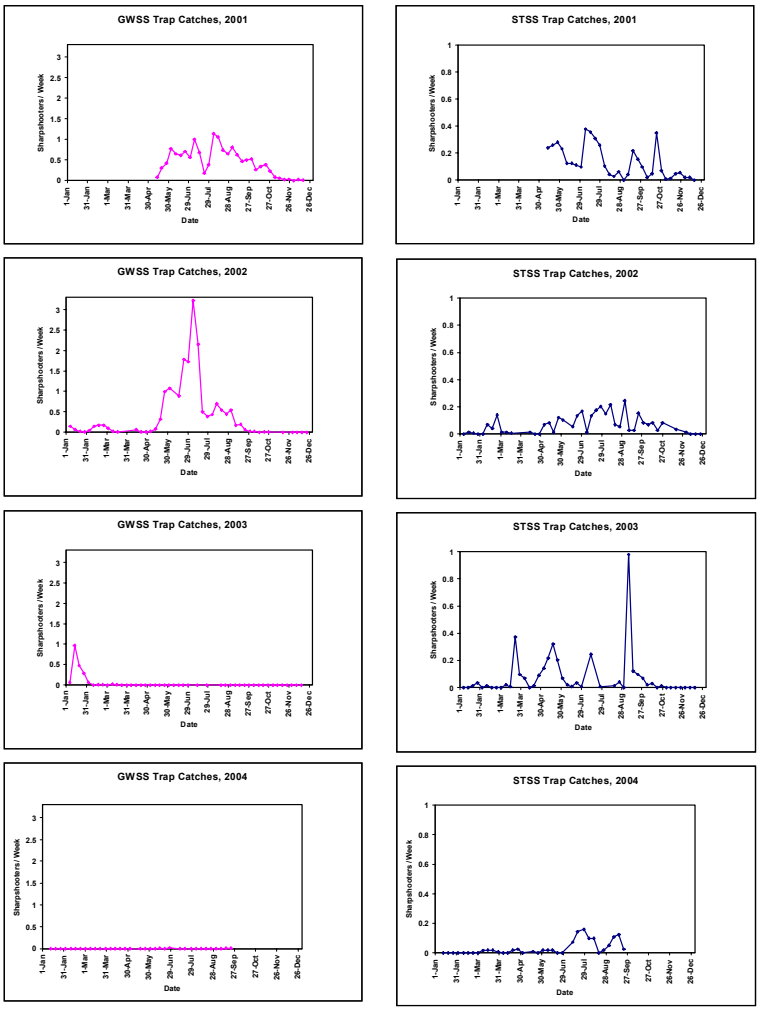


Figure 3. Average number of GWSS (pink) and STSS (blue) trapped per week from 2001 – 2004 in the Coachella Valley displayed for each year.

Describe the epidemiology of PD in the Coachella Valley

Since we have so few sites infected with PD, and the number of infected vines at each site is low, it is difficult to draw conclusions about the epidemiology of PD in this area. However, we calculated the maximum numbers of GWSS and STSS adults caught on yellow traps within one mile of the 7 fields in which we have found PD, to determine if any relationships were apparent. From this exercise, we present several preliminary observations. First, we observe the highest incidence of PD was not in an area where we caught large numbers of GWSS (Figure 4) or STSS (Figure 5). In fact, the heaviest PD vineyard, found in the northwest part of the Valley, has had maximum numbers of GWSS and STSS of 1 per week since we started trapping in 2001. In this field, we suspect other sharpshooter species are involved with PD spread, or our trapping program is too coarse to detect GWSS and STSS. Second, the two vineyards in which we identified PD in 2002 were in areas that were heavily infested with GWSS (Figure 4). If the trend of increasing GWSS from 2001 to 2002 (see Figure 3) had been allowed to continue in 2003 (in the absence of the CDFA spray program) one might have predicted spread of PD from these fields to neighboring vineyards. Because this did not materialize, the evidence suggests that the areawide program effectively impeded PD spread in this area of the Coachella Valley. Finally, while the number of fields in which we have found PD remains low, relative to other areas of the state, each year we have found additional vines with PD. Having learned from the epidemic that occurred in Temecula, we suggest continuing the sharpshooter and PD monitoring efforts to insure that this scenario is not repeated in the Coachella Valley.

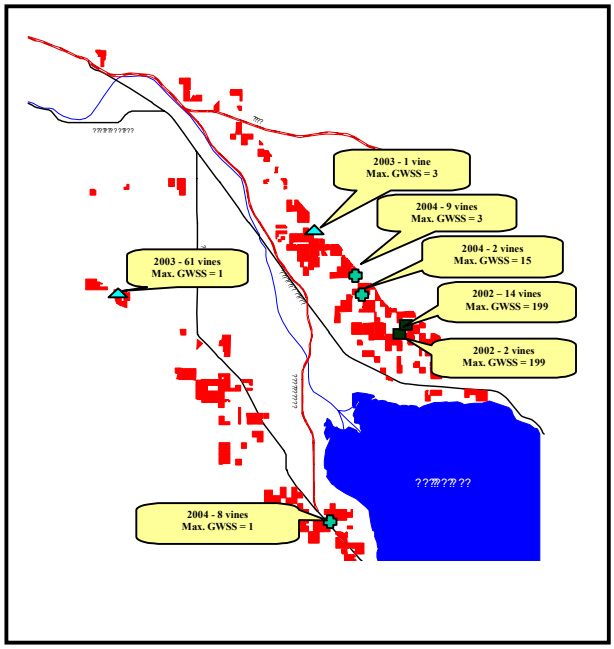


Figure 4. Sites with PD and maximum GWSS numbers in the Coachella Valley from 2001-2004.

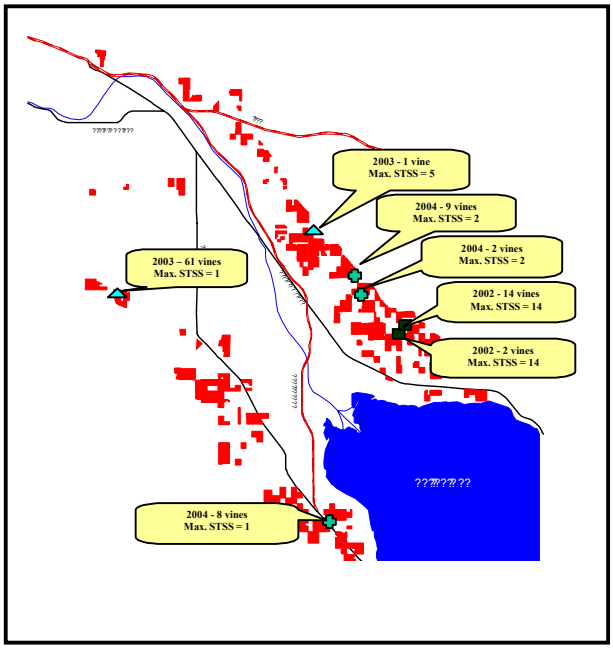


Figure 5. Vineyards (red) in the Coachella Valley, and sites where PD was confirmed in 2002, 2003, and 2004.

REFERENCES

Blua, M. J., P. A. Phillips, and R. A. Redak. 1999. A new sharpshooter threatens both crops and ornamentals. Calif. Agric. 53(2): 22-25.

Goheen, A. 1984. Pierce's disease in Coachella Valley. USDA-ARS Report, Davis, California. 2 pp.

Perring, T. M., C. A. Farrar, and M. J. Blua. 2001. Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. Calif. Agric. 55:13–18.

Riverside County Agric. Commissioner. 2003. Coachella valley acreage and agricultural crop report. Agricultural Commissioner's Office, Indio, CA.

FUNDING AGENCIES

Funding for this project was provided by the California Desert Grape Administrative Committee, the University of California Pierce's Disease Grant Program, and the California Department of Food and Agriculture.

IMPROVING OUR UNDERSTANDING OF SUBSTANCE TRANSPORT ACROSS GRAFT UNIONS

Project Leaders:
Bruce Reisch
Dept. of Horticultural Sciences
NYS Agricultural Experimentation Station
Cornell University
Geneva, New York 14456

Peter Cousins
USDA, ARS, Plant Genetic Resources Unit
NYS Agricultural Experimentation Station
Collier Drive
Geneva, New York 14456

Cooperators:
Julie R. Kikkert & Jose Vidal
Dept. of Horticultural Sciences
NYS Agricultural Experimentation Station
Cornell University
Geneva, New York 14456

Reporting period: The results reported here are from work conducted July 1, 2004 to September 30, 2004.

ABSTRACT
Researchers seeking to genetically-engineer grapevine rootstocks in order to affect Pierce's disease (PD) resistance in scion cultivars know very little about the transport of substances produced by foreign genes across the graft union. Our project seeks to understand how protein size and concentration may affect protein transport from a rootstock to a scion. We possess genetically engineered lines of Chardonnay, Merlot and Chancellor that produced proteins ranging in size from 29 to 97 kDa. These proteins can be readily detected by established techniques. Lines will be identified with low and high protein production potential in their root tissues, and graft combinations will be created with non-transgenic Chardonnay scions. Xylem sap will be collected from the scion and tested for the presence of the transgenic proteins. Given that Xylella fastidiosa causing plugging of xylem tissues, the results of xylem sap testing will be directly applicable to efforts to develop PD resistance inducing rootstocks.

INTRODUCTION
One approach being utilized to develop a long-term solution to Pierce's disease is the development of transgenic PD resistant versions of important wine and table grape varieties. The development of each transgenic cultivar will require a concentrated effort and significant amounts of technical expertise, testing, and funding. To bring each successful product to market, and to pass regulatory agency approval for transgenic crops, also will require a great deal of time and funding. This would be required for each of dozens of scion varieties.

A rootstock-based approach provides a potentially excellent alternative. In theory, a transgenic rootstock would confer PD resistance to its non-transgenic scion. Advantages include: 1) many fewer rootstocks will need to be transformed as compared to the dozens of table grape and wine grape varieties that would need to be altered, 2) consumers might be more accepting of wines produced from non-transgenic scions even if they are grafted on transgenic stocks; and 3) in general, it has been technically easier to transform rootstocks than scion varieties. Before this approach is successful, however, our understanding of the biology of the graft union and the types of substances that can be successfully transported from rootstocks to scions must be improved.

Water, mineral nutrients, hormones, carbohydrates, and other compounds are all known to move, via both xylem and phloem, from rootstocks across graft unions into scions of woody plants. To date, however, there is little evidence available to show whether a transgenic protein can move from the rootstock into the scion in a grafted woody plant. In recent work with grapevines, Meredith and Dandekar (2003) showed that pear polygalacturonase inhibiting protein (PGIP), with a size of 36.5 kDa, could be detected in xylem sap of non-transgenic scions grafted on transgenic stocks engineered to produce this protein. Of great relevance to this proposal, we noted that protein movement into the xylem occurred even <u>without</u> a specific signal targeting it to the extracellular spaces or to the xylem. Imidacloprid (a small compound with molecular weight of approximately 0.25 kDa) and other systemic insecticides applied to the soil are taken up by the roots of grapevines and move from root systems into the scion (Toscano et al. 2003). The present project will investigate aspects of plant physiology critical to determining the potential for deploying transgenic rootstocks for PD management.

It is possible that the size of a transgenic protein produced in a rootstock influences its transport to the scion. For example, large proteins might be less likely to be transported than small proteins. Understanding the relationship between size and movement will allow us to more efficiently test anti-PD compounds. If transgenic proteins are transported across the graft union, their concentration in the roots might be higher than their concentration in the scion. Since there is likely to be a threshold concentration for PD control provided by a given compound, it will be critical to understand the relationship between concentration in the rootstock and concentration in the scion.

By studying non-transgenic scions grafted on transgenic rootstocks in the course of this project, we expect to learn whether the transgenic proteins can move from the rootstock to the scion, whether molecule size affects transport, and whether substance concentration in the rootstock affects levels found in the scion.

OBJECTIVE
Determine the relationship between protein molecule size and concentration in grapevine roots and its ability to move from a grapevine rootstock to a scion across a graft union.

RESULTS
This project is just getting underway, thus, rather than present non-existent research results, an outline of our research plan is presented here.

The following transgenic grapevines are available for use:
1. Two lines of Chancellor transformed with an NPT-II/GUS gene fusion producing a fused protein product. One line strongly expresses the *gus* reporter gene (*uidA*) in all tissues, while the other line shows no GUS expression, even though the gene is present.
2. Multiple lines of Chardonnay and Merlot producing both NPT-II and endochitinase.
3. A series of lines of Chardonnay producing NPT-II along with one of three antimicrobial peptides (AMPs).

All of these lines produce transgenic products under control of constitutive promoters. In cases 1 and 2 above, the CaMV 35S promoter was employed, whereas in case 3, NPT-II was downstream of an *Arabidopsis* ubiquitin promoter. The CaMV 35S promoter was used by Meredith and Dandekar (2003), who showed that PGIP protein from rootstocks could be detected in xylem sap. The NPT-II/GUS gene fusion product in Chancellor was shown to express in root tissues (Striem et al. 2000), but will require re-testing to make sure that protein production has not been lost since these tests were run. We will need to test the other lines (2 and 3 above) to determine the transgenic protein concentration in their roots. The size of the transgenic product molecules varies: NPT-II is ~280 amino acids (aa) (29 kDa); endochitinase is 424 aa (42 kDa); the NPT-II/GUS bifunctional fusion protein has 885 aa (97 kDa).

We will examine root tissues from separate lines of each of the three types of transformed vines listed to determine gene transcription and transgenic protein concentration via established procedures. To test for gene transcription we will use semi-quantitative RT-PCR (Vidal et al. 2003). Transgenic protein concentrations will be determined using standard methods already in use in our lab. We will identify lines with high and low concentrations of transgenic proteins for further use in this project.

The transgenic lines with high and low concentrations of transgenic proteins, along with negative controls, will be bench grafted as rootstocks to non-transgenic Chardonnay scions. The grafted vines will be grown in a greenhouse. Once the grafted vines have been established and their shoots have grown to 50 cm, the non-transgenic Chardonnay scions will be examined for presence of transgenic proteins. Leaf tissue as well as xylem sap will be tested. Samples will be collected under sunny, warm conditions conducive to transpirational pull through the xylem.

Outline of rootstock/scion combination planned:
13 rootstock/scion combinations planned, including control
10 vines of each combination x 13 combinations = 130 vines total planned
Control rootstock: Non-transgenic Chardonnay (to be grafted to non-transgenic Chardonnay)

Experimental rootstocks:
(Each rootstock will be grafted to non-transgenic Chardonnay scions.)
Chancellor, high NPT-II/GUS fused protein product concentration in roots (35S promoter)
Chancellor, transformed vine with no GUS expression in roots (35S promoter)

Chardonnay, high NPT-II concentration in roots (Nos promoter)
Chardonnay, low NPT-II concentration in roots (Nos promoter)

Chardonnay, high NPT-II (*Arabidopsis* ubiquitin promoter)
Chardonnay, low NPT- II (*Arabidopsis* ubiquitin promoter)

Chardonnay, high endochitinase concentration in roots (35S promoter)
Chardonnay, low endochitinase concentration in roots (35S promoter)

Merlot, high NPT-II concentration in roots (Nos promoter)
Merlot, low NPT-II concentration in roots (Nos promoter)

Merlot, high endochitinase concentration in roots (35S promoter)
Merlot, low endochitinase concentration in roots (35S promoter)

Additional controls will include own-rooted transgenic vines to be used to test for presence of foreign protein in the xylem sap.

CONCLUSION

The success of this project will rest on the careful, methodical characterization of foreign gene products. This project will not involve the speculative and lengthy creation of novel transgenic grapevines, but rather uses pre-existing transgenic grapevines in order to investigate the potential for transgenic rootstocks to deliver proteins to their non-transgenic scions.

Based on the evidence from the movement of imidacloprid and PGIP in grafted grapevines, it is likely that transgenic grapevine rootstocks will transmit transgenic proteins to their non-transgenic scions. However, it is premature to speculate concerning the time frame for reduction to practice in the form of a novel PD management strategy. We emphasize that this study is intended to investigate the biological principles of protein transport via xylem in grapevines, a topic that has been studied very little in the past. By understanding the potential of a transgenic grapevine rootstock to move proteins into a non-transgenic scion, scientists will be better equipped to investigate and develop novel PD management strategies.

REFERENCES

Meredith, C. and A. Dandekar. 2003. Directing potential anti-*Xylella* gene products to the xylem of transgenic grapevines. *in* Proceedings of the 2003 Pierce's Disease Research Symposium. Calif. Dept. of Food and Agriculture. 8-11 Dec. 2003. Coronado, CA, USA. pp. 23-25.

Striem, M.J., B.I. Reisch, and J.R. Kikkert. 2000. Differences in GUS expression among grapevine transformants. Proc. ISHS Grapevine Physiology Conference. Acta Horticulturae 526:437-443.

Toscano, N., F. Byrne, and S. Castle. 2003. Laboratory and field evaluations of imidacloprid, thiamethoxam, and acetamiprid against the glassy-winged sharpshooter on grapevines. *in* Proceedings of the 2003 Pierce's Disease Research Symposium. Calif. Dept. of Food and Agriculture. 8-11 Dec. 2003. Coronado, CA, USA. pp. 315-317.

Vidal, J., J. Kikkert, P. Wallace, D. Gadoury, R. Seem, B. Reisch. 2003. Expression of magainin genes in transgenic 'Chardonnay' and disease resistance analyses. International Conference on Plant Molecular Biology. Barcelona, Spain. June, 2003. (abstr.)

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*. PROGRESS REPORT NUMBER TWO: GREEN ISLANDS AND MATCHSTICKS

Project Leaders:

Thomas L. Rost
Section of Plant Biology
University of California
Davis, CA 95616
tlrost@ucdavis.edu

Mark A. Matthews
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
mamatthews@ucdavis.edu

Cooperator:

Josh Stevenson
Dept. of Biology and Chemistry
Texas A&M International University
Laredo, TX, 78041
jstevenson@tamiu.edu

Reporting Period: The results reported here are from work conducted from October 2003 to September 2004.

ABSTRACT

During this period our focus was the comparative xylem anatomy of a resistant species, *Muscadinia rotundifolia* cv Cowart and a susceptible species, *Vitis vinifera* cv Chardonnay. When infected by *Xylella fastidosa* both species produced tyloses (parenchyma ingrowths into tracheary elements) and gums; *M. rotundifolia* tended to have fewer tyloses. The resistant species also had narrower vessels, but otherwise xylem anatomy was similar to *V. vinifera*. Fluorescently tagged beads were loaded into both species. Beads traveled through the stem xylem in both, but did not move into petioles in these experiments. Tyloses were first apparent 24 hours after pruning in both species and most vessels were blocked in both after eight days of pruning. This suggests that the mechanism to form tyloses in both species is similar, although the resistant species tended to show fewer tyloses in response to *Xf*. Two symptoms, green islands and matchsticks are reported in this study. Green islands formed as a result of incomplete initiation of the phellogen. In regions of the stem where a phellogen and subsequent periderm arose, immediately exterior tissue was cut off, causing it to brown. In regions of the stem where no periderm is formed, the exterior tissues remained green. Consequently, the stem is mottled with both green living epidermis and brown dying epidermis as determined by the presence or absence of an underlying periderm. Matchsticks formed when the leaf lamina separated from the petiole, and the petiole remained attached to the stem. Lamina broke off from the petioles consistently in a fracture zone where xylem from the petiole anastomoses into the five major veins of the leaf. No separation layer was found to explain this pseudoabscission.

INTRODUCTION

Xylella inoculation of stem xylem precedes a relatively rapid movement of bacteria through the hydraulic network (system of xylem) to the leaves. Once bacteria moving in the transpiration stream enter regions of the hydraulic network that contain narrow tracheary elements and terminal tracheary elements (i.e. shorter vessels in petioles and leaves), bacteria may be 'filtered out', accumulate, and become embedded in a gel which effectively blocks water flow in that conduit. Tyloses are cell wall extensions of xylem parenchyma cells into tracheary elements. Tylose formation in the stem coincides with bacterial infection, but at least initially, is not present to such a degree that bacterial movement is apparently prevented or that the water supply to distal tissues is restricted to levels causing visual symptoms. Additionally, bacteria can move relatively quickly from an inoculated shoot to another shoot via the subtending trunk.

A similar understanding of the progression of events is needed for resistant varieties and species in order to localize investigations into the mechanism(s) of resistance. The anatomical symptoms of PD, xylem occlusions of gums and tyloses, are well documented in both susceptible (Esau 1948) and resistant plants (Mollenhauer and Hopkins 1976). However, it is not clear whether these occlusions are related to susceptibility or resistance. Only the susceptible plants express leaf scorch and eventual death, and these disease symptoms are widely understood to be water stress (Hopkins, 1989). Sufficient occlusions would produce water deficits downstream. Plants resistant to PD may remain healthy despite systemic populations of *Xylella* present in the vascular tissue because tylose and gum formation are not induced compared to susceptible varieties. Alternatively, the occlusions may prevent the movement of the bacteria, and comparative studies report that the frequency of occlusions is greater in resistant than in susceptible varieties (Fry and Milholland, 1990). Thus, resistant varieties or species may restrict *Xf* to regions of the hydraulic network proximal to the point of inoculation, either by occlusions or other mechanisms described below. In the reported experiments, we have initiated those studies. Regardless of whether resistance is dependent upon controlling the movement of *Xf*, Pierce's Disease is fatal because *Xf* becomes systemic. Host species in which *Xf* is confined to specific tissues, or is otherwise prevented from becoming systemic, do not display symptoms of PD (Hill and Purcell, 1995).

It is generally accepted that the fatal nature of Pierce's Disease is a result of the bacteria becoming systemic and water stress becoming increasingly severe until the plant is no longer able to function (Goodwin et al., 1988). However, the classic PD symptoms: patchy leaf chlorosis, persistent "green islands" on stems, and "matchsticks" (leaf abscission at the petiole/blade junction) are not generally observed in vines exposed to water stress alone. If the symptoms of PD are not, in fact, a result of water deficit, then studies relying on the assumption that water stress is the ultimate killer of plants suffering from PD, may result in misleading information and add years to finding solutions to the PD problem. Our second annual report addresses these concerns.

OBJECTIVES

1. Study the progression of anatomical symptoms created by *Xf* over a time-course in a PD resistant grapevine species, *Muscadinia rotundifolia* cv Cowart.
2. Determine the hydraulic architecture of a PD resistant species, *M. rotundifolia*.
3. Study the integrity of pit membranes of both PD susceptible *Vitis vinifera* cv Chardonnay and resistant *M. rotundifolia* by following the in situ movement of fluorescently tagged beads.
4. Determine the rate of tylose development from wounding in both PD *V. vinifera* and *M. rotundifolia*.
5. Study the developmental anatomy of green island and matchsticks in *V. vinifera*.

RESULTS

1. PROGRESSION OF PD SYMPTOMS IN RESISTANT SPECIES

The progression of anatomical symptoms created by infection by *Xf* was studied along a time-course as was previously conducted with *V. vinifera* (Stevenson, Matthews and Rost, 2004). Similar experiments were conducted with PD resistant *M. rotundifolia* in an attempt to discern quantitative or qualitative anatomical differences in a six-month post-inoculation period. The development of symptoms in the resistant species was qualitatively similar to that in resistant species (development of tyloses in stems, development of gums in petioles), however the rate of development and overall occlusion created by these symptoms was dramatically lower. In the resistant species overall occlusion was minimal (<5% of vessels) after nearly four months (Figure 1), whereas in susceptible species overall occlusion was great (~50% of vessels).

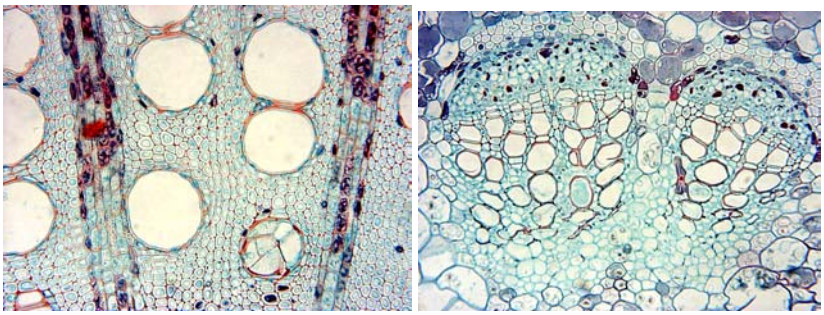


Figure 1. Minimal vessel occlusion in stem (left) and petiole (right) xylem of *M. rotundifolia* 122 days post-inoculation with *Xf*.

2. HYDRAULIC ARCHITECTURE OF RESISTANT SPECIES

The general hydraulic architecture of PD susceptible *V. vinifera* has been presented (Stevenson et al. 2004). Similar studies were conducted with PD resistant *M. rotundifolia* in an attempt to elucidate anatomical differences that may explain PD susceptibility or resistance. Regions of grapevine stem were serially sections to follow xylem arrangement in the node and internode. No significant differences were observed in the organization of stem xylem or in the divergence of xylem to lateral organs between resistant and susceptible species. The only difference found between the species was that *M. rotundifolia* possessed significantly narrower vessels than were found in *V. vinifera*. The difference may be contribute to restricting bacterial movement. Narrow vessels may cause bacterial conglomeration closer to the point of inoculation and prevent long distance bacterial seeding. Additionally, narrower vessels have less overall pit surface, which may further reduce the number of alternative pathways available to bacteria. Both of these proposals require further investigation.

3. PIT PROPERTIES OF SUSCEPTIBLE AND RESISTANT SPECIES

Preliminary investigations were conducted towards the study of the characteristics and integrity of pit membranes in susceptible and resistant grapevine species. The movement of *Xf* bacteria in the host is potentially facilitated by damaged pit membranes of grapevine, compromised either in development, or as a result of frequent cavitation/refilling cycles (Hacke et al. 2001, Sperry et al. 1987).

A. Movement of Fluorescent Beads

Fluorescent beads of similar size to *Xf* bacterial cells were injected into stem xylem of *V. vinifera* and *M. rotundifolia* (Figure 2). The distance of bead travel from the inoculation point was recorded as an indicator of vessel length and pit membrane integrity. Beads were observed to travel similar distances in both species (*V. vinifera* 1.6 ±0.5 nodes, *M. rotundifolia*1.8 ±0.4 nodes). The relatively short distance that these beads traveled indicates a general integrity within the vessel pits and is evidence against pit damage commonly occurring. Beads were never observed to pass into petiole xylem, which suggests

that some pit membrane disruption, is required for bacteria to colonize petiole and leaf tissue (Stevenson, Matthews and Rost, 2004a).

B. Resin-casting and Macerations

Resin casts were made of the internal spaces of vessel lumina and pit surface morphology in both *V. vinifera* and *M. rotundifolia* (Figure 2). Superficially, no differences were seen in pit patterns, pit integrity, or relative pit surface area between the species. Further study is required to investigate subtle characters of pit membranes (ex. total pit membrane area, dimensions of pit apertures) that may facilitate pit membrane disruption by bacteria.

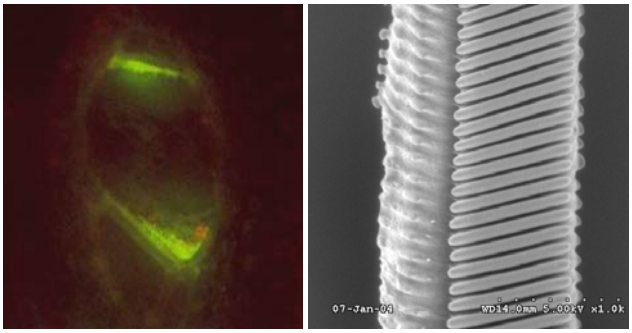


Figure 2. Fluorescent beads within stem xylem of *V. vinifera* used to mimic movement of passive bacterial cells (left), and resin casts of xylem vessels from *M. rotundifolia* to show fine detail of pit surfaces (right).

4. TYLOSE DEVELOPMENT

A. Rate of Tylose Development

A working hypothesis was developed that differential susceptibility to PD among grapevine species may involve differences in the rate of tylose development. The rate of tylose development was studied in both resistant and susceptible grapevines following wounding (pruning) injury. Tylose development was then observed allowing one, four, and eight days for tyloses to develop. Initial tylose development was found within a day, about half of the vessels were occluded by day four, and at day eight, most vessels of the stems were observed to be significantly blocked by tyloses (Figure 3). No superficial difference was seen between the rate of tylosis in PD susceptible *V. vinifera* and resistant *M. rotundifolia* at any of the time intervals, however, further quantitative analysis is necessary.

B. Vitality of Tyloses and Paratracheal Parenchyma

The presence of living cells surrounding the vessels during tylose formation following pruning was studied using the vital stain fluoroscein diacetate. This technique was used to discern a correlation between the amount of tylose occlusion found in the vessel and the number of vital paratracheal cells surrounding that vessel, and whether the number of vital paratracheal cells was significantly greater in PD susceptible grapevine species. Both resistant and susceptible grapevines were observed in this manner over the eight-day time course described in 4A. No superficial differences were seen in the vitality of paratracheal parenchyma surrounding vessels in the two species, however greater quantitative analysis is required. Overall, tyloses fluoresced greatly, indicating vital development, whereas paratracheal cells fluoresced only occasionally (Figure 3). These results suggest that very few active paratracheal cells are required to result in significant tylose development.

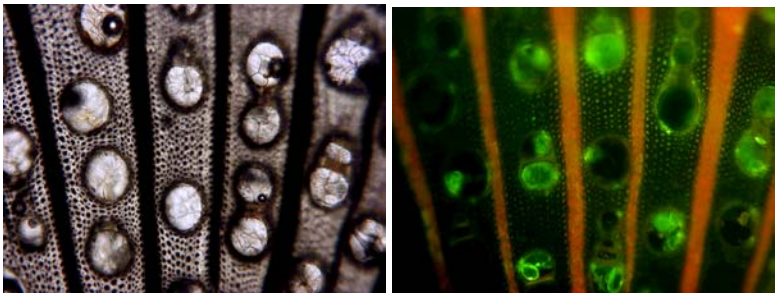


Figure 3. Micrographs of similar grapevine stems eight days following pruning. A bright field light micrograph (left) shows significant occlusion by tyloses at this interval. A fluorescence micrograph (right) shows fluorescent green vital staining predominantly by tyloses, but occasionally by paratracheal

5. DEVELOPMENTAL ANATOMY OF MATCHSTICKS AND GREEN ISLANDS

The development of the external visual PD symptoms of matchsticks and green islands was studied from a anatomical perspective (Stevenson, Matthews and Rost 2004b).

A. Matchsticks

Matchsticks result from pseudoabscission of the leaf lamina from the petiole. Following significant leaf scorching, the lamina breaks from the petiole at a predictable fracture zone. No separation zone develops as is common with typical leaf abscission, and hence this process is described as pseudo-abscission. Following pseudoabscission, exposed petiole tissues dehydrate and blacken to take on the appearance of a burnt matchstick. Occasionally, a wound periderm will form near the fracture zone following pseudoabscission. When this periderm forms, dehydration of the petiole is minimal. The process of matchsticking has never before been described anatomically.

B. Green Islands

Green islands arise from the incomplete development of the deep-seated phellogen (cork cambium) in *V. vinifera*. In regions of the stem where the phellogen arises and produces subsequent phellem (cork), external tissues (phloem, cortex, epidermis) are cut off from their nutrient sources and begin to die and brown. The juxtaposition of stem regions with active phellogen, and the juvenile character of no phellogen, creates green islands. It is unknown whether green regions are delayed in their development, or whether brown regions display advanced development. No obvious correlation was seen in the level of vessel occlusion proximal to green or brown regions. Additionally, periderm formation was observed in *M. rotundifolia*. Periderm formation in this species is subepidermal (vs. deep-seated) and consequently green islands may not form in this species (Stevenson et al. 200xC). This is important point for researchers using green islands as an indicator of PD resistance.

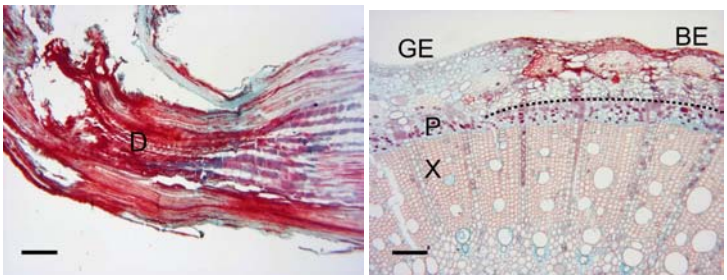


Figure 4. Longitudinal section through a matchsticked petiole (left) displaying basipetal dehydration (D) following pseudoabscission. Transverse section through a stem with green island (right) showing regions of green epidermis (GE) and brown epidermis (BE) created by presence of absence of phellogen initiation.

CONCLUSIONS

1. The development of tyloses and gums in response to *Xf* infection were qualitatively similar in the resistant *M. rotundifolia* cv Cowart and the susceptible *V. Vinifera* cv Chardonnay, although the resistant species tended to form fewer tyloses.
2. The only observable difference in hydraulic architecture was that the resistant species had narrower vessels.
3. Fluorescent beads were loaded into stems of both species. Beads moved approximately the same distance (~1.6-1.8 nodes) and in both cases did not enter into petioles.
4. Tyloses were first seen about 24 hours after pruning in both species. After four days about 50% of vessels were blocked. By eight days most vessels were blocked in both species.
5. Matchsticks formed in *V. vinifera* leaves after several days of *Xf* infection. This symptom consisted of the pseudoabscission of the petiole from the leaf blade. Green islands are green areas of the stem created by incomplete formation of periderm in infected plants.

REFERENCES

Esau K. (1948) Anatomic effects of the viruses of Pierce's disease and phony peach. Hilgardia 18: 423-482.

Fry S.M., Milholland R.D. (1990) Multiplication and translocation of *Xylella fastidiosa* in petioles and stems of grapevine resistant, tolerant, and susceptible to Pierce's disease. Phytopathol 80: 61-65.

Goodwin P.H., DeVay JE, Meredith CP (1988) Physiological responses of *Vitis vinifera* cv. "Chardonnay" to infection by the Pierce's disease bacterium. Physiol Mol Plant Pathol 32: 17-32.

Hacke U.G., Stiller V, Sperry JS, Pittermann J, McCulloh KA. 2001. Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. Plant Physiology 125: 779-786.

Hill B.L., Purcell AH 1995 Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathol 85(11): 1368-1372.

Hopkins D.L. 1989 *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Ann Rev Phytopathol 27: 271-290.

Mollenhauer H.H., Hokins DL 1976 Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. Physiol Plant Pathol 9: 95-100.

Sperry J.S., Holbrook N.M., Zimmermann M.H., Tyree M.T. 1987. Spring filling of xylem vessels in wild grapevine. Plant Physiology 83: 414-417.

Stevenson, JF, Matthews, MA, Rost, T.L. 2004a. Grapevine susceptibility to Pierce's disease I: Relevance of hydraulic architecture. American Journal of Enology and Viticulture 55:228-237.

Stevenson, JF, Matthews, MA, Rost, T.L. 2004b. The developmental anatomy of "green islands" and "matchsticks" as symptoms of Pierce's disease of grapevines. Plant Disease. In Press.

Stevenson, JF,Matthews, MA. Greve, L.C., Labavitch, JM, Rost, TL. 2004. Grapevine Susceptibility to Pierce's Disease II: The progression of anatomical symptoms. American Journal of Enology and Viticulture 55:238-245.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

MAGNETIC RESONANCE IMAGING: A NONDESTRUCTIVE APPROACH FOR DETECTION OF XYLEM BLOCKAGES IN *XYLELLA FASTIDIOSA*-INFECTED GRAPEVINES

Project Leaders:

Ken Shackel
Dept. of Pomology
University of California
Davis, CA 95616

John Labavitch
Dept. of Pomology
University of California
Davis, CA 95616

Cooperators:

Mark Matthews
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

L. Carl Greve
Dept. of Pomology
University of California
Davis, CA 95616

Jeffrey Walton
NMR Facility
University of California
Davis, CA 95616

Alonso Pérez
Dept. of Pomology
University of California
Davis, CA 95616

Reporting period: The results reported here are from work conducted from April 4, 2004 to October 1, 2004. (**Note**: we are now in the second year of a project originally approved in 2003.)

INTRODUCTION

Results from Pierce's disease (PD) research programs led by Matthews, Rost and Labavitch (reported in 2001, 2002 and 2003 in San Diego) have provided substantial support for the idea that obstructions in the vine's water-transporting xylem tissue develop rapidly post-inoculation, before an appreciable bacterial population has been established. The results also strongly suggest that these obstructions, and likely other aspects of the PD "syndrome", result from the grapevine's active responses to the presence of *X. fastidiosa* (*Xf*), rather than to direct "action" by the bacterium. Thus, careful analysis of the timing of changes in xylem element anatomy and function relative to *Xf* introduction, as well as to external symptoms of disease development, is important for establishing reliable indicators of the "stage" of PD development. The analyses done thus far have been based on destructive tissue sampling. Such sampling can be particularly "blind" when it is done on vines in which (based on our earlier results) internal symptoms of PD are present but external, visible symptoms are not yet present.

In the report of the year 1 work of our study (Shackel and Labavitch, 2003), the success of Mr. Pérez and Dr. Walton in imaging non-functional vessels in the stems of PD-infected and ethylene-treated grapevine stems was demonstrated. In this report we elaborate on those studies, showing that locations of reduced vine water transport capacity, as determined by non-destructive MRI analysis, is correlated with the locations of PD and ethylene effects on vessel functionality (destructive analysis). In addition, because interpretation of the meaning of the MRIs with respect to the anatomy and functioning of vessels is a crucial aspect of our work, we have described the methodology used to validate our approach to obtaining the relevant information from the MRIs.

OBJECTIVES

1. Optimize the use of MRI (Magnetic Resonance Imaging) and to spatially visualize altered water movement in grapevines.
2. Test correlations of observed vascular system obstructions (based on grapevine dissection and microscopy techniques) with predictions based on MRI data.
3. Use MRI to follow the development of grapevine obstructions over time in vines infected with *X. fastidiosa* or treated with ethylene, bacterial wall-degrading enzymes or plant cell wall oligosaccharides, all of which may be important intermediates in regulating the vine's response to infection and the eventual development of PD symptoms.
4. Use NMR imaging to determine whether localized xylem cavitation occurs at the site and time of *X. fastidiosa* inoculation or introduction by the glassy-winged sharpshooter.

RESULTS

Optimization of the Use of MRI for Visualizing Water Transport Deficiencies in PD-Infected Grapevines.

Progress on this objective has been delayed because a supplier for a key electronic element of the new MRI probe that has been designed for use with grapevines no longer provided a key part. The parts are all now available and development of the new probe is underway. We are proceeding with the testing of aspects of the PD model using the NMR instrument in its more conventional configuration.

MRI Will Show Non-functional Sections in the Xylem of a PD-infectedGgrapevine Stem.

Usually the techniques to evaluate xylem function are destructive. Magnetic Resonance Imaging (MRI) allows us to visualize vessels that are functional and full of movable water. Functional vessels appear as bright spots in an MRI view of the stem cross-section; non-functional vessels lack water and appear as dark spots in the area of the stem where water-conducting cells are found. Figures 2a & 2b show the difference in the distributions of functional vessels in an infected vine at a point where leaf symptoms of PD are apparent (Figure 2a) and nearer to the stem apex at a point where the leaves show no sign of PD symptoms (Figure 2b). Compare these images with that for a healthy vine (Figure 3a). Cavitation of xylem vessels is also of

potential importance in PD development. Our analysis can reveal vessels that have cavitated. Figure 3 shows functional vessels in an intact stem, and empty vessels after the stem is severed to cause cavitation, and that cavitated vessels can be re-filled with water under pressure. When we have the optimized MRI probe we will develop a series of image sets taken along the lengths of vines at intervals following water (control) and *Xf* inoculation to give a time course of PD development. However, at this point we do not have images for a full time course.

MRI is capable of showing xylem disruption and non-functional vessels well before external symptoms appear in infected plants. Figures 4 and 5 show images for the length of control (buffer-innoculated) and infected (*X. fastidiosa*-innoculated) vines six months after inoculation. MRIs of the control-inoculated vine show defined xylem rays, in which individual vessels can be clearly observed. As in previous experiments, stem cross section MRIs of infected plants (Figure 5) show that major sectors of the xylem appear dark, indicating that they are no longer water-filled (Note: the magnetic signal is lost in cavitated vessels). Furthermore, MRIs of plants infected with *Xf* become less sharp, making it more difficult to discriminate structure, particularly of individual, probably still functional, vessels. Efforts to explain this will be a feature of the work as this project continues. MRI also has been used to follow changes in the functionality of the xylem of plants exposed to ethylene in enclosed chambers (10 ppm for 48 hours). We previously described the progressive development in time of "dark sectors" in the xylem of ethylene-gassed, presumably indicating vessels no longer involved in water transport. This new set of experiments has allowed us to confirm that, after 6 months of exposure to ethylene, gassed plants show progressive xylem disruption along the stem (Figure 6). Most of the damage is localized close to nodes/internodes that had just developed in the stem growth tip at the time of ethylene treatment and had then expanded in the intervening six months prior to our observations. The MRIs show "dark sectors" in those internodes. These sectors decrease are less extensive in internodes below and above the internodes that were in the growth tip at the time of treatment; that is, internodes formed after the time of treatment and already partially elongated, respectively when ethylene as applied. As in *Xf*-infected plants, MRIs of ethylene-treated plants are less sharp than images of control plants (Figure 6).

The impression of a loss in xylem function that is given by the MRIs of *Xf*-inoculated and ethylene-gassed vines can be correlated with a decrease in the hydraulic conductivity of internodes. This is tested by determining the rate of movement of pressurized water through stem segments (Figure 7). Similarly, stems of treated vines showed an increase in the hydraulic resistivity (the inverse of conductivity) relative to the controls (Figure 8), although this difference was statistically significant only for the ethylene experiment. The lack of statistical difference in the inoculation experiment is mainly due to the great variability found in the hydraulic resistivity of inoculated plants. In turn, this might be explained because these vines were in a gradation of early stages of PD infection when examined (they were not showing external symptoms). While there is some correlation between the MRIs showing localized areas of empty vessels and reduced hydraulic conductivity in regions of infected stems, the correlations are not perfect. This is due to at least two factors that will be tested more fully in our continuing work. First, an empty vessel shown in the MRI at one level in the plant's stem could be the result of a vessel obstruction or cavitation above or below the point on the stem where the MRI observation was made. There may be no actual impediment to water flow in the empty vessel at the level at which it is being imaged. Thus, a test of water flux at the imaged level may reveal no water flux difficulty. Second, while cavitation may be an important factor in PD development, because the tests of water conductivity are carried out using water under pressure, cavitated vessels will be re-filled during the test and no reduction in water flux would be revealed. Destructive anatomical work will define which kind of vessel disruption (tylose, gel or air embolism) exists in stems with non-functional vessels as revealed by MRI.

A more quantitative analysis of the MRIs has been attempted in order to characterize objectively the presence of "dark sectors" in the images. For this purpose, the MRIs were processed and analyzed using the ImageJ program (developed at the U.S. National Institutes of Health and available at http://rsb.info.nih.gov/ij). First, the number of functional vessels ($\boldsymbol{N_V}$) was counted in the MRIs of inoculated and control vines (like the one in Figure 9a), based on the assumption that a bright (hence, water-filled) vessel was functional. Next, the xylem-cross sectional area ($\boldsymbol{A_x}$) was measured by isolating in the MRIs (Figure 9b) the ring of tissue that is usually occupied by the xylem. Then, the digital image of the xylem-ring was converted to a binary image (Figure 9c) using a built-in algorithm in ImageJ, in which all the pixels above a set grey intensity threshold are black and the pixels below this value remain white, and the functional xylem-cross sectional area ($\boldsymbol{A_f}$) was determined by measuring the black area. To confirm that the threshold area correctly estimated A_f,,the area of individual functional vessels was selected by hand and measured in a series of MRIs, some with clearly delimited vessel images and others with less distinct ("fuzzy") images such as those often seen when PD-infected grapevine stems are examined. The images from infected vines often do not show vessels as bright or darks spots, rather the images of individual vessels are fuzzy, making determination of vessel functional status difficult. The area of functional xylem measured manually was then correlated with the number of functional vessels (Figure 10), and with the results of the automated routine (Figure11). The regressions confirmed that both the number of functional vessels and the threshold areas depicted in the binary images, are excellent estimators of A_f. Preliminary results of the quantitative analysis described above, in which all the images for an individual plant were averaged; indicate that *Xf*-inoculated vines have a lower mean density of functional vessels (Table 1) than that of controls. Figures 12 and 13 show that the vessel density also correlates positively with the hydraulic conductivity for whole stems, suggesting that the visual assessment of MRIs conveys information about the actual water movement capacity of grapevine stems. Principal components ellipses ($p = 0.5$) in Figures 12 and 13 show that, in both, inoculated and control vines, the hydraulic conductivity for the whole stem is a function of the vessel density, but infected the vines tend to localize

clearly in the lower range of that response. We have shown that cavitated vessels that are air-filled can be re-filled (including restoring an image showing that they are water-filled, see Figure 3). However, attempts at refilling segments of PD-infected stems that showed "dark sectors" in the MRIs generally failed. This indicates that "dark sectors" in MRIs of infected vines are likely a sign of a relatively permanent deterioration of the water movement capacity in the stem, probably a consequence of tylose formation and/or vascular gel development.

Table 1. Mean values for calculated functional vessel densities in healthy and infected grapevine stems.

	Vessel density ± 1 SE	
Treatment	N_V/A_x	N_V/A_f
Control	63.03 ± 4.81	124.88 ±11.93
Xf-inoculation	49.78 ± 4.81	93.25 ±11.93

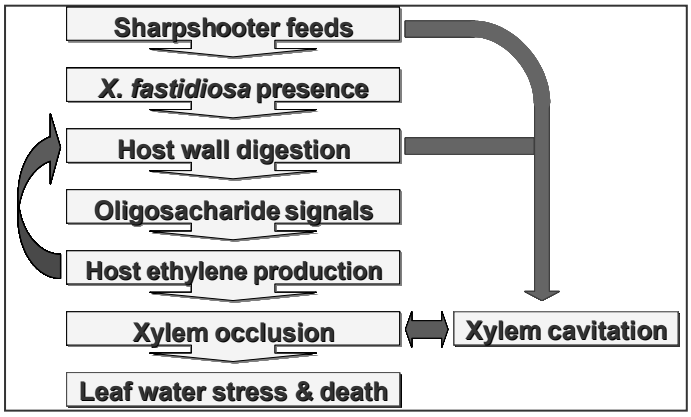


Figure 1. Hypothetical model for PD development. PD starts with a local infection caused by the glassy-winged sharpshooter's introduction of *Xf* locally (i.e., into one or a few vessels). Once *Xf* is in the xylem the bacteria become systemic, which implies that *Xf* must be able to cross (digest away?) the cell wall in the pit membranes that separate two neighboring vessels. The digestion of the cell wall by bacterial enzymes would generate transient oligosaccharides with biological activity. The presence of these oligosaccharides is detected by the plant triggering a series of defensive responses, including a raise in ethylene production. Ethylene has been shown to induce tylose formation. Cavitation of vessels may be also important for the disruption of water transport in the plant. Cavitations may happen during insect feeding or during PD progression. The "bottom line" of our thinking is that PD is primarily caused by the grapevine's responses (local and systemic) to *Xf* presence.

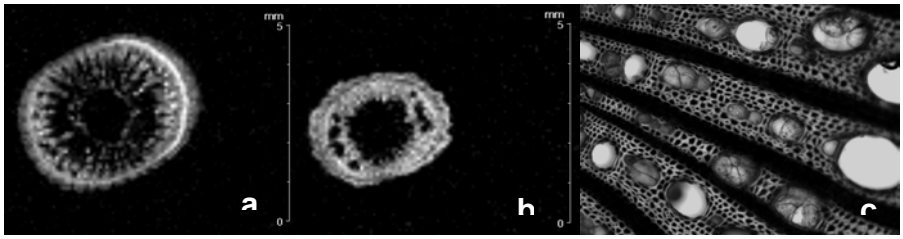


Figure 2. MRI of a PD-infected stem in a basal internode (a), and closer to the apex (b). Bright spots between the central pith (dark) and the ring of vascular cambium show functional vessels. Image b shows dark pockets within the vascular tissue that indicate areas in which vessels are not water-filled (compare the image to the healthy stem in Figure 3a). Tyloses (cellular-physical blockages of the vessels) are often associated with dark spots in MRIs of infected xylem, Tyloses are shown as accumulations of dark, bubble-like structures in vessel seen in the light microscope of an infected stem (c).

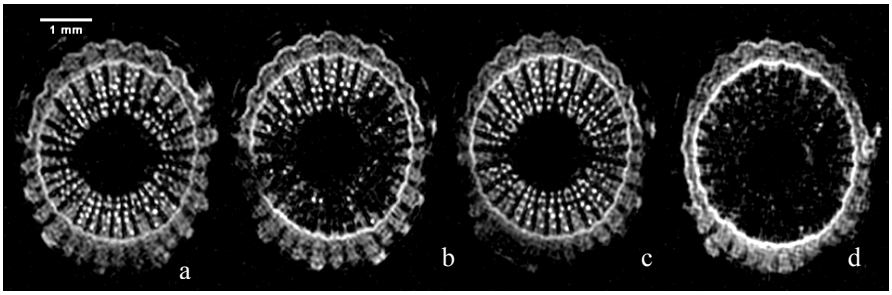


Figure 3. (a) MRI of an intact stem segment in a healthy shoot. (b) Image of the same stem portion after an important part of the cross section below has been severed, thus causing cavitation of many vessels. (c) The same stem segment after it has been refilled with water. (d) Stem segment after flushing with air to completely empty the xylem vessels.

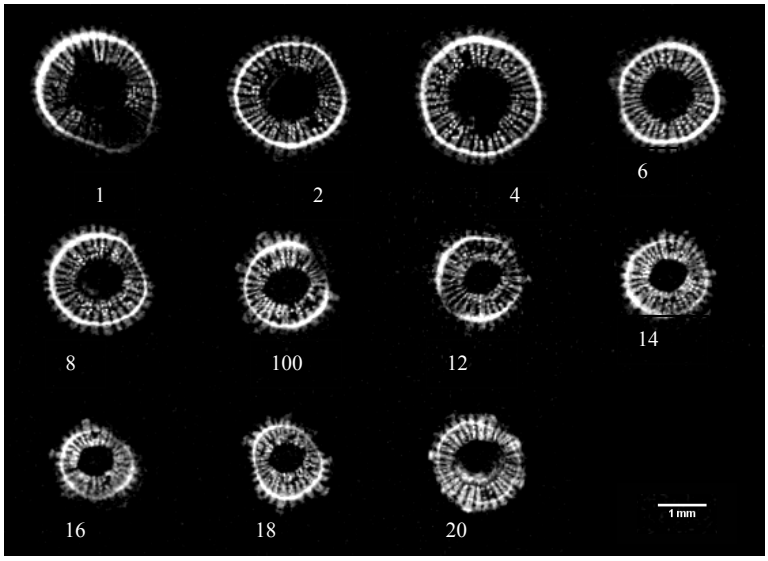
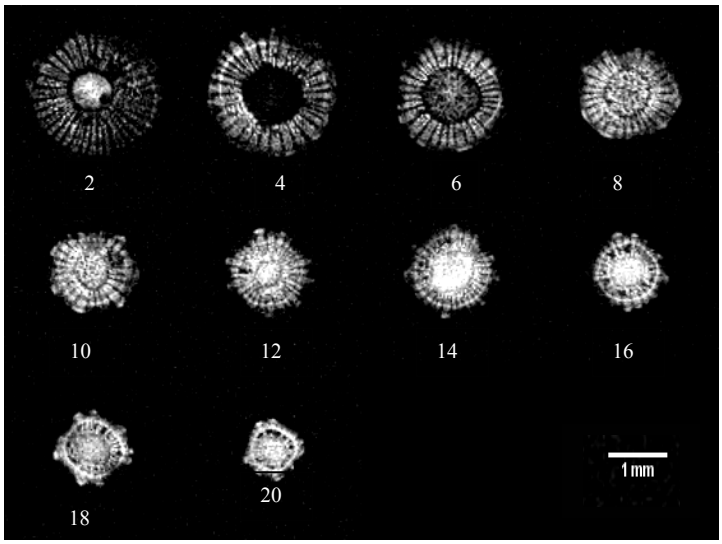


Figure 4. Stem cross section MRIs of a Control (water-inoculated) plant. The numbers indicate the internode position, counting from the base of the stem. In internodes 1-3 it is possible to observe the disruption of the xylem caused by the needle inoculation. The xylem disk looks normal in the other internodes. Note that individual vessels are easily observed as bright spots.

Figure 5. Stem cross section MRIs of an infected plant. This plant was not showing external symptoms after 6 moths of inoculation. The effect of needle inoculation can be seen in internode 2. Dark sectors of embolized vessels can be observed from internodes 10 to 20. Note that in this image it is more difficult to distinguish anatomical features and individual vessel than in MRIs of a Control plant (Figure 4).



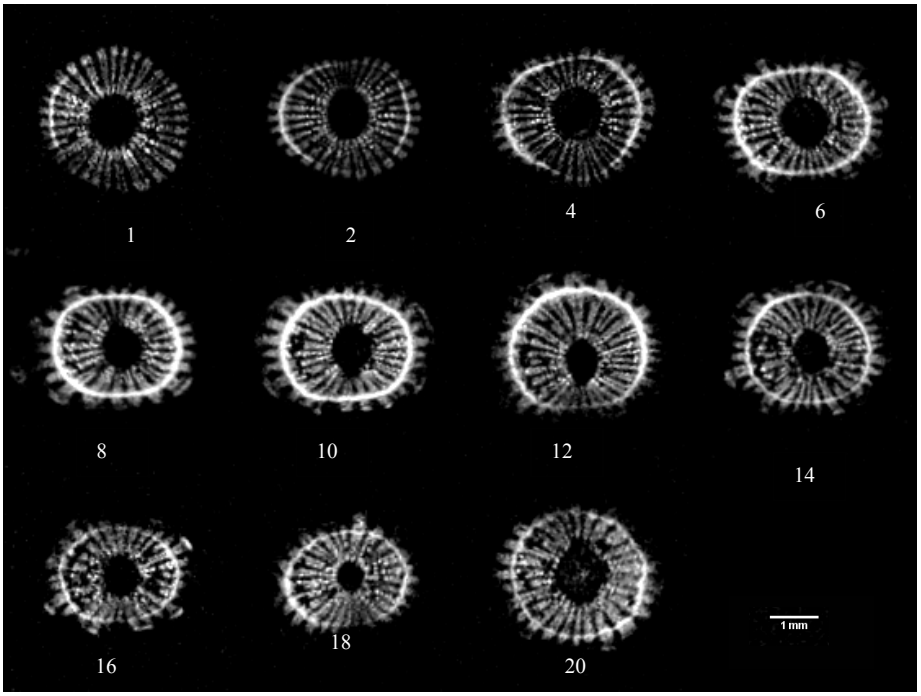


Figure 6. Stem cross MRIs of a plant exposed to ethylene. Numbers indicate the position of the internodes, numbered from the base of the stem. "Dark spots" that show non-functional vessels can be seen increasing in size from the base of the stem. The xylem disk appears to be compromised the most at internode 16, which was approximately the youngest internode in the stem (i.e., in the growing tip) at the time of ethylene treatment.

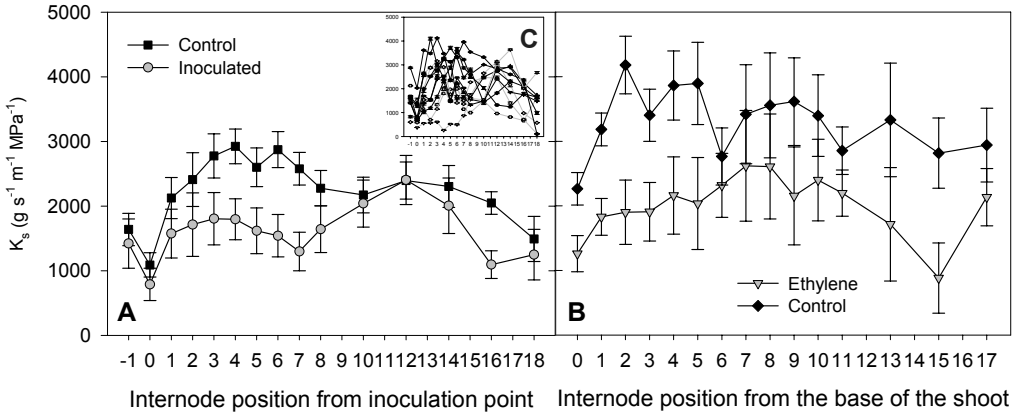


Figure 7. Specific hydraulic conductivities (K_s) for individual internodes of vines (a) inoculated with *Xf* and (b) exposed to ethylene (± 1 SE). Control plants show maximum K_s in middle third of the stem. In contrast, infected plants show a decrease in K_s in the middle portion of the stem. Panel (c) shows K_s ± 1 SD for all the plants analyzed in the inoculation experiment. Although the variation among different plants is high, the error associated with the measurements is negligible. **Note:** These measurements reflect the contribution of water flowing through cavitated vessels because the embolized vessels are filled by the pressurized water that is used in the test.

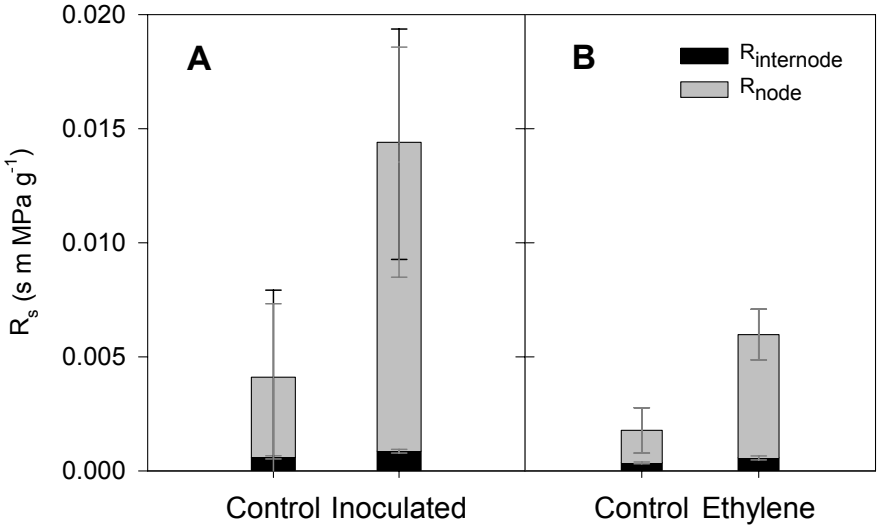


Figure 8. Specific hydraulic resistivity (R_s) for (a) vines inoculated with *Xf* and (b) exposed to ethylene. Total bar height represents R_s ± 1 SE (in black). R_s components, R_{node} and $R_{internode}$, are also shown (± 1 SE in gray). The nodes are a major component of stem hydraulic resistivity (the inverse of conductivity). It can be noted that R_s is about 3 fold higher for stems of infected plants than for controls, even when infected plants have no external symptoms. This observation agrees with the information provided by MRI.

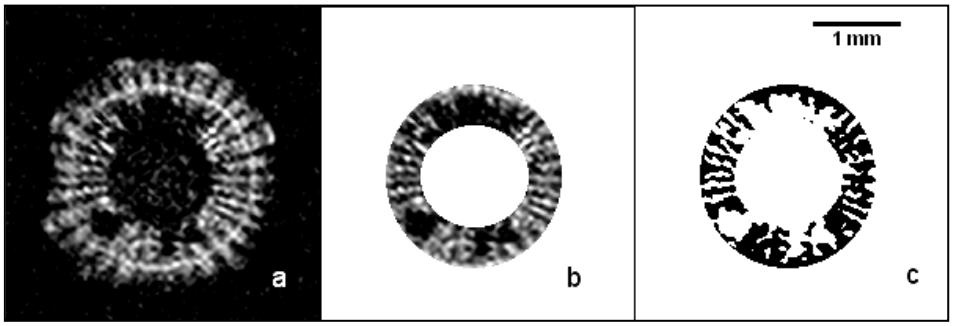


Figure 9. Example of the digital processing and analysis performed on MRIs to evaluate quantitatively the development of dark spots. (a) Original cross section MRI of an infected plant showing dark spots. Individual functional vessels are counted using this type of image. (b) Isolation and quantification of the cross sectional area of the stem that is normally xylem tissue (A_x). (c) Binary analysis of the xylem ring to determine the area of functional xylem (A_f), the black area represents the pixels that are above the threshold defined as the minimum value for a water-filled pixel. The program allows us to vary the threshold value.

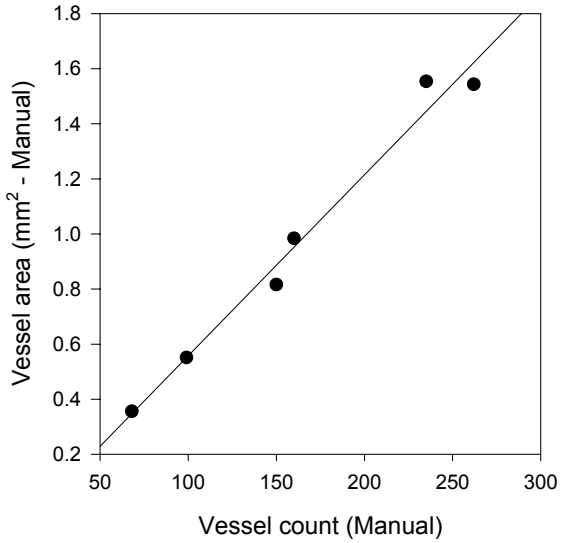


Figure 10. The number of functional vessel (vessel count) is a good predictor of the total area occupied by those vessels. Individual vessel areas were marked on the digitized MRI and summed automatically by ImageJ. Linear regression line $r^2 = 0.98$.

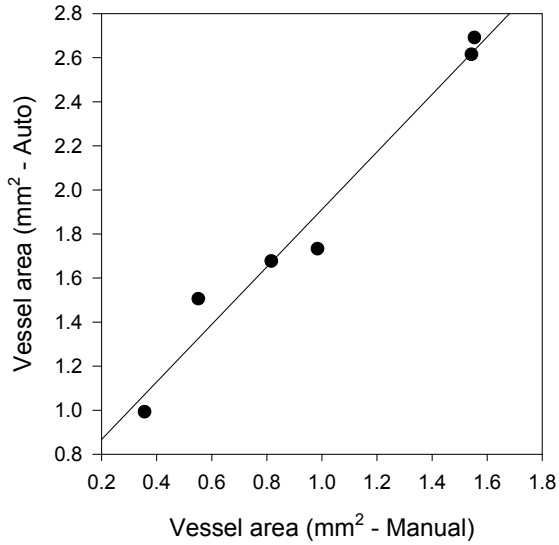


Figure 11. The area of functional xylem (the summation of the areas of individual vessels, see Figure 10 legend) is well correlated with the area calculated using an automated algorithm ($r^2 = 0.97$). A_f is the area calculated using the algorithm.

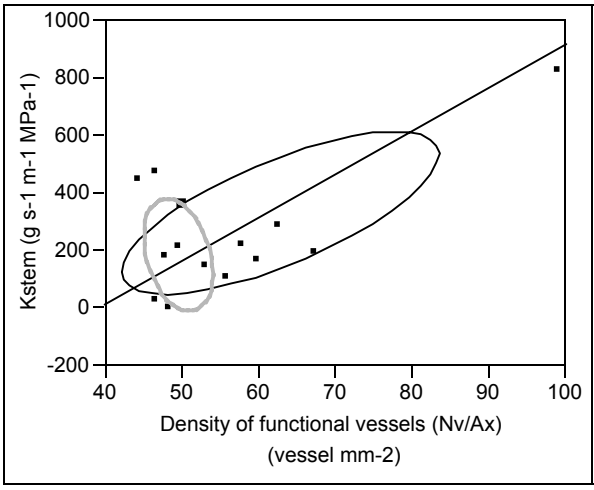


Figure 12. Principal component analysis plotting stem conductivity (y-axis) *vs* functional vessel density calculated as vessel number divided by total xylem area (x-axis). Ellipses enclose values for healthy vines (dashed, light line) and infected vines (heavy, grey line).

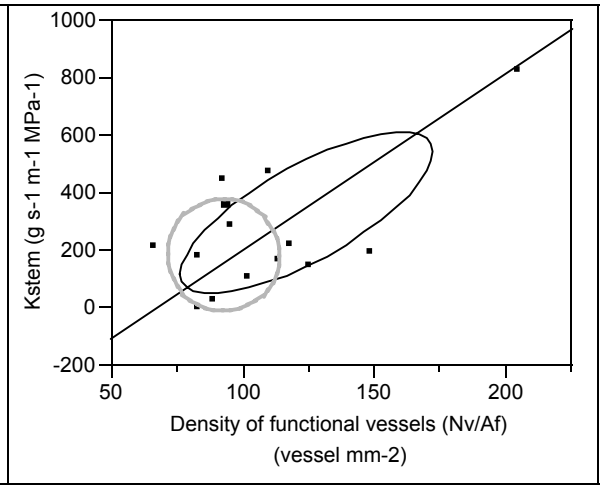


Figure 13. As in the Figure 12 legend, except that functional vessel density is calculated as vessel number divided by functional xylem area.

CONCLUSIONS

MRI will be a powerful adjunct to other, more conventional approaches for characterizing the changes that occur in grapevine xylem following introduction of *Xf*.

REFERENCES

Shackel KA, Labavitch JM (2003). Magnetic resonance imaging: A non-destructive approach for detecting xylem blockages in *Xylella fastidiosa*-infected grapevines. 2003 Pierce's Disease Research Symposium. p. 33-35.

Pérez AG, Greve LC, Walton J, Shackel KA, Thorpe E, Matthews MA, Lurie S and Labavitch JM (2004). *Xylella fastidiosa* infection and ethylene exposure cause xylem disruption and impaired water movement in grapevines shoots. Proceedings of the Annual Meeting of American Society of Plant Biology. Orlando, FL. June 2004.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease Research Grant Program.

IMPACT OF HOST PLANT XYLEM FLUID ON *XYLELLA FASTIDIOSA* MULTIPLICATION, AGGREGATION, AND ATTACHMENT

Project Leaders:

Nick Toscano
Dept. of Entomology
University of California
Riverside, CA 92521

Donald Cooksey
Dept. of Plant Pathology
University of California
Riverside, CA 92521

Cooperators:

Jian Bi
Dept. of Entomology
University of California
Riverside, CA

Korsi Dumenyo
Dept. of Plant Pathology
University of California
Riverside, CA

Rufina H. Martinez
Dept. of Plant Pathology
University of California
Riverside, CA

Reporting Period: The results reported here are from work conducted from October 2003 to August 2004.

ABSTRACT

Research in Temecula Valley indicated that the proximity of citrus groves to vineyards has influenced the incidence and severity of Pierce's disease (PD), *Xylella fastidiosa (Xf)*, in grapes. Although the glassy-winged sharpshooter (GWSS) feeds on and moves back and forth between Temecula citrus groves and vineyards, there are no visible *Xylella fastidiosa (Xf)* symptoms in the citrus. This implies that citrus trees are resistant or tolerant to the *Xf* but may be a reservoir to harbor the pathogen for GWSS acquisition while grape vines are susceptible. We investigated the mechanisms of host plant resistance/susceptibility by examining the impact of xylem fluid of grapefruit, orange, lemon and grape on *Xf* multiplication, aggregation and attachment as well as the related xylem fluid chemistry. Our laboratory experiments revealed that xylem fluid of grapefruit, orange and lemon caused an aggregation of Temecula PD cells to form large white clumps while grape xylem fluid did not cause visible clumping, but created a visible thick biofilm. The numbers of *Xf* cells in grapefruit xylem fluid treatment were significantly higher at 6, 8 and 9 days after culture compared with those in grape xylem fluid treatment. The numbers of *Xf* cells in orange or lemon xylem fluid tests were generally lower than those in grape xylem fluid treatment. Citrus xylem fluid significantly inhibited *Xf* biofilm formation compared to grape xylem fluid. The content of total amino acids in grape xylem fluid was near 9-fold higher than that in grapefruit xylem fluid. Sugar contents were 1.4- to 5.5-fold higher in grape xylem fluid than those in grapefruit xylem fluid. Peroxidase and total thiol levels were also higher in grape xylem fluid than in citrus xylem fluid. Our results indicate that the differences between citrus and grape plants in their responses to *Xylella* may be due to differences in their xylem fluid chemistry.

INTRODUCTION

Xylella fastidiosa (*Xf*) is a xylem-limited, plant pathogenic bacterium that causes Pierce's disease (PD) in grapes (Purcell, 1981). *Xf* is mainly vectored by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata,* in Southern California. Although a comprehensive list of suitable hosts for the GWSS has been identified, comprising 75 plant species in 35 families (Turner and Pollard, 1959), the major crop hosts in Temecula Valley are citrus and grapes. Previous studies in California have identified 94 plant species in more than 28 of plant families as host of *Xf* (Freitg, 1951; Raju et al, 1983; Raju et al., 1980). Most identified *Xf* hosts show no symptoms but serve as inoculum sources of *Xf* for vector acquisition. Perring et al (2001) studied the incidence of PD in the Temecula Valley and found that proximity of citrus groves to vineyards has influenced the incidence and severity of PD in grapes. The PD infection is most severe when the grape vines are adjacent to citrus, and that the damage declines as one moves away from citrus (Perring et al., 2001). Although the GWSS feeds on and moves back and forth between citrus trees and grape vines, there is generally no *Xf* caused disease symptom in citrus in the area. This implies that citrus trees are resistant or tolerant to the *Xf*, but may be a reservoir to harbor the pathogen for GWSS acquisition and transmission while grape vines are susceptible. Little is known about the biochemical mechanisms involved in host plant resistance/susceptibility to *Xf* in the system. Additional information is required to determine if citrus can be suitable reservoirs for *Xf*. Elucidation of the biochemical mechanisms may be useful for developing host plant resistance in grapes as a sustainable component of integrated pest management program.

Xf aggregates to form biofilm inside its host plants and insect vectors. The biofilm formation is considered as a major virulence factor of PD (Marques and Ceri, 2002). Biofilm is defined as structured communities of sessile microbial aggregates enclosed in a self produced polymeric matrix and attached to a surface (Costerton et al., 1995). It was recently reported that a defined medium with some components based on susceptible grape cultivar "Chardonnay" xylem fluid chemistry better supports *Xf* growth and stimulates *Xf* aggregation and biofilm formation in vitro (Leite et al. 2004). However, the effect of citrus xylem fluid on *Xf* multiplication, aggregation and biofilm formation remains unknown.

Xf is a nutritionally fastidious bacterium (Wells et al. 1987). In defined medium certain amino acids are essential for *Xf* growth, glucose stimulates the growth while fructose and sucrose have inhibiting effect (Wells et al. 1987; Chang and Donaldson, 2000). It is not known whether differences in contents of amino acids and the sugars in the xylem fluid of citrus

and grape may differentially affect growth of *Xf*. Redox status also likely affects the tendency for *Xf* aggregation and biofilm formation. Adding reducing agents such as glutathione to artificial medium promotes *Xf* aggregation and biofilm formation (Leite et al., 2004). It was reported that thiols mediate the aggregation and adhesion of *Xf* (Leite et al., 2002). Thiol-containing compounds in xylem fluid include cysteine, methionine and glutathione. The redox status in citrus and grape xylem fluid and its role in *Xf* aggregation and biofilm formation, and host plant resistance/susceptibility to *Xf* need to be further investigated.

OBJECTIVES

1. Investigate the effect of host plant xylem fluid on *Xf* multiplication, aggregation and attachment.
2. Determine the biochemical mechanisms of host xylem fluid influence on *Xf* multiplication, aggregation and attachment.

RESULTS

Commercial citrus (lemon, orange and grapefruit) groves in proximity to vineyards were selected in the Temecula Valley, California. Three blocks of 30 citrus and 30 grape vines were used. A minimum of 15 citrus trees and 15 vines were randomly selected from each block (making a total of 15 trees or vines from each plant species) to extract xylem fluid. Terminal shoots from each plant were used for xylem extraction with a pressure bomb apparatus (Anderson et al., 1989). Upon collection, the xylem fluid was immediately placed on dry ice before final storage in a -80 ^{o}C freezer. The samples were used to test the impact of these xylem fluid on *Xf* resistance and chemical analyses of soluble carbohydrates, free amino acids, and redox status.

Effects of xylem fluid of each plant species on *Xf* attachment were evaluated on the biofilm formation. Formation of biofilm on the abiotic surfaces was assessed as described by Espinosa-Urgel et al. (2000). The analyses of *Xf* multiplication and aggregation were based on the fact that optical density (540 nm) is correlated with bacterial cell numbers and aggregation state as described by Burdman et al. (2000).

Our data indicated that, when the xylem fluid of grapefruit, orange and lemon was added to the PD Temecula strain of *Xf* in PD3 medium in glass culture tubes, there were heavy *Xf* cell aggregations to form large white clumps in suspension of the culture and the culture fluid was clear with no significant turbidity; in contrast, grape xylem fluid added to the same *Xf* culture did not cause visible clumping, but rather a visible thick biofilm was formed on the surface of glass tube and the culture was turbid (Figure 1). After homogenization of the culture, we found that the numbers of *Xf* cells in the grapefruit xylem fluid treatment were significantly higher at 6, 8 and 9 days after culture compared with those in the grape xylem fluid treatment (Figure 2). The numbers of *Xf* cells in orange or lemon xylem fluid treatments were generally lower than those in grape xylem fluid treatment (Figure 3). These data suggest that the citrus species, especially grapefruit, are suitable hosts for *Xf* growth and may serve as a great reservoir of the pathogen for GWSS acquisition. Our assay results revealed that xylem fluid of the citrus species significantly inhibited *Xf* biofilm formation compared to that of grape (Figure 4). Our attempt to investigate the biochemical mechanisms likely to be involved indicated that 96% of amino acids in grape xylem fluid was comprised of glutamine, while 47% of amino acids in grape fruit xylem fluid was proline (Figure 5). The content of total amino acids in grape xylem fluid was near 9-fold higher than that in grapefruit xylem fluid (Figure 5). Sugar contents were 1.4- to 5.5-fold higher in grape xylem fluid than those in grapefruit xylem fluid (Figure 6). Peroxidase and total thiol levels were also higher in grape xylem fluid than in citrus xylem fluid (Figures 7 and 8).

CONCLUSIONS

Xylem fluid of grapefruit, orange and lemon caused PD Temecula strain of *Xf* cells to aggregate and form large white clumps but inhibited the attachment. In contrast, grape xylem fluid did not cause visible clumping but led to heavy attachment. Grapefruit xylem fluid significantly increased multiplication of *Xf* cells compared with grape xylem fluid. Citrus species, especially grapefruit, appear to be suitable hosts for *Xf* growth and may serve as a reservoir of the pathogen for GWSS acquisition and transmission to grape vines. Further research is underway to elucidate the biochemical mechanisms.

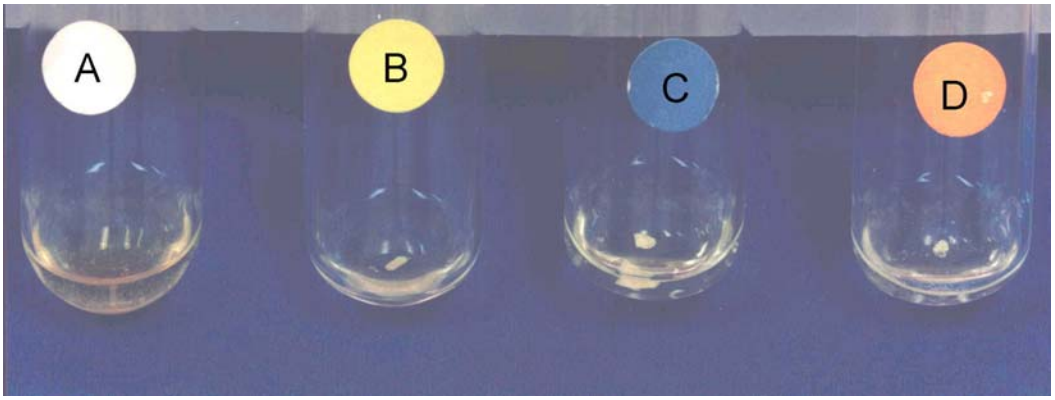


Figure 1. Effect of host plant xylem fluid on *Xf* aggregation. A, treatment with grape xylem fluid. B, treatment with grapefruit xylem fluid. C, treatment with orange xylem fluid. D, treatment with lemon xylem fluid. Note that white clumps of *Xf* aggregates are formed in the grapefruit, orange and lemon xylem fluid treatments.

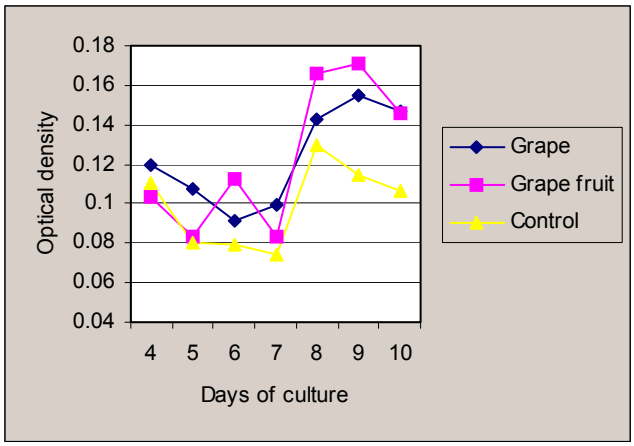


Figure 2. Effect of host plant xylem fluid on *Xf* growth.

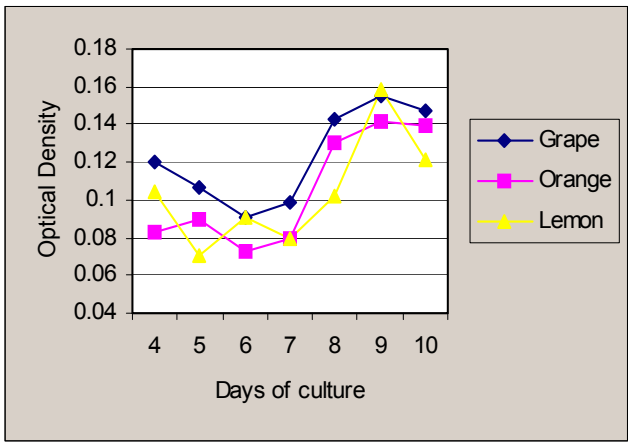


Figure 3. Effect of host plant xylem fluid on *Xf* growth.

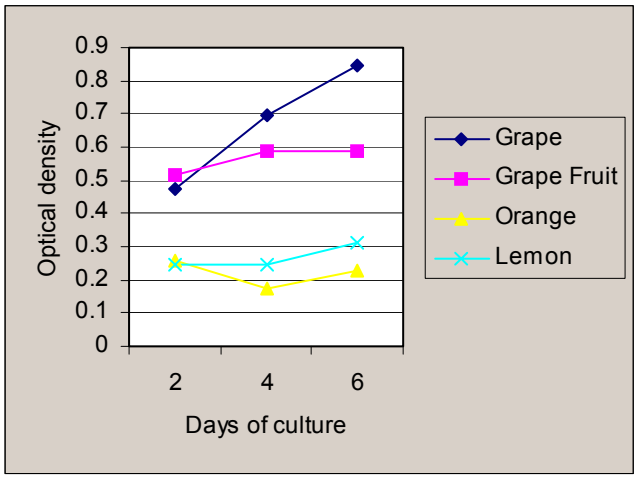


Figure 4. Effect of host plant xylem fluid on *Xf* biofilm formation.

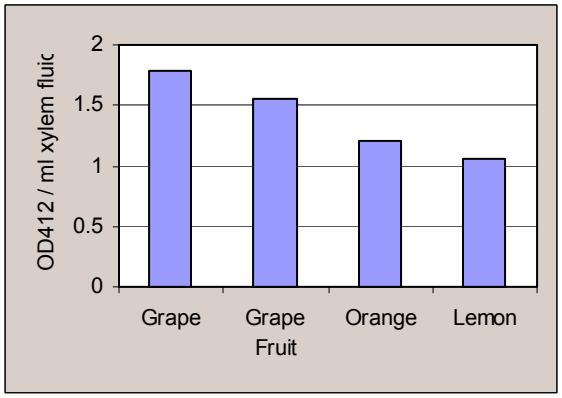


Figure 8. Total thiol contents in host xylem fluid.

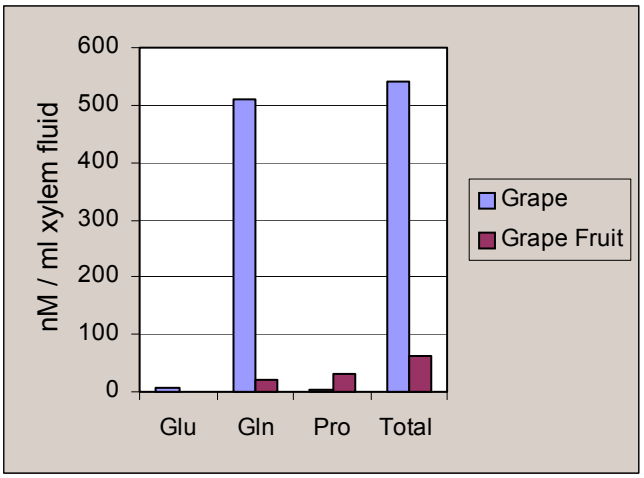


Figure 5. Some amino acid contents in grape and grape fruit xylem fluid.

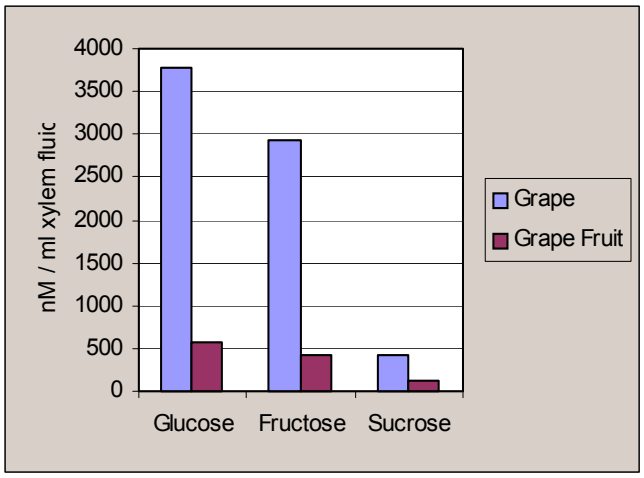


Figure 6. Sugar contents in grape and grape fruit xylem fluid.

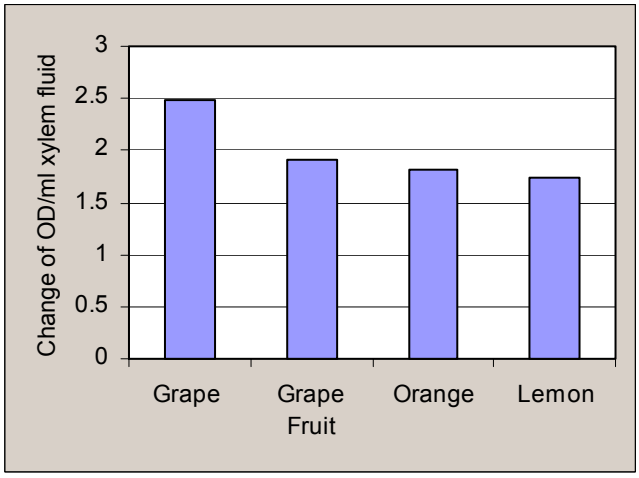


Figure 7. Peroxidase levels in host xylem fluid.

REFERENCES

Anderson, P. C., B. V. Brodbeck and R. F. Mizell III. 1989. Metabolism of amino acids, organic acids, and sugars extracted from the xylem fluid of four host plants by adult *Homalodisca coagulata*. *Entomol. Exp. Appl.* 50-149-160.

Anderson, P. C. 2002. Biologival, cultural, genetic, and chemical control of Pierce's disease: xylem fluid chemistry mediation of resistance to Pierce's disease, pp. 43-44, *in* Pierce's Disease Research Symposium, 2002. CDFA.

Burdman, S., E. Jurkevitch, M. E. Soria-Diaz, A. M. G. Serrano, and Y. Okon. 2000. Extracellular polysaccharide composition of *Azospirillum brasilense* and its relation with cell aggregation. *FEMs Microbiol. Lett.* 189:259-264.

Chang, C. J., and R. C. Donaldson. 2000. Nutritional requirements of *Xylella fastidiosa*, which causes Pierce's disease in grapes. *Can. J. Microbiol.* 46: 291-293.

Costerton, J. W. Z. Lewandowski, D. E. Caldwell, D. R. Corber, and A. M. Lappin-Scott. 1995. Microbial biofilms. *Annu. Rev. Microbiol*. 49:711-745.

Espinosa-Urgel, M., A. Salido, and J. L. Ramos. 2000. Genetic analysis of functions involved in adhesion of *Pseudomonas putida* to seeds. *J. Bacteriol*. 182:2363-2369.

Freitg, J. H. 1951. Host range of Pierce's disease virus of grapes as determined by insect transmission. *Phytopathology* 41: 920-934.

Leite, B., P. C. Anderson, and M. L. Ishida. 2004. Colony aggregation and biofilm formation in xylem chemistry-based media for *Xylella fastidiosa*. *FEMS Microbiol. Lett.* 230:283-290.

Marques, L. L. R. and H. Ceri. 2002. Charaterization of biofilm formation by *Xylella fastidiosa* in vitro. *Plant Disease.* 86:633-638.

Leite, B., P. C. Anderson, and M. L. Ishida. 2004. Colony aggregation and biofilm formation in xylem chemistry-based media for *Xylella fastidiosa*. *FEMS Microbiol. Lett.* 230:283-290.

Perring, T. M., C. A. Farrar and M. J. Blua. 2001. Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. *Calif. Agr.* 55:13-18.

Purcell, A. H. 1981. Pierce's disease. In Flaherty, D. L., Jenson, F. L. Kasamatis, A. N. et al., (eds). Grape Pest Management. UC Berkeley. Division of Agricultural Science. No. 4105. p 62-9

Raju, B. C., A. C. Goheen, and N. W. Frazier. 1983. Occurrence of Pierce's disease bacteria in plants and vectors in California. *Phytopathology* 73: 1309-1313.

Raju, B. C., S. F. Nome, D. M. Docampo, D. M. Goheen, A. C. Nyland, and S. K. Lowe. 1980. Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. *Am. J. Enol. Vitic.* 31: 144-148.

Turner, W. F. and H. N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. *USDA Tech. Bul.* 1188.

Wells, J. M., B. C. Raju, H. Y. Huang, W. G. Weiburg, L. Mandelcopaul, and D. J. Brenner. 1987. *Xylella fastidiosa* gen. Nov.: Gram negative, xylem limited fastidious plant bacteria related to *Xanthomonas* spp. *Int. J. Syst. Bacteriol*. 36: 136-143.

FUNDING AGENCY

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

OPTIMIZING MARKER-ASSISTED SELECTION FOR RESISTANCE TO *XYLELLA FASTIDIOSA* TO ACCELERATE BREEDING OF PIERCE'S DISEASE RESISTANT GRAPES OF HIGH FRUIT QUALITY

Project Leaders:

Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Alan Krivanek
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

Summaira Riaz
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

Reporting Period: The results reported here are from work conducted from October 2003 to October 2004. Research on this project was initiated under the "Genetics of Resistance to Pierce's Disease" of the Long-term American Vineyard Foundation Pierce's Disease Project.

ABSTRACT

Efforts at identifying molecular markers linked to *Xylella fastidiosa* (*Xf*) resistance are continuing. Our primary focus is on resistance derived from b43-17, a *Vitis arizonica/candicans* type collected near Monterrey, Nuevo Leon, Mexico. The '9621' *V. rupestris* x *V. arizonica* hybrid mapping family (PD resistant D8909-15 x PD resistant F8909-17) was used to localize *PdR1*, a primary PD resistance locus within the linkage map of the male parent F8909-17 (progeny of b43-17) and identify candidate linked resistance markers. In more recent research, a comparative mapping strategy between the '9621' linkage map and other SSR maps within *Vitis* was used to identify 9 SSR markers within 10 cM of the resistance locus. Resistance from the female parent D8909-15 has not yet been localized to a genetic map. The strategy of bulk segregant analysis (BSA) in concert with the AFLP marker system has been initiated to saturate the region around the resistance locus and is expected to yield an additional 20 to 50 markers linked to the resistance trait. All candidate resistant markers have been and will continue to be applied to breeding populations derived from '8909' x *V. vinifera* and ('8909' x *V. vinifera*) x *V. vinifera* back-cross generations in order to confirm resistance marker effectiveness in *V. vinifera* backgrounds and continue with marker assisted selection for development of high quality PD resistant grapes.

INTRODUCTION

Several American *Vitis* species are native to the regions where PD is endemic, and resistance from these sources has been introgressed into many different cultivars grown in the south-eastern United States. The acceptance of the new hybrid cultivars has been limited due in part to some undesirable non-vinifera fruit quality traits. The development of high quality PD resistant cultivars will be facilitated by the use of molecular markers to achieve a more precise introgression of the resistance genes into domesticated backgrounds and avoid introgression of undesirable traits (Figure 1). Backcross introgression via molecular markers has been accomplished successfully in other crops (Young and Tanksley 1989). This type of introgression is generally termed Marker Assisted Selection (MAS), whereby indirect selection on a trait of interest (such as disease resistance) is made by screening for the presence of a DNA marker allele tightly linked to the trait. MAS for disease resistance can also be used to eliminate susceptible genotypes in a breeding population early in the selection process, which allows for evaluation of much larger effective populations. Larger effective population sizes increase the opportunity to identify genotypes with high disease resistance and good horticultural qualities (such as good flavor traits, color, berry and cluster size. etc.). Other key aspects of the MAS process include avoiding confounding environmental effects on the trait phenotype and accelerating breeding progress while saving space and time, allowing for more efficient use of resources (Paterson et al. 1991, Kelly 1995). Rapid screening time is particularly valuable when applied to perennial crops such as grape with relatively long generation times (Alleweldt 1988, Striem et al. 1994). To effectively use linked markers in MAS only requires that the markers be highly reproducible, linked in coupling phase i.e. on the same homologous chromosome, and within 5 centimorgan (cM) mapping units of the resistance locus (Kelly 1995).

Within grapevines, markers linked to powdery mildew resistance (Dalbo et al. 2001, Pauquet et al. 2001), downy mildew resistance (Luo et al. 2001) and seedlessness (Lahogue 1998) have been published. In the case of powdery mildew resistance, MAS has already been successfully utilized for screening a grape breeding population. We are successfully developing a MAS system for screening PD resistant genotypes that will greatly benefit our breeding of PD resistant wine grapes.

OBJECTIVES

Our overall objective is to identify DNA markers that are tightly linked to the primary locus or loci required for complete resistance to PD within *Vitis*. Research will focus on PD resistance as inherited from *V. arizonica* and will utilize an established *V. rupestris* x *V. arizonica* genetic map. These markers will be utilized for MAS to eliminate susceptible seedling progeny our continuing PD resistance breeding program.

Sub-objectives

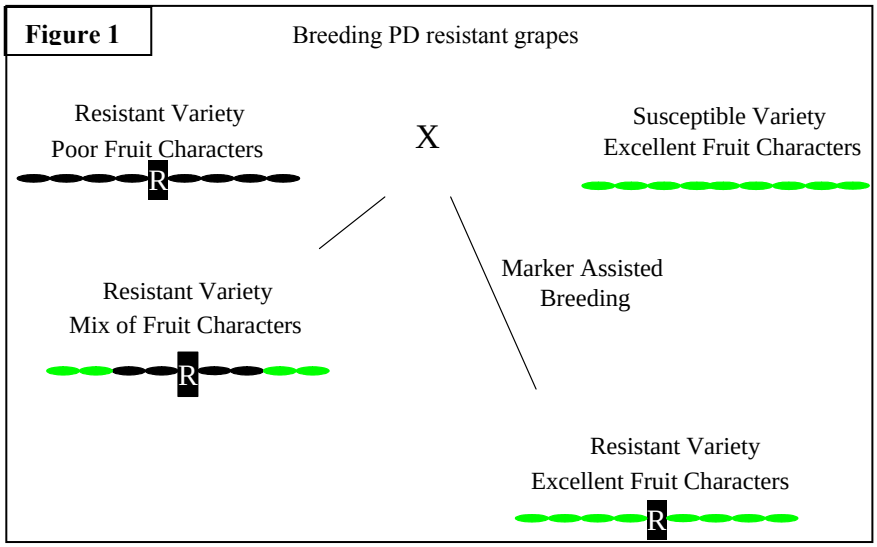
1. Continue with a comparative mapping strategy between the *V. rupestris* x *V. arizonica* 9621 (D8909-15 x F8909-17) linkage map and other SSR maps within *Vitis* in order to identify additional SSR markers linked to resistance.

2. Utilize Bulk Segregant Analysis (BSA) with the AFLP marker system to saturate with markers the region around the previously mapped *Xf* resistance locus and eventually convert confirmed candidate markers to stable SCAR primers.
3. Confirm candidate marker linkage to resistance within families derived from resistant by susceptible crosses such as the '8909' x *V. vinifera* and ('8909' x *V. vinifera*) x *V. vinifera* back-cross generations.

RESULTS AND CONCLUSIONS

Sub-objective 1.

Initial mapping of the PD resistance locus *PdR1* in the male parent F8909-17 of the 9621 family localized it to chromosome 14, and identified 6-8 SSR markers on the same linkage group. Marker placement on published SSR linkage maps of *Vitis* were used to preferentially target chromosome 14, bringing the total number of SSR markers on the linkage group up to 30. Approximately 9 SSR markers are localized within a 10 cM distance of the resistance gene. These SSR markers are reliable and are the easiest of the molecular markers to incorporate within a MAS breeding program. Correlation tests of these candidate markers to PD resistance when functioning within a *V. vinifera* genetic background are underway and described in sub-objective 3. The SSR marker analysis has allowed us to confirm that marker alleles linked in coupling to PD resistance alleles of the *PdR1* locus in another PD resistant progeny of b43-17 (F8909-08) are different than the alleles linked in coupling the resistance alleles in F8909-17. It is apparent from these results that b43-17 is homozygous resistant for the PdR1 locus, and that F8909-17 inherited its resistance allele from one chromosome 14 and F8909-08 inherited its resistance allele from the homologous chromosome 14. In either case the markers linked to resistance will function for MAS, however, different alleles linked in coupling to the resistance alleles will have to be followed through the downstream MAS process. Placement of SSR markers to chromosome 14 via the comparative mapping strategy continue as the markers become available, however, the number of SSR markers that can be targeted to a specific chromosomal region via comparative mapping is limited.



Sub-objective 2.

For high density marker saturation within a narrow window around the *PdR1* locus, a bulk segregant analysis (BSA) strategy (Michelmore et al. 1991) in concert with the AFLP marker system was chosen as the method of choice. Initial BSA was attempted within the 9621 family, however, confounding effects of the resistance loci within the D8909-15 parent made the attempt more difficult than expected. To avoid confounding affects from resistance inherited from other genetic backgrounds and focus the BSA procedure only on the *PdR1* locus, work has begun within two segregating families from susceptible by resistant crosses. The first family, 99217 (C8909-07 x F8909-08) consists of 33 genotypes, has been screened for PD resistance (Krivanek et al. submitted) and segregates 1:1 resistant to susceptible (Table 1). DNA has been extracted from these genotypes, flanking SSR markers were run and a good correlation between resistance and resistance marker alleles has been established (Table 1). A bulk of the DNA from the 12 most susceptible and a bulk of the DNA from the 12 most resistant genotypes are in process and will be tested for AFLP polymorphisms utilizing florescent primers and visualized on a PE 3100 sequencer. The second family derived from a susceptible by resistant cross is a *V. vinifera* x F8909-08 family; it consists of 40 genotypes and has been designated as 0062. Testing of this family for PD resistance is currently underway via our standard greenhouse testing procedure (Krivanek et al. in press; Krivanek and Walker in press). It is expected that the progeny in this family will segregate in a 1:1 manner, and if so, DNA extraction and BSA procedures will be undertaken as with the 99217 family. Candidate AFLP markers will be converted to stable and more reliable SCAR primers before incorporation into the MAS program.

Sub-objective 3.

Work is progressing with two distinct breeding populations for testing of candidate resistance markers and initial application of those markers to MAS. One family is a cross of the PD resistant F8909-08 to a female *V. vinifera* wine grape F2-7 (Cabernet Sauvignon x Carignane) and designated as the 0062 family. A second breeding population consists of a cross of F8909-08 to several elite *V. vinifera* table grape genotypes (the 500 series). A subset of the 500 series has been screened for PD resistance and screened for markers flanking the *PdR1* locus. Five confirmed resistant genotypes have been utilized in the development of the first backcross generations BC1 (backcrossed to additional elite *V. vinifera* genotypes). The BC1 population (25000 series) consists of approximately 200 individuals and was planted in the field in 2003. Marker analysis for flanking markers to the *PdR1* locus has been completed for the 25000 series and the marker information was utilized in selection of genotypes for the spring of 2004 crosses for the development of the BC2 generations. Subsets of candidate

resistant and susceptible genotypes within the 25000 series have shown improved fruit quality (Figure 2) and are currently being screened to confirm the correlation between the resistance markers and the PD resistance trait. We are also utilizing these populations to confirm the effectiveness and economics of the MAS relative to our greenhouse screening procedure.

Table 1. Resistance classification and marker genotypes for the individuals of the full-sib family derived from the susceptible by resistant cross of C8909-07 x F8909-08. * = Genotypes selected for Bulk Segregant Analysis procedure.

Genotype	Overall resistance level to PD	Mean natural log (cells/ml)	Mean CMI score	Mean % leaf scorch	Alleles of SSR markers flanking the PdR1 resistance
99217-21 *	Resistant	9.51	1.00	58.3	Rr / Rr
99217-40 *	Resistant	9.70	1.33	75.0	rr / Rr
99217-18 *	Resistant	9.77	2.75	95.0	Rr / Rr
99217-41 *	Resistant	10.19	4.25	76.3	Rr / Rr
99217-35 *	Resistant	10.55	1.33	100.0	rr / Rr
99217-19 *	Resistant	11.08	2.50	76.7	rr / Rr
99217-01 *	Resistant	11.52	2.25	90.0	rr / Rr
99217-23 *	Resistant	11.57	3.00	87.5	Rr / Rr
99217-34 *	Resistant	11.83	3.75	65.0	Rr / Rr
99217-46	Resistant	11.87	5.75	100.0	Rr / Rr
99217-27 *	Resistant	12.20	4.25	100.0	Rr / rr
99217-22 *	Resistant	12.29	4.00	100.0	Rr / Rr
99217-12 *	Resistant	12.50	4.00	95.0	Rr / Rr
99217-38	?	12.69	5.00	100.0	Rr / Rr
99217-36	?	13.09	5.00	100.0	rr / rr
99217-50	?	13.52	4.25	83.8	Rr / Rr
99217-14	Susceptible	14.06	5.50	88.8	rr / Rr
99217-07	Susceptible	14.87	5.50	100.0	rr / rr
99217-04 *	Susceptible	15.42	6.00	100.0	rr / rr
99217-33 *	Susceptible	15.59	5.75	100.0	rr / rr
99217-06 *	Susceptible	15.80	5.25	68.3	rr / rr
99217-09 *	Susceptible	15.81	5.75	100.0	rr / rr
99217-10	Susceptible	15.82	4.75	100.0	rr / rr
99217-13 *	Susceptible	15.84	5.50	100.0	rr / rr
99217-42	Susceptible	15.85	4.25	75.0	rr / Rr
99217-15 *	Susceptible	15.87	5.25	100.0	rr / rr
99217-32 *	Susceptible	15.87	5.50	100.0	rr / rr
99217-28 *	Susceptible	15.91	5.75	100.0	rr / rr
99217-05 *	Susceptible	15.91	5.75	100.0	rr / rr
99217-37 *	Susceptible	15.92	5.25	100.0	rr / rr
99217-26 *	Susceptible	15.95	5.50	100.0	rr / rr
99217-24 *	Susceptible	16.04	6.00	100.0	rr / rr

Figure 2.

Vitis arizonica PD Resistant poor fruit quality

Hybrid BC1-25017 with flanking PD resistance markers Improved fruit quality

Vitis vinifera PD Susceptible Excellent fruit quality

REFERENCES

Alleweldt G. and Possingham J.V. 1988. Progress in grapevine breeding. Theor. Appl. Genet. 75:669-673.

Dalbo M.A., Ye G.N., Weeden N.F., Wilcox W., and Reisch B.I. 2001, Marker-assisted selection for powdery mildew resistance in grapes. J. Am. Soc. Hort. Sci. 126: 83-89.

Kelly J.D. 1995. Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogen. HortScience 30:461-465.

Krivanek A.F., Famula T.R., Tenscher A. and Walker M.A. (Submitted) Inheritance of resistance to Pierce's disease within a *Vitis rupestris* x *Vitis arizonica* hybrid population. Theor Appl Genet.

Krivanek A.F., Stevenson J.F. and Walker M.A. (In Press) Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology.

Krivanek A.F. and Walker M.A. (In Press) *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology.

Lahogue F., This P. and Bouquet A. 1998. Identification of a codominant SCAR marker linker to the seedlessness character in grapevine. Theor. Appl. Genet. 97: 950-959.

Luo Su-Lan, He Pu-Chao, Zhou P. and Zheng Xue-Qin. 2001. Identification of molecular genetic markers tightly linked to downy mildew resistant genes in grape. Acta Genet. Sinica 28: 76-82.

Michelmore R.W., Paran I. and Kesseli R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828-9832

Paterson A.H., Tanksley S.D. and Sorrells M.E. 1991. DNA markers in plant improvement. Adv. Agr. 46:39-90.

Pauquet J., Bouquet A., This P. and Adam-Blondon A-F. 2001. Establishment of a local map of AFLP markers around the powdery mildew resistance gene Run1 in grapevine and assessment of their usefulness for marker assisted selection. Theor. Appl. Genet. 103:1201-1210.

Striem M.J., Ben-Hayyim G. and Spiegel-Roy P. 1994. Developing molecular genetic markers for grape breeding, using polymerase chain reaction procedures. Vitis 33:53-54.

Young N.D. and Tanksley S.D. (1989) RFLP analysis of the size of chromosomal segements retained around the *Tm-2* locus of tomato during backcross breeding. Theor Appl Genet 77:353-359.

FUNDING AGENCIES

Funding for the 2004-2005 funding year was received in mid-September 2004. This proposal was not submitted to other funding agencies. However, it is linked to the Walker/Tenscher Pierce's disease resistance breeding project funded by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board (and formerly by the California Table Grape Commission and the California Raisin Advisory Board), and the Walker/Riaz mapping project. This project was initiated through funding by the American Vineyard Foundation and CDFA for the Genetics of Resistance to Pierce's disease, a project that developed a framework map for the 9621 population. Funding from the Louis P. Martini Endowed Chair in Viticulture has also supported Pierce's disease mapping and marker development projects.

MAP BASED IDENTIFICATION AND POSITIONAL CLONING OF *XYLELLA FASTIDIOSA* RESISTANCE GENES FROM KNOWN SOURCES OF PIERCE'S DISEASE RESISTANCE IN GRAPE

Project Leaders:
Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Summaira Riaz
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

Reporting Period: The results reported here are from work conducted from November 2003 to October 2004.

ABSTRACT
Development of an SSR genetic linkage map based on the 9621 family is continuing. The family segregates for PD resistance and is based on the cross of PD resistant D8909-15 x PD resistant F8909-17. We expanded the mapping population size from 116 to 188 genotypes. The current genetic linkage map consists of 217 non-AFLP markers (SSR, EST-SSR and ESTP) in 19 linkage groups. The PD resistance locus *PdR1* maps to linkage group 14 of the male parent (F8909-17), which now consists of 30 markers, 9 of which are localized within 10 cM of *PdR1*. To avoid confounding affects from resistance inherited from D8909-15 additional families derived from a susceptible by resistant cross are currently being evaluated for map based cloning of the *PdR1* locus. A family from the cross of F2-7 (a cross of two *V. vinifera* wine grapes, Cabernet Sauvignon x Carignane) x F8909-08 (a PD resistant sibling of F8909-17) has been made and is currently being screened for PD resistance via our standard greenhouse testing procedure. To saturate a narrow region around the resistance locus with molecular markers, bulk segregant analysis (BSA) in concert with the AFLP marker system has been initiated in cooperation with our report titled "Optimizing marker-assisted selection (MAS) for resistance to *Xylella fastidiosa* to accelerate breeding of PD resistant grapes."

INTRODUCTION
This project expands upon and continues a genetic mapping effort initiated with funding from the California Grape Rootstock Improvement Commission, the Fruit tree, Nut tree and Grapevine Improvement Advisory Board, the California Table Grape Commission and the American Vineyard Foundation. The project has been mapping resistance to *Xiphinema index*, the dagger nematode, and *Xylella fastidiosa* (*Xf*) in an "F2" population designated as the 9621 family (D8909-15 x F8909-17). A genetic map of 116 individuals from the 9621 population was created primarily with AFLP markers (Doucleff et al. 2004). Our efforts were expanded to informative markers, such as microsatellites or simple sequence repeats (SSR) for two main reasons. First, a genetic map based on SSR markers provides a reliable and repeatable framework for initial mapping of candidate genes and quantitative trait loci (QTLs). Secondly, SSR markers tightly linked to resistance and phenotypic traits of interest are ideal for marker-assisted selection due to their applicability across different genetic backgrounds and ease of use. The grape genetic research community formed the International Grape Genome Program (IGGP) to increase coordination and cooperation and to enhance knowledge of the grape genome. Use of the SSR marker system is common among the different research groups so that our mapping efforts can be linked to others. Integrating the 9621 genetic linkage map to other mapping populations will facilitate targeting genomic regions that harbor quantitative trait loci. Comparison to other maps will allow us to identify more markers that are linked to *Xf* resistance and optimize marker-assisted selection strategies applied to breeding programs. For fine scale mapping a narrow region around the primary resistance locus, we include procedures here. The proposal will expand to include construction and utilization of a genomic library of a resistant parental genotype for eventual cloning of the PD resistance gene.

OBJECTIVES
1. Increase the base population from 116 to 188 genotypes within the 9621 family and expand to a family based on a susceptible by resistant cross of 2,000 to 4,000 genotypes.
2. Increase the number of SSR and EST markers on the core genetic linkage map from 100 to 300 markers.
3. Screen an additional 100-150 EST derived SSR markers for which functions are known after their comparison to homologues in available EST databases.
4. Develop core framework map with an average distance of 2 to 5 cM between markers and utilize Bulk Segregant Analysis (BSA) with the AFLP marker system to saturate a 1 cM region around the *PdR1* resistance locus.

RESULTS AND CONCLUSIONS
Objective 1
The original starting material for this project was a molecular marker linkage map of the 9621 population based on 116 individuals (Doucleff et al. 2004). We expanded the core set of individuals from the 9621 to 188 genotypes to take advantage of 96-well plate based techniques and to increase resolution on the map to improve marker association with PD resistance. A second family derived from a susceptible by resistant cross of F2-7 (a *V. vinifera* wine grape, Cabernet Sauvignon x Carignane) x F8909-08 (a PD resistant sibling of F8909-17) has been made, and 40 individuals are currently being screened for PD resistance via our standard greenhouse testing procedure. An expansion of the family was made in the

Spring 2004 and a total of 4,500 seeds have been collected and placed into cold stratification. Should the initial subset of the family segregate in a 1:1 resistant to susceptible ratio as expected the expanded family of approximately 2,000 to 3,000 genotypes will be an excellent choice for fine resolution placement of the *PdR1* resistance gene. This would be the first step toward placement of resistance markers (flanking the *PdR1* locus) onto a bacterial artificial chromosome (BAC) within a genomic library in a procedure termed "chromosome landing" (Tanksley et al. 1995). Plans for construction of the library are underway.

Objective 2

The original genetic linkage map was based primarily on AFLP markers with 375 placed on the map, with an additional 32 ISSR, 25 RAPD and 9 SSR markers (Doucleff et al. 2004). Our efforts expanded to more reliable SSR markers in order to construct a repeatable framework map useful for more precise placement of primary resistance genes, QTL analysis and marker-assisted selection. Among the marker classes added to the map 310 SSR markers have been tested, 155 were polymorphic in the parents and all have been added to the map; 90 EST derived SSR markers have been tested, 60 of them were polymorphic and 46 have been added to the map; 20 EST markers (provided by Doug Adams) have been tested and 16 were added to the map (Table 1). A total of 217 markers (SSR, EST-SSR and ESTP) tested on 188 genotypes have now been utilized for map construction.

The 217 SSR markers included some that have been previously published and many that were developed by Vitis Microsatellite Consortium and are as yet unpublished. All markers were tested on a small set of 8 DNA samples including both parents and run on 6 % polyacrylamide gels. DNA on the gels was visualized by silver staining with a commercial kit (Promega). We have tested and used all available informative genomic microsatellite markers for the 9621 population. Meanwhile, we also initiated collaboration efforts with the research group at INRA (Montpellier, France) to obtain primer sequences of SSR markers developed at their facility.

To develop ESTP (expressed sequence tagged polymorphism) markers, sequences of grape cDNA were obtained from Dr. Doug Adams (Department of Viticulture and Enology, UC Davis). Potential PCR primers were designed using the computer program PRIMER 0.5. Primers were selected to have similar properties to facilitate standard conditions for PCR reactions. Primers are 20 to 23 nucleotides long with GC contents of 50-60% and melting temperature ranging from 59-64°C. Amplification and polymorphism for each EST was tested on 2% agarose gels. If length base polymorphisms were not revealed, then a set of 10 different restriction enzymes (*Hind*III, *EcoR*I, *Ava* II, *Bst*NI, *Dra*I, H*ae* III, H*inf*1, M*sp* I, *EcoR*V, R*sa* I) were tested to find restriction site based polymorphism among parents D89090-15 and F8909-17.

Objective 3

There are now a large number of EST derived SSR markers available, in addition to the genomic SSR markers from the Vitis Microsatellite Consortium. The EST derived SSR markers are more valuable if the cDNA sequence from which the EST was derived has a known function as determined by comparisons with homologs from other EST databases. We plan on selecting EST-SSR markers that show homology to genes which control disease resistance along with those that control other important morphological, physiological and agronomic traits. So far we have tested 90 EST-SSR markers from three different sources (Table 1) and 45 of informative markers were added to the entire core set of 9621 population. Our goal is to screen an additional 100-150 EST-SSR markers with putative known function and we are adding to the map as they are completed.

Objective 4

In order to develop the core framework map based on SSR markers, preliminary linkage analysis for each parent was carried out with MAPMAKER 2.0. Each segregating locus was paired with a "dummy" locus, resulting in a doubled data set. Linkage groups obtained from the doubled data set were then divided into two symmetrical sets of groups and one set was chosen for further detail. The "first order" and "compare" commands were used to determine the probable order of all markers in each linkage group. The integrated linkage analysis to obtain the sex-average map was performed with JOINMAP 2.0 (LOD 5.0 and recombination frequency 0.45). Using the fixed sequence command, the order of markers was determined relative to the established order obtained from the initial MAPMAKER analysis. Map units in centimorgans (cM) were derived from the Kosambi (K) mapping function. The integrated consensus map analysis was carried out with JOINMAP 3.0. The consensus linkage map was developed with 217 markers (155 SSR markers, 45 EST-SSR, 16 ESTP markers and the Pierce's disease resistance locus). A total of 214 markers fall in 19 linkage groups and only 3 markers were unlinked. Total map length is 1300 cM with average distance between markers of 5.9 cM. All markers were evenly distributed. The current map is depicted in Figure 1. The largest linkage group was comprised of 30 markers and smallest group consisted of 4 markers (Table 2). The locus for Pierce's disease resistance mapped to linkage group 14 with flanking markers on each side (Figure 1). Many additional markers have been added but have not been included on the map.

To saturate a narrow region around the *PdR1* locus resistance locus with molecular markers, the strategy of bulk segregant analysis (BSA) (Michelmore et al. 1991) in concert with the AFLP marker system has been initiated in cooperation with our report titled "Optimizing marker-assisted selection (MAS) for resistance to *Xylella fastidiosa* to accelerate breeding of PD resistant grapes." Work has begun within two segregating families from susceptible by resistant crosses. One family, C8909-07 by F8909-08, segregates 1:1 resistant to susceptible and a good correlation between resistance and resistance marker alleles has been established. A bulk of the DNA from the 12 most susceptible and a bulk of the DNA the 12 most

resistant genotypes are in process and will be tested for AFLP polymorphisms utilizing florescent primers and visualized on a PE 3100 sequencer.

Table 1. Data on number of markers mapped for the 9621 (D8909-15 x F8909-17) mapping population.

Molecular Markers		
Genomic SSR	VMC published/unpublished	134
	VVMD	10
	VVS	2
	INRA	9
EST derived SSR	Southern Cross University, Australia	4
	INRA, France	7
	Genome Facility (U.C. Davis)	35
ESTP markers	Doug Adams/NCBI data base	16
Grand Total		217

Table 2. Details of the 9621 genetic linkage map.

Linkage groups	19
Linked markers	214
Total map length	1300 cM
Average distance between markers	5.98 cM
Largest group (PD linkage group)	30 markers 80cM (group14)
Smallest group	4 markers 18cM (group 15)

Figure 1a. Riaz & Walker2004 SSR based genetic linkage map of 9621 (8909-15 X8909-17)

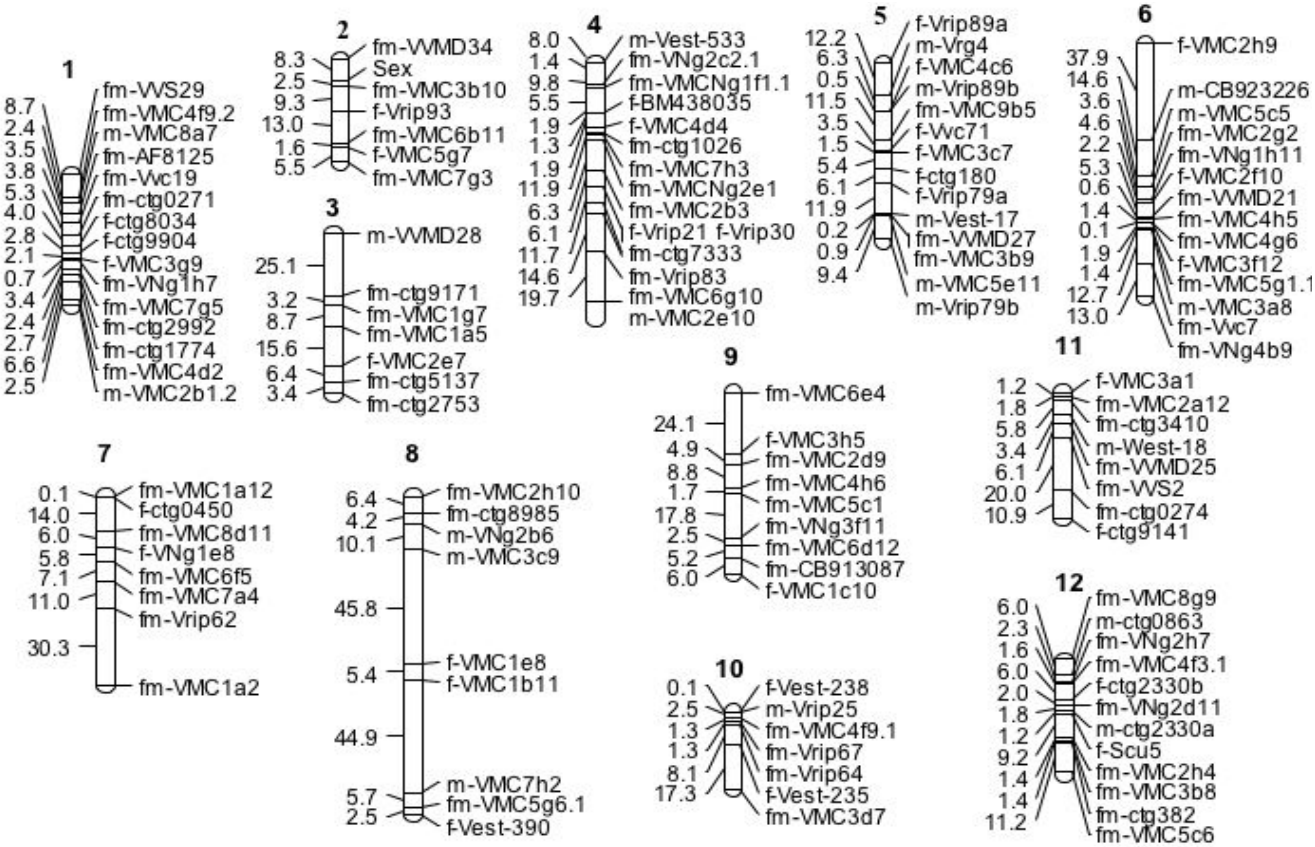
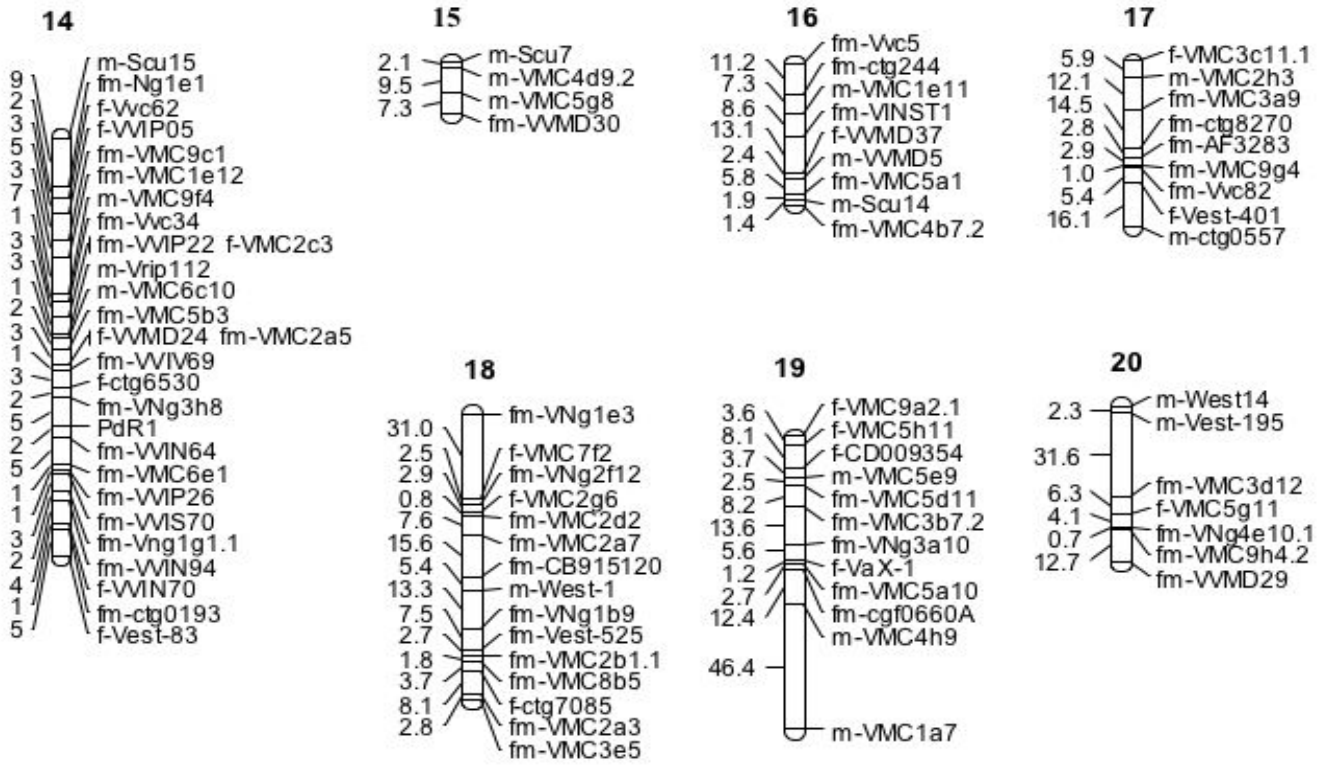


Figure 1b. Riaz & Walker2004 SSR based genetic linkage map of 9621 (8909-15 X8909-17)



REFERENCES

Doucleff M, Jin Y, Gao F, Riaz S, Krivanek AF, Walker MA (2004) A genetic linkage map of grape utilizing *Vitis rupestris* and *Vitis arizonica*. Theor Appl Genet (Published on-line Aug 4).

Michelmore RW, Paran I, Kesseli RV 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828-9832

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11:63-68.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. Previous mapping efforts upon which this research is based received funding from the American Vineyard Foundation, the California Grape Rootstock Improvement Commission, and the Louis P. Martini Endowed Chair in Viticulture.

BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

Project Leaders:
Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616-8749
awalker@ucdavis.edu

Alan Tenscher
Dept. of Viticulture and Enology
University of California
Davis, CA 95616-8749

Reporting Period: The results reported here are from work conducted from November 2003 through October 2004.

ABSTRACT
Strong and continued progress is being made in breeding Pierce's disease (PD) resistant grapes. Fruit quality has markedly improved while maintaining high levels of PD resistance. We continue to make many crosses, produce thousands of seeds, and plant about two thousand plants in the field each year. We have been increasing the number of seedlings and high fruit quality selections we test under our greenhouse screen. This screening is very severe, but material that passes the screen is reliably resistant and dramatically restricts *Xylella fastidiosa (Xf)* movement. We are also co-screening for powdery mildew resistance. The heritability of *Xf* resistance from a range of resistant southeast US (SEUS) cultivar and species parents is not consistent – some parents produce few resistant offspring, while others produce a large percentage – making careful parental screening very important. We have been able to expand our *Xf* screening the past few years and have tested hundreds of potential parents before we need to make breeding decisions the following year.

INTRODUCTION
Renewed and intensified PD outbreaks in historic PD zones in wine regions around the state and the introduction of GWSS into the southern San Joaquin Valley demonstrate the vulnerability of *V. vinifera* wine grape culture in California. All of California's wine grapes are susceptible to PD and no effective prevention or cure currently exists. Under severe PD pressure, culture of *V. vinifera* grapes is not possible. We are currently breeding PD-resistant wine grape cultivars for localized use in traditional PD "hot-spots" that are common in the North Coast, and it is likely that acceptable white and red wine grapes for these areas can be produced in two generations of crosses with our current *Xf* resistant selections. To further improve the utility of these *Xf* resistant cultivars, we are co-selecting for high levels of powdery mildew resistance. Unlike wine varieties for widespread use where the need for "pure *V. vinifera*" cultivars is enforced by marketing, given adequate quality (neutrality, color, season, cultural characteristics) varieties for localized use should prove useful to industry as blenders and by keeping "hot-spot" vineyard acreage in production. Our concurrent efforts to identify *Xf* resistance genes (see companion proposal – Walker and Riaz) will make it possible in the future to transform wine grapes with grape-derived resistance genes. Using grape genes to transform grapes should help overcome public reluctance about GM grapes and provide durable PD resistance.

PD resistance exists in a number of *Vitis* species and in the related genus, *Muscadinia*. Resistant cultivars have been developed in public and private breeding programs across the southeastern United States (SEUS). These cultivars have high PD resistance, but relatively low fruit quality relative to *V. vinifera* grapes. In the southeastern US, they must also resist downy and powdery mildew, black rot and anthracnose, which have as great an effect on viticulture in the southeast as PD does. Most of these diseases are not found in California, allowing breeders to incorporate more high quality *V. vini*fera into their breeding efforts and enabling the production of much higher quality PD resistant cultivars in a shorter time span. We have characterized (see past reports) and employed a wide range of PD resistant germplasm from the collections at the National Clonal Germplasm Repository, Davis; selections obtained from breeders in the southeastern U.S.; from *V. rupestris* x *V. arizonica* selections that have exceptional PD resistance; and from several *V. vinifera* x *M. rotundifolia* hybrid winegrape types that have some fertility. These breeding efforts have already resulted in relatively high quality selections with excellent PD resistance.

At UC Davis we are uniquely poised to undertake this important breeding effort. We have developed rapid screening techniques for *Xf* resistance and have optimized ELISA and PCR detection of *Xf* (Buzkan et al. 2003, Buzkan et al. 2004, Krivanek et al. 2004, Krivanek and Walker 2004). We have unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southeastern grape hybrids, that offer the introduction of extremely high levels of *Xf* resistance into commercial grapes. We also have several years' worth of seedlings in the ground that need evaluation as winegrape types.

OBJECTIVES
The objectives of our PD breeding project are divided into two primary parts. The first is the breeding of *Xf* resistant wine grapes through backcross techniques using *V. vinifera* wine grapes and *Xf* resistant selections and sources characterized from our previous breeding efforts. The second is the continuing characterization of *Xf* resistance and winegrape quality traits (color, tannin, ripening dates, flavor, productivity, etc.) in novel germplasm sources, in our breeding populations, and in our genetic mapping populations. These efforts support both the breeding program and the genetic mapping program.

Completion of these objectives is tied to the speed with which seedlings can be produced, fruited and evaluated and subsequent generations produced.

- Develop multiple lines of *Xf* resistant wine grapes using 8909 (*V. rupestris* x *V. arizonica* selections; *Xf* resistant breeder selections (DC1-39, Zehnder selections, etc); and southern grape species (*V. arizonica*, *V. champinii*, *V. shuttleworthii*, *V. simpsonii*, *M. rotundifolia*, and others).
- Continue backcross generations with 8909-08, DC1-39, and other lines to advanced *vinifera* selections and select for high quality wine grape characteristics.
- Continue to identify and characterize additional sources of *Xf* resistance with high levels of powdery mildew resistance.
- Maintain current and produce additional populations for genetic mapping efforts aimed at characterizing *Xf* resistance genes, and identifying and mapping fruit quality traits such as color, tannin content, flavor, production, etc. in *Xf* resistant backgrounds.
- Study the inheritance of *Xf* resistance from a broad range of resistance sources.

RESULTS AND CONCLUSIONS

Shift From Table Grape Breeding to Wine Types

Because the California Table Grape Commission's decision to not fund the breeding of PD resistant grapes, as of May 2004 we are now solely breeding PD resistant wine grapes. This year we evaluated 4,042 seedlings from 39 different crosses made in the last three years for use as wine grapes. From this number, four subgroups based on different resistance source were identified as particularly promising (Table 1). Promise was based on resistance to *Xf* and powdery mildew, fruit quality parameters, and viticultural characteristics such as yield and growth habit.

Evaluation of Fruit Quality

Within a cross we observed useful segregation of wine grape quality factors such as quality and quantity of color, acidity, pH, flavor, and skin and seed tannin. Table 2A and 2B present data for typical genotypes from three of the four resistance groups. These were harvested on August 26, 2004. Figure 1 displays clusters from two of the four promising *Xf* resistance subgroups listed in Table 1. Their morphology is becoming very vinifera-like in the first generation. Figure 2 displays juice extracted from some of the *Xf* resistant crosses in comparison with the juices from Cabernet Sauvignon and Pinot noir. There are a wide variety of colors that should allow matching enological needs with our selection process.

Planting of 2003 Crosses

Table 3 summarizes the field planting of wine crosses made in 2003. We did not germinate the 2,150 seeds of the cross of a SEUS cultivar by Syrah since our GH screening of progeny from the same SEUS female by pure *V. vinifera* indicated only 1 in 12 of the seedlings was likely to be resistant. Crosses made in Spring 2003 contained efforts directed at table and raisin grape production. This year's crosses were entirely devoted to wine grape efforts.

Wine Crosses Made in 2004

Table 4 details the wine grape crosses made during Spring 2004. We were able to tailor our choices for PD resistant parents with our previous experiences directed at table grape breeding. The assays of subsets of progeny from crosses with various parental sources found that the expression of PD resistance in progeny varies. *Vitis arizonica/candicans* selections from near Monterey, Mexico (b43-17, b43-36, and b43-56) produced 100% resistant progeny in the testing of the subset and should therefore be homozygous resistant. F8909-08 and F8909-17 were both derived from b43-17. The heritability of selections from Florida varied: BO2SG, BD5-117 and Midsouth produced 50% resistant progeny; while only 20% of the progeny of BO3SG was resistant, so progeny from it will be planted sparingly. NC-11J x UCD0124-01 represents a resistant x resistant cross from two different resistant backgrounds. B55-1 and NC6-15 are opportunities to ingress resistance from *Muscadinia rotundifolia* into wine crosses. We plan to plant between two and three thousand of the most promising seedlings from the crosses detailed above in Spring 2005.

Greenhouse Screen Results

We screened 474 genotypes with our greenhouse screen. The tested genotypes included cultivars and species from the SEUS, many Olmo Vinifera/Rotundifolia (VR) hybrids with potential PD resistance and for use as parents, table and wine grape crosses, and possible *Xf* resistant wine grape selections from a private breeder in North Carolina. Several promising *Xf*-resistant SEUS genotypes were identified. Six of 19 Olmo VR hybrids tested resistant. Two may be promising parents. None of the wine grape selections from North Carolina proved to be adequately resistant.

Table 5 presents the ratio of resistant to susceptible (R:S) progeny from crosses of highly susceptible *V. vinifera* parents crossed with a variety of *Xf* resistance sources. One *V. smalliana* and one *V. champinii* F1 hybrid progeny had R:S ratios of close to 1:1, suggesting that the resistance in these parents was heterozygous and controlled by a single gene. Other parents had ratios ranging from 1:3 through 1:11. Details are summarized in Table 5. We made crosses onto the *V. champinii* hybrid this year and they will be tested to see if the inheritance ratio remains 1:1, as does our F8909-17 resistance source (see Walker-Krivanek report). In other backgrounds, resistance seems to erode with continued backcrossing to *V. vinifera*, thus these stable resistance sources are very valuable and are easily adapted to marker-assisted selection.

Progeny from crosses of field resistant parents, like JS23-416 – judged resistant in Florida (Herb Barrett, personal communication) yet has been susceptible in our greenhouse tests, to *V. vinifera* do not seem to be resistant (<100,000 fu/ml). However, they do produce a broad and relatively even distribution of progeny from 170,000 to almost 6,500,000 cfu/ml. Although we would not consider those at the low end of this scale to be resistant, they have as low or lower bacterial levels than do some of the field resistant genotypes from the SEUS we have tested. We have avoided these progeny and using these parents to prevent release of field resistant cultivars that may survive PD infection, but allow vine-to-vine movement in vineyards.

We are beginning testing of about 200 genotypes with results expected in March 2005. These results will be used to direct backcrossing of the most resistant genotypes to *V. vinifera* wine grapes.

Napa Field Trial

This year we planted another block in our field trial at Beringer Vineyards in Yountville. We expanded the plot by adding 6 vine replicates of 20 different genotypes from 4 different resistant sources. Based on our GH screen results, both highly resistant and highly susceptible genotypes from each resistant source were planted. These will be inoculated with *Xf* next April and ELISA tested in October 2005.

This fall we observed the most pronounced visual PD symptoms to date in the 2001 and 2003 plantings following inoculation with *Xf* early this spring. We used a mixture of 5 different Napa PD strains as inoculum. The 2001 planting consists of known field resistant selections from the SEUS, and the 2003 planting consists of 3 vine reps of some of our early crosses and a few more SEUS field resistant types. On October 8, 2004 we scored these vines for visual symptoms and took samples for ELISA testing from 291 vines in these blocks. Results will be reported in December.

REFERENCES

Buzkan, N. A.F. Krivanek, A. Eskalen and M.A. Walker. 2003. Improvements in sample preparation and polymerase chain reaction detection techniques for *Xylella fastidiosa* in grapevine tissue. American Journal of Enology and Viticulture 54:307-312.

Buzkan, N. L. Kocsis and M.A. Walker. 2004. Detection of *Xylella fastidiosa* from resistant and susceptible grapevine by tissue sectioning and membrane entrapment immunofluorescence. Microbiological Research 159 (In Press).

Krivanek, A.F, Stevenson, J. and Walker, M.A. 2004. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology (In Press).

Krivanek, A.F. and Walker, M.A. 2004. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology (In Press).

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. In the past, funding has also been received from the California Raisin Marketing Board, the California Table Grape Commission, and the USDA Animal and Plant Health Inspection Service.

Table 1. Summary of different crosses within the subgroups and the relative number of genotypes within each group that merit further evaluation.

Resistance Source	*V. vinifera* Parent	Genotypes Evaluated	Genotypes Selected
BO2SG (*V. smalliana*)	C1020	36	10
	Princess	21	9
BO3SG (*V. smalliana-simpsonii*)	C67-129	30	7
	Princess	81	14
AW C52-94 (*V. simpsonii*)	C51-63	353	71
Midsouth	B90-116	39	4
	C67-129	46	1
	Princess	8	1
Total		614	117

Table 2A. Analytical evaluation of representative progeny from three different sources of *Xf* resistance.

Genotype	Species or Cross	Cluster Wt. (g)	Brix	pH	TA (g/L)	Berry Wt. (g)	Est. Yield (gal/ton)
BO2SG	*V. smalliana*	45	24.5	3.28	19.7	0.3	129
BO3SG	*V. smalliana-simpsonii*	66	25.0	3.53	12.1	0.3	90
Cab Sauv	*V. vinifera*	269	23.0	3.52	6.8	1.0	160
Pinot noir	*V. vinifera*	299	25.5	3.72	6.1	1.2	182
J13-09	BO2SG x Melissa	184	24.2	3.16	12.1	1.3	160
J13-13	BO2SG x Melissa	62	25.5	3.22	9.8	1.4	162
J14-09	BO2SG x C1020	90	25.2	3.36	9.1	1.2	176
J14-12	BO2SG x C1020	125	27.0	3.46	8.3	1.0	167
J14-16	BO2SG x C1020	120	26.0	3.38	9.8	1.4	170
J17-3	BO3SG x C67-129	100	25.0	3.32	7.1	1.3	150
J17-06	BO3SG x C67-129	102	25.8	3.53	6.4	1.4	149
J17-08	BO3SG x C67-129	117	26.5	3.43	7.7	1.0	135
J17-14	BO3SG x C67-129	200	27.0	3.68	5.9	0.9	148
J17-24	BO3SG x C67-129	224	26.0	3.62	6.7	1.1	137
J17-25	BO3SG x C67-129	70	27.0	3.65	5.9	1.0	146
J17-36	BO3SG x Melissa	110	26.5	3.76	4.5	0.9	154
J17-39	BO3SG x Melissa	70	25.0	3.33	7.4	0.8	176
J17-50	BO3SG x Melissa	185	24.0	3.32	6.8	1.2	165
J18-18	BO3SG x Melissa	195	23.0	3.14	9.8	1.1	143
J18-24	BO3SG x Melissa	60	26.5	3.54	5.5	1.1	148
J18-35	BO3SG x Melissa	93	26.2	3.55	6.2	0.9	152
J18-37	BO3SG x Melissa	100	23.5	3.14	9.7	0.7	158
J18-38	BO3SG x Melissa	101	25.0	3.23	8.6	1.0	154
J27-03	Midsouth x B90-116	99	23.5	3.85	8.3	1.2	168
J27-06	Midsouth x B90-116	125	25.0	3.76	5.2	1.2	145

Table 2B. Sensory evaluation of representative progeny from three different sources of *Xf* resistance.

Genotype	Species or Cross	Skin Tannin Intensity[a]	Seed Color[b]	Juice Hue	Juice Color Intensity	Juice Flavor
BO2SG	*V. smalliana*	2	4	red	dark	fruity, peppery
BO3SG	*V. smalliana-simpsonii*	1	4	red	dark	fruity, peppery
Cab Sauv	*V. vinifera*	3	2.5	pink	light	slightly vegetal
Pinot noir	*V. vinifera*	1	4	pink	very light	fruity
J13-09	BO2SG x Melissa	2	4	red	medium +	tart, red fruit
J13-13	BO2SG x Melissa	2.5	4	red-purple	medium +	fruity, slight hot pepper
J14-09	BO2SG x C1020	2	4	red	medium	tart, jammy, very slight hot pepper
J14-12	BO2SG x C1020	2	4	pink	light	slightly jammy, broad fruity
J14-16	BO2SG x C1020	2	4	green		green pepper, hot pepper
J17-3	BO3SG x C67-129	1.5	4	red-purple	medium +	slightly fruity, hot pepper
J17-06	BO3SG x C67-129	2	3.5	pink-red	medium	hay, hot pepper
J17-08	BO3SG x C67-129	1.5	4	pink-orange	light +	vinifera-like, acidic, hot pepper
J17-14	BO3SG x C67-129	2	4	red	medium	slightly jammy, fruity
J17-24	BO3SG x C67-129	4	4	red	medium +	fruity, hot pepper
J17-25	BO3SG x C67-129	1.5	4	red	medium	very slightly vegetal-herbal
J17-36	BO3SG x Melissa	2	4	pink	medium -	slight hay, hot pepper
J17-39	BO3SG x Melissa	2	4	red	medium +	tart, raspberry, very slight hot pepper
J17-50	BO3SG x Melissa	2	4	pink-red	medium	simple fruit, berry
J18-18	BO3SG x Melissa	3	4	pink-red	medium -	slight hay, canned
J18-24	BO3SG x Melissa	2	4	red	medium	slight hay, fruity
J18-35	BO3SG x Melissa	2	3.5	pink-red	medium -	hay, hot pepper
J18-37	BO3SG x Melissa	2	4	pink-brown	light	tart berry, slightly buttery
J18-38	BO3SG x Melissa	1	4	red	medium -	berry, slight hot pepper
J27-03	Midsouth x B90-116	1	4	purple	dark	current, vegetal
J27-06	Midsouth x B90-116	1	4	red	medium-	strawberry, herbal

a = (1=low, 4= high); b = (1=green, 4= brown)

Table 3. UC Davis field plantings of wine crosses made in 2003. F2-7 and F2-35 are respectively a black and a white female seedling of the cross Cabernet Sauvignon x Carignane. B34-82 is a USDA cross.

Cross	Resistance Source	Seedlings Planted
F2-7 x F8909-08	*V. arizonica*	10
F2-35 x F8909-08	*V. arizonica*	38
F2-35 x BD5-117	SEUS complex	164
F2-7 x BD5-117	SEUS complex	149
BD5-117 x B34-82	SEUS complex	141
	Total	502

Table 4. Wine grape crosses made at UCD in 2004.

Female Parent	Male Parent	Resistance Source	# Seeds
BO2SG	Cabernet Sauvignon	*V. smalliana*	376
BO2SG	Carignane	*V. smalliana*	196
BO2SG	Sauvignon blanc	*V. smalliana*	404
BO3SG	Chambourcin	*V. smalliana-simpsonii*	412
BO3SG	Petite Sirah	*V. smalliana-simpsonii*	419
BO3SG	Cabernet Sauvignon	*V. smalliana-simpsonii*	371
BO3SG	Carignane	*V. smalliana-simpsonii*	350
BO3SG	Sauvignon blanc	*V. smalliana-simpsonii*	223
F2-7 (CabS x Carig.)	BD5-117	SEUS complex	1131
F2-7	Midsouth	*V. champinii*	522
F2-7	F8909-08	*V. arizonica - candicans*	4,500
F2-7	F8909-17	*V. arizonica - candicans*	300
F2-35 (CabS x Carig.)	B55-1	*M. rotundifolia*	18
F2-35	B43-17	*V. arizonica-candicans*	323
F2-35	B43-36	*V. arizonica*	141
F2-35	B43-56	*V. arizonica*	56
F2-35	BD5-117	SEUS complex	783
F2-35	Midsouth	*V. champinii*	522
NC-11J	UCD0124-01	*M. rotundifolia*-SEUS complex	175
Midsouth	Midsouth	*V. champinii*	500
NC6-15	Sauvignon blanc	*M. rotundifolia*	50
Total			11,772

Table 5. Ratios of *Xf*-resistant: susceptible (R:S) progeny in populations from various resistance sources by *V. vinifera* parents based on a greenhouse screen. Resistance is defined as a mean value less than 100,000 cfu/ml (colony forming ***units per ml).***

Resistant Parent	Resistance Source	Number Resistant	Number Tested	Percent Resistant	Approx: R/S ratio
Midsouth	*V. champinii*	9	17	53%	1:1
BO2SG	*V. smalliana*	11	23	48%	1:1
Cha3-48	*V. champinii*	8	26	31%	1:2
DC1-39	Complex	9	33	27%	1:3
BO3SG	*V. smalliana-simpsonii*	1	6	17%	1:5
F901	*V. shuttleworthii*	1	7	14%	1:6
AW c52-94	*V. simpsoni*	2	15	13%	1:6
Z 71-50-1	Complex	2	25	8%	1/11
AT0023-019	*V. arizonica* (La Paz)	2	29	7%	1/11
F902	*V. shuttleworthii*	0	16	0%	-
Roucaneuf	Complex	0	22	0%	-
Villard blanc	Complex	0	6	0%	-
JS23-416	Susceptible	0	19	0%	-
Total			244		

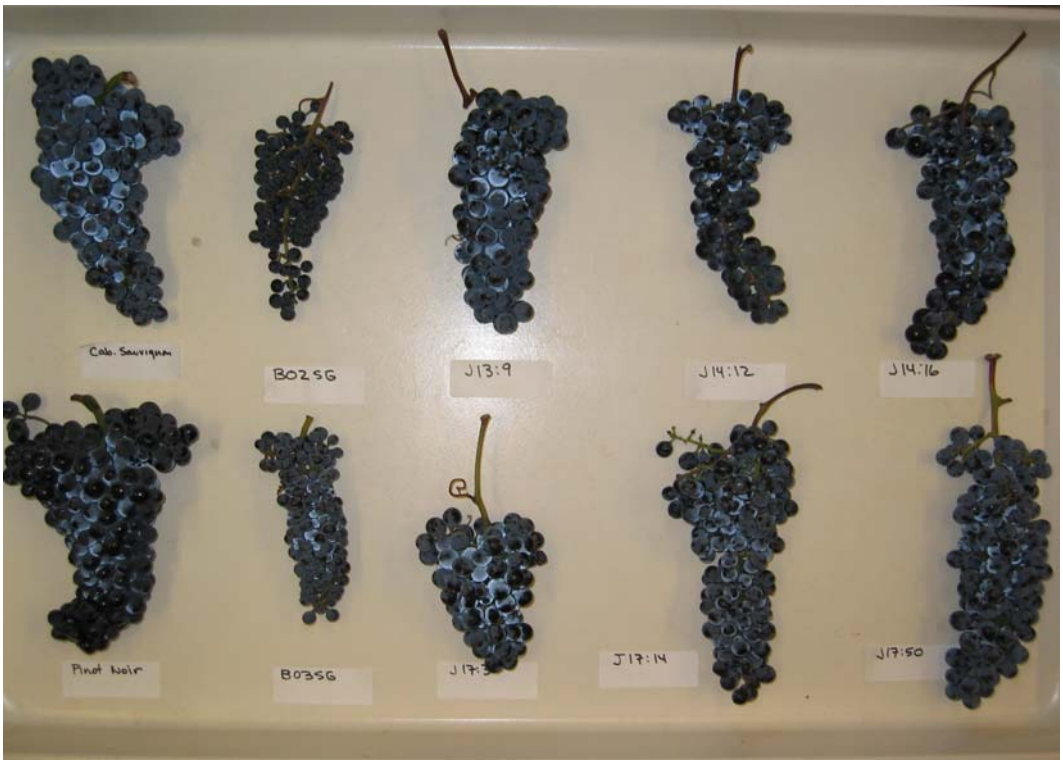


Figure 1. Representative clusters from two promising *Xf* resistance source subgroups. BO2SG and BO3SG are the resistant female parents. Cabernet Sauvignon and Pinot noir are shown for size/shape comparisons. Crosses to BO2SG are in the top row while crosses to BO3SG are in the bottom row. The other clusters are from first generation crosses. Analytical details can be found in Table 2.

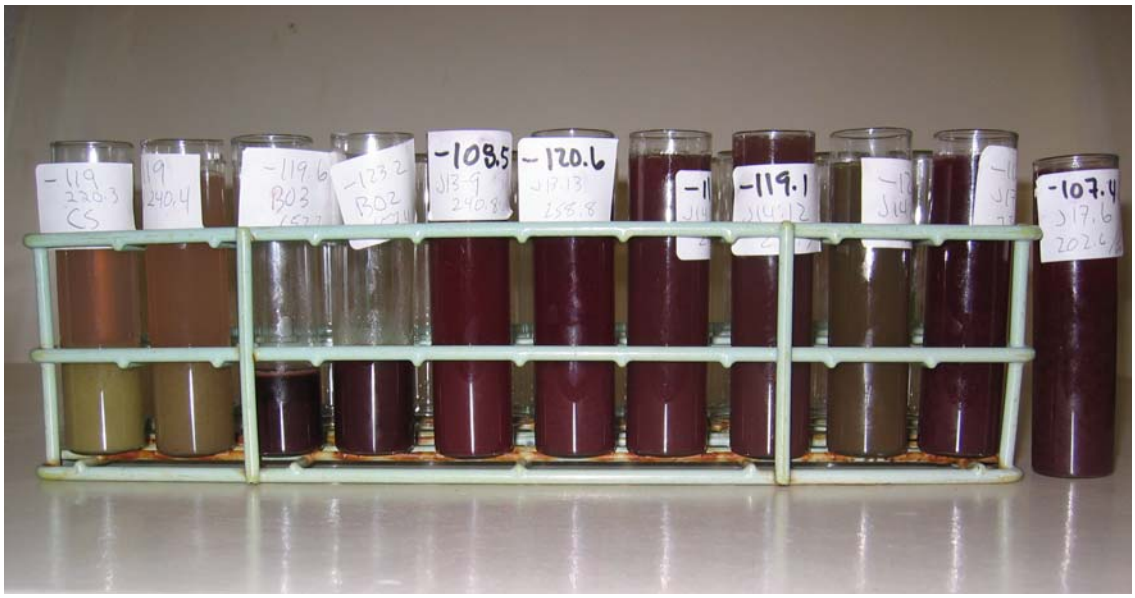


Figure 2. Juice extracted from selected clusters of *Xf*-resistant crosses shown in Figure 1 and detailed in Table 2. Note the high quantity of red color and the variation in hue from some of the crosses. This variation allows for tailoring varieties to meet particular enological needs. Juice from Cabernet Sauvignon and Pinot noir are on the left in the first two vials respectively.

Section 2: Vector Biology and Ecology

PLANT AND PREDATOR EFFECTS ON INTERPLANT MOVEMENT BY THE GLASSY-WINGED SHARPSHOOTER

Project Leaders:
Christine Armer and Sharon Strauss
Ecology and Evolution
University of California
Davis, CA 95616

Cooperator:
David Morgan
California Dept. of Food and Agriculture
Mount Rubidoux Field Station
Riverside, CA 92501

Reporting period: The results reported here are from work conducted from May 2004 through September 2004.

ABSTRACT
Adult GWSS in caged habitats were monitored hourly to determine the effects of plant species availability and predator presence on intra- and inter-plant movement, as these factors are directly related to the acquisition and spread of Pierce's Disease. GWSS were placed in caged habitats with either a monoculture of beans or polyculture of bean, sunflower, and tree tobacco, and either with or without spiders, in a 2x2 factorial design. Origin of the GWSS (field-caught or laboratory-reared) was also included as a third factor in the multi-factor MANOVA to determine the importance of each treatment on GWSS feeding, resting, and intra- and inter-plant movement. Approximately 85-90% of the day was spent feeding or resting on plants. Only 0.5-1.5% of the observations recorded flying GWSS, and another 1-2% found GWSS walking between plants. More insects moved between plants in the mixed-plant cages than in the bean-only cages, suggesting the GWSS are able to detect the presence of other species of plants in the vicinity. This increase in interplant movement would probably correspond to an increase in Pierce's disease transmission. Field-collected insects spent less time feeding and more time resting on plants than did laboratory-reared insects. Both sets of insects spent more time feeding in bean-only cages than in mixed-plant cages. Beans may not have provided optimal nutrients, and GWSS may have moved to other plants to supplement nutrient intake. GWSS fed on sunflower and tobacco readily, although preferences have not yet been calculated. No predator-mediated spread of Pierce's Disease is expected to occur, as the presence, activity levels, and predation by spiders had no affect on GWSS behavior. Further analysis of feeding times and movement between plant species may clarify the relative importance of toxin dilution (nicotine from tree tobacco) and nutrient balancing from bean and sunflower plants.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* Say, is primarily of economic importance because it vectors the Pierce's disease-causing bacterium, *Xylella fastidiosa* (Blua et al. 1999). The insect feeds on hundreds of species of plants (Adlerz 1980; Hoddle et al. 2003), many of which harbor asymptomatic populations of *X. fastidiosa* (Purcell and Hopkins 1996). Every time a GWSS moves to a new plant to feed, the chances of acquiring and transmitting Pierce's Disease increase. Therefore, the factors causing GWSS to move between plants are directly related to the spread of Pierce's disease.

Generalist herbivores such as the GWSS may move to new plants to balance nutrients, to avoid intra- or inter-specific competition, to dilute plant defensive toxins, or to avoid predation. GWSS feeds primarily, if not exclusively, on the xylem, where nutrients are very dilute (Andersen et al. 2003). The nutritional requirements of GWSS have been determined (Andersen et al. 1992; Brodbeck et al. 1996), and only cowpea and soybean have been found to reliably sustain GWSS throughout a complete generation (D.J.W. Morgan, pers. comm.; Brodbeck et al. 1999). However, why GWSS move between plants, especially when a nutritionally adequate host such as bean is available, is unknown. Interspecific competition is rarely a concern for GWSS, as few other organisms feed on the xylem on the host plants on which GWSS can feed. Intraspecific competition may occur, as GWSS move off plants when present in very high densities (Armer, pers. obs.), but these densities will not occur frequently when biological control is in place. Plant defensive compounds are not common in the xylem (Raven 1983), but alkaloids and quinones are present in certain plant families and may be more prevalent than scientists have previously expected. For example, solanaceous plants carry defensive compounds from synthesis sites in the roots to the leaves via the xylem. Tree tobacco is one such solanaceous plant, which contains nicotine in the xylem. Finally, predators may affect herbivore behavior, as some herbivores can detect and respond to the presence of predators by halting feeding or altering host plant selection (Schmitz et al. 1997; Schmitz and Suttle, 2001). Alternately, an herbivore that moves frequently between plants to optimize feeding may be more apparent to visual predators.

OBJECTIVE
Determine the effect of plant species variety and predators on GWSS interplant movement.

RESULTS
Caged habitats of 0.56m^2 contained 6 plants in soil. Plants and predators were set up in a 2x2 factorial design, with either a monoculture (all bean plants) or polyculture (2 bean, 2 sunflower, and 2 tree tobacco plants) and with or without spiders. Sixteen adult GWSS were placed in each cage and their location and behavior were monitored every hour throughout as daylight was available, for 10-14 hours. The behaviors are shown on the x-axis of Figure 1. The percent of adult GWSS in a cage performing each activity was averaged over all hours observed. The data were compared by a 3-factor MANOVA (SAS

v.8) for differences due to the plant availability (beans-only or mixed plants), spiders (presence or absence), and whether the GWSS were field-collected as adults or lab-reared. Adults that had been reared from birth only on bean plants in laboratory colonies were used in 27 cages, and GWSS that had been captured in the wild as adults were used in 9 cages. One behavior was omitted from the analysis to allow independence of the observations (see Cisneros and Rosenheim 1998).

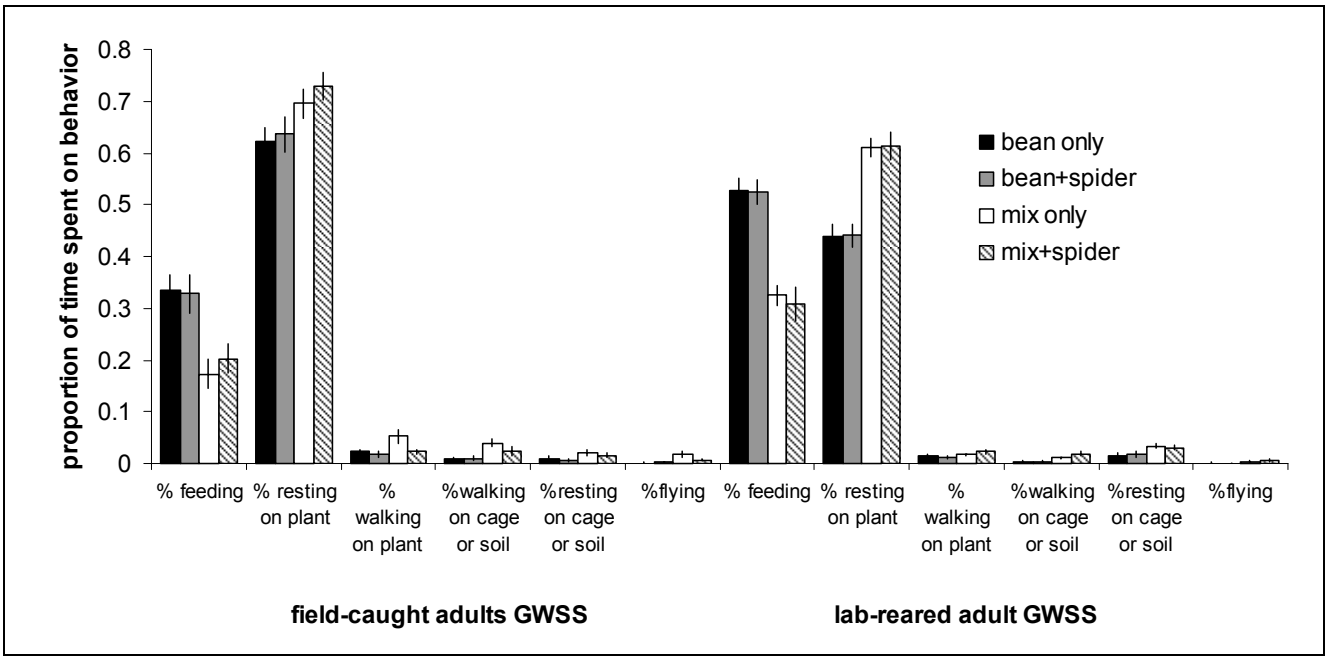


Figure 1. Behaviors performed by caged GWSS adults observed during daylight hours. The average time spent by individuals in each cage on each behavior is shown; error bars indicate standard error.

GWSS spent nearly all of their time either feeding or resting on plants (Figure 1). About 2-5% of the time was devoted to walking on a plant, 1-5% to walking on the cage or soil, 2-5% to resting on the cage or soil, and 0-2% to flying. Plant treatment (bean-only or mixed species) affected all behaviors ($F=13.87$, $df=5,132$, $P<0.0001$). Individuals on beans spent more time feeding and less time resting than insects did on plants in mixed-species cages. Field-caught insects varied significantly from laboratory-reared individuals in their behaviors ($F=16.20$, $df=5, 132$, $P<0.0001$), feeding less and resting more than laboratory insects. However, both groups of insects showed similar time budgets. Both spent less time feeding on beans than on mixed plants. However, lab-reared insects spent less time resting than feeding on beans, and field-reared insects rested more than feeding on beans. This interaction between plant treatment and insect origin (field-caught vs. lab-reared) was significant ($F=2.58$, $df=5,132$, $P=0.029$). Both plant treatment and insect origin significantly affected all insect behaviors at the $p=0.01$ level or greater.

Interplant movement, either by walking or by flying, was higher in the mixed-species cages. GWSS also spent more time resting on the cage or on the soil in the mixed-plant treatment cages, although such a small amount of time was spent in this behavior that it was probably not biologically significant. However, the increase in movement between plants in the mixed cages, although small, is significant in that such behavior increases the GWSS' opportunities to acquire and transmit Pierce's disease.

The three plant species were selected because one provided a host on which GWSS can complete multiple generations (bean), one was an alternate host favored in the field (sunflower), and the final plant contains potentially toxic nicotine in the xylem (tree tobacco), and so may be preferentially avoided. All three plant species were used as host for feeding, but the amount of time spent feeding on each species has not yet been calculated. Both the time spent feeding, and the frequency of leaving each species of plant, will indicate the GWSS' preference for the 3 species.

The presence of spiders did not affect GWSS behaviors ($F=1.08$, $df=5, 132$, $P=0.376$). There were no interactions between spiders and plant species or origin of GWSS. Spiders used in the experiments were field-collected, and the species changed as the season progressed. Predation activity also varied within species, perhaps due to hunger levels of each individual. The presence of spiders did not affect GWSS, but wide variation in spider activity level might hide predation effects. We therefore examined spider activity levels (% of observations in which the spider moved), based on intra- and inter-plant movements, to correlate predation pressure to GWSS movement and feeding behavior. GWSS did not show a behavioral response to spider activity levels (spider activity not correlated to GWSS time spent feeding, moving on the same plant, resting on the plant, moving on the soil or cage, flying) in either plant treatment, nor was the number of GWSS eaten related to spider activity (all non-significant in direct regressions). The spiders were equally active in the two plant treatments,

moving an estimated 28±3% (mean±SE) of the observation period in both treatments. Spiders in the bean treatment caught and fed on 0.22±.07 GWSS per day, whereas those in the mixed-plant treatment fed on 0.33±.09 GWSS.
All GWSS were sexed after observation, and data were examined for possible behavioral differences. However, there were no differences between the sexes in terms of their behavior (MANOVA with sex and plant-spider treatment as the factors; $F=1.29$, $df=5,276$, $p=0.27$).

CONCLUSIONS

The availability of multiple plant species increased GWSS interplant movement, and feeding times were reduced in these cages, suggesting GWSS 1) can detect the presence of other host species in the vicinity, probably through olfaction, and 2) that diet-mixing helps GWSS obtain needed nutrients more rapidly. However, the increased movement between plants also may correspond to an increased in acquisition and spread of the bacterium that causes Pierce's Disease. The effects of potentially toxic plants, such as tree tobacco, are not currently understood on GWSS interplant movement. Further data analysis should help clarify the insects' response. Spiders did not affect GWSS feeding and intra- and inter-plant behavior in the observations described here. Thus, these (and possibly other arthropod) predators should not affect the GWSS' acquisition and spread of Pierce's Disease.

REFERENCES

Adlerz, W.C. (1980) Ecological observations on two leafhoppers that transmit the Pierce's disease bacterium. Proc. Flor. State Hort. Soc. 93:115-120.

Andersen, P.C., Brodbeck, B.V., and Mizell, R.F. III (1992) Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. J. Insect Physiol. 38:611-622.

Andersen, P.C., Brodbeck, B.V., and Mizell, R.F. III (2003) Plant and insect characteristics in response to increasing densities of *Homalodisca coagulata* on three host species: a quantification of assimilate extraction. Entomol. Exp. Appl. 107:57-68.

Blua, M.J., Phillips, P.A., and Redak, R.A. (1999) A new sharpshooter threatens both crops and ornamentals. Calif. Agric. 53:22-25.

Brodbeck, B.V., Andersen, P.C., and Mizell, R.F. III (1996) Utilization of primary nutrients by the polyphagous xylophage, *Homalodisca coagulata*, reared on single host species. Arch. Insect Biochem. Physiol. 32:65-83.

Brodbeck, B.V., Andersen, P.C., and Mizell, R.F. III (1999) Effects of total dietary nitrogen and nitrogen form on the development of xylophagous leafhoppers. Arch. Insect Biochem. Physiol. 42:37-50.

Cisneros, J.J. and Rosenheim, J.A. (1998) Changes in the foraging behavior, within-plant vertical distribution, and microhabitat selection of a generalist insect predator: an age analysis. Environ. Entomol. 27:949-957.

Hoddle, M.S., Triaptisyn, S.V., and Morgan, D.J.W. (2003) Distribution and plant association records for *Homalodisca coagulata* (Hemiptera: Cicadellidae) in Florida. Florida Entomol. 86:89-91.

Purcell, A.H. and Hopkins, D.L. (1996) Fastidious xylem-limited bacterial plant pathogens. Ann. Rev. Phytopathol. 34:131-151.

Raven, J.A. (1983) Phytophages of xylem and phloem: a comparison of animal and plant sap-feeders. Adv. Ecol. Res. 13:135-234.

Schmitz, O.J., Beckerman, A.P., and O'Brien, K.M. (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78:1388-1399.

Schmitz, O.J. and Suttle, K.B. (2001) Effects of top predator species on direct and indirect interactions in a food web. Ecology 82:2072-2081.

FUNDING AGENCIES

Funding for this project was provided by an Invasive Species NSF-IGERT postdoctoral fellowship to C. Armer at the University of California, Davis.

SHARPSHOOTER FEEDING BEHAVIOR IN RELATION TO TRANSMISSION OF THE PIERCE'S DISEASE BACTERIUM

Project Leader:
Elaine Backus
USDA, ARS, PWA
Exotic & Pests Dis.
Parlier, CA 93648

Researchers:
P. Houston Joost
Dept. of Entomology
University of California
Riverside, CA 92521

Javad Habibi
Dept. of Entomology
University of Missouri
Columbia, MO 65211

Holly Shugart
USDA, ARS, PWA
Exotic & Pests Dis.
Parlier, CA 93648

Cooperators:
David Morgan
Calif. Dept. of Food & Agric.
Mt. Rubidoux Field Stn.
Riverside, CA 92501

William H. Bennett
(Private consultant)
7441 Elkstown Rd.
Otterville, MO 65348

Edwin Civerolo
USDA, ARS, PWA
Exotic & Pests Dis.
Parlier, CA 93648

Russell Groves
USDA, ARS, PWA
Exotic & Pests Dis.
Parlier, CA 93648

Reporting Period: The results reported here are from work conducted from November 1, 2003 to September 30, 2004.

ABSTRACT
Progress this year consisted of completing past projects as well as building infrastructure for future research. Backus's new lab in Parlier was renovated, upgraded and equipped with state-of-the-art facilities for electrical penetration graph (EPG) monitoring of insect feeding and histology of plant and insect tissues. Extensive colonies of glassy-winged, smoke tree, green, and red-headed sharpshooters were established in Fresno and Parlier (with R. Groves, ARS Parlier). New personnel were hired; data was intensively analyzed and grant proposals written. Much effort was also expended in developing new protocols and preliminary findings for feeding waveform correlations with bacterial expulsion and muscle contraction, as well as AC and DC waveforms for several species in colony. Stylet activities and salivary sheath-cell type correlations for the major GWSS waveforms were completed (Objective 1), as was all of the plant histology for the GWSS inoculation test (Objective 2). Results to date support a modified version of last year's hypothesis for the mechanism of *Xf* inoculation to grape. *Xf* bacteria may exit the stylets during brief stylet activities represented by the B1spikelet burst, B1-like portions of N and/or C, probably within seconds of the first puncture of any penetrated cell, both along the pathway to and within xylem. Proper placement of the bacteria appears to be crucial; placement in xylem leads to growth of the bacteria sufficient for detection by less sensitive methods such as culturing. Otherwise, when more sensitive detection methods such as immunocytochemistry of the tissues immediately surrounding the salivary sheath are used, they can detect *Xf* in non-xylem tissues. Three papers from this research are in preparation for submission in late 2004 – early 2005. This work will help solve the PD/GWSS problem by identifying the mechanism of *Xf* inoculation and crucial aspects of inoculation efficiency, and eventually aid host plant resistance through the development of the Stylet Penetration Index.

INTRODUCTION
Almost nothing was known, until this work, about the stylet penetration behaviors of the glassy-winged sharpshooter (GWSS), and how they interact with populations of *Xylella fastidiosa* (*Xf*) to facilitate transmission to grapevine. This project is combining the three most successful methods of studying leafhopper feeding (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electrical penetration graph [EPG] monitoring) to identify most details of feeding.

OBJECTIVES
1. Identify and quantify all feeding behaviors of GWSS on grapevine, and correlate them with location of mouthparts (stylets) in the plant and presence/ population size of *Xf* in the foregut.
2. Identify the role of specific stylet activities in *Xf* transmission, including both the mechanisms of acquisition and inoculation, and their efficiency. This project's emphasis is on inoculation.
3. Begin to develop a simple, rapid method to assess feeding, or detect the likelihood of *X. fastidiosa* transmission (an "inoculation-behavior detection method"), for future studies.

RESULTS
During the first six months of this reporting period (Nov. 2003 – April 2004), Backus's new lab at USDA-ARS in Parlier was closed due to extensive renovation construction underway. Notwithstanding this delay, we made significant progress on several sharpshooter research fronts during this time. We hired new personnel (a post-doc and a second technician), purchased many supplies and pieces of equipment (including a new confocal microscope), and trained in the use of the equipment. Also, we received CDFA importation permits and permission for a GWSS maintenance colony to be established in Fresno Co., at a site on the campus of CSU-Fresno. A trailer was rented, retrofitted for quarantine infrastructure, and inspected by officers of the Fresno Co. Agricultural Commissioner's office. Insect maintenance and research rooms were built and outfitted with lighted shelves, cages, growth chambers, and research equipment. Also, a contract was arranged by Groves and Civerolo with Morgan to supply greenhouse-reared GWSS on a monthly basis. Acquisition of insects began in

September 2004. The new USDA-ARS/CSU-Fresno Insect Maintenance and Research facility went into full operation in October 2004. Also during this time we established colonies in the greenhouse in Parlier of the following species: smoke tree sharpshooter, *H. liturata* (STSS), as well as (with Groves) red-headed sharpshooter, *Xyphon fulgida* (RHSS), green sharpshooter, *Draeculacephala minerva* (GSS) and three-cornered alfalfa hopper, *Spissistilus festinus* (3CAH) (collected locally). Preliminary studies of the feeding behavior and EPG waveforms of all of these species are underway.

In addition to major infrastructure improvements in the first 6 months, we also analyzed past data, and Joost performed extensive preliminary tests to develop new protocols in electromyography and real-time imaging of sharpshooter muscles controlling feeding. We also wrote papers, and reviewed and wrote grant proposals. Among these were revisions of the Almeida & Backus paper on blue-green sharpshooter waveforms, now in print [1] and a newly funded UC PD proposal to continue research on mechanisms of *Xf* transmission and details of ingestion behavior. Once we had moved back into the lab and set up, progress resumed on existing objectives during the last four months of the reporting period (July – October 2004).

Objective 1 - Waveform Correlations

Experiment 1: AC-DC Correlation Monitor

Significant progress was made this year in the continuing development of this technology. Bennett built two new prototype monitors, the last of which included design suggestions developed by Backus in consultation with W. F. Tjallingii, Wageningen Agricultural University, The Netherlands. These prototypes for the first time succeeded in achieving waveform fidelity with the original, separate AC and DC waveforms, a goal sought for the last two years of work developing these instruments [2].

Experiment 2: Salivary Sheath-Cell Type Correlation

Backus analyzed histological images produced last year by Habibi from recordings made by Yan (see methods and preliminary findings in [2, 3]). Preliminary findings and waveform appearances are the same as those pictured in the 2002 and 2003 progress reports [2, 3], but waveform names are as in [3]. Results show that early pathway activities, especially A1, occur in the shallow epidermal/parenchyma tissues, A2 and continuous B1 usually occur in the parenchyma peripheral to the vascular bundle (although the sample size of tissues collected for B1 is very small). B2 usually occurs in the parenchyma or phloem, and is often associated with a large deposit of sheath saliva sometimes at a branching point in the sheath. The number of B2 events is also correlated with the number of sheath branches. Short, early C and N events can occur variably, in parenchyma, phloem or xylem; however, longer later C and N events are almost always in mature xylem cells. It is still uncertain whether B1 or C may represent the first penetration of a xylem cell. Correlations were completed and a manuscript is *in prep* for submission in late November [4]. Appendix Table A further summarizes the plant tissue/cell correlations known at the end of the reporting period (late Sept. 2003).

Experiment 3: Stylet Activities Correlation

Joost analyzed the videomicrography data collected by Yan of the stylet activities in artificial diet (see methods and preliminary findings in [2, 3], as well as a schematic of the equipment in the Backus et al. 2004 poster). Stylets could clearly be seen performing stereotypical behaviors during three waveform types frequently seen on grape, i.e. A1, A2 and B1. Results are summarized in Figures 1 – 4 below, Table A and in the Backus et al. 2004 poster. They reveal for the first time that A1 represents the primary formation of the salivary sheath (Figures 1, 2), B1 represents stylet tip fluttering (Figures 1, 3), and B2 represents stylet sawing through the hardened sheath (and, we speculate, perhaps also through tough plant material) (Figures 1, 4). It is particularly interesting that the B1 spikelet burst is dispersed intermittently throughout other pathway waveforms, e.g. between peaks of A1 (Figure 2), as well as in continuous durations by itself (Figure 3). This dispersion, plus last year's Experiment 4 finding [3] that B1 was the only pathway waveform associated with *Xf* inoculation, suggest that the spikelet bursts might represent precibarial valve movement, an important component of a hypothesized inoculation behavior [4]. A manuscript describing these results is *in prep* for submission in late November [5].

Objective 2 - Inoculation Behavior:

Experiment 4: EPG Waveforms Associated with Inoculation

Habibi completed sectioning and photomicrography of the remaining grape tissues probed by EPG-recorded GWSS, i.e. those during the short probe treatment (see the 2003 progress report [3] for methods and preliminary findings). Results from each of the three bacterial detection methods used (Table 1) continue to support that immunocytochemistry may be the most sensitive detection method; 56% of probes showed positive detection of *Xf* near the salivary sheath, while 45% were positive with PCR, and only 10% with culturing. These findings continue to support the interpretations discussed in the 2003 progress report [3]. Unlike PCR, immunocytochemistry results suggest that detectable bacteria are inoculated more often during long than short probes (Table 1). However, it will be important to determine how many insects were actually inoculative before we can state that conclusively. We have begun to dissect the fixed, dried heads of the recorded sharpshooters for scanning electron microscopy, to determine how many of them contained *Xf* and in exactly which areas in the precibarium/cibarium. This information will be

Table 1: Number of EPG-GWSS-probed grape samples that was positive for *Xf* near the probe out of the total number tested, for each of the three bacterial detection methods.

Probing Treatment	PCR	Culture	Immunocyt.
3 short probes	5/10	0/10	3/8
1 long probe	4/10	1/8	6/8

correlated with all other findings to determine how often the inoculation behavior, when performed by bacteria-laden insects, actually results in expulsion of *Xf*. Present findings [3] still implicate waveforms B1, C and N, especially during long probes. All data analysis will be completed and a manuscript submitted in early 2005 [6].

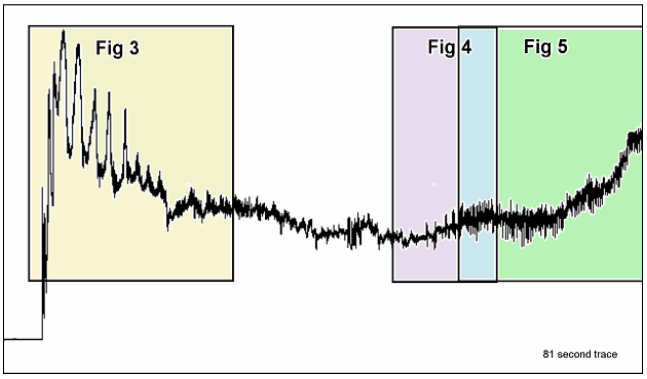


Figure 1. Waveform of GWSS probe in artificial diet compressed 35 times. Box labels indicate where Figures. 3-5 were taken from this trace.

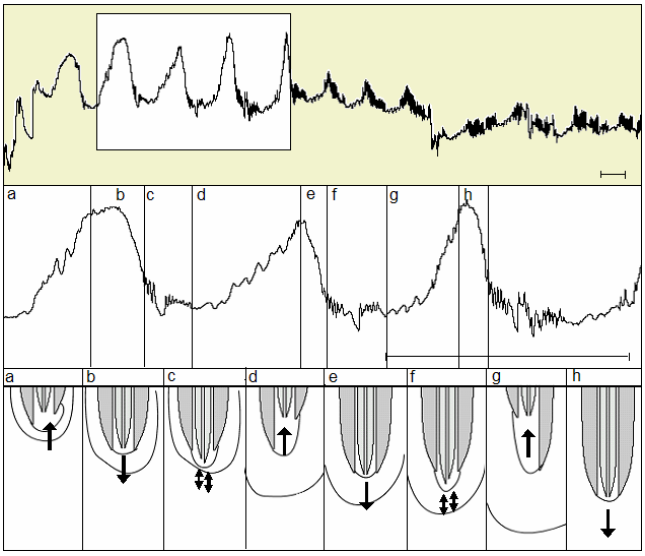


Figure 2. A1 waveforms were correlated with GWSS stylet activities in artificial diet. Top panel trace contains an A1 waveform compressed 5 times. The middle panel is an uncompressed A1 waveform trace that corresponds to the boxed waveform trace in the top panel. Subdivisions, a-h, in middle panel are correlated with stylet activities in the bottom panel with the same subdivision letters. Time marks in the lower right hand corner of the top and middle panel equal one second.

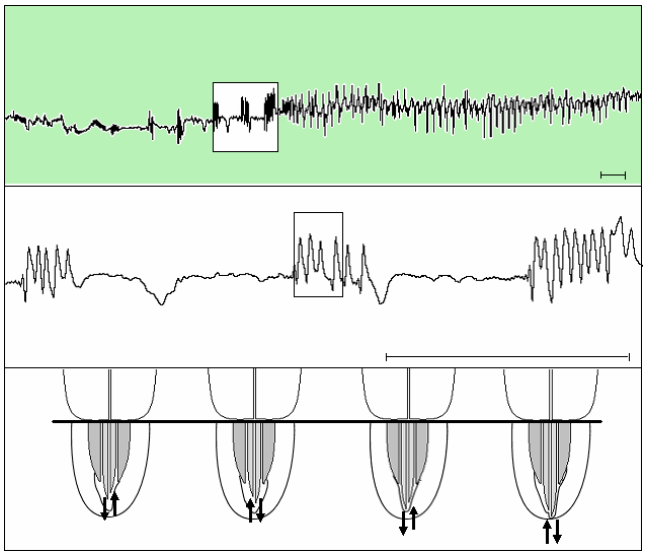


Figure 3. Correlation of B1 waveforms with GWSS stylet activities in artificial diet. Top panel is a waveform trace with B1 compressed 5 times. The middle panel is an uncompressed B1 waveform trace that corresponds to the boxed waveform portion in the top panel. The boxed waveform portion of the middle panel is a B1 spikelet burst and correlates with the stylet activities in the bottom panel. Time marks in the lower right hand corner of the top and middle panel equal one second.

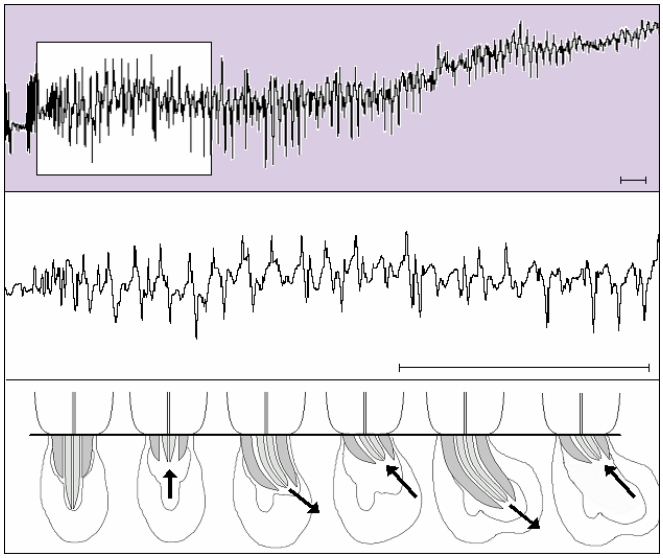


Figure 4. Correlation of B2 waveform with GWSS stylet activities in artificial diet. Top panel is a B2 waveform trace compressed 5 times. The middle panel is an uncompressed B2 waveform trace that corresponds to the boxed portion of the waveform in the top panel. The bottom panel are the stylet activities that were observed at the onset of the B2 waveform and through out the waveform. Time marks in the lower right hand corner of the top and middle panel equal one second.

CONCLUSIONS

These findings will help solve the PD/GWSS problem by:

- Identifying the mechanism of *Xf* inoculation and using EPG to observe it real-time as it occurs,
- Identifying one determinant of inoculation efficiency, i.e. the role(s) of inoculation behavior vs. bacterial presence and/or detachment in the foregut,
- Developing protocols for further tests of transmission biology and efficiency, especially with respect to acquisition.
- Developing a Stylet Penetration Index for testing among host and non-host species or cultivars, diets, etc. for performance of transmission behaviors, ultimately leading to improved host plant resistance.

REFERENCES

1. Almeida, R. and E.A. Backus. 2004. Stylet penetration behaviors of *Graphocephala atropunctata* (Say): EPG waveform characterization and quantification. Ann. Entomol. Soc. Amer. 97: 838-851.
2. Backus, E.A. 2002. Sharpshooter feeding behavior in relation to transmission of Pierce's Disease bacterium. Proceedings of the 2002 Pierce's Disease Research Symposium. pp. 67-69.
3. Backus, E.A. 2003. Sharpshooter feeding behavior in relation to transmission of the Pierce's Disease bacterium. Proceedings of the 2003 Pierce's Disease Research Symposium. pp. 127-131.
4. Backus, E.A., J. Habibi, and F. Yan. 2005. Stylet penetration behaviors of the glassy-winged sharpshooter on grape: AC and DC EPG waveform characterization, tissue correlation, and possible role in *Xylella* transmission. Special Feature edition of Ann. Entomol. Soc. Am., in honor of L. R. Nault and A. H. Purcell.: *In prep.* for Nov. submission.
5. Joost, P.H., E.A. Backus, and F. Yan. 2005. Specific stylet activities by the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), are correlated with AC EPG waveforms. Environ. Entomol.: *In prep.* for Nov. submission.
6. Backus, E.A., F. Yan, J. Habibi, and H. Shugart. 2005. Certain stylet penetration behaviors recorded by EPG are correlated with inoculation of *Xylella fastidiosa* into grape by the glassy-winged sharpshooter. Entomol. Exp. Appl.: *In prep.* for early 2005 submission.

Appendix Table A. Current definitions of the AC EPG waveform phases, families and types of GWSS on grape.

Waveform Phase	Waveform Family	Waveform Type	Waveform Characteristics	Proposed Biological Meanings	
				Plant Tissue/Cell	Insect Activity
Pathway	A	A1	Highest amplitude, hump-like waveform at beginning of probe; usually with spike at the top	Parenchyma or mesophyll	Major salivary sheath formation; deep extension/ retraction of stylets; some watery salivation
		A2	Medium amplitude, variable slope; irregular, high frequency with occasional trenches and/or potential drops	Parenchyma or mesophyll	Lengthening and/or hardening of salivary sheath; cell membrane breakage; some watery salivation
	B	B1	Short, single- or multi-peak "spikelet bursts" (20-28 Hz) separated by flatter, wave-like sections	Parenchyma or xylem or pith	Stylet tip fluttering; possible internal muscle/valve movement; involved in inoculation
		B2	Extremely regular, stereotypical pattern of peaks (6 Hz), with distinct phrases	Parenchyma or xylem or pith	Stylet sawing through salivary sheath or tough wood; sheath branching; sheath salivation
Ingestion	C	C (to be subdivided)	Very regular, low rep. rate (3 Hz) with with distinct phrases	Parenchyma or xylem or pith	Trial (short) or sustained (long) ingestion (watery excretory droplets correlated)
Interruption	N	N (to be subdivided)	Irregular, appearing A-like at times, but interrupting continuous C; ave. dur. 16 sec.	Parenchyma or xylem or pith	Sheath or watery salivation in ingestion cell; sheath extension

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

EFFECTS OF FEEDING SUBSTRATE ON RETENTION AND TRANSMISSION OF *XYLELLA FASTIDIOSA* STRAINS BY THE GLASSY-WINGED SHARPSHOOTER

Project Leaders:
Heather S. Costa
Dept. of Entomology
University of California
Riverside, CA 92521

Donald A. Cooksey
Dept. of Plant Pathology
University of California
Riverside, CA 92521

Cooperator:
Blake Bextine
Dept. of Entomology
University of California
Riverside, CA 92521

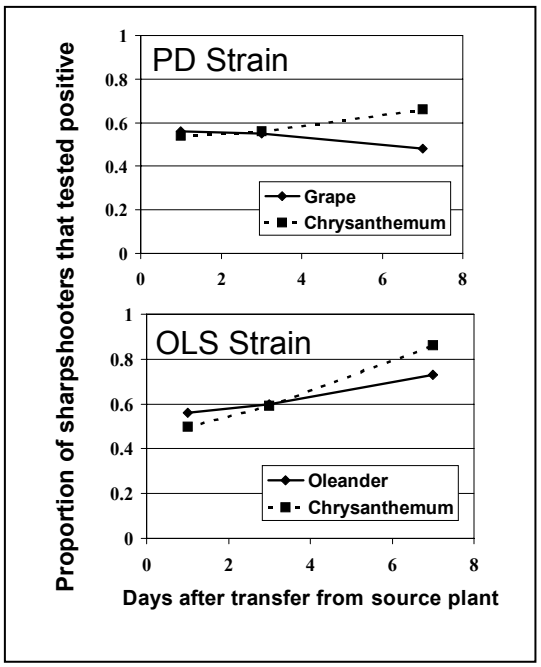
Reporting Period: The results reported here are from work conducted from October 2003 to September 2004**.**

ABSTRACT
In this project we are testing the effects of feeding substrate on the acquisition and retention of *Xylella fastidiosa* by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*. We are using two strains of *X. fastidiosa* that are present in California: a Pierce's disease (PD) strain that infects grape, and an oleander leaf scorch (OLS) strain that infects oleander. A series of experiments were conducted to compare the retention of PD or OLS strains after acquisition, when insects were subsequently maintained on a plant species that was either a host or non-host of that particular strain. In these studies, we found no significant difference in the mean proportion of insects testing positive for the PD or OLS strains, regardless of whether the insects were subsequently fed on either a host or a non-host of the PD or OLS strain. Thus, retention of a particular strain of the pathogen by an individual insect does not appear to be dependant on the xylem content of the plant host on which it is feeding. In a second study transmission efficiency of adult GWSS fed for 24 h on *X. fastidiosa*-infected plants was compared to those fed for 24 h on *X. fastidiosa* from pure media-grown cultures delivered through a cut stem system. In these experiments insects transmitted PD and OLS strains when they acquired the bacteria from a plant, but did not transmit either strain when media-grown bacteria were delivered through the cut-stem system.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is capable of acquiring and transmitting several different strains of *X. fastidiosa* from a variety of host plants. In this project we are testing the effects of feeding substrate on the acquisition, retention and transmission of *X. fastidiosa* by GWSS. Two strains of the pathogen present in California are being used in these experiments: a Pierce's disease (PD) strain that infects grapevine, and an oleander leaf scorch (OLS) strain that infects oleander. These two strains have different host ranges; the PD strain does not infect oleander, and the OLS strain does not infect grape.

OBJECTIVES
1. Compare retention times of *X. fastidiosa* when infected glassy-winged sharpshooter (GWSS) are subsequently fed on plants that are either hosts or non-hosts of the strain they carry.
2. Compare acquisition and transmission efficiency of insects fed on infected plants to those fed on media-grown cultures delivered through cut stems.
3. Compare retention times of two strains of *X. fastidiosa* in GWSS when simultaneously acquired through cut stems, then subsequently fed on either (a) a non-host of both strains, (b) on a host of only one strain, or (c) alternating hosts of each strain.
4. Test the effects of antibacterial materials on acquisition and transmission of *X. fastidiosa* by GWSS.
5. Test the effects of variation in substrate pH and free ion availability on the acquisition and transmission of *X. fastidiosa* by GWSS.

RESULTS
Objective 1
We began by comparing the relative proportion of insects that tested positive after acquisition of a given strain of *X. fastidiosa,* when they were subsequently maintained on a plant species that was either a host or non-host of that strain. Grape plants (*Vitis* spp.) infected with a Pierce's disease (PD) strain of *Xylella fastidiosa*, and oleander plants *(Nerium oleander*) infected with an oleander leaf scorch (OLS) strain were used as sources of inoculum. The strain of *X. fastidiosa* infecting plants was confirmed by PCR. Groups of GWSS adults were caged on either an OLS infected oleander plant, or a PD infected grapevine for 2 days. Insects were then moved to an uninfected plant of the same species as the source plant (oleander or grape), or to a non-host of the strain (chrysanthemum). Samples of insects were collected at 1, 3, and 7 days after transfer to uninfected hosts and frozen. Insects were subsequently tested for the presence of *X. fastidiosa* using PCR.



Results from retention experiments using the OLS strain acquired from oleander showed no significant difference in the mean proportion of insects testing positive when insects were subsequently fed for on either a host (oleander), or a non-host (chrysanthemum) of the OLS strain for 1, 3, or 7 days after acquisition. Similarly experiments using the PD strain acquired from grapevine also found no significant difference in the mean proportion of insects testing positive at 1, 3 or 7 d after acquisition regardless of whether the insects were subsequently fed on either a host (grapevine) or a non-host (chrysanthemum) of the PD strain. Thus, both PD and OLS strains of *X. fastidiosa* remained detectable in GWSS, even when the insects fed on a non-host of the strain for 7 d.

Objectives 2 and 3

To test if feeding substrate can influence the ability of insects to acquire and transmit a particular strain of *X. fastidiosa*, we plan to use a pathogen delivery system to allow us to either maintain or manipulate the feeding substrate as desired. The method described by Bextine and Miller (2002) was originally used for an *Alcaligenes* sp. of bacteria. This technique was modified to provide an environment suitable to survival of *Xylella fastidiosa* and the test plants used. We are using sections of chrysanthemum stem about 10 cm long that are connected by tubing to a syringe with a suspension of *X. fastidiosa* in PBS. The distal end of the stem is also cut and left open. The syringe is depressed until liquid is extruded from the distal end of the cut stem. Then GWSS are allowed to feed on these stems.

To demonstrate that live *X. fastidiosa* cells could survive movement through a cut stem, *X. fastidiosa* was suspended in a PBS buffer, and the syringe was depressed until liquid was extruded from the distal end of the cut stem. Droplets forming on the distal end were collected and analyzed using PCR to determine if *X. fastidiosa* cells were present. In all cases, *Xylella* was detected within the first 10 drops extruded. Thus, in these experiments, material was injected into stems until at least 10 drops of material was extruded from the distal cut end to ensure that the bacteria have been moved the entire distance of the stem.

In transmission experiments, adult insects were fed for 24 hours on either infected plants, or media-grown bacteria delivered through the cut stem system as described above. Adults were then individually moved to uninfected test plants and allowed to feed for 4 d. When GWSS adults were fed on PD-infected grapevines, 12/26 (46%) transmitted the pathogen to healthy grapevine test plants. In contrast, when insects were fed on media-grown PD bacteria through the cut stem method, no individuals (0/48) transmitted the pathogen to test pants. Similar results were found with OLS-infected plants (9/37,or 24% of individuals transmitted) compared to media-grown OLS delivered through cut stems (0/22 transmitted). Thus, insects did not transmit PD or OLS strains when media-grown bacteria were delivered through the cut-stem system. Purcell et al. (personal communication) found similar results when leafhoppers were fed *X. fastidiosa* through parafilm sachets.

Additional studies are being conducted to determine why insects are unable to transmit the pathogen from the cut stem delivery system. For example, a recent study demonstrated that *X. fastidiosa* cultures will produce different levels of "biofilm formation" when grown on different types of media (Leite et al. 2004). We will test if growing our strains on different media may help induce transmissibility by insects. In addition, we will conduct further studies to determine the pathway of the bacteria through the system. For example, by testing the honeydew of insects feeding on the cut stem system, we can determine if the bacteria are successfully passing through the insect. In the interim, work on the remaining objectives will continue using insects fed on PD and OLS infected plants.

CONCLUSIONS

In retention experiments (Objective 1) for both the PD and OLS strains, we found the proportion of insects retaining the pathogen was the same, regardless of whether insects subsequently fed on a host or a non-host of that strain. This indicates that retention of a particular strain of the pathogen by an individual insect is not dependant on host-specific xylem content of the plant on which it is feeding. In transmission experiments (Objectives 2 and 3) insects successfully transmitted the PD and OLS when they acquired the pathogen from infected grapevine and oleander plants respectively, but did not transmit either the PD or OLS strains when the media-grown bacteria were delivered through the cut-stem system. This could be the result of biological characteristics of media-grown bacteria that contribute to non-transmissibility by insects, or failure of the cut stem system to properly deliver bacteria to the insect. Further experiments are being conducted to determine the basis for lack of transmission of media-grown bacteria by GWSS.

REFERENCES

Bextine, B. and T. Miller. 2002. Insect-symbiotic bacteria inhibitory to *Xylella fastidiosa* in sharpshooters: pressure bomb extraction of xylem fluid to improve bacterial detection of *Xylella* in plants. Symposium Proceedings: Pierce's Disease Research Symposium, Dec. 2002, California Department of Food and Agriculture, Sacramento. Pp. 29-30.

Leite, B., P. C. Andersen, and M. L. Ishida. 2004. Colony aggregation and biofilm formation in xylem chemistry-based media for *Xylella fastidiosa*. FEMS Microbiol. Lett. 230: 283-290.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

Project Leader:
Thomas A. Coudron
USDA, ARS, BCIRL
Columbia, MO 65203

Researcher:
Cynthia L. Goodman
USDA, ARS, BCIRL
Columbia, MO 65203

Collaborators:
Walker A. Jones
USDA, ARS, KDLG Subtropical Agric. Res. Center
Beneficial Insects Research Unit
Weslaco, TX 78596

Elaine Backus
USDA, ARS, PWA
SJV Agricultural Sciences Center
Parlier, CA 93648

Wayne Hunter
USDA, ARS
U.S. Horticultural Research Laboratory
Subtropical Insects Research
Ft. Pierce, FL 34945

Reporting Period: Funding for the study was initiated in October, 2004 and the project is in the start-up phase at the time of this reporting.

ABSTRACT
The intent of this project is to develop an artificial rearing system for the glassy-winged sharpshooter (*Homalodisca coagulata*) (GWSS), the primary vector of Pierce's Disease (*Xylella fastidiosa*) (PD). In order to accomplish this, a diet delivery system will first be developed and then used to test artificial diets. Diet formulations will be based, in part, on previous studies performed by Cohen (2002) using GWSS, as well as on artificial diets developed for other Hemiptera (Mitsuhashi, 1979; Coudron *et al*., 2002) and on the xylem chemistry of GWSS host plants (Andersen, et al., 1992). Diets will be evaluated based on their effects on life history analyses, reproductive rate and intrinsic rate of increase of GWSS. Another aspect of our project involves investigating nitrogen source(s) for GWSS, as that may represent a nutrient limitation for xylem feeders. Two potential sources for nitrogen, i.e. proteins or peptides, will be studied by determining the fate of dietary proteins/peptides (Brandt, et al., 2004) and the ability of salivary and midgut proteolytic enzymes to digest proteins/peptides (Wright, et al., 2004). In this way, we will identify the role(s) proteins and peptides play in GWSS nutrition and their potential uses in artificial diet formulations.

INTRODUCTION
The formulation of an artificial diet for GWSS will greatly enhance the ability of researchers to rear this insect. Presently, the rearing of GWSS is labor-intensive and costly because of its dependence on the propagation of appropriate host plants, with researchers often needing to propagate several species of plants to enable them to rear GWSS under optimal conditions. The development of an artificial diet would likely be more cost effective and portable, increasing the availability of high quality insects for Pierce's disease researchers and decreasing the costs and time-constraints associated with maintaining the insect in culture. The increased accessibility of GWSS to researchers can lead to more rapid developments in novel control measures for this major vector of PD, with these new measures being directly applied by growers. Furthermore, the coupling of an artificial diet with a suitable delivery system can lead to an improved understanding of the relationship between GWSS nutrition and other PD-related issues (including GWSS' varying abilities to acquire/maintain/transmit infectious *Xf* under different circumstances, e.g., via artificial membranes vs. plants, Redak et al., 2004). In addition, the diet delivery system alone would have other potential uses such as in studying the interactions between GWSS, *Xf*, and the host plant, as well as in testing potential anti-GWSS and anti-*Xf* control agents. This could be accomplished by incorporating into the feeding system: 1) selected host plant-associated compounds; 2) media containing the causative agent of PD (*Xylella fastidiosa*, *Xf*) (although some studies have suggested that *Xf* acquired via an artificial membrane by GWSS may not be infectious, Redak et al., 2004); 3) control agents including anti-GWSS or -*Xf* compounds (such as proteins to be engineered into host plants to control either GWSS or *Xf*; Dandekar et al., 2003; Lin, 2003; Meredith and Dandekar, 2003; Reisch et al., 2003) or anti-GWSS microbials (Kaya, 2003; Mizell & Boucias, 2003). In summary, the development of an artificial diet and a corresponding delivery system for GWSS could lead to insights that can be used to generate improved methods for controlling GWSS and, therefore, Pierce's disease.

An important part of our project also involves gaining a better understanding of the digestive physiology of GWSS. This will be investigated by focusing on the role proteins and peptides play in GWSS nutrition, as these or similar compounds have been isolated from some xylem fluids (Cohen, 2002; Jain and Basha, 2003; Rep et al., 2003). We will accomplish this by determining the extent to which GWSS can digest proteins and peptides, as well as elucidating the fate of specific ingested proteins in GWSS. This information will be directly used in the generation of an optimal artificial diet for GWSS. Furthermore, GWSS' ability to degrade proteins/peptides will also shed light on the degree to which GWSS can disable defensive proteins/peptides in plants, which is important when dealing with salivary enzymes that are secreted into plant tissues and could alter anti-*Xf* defense components (e.g., either naturally occurring or genetically engineered proteins/peptides; Lin, 2003; Meredith and Dandekar, 2003; Reisch et al., 2003). This knowledge could be used when modifying target plants such as grapevines to improve their resistance against Pierce's disease (PD). Therefore, our investigation into nutritional requirements will not only aid us in the development of a suitable artificial diet for GWSS, but

will also provide insights into the potential efficacies of anti-PD plant modifications.

OBJECTIVES

1. Develop an artificial diet delivery system for rearing the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata.*
2. Formulate and evaluate an artificial diet for the development and reproduction of GWSS.
3. Investigate the utilization of proteinaceous components in the food stream of GWSS in order to refine and improve the artificial diet using physiological and proteomic/genomic approaches.

RESULTS AND CONCLUSIONS

This project has just been funded. Preparation of quarantine facilities is complete and the identification of insect cultures to be used in our studies is underway. The process to hire an additional researcher has been initiated. Preliminary experiments, in collaboration with Jones and Setamou at ARS in Weslaco, have demonstrated continuous feeding by adult GWSS for over 30 days on artificial diets presented through a specialized feeding tube. Additionally, differences in survival have been noted as a result of changes in amino acid concentration and composition within the diet.

REFERENCES

Anderson PC, Brodbeck BV, Mizell RF, 1992. J. Insect Physiol. 38:611-612.

Brandt SL, Coudron TA, Habibi J, Brown GR, Ilagan OM, Wright MK, Wagner RM, Backus EA, Huesing JE, 2004. Curr. Microbiol. 48:1-9.

Cohen AC, 2002. Proc. Pierce's Disease Research Symp., pp. 83-85.

Coudron TC, Wittmeyer JL, Kim Y, 2002. J. Econ. Entomol. 95(6), 1159-1168.

Dandekar A, Gupta G, McDonald K, Hong-Geller E., 2003. Proc. Pierce's Disease Research Symp., pp. 87-88.

Jain AK, Basha SM, 2003. African J. Biotechnol. 2:66-70.

Kaya H., 2003. Proc. Pierce's Disease Research Symp., pp. 263-264.

Lin H, 2003. Proc. Pierce's Disease Research Symp., 158-159.

Meredith C, Dandekar A, 2003. Proc. Pierce's Disease Research Symp., pp. 23-25.

Mitsuhashi J, 1979. In: Leafhopper Vectors and Plant Disease Agents (Maramarosch, K, Harris, KF, eds). Academic Press: NY; pp. 369-412.

Mizell RF, Boucias DG, 2003. Proc. Pierce's Disease Research Symp., pp. 274-275.

Reisch B, Walker A, Kikkert JR, 2003. Proc. Pierce's Disease Research Symp., pp. 30-32.

Rep M, Dekker HL, Vossen JH, de Boer AD, Houterman PM, de Koster CG, Cornelissen BJC, 2003. FEBS Letters 534, 82-86.

Wright, M.K., Wagner, R.M., Huesing, J.E., Brandt, S.L., Habibi, J., Coudron, T.A., Thoma, R., Backus, E.A. 2004. J. Insect Physiol. (submitted).

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

BIOLOGY AND ECOLOGY OF THE GLASSY-WINGED SHARPSHOOTER IN THE SAN JOAQUIN VALLEY

Project Leaders:

Kent M. Daane
Division of Insect Biology, Dept. of Environmental Science, Policy and Management
University of California,
Berkeley, CA 94720

Marshall W. Johnson
Dept. of Entomology
University of California
Kearney Agricultural Center
Parlier, CA 93648

Cooperators:

Glenn Yokota
Division of Insect Biology
University of California
Berkeley, CA

Jennifer Hashim
University of California CE
Kern County
Bakersfield, CA

Elaine Shapland
Division of Insect Biology
Univ. of California
Berkeley, CA

Alexander Purcell
Division of Insect Biology
University of California, Berkeley, CA

James Hagler
USDA-ARS, WCRL
Phoenix, AZ

Valerie Fournier
University of California, Berkeley
(at the USDA-ARS, WCRL)

Russell Groves
USDA-ARS, PWA, SJVASC
Parlier, CA

Robert Luck
Dept. of Entomology
Univ. of California
Riverside CA

Youngsoo Son
University of California
KAC, Parlier, CA

Reporting Period: The results reported here are from work conducted from November 1, 2002 to October 1, 2004.

ABSTRACT

We followed glassy-winged sharpshooter (GWSS) preference and age structure on ornamental host plants in Bakersfield, California. Results of an urban survey showed GWSS host utilization varied greatly. This was especially true during the growing season when the mobile GWSS nymphs and adults would frequently shift amongst abutted host plants. While host plant utilization was dynamic, yet there were clear seasonal patterns. In late-fall through mid-winter, GWSS were most commonly found on privet, oleander, and citrus. In late-winter through spring, the preferred hosts were Xylosma, photinia, and flowering pear. In summer, host utilization was most dynamic and often dependent on host condition (such as irrigation). Nevertheless, GWSS adult and nymph summer and early-fall populations were consistently found on Xylosma, photinia, oleander, star jasmine, and Crape myrtle. Controlled experiments with potted host plants found similar results and highlight differences in GWSS feeding and oviposition preferences. Throughout all studies, we sampled the numbers of predators and parasitoids. Emerged parasitoids show *Gonatocerus ashmeadi* and *G. triguttatus* were reared from egg masses collected on most host plants, and accounted for a large percentage of summer GWSS mortality. Predators were present, especially spiders, and often observed feeding on GWSS. However, our data has not yet found any one predator species to be consistently associated with GWSS or with a reduction in GWSS densities. Collected predators are being analyzed using immunologically-based assays that employ pest-specific monoclonal antibodies (MAbs) to help identify the key predators of GWSS. During the urban surveys, we collected plant material (e.g., potential vector host plants) and potential insect vectors to determine the incidence of *X. fastidiosa*. This material was processed in the laboratory using "immunocapture DNA extraction" to determine the presence of *X. fastidiosa*. Results show that GWSS collected in urban regions often (>10%) carry *Xylella fastidiosa*, however, it is not the strain that cause PD.

INTRODUCTION

The primary focus of this research is the description of glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, GWSS preference, egg deposition, age structure, population dynamics and levels of natural regulation on different host plants in the urban / agricultural interface in the San Joaquin Valley (SJV). Currently, such a description of GWSS biology and ecology in the SJV is lacking. The developed information from this research will help understand GWSS seasonal movement and infestation foci. Of primary concern to regional control programs is whether or not untreated urban GWSS populations serve as an inoculum source for either the insect vector or the bacterial pathogen, *Xylella fastidiosa* (*Xf*).

To develop a more complete description of host plant influence on GWSS age structure and natural enemy impact, we conducted both urban surveys and manipulative experiments. Specifically, we sought to determine the potential of common plant species used in residential landscaping to either reduce or increase GWSS densities. We further screened common plants and GWSS collected for the presence of *Xylella fastidiosa*. When completed, information on the abundance, host plant use, and seasonal dispersal patterns of GWSS and natural enemies in urban better enable researchers to determine GWSS movement and host plant succession in the SJV, and the data may be useful for modification of surrounding vegetation, such as trap crops, to suppress GWSS movement into a vineyard.

OBJECTIVES

1. Determine glassy-winged sharpshooter biology and ecology throughout the season, particularly its age structure on and utilization of the different host plants that represent common breeding or dispersion refuges for glassy-winged sharpshooter in the San Joaquin Valley.
2. Determine the contribution of resident natural enemies on glassy-winged sharpshooter mortality and whether natural enemy abundance or species composition varies significantly on different GWSS host plants or ecosystems in the San Joaquin Valley.
3. Determine the presence of *Xylella fastidiosa* in glassy-winged sharpshooter collected from different host plant species and in selected ecosystems in the San Joaquin Valley.

RESULTS

Objective 1 - Survey.

GWSS numbers, age structure and natural enemies were surveyed in residential areas in Bakersfield, California. In the 2003-2004 season, six residential sites were sampled. Each site was selected for its combination of different GWSS and *Xf* host plants; most of the sampled sites had 3-8 individual plants of each plant species, with 3 or more GWSS host plant species in close proximity. Host plants surveyed included: carob, rose, star jasmine, Chinese elm, flowering pear, apple, escallonia, pink lady, ivy, nectarine, photinia, citrus, gardenia, privet, euonymous, hibiscus, agapanthus (lily of the Nile), grape, crape myrtle, eucalyptus, mock orange, oleander, Xylosma and Wheeler's dwarf. Each month, samples were taken for GWSS and natural enemies. We also recorded plant condition. From April 2003 to October 2004, we made >3000 plant samples (sample plant × sample date).

A thorough analysis of this data set will be made at the end of the residential survey (April 2005) when we project to have >5000 samples, each with information on host plant species, condition and phenology; GWSS density and age structure; and potential natural enemies present. An initial analysis show strong host plant preferences GWSS adults and nymphs, especially towards oleander, crape myrtle and Xylosma during the spring and summer months (Figure 1). Host plant preference for adult and nymph feeding sites was not always the same as those preferred for egg deposition – especially with respect to oleander, as reported by other researchers.

The seasonal population dynamics showed a strong spring GWSS population on all hosts followed by a summer decline, which is largely attributed to egg parasitism of the summer brood. We believe that the winter period is critical for GWSS population dynamics as this period represents the low point in the population density. Oleander and privet may be the most important overwintering hosts in the urban regions. In contrast, host plants as crape myrtle and crabapple are dormant throughout winter and, according to our samples, play no role in the GWSS overwintering. However, they are excellent hosts for oviposition and nymphal development during late spring and summer time. For some host, GWSS are confined to specific sections. For example, the flowering pear trees brake dormancy early in the year and start blooming by the first week of February. GWSS adults have been found on the twig tips in the middle of the winter in these trees. It is unknown whether they survive the entire winter in this plant or the early physiological activity of the flowering pear attracts the GWSS. We also found GWSS overwintering exclusively on the "suckers" of the following tree species: eucalyptus, carob tree, Chinese elm, and olive.

Objective 1 – Manipulative Experiments

To categorize GWSS age structure, ecology, and resident natural enemies (particularly predators) on different host plants common in urban areas, potted (6.6 L) plants were used to provide a replicated array of similarly-conditioned (e.g., age, size, irrigation) GWSS host plant species. These preference studies were conducted in an unsprayed, GWSS infested citrus orchard, and two unsprayed residential areas in Bakersfield, California. Perennial species included ivy, photinia, citrus, gardenia, privet, euonymous, hibiscus, agapanthus (lily of the Nile), grapevine, crape myrtle, eucalyptus, and oleander. Annual (or weed) species included prickly lettuce, little mallow, annual sowthistle, coast fiddleneck, common groundsel, London rocket, fox tail brome, lambsquarters, blue grass, and shepperd purse. Both perennial and annual species were set in a randomized block design. Results show GWSS seasonal-long densities were influenced by host plant species, with a significant difference (ANOVA, $P < 0.001$) among host plants, for both perennial and annual categories (Daane et al. 2003. 2004a). Results are provided for perennial host plants in the citrus orchard (Figure 2), which shows a 20-fold difference in the number of GWSS on ivy, the least preferred host planted tested, and grape, the most preferred. We found a relatively similar pattern in the 2002/03 and 2003/04 seasons. Interestingly, GWSS egg mass density was not related to adult or nymphal densities ($P = 0.25$, $r^2 = 0.03$; $P = 0.35$, $r^2 = 0.01$, respectively). As with the urban survey, we conclude that GWSS adults have oviposition preferences that may be different from the nymphal feeding preference. We believe this difference is a result of both GWSS adults and nymphs switching among host plants, and to a disparate level of predator and parasitoid activity.

In a second experiment, we manipulated combinations of GWSS host plant species in cages. Four plant species have been planted in different combinations (e.g., citrus only, citrus and oleander, oleander only, oleander, citrus and crape myrtle), with a total of 7 plant species (4 replicates). Initial progress was slowed by the difficulty we encountered in transferring field-

collected GWSS material to the experimental site – basically, many of the GWSS nymphs died or left the tested host plant almost immediately after being transfer. We are currently improving inoculation techniques.

Objective 2 – Natural Enemies

During the surveys of GWSS population dynamics in non-agricultural regions, described previously, we collected information on GWSS natural enemies, using sampling techniques such as GWSS egg mass collections (>100 leaves per perennial plant species per collection) and potential GWSS predator collections (beat and sweep samples). As in all studies, we recorded host plant species and seasonal period. We found *Gonatocerus ashmeadi* and *G. triguttatus* (Triapitsyn et al. 1998) comprised about 95 and 4%, respectively, of collected parasitoids. As has been suggested, these parasitoids kill >90% of the summer GWSS population. Parasitoid numbers drop during the winter, when most GWSS are in the adult stage – although large nymphs were present as well. No egg masses or recently hatched nymphs were found from November through February. The first fresh egg masses were collected in April (2003) and March (2004), and we found parasitized eggs within as soon as April (2004). Our results suggest that egg parasitoids are the primary biological control factor. Combined with the winter / spring area wide insecticide control programs (which dramatically reduce the over-wintered population on citrus, the primary GWSS host plant during this period, and lower the overall GWSS population levels in the SJV) the egg parasitoids reduce the GWSS population in the urban regions to such an extent that GWSS can be difficult to find in large numbers in late summer samples.

Predators may play a small role controlling GWSS nymphs. Spiders were the most common predator found, and there was a significantly positive relationship between the number of spiders found and the number of GWSS egg masses ($P < 0.001$, $r^2 = 0.28$). Still, there has not yet been any concrete evidence that links these generalist predators with the regulation or suppression of GWSS. During the GWSS urban surveys, predators were collected, identified to family or genus, and stored at -80ºC. These specimens have been shipped to the Western Cotton Research Laboratory, where the predator gut content is being assayed with immunologically-based assays that employ pest-specific monoclonal antibodies (MAbs) for the presence of GWSS egg protein using the ELISA by Drs. Hagler, Fournier and Leon (Hagler et al. 2003). These studies will provide direct evidence of predation by generalist predators.

Objective 3 - Xylella

How important are glassy-winged sharpshooter populations in the urban regions as vectors of *Xf* in nearby agricultural areas? First, GWSS population densities have been relatively low in the SJV urban centers, as previously described. Second, GWSS has a relatively low *Xf* transmission efficiency. Together, the low density and poor transmission efficiency would suggest few GWSS would have *Xf* in their mouthparts and play any role in the movement of the pathogen. We tested adult GWSS collected from ornamental plants in Bakersfield and, to our surprise, found *Xf* in GWSS (mouthparts) collected from oleander, Xylosma, and Chinese elm. The positive results do not necessarily mean that the GWSS acquired the *Xf* from the plants that they were collected on as the adults move between host plants often.

How important are GWSS nymphs in the movement of *Xf* among ornamental plants and to vineyards? Nymphs shed the lining of their gut with each molt before adulthood, loosing any *Xf* living there and therefore provide a better indication of acquisition. The initial screening of GWSS nymphs used a "presence" or "absence" of groups of nymphs collected and therefore data are presented as such, rather than a percentage. In the initial collections, *Xf* was found only in GWSS nymphs collected from oleander (in the Bakersfield region). It is also important to note that all GWSS samples testing positive for *Xf* were analyzed for bacterial strain differences and analyses showed that the bacteria present are not of the PD type, but could be oleander, almond, oak, peach or plum. Most likely the *Xf* is oleander strain, which does not pose an immediate threat to nearby vineyards because this strain does not cause PD in grapes

CONCLUSIONS

We have described GWSS population density and age structure on ornamental plants common in residential landscaping in the SJV. We have further described natural enemy presence. This research can be added to information collected in Riverside and Ventura counties to help predict GWSS movement and develop control programs. The research has broader implications for use of ornamental landscape and riparian plants within agricultural settings (e.g., landscaping around farm buildings and homes). Plants which act as preferred hosts for both vector and pathogen can be target for control. By testing GWSS for the presence of *Xf*, researchers will identify potential sources of the pathogen, thereby preventing potential epidemic spread of Pierce's disease causing *Xf* throughout a reservoir of ornamental host plants. To see a list of host plants, for both *Xf* and GWSS) go to: http://nature.berkeley.edu/xylella.

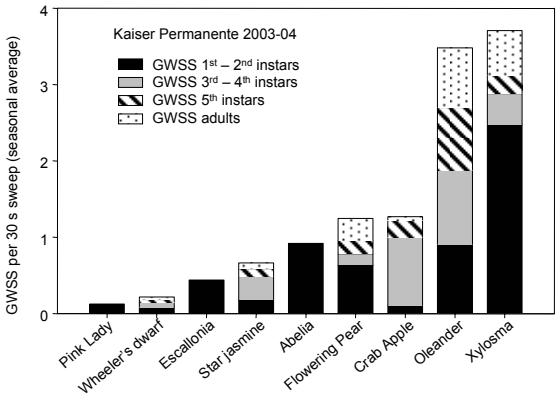


Figure 1. The seasonal average for host plant preference GWSS adults and nymphs was clearly towards oleander and Xylosma at this sampling site. Data of the seasonal average are skewed by the large spring GWSS population density.

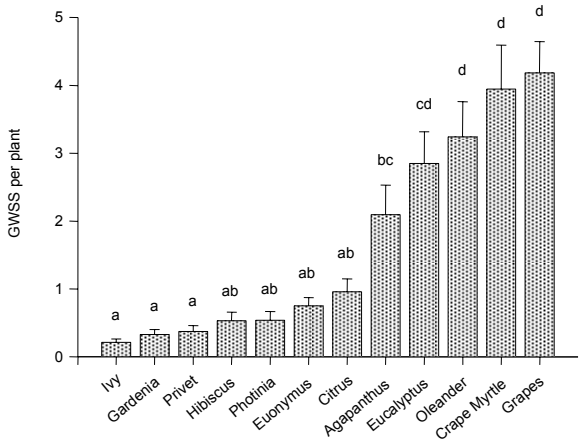


Figure 2. Average densities (± SEM) of GWSS (nymphs and adults) were significantly different among perennial host plants, Tukey's HSD at $P < 0.05$. Data are seasonal averages, and biased towards host species preferred in June and July, when GWSS densities were the highest.

REFERENCES

Hagler, J., K. Daane, and H. Costa. 2003. Progress on the development of a monoclonal antibody specific to glassy-winged sharpshooter egg protein: a tool for predator gut analysis and early detection of pest infestation. *In* M. Athar Tariq et al. [eds.], Proceedings, Pierce's Disease Research Symposium. Calif. Dept. Food and Agricul., Digital Logistix, Sacramento, CA.

Daane, K. M., M. W. Johnson 2003. Biology and Ecology of the glassy-winged sharpshooter in the San Joaquin Valley *In* M. Athar Tariq et al. [eds.], Proceedings, Pierce's Disease Research Symposium. Calif. Dept. Food and Agricul., Digital Logistix, Sacramento, CA.

Daane, K. M., M. W. Johnson, T. Ruiz, T., and J. Hashim. 2004a. Research shows GWSS have their urban preferences. Kern/Tulare GWSS Update. March 5, 2004.

Daane, K. M., E. Shapland, T. Ruiz, M. W. Johnson, and J. Hashim. 2004b. Identifying the role of weeds in the overwintering of *Xylella*. Kern/Tulare GWSS Update. April 20, 2004.

Shapland, E., K. M. Daane, G. Y. Yokota, A. H. Purcell, C. Wistrom, M. W., Johnson. 2004c. Researchers follow *Xylella* pathways. Kern/Tulare GWSS Update. August 6, 2004.

Triapitsyn S. V., R. F. Mizell III, J. L. Bossart, and C. E. Carlton. 1998. Egg parasitoids of *Homalodisca coagulata* (Homoptera: Cicadellidae). Fl. Entomol. 81: 241-243.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

IDENTIFYING KEY PREDATORS OF THE VARIOUS GLASSY-WINGED SHARPSHOOTER LIFESTAGES

Project Leaders:

Valerie Fournier
Division of Insect Biology
University of California
Berkeley, CA 94720

James Hagler
Western Cotton Research Lab
USDA-ARS
Phoenix, AZ 85040

Kent Daane
Division of Insect Biology
University of California
Berkeley, CA 94720

Jesus de León
Beneficial Insects Research Unit
USDA, ARS
Weslaco, TX 78596

Russell Groves
Exotic & Invasive Diseases & Pests Lab
USDA, ARS
Parlier, CA 93648

Cooperators:

Nilima Prabhaker
Dept. of Entomology
University of California
Riverside CA 92521

Heather Costa
Dept. of Entomology
University of California
Riverside CA 92521

Reporting Period: The results reported here are from work conducted from November 1, 2003 to October 1, 2004.

ABSTRACT

Glassy-winged sharpshooter (GWSS) egg-specific monoclonal antibody (MAb) and GWSS-specific genetic markers have been developed for use as diagnostic tools for predator gut content analysis. Feeding trials were conducted to determine how long a MAb-based ELISA can detect GWSS remains in the guts of *Chrysoperla carnea* and *Harmonia axyridis*. We found that *C. carnea* can yield positive ELISA reaction for the presence of GWSS egg antigen for up to 24 hours after eating an egg. Further results showed that the detection period of GWSS egg antigen in *H. axyridis* is less than 6 hours. Using mitochondrial COII primers specific to GWSS, we obtained successful amplification of GWSS DNA fragments from *H. axyridi* that consumed six GWSS eggs. Optimization tests are underway to increase the efficacy of GWSS-specific genetic primers to detect pest DNA in predator guts. Feeding trials with additional predators (*Zelus renardii*, *Sinea diadema*, and several spider species) are currently being performed.

INTRODUCTION

Effective control of GWSS will require an areawide integrated pest management approach (AW-IPM). A major component of AW-IPM is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can increase the effectiveness of other control tactics. Unfortunately, very little information exists on GWSS's predaceous natural enemies. Evidence of predation of GWSS eggs and adults has been observed in the field (JH pers. obs.); however, the composition of the predator complex, and the relative impact of each predator on GWSS mortality is unknown. A major obstacle is the difficulty of studying predators in their natural environment. Unlike parasitoids, predators rarely leave evidence of attack. Laboratory experiments can be used to evaluate the suitability of particular prey and the rates of predation. However, lab studies seldom translate to field situations. Direct field observations are sometimes used to identify predators of key pests, but the small size and cryptic nature of predators and GWSS make direct observations difficult and laborious. Predator gut content analysis represents a valid approach to investigate predation. Currently, the state-of-the-art predator stomach content assays include enzyme-linked immunosorbant assays (ELISA) for the detection of prey-specific proteins (Hagler 1998; Hagler & Naranjo 1994ab) and polymerase chain reaction (PCR) assays for the detection of prey-specific DNA (Symondson 2002). To this end, we have developed GWSS egg-specific MAbs (Hagler et al. 2002; Fournier et al. submitted) and GWSS-specific primers (de León & Jones 2004). Both assays provide an avenue to qualitatively assess the impact of predator species on GWSS populations.

OBJECTIVES

Our main objective is to identify the composition of the GWSS predator complex using pest-specific ELISA and PCR assays. However, several optimization studies are needed (e.g. detectability half-life) before these assays can be used to examine field-collected predators. Here we report results of laboratory tests on detection periods of GWSS egg antigen in the guts of two generalist predators, the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using a GWSS egg-specific ELISA. We also present preliminary results on predator gut content analysis using PCR.

RESULTS:

ELISA Response to Lacewing that Consumed GWSS Eggs

Predators were placed individually in Petri dishes and starved for 36 h. Lacewings were then fed one or two GWSS eggs (within a 30-min time frame) and isolated from food for 0, 6, 9, 12, 24, or 36h at 25°C, photoperiod of 16:8h (L:D), and then frozen (-80ºC). Negative controls were individuals that did not eat any GWSS eggs. Each lacewing was analyzed by indirect

ELISA for the presence of GWSS egg antigen (methods described in Hagler et al. 2002). Data indicate that the number of ELISA positive reactions decreased over time (Table 1). All negative controls yielded negative ELISA absorbance values. Significant differences between the mean absorbance of values of the lacewings fed GWSS eggs and their negative control counterparts was found in all post-feeding time intervals, except for time=24 and 36 h.

Table 1. ELISA results testing for the presence of GWSS egg antigen in the guts of *Chrysoperla carnea* (3rd instar larva).

Treatments [a]	Negative Control			Lacewing fed with GWSS eggs		
	Absorbance at 405 nm, mean ± SD	Critical value[b]	% positive reactions (N) [c]	Absorbance at 405 nm, mean ± SD	% positive reactions (N)	Significance[d]
0h	0.089±0.003	0.098	0 (15)	0.526±0.488	95 (19)	***
6h	0.072±0.006	0.090	0 (22)	0.176±0.142	62 (21)	***
9h	0.076±0.004	0.088	0 (19)	0.197±0.167	76 (21)	**
12h	0.074±0.007	0.095	0 (21)	0.147±0.149	43 (23)	*
24h	0.077±0.008	0.101	0 (14)	0.170±0.180	36 (22)	N.S.
36h	0.073±0.005	0.088	0 (22)	0.072±0.011	0 (22)	N.S.

[a] post-GWSS egg consumption intervals (hour).
[b] Mean + 3SD of the negative controls (Sutula et al. 1986).
[c] Based on the critical value of the negative control predators. N=total no. of individuals assayed for each treatment.
[d] Significant differences (*t* test) between negative control predators and their counterparts fed GWSS eggs: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; N.S., not significant.

ELISA Response to Multicolored Asian Lady Beetle that Consumed GWSS Eggs

Adult beetles were placed in individual Petri dishes and starved for 36 h. Each adult was fed six GWSS eggs (within a 60-min time frame) and isolated from food for 0 or 6h and then frozen (-80ºC). Negative controls were individuals that did not eat any GWSS eggs. We analyzed the dissected gut of each individual by indirect ELISA for the presence of GWSS egg antigen. All negative controls yielded negative ELISA absorbance values. We found that 65% of the individuals that ate GWSS eggs scored positive at time=0 h, and 8% at time=6h. A significant difference between the mean absorbance values of the beetles fed GWSS eggs and their negative control counterparts only occurred for the time=0h treatment.

Predator Gut Content Analysis Using PCR Assays

We are currently optimizing a PCR assay to detect GWSS DNA in the guts of various species of predators. Several pairs of primers were designed to amplify GWSS-specific fragments from: (1) randomly amplified polymorphic DNA (RAPD) based on sequence characterized amplified regions (SCAR); and (2) the mitochondrial cytochrome oxydase subunit I (COI) and subunit II (COII) genes (de León & Jones 2004). The size of amplified fragments of GWSS DNA varies from 166 to 302 bp. Adult *H. axyridis* fed six GWSS eggs were immediately frozen (-80ºC) after eating. Negative controls were beetles that did not eat any GWSS eggs. Each individual was homogenized in a lysis buffer solution, DNA was extracted using a DNeasy kit (Qiagen Inc., Valencia CA) and subjected to PCR using GWSS-specific COII primers. GWSS DNA was successfully amplified from *H. axyridis* extracts (Figure 1). Further tests are underway comparing the efficacy of different primer sets and determining the half-life detection interval of GWSS DNA in the guts of several predator species (*C. carnea*, *Z. renardii*, *S. diadema*, and several species of spiders).

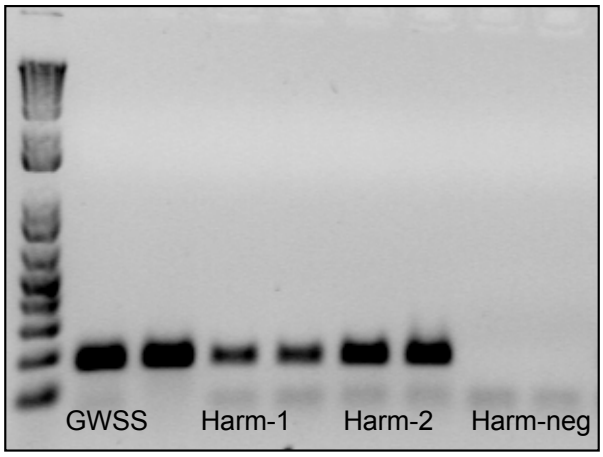


Figure 1. PCR assays were performed using GWSS-specific COII primers on *Harmonia axyridis*. This 2% agarose gel shows that GWSS DNA fragment (178bp) was amplified from the following samples (duplicates): positive control (GWSS), predators fed six GWSS eggs (Harm-1, Harm-2). No amplification occurred for the *H. axyridis* individual that did not consumed any GWSS eggs (Harm-neg).

CONCLUSIONS

We showed that molecular gut content assays can be used to detect GWSS remains in the guts of predators. Once optimization tests are complete we will assay extensive numbers of field-collected predators. We will be able to distinguish specimens that preyed upon immature and adult life stages of the GWSS via the PCR assay and those that consumed eggs via the ELISA assay. An understanding of the key natural enemies of GWSS will contribute to an areawide IPM approach for GWSS control. Once key predators are identified they can be better exploited for conservation and augmentative biological control programs.

REFERENCES

de León, J. H., & W. A. Jones. 2004. Detection of DNA Polymorphisms in *Homalodisca coagulata* (Homoptera: Cicadellidae) by Polymerase Chain Reaction-based DNA Fingerprinting Methods. Ann. Entomol. Soc. Am. 97: 574-585.

Hagler, J.R. 1998. Variation in the efficacy of several predator gut content immunoassays. Biol. Cont. 12: 25-32.

Hagler, J.R., K. Daane, & H. Costa. 2002. Progress on the development of a monoclonal antibody specific to glassy-winged sharpshooter egg protein: A tool for predator gut analysis and early detection of pest infestation. Pierce's Disease Symposia Proceedings, CDFA Sacramento. pp. 79-80.

Hagler, J.R. & S.E. Naranjo. 1994a. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. Entomol. Exp. et Applicata. 72: 59-66.

Hagler, J.R. & S.E. Naranjo. 1994b. Qualitative survey of two Coleopteran predators of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) using a multiple prey gut content ELISA. Biol. Cont. 23:193-197.

Sutula, C.L., J.M. Gillett, S.M. Morrissey, & D.C. Ramsdell. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70: 722-726.

Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Mole. Ecol. 11: 627-641.

FUNDING AGENCIES

Funding for the project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, the University of California's Pierce's Disease Grant Program, and the USDA Agricultural Research Service.

ULTRASTRUCTURAL CONTRIBUTIONS TO THE STUDY OF THE GLASSY-WINGED SHARPSHOOTER AND PIERCE'S DISEASE

Project Leader:
Thomas P. Freeman
Electron Microscopy Laboratory
North Dakota State University
Fargo, ND 58105

Cooperators:
Roger A. Leopold, Dennis R. Nelson, James S. Buckner
USDA, ARS, Biosciences Research Laboratory
Fargo, ND 58105

Thomas J. Henneberry
Western Cotton Research Laboratory
Phoenix, AZ 85040

Reporting Period: The results reported here are from work conducted from October 2003 to October 2004.

ABSTRACT
A variety of microscopic techniques including light microscopy, confocal scanning light microscopy, transmission electron microscopy, and scanning electron microscopy are helping to elucidate the structure and function of the mouthparts and the salivary sheath of the glassy-winged sharpshooter, a vector of Pierce's disease.

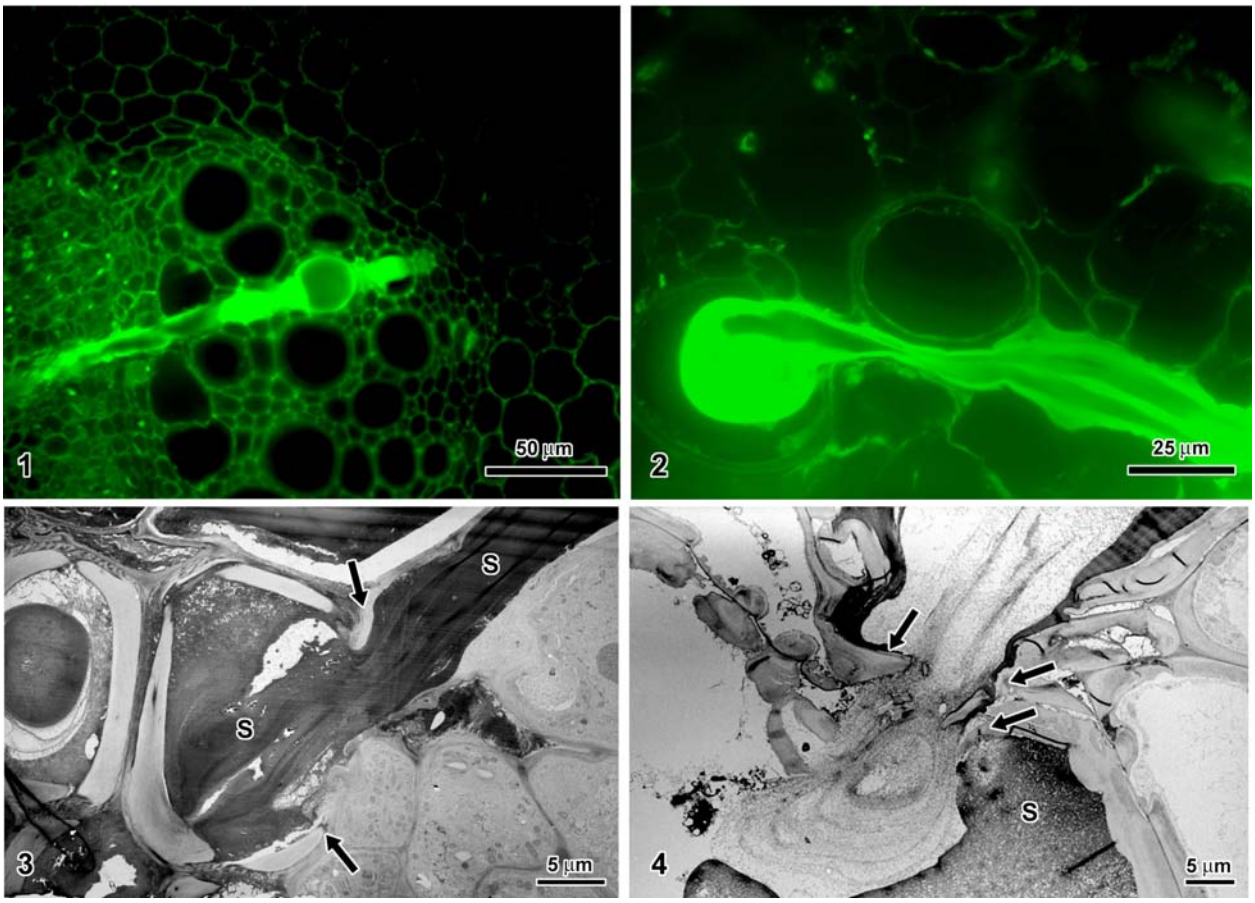
OBJECTIVES
1. Describe the morphology and ultrastructure of the glassy-winged sharpshooter mouthparts.
2. Describe stylet penetration and the function of each stylet pair during feeding.
3. Ascertain the path of mouthparts from the epidermal layer to the vascular tissue of the host plant, and to ascertain if the sharpshooter has fed in parenchymatous or phloem tissue en route to xylem tissue.
4. Determine the ultrastructure of the salivary sheath and its association with all plant tissues encountered from the epidermal layer to the xylem tissue.

RESULTS AND CONCLUSIONS
The glassy-winged sharpshooter (GWSS) has a significant economic impact as the vector for the transmission of *Xylella fastidiosa*, which causes Pierce's disease in grapes, leaf scorch in oleander and almonds, and variegated chlorosis in citrus. Different strains of the bacterium also cause diseases of avocados, peaches, plums, apricot, cherries, and many other trees and ornamentals (Purcell and Saunders 1999, Purcell et al. 1999). The GWSS feeds primarily on the xylem fluid of more than 100 different host plants from more than 35 plant families.

In response to the tremendous economic importance of this insect, a variety of research avenues are under investigation to develop control or management strategies. One important research area that has not received adequate attention is the interaction between the GWSS and the host plants. Until very recently we knew very little regarding the structure of the GWSS mouthparts, and simply assumed that they were similar to those of other leafhoppers. During the last two years, we have provided extensive ultrastructural descriptions of the GWSS mouthparts, including several new sensory structures associated with the sharpshooter stylets and labium (Leopold et al. 2003, Freeman et al. 2002, 2003).

Many unbranched salivary sheaths and branches of very complex sheaths, formed by nymph and adult sharpshooters, do not always extend directly from the host-plant epidermis to the xylem tissue. GWSS stylets may penetrate only as far as the vessel element wall or they may actually fragment the lignified wall and enter the cell lumen (Figures 1-4). Several vessel elements in a vascular bundle or secondary xylem may be damaged during a single sharpshooter probe (Figure 1). Fragmented vessel elements (Figures 2-4) would change the dynamics of water translocation. Penetrated vessel elements are only infrequently surrounded by salivary sheath material, which raises questions as to the function of the sheath in reducing or preventing cavitation. Penetrated vessel elements can, however, become partially or completely occluded with GWSS salivary sheath material (Figures 1-3), a situation that would also disrupt water translocation even in the absence of *X. fastidiosa*.

The glassy-winged sharpshooter ingests large volumes of xylem fluid during feeding, most of which is quickly excreted. We have noted that both nymph and adult sharpshooters produce exudates during probes that do not reach the xylem, suggesting that they may be feeding in host cells located between the epidermal layer and the xylem. The transfer of *Xylella* to parenchyma cells outside of the xylem (Backus et al. 2003) might be another indicator that sharpshooters are feeding in non-xylem tissues. With a high assimilation efficiency of carbon (Brodbeck et al. 1993, 1995, 1996), there may be a nutritive advantage for even limited feeding in parenchymatous tissues. We now have preliminary data showing that first, second, and third-instar nymphs successfully feed on sunflower stems where the xylem is located too distant from the epidermis to be reached by the length of their stylets. We note that less than 50% of first and second instars have salivary sheaths terminating in the xylem even when the xylem is within the reach of their stylets. Third and fourth instars are only slightly more successful.



Figures 1, 2. Confocal scanning light micrographs. Figure 1. Several vessel elements damaged by a single GWSS stylet probe.

Figure 2. Salivary sheath material occluding a fragmented vessel element

Figures 3, 4. Transmission electron micrographs showing fragmented vessel element walls (arrows) and salivary sheath occlusions (s).

In our greenhouse and laboratory studies, host plants fed on by sharpshooters for several days to weeks begin to show symptoms similar to those of plants infected with the bacterium *X. fastidiosa.* These symptoms occur in our host plants even though the sharpshooters we are studying are free of *Xylella.* Previous reports indicated that the symptoms of Pierce's disease may occur very shortly after inoculation with *X. fastidiosa*, long before there is a significant increase in the population of the bacteria to a level believed necessary to produce symptoms (Labavitch *et al.* 2002). Many plant species infected by strains of *X. fastidiosa* show no symptoms of Pierce's disease (Purcell and Saunders 1999). Our research is ongoing to determine the correlation of mechanical damage and occlusion of vessel elements to the onset of symptoms in non-infected host plants

REFERENCES

Backus, E. A., F. Yan, J. Habibi, W. Bennett, M. Blua, A. Purcell, and E. Civerolo. 2003. Sharpshooter feeding behavior in relation to transmission of the Pierce's disease bacterium. Pierce's Disease Research Symposium. Dec. 8-11. Coronado, CA.

Brodbeck, B.V., R.F. Mizell III, and P.C. Andersen. 1993. Physiological and behavioral adaptations of three species of leafhoppers in response to dilute nutrient content of xylem fluid. J. Insect Physiol. 39:73-81.

Brodbeck, B.V., P.C. Andersen, and R.F. Mizell III. 1995. Differential utilization of nutrients during development by the xylophagus leafhopper, *Homalodisca coagulata*. Entomol. Expt. et Applicata 75:279-289.

Brodbeck, B.V., R.F. Mizell III, and P.C. Andersen. 1996. Utilization of primary nutrients by the polyphagus xylophage, *Homalodisca coagulata*, reared on single host species. Arch. Insect. Biochem. Physiol. 32:65-83.

Freeman, T. P., R. A. Leopold, J. S. Buckner, and D. R. Nelson. 2002. Ultrastructural contributions to the study of the glassy-winged sharpshooter and Pierce's disease. Proceedings of the Pierce's Disease Research Symposium, Dec. 15-18. Coronado, CA, pp 116-118.

Freeman, T. P., R. A. Leopold, D. R. Nelson, and J. S. Buckner. 2003. Ultrastructural contributions to the study of the glassy-winged sharpshooter and Pierce's disease. Proceedings of the Pierce's Disease Research Symposium, Dec. 8-11. Coronado, CA, pp 215-216.

Leopold, R. A., T. P. Freeman, J. S. Buckner, and D. R. Nelson. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera; Cicadellidae). Arthropod Struct. & Develop. 32:189-199.

Labavitch, J. M., M. A. Matthews, L. C. Greve. 2002. The development of Pierce's disease in xylem: The role of vessel cavitation, cell wall metabolism, and vessel occlusion. Proceedings of the Pierce's Disease Research Symposium, Dec. 15-18. Coronado, CA

Purcell, A.H., and S. R. Saunders. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant-dis. 83:825-830.

Purcell, A.H., S.R. Saunders, M. Henderson, M.E. Grebus, and M.J. Henry. 1999. Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. Phytopath. 89:53-58.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

EPIDEMIOLOGY OF PIERCE'S DISEASE IN THE CENTRAL SAN JOAQUIN VALLEY OF CALIFORNIA: FACTORS AFFECTING PATHOGEN DISTRIBUTION AND MOVEMENT

Project Leaders:

Russell Groves
USDA, ARS, SJVASC
Parlier, CA 93648

Jianchi Chen
USDA, ARS, SJVASC
Parlier, CA 93648

Cooperators:

Marshall Johnson
Dept. of Entomology
University of California, Riverside
Kearney Agricultural Center
Parlier, CA 93648

Kent Daane
Division of Insect Biology
Dept. of Environmental Science Policy and Management
University of California
Berkeley, CA 94720

Dennis Haines
Tulare County Agric. Commissioner
Tulare, CA 93274

Dan Bigham
Tulare County Agric. Commissioner
Tulare, CA 93274

Reporting Period: The results reported here are from work conducted November 2003 to September 2004.

ABSTRACT

The primary objective of this research was to characterize the seasonal abundance, dispersal, and overwintering biology of the glassy-winged sharpshooter (GWSS), a primary vector of *Xylella fastidiosa (Xf*). Moreover, to identify where the vector(s) acquire the pathogen, to determine when vectors move into vineyards and transmit the pathogen to grapes, and to genetically characterize the populations of *Xf* isolated from GWSS collected in different perennial cultivated and non-cultivated plant species. Based on results of seasonal plant utilization by GWSS in our study through the winter of 2003-04 and into the subsequent growing season, we conclude that host plant species can significantly influence GWSS population biology. GWSS adult, nymph, and egg mass densities varied among perennial, cultivated crop plant species and non-cultivated weed species examined in this study. Perennial crop species examined included sweet cherry, navel, lemon, olive, avocado, peach, plum, pomegranate, pistachio, and grape. Adult GWSS dispersed into and fed upon a wide range of these crop species with the largest dispersing populations observed in citrus (lemon and navel) and pomegranate, similar to our findings in 2003. Adult GWSS were also regularly collected from and observed feeding upon a wide range of non-crop weed species within and surrounding experimental orchard crops. Nymph populations were not equally represented across all perennial tree crops with increased populations collected from citrus, pomegranate, and also non-crop annual weed species. Overwintering adult GWSS were consistently collected in relatively low population densities on citrus, pomegranate, avocado, plum, peach, and non-crop annual weed species. Patterns of adult GWSS capture among the distances sampled along linear transects extending into perennial crops were dissimilar among perennial crops. The presence of *Xf* in a subsample of vectors collected from different perennial crops and on non-crop species is underway using a multiplex PCR protocol to differentiate genomic populations.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, was introduced into Southern California in the late 1980's and later identified in 1994 (Blua et al. 1999). The insect regularly occurs in most of Southern California and has become established along eastern portions of the San Joaquin Valley of central California. Large populations of the GWSS are becoming widely distributed and will reportedly feed and oviposit on a wide range of perennial crop and ornamental plant species as well as numerous non-crop wild plant species (Adlerz and Hopkins 1979, Daane and Johnson 2003). This sharpshooter has continued to expand its range in the state and is expected to affect the overall increase in plant diseases caused by *Xylella fastidiosa* (*Xf*) (Purcell and Saunders 1999a). Strains of *Xf* have a complex pathogenic relationship with a diverse host range including members of both monocots and dicots (Chen et al. 2000). Analyses of the genetic diversity of *Xf* have begun to elucidate differences between many of the strains (Chen et al 1995, Hendson et al. 2001, Pooler and Hartung 1995). Knowledge of the genetic diversity of strains that comprise the population of *Xf* in the central San Joaquin Valley (SJV) of CA, especially as it relates to insect vectors, will help in devising effective strategies for managing Pierce's disease (PD), as well as other diseases caused by this bacterium.

Xylella fastidiosa is transmitted by xylem feeding sharpshooters (Cicadellidae) and spittlebugs (Cercopidae) (Hill and Purcell 1997, Purcell and Frazier 1985). In California, there are at least 20 species capable of transmitting the pathogen, although only four species are considered to be epidemiologically important in grapes (Pearson and Goheen 1988). Based on the population dynamics of native sharpshooter species in coastal California vineyards, much of the spread of *Xf*, especially early in the season when it is most damaging to grapevines, are by adults that move into the vineyard from outside host sources (Purcell and Saunders 1999b). Knowledge of which vector species transmit *Xf* in the central SJV, where they acquire the

pathogen, when they move into vineyards, and when they spread the pathogen to grapes is critical to understanding and managing the spread of PD in this area.

OBJECTIVES

1. To identify and characterize the seasonal abundance of the primary vectors of *Xf* and seasonal patterns of insect dispersal.
2. Compare the genetic structure of *Xf* strains isolated from GWSS collected from perennial, cultivated and non-cultivated plant species.

RESULTS

Objective 1

Examination of the seasonal host utilization patterns and dispersal biology of the glassy-winged sharpshooter, *Homalodisca coagulata* (GWSS) within and among a variety of perennial crop plant species has been monitored through the winter (2003-04) and following spring and summer seasons of 2004. Experimental sites are located in GWSS-infested areas of Tulare County, California. The results of these studies continue to provide valuable insight into the relative importance of different crop types as predominant overwintering habitats, ovipositional substrates, and preferred feeding hosts for GWSS. Patterns of crop utilization were monitored within perennial crop species including grape, citrus (navel and lemon), stonefruit (sweet cherry, peach, and plum), olive, and avocado at each of three locations for each crop type. Additionally, non-crop weed vegetation was monitored throughout the season at three experimental sites along with riparian vegetation. Host utilization was assessed monthly at each of three locations for each crop type based on sweep/beat-net sampling for adult and immature GWSS and visual inspections for GWSS egg masses. Results from our second year again indicate that host plant species influences GWSS population biology. Similar to our findings in 2003, the largest mean number of adult GWSS were collected from citrus (navel and lemon) and pomegranate whereas mean nymphal population densities were lower than the previous season. More nymphs were present in navel orange and pomegranate with fewer nymphs collected in olive, avocado, cherry, plum, and peach. Non-crop plant species upon which adult and nymphal GWSS were collected included red-root pigweed, prickly lettuce, annual sowthistle, little mallow, lambsquarters, field bindweed, blue morning glory, curly dock, evening primrose, johnsongrass, and ground cherry. The greatest mean number of GWSS egg masses were collected from both citrus and pomegranate.

Seasonal dispersal of adult GWSS was again monitored within and among the previously indicated perennial crop plant species Traps were suspended 2 m above the ground between tree canopies along 4 linear transects at each of 3 experimental locations for each crop sampled. Beginning November 2003, a total of 11,677 adult GWSS, 29 green sharpshooters (GSS, *Draeculacephala minerva*), and 351 spittlebugs (Cercopidae) were captured on yellow sticky cards. Temporal patterns of GWSS capture were similar in citrus and pomegranate throughout the 2004 sampling season representing dispersal of both overwintered and 1st generation adult GWSS. Seasonal patterns of GWSS capture in olive, avocado, and plum was dissimilar to that of either citrus or pomegranate similar to the patterns observed in 2003. Beginning November 2003, we have begun to closely monitor the overwintering host utilization patterns of adult GWSS among the variety of perennial crop and non-crop weed species previously listed. Overwintering adult GWSS have been sampled monthly (Nov – Feb, 2003) in perennial tree crops by beating/shaking all scaffolds over two, 80 ft^2 white, PVC tarps that flank both sides of the tree stem and in non-crop weed species using sweep net collections described previously. Adult GWSS have been collected overwintering on citrus (lemon and navel), pomegranate, peach, plum, and avocado averaging 0.2, 0.4, 0.9, 0.02, 0.05, and 0.5 adult GWSS/tree, respectively, over the four month sample interval. Mean populations of adult GWSS swept from non-crop annual vegetation have averaged 1.1, 2.4, 0.9, and 0.3 adult GWSS/50-sweep sample over the four month sample interval, respectively. To examine the seasonal population biology of GWSS utilizing non-crop host species, GWSS, native sharpshooters, and all spittlebugs have been sampled monthly from the ground cover and surrounding vegetation at each of the 3 experimental locations with high populations of GWSS present in 2003. At each location, sharpshooter and spittlebug adults and nymphs associated with the ground cover and surrounding non-crop vegetation are sampled using a standard sweep net (100 sweeps at each of 10 sites per location for ground cover).

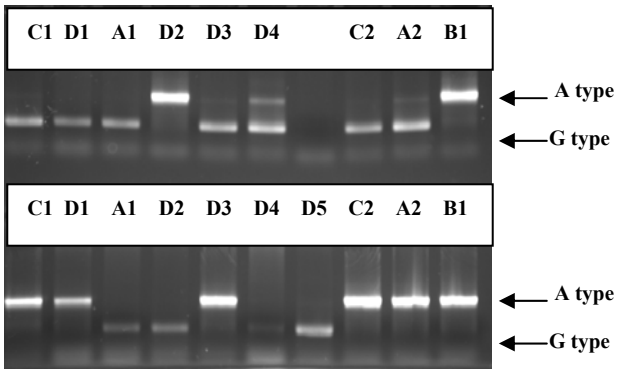


Figure 1. Polymerase chain reaction products from 18 *Xf* isolations collected in 2003 illustrating 2 genotypes (A = almond; G = grape).

Objective 2

The presence of *Xf* in a subsample of vectors captured among the different perennial crops and on non-crop species has begun using PCR. Genomic DNA is first isolated and initially screened against RST 31/33 universal primers to detect all *Xf* strains. The diversity of the chosen *Xf* isolates will be assessed using RAPD-based protocols and single nucleotide polymorphisms (SNPs) from genome loci of taxonomic importance deduced from the available genome sequences. Previous studies have demonstrated that these

protocols generate sufficient polymorphisms within *Xf* to enable grouping of strains according to host associations. SNP analyses represent one of the most recent technologies used for comparative studies of closely related bacteria. Based on published genomic information, strain specific primers recently will be used to investigate the pathotype profile using the 16S rDNA intergenic region. Results from our current season's research indicate that this multiplex PCR protocol can differentiate genomic populations which might co-exist in infectious vectors (Fig. 1). Here again, attempts will also be made to quantify *Xf* in selected insect vectors to identify the population dynamics of *Xf* within a vector population.

CONCLUSIONS

The results obtained from the second year of this project remains consistent with our first year observations and has generated significant new information regarding the seasonal host utilization patterns, dispersal, and overwintering biology of GWSS in the central SJV of California. This information will improve our understanding of the epidemiology of Pierce's disease which will also be useful in understanding the epidemiology of other economically important diseases caused by *Xf* for which GWSS may become an important vector. This objective directly addresses gaps in our present understanding that must be filled in order to develop comprehensive PD and GWSS management strategies. This research has expanded on previous work by documenting important aspects of the population biology of GWSS in the agricultural landscape of the central San Joaquin Valley of California. An improved knowledge of the genetic diversity of strains that comprise the population of *Xf* detected from potentially infectious GWSS will further help in devising effective strategies for managing Pierce's Disease, as well as other important diseases caused by this bacterium.

REFERENCES

Adlerz, W.C., and Hopkins, D.L. 1979. Natural infectivity of two sharpshooter vectors of Pierce's disease in Florida. J. Econ. Entomol. 72: 916-919.

Blua, M. J., Phillips, P. A., and Redak, R. A. 1999. A new sharpshooter threatens both crops and ornamentals. Calif. Agric. 53:22-25.

Chen, J., Lamikanra, O., Chang, C.J., and Hopkins, D.L. 1995. Randomly amplified Polymorphic DNA analysis of *Xylella fastidiosa* Pierce's disease and oak leaf scorch pathotypes. Appl. Environ. Microbiol. 61: 1688-1690.

Chen, J., Banks, D., Jarret, R.L., Chang, C.J., and Smith, B.J. 2000. Use of 16S rDNA sequences as signature characters to identify *Xylella fastidiosa.* Curr. Microbiol. 40: 29-33.

Daane, K. M. and Johnson, M. W. 2003. Biology and ecology of the glassy-winged sharpshooter in the San Joaquin Valley, pp. 247-249. *In* Proceedings, Pierce's Disease Research Symposium, M. Athar Tariq, S. Oswalt, P. Blincoe, R. Spencer, L. Houser, A. Ba, and T. Esser (eds.), California Dept. of Food and Agric., 8-11 Dec 2003, San Diego, CA.

Hendson, M., Purcell, A.H., Chen, D., Smart, C., Guilhabert, M., and Kirkpatrick, B. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. Appl. Environ. Microbiol. 67:895-903.

Hill, B.L., and Purcell, A.H. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopath. 87: 1197-1201.

Pearson, R.C., and Goheen, A.C., eds. 1988. Compendium of Grape Diseases. American Phytopathological Society, St. Paul, MN. 93 pp.

Pooler, M.R., and Hartung, J.S. 1995. Genetic relationships among strains of *Xylella fastidiosa* from RAPD-PCR data. Curr. Microbiol. 31:134-137.

Purcell, A.H. and Frazier, N.W. 1985. Habitats and dispersal of the principal leafhopper vectors of Pierce's disease in the San Joaquin Valley. Hilgardia. 53:1-32.

Purcell, A.H. and Saunders, S.R. 1999a. Glassy-winged sharpshooters expected to increase plant disease. Calif. Agric. 53:26-27.

Purcell, A.H. and Saunders, S.R. 1999b. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant Dis. 83:825-830.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

A NOVEL IMMUNOLOGICAL APPROACH FOR QUANTIFYING PREDATION RATES ON GLASSY-WINGED SHARPSHOOTER

Project Leaders:
James Hagler and Thomas Henneberry
USDA, ARS, Western Cotton Research Lab
Phoenix, AZ 85040

Kent Daane and Valerie Fournier
University of California
Berkeley, CA 94720

Russell Groves
USDA, ARS
Parlier, CA 93648

Cooperators:
Nilima Prabhaker, Heather Costa, and Mark Hoddle
University of California
Riverside, CA 92521

Reporting Period: The results reported here are from work conducted from August 15, 2004 to October 12, 2004.

ABSTRACT
A glassy-winged sharpshooter (GWSS) protein marking system is being developed for use as a diagnostic tool for predator gut content analysis. We determined that GWSS can be marked with 100% efficiency for at least 7 days after feeding on protein-marked plant material or spraying with a topical protein solution. Moreover, feeding trials have shown that protein marked insects can be detected by a protein-specific ELISA in the guts of predators that consumed them. Field studies are being initiated that will quantify the predation rates of an assemblage of predators on GWSS using a multitude of protein-specific ELISAs.

INTRODUCTION
Very little information exists on predaceous natural enemies of GWSS. While predaceous arthropods are important regulators of arthropod populations (Luff, 1983; Sabelis, 1992; Symondson et al., 2002); identifying the feeding choices and amount of prey consumed by generalist predators is very difficult. Predators and GWSS are small, elusive, cryptic (Hagler et al., 1991), and the predators may feed exclusively at night (Pfannenstiel & Yeargan, 2002). Hence, visual field observations of predation are extraordinarily difficult to obtain. Moreover, predators do not leave evidence of attack. Perhaps the most frequently used experimental approach for evaluating natural enemies in the field are through studies conducted in field cages (Luck et al., 1988). Such studies require manipulation of either the natural enemy or the targeted prey population(s) within the cage (e.g., the removal or introduction of the organism of interest). Mortality of the pest can be estimated based on the presence or absence of the pest (Smith & De Bach, 1942; Leigh & Gonzalez, 1976; Luck et al., 1988; Lang, 2003). Such studies have documented the qualitative impact of manipulated predator assemblages on many types of pests, but they do not provide quantitative information on predation rates or evidence of which predator in the assemblage is exerting the greatest biological control. Often the only direct evidence of arthropod predation can be found in the stomach contents of predators. Currently, the state-of-the-art predator stomach content assays include enzyme-linked immunosorbent assays (ELISA) for the detection of pest-specific proteins (Hagler, 1998) and PCR assays for the detection of pest-specific DNA (Agustí et al.; 1999; Symondson, 2002; Greenstone & Shufran, 2003).

ELISAs have been widely used to identify key predators of certain pests, including GWSS (Ragsdale et al., 1981; Sunderland et al., 1987, Hagler et al., 1992, 1993, 1994; Hagler & Naranjo, 1994ab; Bacher et al., 1999; Fournier et al., in prep). The simplicity and low cost of conducting an ELISA lends itself to the efficient screening of hundreds of field-collected predators per day. However, polyclonal antibody-based ELISAs often lack species specificity and monoclonal antibody-based ELISAs are too technically difficult, costly, and time consuming to develop for wide scale appeal (Greenstone, 1996). Moreover, pest-specific ELISAs share the same limitation as the other predator evaluation methods; the quantification of predation rates is impossible (see Hagler & Naranjo, 1996; Naranjo & Hagler, 1998 for reviews). PCR assays using pest-specific DNA probes might be less expensive to develop (Greenstone & Shufran, 2003), but PCR assays are also not quantifiable and they are more costly, technical, tedious, and time consuming to conduct than ELISAs (pers. obs.).

Due to the reasons discussed above, quantifying predation rates is extremely difficult. These difficulties have resulted in a dearth of information on the quantitative impact that generalist predators have on suppressing pest populations. The many shortcomings of each method of predator assessment described above were the impetus for us to develop a technique to quantify predator activity. The technique combines our previous research using pest-specific MAb-based ELISAs to detect predation (Hagler et al., 1991, 1993, 1994, 2003) with protein marking ELISAs we developed to study arthropod dispersal (Hagler & Miller, 2002; Hagler, 1997a, b; Hagler & Naranjo, 2004; Hagler & Jackson, 1998; Hagler et al., 2002). Here we describe a technique for marking individual GWSSs, each with a unique protein. In turn, the gut contents of each predator in the assemblage can be examined by a multitude of protein-specific ELISAs to determine how many GWSS were consumed and which predator species consumed them. The advantages of immunomarking prey over prey-specific ELISAs are: (1) prey-specific antibodies (or PCR probes) do not need to be developed, (2) the protein-specific sandwich ELISAs are more sensitive than the indirect prey-specific ELISAs (Hagler et al., 1997), (3) a wide variety of highly specific protein/antibody complexes are available, (4) the specificity of each antibody to its target protein facilitates the marking and examination of

the gut contents of every predator in the assemblage by a myriad of protein-specific ELISAs, and (5) all of the proteins and their complimentary antibodies are commercially available at an affordable price.

OBJECTIVES

We are in the preliminary phase of a research project dedicated to quantifying predation rates on GWSS nymphs and adults and qualifying predation on eggs. There are enough protein/antibody complexes commercially available that each GWSS in a field cage can be marked with a specific protein. We will mark individuals (e.g. adults and nymphs) and release them for 6 hours into a cage containing an assemblage of predators. The experiment will contain a day and night treatment. Observed mortality for each GWSS life stage will be determined by simply counting the number of GWSSs remaining in each cage. Each predator will then be examined by a multitude of protein-specific ELISAs to determine which predators ate GWSS nymphs and adults and how many each predator consumed. Then, each predator will be examined by a GWSS egg-specific ELISA to determine the frequency of predation on GWSS eggs (see Fournier et al. in this volume). Specifically, this study will: (1) quantify predation on GWSS nymphs and adults, (2) qualify predation on GWSS eggs, and (3) determine the circadian feeding activity of predators. Results obtained from this research will enhance our basic understanding of predator-prey interactions and aid in evaluating the efficacy of generalist predators for a conservation biological control program or an inundative biological control program.

RESULTS

We (JRH) conducted feasibility studies to determine if protein markers can be substituted for pest-specific MAbs for the immunological detection of prey in predator guts. In a series of lab studies, we fed a wide variety of predators (e.g., chewing and piercing/sucking type predators) both large and small prey marked with rabbit immunoglobulin G (IgG). In turn, the gut contents of each predator was analyzed by a rabbit IgG-specific ELISA. The results showed that, regardless of the predator species and the size of prey consumed, the rabbit IgG ELISA could easily detect the mark in the predator's stomach for at least 6 hours after feeding (Figure 1).

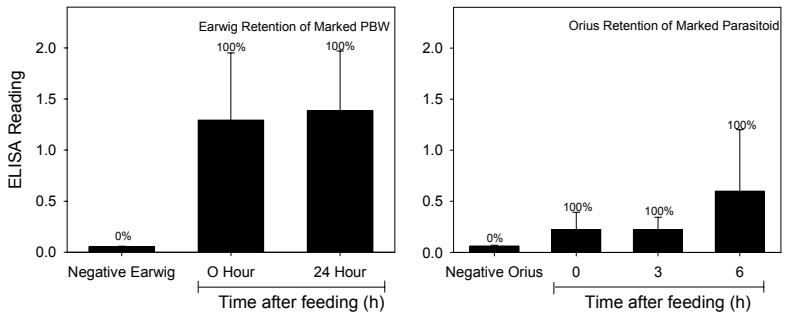


Figure 1. Mean (±SD) ELISA readings for the retention of rabbit IgG in the gut of two types of predators that consumed either a single 2nd instar pink bollworm larva or an adult parasitoid (*Eretmocerus emiratus*) marked with 5.0 mg/mL of rabbit IgG. The numbers above the error bars are the percentage of individuals positive for rabbit IgG. The negative predators consumed unmarked prey. Note: these data were chosen for display because they represent the extreme case scenarios (e.g., a large chewing predator eating a relatively large marked prey and a small piercing/sucking predator eating a very small marked prey). Similar studies are being conducted on GWSS.

The next study was designed to determine if we could mark adult GWSSs. In a pilot study, we marked (internally and externally) adult GWSS with rabbit IgG protein using the techniques described below.

Internal Marking

GWSSs were provided a chrysanthemum (mum) that was previously marked with a topical spray of a 5.0 mg/mL rabbit IgG solution. Individuals were allowed to feed on a protein-marked mum for 48 h. The GWSSs were removed from the protein-marked mum and placed on unmarked mums for 3, 5, or 7 days after marking and then analyzed for the presences of rabbit IgG by the anti-rabbit IgG ELISA described by Hagler (1997a). The efficacy of the marking procedure is given in Figure 2.

External Marking

We applied an external mark to individual GWSSs by spraying them with 1.0 ml of a 0.5 mg/mL rabbit IgG solution using a medical nebulizer (Hagler 1997b). The GWSS were air-dried for 1 h and then placed on mums for 3, 5, or 7 days after marking and then analyzed for the presence of rabbit IgG by ELISA. The efficacy of the marking procedure is given in Figure 2.

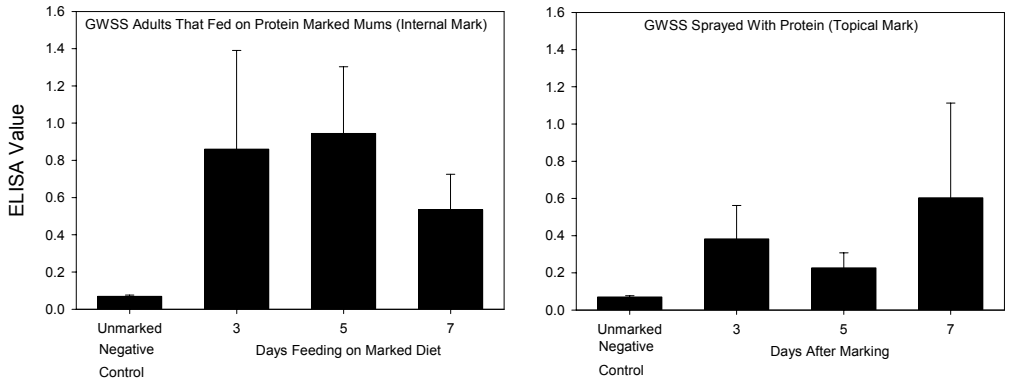


Figure 2. The efficacy of the internal (left graph) and external marking procedure (right graph) (n=8 to 16 per treatment). All of the GWSSs assayed 3, 5, and 7 days after marking yielded positive ELISA responses for the presence of rabbit IgG. All of the unmarked GWSSs yielded negative ELISA responses.

Results indicate that the protein marking procedure works for at least 7 days after marking GWSS. The next phase of our research (in progress) will be to mark individual GWSSs using the methods described above. Specifically, 10 individual GWSSs will be marked, each with a unique protein (see Table 1). The 10 GWSSs will then be placed in a field cage containing various predator species. The predator assemblage examined will represent those predators commonly found in areas inhabited by GWSS (JRH, pers. obs.). A partial list of the predator assemblage that will be examined and their probable feeding behaviors is given in Table 2. After 6 h in the cage, every remaining predator will be collected and analyzed by 10 different protein-specific ELISAs. A hypothetical example of the data we will generate over the next year is given in Table 3.

Table 1. A listing of the proteins that will be used to mark 10 individual GWSS.

Individual GWSS	Protein marker
1	Rabbit IgG
2	Guinea pig IgG
3	Equine IgG
4	Mouse IgG
5	Dog IgG
6	Pig IgG
7	Bovine IgG
8	Cat IgG
9	Rat IgG
10	Sheep IgG

Table 2. A listing of the arthropod assemblage to be examined.

Species	Stage[1]	Classification[2]	Likely GWSS prey[3]
H. convergens	Adult/immature	Carnivore	Egg
Zelus renardii	Adult/immature	Carnivore	Nymph/Adult
Geocoris punctipes	Adult	Omnivore	Egg/early instar nymph
Spiders Salticidae Clubionidae Agelenidae Araneidae	Adult/immature	Carnivore	Nymph/Adult
Earwig	Adult/immature	Omnivore	Egg, nymph, adult
Chrysoperla carnea	Immature	Carnivore	Egg
Preying mantis	Adult/immature	Carnivore	Nymph, adult
Syrphid fly	Immature	Carnivore	Egg
Coccinella septempunctata	Adult/immature	Carnivore	Egg

[1/]The predator life stage that will be examined.
[2/]The primary feeding habit of each species.
[3/]The most likely GWSS life stage that will be attacked.

Table 3. A hypothetical example of results yielded from a multitude of IgG-specific gut content ELISAs conducted on an individual predator (e.g., *Zelus renardii*). The number of positives yielded in all the assays indicates the number of prey consumed by this single predator.

Predator	Targeted GWSS	Protein marker designated in Table 1	Protein-Specific ELISA	ELISA result[1/]
Z. renardii	1	Rabbit IgG	Anti-Rabbit IgG	-
	2	Guinea pig IgG	Anti-Guinea pig IgG	-
	3	Equine IgG	Anti-Equine IgG	-
	4	Mouse IgG	Anti-Mouse IgG	-
	5	Dog IgG	Anti-Dog IgG	-
	6	Pig IgG	Anti-Pig IgG	+
	7	Bovine IgG	Anti-Bovine IgG	-
	8	Cat IgG	Anti-Cat IgG	+
	9	Rat IgG	Anti-Rat IgG	-
	10	Sheep IgG	Sheep IgG	-

[1/]This individual predator scored positive in the anti-pig and anti-cat ELISAs; therefore it consumed 2 marked GWSSs.

CONCLUSIONS

Although it is widely accepted that predators play a role in pest regulation, we still have an inadequate understanding of, and ability to predict their impact in cropping systems. Frequently parasitoids are given major credit for suppressing pest populations; however, the impact that predators have on suppressing GWSS populations goes unrealized due to the difficulties of assessing arthropod predation as discussed above. The prey marking technique described here circumvents many of the shortcomings of the current methods used to study predation. The preliminary studies described here prove that prey marking can be a powerful method for the immunological detection of predation and can be used to study various aspects of predator feeding behavior. Over the next 2 years we plan to quantify predation rates on GWSS. Ultimately, this information can be used to improve the efficacy of conservation and inundative biological control of GWSS. This research is designed to determine which predators are exerting the greatest biological control on GWSS eggs, nymphs and adults. This information can then be used to develop a comprehensive biological control program that better conserves the populations of those predators exerting the greatest control on the various GWSS life stages.

REFERENCES

Agustí, N., M.C. De Vicente & R. Gabarraa. 1999. Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: A new polymerase chain reaction-based technique for predator gut analysis. Mole. Ecol. 8: 1467-1474.

Bacher, S., K. Schenk & H. Imboden. 1999. A monoclonal antibody to the shield beetle *Cassida rubiginosa*: A tool for predator gut analysis. Biol. Cont. 16: 299-309.

Greenstone, M.H. 1996. Serological analysis of arthropod predation: Past, present and future. In: The Ecology of Agricultural Pests: Biochemical Approaches. W.O.C. Symondson, W.O.C. & J.E. Liddell, eds. Chapman & Hall, New York, NY. Pp. 265-300.

Greenstone, M.H. & K.A. Shufran. 2003. Spider predation: Species-specific identification of gut contents by polymerase chain reaction. J. Arachnology. 31: 131-134.

Hagler, J. R. 1997a. Field retention of a novel mark-release-recapture method. Environ. Entomol. 26:1079-1086.

Hagler, J. R. 1997b. Protein marking insects for mark-release-recapture studies. Trends Entomol. 1:105-115.

Hagler, J. R. 1998. Variation in the efficacy of several predator gut content immunoassays. Biol. Control 12:25-32.

Hagler, J. R. & C. G. Jackson. 1998. An immunomarking technique for labeling minute parasitoids. Environ. Entomol. 27:1010-1016.

Hagler, J.R. & E. Miller. 2002. An alternative to conventional insect marking procedures: Detection of a protein mark on pink bollworm by ELISA. Entomol. Exp. et Appl. 103: 1-9.

Hagler, J. R. & S. E. Naranjo. 1994a. Determining the frequency of Heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. Entomol. Exp. et Appl. 72:59-66.

Hagler, J. R. & S. E. Naranjo. 1994b. Qualitative survey of two Coleopteran predators of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) using a multiple prey gut content ELISA. Biol. Cont. 23:193-197.

Hagler, J. R. & S. E. Naranjo. 1996. Using gut content immunoassays to evaluate predaceous biological control agents: a case study. In: The Ecology of Agricultural Pests. W.O.C Symondson & J. E. Liddell, eds. Chapman & Hall, New York, NY. pp. 383-399

Hagler, J. R. & S. E. Naranjo. 1997. Measuring the sensitivity of an indirect predator gut content ELISA: Detectability of prey remains in relation to predator species, temperature, time, and meal size. Biol. Cont. 9:112-119.

Hagler, J.R. & S.E. Naranjo. 2004. A multiple ELISA system for simultaneously monitoring intercrop movement and feeding activity of mass-released predators. International Journal of Pest Management. 50: 199-207.
Hagler, J. R., A. C. Cohen, F. J. Enriquez & D. Bradley-Dunlop. 1991. An egg-specific monoclonal antibody to *Lygus hesperus*. Biol. Cont. 1:75-80.
Hagler, J. R., S. E. Naranjo, D. Bradley-Dunlop, F. J. Enriquez & T. J. Henneberry. 1994. A monoclonal antibody to pink bollworm (Lepidoptera: Gelechiidea) egg antigen: A tool for predator gut analysis. Ann. Entomol. Soc. Am. 87:85-90.
Hagler, J. R., A. G. Brower, Z. Tu, D. N. Byrne, D. Bradley-Dunlop & F. J. Enriquez. 1993. Development of a monoclonal antibody to detect predation of the sweetpotato whitefly, *Bemisia tabaci*. Entomol. Exp. et Appl. 68:231-236.
Hagler, J. R., A. C. Cohen, D. Bradley-Dunlop, and F. J. Enriquez. 1992. Field evaluation of predation on *Lygus hesperus* (Hemiptera: Miridae) using a species- and stage-specific monoclonal antibody. Environ. Entomol. 21:896-900.
Hagler, J.R., C.G. Jackson, T.J. Henneberry & J.R. Gould. 2002. Parasitoid mark-release-recapture techniques. II. Development and application of a protein marking technique for *Eretmocerus* spp., parasitoids of *Bemisia argentifolii*. Biocont. Sci. Tech. 12: 661-675.
Hagler, J.R., D. Daane, & H. Costa. 2003. A monoclonal antibody specific to glassy-winged sharpshooter egg protein. Proceeding, Pierce's Disease Research Symposium, 9-11 December 2003, San Diego, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, and Tom Esser, Sacramento, CA. pp. 188-191.
Lang, A. 2003. Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. Oecologia. 134: 144-153.
Leigh, T.F. & D. Gonzalez. 1976. Field cage evaluation of predators for control of *Lygus hesperus* Knight on cotton. Environ. Entomol. 5: 948-952.
Luff, M.L. 1983. The potential of predators for pest control. Agric. Ecosystems Environ. 10:159-181.
Luck, R.F., B.M. Shepard & P.E. Kenmore. 1988. Experimental methods for evaluating arthropod natural enemies. Ann. Rev. Entomol. 33: 367-391.
Pfannenstiel, R.S. & K.V. Yeargan. 2002. Identification and diel activity patterns of predators attacking *Helicoverpa zea* eggs in soybean and sweet corn. Environ. Entomol. 31: 232-241.
Ragsdale, D.W., A.D. Larson & L.D. Newsome. 1981. Quantitative assessment of the predators of *Nezara virdula* eggs and nymphs within a soybean agroecosystem using ELISA. Environ. Entomol. 10: 402-405.
Sabelis, M. 1992. Predatory arthropods. In: Natural Enemies: The Population Biology of Predators, Parasites, and Diseases. In: Crawley, M.J., ed. Blackwell Scientific Publishing. pp. 225-264.
Smith, H.S. & P. De Bach. 1942. The measurement of the effect of entomophagous insects on population densities of their hosts. J. Econ. Ent. 35: 845-849.
Sunderland, K.D., N.E. Crook, D.L. Stacey & B.J. Fuller. 1987. A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. J. Appl. Ecol. 24: 907-933.
Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Molecular Ecol. 11: 627-641.
Symondson, W.O.C., K.D. Sunderland & M. Greenstone. 2002. Can generalist predators be effective biological control agents? Ann. Rev. Entomol. 47: 561-594.

FUNDING AGENCIES
Funding for this project was provided by the University of California's Pierce's Disease Grant Program and the USDA-Agricultural Research Service.

IDENTIFICATION OF THE NATIVE PARASITOID FAUNA ASSOCIATED WITH *GRAPHOCEPHALA ATROPUNCTATA* AND HOST SPECIFICITY TESTING OF *GONATOCERUS ASHMEADI* ON *HOMALODISCA LITURATA*

Project Leader:
Mark S. Hoddle
Dept. of Entomology
University of California
Riverside, CA 92521

Cooperators:
Elizabeth A. Boyd
Dept. of Entomology
University of California
Riverside, CA 92521

Serguei Triapitsyn
Dept. of Entomology
University of California
Riverside, CA 92521

Reporting Period: The results reported here are from work conducted from May 2003 to October 2004

ABSTRACT
To determine the oviposition preference of female blue-green sharpshooters (BGSS), *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), a survey was conducted on southern California wild grape, *Vitis californica* Benth (Vitaceae) growing near Temecula, California in August 2003 where populations of BGSS were known to occur. Female BGSS oviposited into new growth, primarily the succulent tendrils and stems. The under sides of small leaves and petioles were also used for oviposition, but to a lesser extent. Mature stems, large and medium sized leaves and petioles were not utilized for oviposition. Two parasitoids, *Gonatocerus latipennis* Girault and a *Polynema* sp. (Hymenoptera: Mymaridae) were reared from BGSS eggs. Literature reviews revealed a deficiency of known natural enemies for *G. atropunctata*. A sentinel plant study was conducted to further confirm the parasitization of BGSS eggs by these parasitoids. Collectively the *Polynema* sp. and *Gonatocerus latipennis* constitute the first documented parasitic natural enemies of BGSS eggs. A further examination, commencing in January 2004, of the activity of BGSS and its parasitoids in southern California is currently underway. Blue-green sharpshooter adult activity reached its peak in July while bi-weekly samples of wild grape canes and tendrils revealed peak emergence of blue-green nymphs and parasitoids occurred from mid-July to mid-August. No-choice tests with *Gonatocerus ashmeadi* Girault, a parasitoid of the galssy-winged sharpshooter, *Homalodisca coagulata*, and BGSS eggs as part of a non-target impact assessment have yielded few results thus far. However, no-choice tests with *G. ashmeadi* and the native smoke-tree sharpshooter (STSS), *Homalodisca liturata* Ball, yielded no significant differences in percent parasitism of eggs when compared to the GWSS control.

INTRODUCTION
The native BGSS has been a threat to California grape growers for nearly a century due to its excellent transmission efficiency (Hill and Purcell 1995) of the bacterium that causes Pierce's Disease, a severe malady of commercially grown grapes. While much research has been devoted to epidemiologically related issues concerning this insect, little has been done to examine some of the most fundamental life history traits of this native pest, specifically oviposition preference (Severin 1949) and the native Californian parasitoids attacking the eggs of this pest. Further, we intend to investigate possible non-target effects of the exotic egg parasitoids that have been released to control another hemipteran pest, the GWSS, on BGSS and other native California sharpshooters and to identify the native parasitoid fauna associated with these native sharpshooter species. To address these issues, we need to know the oviposition preferences of native sharpshooters associated with particular host plants and their respective natural enemy fauna attacking oviposited eggs. The studies outlined below have determined the oviposition preferences of BGSS on wild grape, have documented its associated egg parasitoids, and provide data on host specificity of *G. ashmeadi,* a parasitoid being used as part of the classical biological control program against GWSS on the targets congener, the native STSS.

OBJECTIVES
1. Classify the native egg parasitoid fauna in California associated with sharpshooters native to California, primarily the smoke-tree sharpshooter (STSS): *Homalodisca liturata* Ball (Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Proconiini), blue-green sharpshooter (BGSS): *Graphocephala atropunctata* (Signoret), red-headed sharpshooter (RHSS): *Xyphon fulgida* (Nottingham), and green sharpshooter (GSS): *Draeculocephala minerva* Ball (the latter three, all Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Cicadellini).
2. Assess the possible non-target impacts of *Gonatocerus ashmeadi*, *G. trigutattus*, and *G. fasciatus*, parasitioids being used for the classical biological control of GWSS, on the above mentioned native sharpshooters.

RESULTS:

Oviposition Survey

Wild grape plant material collected on 5 August 2003 consisted of: 50 canes (terminal 25 cm of cane), 50 tendrils, 100 large, 100 medium, and 100 small leaves with petioles. The tendrils and small leaves with petioles were selected from the terminal 25 cm sections of the canes. Each of the 50 canes was cut into thirds: upper, middle and lower. No insects emerged from large or medium leaves and their petioles and are thus excluded from further discussion. A total of 49 insects (26 *G. atropunctata*, 18 *Polynema* sp. and five *G. latipennis* parasitoids, Figures. 1 and 2) emerged from plant material collected. The highest percentage of BGSS nymph emergence (18%) occurred in the apical-most portion of the stem, with less emerging from tendrils (14%), and middle (10%) and lower (2%) stems, respectively. A very small percentage of *G. atropunctata* nymphs emerged from small leaves and their petioles. For the parasitoids the highest percent emergence occurred from the tendrils (38%). Collectively, the tendrils and stems yielded the greatest emergence (Figure 3).

G. latipennis

Polynema sp.

Figures. 1 and 2. Parasitoids of the BGSS.

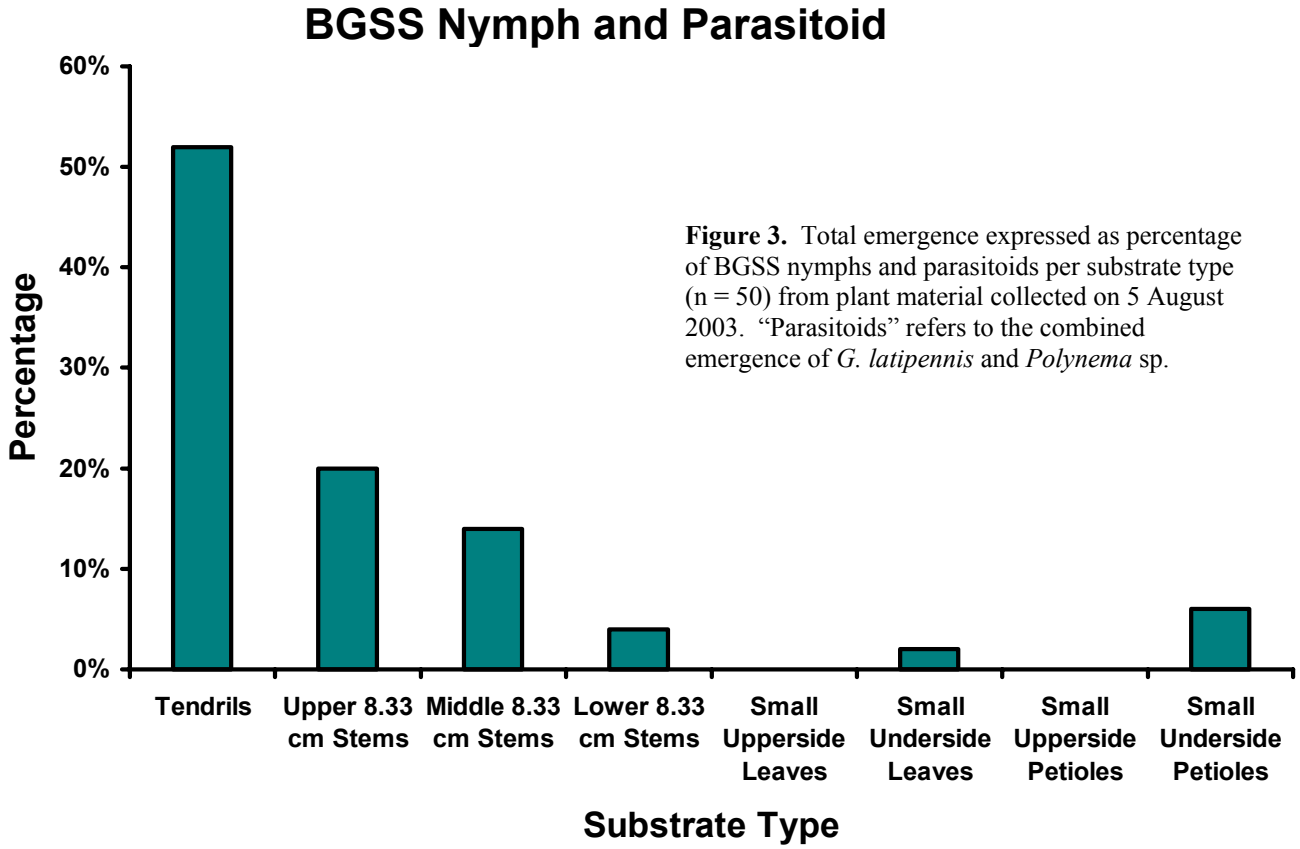


Figure 3. Total emergence expressed as percentage of BGSS nymphs and parasitoids per substrate type (n = 50) from plant material collected on 5 August 2003. "Parasitoids" refers to the combined emergence of *G. latipennis* and *Polynema* sp.

Ten entire grape canes were sampled on 14 August 2003 to account for any possible oviposition substrate not sampled in the previous survey. These canes were cut into thirds (apical, middle and basal), then placed into 10 cm of water in a Mason jar which left approximately 25 cm of cane exposed for emergence of nymphs and parasitoids. Canes and mason jars were then placed into three separate cages, according to their stem position. Cane sections were examined daily for emergence. In total, two BGSS nymphs and 16 *Polynema* sp. emerged from the canes. As there were so few insects emerged from these cane sections, the stems, leaves, petioles and tendrils were examined under the microscope for recent emergence holes from both BGSS nymph and parasitoids. A total of 65 emergence holes were counted. The majority of emergence holes were on the apical stems (n = 37) and on tendrils (n = 6, 13, 7, for apical, middle and basal portions, respectively) occurring along the length of the entire canes. Only two emergence holes were counted from leaf petioles and none were counted from middle and basal stems and leaves.

Sentinel Plant Study

To confirm the host association of the emerged parasitoids with the BGSS, three sweet-basil, a chrysanthemum and two wild grape plants were exposed to BGSS lab colonies for 3 days to allow for oviposition. Plants were removed from the colonies and transported to the oviposition survey site to allow for parasitization of BGSS eggs. After three days, the plants were brought back from field, cleaned of any insects and placed into separate cages. Plants were observed daily for any emerging insects. A combined total of 197 BGSS and *Polynema* sp. emerged from the five sentinel plants. Of these, 55 were BGSS nymphs and 142 were *Polynema* sp. (54 males, 88 females). Parasitism rates of BGSS eggs by *Polynema* sp. ranged from 33% on the mum to 78% and 86% on wild grape and basil, respectively.

BGSS and Parasitoid Activity

A total of 12 yellow sticky card traps (11 x 15 cm), were placed at the 2003 oviposition survey site to monitor BGSS adult and parasitoid flight activity. Traps were set up on 9 January 2004 and collected at bi-weekly intervals. Peak trap catch of BGSS adults occurred over the two week period of 11 June to 25 June 2004. Additionally, as soon as wild grape had sprouted and was available for collection, starting on 16 April 2004, twelve 30 cm cane sections were collected at the same bi-weekly sampling intervals. Tendrils were severed from the cane and placed into individual Petri dishes while stems were placed into dual 50 dram vials (25 cm of cane above water to allow for emergence). Plant material was checked daily for emergences of nymphs and parasitoids. Peak emergence of BGSS nymphs and parasitoids was spread over a four week period from 24 July to 20 August 2004. Data compilation is still in progress, however some of the results are shown below in Figure 4.

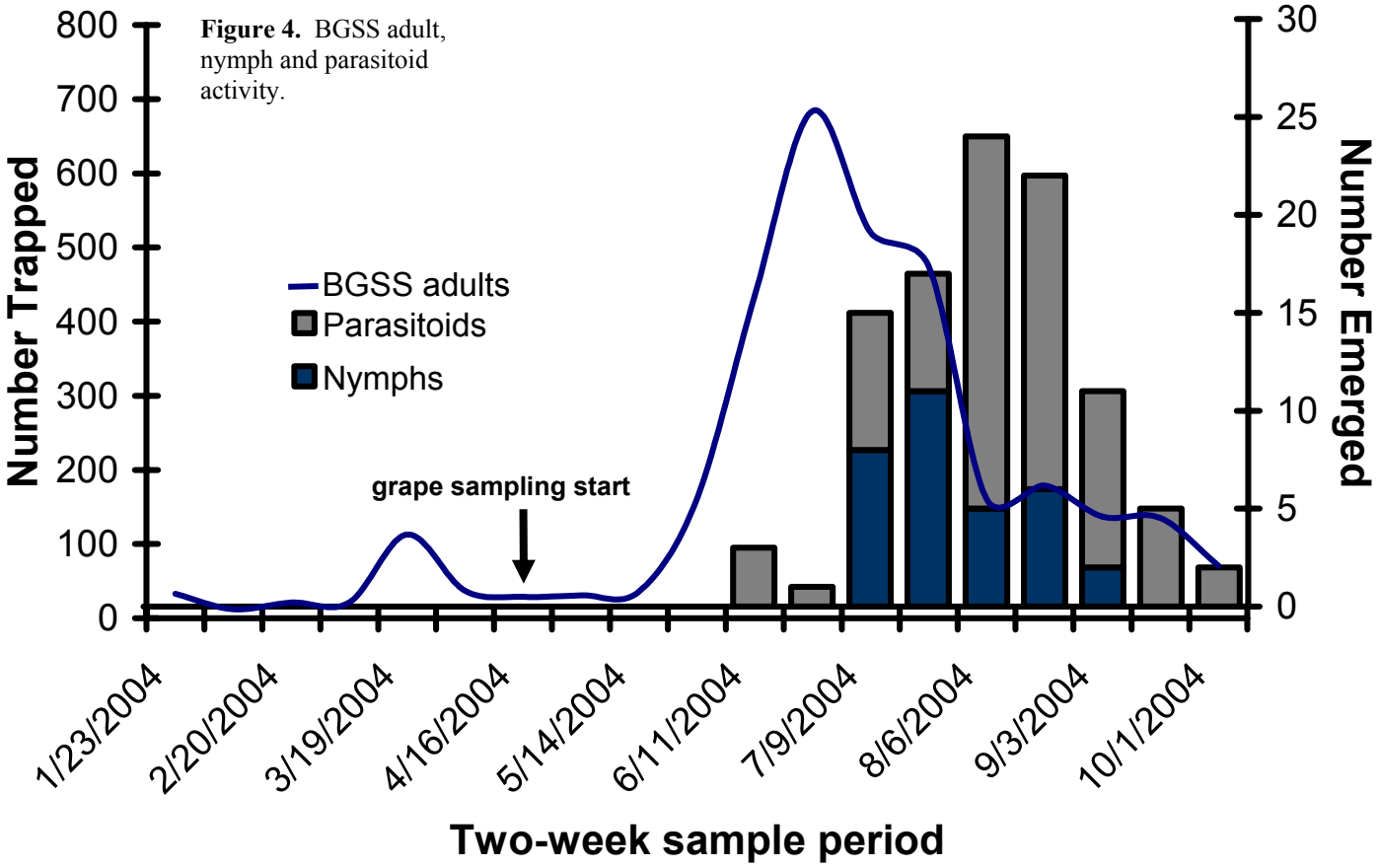


Figure 4. BGSS adult, nymph and parasitoid activity.

***Host specificity testing**:* No-choice tests were conducted with *G. ashmeadi* and STSS eggs. Single, one day old, mated, fed *G. ashmeadi* were exposed to STSS (n = 40 egg masses) and control (GWSS, n = 7 egg masses) eggs on chrysanthemum leaves in individual 100 x 15 mm Petri dishes. Each wasp was supplied one egg mass less than 48 hours of age and allowed 24 hr to parasitize the eggs before removal from the dish. The number of eggs per egg mass ranged from 2-14 ($\bar{x}$ = 5.65) for STSS and 2-19 ($\bar{x}$ = 5.89) for GWSS. Percent parasitism of egg masses ranged from 0-100% for both STSS ($\bar{x}$ = 84.58%) and GWSS ($\bar{x}$ = 71.43%) and was not found to be significantly different (Figure 5, Student's t-test, alpha = 0.05, P = 0.37702).

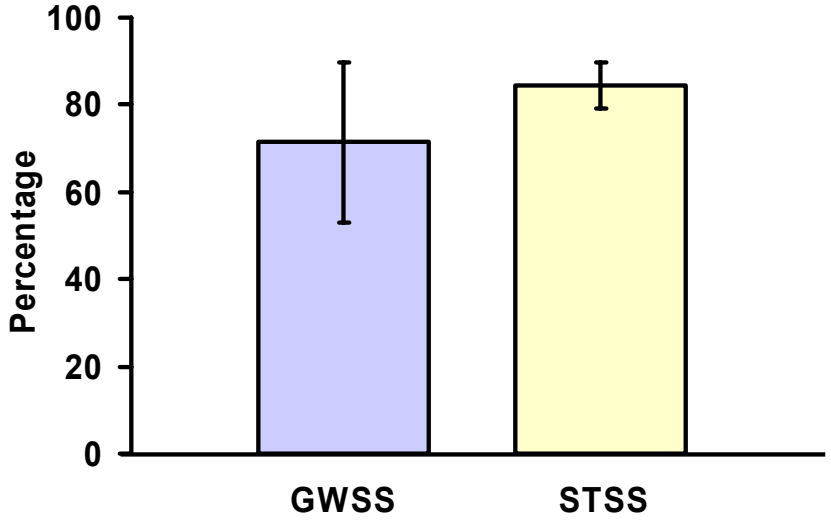


Figure 5: Percent parasitism of STSS and GWSS eggs by *G. ashmeadi* in Petri dish no-choice studies.

CONCLUSIONS

Clearly we now know BGSS oviposition preference on wild grape is for new growth, consisting primarily of the terminal 25 cm of succulent stems and tendrils that occur along the entire length of the grape cane. Additionally we have confirmed two new natural enemy host associations for the BGSS, *G. latipennis* and *Polynema* sp. While these studies were conducted on wild grape, the information acquired may have implications in developing a more complete IPM program involving this native pest species and its associated natural enemies. Overall, the new knowledge of BGSS oviposition preference provides essential information for conducting future non-target effect studies involving the exotic GWSS egg-parasitoids which we have started to investigate. Peak BGSS adult activity measured through trap catches occurred from mid-June to early August while peak emergence of nymphs and parasitoids was spread over a four week period from 24 July to 20 August 2004. Another peak of adult activity may be expected in October once the nymphs have matured into adults. No-choice tests with *G. ashmeadi* and the STSS yielded no significant differences in percent parasitism as compared with GWSS control. It is likely there will be non-target impacts by *G. ashmeadi* in STSS habitats where this parasitoid is able to successfully infiltrate and compete with other resident natural enemies such as *Ufens* and *Zagella* sp. (both Trichogrammatidae)

REFERENCES

Hill, B. L., Purcell, A. H. (1995). Acquisition and retention of *Xylella fastidiosa* by an efficient vector. Phytopath. 85: 209-212.

Severin, H.H.P. (1949) Life history of the blue green sharpshooter, *Neokolla circellata*. Hilgardia 19: 187-189.

FUNDING AGENCIES

Funding for this project was provided by the University of California Agriculture and Natural Resources.

IS THE GLASSY-WINGED SHARPSHOOTER PARASITOID *GONATOCERUS MORRILLI* ONE SPECIES OR A COMPLEX OF CLOSELY RELATED SIBLING SPECIES?

Project Leaders:
Mark Hoddle
Dept. of Entomology
University of California
Riverside, CA 92521

Richard Stouthamer
Dept. of Entomology
University of California
Riverside, CA 92521

Cooperator:
Serguei Triapitsyn
Dept. of Entomology
University of California
Riverside, CA 92521

Reporting period: The results reported here are from work conducted from July 2004 to October 2004.

INTRODUCTION

This is a new proposal that was officially funded in July 2004. This project objective is to determine the status of different *Gonatocerus morrilli* populations. We intend to use three approaches to determine the species identity of different *G. morrilli* populations: (1) Reassessment of key morphological features using scanning electron microscopy to determine if subtle morphological differences exist between *G. morrilli* populations which could possibly indicate species differences (Triapitsyn to conduct this work). (2) Conduct mating compatibility studies to determine if different populations of *G. morrilli* are reproductively isolated, or if mating occurs, whether offspring are viable thereby defining species groups on the basis of successful interbreeding (Hoddle). (3) To determine if molecular differences exist between *G. morrilli* populations collected from different regions by comparing mitochondrial and ribosomal DNA sequences. Molecular dissimilarities of key regions could potentially indicate the existence of different species (Stouthamer). Results from these three areas (morphology, behavior, and molecular) of investigation will be evaluated together to determine whether *G. morrilli* as it is currently viewed is a valid species or whether it is an aggregate of morphologically similar cryptic species.

A classical biological control program is currently underway for glassy-winged sharpshooter (GWSS), which is an exotic pest in California. The native range of GWSS is the southeastern United States and northeastern Mexico (Triapitsyn & Phillips, 2000). GWSS is thought to have invaded California around 1990 as egg masses that were accidentally imported on ornamental plants from Florida. Species of GWSS egg parasitoids not present in California are currently being prospected for in the native range of GWSS. Promising candidate natural enemy species that attack eggs are being imported and released in California for GWSS control (Triapitsyn et al., 1998; Triapitsyn & Hoddle, 2001). Interestingly, one species of egg parasitoid associated naturally with GWSS in California, *Gonatocerus morrilli* (Howard) (Hymenoptera: Mymaridae), is also widely distributed in the home range of GWSS, but at the time of its initial discovery in California, *G. morrilli* had not been intentionally released here and was thought to be native to California. A potential host for *G. morrilli* in California prior to the arrival of GWSS could have been the native *Homalodisca liturata* (Ball) which has had unidentified *Gonatocerus* spp. reared from its egg masses collected in the San Diego area (Powers, 1973). The presence of *G. morrilli* in Riverside in 1980-1984 has been documented (Huber 1988). *Gonatocerus morrilli* is now the second most important natural enemy of GWSS egg masses in California (Al-Wahaibi, 2004).

The success and failure of a number of biological control projects against insect pests and weeds has hinged on the correct taxonomic identification of the target and its natural enemies (Gordh and Beardsley, 1999). Incorrect understanding of the taxonomy and subsequent interrelationships between the target and its natural enemy guild are serious impediments to an efficacious biological control program. For example, *Trichogramma minutum* and *T. platneri* are important commercially available biological control agents that are morphologically indistinguishable but reproductively incompatible (Nagarkatti, 1975). Experimental work and subsequent modeling with these two species of *Trichogramma* has indicated that because pre-mating isolation mechanisms are absent (e.g., pre-mating courtship behaviors that prevent coupling of males and females from different species) severe negative effects on biological control can occur. Negative effects manifest themselves because females that mate with males from different species fail to produce female offspring. This occurs because *Trichogramma* like *Gonatocerus* are haploid-diploid parasitic Hymenoptera. In this haplo-diploid system, fertilized eggs produce female offspring and unfertilized eggs produce male offspring. In situations where incompatible interspecies matings are occurring both species fail to produce females and the potential population growth of both parasitoid species is reduced to levels below the growth rate expected for either species in the absence of the other (Stouthamer et al., 2000).

If different populations of morphologically similar *G. morrilli* from Florida, Louisiana, Texas, and Mexico are indeed valid species that lack pre-mating isolation mechanisms, then the current biological control program against GWSS in California that is attempting to establish these new agents may reduce the current level of control achieved by the precinctive populations of *G. morrilli* in California. This could occur because of male-biased offspring production resulting from incompatible matings across species. The rationale for introducing new strains or races of *G. morrilli* into California is based on the idea that different biotypes of this parasitoid may exist and fill niches not currently occupied by the strain of *G. morrilli* already present in California.

In this grant we propose to determine if geographically distinct populations of *G. morrilli* are part of one continuous interbreeding population or if populations of *G. morrilli* are separate species that can't be easily separated on the basis of

currently employed morphological characters. To do this we intend to combine three separate approaches to determine the species identity of different *G. morrilli* populations: First, we'll reassess key morphological features used to characterize *G. morrilli* with scanning electron microscopy to determine if subtle morphological differences exist between *G. morrilli* populations which could possibly indicate species differences. Such differences - should they exist - may not be easily observed with light microscopy. Second, we'll conduct mating compatibility studies to determine if different populations of *G. morrilli* are reproductively isolated, or if mating occurs, whether offspring are viable thereby defining species groups on the basis of successful interbreeding. Third, we'll determine if molecular differences exist between different *G. morrilli* populations by comparing mitochondrial and ribosomal DNA sequences. Molecular dissimilarities of key regions could potentially indicate the existence of different species, and at the same time allow their identification. Results from these three areas (morphology, behavior, and molecular avenues) of investigation will be evaluated together to determine whether *G. morrilli* as it is currently viewed is a valid species or whether it is an aggregate of morphologically indistinguishable cryptic species.

RESULTS

This project has not commenced. The reason for this is that the recruitment of the post-doc has taken some time. We expect the post-doc to be on-line in early December 2004. We will be formally requesting a no-cost extension for this project.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

SPATIAL POPULATION DYNAMICS AND OVERWINTERING BIOLOGY OF THE GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA'S SAN JOAQUIN VALLEY

Project Leaders:

Marshall W. Johnson
Dept. of Entomology
University of California, Riverside
Kearney Agricultural Center
Parlier, CA 93648

Kent M. Daane
Division of Insect Biology
Dept. of Environmental Science
Policy and Management
University of California
Berkeley, CA 94720

Elaine Backus
USDA, ARS, PWA, SJVASC
Parlier, CA 93648

Russell Groves
USDA, ARS, PWA, SJVASC
Parlier, CA 93648

Collaborators:

Youngsoo Son
Dept. of Entomology
University of California
Kearney Agricultural Center
Parlier, CA 93648

David Morgan
CDFA Mount Rubidoux Field Station
Riverside, CA 92501

Andrew Larson
Dept of Plant Science
California State University
Fresno, CA 93740-8033

Reporting Period: The results reported here are from work conducted from June 2004 through September 2004.

ABSTRACT

The purpose of this project is to define specific environmental constraints that influence glassy-winged sharpshooter (GWSS) population dynamics and overwintering success. We are beginning experiments to determine the temperature-dependent feeding biology of GWSS in temperature-controlled chambers. Experiments are underway in the recently established GWSS Experimental Laboratory on the campus of California State University, Fresno. Adult GWSS feeding and survival under different combinations of host plant type and temperature regimes will be monitored to determine the temperature thresholds for adult feeding activity. Complementary experiments measuring honeydew excretion rates have begun to determine the amounts of excreta collected upon exposed surface(s) of water-sensitive paper and will be compared among different temperature and exposure regimes. Electro-penetration feeding monitoring assays are underway at different temperatures on individually tethered and feeding GWSS adults. Time course examinations of waveforms reveal the frequency and duration of insect feeding behavior under varying environmental conditions. The seasonal population dynamics of GWSS will be monitored on selected host plants placed in different micro-climatic areas of the San Joaquin Valley. Results from these experiments will be coupled with climatological data to help to spatially define where GWSS can be expected to persist in the agricultural landscape and identify where continued management efforts should be directed to limit introductions into currently non-infested areas.

INTRODUCTION

The bacteria *Xylella fastidiosa* (*Xf*) causes economically important diseases of several agronomic, horticultural, and landscape ornamental crops (Pearson and Goheen 1988). The bacterium is transmitted by xylem feeding sharpshooters (Cicadellidae) and spittlebugs (Cercopidae) (Adlerz and Hopkins 1979, Purcell and Frazier 1988). In California, Pierce's disease incidence has been exacerbated following the introduction, establishment and continued spread of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, which is an effective vector of *Xf*. GWSS was first detected in southern California in the early 1990's and populations have since become established in many locations throughout southern portions of the state. First detected in Kern County in 1998, GWSS is now present in the San Joaquin Valley. However, the rapid population expansion first observed in southern California appears to be constrained to discrete regions within agricultural areas of the San Joaquin Valley and incipient, localized populations in urban areas of Fresno, Sacramento, Chico, and San Jose. The continued spread of GWSS into other California localities will almost certainly threaten the economic viability of grapes and other crop species susceptible to infection by various *Xf* strains.

Climate appears to play a significant role in the geographic distribution of diseases caused by *Xf* strains in California and throughout the southeastern U.S. (Purcell 1977, 1980, 1997). Similarly, populations of GWSS in the southeastern US appear to be constrained by climatic factors that limit the pest's establishment and persistence (Pollard and Kaloostian 1961, Hoddle 2004). Presently, limited information exists on the overwintering biology and ecology of GWSS in the San Joaquin Valley of California. An emerging hypothesis is that GWSS may be limited by certain temperature thresholds at, or below, which feeding may be discontinued. In turn, we are designing experiments to carefully determine the thresholds below which feeding discontinues. Additionally, we will determine the critical duration of time spent in this non-feeding state, which may result in increased mortality. The results of the outlined experiments will advance our ability to define the specific environmental constraints that influence GWSS population dynamics and overwintering success. This information will by

increase our present understanding of the overwintering requirements of GWSS with a focus on critical environmental and host species factors that may limit population distribution in the Central Valley of California.

OBJECTIVES

1. Identify the critical environmental constraints that influence the spatial population dynamics and overwintering success of GWSS in California's Central Valley.
2. Characterize the impact of host plant species succession on the overwintering survivorship of GWSS populations that constrain the insect's ability to become established and persist throughout the San Joaquin Valley.

RESULTS

Objective 1

Experiments designed to define the temperature-dependent feeding biology of GWSS are underway at the GWSS Experimental Laboratory on the campus of California State University Fresno (CSUF). Colonies of adult GWSS are maintained at this newly established USDA-ARS research facility in cooperation with research personnel from CSUF, the University of California (Riverside, Berkeley), and the California Department of Food and Agriculture. Plans are to characterize adult GWSS feeding and survival in climate-controlled growth chambers to determine the temperature threshold for adult feeding activity under different combinations of host type and temperature regimes. Adult insects from the rearing colonies, as well as field collected insects in reproductive diapause, will be caged on selected plant species at varying temperatures for different exposure periods in environmental chambers. At the completion of the exposure period(s), the three infested treatments of each plant species will be removed from the chamber and adult GWSS performance and survivorship monitored through the remainder of the adult insect life on the respective test plants in individual screen cages.

In preliminary trials designed to indirectly measure feeding rates, water sensitive paper placed under caged adult GWSS on cowpea collected varying levels of excreta at temperatures of 15.6, 10.0, and 4.6°C (Figure 1). Water sensitive paper strips (2" X 3"), which collect excreted honeydew, are placed adjacent to the plant stem and immediately below a 2" diameter cylindrical Lexan® cage in which adult GWSS are confined on a test plant. In future experiments, the paper will be notched and fit to the plant stem and will be manually replaced on a 4 hour interval over 24 hour intervals. Over the 24 h observations, 12 honeydew clocks will be used for each variety at each of 3 start times corresponding to 0600, 1400, and 2200 h to determine any influence of time of day (Padgham and Woodhead 1988). The amount of excreta collected upon the exposed surface(s) of water-sensitive paper will be compared among different, replicated temperature and exposure regimes to better refine the environmental conditions in which GWSS feeding is restricted or discontinued.

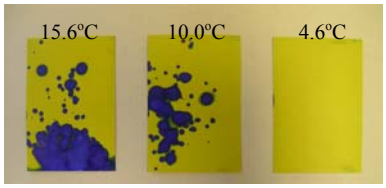


Figure 1. Adult GWSS honeydew collected on water-sensitive paper at varying temperatures over a 24 h interval.

A third set of laboratory experiments are underway using an electro-penetration feeding (EPG) monitoring apparatus to perform waveform analysis at different temperatures. Ten day old adult female GWSS are used in these EPG experiments and are initially placed in separate acclimation cages for 2 hours at the appropriate temperature upon which they will be tested. Preliminary results illustrate differences in the frequency and duration of probing events (green-shaded boxes) of adult GWSS held at temperatures of 15.6, 10.0, and 4.6°C for 12 hour testing intervals on cowpea test plants (Figure 2). Waveform excerpts were taken approximately 225 seconds after the recording began and compressed 2000 times to represent 6.5 hours of recording. These preliminary results indicate that temperature grossly affects GWSS probing behavior between 4.4-15.5 °C. In planned experiments, a total of 5 tethered insects will be simultaneously monitored as experimental replicates at temperatures of 12.2, 10.0, 8.9, and 6.7 °C for exposure intervals of 6, 12, and 24 hour periods. Time course examination of waveforms will reveal the frequency and duration of insect feeding behavior and will help to accurately define the temperature threshold at which ingestion and other waveforms are halted (Serrano et al. 2000).

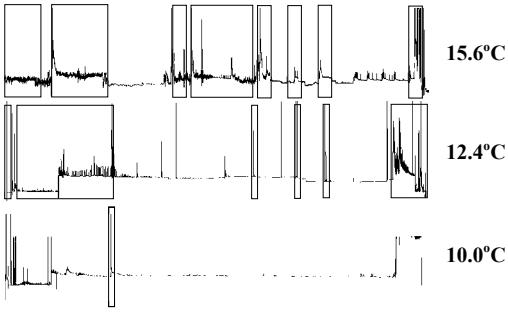


Figure 2. Waveform excerpts (ca. 6.5 h) from a preliminary experimental replicates illustrating the effects of temperature on GWSS probing behavior.

Objective 2

Seasonal population dynamics of GWSS will be monitored on selected host plants placed in different micro-climatic areas of the San Joaquin Valley: 1) the citrus-growing, foothill region of Tulare County; and 2) a GWSS-infested region of the valley floor just west of Porterville in Tulare County. In these experiments, we will examine GWSS survivorship in caged experiments on a selected host plant species. In each cage, fifty second generation GWSS adults, nearing reproductive diapause in the fall season, will be collected from natural infestations and released onto caged plants in late summer. Insects

will be introduced onto potted plants placed in cages and populations monitored monthly throughout the winter period and in the subsequent spring. At each location, four caged replicates of host plant species including the plant species navel orange, grape, and peach will be evaluated individually and in combination. A detailed record of adult GWSS feeding and resting preference will be observed twice monthly throughout the 20 week duration of the experiment beginning November and lasting through March.

CONCLUSIONS

We believe that this recently funded project has a high probability of success both in terms of generating significant new information regarding the overwintering population dynamics of GWSS in California and in providing practical guidance towards management of this pathosystem. This information will further be useful in accurately identifying specific regions of the Central Valley where GWSS overwintering survivorship is greatest and a significant threat of reinfestation is posed. Our research will expand on previous work that has characterized the role of climatic factors in the distribution of *Xf* diseases by defining the specific environmental constraints that influence GWSS population dynamics. Moreover, results from these experiments will be coupled with climatological data in an effort to spatially define those locations where GWSS populations may be unable to successfully overwinter or conversely where populations may find overwintering refuges from extended periods of temperatures that limit adult feeding (Figure 3). Combined with our findings in laboratory bioassays, high resolution (i.e., 1 km scale) raster-based data can be queried to generate predictive maps revealing areas within the Central Valley that may function as "thermal islands", which could favorably support GWSS overwintering populations compared to adjacent agricultural landscapes. As an example, Figure 3 illustrates results of a raster file generated from data collected in January 1993 portraying the number of occurrences where daily maximum temperatures never exceeded 10°C (50°F) for periods of 48 and 96 hours, respectively. With an improved understanding of the climatological limits of GWSS overwintering survivorship, these data can help to spatially define where GWSS can be expected to persist in the agricultural landscape and identify where continued management efforts should be directed to limit introductions into currently non-infested areas. The proposed research will generate critical new information about GWSS spatial population dynamics, thereby contributing towards the development of long-term, economically, and environmentally sustainable management solutions that will directly benefit agricultural producers, crop consultants, and other stakeholders.

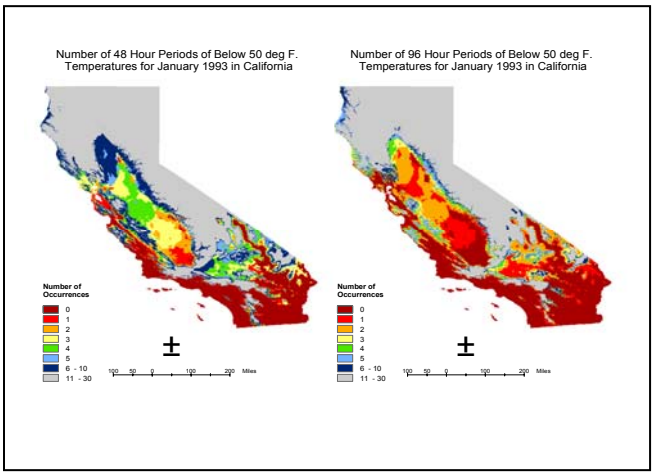


Figure 3. Extended intervals of cool temperatures (< 50°F) January 1993 illustrating microclimatic differences in the San Joaquin Valley

REFERENCES

Adlerz, W.C., and Hopkins, D.L. 1979. Natural infectivity of two sharpshooter vectors of Pierce's disease in Florida. J. Econ. Entomol. 72: 916-919.

Hoddle, M. S. 2004. The potential adventive geographic range of glassy-winged sharpshooter, *Homalodisca coagulata* and the grape pathogen *Xylella fastidiosa*: implications for California and other grape growing regions in the world. Crop Protec. 23:691-699.

Padgham, D. E. and Woodhead, S. 1988. Variety-related feeding patterns in the brown planthopper, Nilaparvata lugens (Stål) (Hemiptera: Delphacidae), on its host, the rice plant. Bull. Ent. Res. 78:339-349.

Pearson, R.C., and Goheen, A.C., eds. 1988. Compendium of Grape Diseases. American Phytopathological Society, St. Paul, MN. 93 pp.

Pollard, H. N., and Kaloostian, G. H. (1961). Overwintering habits of Homalodisca coagulata, the principal natural vector of phony peach disease. J. Econ. Entomol. 54: 810-811.

Purcell, A. H. (1977). Cold therapy of Pierce's disease of grapevines. Plant Dis. Reptr. 61: 514-518.

Purcell, A. H. (1980). Environmental therapy for Pierce's disease of grapevines. Plant Dis. 64: 388-390.

Purcell, A.H. and Frazier, N.W. 1985. Habitats and dispersal of the principal leafhopper vectors of Pierce's disease in the San Joaquin Valley. Hilgardia. 53:1-32.

Purcell, A. H. (1997). Xylella fastidiosa, a regional problem or global threat? J. Plant Path. 79: 99-105.

Serrano, M. S., Backus, E. A., and Cardona, C. 2000. Comparison of AC electronic monitoring and field data for estimating tolerance to *Empoasca kraemeri* (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796-1809.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

BIOLOGY AND MORPHOMETRIC ANALYSIS OF GLASSY-WINGED SHARPSHOOTERS REARED ON COWPEA

i

Project Leader:
Walker A. Jones
USDA, ARS
Beneficial Insects Research Unit
Weslaco, Texas 78596

Cooperator:
Mamoudou Sétamou
USDA, ARS
Beneficial Insects Research Unit
Weslaco, Texas 78596

Reporting period: The results reported here are from work conducted from October 1, 2003 to September 30, 2004.

ABSTRACT

Stage specific survival, growth, developmental biology, and morphometric analysis of individual glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), were studied in the laboratory at 27 ± 1 ºC, 65 ± 5 RH and 14:10 L:D photoperiod regime, on excised cowpea leaves and stems. Embryonic development of eggs was completed in 7.1 days with 92.6% of the eggs incubated being fertile. The total nymphal period for females (61 ± 3.0 days) was significantly longer than that of males (53 ± 1.5 days). Significant differences were observed between the duration of the 5 nymphal stages, with the 2nd being the shortest and the last (5th) the longest for both sexes. Stage specific mortality was similar between instars, ≈ 36.4% of the nymphs reached adult stage, and adult sex ratio was not different from a 1:1 ratio. Based on a cohort of 15 pairs, analysis of life table parameters indicated that populations of *H. coagulata* increased at a rate of 1.045 per day and doubled within 15.6 days. Biometric data comprising body length, head capsule width and hind tibia length were recorded on a total of 276 individuals. The different growth stages were well described by the three biometric parameters. However, analysis of frequency distribution showed that head capsule width was the most suitable parameter for distinguishing the immature developmental stages of GWSS.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is a highly polyphagous xylem-feeder that is indigenous to the southern United States, from Florida to Texas, and northerneastern Mexico (Turner and Pollard 1959). Other than being a minor nuisance in urban environments, the glassy-winged sharpshooter itself causes relatively little direct economic damage or plant loss except for the cosmetic damage to citrus fruits from egg masses deposited into fruits when populations of *H. coagulata* are high (Hix et al. 2003). The most destructive characteristic of GWSS lies in its ability to transmit a plant bacterial pathogen, *Xylella fastidiosa*, one of the causal agents of Pierce's disease (PD) (Redak et al. 2004). However, the recent invasion and establishment of *H. coagulata* in California has dramatically changed the ecology of *X. fastidiosa* and the epidemiology of Pierce's disease (Almeida and Purcell 2003).

Despite the importance and vector status of GWSS, few studies have evaluated its reproductive biology. Little is known about its life table statistics, as published biological studies have not covered the entire life cycle of GWSS. The reasons of the paucity of knowledge on the reproductive biology of GWSS might be the lack of artificial diet-based rearing method for GWSS, as well as the different nutrient requirements of nymphs and adult (Brodbeck et al. 1996).

The present study is focused on developing a simple rearing method for following the development of individual GWSS from egg to adult emergence. We also recorded the longevity and fecundity of adults, and determined the life table statistics of GWSS. Life tables and fertility tables are powerful tools for analyzing and understanding the impact that an external factor has on growth, survival, reproduction, and rate of increase of an insect population (Bellows et al. 1992). As the GWSS undergoes five ecdyses during its development (Turner and Pollard 1959, Brodbeck et al. 1999), it is of significant importance to develop reliable morphological criteria for distinguishing the various nymphal stages.

OBJECTIVES

1. Develop a simple method for rearing individual GWSS from egg to adult on cowpea.
2. Determine the survivorship, egg to adult development time, and reproduction potential of GWSS on cowpea.
3. Examine the growth pattern of this sharpshooter based on three selected biometric parameters that could be used to distinguish the different developmental stages.

RESULTS AND CONCLUSIONS

Biology and Life Table Statistics

The ultimate survivorship of *H. coagulata* on cowpea was 36.4% (Figure 1). The duration of the five instars ranged from 6 to 24 d and was significantly affected by nymphal stage, sex and the sex by developmental stage interaction (Table 1). Within each sex group, the first three instars had the shortest development time, while the last instar (5th) took the longest time to complete for females only (Table 1). The mean total nymphal period of *H. coagulata* on cowpea was 8 d longer for females (61 d) than males (53 d) (Table 2). Out of the 32 *H. coagulata* adults that emerged, 18 were females but the sex ratio was not different from a 1:1 ratio.

Adult longevity was comparable for males (47 d) and females (52 d). For both males and females, no mortality occurred until 20 d after adult emergence. There was a 5 d pre-oviposition period (3 - 9 d) and a 3 d post-oviposition period (0 - 7 d).

A high proportion of females (88%) deposited eggs, with a mean total of 194 eggs per female. The eggs were deposited in clusters under the epidermis layer of cowpea leaves and were mostly in even numbers (93%). Most of the eggs incubated (92.6%) were fertile, and took from 5 to 8 d, with a mean value of 7.1 d, to emerge at 27 ºC.

Life table statistics of GWSS on cowpea are presented on Table 2. Populations of GWSS could multiply at a rate of 33.6 times per generation on cowpea, thus doubling in 15.6 d. Analysis of natality pattern of GWSS revealed that the number of offspring per female was independent of female age, suggesting that food availability might determine the fecundity potential of females.

The successful completion of GWSS life cycle on cowpea suggests that the xylem fluid of this plant has a nutrient profile suitable for both immature and adult stages. The rearing approach used here is quite sample and allowed us to follow each individual GWSS during its development.

Biometric analysis

Values of the three biometric parameters, BDL, HTL, and HCW, varied significantly with the developmental stage (Table 1). Only the grouping for the HCW did not overlap between nymphal stages as indicated by the mean comparison and the distribution of frequency analysis (Table 1, Figure 2). Thus, the HCW could be used as a reliable parameter for distinguishing the five nymphal stages of GWSS.

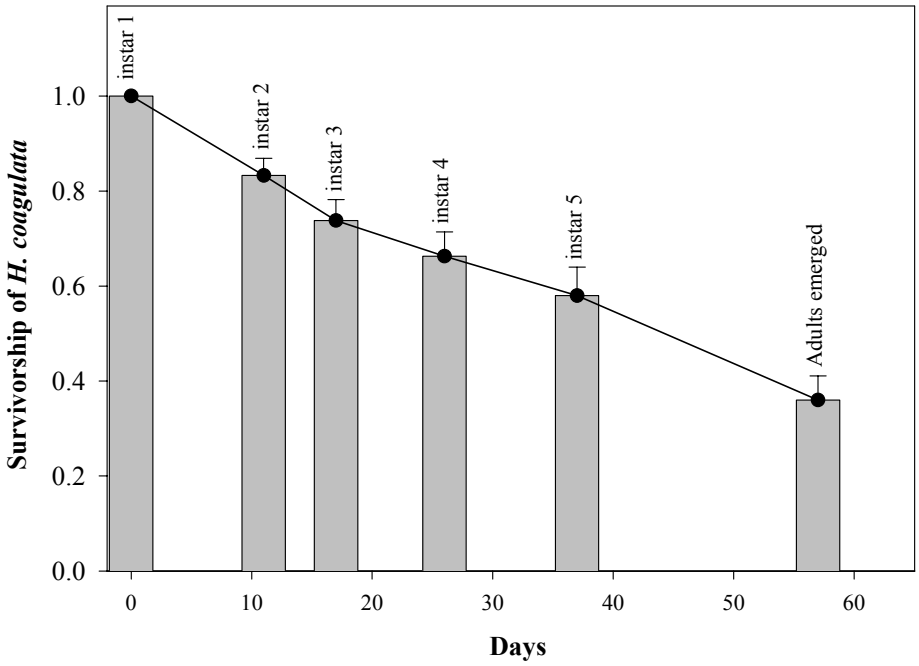


Figure 1. Survival of *H. coagulata* nymphal stages on excised cowpea leaves maintained at 27 °C.

Table 1. Mean[a] developmental duration and size of three biometric parameters of immature stages of GWSS reared on excised cowpea leaves.

Instar	Immature duration ± SE (days)			Biometric parameter ± SE (mm)			
	N	Female	Male	*N*	HCW	BDL	HTL
1	90	10.8 ± 0.9 a BC	10.1 ± 0.9 a BC	76	0.63 ± 0.004 g	2.30 ± 0.03 f	0.86 ± 0.01 g
2	76	6.1 ± 0.5 a C	5.8 ± 0.8 a C	21	0.82 ± 0.015 f	2.98 ± 0.0.09 f	1.19 ± 0.02 f
3	57	8.2 ± 0.9 a BC	8.9 ± 1.2 a BC	27	1.33 ± 0.034 e	5.61 ± 0.24 e	2.03 ± 0.08 e
4	40	12.1 ± 0.7 a B	12.9 ± 0.9a AB	27	1.87 ± 0.013 d	8.63 ± 0.14 d	3.01 ± 0.04 d
5	32	23.7 ± 2.5 a A	14.6 ± 1.8 b A	40	2.13 ± 0.031 c	9.58 ± 0.15 cd	3.19 ± 0.04 d
Total	32	60.9 ± 2.9 a	53.0 ± 1.5b	-	-	-	-

[a] Means followed by the same small case letter within each row and by the same capital letter within each column are not significantly different (P > 0.05), Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ). N, represents the sample size.

Table 2. Fecundity and life table parameters of GWSS reared on excised cowpea leaves.

Parameter	*n*	*Fecundity**	r_m	R_o	*G*	*DT*	λ
Mean	15	193.7	0.044	33.6	79.3	15.6	1.045
95% LCI		154.2	0.040	22.38	74.7	14.1	1.041
95% UCI		233.2	0.049	44.75	83.8	17.0	1.050

* Mean fecundity of gravid females only, i.e., 13 females; *n*, number of pairs included in analysis; r_m, jackknife estimate of the intrinsic rate of increase; R_o, net reproductive rate; *G*, mean generation time (in days); *DT*, population doubling time (in days); and λ, finite rate of increase; LCI = lower confidence limits and UCI = upper confidence limit

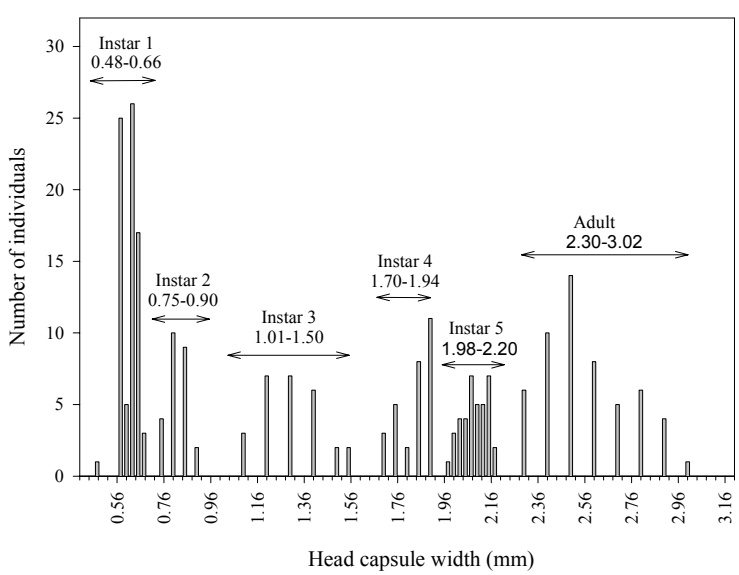


Figure 2. Distribution of head capsule widths of GWSS nymphs and adults.

REFERENCES

Almeida, R. P. P. and A. H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). J. Econ. Entomol. 96: 264–271.

Bellows, T. S. Jr., R. C. Vab Driesche, and J. S. Elkinton. 1992. Life table construction and analysis in the evaluation of natural enemies. Annu. Rev. Entomol. 37: 587–614.

Brodbeck, B. V., P C. Andersen, and R. F. Mizell, III. 1999. Effects of total dietary nitrogen and nitrogen form on the development of xylophagous leafhoppers. Arch. Insect Biochem. Physiol. 42: 37–50.

Brodbeck, B. V., P C. Andersen. , and R. F. Mizell, III. 1996. Utilization of primary nutrients by the polyphagous xylophage, *Homalodisca coagulata*, reared on single host species. Arch. Insect Biochem. Physiol. 32: 65–83.

Hix, R. L., M. L. Arpaia, B. Grafton-Cardwell, C. Lovatt, and P. Phillips. 2003. Glassy-winged sharpshooter impact on orange yield, fruit size, and quality. In: Pierce's Disease Program: Symposium Proceeding, Coronado, CA.

Redak, R. A., A. H. Purcell, J. R. S. Lopes, M. J. Blua, R. F. Mizell, III, and P. C. Andersen. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annu. Rev. Entomol. 49: 243–270.

Turner, W. F., and H. N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. U.S. Dep. Agric. Tech. Bull. 1188:28.

FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service.

EFFECTS OF USING CONSTANT AND CYCLICAL STEPWISE-INCREASING TEMPERATURES ON PARASITIZED AND UNPARASITZED EGGS OF THE GLASSY-WINGED SHARPSHOOTER DURING COLD STORAGE

Project Leader:
Roger A. Leopold
USDA, ARS
Biosciences Research Laboratory
Fargo, ND 58105

Cooperators:
Wenlong Chen
Dept. of Entomology
North Dakota State University
Fargo, ND

George D. Yocum
USDA, ARS
Biosciences Research Laboratory
Fargo, ND

Reporting Period: The results reported here are from work conducted from December 1, 2003 to October 1, 2004.

ABSTRACT
Glassy-winged Sharpshooter (GWSS) egg masses, deposited on *Euonymus japonica* cuttings, were stored 1d after oviposition at either a constant temperature of 12ºC or under a regime that cycled daily, stepwise, (10, 11, 12, 13ºC @ 6h intervals) under an 8L:16D photoperiod. After storage under the cycled temperature regime for 15 and 20d, the hatch was 74 and 63%, respectively. Control hatch at 20d was about 80% and 50% after storage at a constant 12ºC. The survival to adulthood, length of the nymphal stage, and the fecundity of the adult females were all affected by cold storage during the egg stage, regardless whether the temperature was held constant or cycled. Survival to adulthood was reduced 30 to 40% and the time required to complete the nymphal stages was significantly longer than the control. The number of eggs oviposited by females and length of the ovipositional period after being held at 12ºC during the egg stage was about one-half that of the control group, while the values for the 20d cycled group are yet to be determined. The rates of parasitism and emergence by *Gonatocerus ashmeadi* decreased with the length of time that 1-d-old unparasitized GWSS eggs were stored under the cycled regime. When held up to 25d in storage, parasitism by wasps and emergence of their progeny remained statistically similar. After 50d of storage, parasitism and progeny emergence dropped 30% and 20%, respectively. After a storage period of 25d, parasitoid emergence from parasitized eggs stored at a constant 4.5ºC was significantly higher than those stored similarly at 4ºC. The cycled stepwise-increasing temperature regime of 4.5, 6.0, and 7.5ºC changing at 8h intervals yielded a significantly higher parasitoid emergence than a cycled regime of 4, 6, and 8ºC. When stored under the regime starting at 4.5ºC, for 10, 20 and 25d, the emergence of wasps was 66%, 59% and 59%, respectively. Parasitized eggs stored under this regime for 80d produced no wasps.

INTRODUCTION
Studies on cold storage of insects and their eggs have shown that developmental age, storage temperature, time in storage, and inherent species tolerance are the factors which influence survival after a cold storage period (Leopold 1998). The most effective temperature for storage of GWSS eggs was determined to be 12ºC (Leopold et al. 2003). Storage of 1-d-old GWSS eggs at 10ºC resulted in no survival after only 8d period. Storage at 13 and 14ºC resulted in high survival and parasitism by *Gonatocerus ashmeadi* and *G. triguttatus* at 20d, but in-storage hatching of the GWSS eggs occurs after 30d and successful parasitism by the wasps decreases under these constant temperature regimes. The within-host cold tolerance of the *Gonatocerus spp*. is significantly greater than that of the unparasitized GWSS eggs. Emergence of the wasps occurs at temperatures $\geq$ 5ºC when the parasitized eggs are stored < 20d. Since certain conditions, such as temperature variation and fluctuation and high or low humidities have been reported to enhance survival of insects and their parasites during cold storage (Iacob and Iacob 1972, Gautum 1986, Liu and Tian 1987, Leopold et al. 1998), the present study was initiated to determine whether we could lengthen the survival time of GWSS eggs and the egg parasitoid by varying the temperature while in storage. We were especially interested in determining whether any latent damaging effects of chilling would be expressed, beyond diminished emergence, that might affect the quality of previously cold-stored insects.

OBJECTIVES
1. Compare the cold tolerance of GWSS eggs stored at a constant temperature with eggs stored under a cycled stepwise temperature regime and evaluate the post storage developmental time of nymphs and reproduction of adults.
2. Compare the effects of cold storage of unparasitized GWSS eggs under constant and cycled stepwise low temperatures regimes on the subsequent parasitism and emergence of *G. ashmeadi*.
3. Determine whether a cycled stepwise cold temperature regime enhances the shelf-life of parasitoids while in host eggs.

RESULTS AND CONCLUSIONS
Cold storage of Unparasitized GWSS Eggs
GWSS egg masses deposited on *Euonymus* cuttings were stored in incubators set at constant (12ºC) and cycling stepwise-increasing temperatures (10, 11, 12, and 13 ºC @ 6h intervals) under an 8L:16D photoperiod for varying lengths of time. After removal from storage, the cuttings bearing GWSS egg masses were incubated at room temperature (ca. 22 ºC) to record egg hatch. After storage at 12ºC for 30d, 52.7 ± 10.2% of 1-d-old eggs (n = 102), 50.7 ± 7.1% of 3-d-old eggs (n = 87) and 44.7 ± 5.1% of 5-d-old eggs (n =61) hatched. However, no hatching was observed after 30d storage. When stored at the stepwise cycling temperature (10-13 ºC) for 15, 20, and 25d, the hatch of 1-d-old eggs was 73.9 ± 11.1% (n = 142),

62.6 ± 9.1% (n = 98) and 44.6 ± 9.1% (n = 104), respectively. There was a significant difference in percentage hatch of 1-d-old GWSS eggs between the control eggs (83.0 ± 7.4%, n = 317) and those eggs stored for 25d (F = 3.939, df = 3,45, P = 0.014), but no significant differences were found between those groups stored in the cold for 15 or 20 days and the control. After storage for 80d under the daily cycled regime, no hatching was observed.

To determine effect of cold storage during GWSS egg stage on nymphal development and adult reproduction, newly hatched nymphs from eggs stored at 12 ºC for 20 days, and at the daily cycled temperature regime for 15 and 20 days were reared on sunflower plants until they emerged as adults. When the characteristic patch of brochosomes was observed on the forewings of the adult females (brochosomes were considered as the sign that females had mated), they were then individually maintained on sunflower plants and their egg mass output recorded until death occurred. Our preliminary data (Table 1) shows that 50% of nymphs from eggs stored at 12ºC for 20 d and 50% and 40% of nymphs held under the stepwise temperature regime for 15 days and 20 days, respectively, successfully developed into adults. In comparison with the control groups, GWSS males and females from those eggs that had been exposed to either cold storage regime took significantly longer to complete their nymphal stages (Table 1). There were no differences in male and female developmental times among the nymphs that hatched from GWSS eggs that had undergone cold storage. The number of eggs produced/female and the ovipositional period was considerably greater for the control groups and approached 2-fold differences.

Effects of Cold Storage of GWSS Eggs on Parasitism and Emergence by G. ashmeadi

Following storage in incubators set at a constant 12 or 12.5ºC and also at the stepwise cycled regime as described above, GWSS egg masses were exposed to caged *G. ashmeadi* colonies for 2 days at room temperature (ca. 22 ºC) and under an 10L:14D photoperiod. Before statistical analysis, the data recorded for parasitism and emergence were square-root transformed to correct non-normality because the number of eggs/mass was not constant.

After storage at 12ºC for 30d, 69.6 ± 11.7% of the 3-d-old GWSS eggs (n = 90) and 47.7 ± 11.7% (n = 106) of the 1-d-old eggs were successfully parasitized by *G. ashmeadi*. The percentage wasp emergence was 68.5 ± 11.3 for 3 day-old eggs and 35.3 ± 10.0% for the 1 day-old eggs. There were no significant differences in the incidence of parasitism, as determined by egg dissection, ($F = 4.034$, $df = 1,14$, $P = 0.066$) and emergence ($F = 1.728$, $df = 1,14$, $P = 0.211$). Further, *G. ashmeadi* successfully parasitized about 77% of the 4-d-old , 52% of the 5-d-old, and 45% of the 3-d-old GWSS eggs stored at 12.5ºC for 30d, and 46% of 3-d-old eggs stored for 50d. As above, there were no significant differences between parasitism and emergence in any of the comparable groups (data not shown).

When stored under the cycled stepwise temperature regime (10-13 ºC), the parasitism ($F = 14.934$, $df = 8,137$, $P < 0.001$) and emergence ($F = 13.661$, $df = 8,137$, $P < 0.001$) of 1-d-old GWSS eggs by *G. ashmeadi* varied significantly with storage time (Table 2). More than 75% of GWSS eggs stored up to 25d were successfully parasitized and there was no significant difference in the incidence of parasitism between the control (92.1 ± 9.9%, n = 172) and the eggs stored for 15, 20 or 25d ($F = 1.764$, $df = 3,35$, $P = 0.172$). However, percentage emergence for the eggs stored for 25d was significantly lower than that for the control (91.7 ± 2.7%, n =172) ($F = 3.250$, $df = 3,35$, $P < 0.033$). Further, there were no significant differences in percentage emergence between the control eggs and the eggs stored for 15 or 20d ($P = 0.099$). After storage for 65d, < 44% eggs were parasitized by *G. ashmeadi*, and about 23% of wasps emerged, which was significantly lower than for eggs for stored for 25d or less. When stored for over 80d, the percentage parasitism and emergence were less than 12% and 7%, respectively. When these data were analyzed via a regression analysis, the percentage parasitism and emergence vs. storage time was found to be inversely correlated (Figures 1 and 2).

Cold Storage of GWSS Eggs Parasitized by G. ashmeadi

The experimental conditions for this study consisted of a constant 4 or 4.5ºC storage temperature and 2 daily cycled stepwise-increasing regimes (4, 6, and 8ºC or 4.5, 6, and 7.5ºC - each temp. changing at 8h intervals) under an 8 L: 16 D photoperiod. After the parasitized eggs were stored at 4 ºC for 10d, only 7.2 ± 5.0% (n = 85) of the wasps emerged, which was significantly lower than those parasitoids similarly stored at 4.5ºC (33.5 ± 7.2%, n = 280), 20 days (33.9 ± 6.9%, n = 114) or 25 days (21.7 ± 5.2%, n = 125) ($F = 11.962$, $df = 4,66$, $P < 0.001$). No parasitoids (n = 164) emerged from host eggs stored at 4ºC for 20d (Figure 3). When parasitoids were stored within hosts under the cycled stepwise temperature regime starting at 4 ºC, percentage emergence was 42% (n =126) at 10 d, 8 % (n = 420) at 20d and 0% (n = 184) at 25d (Figure 4). However, for parasitized eggs stored at the other cycled regime starting at 4.5ºC, the wasp emergence was at or above 60% throughout the 25d of storage. Thus, the percentage emergence for the parasitoids stored under the stepwise regime starting at 4.5ºC for 10-25d was significantly higher than that for the eggs stored for 15d under the regime starting at 4ºC ($F = 48.237$, $df = 5, 114$, $P < 0.0001$). Parasitoids within GWSS eggs did not emerge after storage for 80 days, but further research is needed to ascertain if maintenance of the *Euonymus* cuttings that bear the egg masses during the storage period is causing a problem.

REFERENCES

Gautam, R. D. 1986. Effect of cold storage on the adult parasitoid *Telenomus remus* Nixon (Scelionidae: Hymenoptera) and the parasitized eggs of *Spodoptera litura* (Fabr.) (Noctuidae: Lepidopetra). J. Entomol. Res. 10: 125-31

Iacob, M. and N. Iacob. 1972. Influence of temperature variation on the resistance of *Trichogramma evanescens* Westw to storage with a view to field release. Anal. Inst.de Cercetari pentru Protctia Plantelor 8: 191-199.

Leopold, R. A. 1998. Cold storage of insects for integrated pest management. In: Temperature sensitivity in insects and application in integrated pest management. G. J. Hallman and D. L. Denlinger (eds.). Westview Press, Boulder. pp. 235-267.

Leopold, R.A., R.R. Rojas, & P.W. Atkinson. 1998. Post pupariation cold storage of three species of flies: increasing chilling tolerance by acclimation and recurrent recovery periods. Cryobiology 36: 213-224.

Leopold, R. A, W. Chen , D. J. W. Morgan & G. D. Yocum. 2003. Cold storage of parasitized and unparasitized eggs of the glassy-winged sharpshooter, *Homalodisca coagulata*. pp 221-224. *In* M. Athar Tariq, S. Oswalt, P. Blincoe & T. Esser (eds). Proc. of Pierce's Disease Research Symposium, Dec. 8-11, San Diego

Liu, J.J. & Y. Tian. 1987. Cold storage of *Encarsia Formosa* Gahn. Chin. J. Biol. Cont. 36:4-6.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service and the USDA Agricultural Research Service.

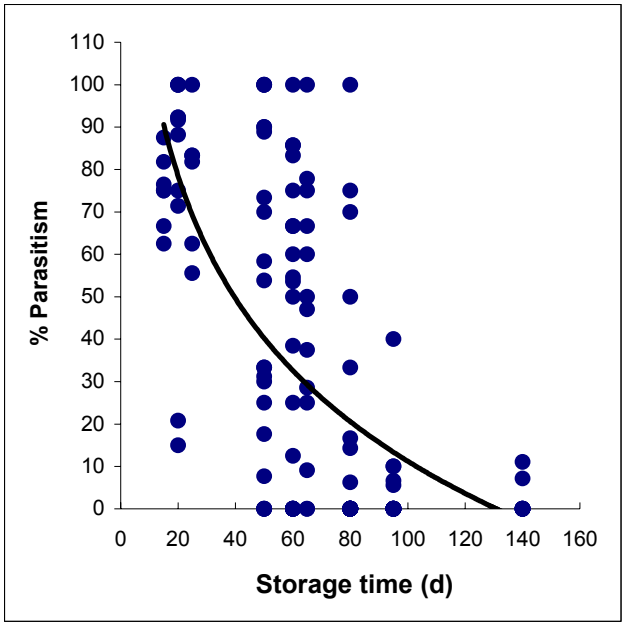


Figure 1. Relationship of the % parasitism (y) of *G. ashmeadi* to storage time (x) of the GWSS eggs at stepwise temperatures (10~13ºC)(y = 5.10 + 1393.18/x, $r = 0.58$) ($F = 68.24$, $df = 136$, $P < 0.001$)

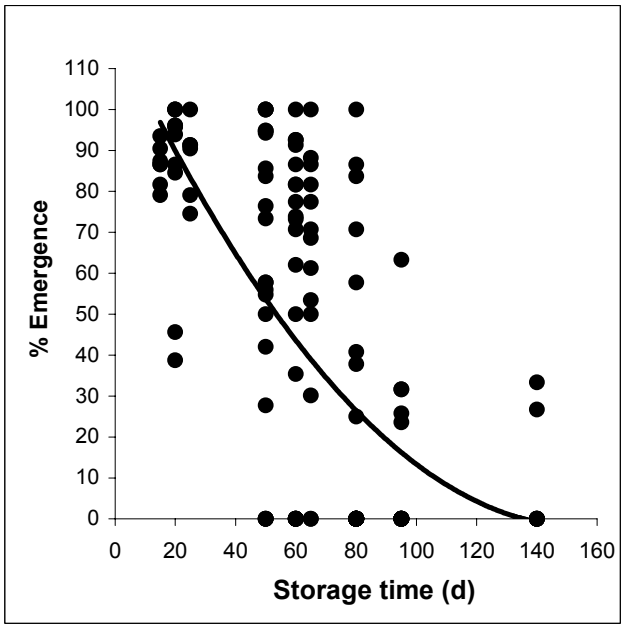


Figure 2. Relationship of the % emergence (y) of *G. ashmeadi* to storage time (x) of the GWSS eggs at stepwise temperatures (10~13ºC) (y = -0.35 + 1286.50/x, $r = 0.59$) ($F = 79.01$, $df = 136$, $P < 0.001$).

Table 1. Egg hatch, development time of nymphs and reproduction of adults for GWSS eggs stored under different temperature conditions (mean ± SE).

Storage conditions	Egg hatch (%)	Development time of nymphal stage			Adult reproduction	
		% survival	Male (d)	Female (d)	No. eggs/ female	Ovipositional period (d)
Control (25ºC)	82.9 ± 7.4	80.2	35.9 ± 0.5 a	35.3 ± 0.6 a	1068.8 ± 187.7	113.4 ± 49.6
12ºC for 20 d	52.7 ±10.2	50.0*	43.9 ± 0.9 b	42.5 ± 0.7 b	589.3 ± 81.9	65.7 ± 26.0
10-13ºC for 15 d	73.9 ± 5.4	50.0*	43.0 ± 0.7 b	41.0 ± 1.2 b	662.7 ± 111.1	65.0 ± 11.2
10-13ºC for 20 d	62.6 ±10.3	40.0*	43.0 ± 3.5 b	41.9 ± 0.4 b	In progress	In progress

Only 1 replicate. Means within a column followed by different letters were significantly different at the significant level of 0.05 (SAS Proc GLM with LSD). Data for egg hatch were square-root transformed before analysis.

Table 2. Parasitism and emergence by *G. ashmeadi* on the GWSS eggs exposed to the daily stepwise temperature regime (10, 11, 12, 13ºC - changing at 6h intervals) for 15 to 140 d.

Storage time	No. egg masses	No. eggs	Parasitism (mean % ±SE)	Emergence (mean % ± SE)
15 d	7	88	74.99 ± 3.20 a	68.25 ± 3.11 a
20 d	11	106	76.98 ± 9.26 a	67.18 ± 9.23 ab
25 d	6	69	77.76 ± 6.58 a	57.15 ±13.49 ab
50 d	21	226	47.75 ± 8.15 b	41.89 ± 8.05 bc
60 d	23	208	37.27 ± 7.49 b	28.69 ± 6.61 c
65 d	13	126	44.36 ± 8.69 b	22.58 ± 7.11 c
80 d	31	253	11.79 ± 4.68 c	7.31 ± 3.84 d
95 d	17	193	4.25 ± 2.40 c	1.90 ± 0.89 d
140 d	9	96	2.02 ± 1.38 c	2.02 ± 1.38 d
			$F = 14.934$	$F = 13.661$
			$df = 8,137$	$df = 8,137$
			$P < 0.001$	$P < 0.001$

Means within a column followed by different letters were significantly different at the significant level of 0.05 (SAS Proc GLM with LSD). Data were square-root transformed before analysis.

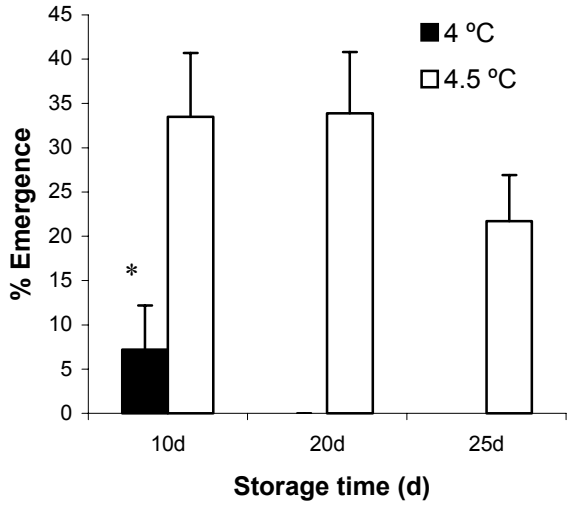


Figure 3. Percentage emergence of *G. ashmeadi* from GWSS eggs stored at constant temperatures for 10-25 d. Bar marked by an asterisk represents a significant difference (P < 0.05).

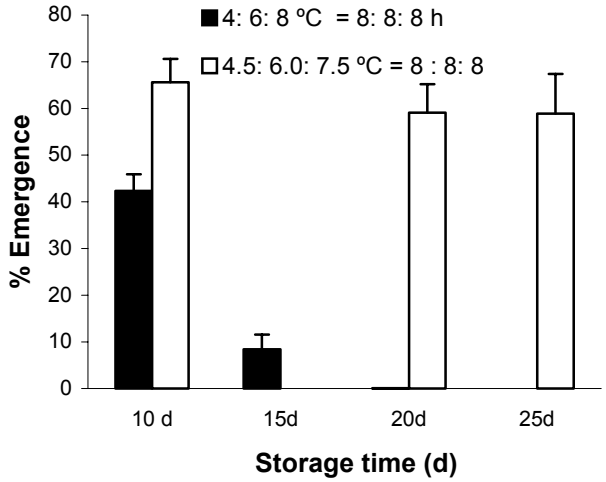


Figure 4. Percentage emergence of *G. ashmeadi* from the GWSS eggs stored at stepwise temperatures for 10-25 d. Bar marked by an asterisk represents a significant difference (P < 0.05).

PARASITISM OF THE GLASSY-WINGED SHARPSHOOTER: FUNCTIONAL RESPONSES AND SUPER-PARASITISM BY THE EGG PARASITOID *GONATOCERUS ASHMEADI.*

Project Leader:
Roger A. Leopold
USDA, ARS
Biosciences Research Laboratory
Fargo, ND 58105

Cooperators:
Wenlong Chen
Dept. of Entomology
North Dakota State University
Fargo, ND

David J. Morgan
CDFA
Mt. Rubidoux Field Station
Riverside, CA 92504

Reporting Period: The results reported here are from work conducted from December 1, 2003 to October 1, 2004.

ABSTRACT
The functional responses and super-parasitism by the egg parasitoid, *Gonatocerus ashmeadi*, on *Homalodisca coagulata* eggs were related to host age and density when studied under laboratory conditions. Parasitism of Glassy-winged Sharpshooter (GWSS) eggs, 1-, 3-, 5-, 7- and 9-d-old, was measured at 22 ± 1ºC and under 10L:14D regime. For each host age, 10-60 eggs were exposed to an individual parasitoid for 24 h. The functional responses for the parasitoids to host eggs of all age groups most closely fit the type II and III models of Hollings (1959) and Hassell (1978) which relate to the elapsed time for accomplishing the behavioral events associated with parasitism of the host as modified by host density. The instantaneous attack rate by parasitoids on 1-d-old host eggs, as specified in the type III model, was significantly greater from that of the other ages. This rate was also greater in the type II model but was not statistically significant. The total number of host eggs parasitized varied significantly with host density and age of the eggs, but not when analyzed by a host x density interaction. Host age and density, as well as the host x density interaction, contributed significantly to the differences found in length of development time of *G. ashmeadi* within host eggs. The wasps exhibited a tendency towards super-parasitism at relatively high parasitoid-to-host ratios. The maximum number of parasitoid eggs found in a single host egg was 18. The development time and eclosion of the parasitoids had no correlation with parasitoid-to-host ratios. Frequencies of super-parasitism for *G. ashmeadi* displayed an aggregated distribution over all observed host densities.

INTRODUCTION
The effectiveness of parasitoids in regulation of a pest population is highly dependent on their ability to search for and handle hosts in a varying ecosystem. This effectiveness has been traditionally related to the functional response of a parasite or predator (Hassell 1978, Fujii *et al.* 1986). The functional response is defined as the relationship between the numbers of prey taken by the predator as a function of prey density (Holling 1959). The functional response is an essential component of the dynamics of host-parasitoid relationship, and is an important determinant of the stability of the system (Oaten and Murdoch 1975). Functional response analyses are commonly used to help predict the potential for parasitoids to regulate host population (Solomon 1949, Oaten and Murdoch 1975). Successful parasitoids have the ability to discriminate among parasitized eggs, avoid super-parasitism and minimize the waste of time and energy associated with their searching and parasitizing behaviors (Godfray 1994). However, under certain circumstances, superparasitism might be adaptive (van Alphen & Visser 1990). Further, when mass-rearing solitary parasitoids for use in an augmentative release program, super-parasitism represents a waste of the production colony's potential output. This report presents the progress on investigations determining certain aspects of the functional responses and super-parasitism by the parasitoid, *G. ashmeadi*.

OBJECTIVES
1. Investigate the response of *G. ashmeadi* to GWSS eggs of different ages and determine the effects of host egg age on functional response parameters and parasitism.
2. Determine effect of host densities and ages with respect to developmental time of wasps.
3. Investigate relationship between super-parasitism by the wasp at different host densities and effect of super-parasitism on wasp emergence and development time.

RESULTS
Functional Responses
There was a significant increase in the numbers of *H. coagulata* eggs of different ages parasitized by egg parasitoid, *G. ashmeadi,* with an increase in host density (Table 1). At the host densities of 40, 50, and 60, the numbers of eggs parasitized were significantly higher than that of relatively low densities of 10 and 20 over all host ages. The number of 1-d-old eggs parasitized was slightly greater than that of 5-, 7- and 9-d–old-eggs. A two-way ANOVA, with age and density as factors, revealed that the number of eggs parasitized varied significantly with host age ($F = 3.64$, $df = 4,299$, $P = 0.007$) as well as host densities ($F = 88.43$, $df = 5,299$, $P < 0.0001$). There was no significant effect of age × density interaction on the number of host eggs parasitized ($F = 0.44$, $df = 20, 299$, $P = 0.899$).

The functional responses of *G. ashmeadi* parasitizing host eggs at the various ages showed that the shape of the functional response curves were affected by differences in the parasitization rates of *G. ashmeadi*. At all host ages, the *G. ashmeadi* functional response data most closely fit the type II and III models. Coefficients of determination (r^2 values) for type II and III curves were very similar (Table 2). The instantaneous attack rates (a) and handling time (T_h) estimated

from type II functional response models varied slightly but were not significantly different among host ages (Table 3). The *a* value for 1-d-old eggs was slightly higher than that for other ages when data were fit to a type II functional response model. The estimate for handling time (time spent on eggs) by the wasps for all host egg ages did not vary significantly. When the data were fitted to a type III functional response model, the *a* value estimated for 1-d-old eggs was significantly higher than that for host eggs of 3-, 5-, 7- and 9-d-old. However, the handling time of *G. ashmeadi* for all egg ages was similar, ranging from the value of 0.032 to that of 0.040.

Effect of Host age on Parasitoid Development Time

The development time of *G. ashmeadi* within host eggs varied significantly with host density and host age (Table 4). Within the 1-, 3-, 5-, 7- and 9-d-old host eggs, the mean development time (± SE) of the parasitoid was 16.0 ±1.0 d (n = 1435), 18.9 ± 1.8 d (n = 996), 18.3 ± 1.5 d (n = 1181), 17.6 ± 1.2 d (n = 961) and 17.8 ± 1.5 d (n = 1254), respectively. The parasitoid within 1-d-old sharpshooter eggs developed significantly faster than that within other ages ($F = 766.41$, $df = 5, 5826$, $P < 0.0001$). A two way ANOVA further showed that host age ($F = 999.47$, $df = 4, 5826$, $P < 0.0001$) and density ($F = 58.26$, $df = 5, 5826$, $P < 0.0001$) contributed significantly to the development time of *G.ashmeadi*. The significant interactive effect on development time occurred between host age and density ($F = 62.82$, $df = 20, 5826$, $P < 0.0001$).

Super-parasitism.

Maximum number of parasitoid eggs in one host egg was 18. The level of super-parasitism of *G. ashmeadi* (Table 5) varied significantly with increasing host density ($F = 225.17$, $df = 5,549$, $P < 0.0001$). The mean number of parasitoid eggs per sharpshooter egg at 1:1 parasitoid-to-host ratio is significantly greater than that at other ratios. When the parasitoid-to-host ratio increased to > 1:15, host eggs pooled from each host density were almost all parasitized. There was a significant positive correlation between the number of parasitoid eggs per host egg and parasitoid-to-host ratio ($F = 1231.69$, $df = 548$, $r = 0.8319$, $r^2 = 0.692$, $P < 0.0001$). *G. ashmeadi* is a solitary parasitoid and normally only one wasp emerges from each egg of its host. In treatments with high host densities such as at 1:1 and 1:5 parasitoid ratios, the percentage of parasitoid eclosion was significantly higher than in low-density treatments ($F = 3.996$, $df = 4,243$, $P = 0.004$)(Table 5). However, there is no correlation between parasitoid-to-host ratio and percentage of parasitoid eclosion ($F = 3.29$, $df = 242$, $r = 0.1140$, $r^2 = 0.013$, $P = 0.071$). Although there was a significant statistical difference in development time of the parasitoid within the host egg among different parasitoid-to-host ratios ($F = 46.851$, $df = 4, 1862$, $P < 0.0001$), the maximum difference was only about 0.7d.

For *G. ashmeadi*, x^2 goodness-of-fit analyses of parasitoid egg numbers per host egg revealed that frequencies of super-parasitism were significantly different from the expected Poison distribution over all host densities ($x^2 = 231.291$, $df=4$, $P < 0.0001$). The relationship between the variances (S^2) and means (m) was described by Taylor's power law (Taylor 1961) as: $\log S^2 = -0.4384 + 1.0288 \log m$ ($r^2 = 0.604$, $df = 28$, $F = 42.78$, $P < 0.0001$, where $b = 1.0288 > 1$, indicating an aggregated distribution of super-parasitism for *G. ashmeandi* over all experimental parasitoid-to-host ratios.

CONCLUSIONS

The studies on the functional responses of *G. ashmeadi* to GWSS eggs of different ages and densities in the laboratory have improved our understanding of the interactions between the parasitoid and host egg. Because this parasitoid fits the II and III functional response models in relation to different host ages, it further confirms that the wasp has the capacity of effectively parasitizing eggs throughout most of the embryonic development of the GWSS. Further, studies on super-parasitism of *G. ashmeadi* provide valuable information for the mass-rearing and field release of this parasitoid. Our results indicate that super-parasitism occurs when the parasitoid-to-host ratio is greater than 1:15. Super-parasitism results in a waste of the reproductive potential of this species because *G. ashmeadi* is a solitary-developing wasp and usually only one parasitoid emerges from one GWSS egg.

REFERENCES

Fujii, K., C. S. Holling and P. M. Mace. 1986. A simple generalized model attack by predators and parasites. *Ecol. Res*. 1: 141-156.

Godfray, H. C. J. 1994. Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ.

Hassell, P. M. 1978. The dynamics of arthropod predator-prey systems. Princeton University Press, Princeton, NJ.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. *Can. Entomol.* 91: 317-324.

Oaten, A. and W. W. Murdoch. 1975. Functional response and stability in predator-prey systems. *Am. Nat*. 109: 289-298.

Solomon, M. E. 1949. The natural control of animal populations. *J. Anim. Ecol.* 18: 1-45.

Taylor, L.R. 1961. Aggregation, variance and the mean. *Nature.* 189: 732-735.

van Alphen, J. J. M., and M. E. Visser. 1990. Superparasitism as an adaptive strategy for insect parasitoids. *Ann. Rev. Entomol*. 35: 59-79

Table 1. Parasitism by *G.ashmeadi* on *H. coagulata* eggs of different ages at varying densities.

Density	Mean No. Parasitized (SE)				
	1d	**3d**	**5d**	**7d**	**9d**
10	9.5(1.3) a	8.7(2.2) a	8.9(1.6) a	9.0(2.2) a	9.1(1.1) a
20	18.1(1.6) b	15.5(3.2) b	14.8(3.4) b	14.6(3.9) ab	14.7(3.3) ab
30	22.9(3.0) c	17.9(8.4) b	22.0(5.8) c	19.8(7.7) bc	18.7(4.1) b
40	26.5(4.7) cd	22.2(9.8) bc	25.1(7.3) cd	22.7(5.6) c	25.8(6.2) c
50	30.3(7.5) d	25.6(10.0) cd	29.4(5.1) de	23.9(11.9) cd	29.5(13.1) c
60	34.8(4.7) e	30.7(6.9) d	32.2(4.5) e	29.9(7.3) d	30.1(3.4) c
	$F = 43.12$	$F = 11.02$	$F = 31.69$	$F = 10.59$	$F = 16.96$
	df = 5,59	df = 5,59	df = 5,59	df = 5,59	df = 5,59
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.001$

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan).

Table 2. Coefficients of determination for functional response regression models of *G. ashmeadi* to *H. coagulata* eggs of different ages.

Age of Eggs (d) [a]	Type I (r^2)	Type II (r^2)	Type III (r^2)
1	0.7776	0.9729	0.9727
3	0.4979	0.8993	0.8992
5	0.7260	0.9607	0.9608
7	0.4783	0.9038	0.9036
9	0.5872	0.9280	0.9280

[a] *G. ashmeadi* targeted host densities ranged from 10 to 60 sharpshooter eggs per experimental container. Type I functional response model was evaluated using SAS PROC GLM whereas Type II and III models were evaluated using SAS PROC NILN to generate r^2 values indicating best fit.

Table 3. Type II and III functional response parameters of *G. ashmeadi* when parasitizing *H. coagulata* eggs of different ages.

Functional response model	Host age (d)	Instantaneous attack rate (a ± SE)[a]	Handling time (T_h ± SE)[a]
Type II	1	0.5782 ± 0.0626 a	0.0300 ± 0.0004 a
	3	0.4544 ± 0.0959 a	0.0315 ± 0.0105 a
	5	0.5013 ± 0.0640 a	0.0286 ± 0.0058 a
	7	0.5064 ± 0.1088 a	0.0377 ± 0.0099 a
	9	0.4831 ± 0.0849 a	0.0296 ± 0.0082 a
Type III	1	2.8131 ± 2.2011 a	0.0342 ± 0.0056 a
	3	1.0137 ± 0.5410 b	0.0333 ± 0.0117 a
	5	1.4394 ± 0.6301 b	0.0316 ± 0.0067 a
	7	1.3858 ± 0.9508 b	0.0403 ± 0.0113 a
	9	1.2495 ± 0.6620 b	0.0322 ± 0.0094 a

[a] Instantaneous attack rate (a) and handling time (T_h) estimated by SAS PROC NLIN and pairwise compared among host ages using indicator variable (0 or 1) for age.

Table 4. Development time of *G. ashmeadi* within *H. coagulata* eggs of different ages when parasitized at varying densities.

	Development time (SE) at age:				
Density	**1d**	**3d**	**5d**	**7d**	**9d**
10	15.9(0.6) d	21.0(2.1) a	17.9(1.6) c	15.7(1.4) e	17.6(1.4) c
20	16.5(0.8) a	18.5(1.6) c	18.0(1.1) c	18.3(0.9) a	18.3(0.8) b
30	16.5(0.7) b	18.6(2.2) c	18.8(1.3) b	18.1(0.9) ab	19.1(1.2) a
40	16.1(0.8) c	18.3(2.2) c	17.8(1.5) c	18.0(1.0) bc	16.9(1.6) d
50	16.0(1.4) cd	18.6(1.5) c	19.5(1.2) a	17.8(0.6) c	17.4(1.4) c
60	15.5(0.7) e	19.4(1.0) b	17.4(1.0) d	17.2(1.2) d	18.1(1.2) b
	$F = 45.39$	$F = 37.00$	$F = 88.13$	$F = 84.08$	$F = 73.93$
	df = 5,1434	df = 5,995	df = 5,1180	df = 5,960	df = 5,1253
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan).

Table 5. Number (mean ±SE) of G. ashmeadi eggs per host egg, percentage of emergence and development time at different parasitoid-to-host egg ratios.

Parasitoid-host ratio	**No. parasitoid / host**		**% Emergence**		**Development time**	
	N_1	**Mean ±SE**	**N_2**	**Mean ±SE**	**N_3**	**Mean ±SE**
1:1	50	10.40 ± 4.86 a	NA	NA	NA	NA
1:5	100	3.02 ± 1.69 b	11	97.6 ± 1.7 a	141	18.02 ± 0.07a
1:10	100	2.24 ± 1.16 c	15	98.9 ± 0.6 a	136	18.20 ± 0.06 b
1:15	100	1.66 ± 0.89 d	77	93.6 ± 1.5 b	490	18.30 ± 0.04 b
1:20	100	1.20 ± 0.59 d	70	91.7 ± 1.0 b	263	18.25 ± 0.04 b
1:25	100	1.15 ± 0.58 d	71	90.0 ± 1.7 b	833	18.77 ± 0.03 c

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan). N_1 represents the number of dissected host eggs, N_2 represents the number of egg masses observed, and N_3 is the number of parasitoid emerging from host eggs.

FUNDING AGENCIES
Funding for this project was provided by the USDA Animal and Plant Health Inspection Service and the USDA Agricultural Research Service.