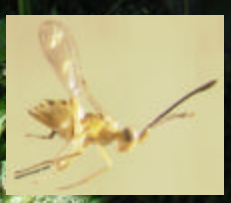
Glassy-winged Sharpshooter & Pierce's Disease Research Summaries

FY 00-01/01-02



Glassy-winged Sharpshooter and Pierce's Disease Research Summaries

FY 2000-2001 and FY 2001-2002

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California Table Grape Commission (Table)

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City of Temecula (Temecula)

County of Riverside (Riverside)

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UC Pierce's Disease Grant Program (UC - PD)

UC Pierce's Disease Integrated Pest Management Program (UC - IPM)

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GWSS and Pierce's Disease Research Summary
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2000-2001 and FY 2001-2002

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			TOTAL	$12,172,181

* - Multi-Year Program

Funding Agency Key	
Almond	Almond Board of California
AVF	American Vineyard Foundation
CA Citrus Nursery	California Citrus Nursery Advisory Board
Cal Trans	California Department of Transportation
CCGPRVE	California Competitive Grant Program for Research Viticulture/Enology
CDFA	California Department of Food and Agriculture
CDFA - 1232	California Department of Food and Agriculture - Funds From Assembly Bill 1232
Citrus Board	Citrus Research Board
Kern/Tulare	Kern/Tulare GWSS Task Force
Raisin	California Raisin Marketing Board
Riverside	County of Riverside
Table	California Table Grape Commission
UC - IPM	University of California Integrated Pest Management Project
UC - PD	University of California Pierce's Disease Grant Program
USDA	United States Department of Agriculture
USDA-APHIS	United States Department of Agriculture - Animal Plant Health Inspection Service
VC	Viticulture Consortium

Project Title :
Identification of Molecular Markers in the Grapevine's Response to Infection by *Xylella fastidiosa*

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Objectives of Proposed Research:

1. We have identified numerous genes that will be important in physiological and biochemical approaches to understanding ripening.
2. Many of the ripening-expressed sequence tags (ESTs) we found were related to cellular housekeeping functions such as protein synthesis, protein processing and turnover, and enzymes of primary and secondary metabolism.
3. Several others are pathogenesis related (PR) proteins, and while the number of such genes was unexpected, they provide an opportunity identify molecular markers in the grapevines response to infection by the pathogen that causes Pierce's Disease.

Justification and Importance of Proposed Research:

Goodwin et al. (1988a, 1988b) have studied the physiological responses of *Vitis vinifera* to infection by *Xylella fastidiosa* and have shown that water stress plays a major role in symptom development in diseased vines. Marginal necrosis and accelerated leaf senescence associated with the disease were both attributed to vascular dysfunction (Goodwin et al., 1988a). While these visible symptoms are useful for identifying affected vines in the field, they occur very late in the course of the disease and are thus of limited utility in studying the early events in the vine's response to infection.

In a second study Goodwin et al. (1988b) compared solutes, minerals and plant hormones in diseased leaves and healthy leaves. They found that diseased leaves had higher levels of glucose and fructose, and calcium and magnesium, but lower levels of potassium. They also showed that diseased leaves contained higher concentrations of the plant hormone abscisic acid (ABA), which is consistent with its role as a mediator of water stress in plants. Even leaves with low levels of symptoms (2 to 5% marginal chlorosis and necrosis) had elevated levels of ABA compared to healthy leaves. Symplastic ABA was nearly twice that in healthy leaves and apoplastic ABA levels were more than five times higher in diseased than healthy leaves. This is consistent with previous work showing increased levels of ABA in grapevine leaves with decreases in leaf water potential. Thus they concluded that "High ABA concentrations in diseased leaves appear to reflect water stress" (Goodwin et al, 1988b).

Many of the grape genes that we uncovered in our work on fruit ripening are related to water stress and the plant hormone ABA, which is thought to mediate some of the fruit's responses to decreased water potential. In the case of fruit the reduced water potential is probably related to the rapid accumulation of solutes in the fon-n of glucose and fructose, but in Pierce's Disease it would result from xylem blockage by the pathogen. Even though Goodwin et al. (1988b) showed that diseased leaves also contained elevated glucose and fructose levels compared to healthy leaves, they proposed that changes in the sugar concentration was a result of water stress and provided some degree of osmotic adjustment in the affected leaf. Thus we might expect that some of the genes we have identified in fruit that respond to solute accumulation and the concomitant decreased water potential, might also be found in leaves under water stress conditions. If we can identify a set of genes that respond to water stress in grapevine tissues, they could be used in several ways. For example, they could be used in conjunction with pathogen titers to determine the infection level required to elicit a response in the plant. Such a marker could provide a very early and very sensitive detection method for the disease in terms of the plant's response to infection. This would be a valuable tool to study the mechanism of resistance to the disease and could perhaps be useful in screening seedlings for resistance to the pathogen. The possibility of identifying a plant response marker for Pierce's Disease in the short term by taking advantage of genes we have already identified would seem to be one of the strong justifications for this project.

Project Title :
Sharpshooter Feeding Behavior in Relation to Transmission of Pierce's Disease Bacterium

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Objectives of Proposed Research:

1. To identify and quantify (by both frequency and duration) all feeding behaviors of GWSS on grapevine, and correlate them with location of mouthparts (stylets) in the plant and presence/ population size of *X. fastidiosa* in the foregut.
2. To identify the role of specific stylet activities in *X. fastidiosa* transmission, including both the mechanisms of acquisition and inoculation, and their efficiency.
3. 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.

Justification and Importance of Proposed Research:

For this proposal's 2-year time frame:
GWSS feeding behavior
Fundamentals of *Xylella* transmission
Mechanism and efficiency of pathogen transmission by vectors
Proportion of GWSS population carrying *Xylella.*

For future studies allowed by this work:
Identification and evaluation of sources of host plant resistance
Chemical, biological and chemotherapy control tactics

Much is known of the nutritional and physiological ecology of GWSS. However, almost nothing is known of its exact feeding behaviors, and how they interact with the population dynamics and colony behavior of *X. fastidiosa* (within the sharpshooter's foregut) to facilitate transmission to grapevine. The research proposed herein will combine all three of the most important and successful methods of studying leafhopper feeding behavior (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electropenetration graph monitoring) to definitively identify all details of feeding. This effort will provide much more information about feeding and *X. fastidiosa* transmission than can be provided by any one of these methods, used in isolation. In addition, the work will also answer other key questions, such as:

What specific "inoculation behaviors", performed how many times and by how many insects, will lead to inoculation of *X. fastidiosa* to grapevine, if the *X. fastidiosa* bacteria are present in the insects' foreguts? Can we detect the exact instant of *X. fastidiosa* inoculation to grapevine? What is the precise nature of a successfully inoculating "bug visit."

Project Title :
Sampling, Seasonal Abundance and Distribution of GWSS in Citrus and Grapes

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Objectives of Proposed Research:
1. Develop, test and deliver statistically-sound sampling plans for estimating density and inoculum potential of GWSS for research and management application.
2. Compare rates of movement between GWSS and the native smoke-tree sharpshooter to help understand changes in spread of Pierce's disease.
3. Correlate the effects of crowding, sex ratio, reproductive status, host-plant quality and environmental variables with population dynamics and movement of GWSS as an aid to predicting insect and disease spread.

Justification and Importance of Proposed Research:
The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), was first detected in California in 1990 (Sorenson and Gill 1996). Since that time it has spread throughout southern California and into parts of Kern County (Blua et al. 1999). This insect feeds on a variety of ornamental and crop plants, and in the process transmits the bacterium, *Xylella fastidiosa*, which is the causal agent of Pierce's disease (PD), as well as several other diseases. It is estimated that these diseases will cost the state of California millions of dollars and their spread now threatens the viticulture and ornamental industries of California. The work proposed here addresses two important and interrelated research areas crucial to the development of robust management strategies, sampling tools for estimating pest density and knowledge of biological and ecological factors affecting pest movement within an agroecosystem.

Crop protection begins with an understanding of how different densities of a pest impact the growth and development of a crop. The concepts of economic injury level and economic threshold were developed decades ago to relate pest densities to damage levels in crops and provide a rational basis for initiating protective action to avoid further damage (Stern et al. 1959; Poston et al. 1983; Pedigo et al. 1986). The key to incorporating these concepts into pest management is being able to precisely appraise relative densities of the target population on the crop so that timely counteractive measures can be imposed when economic thresholds have been attained. Further, evaluation of experimental treatments in basic and applied field research often depends upon reliable and repeatable estimates of pest density. Optimal sampling methods and plans should detect all key stages of interest, be representative and repeatable, be rapid, simple to use and sampler-independent, and provide density estimates with acceptable levels of confidence.

Little effort has been expended towards understanding the spatial distribution of GWSS and what densities of the pest on citrus and grape are important from a crop protection standpoint. Yellow-trap surveys have focused on relative density differences between citrus and grapes, or differences between perimeter and core locations within orchards and vineyards. Such comparisons are both valid and useful for understanding distribution patterns and inferring movement of GWSS and underscore the need for an efficient and sensitive trap that can detect early stages of infestation. At this time, however, yellow-trap catches can only be related to other yellow-trap catches, but cannot provide insight into a local area's population densities. Moreover, the most basic questions concerning trap design and efficiency cannot be reliably addressed without some comprehension of how many GWSS occur in a local area and how they are distributed. Similarly, basic questions of the occurrence and distribution of GWSS in a local area cannot be addressed without a statistically-robust sampling program based on vegetation samples. Elucidation of the distribution and abundance of GWSS on citrus and grapevines through intensive sampling and comparison to yellow-trap catches will advance efforts to identify critical densities of GWSS from a crop protection standpoint.

An additional goal of a sampling program would be to provide a methodology by which certain epidemiological parameters of GWSS problems in association with PD might be revealed. There is currently a poor understanding of

interrelationships among vector numbers, inoculum sources, and transmission rates that underlie the ongoing epidemic of PD in the Temecula region. Do numbers of GWSS in the region need to be reduced 10-fold or 1,000-fold before the epidemic is arrested? Or is it possible that modest reductions in GWSS populations in combination with protective treatments of vineyards could substantially slow the spread of *X. fastidiosa* and incidence of PD? Educated answers to these and other questions will be possible if more basic information is available on the distribution and abundance of GWSS in orchards and vineyards. One critical issue is how to sample GWSS to determine the proportion of the population that is inoculative with *X. fastidiosa.*

GWSS differs from native vectors of *X. fastidiosa* because it appears to be moving further into the vineyards and it can feed on the lower portions of the grapevine, which enhances vine-to-vine spread of PD. To date, most of the evidence regarding its dispersal ability is circumstantial, and the studies that have been conducted have not been done in a comparative manner. The fact that GWSS appears to be spreading faster than native vectors may not be the result of a higher propensity to engage in flight, but may simply be due to a higher reproductive potential and an expanded host range resulting in larger populations that are more easily detected with yellow sticky traps. Nevertheless, insect dispersal is a species-specific trait that can be influenced by numerous factors such as increasing population densities, reproductive status, biased sex ratios, host breadth, declining host quality and changing environmental conditions (Denno 1979, Taylor 1985, Blackmer and Phelan 1991, Blackmer and Byrne 1993a,b; 1999, Blackmer and Cross 2001). A better understanding of the factors that influence the dispersal of GWSS relative to other native sharpshooters will facilitate the development of sound strategies for managing GWSS.

One method for studying insect dispersal is the mark-release-recapture (M-R-R) technique (Southwood 1978). M-R-R involves marking insects with a stable, long-lasting material, releasing them in the field, and recapturing them at a given time interval after they disperse. Researchers have used a variety of methods to mark insects (Southwood 1978, Hagler and Jackson 2001), but several of these techniques interfere with the insects ability to disperse.

We have developed an insect marking procedure that is easy, rapid, safe, inexpensive, invisible, and stable (Hagler and Jackson 1998). The insect is marked externally by submerging them in or misting them with rabbit immunoglobulin G (IgG) solution or internally by allowing them to feed on the IgG solution. In field trials, the retention of two different IgG protein markers (rabbit IgG and chicken IgG) was compared with Day-Glo™ dust. All of the adult convergent lady beetles remained positive for 20 days and 75% were positive 30 days after their release. In contrast, only 50% of Day-Glo™ labeled individuals remained marked after 1 week (Hagler 1997a).

This marking technique, in combination with behaviorally active yellow-sticky traps and passive-interference traps, will be used to examine the dispersal of *H. coagulata* relative to other native sharpshooters (specifically the smoke tree sharpshooter). This study will help us better define whether the increased incidence of PD is partially explained by differences in flight activity. As numerous variables can influence insect dispersal, we also hope to determine how host-plant quality, crowding, sex ratio, reproductive status, and environmental factors influence the timing of and propensity to disperse by *H. coagulata*.

As with any biological problem, the nature of the problem ultimately comes down to an issue of numbers and movement as they relate to the incidence and spread of the problem. We have identified one research objective that follows a logical sequence in terms of developing an efficient and reliable sampling methodology and two objectives that will provide fundamental knowledge of pest dispersal and the factors influencing movement and distribution within the citrus and grape system. Together this research will provide a robust foundation for the development of management systems to combat this serious pest.

Project Title :
Impact of Sub-Lethal Doses of Neonicotinoids on GWSS Feeding and Transmission of Pierce's Disease

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Objectives of Proposed Research:
1. To compare GWSS feeding behaviors among healthy and PD-infected grapevines,-either treated or not with sub-lethal doses of neonicotinoids.
2. To compare rates of acquisition of *Xylella fastidiosa*. by GWSS from PD-infected grapevines treated with sub-lethal doses of neonicotinoid to acquisition from PD-infected grapevines not treated.
3. To compare inoculation rates of infective GWSS on healthy grapevines treated with sub-lethal doses of neonicotinoids to inoculation rates on grapevines not treated.

Justification and Importance of Proposed Research:
The current epidemic of Pierce's disease (PD) in Temecula has characteristics that are remarkably different from outbreaks previously known in California since the 1880's. At the crux of this difference are the remarkable speeds of the epidemic there, and the distance of disease spread from the edge of the vineyard. These differences can be accounted for by a new vector, the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata,* spreading PD. The most important aspects of the GWSS that account for the rapid epidemic of PD are twofold: (1) the GWSS has a propensity to fly long distances into a vineyard, thus spreading PD far and affecting a large percentage of grapevines, in contrast to the traditional spread of PD to the first several rows of a vineyard. (2) GWSS can feed on woody grapevine branches, accounting for what appears to be vine-to-vine spread of PD in California, a phenomenon previously unknown.

Management of PD that is spread by the GWSS is lacking a fundamental strategy and solid tactics. Although grape growing in areas with "traditional" California vectors (e.g. blue-green, red-headed, and green sharpshooters) is possible and practical, growing in the presence of high populations of the GWSS is not. Moreover, the GWSS is currently spreading throughout California, and will without doubt spread PD in other areas, thus putting grape-growing areas in the entire state at risk. Already, PD has been identified in table grapes in the Arvin area, north of Bakersfield (A. Purcell, unpublished data). This area has not been known for PD in at least 50 years, and whether or not those infected vines are associated with the GWSS now, they will be soon as the already-stable population in that area increases and spreads. An important goal to minimize PD spread in this area will include minimizing numbers of GWSS in vineyards, in order to reduce their ability to acquire the bacterium from infected vines and to inoculate uninfected vines. Removing diseased vines will be an important way of minimizing acquisition, but chemicals that reduce vector feeding may also keep infectivity of vectors at a low level. Although research is exploring avenues that attack the plant-pathogen interface (B. Kirkpatrick & E. Civorolo, U.C.Davis), there are no therapies available to "cure" infected plants. This makes vector control not only helpful, but also imperative. Of all the interactions that can be affected to control GWSS-spread PD, current technologies are closest to developing tactics that disrupt the interaction between grapevines and the GWSS (Blua et al. 2000).

Insecticides to control numbers and movement of the GWSS in vineyards will undoubtedly be part of a management strategy to control outbreaks of PD. Two aspects of insecticides are necessary: (1) they must affect GWSS immediately after they arrive on a vine; and (2) they must remain efficacious for a long time. Since 1998, we have experimented with insecticides to kill GWSS on grapevines (Blua et al. 2000). We chose to study insecticides of the chemical class known as neonicotinoids because of their reputed efficacy against sucking-insects, and their long residual activity.

The three neonicotinoids we examined that are the most effective are imidacloprid (Admire, Bayer Corp, Kansas City MO) and thiamethoxam (Platinum, Novartis Corp., Greensboro NC) applied to the soil, and foliar applications of acetamiprid (Assail, Aventis Corp. Research Park NC). Imidacloprid is registered for use in grapevines against leafhoppers, which includes sharpshooters. Acetamiprid and thiamethoxam are not registered for use in grapevines, yet representatives from Aventis and Novartis corporations have indicated that these products currently are in the process of registration in California for use in vineyards against leafhoppers.

Acetamiprid was exceptional in that it induced a quick “knockdown” because of its foliar-application. Yet, it maintained excellent efficacy to the end of the experiment, 8 weeks after application, inducing mortality in 81% (+ 8.3%) of the GWSS after 24 h of exposure to the treated vines. Imidacloprid at a full rate was the second most efficacious treatment, and the most efficacious soil-applied insecticide. After 24h of exposure, imidacloprid induced 63% (+ 8.2%) GWSS mortality in 1999 trials even 8 weeks after application. In an experiment conduced last year (1998), imidacloprid induced 93% (+ 6.2%) mortality under similar circumstance. Thiamethoxam was less effective in 1999, but more effective in 2000 trials.

In addition to killing sharpshooters on treated vines, A. Purcell's lab has been exploring the effects of imidacloprid on sharpshooter flight, movement, and transmission of *X. fastidiosa* to grape. Insecticides that increase vector movements from plant to plant could increase transmission, so determining the effects on plant acceptance are important. Preliminary results indicate that very high doses of imidacloprid have a repellent effect in lab assays, but only at doses that exceed currently approved rates of application. At "sublethal doses" that kill less than about 20% of GWSS within 1-3 days, there was no evidence for increased plant to plant movements or movements away from treated towards untreated plants positioned in 60 cm cages.

Anti-feedant qualities are one of the important aspects of neonicotinoids. In a 1999 experiment conducted at the University of California, Riverside, sharpshooters caged on field-grown grapevines treated with imidacloprid did not feed enough to generate visible amounts of excreta, a trait for which sharpshooters are known. Yet, sharpshooters on untreated vines generated a significantly larger volume of excreta than did controls. This strongly suggests they imbibed less xylem fluid. Our most recent experiment shows this effect for soil-applied imidacloprid and thiamethoxam, as well as for plants treated with foliar-applied acetamiprid. Most striking is our observation that imidacloprid applied to grapevines in September 1999 had a substantial impact on GWSS feeding a year later. This may, in fact, be more important to protecting plants from *X. fastidiosa* -carrying sharpshooters then inducing mortality. However, we have yet to document the impact of neonicotinoids on bacterial acquisition or inoculation.

Project Title :
Epidemiology of *Xylella fastidiosa* Diseases in California

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Objectives of Proposed Research:

1. Expand knowledge of the genotypic diversity, as well as distribution of genotypes, of *Xylella fastidiosa* strains isolated from grapevines and almonds in California.
2. Expand knowledge and understanding of grapevine, almond, stone fruits and citrus as hosts for California strains of *Xylella fastidiosa*.

Justification and Importance of Proposed Research:

Several diseases of agronomic, horticultural and landscape ornamental plants in California are caused by *Xylella fastidiosa* (Wells, et a], 1987; Hopkins, 1989; Purcell and Hopkins, 1996; Purcell, et al, 1999). Information about the epidemiology of these diseases is critical for development of effective disease management strategies, and is the basis for management of Pierce's disease (PD) of grapevines (Purcell and Hopkins, 1996; Purcell and Saunders, 1999; Purcell, et al, 1999). However, information about the epiderniologies of *X. fastidiosa* -caused diseases of other major agricultural commodities, and the epidemiological relationships among these diseases in California specifically, is not well understood. Moreover, it is not clear if the epidemiological information about PD in northern California is applicable to management of PD and other *Xylella*-caused diseases in other parts of the State. In addition, it appears that the introduction, establishment and spread of the glassy winged sharpshooter (GWSS) in California already have changed the epidemiology of PD and may be major a factor in *X. fastidiosa* transmission and increased incidence of PD and other *X. fastidiosa* diseases.

Project Title :
Development of An Artificial Diet for the Glassy Winged Sharpshooter (GWSS)

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Objectives of Proposed Research:
1. Development of an artificial diet for the glassy winged sharpshooter, (*Homalodisca coagulata*).
2. Development of a feeding system for presenting the diet as an in vitro procedure.
3. Development of a system of oviposition/ egg harvesting for perpetuating the colony that is to be reared on artificial diet.
4. Development of the other components of the rearing system that can be applied to mass rearing.

Justification and Importance of Proposed Research:
The glassy winged sharpshooter (*Homalodisca coagulata*) is a devastating pest in several cropping systems where it transmits the bacterium (*Xylella fastidiosa*) that is the causative agent for Pierce's disease. One of the chief factors behind the tremendous vectoring potential of this pest is its cosmopolitan selection of host plants from well over 100 species, representing dozens of families. For example, in the southeastern US where GWSS is native, it feeds on crepe myrtle, peach, grape, and a wide variety of other ornamentals and crops. Likewise, in its new habitats in California, it has been found to exploit a wide range of native plants (including the widespread live oak, willows, even xerically adapted yuccas), weeds that are virtually ubiquitous in California (such as tree tobacco and ragweed) and crops of a vast variety of growth forms and families. This cosmopolitan capability of GWSS makes it a most capable opportunist that can always find a "green bridge" from one host plant to another, increasing its likelihood of not only infesting the plants but also of vectoring the Pierce's disease bacterium, which is also widely distributed, but prior to the introduction of the GWSS was not efficiently carried from plant to plant.

These problems are coupled with the need to find alternatives to insecticides that are undesirable in some cropping systems because of concerns for development of resistance by target pests, damage to non-target arthropods, and toxicity to non-target organisms, in general. Virtually all alternatives measures for control of GWSS require or would profit from an ability to mass produce the target insect. Mass production of natural enemies for inundative (mass) release or even moderate scale production for inoculative release would be greatly simplified, if an artificial diet based rearing system for GWSS existed to support rearing of the natural enemy. It has been long understood that having to maintain plants to rear pests to serve as hosts for soon to be released natural enemies is not economical and therefore not practical. While it would be most economical to rear the natural enemies directly on artificial diets, the technology for this type of effort is still not developed enough for most parasitoids. Also, mass reared GWSS, produced on an inexpensive artificial diet and diet based rearing system would be potentially useful for other biologically based control technologies such as sterile insect release and sterile hybrid techniques. Also, experiments with genetically modified hosts, novel pesticides, and all other control innovations would be greatly enhanced by the availability of a ready supply of healthy pest insects during all seasons and throughout the world. The underlying need for these needs is the development of an artificial diet that Supports production of healthy, robust insects. The characteristics of a suitable artificial diet for the purposes described here are that the insects must be able to complete their life cycle on the diet, displaying normal growth, fecundity (production of offspring), fertility (egg hatch), and the insects should be able to resume their normal activities on the range of host plants to which their feral counterparts are adapted to use. The development of such a diet has not been achieved for GWSS (nor for many other homopterans). In fact, the xylem sap feeding Homoptera have proved to be very difficult to rear on artificial diet based systems.

Project Title:
Functional Genomics of the Grape-*Xylella* Interaction: Towards the Identification of Host Resistance Determinants

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Objectives of the Proposed Research:

1. Construction of cDNA libraries from infected and non-infected grape plants of both susceptible *V. vinifera* and tolerant/resistant *Vitis* species (e.g., *V. shuttleworthii* or *V. aestivalis complex*).
2. A total of 30,000 DNA sequencing reactions will be completed in the first year of the project from cDNA products of the above libraries. The resulting sequence information (i.e., Expressed Sequence Tags (ESTs) will be submitted to the National Center for Biotechnology Information (NCBI) in a simple annotated format.
3. An on-online relational database will be developed in Oracle 8 to distill relationships within the data, and in particular to estimate a minimum gene set expressed during *Xylella*-grape interactions. A Web-based interface to the project database will make the results of this project available to all Pierce's Disease researchers, with the intent of stimulating interaction among scientists and accelerating progress towards control of the *Xyella* pathogen in cultivated grapes.
4. Subsequent to EST sequencing and electronic data mining, we will employ functional genomics strategies to first verify and then dissect host gene expression in both susceptible and tolerant/resistant grape genotypes.

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

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

In the first phase of the project, a total of 60 individuals each from both chardonnay and cabernet plants in the Napa valley of California were randomly selected. For each grape variety, thirty plants were located close to riparian areas with a previous history of PD infection and 30 plants were located distally from the riparian habitat, in areas without previous PD infection. At two-week intervals, plants were analyzed for PD using a PCR-based approach with *Xylella*-specific primers. By early July the first symptoms of PD infection were observed, and PCR analysis confirmed that two chardonnay and three cabernet plants were infected by *Xylella*. These same plants gave positive PCR results in subsequent weeks, thus confirming the original diagnosis. By late September the frequency and severity of PD symptoms had increased, and tissue was again sampled for cDNA library construction immediately prior to the grape harvest. cDNA libraries are being constructed from these infected and non-infected plants, at time points corresponding to early and late disease development (i.e., early July and late September). DNA sequencing reactions are being carried out at the UC Davis College of Agricultural and Environmental Sciences Core Genome Facility (http://cgf.ucdavis.edu). By March 2002 a total of 30,000 cDNAs will be sequenced and analyzed. These data, corresponding to differences in the transcriptional profiles between infected and non-infected plants, are expected to include host resistance and susceptibility factors. Thus, they will provide the basis for new lines of experimental inquiry focused on testing the efficacy of specific host genes for PD resistance.

Project Title :
Biological Control of Pierce's Disease with Non-pathogenic Strains *of Xylella fastidiosa*

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Objectives of Proposed Research:
1. Construct deletion mutations in putative virulence genes of *Xylella fastidiosa.*
2. Test mutant strains for virulence in grapevines.
3. Test mutant strains for biological control of pathogenic strains in grapevines.
4. Compare sequences of virulence genes between the Pierce's disease strain and the CVC strain.

Justification and Importance of Proposed Research:
Competitive exclusion of plant pathogens with nonpathogenic or less virulent strains has been demonstrated for a number of bacterial, fungal, and viral pathogens. Many nonpathogenic mutants retain the ability to colonize either external or internal plant tissues, and if established first, can effectively compete for colonization and establishment of pathogenic strains. One advantage of this approach for biological control is that the biocontrol agent and target pathogen occupy the same niche and have similar requirements for growth and survival. In addition, the specificity of the biocontrol interaction reduces the possibility of undesirable non-target effects. We propose to construct several nonpathogenic derivatives of *Xylella fastidiosa* and test them for preemptive competitive exclusion of pathogenic strains in grape. In practice, such strains could be established in plants at the nursery level or potentially inoculated to mature vines. To construct nonpathogenic mutants, we will take advantage of the full enome 9 sequence of the citrus variegated chlorosis (CVC) strain of *X. fastidiosa* that will be published within a few months by a Brazilian consortium. We predict that genes that are likely to be required for pathogenicity can be identified through comparison of the CVC sequence with known pathogenicity gene sequences from its nearest relative, *Xanthomonas campestris*, or other plant pathogens. PCR methods will then be used to amplify these genes from the Pierce's disease strain of *X. fastidiosa*. Deletions will be created in the genes, and homologous recombination will be used to introduce each deletion independently into the Pierce's disease strain by a method that results in unmarked deletions. Each mutant will be tested for virulence and systemic colonization of grapevines, as well as the ability to competitively reduce populations of a pathogenic strain and reduce expression of symptoms. Comparative sequencing of pathogenicity genes from the Pierce's disease strain and the CVC strain will also provide important fundamental information related to virulence host specificity.

Project Title :
Epidemiology of Pierce's Disease in Southern California: Identifying Inoculum Sources and Transmission Pathways

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Objectives of Proposed Research:

1. Determine which plant species near vineyards harbor *Xylella fastidiosa* and serve as potential reservoirs of inoculurn for the spread of Pierce's disease to grapes.
2. Analyze samples of glassy-winged sharpshooter populations on grape and alternate hosts to detect the presence of *Xylella fastidiosa*, and identify the major sources of vectors that move the Pierce's disease pathogen into grapes.
3. Measure the ability of the glassy-winged sharpshooter to acquire and transmit *Xylella fastidiosa* to and from grape, citrus, and other plant species identified as potential hosts and sources of inoculum for the spread of Pierce's disease.
4. Develop and optimize methods to screen large numbers of plant and insect samples for the presence of Pierce's Disease.

Justification and Importance of Proposed Research:

Previous studies on the epidemiology of Pierce's disease of grape in Northern California have described systems dealing with different primary vector species and different alternate host plants than those that are found in the Southern California systems. Understanding the role that other plant species in the Temecula area may play in spread of Pierce's disease of grapes could be critical to management decisions. In addition to dealing with different host plants, the feeding habits and host range of the primary vector of the pathogen in Southern California differ from other primary vector species in California. Studies with the insect vector species present in Northern California suggest that the pathogen was primarily spread by vectors moving into vineyards from outside habitats, rather than spreading from vine to vine. There is little information available on the relative ability of the glassy-winged sharpshooter to acquire or transmit the Pierce's disease pathogen from vine to vine, or from alternate hosts to grape. Because in many cases the vineyards of the Temecula area are in close proximity to citrus groves, it is critical to know the relative inoculurn pressure that citrus and other plant hosts may provide in that area. Knowledge of the source of disease inoculum from vectors, whether from inside or outside the vineyard, will be critical to development of management strategies for disease control, such as the choice and management of plant species surrounding vineyards. Results of these studies, combined with data on seasonal fluctuations of sharpshooter populations, will also allow us to estimate the time of year and the regions where pathogen pressure is the greatest, and management strategies can be adjusted appropriately.

Project Title :
Control of Pierce's Disease Through Degradation of Xanthan Gum

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Objective of Proposed Research:

1. The goal of this proposed project is to exploit the natural occurrence of xanthan degrading bacteria in attempts to reduce the symptoms caused by *Xylella fastidiosa* in grapevines or its transmission by the glassy-winged sharpshooter.

Justification and Importance of Proposed Research:

Pierce's disease of grapevine, caused by *Xylella fastidiosa,* has been known in California for over 100 years (Gardner and Hewitt, 1974), but the recent arrival of a much more efficient vector, the glassy-winged sharpshooter, has greatly increased the threat of this pathogen to the grape industry (Blua et al., 1999). The CDFA Glassy-Winged Sharpshooter/Pierce's Disease Task Force list of research priorities to manage this threat included the testing of bactericides against *X. fastidiosa* as a high priority, and the use of endophytic bacteria that are antagonistic to *X fastidiosa* was listed as a medium priority. Our proposed use of bacteria that produce xanthandegrading enzymes combines these two CDFA research priorities through the use of endophytic bacteria, or potentially grape cultivars, that produce enzymes that target a specific virulence factor of *X. fastidiosa.* This approach has the potential to significantly reduce damage caused by Pierce's disease in grapes and potentially in other hosts of *Xylella fastidiosa,* such as almonds and oleander. If xanthan gum is important in the aggregation of the pathogen in the insect vector, then our approach may also reduce the efficiency of transmission of Pierce's disease. We have discussed this approach with other researchers involved in related research, particularly Dr. Bruce Kirkpatrick of UC Davis, who is exploring the use of antagonistic endophytes for biological control of Pierce's disease. He felt that this was clearly different than his approach, which involves the use of endophytes producing antibiotic substances rather than an enzyme that targets a specific virulence factor. We will, of course, continue to communicate and share information with Dr. Kirkpatrick, as we routinely do for our other work on genetic analysis of *Xylella* virulence. Both of us feel that the use of endophytic bacteria may be one of the most efficient ways of delivering substances that interfere with *Xylella* growth and virulence in the grapevine xylem. Another approach is to engineer grape plants to produce these substances. Our project is designed to first explore the use of xanthan-degrading endophytes, but through the cloning and characterization of genes encoding xanthan lyases, we will also facilitate possible efforts to transform grapevines to produce these enzymes.

Project Title :
Impact of Multiple Strain Infections of *Xylella fastidiosa* on Acquisition and Transmission By the GWSS

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Objectives of Proposed Research:
1. Analyze samples of glassy-winged sharpshooter populations on grape and alternate hosts to detect the presence of *Xylella fastidiosa*, and identify the major sources of vectors that move the Pierce's disease pathogen into grapes.
2. Assess the ability of glassy-winged sharpshooter to acquire multiple strains of *Xylella fastidiosa*.
3. Assess the ability of glassy-winged sharpshooter inoculated with multiple strains of *Xylella fastidiosa* to transmit either strain of the pathogen.

Justification and Importance of Proposed Research:
Xylella fastidiosa is a xylem-limited bacterium that causes a number of plant diseases such as Pierce's disease (PD) of grapevines, almond leaf scorch disease, alfalfa dwarf, citrus variegated chlorosis, leaf scorch of live oak, pear leaf scorch, and oleander leaf scorch (OLS) (Brlansky et al., 1982; Purcell and Hopkins, 1996; Purcell et al., 1999). Recent studies have shown that oleander leaf scorch is caused by a different strain of *Xylella fastidiosa* than the strain that causes PD (Purcell et al., 1999). Thus far, two strains of *X. fastidiosa* have been identified in Southern California, one that cause Pierce's disease of grapevines and almond leaf scorch, and another one that causes oleander leaf scorch (OLS). The strain that infects oleander does not appear to infect grape, and the strain that infects grape, does not appear to infect oleander.

Project Title :
Rootstock Variety Influence on Pierce's Disease Symptoms in Grafted Chardonnay (*Vitis vinifera* L.) Grapevines

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Objective of Proposed Research:
1. To evaluate the influence of rootstock variety on Pierce's disease in grape.

Justification and Importance of Proposed Research:
Pierce's disease of grapes (PD) is caused by the bacterium *Xylella fastidiosa*. The disease limits cultivation of *Vitis vinifera* grapes in parts of California and across the southeastern United States, Mexico, and Central and South America. Once infected, susceptible vines typically show symptoms of drought stress, beginning with necrosis of leaves (dried leaves and berries). Leaves dry up and fall, and roots, canes and entire vines eventually die in a severe PD infection. Indeed, this disease has wiped out entire vineyards. *Xylella fastidiosa* is spread by xylem feeding insects, including sharpshooters. The glassy-winged sharpshooter (*Homalodisca coagulata*), a Florida native, is an especially effective vector because of its large size and powerful flight. Other xylem feeding insects are also vectors. Viticulture in warm regions with summer rainfall is especially at risk, since *X. fastidiosa* can be moved from vegetated row middles to vines by insects that feed on the vegetation in the rows.

Some wild grapes native to PD endemic areas demonstrate resistance or tolerance to the disease. Grape varieties resistant or tolerant to PD are available. However, commercial acceptance of wines and table grapes from these varieties is low, due in part to flavors considered foreign and undesirable to palates trained to appreciate *V. vinifera.* Consumer wine perception and sales in the U.S. are often tied closely to varietal identification, and new varieties of grapes are usually only adopted slowly if at all.

California viticulture in particular is dependent on elite *V. vinifera* varieties. In wine grape production, premium fine wine varietals such as Chardonnay and Cabernet Sauvignon increasingly are being planted in warm interior valleys more viticulturally suited to "bulk wine" varietals such as Emerald Riesling, Colombard, and Ruby Cabernet. This is due to the high value consumers place on varietal identification—consumers are demanding recognized varietal wines, even in lower price categories. This market trend suggests high industry and consumer resistance to new varieties—even if new varieties are resistant or tolerant to PD.

Chemical control of the insect vectors of *X. fastidiosa* has been used to manage PD infection. Reductions in PD in vineyards adjacent to treated wilderness have been reported. However, only a few insect vectors are needed to saturate the vineyards with *X. fastidiosa* and bring about economic losses from PD. Insecticides may be hazardous to humans and other vertebrates or may kill beneficial insects in their wilderness habitat. Vegetation control to eliminate vector habitat is restricted in many areas due to deleterious effects including increased erosion and runoff. Control of the bacteria with tetracycline antibiotics has been considered, but is considered commercially unfeasible (Goheen and Hopkins 1988).

Rootstocks have been proposed as a possible tool to manage PD in grapes. Rootstocks are already in use in many vineyards for the management of soil-borne pests and diseases, such as phylloxera and nematodes. If grape rootstocks could contribute PD resistance or tolerance to their scions, this would be a major benefit to viticulture in areas prone to PD infection. Elite wine, juice, and table grape varieties could be grown in areas where viticulture is currently restricted to PD resistant and tolerant varieties whose consumer appeal is low.

In peach, also subject to *X. fastidiosa*-caused diseases, rootstock variety influenced insect vector occurrence and concentration of *X. fastidiosa* in the xylem (Gould et al. 1991), with lower levels of both found in scions grafted on a particular rootstock. In grape, Hewitt (1958) notes Pierce observed Mataro (*V. vinifera*) to be resistant to PD when grafted on St. George and cites Loomis as saying that the grape *V. champinii* does not develop PD symptoms and "when used as a rootstock, it also lengthens the life of the variety grafted upon it." Lu (personal observation) has observed *V. vinifera* cultivars including Thompson Seedless and Merlot to survive longer under high PD pressure (at Tallahassee, Florida) when grafted onto PD resistant rootstocks than when ungrafted.

Several investigators have examined the progress of Pierce's disease in ungrafted rootstocks. Magoon and Magness (1938) found Ramsey and St. George to be among the best performing rootstocks at Poplarville, Mississippi. Loomis (1958) specifically examined the response to PD of grape species selections and rootstocks grown ungrafted at Meridian and Poplarville, Mississippi and also identified St. George for its good performance under PD pressure.

Evaluations by Loomis (1965, 1952) particularly highlight the potential impact of rootstock variety on PD in scions. Loomis investigated the influence of rootstock variety on vine survival, not specifically on the impact of rootstock on scion PD symptoms. In his trials involving the PD susceptible juice grapes Concord and Catawba, no own-rooted vines survived in Mississippi at the end of an eight year trial period (1952). In contrast, all of the Concord and two-thirds of the Catawba vines grafted onto Dog Ridge survived, with many vines growing vigorously and producing good yields. Loomis studied a selection of rootstocks further (1965) and found that Concord grafted on Dog Ridge was still producing well at the end of a twelve year experiment. He specifically recommended Dog Ridge for rootstock use in the South because of its "tolerance" to PD (1965). Dog Ridge is recognized as not susceptible to PD (Loomis 1958) and has been used in breeding varieties with resistance to PD (Mortensen et al. 1994, Overcash et al. 1981).

Loomis pioneered the use of muscadine grape hybrids as grape rootstocks in his studies of rootstock influence on vine survival and productivity in the South (1952). A selection used in his trials, B4-5, promoted the yield and longevity of PD susceptible scion varieties. While B4-5 is not used currently as a rootstock, the muscadine hybrid rootstock O39-16 currently is available and is recommended for specific pest situations. Many muscadine varieties show excellent resistant to PD, but muscadines are characteristically difficult to graft to *V. vinifera* scions. Due to its muscadine grape parentage, O39-16 should be evaluated for its impact on PD expression in its scions.

Greenhouse screening has been used to investigate the PD resistance, tolerance, and susceptibility of grape plants and could be used to evaluate the influence of rootstocks on PD in grapes. 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 *X. fastidiosa* or insect vectors. Greenhouse screening based on leaf and stem symptom expression is tenuous—accidental drying out of the soil mix in a small pot can result in leaf scorching symptoms that closely mimic PD. Greenhouse screening often relies on tests that directly detect the presence of *X. fastidiosa* in the grapevine, whether through ELISA or PCR-based testing or by plating onto selective media. These tests are costly and time consuming to administer and require skilled personnel who are expensive to train and employ. Additionally, the presence of *X. fastidiosa* in the vine is not necessarily indicative of whether any symptoms will develop. Field screening is relatively cheaper, requiring no specialized equipment and can be accomplished quickly, with symptom expression being used as the main criterion. Mortensen, a grape breeder who focused on the development of PD resistant varieties, demonstrated the efficacy of field screening by using natural infection in Florida to identify PD resistant seedlings for selection and investigate the inheritance of resistance to PD (Mortensen 1967). Rootstocks, including those identified by previous researchers as being resistant, tolerant, or susceptible to PD, should be screened to assess their influence on PD expression. Disease expression in grafted and ungrafted vines of each rootstock should be compared to determine any correlation. If there is a close correlation between PD symptom expression in ungrafted rootstocks and PD that develops in the scions grafted to those rootstocks, rootstocks with potential for PD management could be selected more easily.

Project Title :
Biology and Ecology of GWSS in the San Joaquin Valley

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Objectives of Proposed Research:
1. Determine glassy-winged sharpshooter (GWSS) 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 GWSS in the San Joaquin Valley.
2. Determine the presence of *Xyella fastidiosa* in GWSS collected from different host plant species and in selected ecosystems in the San Joaquin Valley.
3. Begin to evaluate predator release as an additional suppression tactic (to be used where insecticide sprays are prohibited).

Justification and Importance of Proposed Research:
Table, raisin, and wine grapes grown in the San Joaquin Valley (SJV) comprise some of California's largest and economically most productive agricultural commodities. Their commercial existence is now threatened by presence of both the glassy-winged sharpshooter's (GWSS), *Homalodisca coagulata*, in the SJV (Phillips 1998, Blua et al. 1999) and the bacterial pathogen, *Xyella fastidiosa,* which it can carry. Xyella fastidiosa is a xylem-limited bacterium (Wells et al. 1987) that, in highly susceptible host plants, will clog the xylem and result in such severe water stress that the infected plant may die (Hopkins 1989). In grapes, *X. fastidiosa* is the causal agent of Pierce's disease (PD) (Goodwin and Purcell 1992).

Prior to the arrival of GWSS, the most common vectors of PD in California were native sharpshooters (Cicadellidae: Cicadellinae: Proconiini): the green sharpshooter (Draeculacephala minerva), the red-headed sharpshooter (Carneocephala fulgida) and the blue-green sharpshooter (Graphocephala atropunctata) (Freitag and Frazier 1954, Purcell 1990, Goodwin and Purcell 1992). While PD has long been present in the SJV (Goodwin and Purcell 1992), its spread and damaging effects were limited because breeding habitats of these sharpshooters feed on grapevines only accidentally (Purcell and Fraizer 1985). However, where PD coexists with efficient vectors in the southeastern U.S., it has precluded or severely limited grape production (Gardner and Hewitt 1974, Adlerz and Hopkins 1979).

GWSS may not be a more "efficient" vector of *X. fastidiosa* than the California sharpshooters (Purcell and Saunders 1999a), but it is certainly a more important vector for other reasons. It has a wide host range (Turner and Pollard 1959, Sorensen & Gill 1996, Blua et al. 1999), and feeds on many of the same plant species that host X. fastidiosa. It is a strong flyer that can carry (Brlansky et al. 1983) and move the bacterium great distances. It will feed on mature grapevine canes, as well as leaves, which maximizes the degree of PD transmission and decreases the likelihood that the bacterium will be lost during winter pruning.

The arrival of GWSS has dramatically changed the epidemiology of PD in California. This was clearly demonstrated in the Temecula Valley (Riverside County), where the combined presence of GWSS and PD resulted in millions of dollars of damage to vineyards in a very short period (Blua et al. 1999). For these reasons, initial control efforts against GWSS in the SJV will most certainly be directed at chemical suppression or spot eradication. Nevertheless, there are a number of questions on GWSS biology and ecology in the SJV that should be addressed in order to improve control programs and/or increase control options.

The primary focus of this research will be to describe GWSS age structure and population dynamics on different host plants in the SJV. Further, we will test sampled GWSS, from these different host plants and ecosystems, for the presence of S. fastidiosa. Research outlined in this proposal will improve suppression programs because there is, currently, little

information on GWSS age structure, ecology, or resident natural enemies (particularly predators) in the SJV. We expect important information garnered will include:

A description of GWSS biology and ecology on host plants resident to the SJV. This information will help redict GWSS seasonal movement. For example, information on the abundance, host plant use, and seasonal dispersal patterns of resident sharpshooters (e.g., blue-green sharpshooter) (Purcell 1975, 1979; Purcell and Frazier 1985) has greatly improved control of PD (Goodwin and Purcell 1992). The same critical information for GWSS is lacking for the SJV. Information from the proposed research will help describe important host plant associations, thereby providing information on the contribution of different host plants to the migrating or overwintering GWSS populations.

Second, indentification of GWSS phenology and ecology on non-agricultural host plants will provide some measure of its potential activity in Coastal and Northern wine growing regions. To this goal, it will be especially important to survey GWSS in riparian zones to provide an early indication of GWSS feeding preference and population structure in habitats that more closely mimic important PD areas elsewhere in the state. For example, plant species found in SJV riparian zones have previous been studied as PD hosts (Purcell and Saunders 1999, Freitag 1951, Raju et al. 1983), which will aid in studies of the role of GWSS in spreading plant diseases.

Third, this work will provide a needed baseline on resident natural enemies of GWSS in the SJV and their contribution to GWSS mortality. A research focus has been directed to eff parasitoids, (e.g., Gonatocerus ashmeadi Hymenoptera: Mymaridae), which has been found to parasitize as much as 80-95% of the eggs by theend of summer (Phillips 1998, Triapitsyn et al. 1998). However, there are numerous natural enemies that have been observed feeding on GWSS. This research will document densities of potential GWSS natural enemies among different host plant species or ecosystems. This work will benefit proposed programs on GWSS natural enemies (e.g. Hodler et al., Hagler et al.).

Fourth, information collected on GWSS movement and host plant succession in the SJV may be useful for future suppression tactics. For example, modification of surrounding vegetation or traps crops can potentially suppress GWSS movement into a vineyard. Data collected on GWSS seasonal abundance and host plant preference may be used to determine the potential of a "trap crop" program (see Hokkanen 191). The selection of a trap crop species will greatly depend on GWSS seasonal patterns and flight behaviors. GWSS appears to shift its host6 plant without regard to plant taxonomic grouping and may be more influenced by host plant condition. Therefore, the developmental stages and maturation sequence of the local flora may be critical factors in host preferences shown by GWSS in any given region.

Finally, identifying the incidence of S. fastidiosa in GWSS adults collected from different habitats in different geographic regions will aid researchers currently mapping out PD and S. fastidiosa sources in the SJV.

Project Title :
Genome Sequence of a Pierce's Disease Strain of *Xylella fastidosa*

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Objectives of Proposed Research:
1. The objective of this cooperative research project is to determine the complete sequence of the genome of a Pierce's disease strain of *Xylella fastidiosa.*

Justification and Importance of Proposed Research:
Isolate a strain of *Xylella fastidiosa* from Pierce's disease affected wine grapes in the Temecula area of southern California. Prepare total genomic DNA from this strain. Construct DNA libraries of DNA fragments from this strain. Determine the complete sequence of the genome of this strain with 8-fold redundancy using standard technology.

Several plant diseases are caused by different strains or variants of *X. fastidiosa* including diseases of important agronomic and horticultural crops such as Pierce's Disease (PD) of grapes, citrus variegated chlorosis (CVC), almond leaf scorch (ALS), phony peach, plum leaf scald, and oleander scorch (OS). PD, ALS and OS occur in California. Other *X. fastidiosa* caused diseases are potential serious threats to California citrus and stone fruit industries.

The complete sequence of the genome of a CVC strain of *X. fastidiosa* has recently been determined in Brazil. In addition, putative functions have been assigned to about 47% of the 2,904 predicted coding regions in this strain.

The overall objective of this cooperative research is to obtain fundamental information about the genome of *X. fastidiosa* in order to develop new, innovative strategies to manage diseases caused by this pathogen. Specifically, the sequence of the genome of an *X. fastidiosa* strain that causes PD in the Temecula Viticulture area in southern California will be determined, and functional genes will be identified by comparison with available data from the CVC strain of *X. fastidiosa*.

Project Title :
Xylella fastidiosa Genome Analysis – Almond and Oleander Comparison to Pierce's Disease Temecula1 and Citrus Strains

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Objectives of Proposed Research:
1. Close the genome of Oleander and Almond *Xylella fastidiosa* strains.
2. Compare the general genome structure of the Pierce's disease and citrus *Xylella* strains with both the almond and oleander strains.

Justification and Importance of Proposed Research:
Xylella fastidiosa is a bacterium that causes disease in several plant species. One strain causes Citrus Variegated Chlorosis in citrus trees (henceforward called CVC-strain); another causes Pierce's disease in grapevine (PD-strain); two other strains (among others) cause diseases in almond (a-strain) and oleander (o-strain). The Brazilian ONSA Network has undertaken a jointly funded project between the US Department of Agriculture (USDA) and FAPESP (Brazil) in order to sequence the genome of a grapevine derived *X. fastidiosa* clone that is responsible for the potentially devastating outbreak of Pierce's disease in Californian vineyards. This strain named Temecula1 has been sequenced by the Brazilian group generating over 15 fold coverage plus around 2000 paired cosmid ends (http://onsona.lbi.ic.unicamp.br/xf-grape/). The contigs and the cosmid ends were used to build a scaffold from which plasmid and cosmid clones are being selected to fill the gaps.

The CVC-strain genome has been sequenced (Simpson et al. 2000.) by the ONSA consortium (http://onsona.lbi.ic.unicamp.br/xf/). The PD-strain genome is currently being sequenced and analyzed by the AEG Network. The DOE Joint Genome Institute, in Walnut Creek, CA, has partially sequenced genomes (8-fold coverage) of the almond and oleander strains. The goal of this project is to complete the sequence of these two strains and compare them to the genomes of the PD and CVC strains. The sequences already available suggest that all *X. fastidiosa* subspecies are very closely related. This implies that the completion of the genome of four strains of this species would enormously increase our knowledge of *X. fastidiosa* genome structure and informational content since little genetic tools are available. Also for functional genomics and disease control a more definite comparison could be made in order to determine specific targets for future studies.

The proposed research is important to the grape and wine industry because it will increase our understanding of the pathogenicity mechanisms used by *X. fastidiosa*. This understanding will in turn get us closer to the goal of finding more cost-effective ways to deal with the various diseases caused by *X. fastidiosa*, and in particular with Pierce's disease.

By comparing and contrasting the PD-strain genome to the other three genomes we should get a very good picture of what the PD-strain has in common with the other three as well as what is unique about it. This will facilitate research aiming at PD control. On the other hand, this compare-and-contrast methodology requires that all four genomes be essentially complete. We cannot say that a gene is or is not present in a genome unless we are sure that the genome sequence is complete. This is the basic justification for going beyond the 8-fold coverage of the oleander and almond strains already achieved by the JGI.

Project Title :
Role of Type I Secretion in Pierce's Disease

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Objectives of Proposed Research:
1. Develop an effective functional genomics tool kit for efficient transformation and gene knock-out experiments in a PD strain.
2. Determine culture conditions for activation of type I secretion.
3. Determine the effect of type I secretion gene knockout experiments on pathogenicity of a PD strain on grape.

Justification and Importance of Proposed Research:
The results of obtaining the complete genome sequence a citrus variegated chlorosis (CVC) strain 9a5c of Xylella fastidiosa (Simpson et al., 2000) are at once both exciting and disappointing. Exciting because it is a first, ad it should serve as a continuing resource as more data is discovered that is useful for gene annotation. Disappointing because it is not obvious how the CVC strain 1) causes such severe damage to citrus; 2) nor how it achieved a host range on citrus or how it arose in the first place, and 3) where it came from. To determine the answers to these questions require functional genomics analyses, and this will be true of the Pierce's Disease (PD) strain, when its sequence is completed. The primary sequence data allows the formulation of hypotheses, but functional tools are required to test the hypotheses.

The PI has been privileged to be one of four international advisors on the Xylella fastidiosa Functional Genomics Steering Committee for Fundacao de Ampara a Pesquisa do Estado de Sao Paulo (FAPESP), which assembled and financed the group responsible for sequencing the first plant pathogen genome. I have evaluated all functional genomics proposals related to CVC submitted for FAPESP funding, and became aware of a severe limitation in most of the proposals: to date, no one has been able to move DNA into the CVC strain that was sequenced, 9a5c. This includes cloning vectors, marker-exchange/interruption vectors and transposons. That is, none of the most basic tools and techniques needed for functional genomics analyses have been applied to the 9a5c strain.

There are two practical difficulties in performing functional genomics analyses on any *X. fastidiosa* strain. First, *X. fastidiosa* strains are appropriately named and nearly fastidious; it takes roughly five days for a streak to appear on an agar plate using the best medium, and it takes about ten days for a liquid culture to grow to sufficient density to use the cells for most routine purposes (DNA extractions, conjugation, etc.). This problem definitely slows the pace of experimentation and should not be underestimated; there are no "overnight" cultures. The second problem requires work: it is not a simple matter to get standard DNA cloning vectors into any of the CVC or PD strains. Several labs around the world, including my own, have tried multiple times to introduce a variety of standard shuttle vectors, such as the pLAFR series (repP), the pUFR series created in my lab (DE Feyter et al., 1990); (rep W) and even tolling circle replicons, such as pKT230 (repQ) into either PD or CVC strains. Our lab successfully introduced pUFR047 into a PD strain one time by conjugation and only recently repeated the result. John Hartung reported introduction of a gybrid plasmid (a pUC derivative fused with a plasmid replicon derived from a CVC strain) back into a CVC strain (personal communication; manuscript submitted). However, this plasmid was introduced by electroporation into only one CVC strain, even though many were tried.

The problem with electroporation or transformation may be restriction-modification enzymes, which cleave introduced double stranded DNA. There are four Type I restriction endonucleases found in the CVC strain 9a5c genome (all gene numbers referenced here and throughout the remainder of the proposal are found at http://onsona.lbi.dcc.unicamp.br/xf/): XF0295, XF2721, XF2725, and XF2739). Each restriction enzyme present reduces conjugation frequency about two to three orders of magnitude, depending on the size of the plasmid (De Feyter and Gabriel, 1999). In our experience,

transformation/electroporation frequencies are reduced by at least another two orders of magnitude below conjugational frequencies. This is likely because restriction enzymes recognize double stranded templates and transformation involves only double stranded DNA, while conjugation involves single stranded DNA that is then made double stranded and may be hemi-methylated in the process. In any case, four restriction enzymes would therefore make it practically impossible to conjugate, and certainly not electroporate, any plasmid vectors, let alone vectors with inserts. There are several ways to circumvent this problem; my lab has considerable experience in this area and the first part of this proposal is devoted to solving this general problem in the first year and to make the results available to everyone as soon practical.

After the DNA entry barrier is solved, we can move on to functional analyses, proposed to begin in the second year. We propose to determine how the PD strain causes leaf scorch symptoms, on the belief that understanding how it causes symptoms should lead to methods to suppress pathogen growth, suppress the symptoms and control the disease.

Project Title :
Application of *Agrobacterium rhizogenes*-Mediated Transformation Strategies for a) Rapid High Through Put Screen for Genetic Resistance to Pierce's Disease in Grape that Maintains Clonal Integrity of the Recipient Host, and b) Rapid Screening for Virulence Determinants in *Xylella fastidiosa*

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Objectives of Proposed Research:
1. To provide a tool for understanding the action, and therefore the probable effectiveness and longevity, of selected genes conferring resistance against *X. fastidiosa*.
2. To take advantage of a rapid visual screen for *X. fastidiosa* recently developed by Drs. Bruening and Civerolo.
3. This assay will be evaluated for its ability to detect transposon-induced mutants of *X. fastidiosa* that Piffer in relative virulence.
4. This work is expected to be useful in predicting what types of functional genes in grape may prove useful in blocking pathogen virulence in grape, if individual virulence determinants can be identified.

Justification and Importance of Proposed Research:
Genetic resistance to plant disease is generally the most cost-effective control strategy, providing resistance genes can be identified and introgressed into susceptible lines. The ultimate goal of this project is to identify novel genes from either grape or heterologous plants that, when expressed in grape, will lead to disruption of infection or spread of the xylem-limited bacteria, *X fastidiosa.* There are several limitations currently to the rapid identification and deployment of genetic resistance to PD in grape. The bacterium cannot be studied directly in living xylem tissue, there is no useful genetic resistance in commercially used gape clones, and introgression of resistance from grape relatives by sexual crossing introduces substantial genetic variation. Introgression of resistance would be most useful if it were introduced directly into vegetative tissue from which that could be cloned directly without requiring recurrent selection to attempt to return to the original host genotype. From our perspective it appears that the best solution to the problem of resistance gene integration is to use plant transformation technology that maintains the clonal integrity of the recipient host. We have developed at CEPRAP a functional screen and assay for resistance genes in tomato using *A. rhizogenes* mediated transformation that also enables the direct introgression of cloned resistance genes into a susceptible host plant while maintaining the clonal integrity of the recipient plant following transformation. This technology was developed at CEPRAP as part of our ongoing studies to identify novel resistance genes from plant cDNA libraries. It should also be noted that, as part of this project, we will develop several techniques and tools that should be of use to other researchers.

Project Title :
Efficacy of Insecticides used for GWSS Control in Citrus

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Objective of Proposed Research:
1. Conduct pesticide trials in commercial citrus in Kern County to determine the efficacy of various products against GWSS.

Justification and Importance of Proposed Research:
Using registered insecticides and label rates, conducted a series of pesticide trials in which we treated trees in commercial citrus orchards and observed GWSS survival for 6 weeks post treatment. For each experiment, several rows of citrus were treated per insecticide with a commercial spray rig. After treatment, fresh GWSS egg masses were enclosed in cloth bags. The caged branches were examined each week for numbers of live and dead nymphs and the stages of nymphs are noted. Insecticides tested included Lorsban, Danitol, Success, Nexter, Admire, Baythroid, Sevin, and Agri-Mek.

Project Title :
Evaluation of Efficacy of Sevin Treatments in Porterville GWSS Infestation

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Objectives of Proposed Research:

1. Cloth bags enclosing egg masses: In the first survey, all of the trees and plants in two yards one mall front, and two highway exits are searched for GWSS egg masses. After treatment by the county with Sevin, the egg masses are enclosed in cloth bags. At weekly intervals, the bags are opened and the egg masses examined for emergence of nymphs and survival of nymphs. This post treatment sampling will be followed for 4 weeks.

2. Visual survey for live insects 3 weeks post treatment: In each of two suburban blocks in Porterville, all of the trees and plants in 8 treated and 8 untreated yards in each neighborhood will be surveyed for any live stage of GWSS approximately 3 weeks after the yards were treated with Sevin. These blocks were both heavily infested at the start of the spraying program.

3. Visual survey for live insects 3-4 weeks after the second treatment: In the same two suburban blocks in Porterville, repeat the surveys 3-4 weeks after the second round of Sevin sprays have been applied.

Justification and Importance of Proposed Research:
Three sets of glassy-winged sharpshooter (GWSS) surveys in the Porterville infestation are planned. The purpose of this program is to determine the efficacy of the Sevin pesticide treatment program in the Porterville area.

Project Title :
Screening Insecticides in Nursery Citrus for Efficacy Against Glassy-winged Sharpshooter

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Objectivesof Proposed Research:
1. Using commercial citrus nursery trees, evaluate the effects of organophosphate, carbamate, pyrethroid, and neonicotinoid insecticides on survival of eggs, nymphs, and adults of GWSS.

Justification and Importance of Proposed Research:
We have found that the neonicotinoids and pyrethroids are the most effective and longest residual insecticides for use in nursery citrus. The results of the experiments will be used to help guide citrus nurserymen in their treatment program for nursery stock that is to be shipped to un-infested areas.

Project Title :
A Monoclonal Antibody Specific to GWSS Egg Protein: A Tool for Predator Gut Analysis and Early Detection of Pest Infestation

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(Kent Daane, University of California at Berkeley; Heather Costa, University of California at Riverside)

Objectives of Proposed Research:
1. We plan to develop a monoclonal antibody specific to glassy-winged sharpshooter (GWSS) egg protein to use in an enzyme linked immunoassay (ELISA) to:
2. Identify key predators of GWSS by analyzing their gut contents for GWSS remains.
3. Differentiate GWSS eggs from taxonomically and visually similar species.

Justification and Importance of Proposed Research:
Pierce's disease is caused by a xylem-limited bacterium, *Xyella fastidiosa*, (Wells et al., 1987) that kills grapevines by blocking water movement within the plant (Hopkins, 1989). The bacterium spreads from plant to plant by xylem-feeding insects. Over the past 100 years Pierce's disease has been a periodic problem to grape growers in California. However, in the late 1990's a new epidemic began in California vineyards as a direct result of the introduction and establishment of the glassy-winged sharpshooter, *Homalodisca coagulata*. Several characteristics of GWSS make it potentially a more important vector of Pierce's disease than the native sharpshooters: the green sharpshooter (*Draeculacephala minerva*), the red-headed sharpshooter (*Carneocephala fulgida*) and the blue-green sharpshooter (*Graphocephala atropunctata*) (Purcell, 1990). First, GWSS appear to have greater dispersal ability than other vectors of Pierce's disease. Second, GWSS thrive on a wider range of host plants than other vectors. Finally, GWSS can feed low on the cane, thus increasing the number of chronically infected vines (i.e., the infection is not mechanically removed during normal pruning practices) (Varela et al., 2001).

Effective control of GWSS will require an integrated pest management approach. A major component of true integrated pest management is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can increase the effectiveness of other control tactics (e.g., chemical, mechanical, cultural, etc.). Unfortunately, very little information exists on GWSS natural enemies (Triapitsyn et al., 1998). This is especially true for their predaceous natural enemies (i.e., we could not find any peer-reviewed papers). Evidence of predation of GWSS eggs has been observed in the field (D. Morgan pers. comm.); 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. Historically, the study of insect predation has relied mainly on inexact and indirect techniques for measurement and analysis (Luck et al., 1988). This stems directly from the very nature of predation. Unlike parasitoids and pathogens, predators rarely leave evidence of attack. Highly artificial laboratory experiments can be used to evaluate the suitability of particular prey and the rates of predation (Orphanides et al., 1971; Henneberry and Clayton, 1985; Hagler and Cohen, 1991). However, these types of studies seldom translate to the field where the requirements of predator search are more demanding, many potential prey species are present, and predator and prey are subject to changing environmental conditions. Direct field observations are sometimes used to identify predators of key pests, but the small size and cryptic nature of predators and prey make direct observations difficult (Cisneros and Rosenheim, 1998). Furthermore, direct field observations are time consuming, labor intensive, and disruptive to the normal predator foraging process.

Other direct techniques such as the microscopic analysis of predator gut contents have been used but the process is highly labor intensive, inexact, and not suitable for predator species that liquefy prey contents for consumption (James 1961;

Hengeveld, 1980). Indirect techniques of gut analysis including the use of electrophoresis for identifying prey-specific enzymes in predator guts have also been used, but the technique is time-consuming, insensitive, and non-specific (Murray and Soloman, 1978). These difficulties have resulted in a deficiency of information on the impact that predators have on suppressing key insect pest populations. The most promising technique for measuring predation is use of immunologically-based assays employing pest-specific monoclonal antibodies (MAbs) (Whitten and Oakeshott, 1990; Greenstone, 1996).

Insect pest antigens can stimulate an immunological response in a vertebrate, which culminates in the production of serum polyclonal antibodies to the insect antigen. Once the antibodies have been produced in the vertebrate, they can be harvested and used as a diagnostic tool using any standard immunoassay format (Hagler, 1998). Unfortunately, polyclonal antibodies cross-react with other insect species (Davies, 1969; Lund and Turpin, 1977; Miller, 1981; Gardner et al., 1981; Doane et al., 1985). With advances in hybridoma technology, investigators can now isolate individual antibody-producing cells grown *in vitro* and harvest antibodies of single specificity (Kohler and Milstein, 1975). This is accomplished through cloning by limiting dilution until only one antibody with a single antigenic site is recognized in the cell culture (i.e., monoclonal). The result is an insect MAb that offers species and stage specificity unachievable with conventional polyclonal antiserum.

We have developed a library of MAbs specific to the egg stage of *Lygus hesperus*, *Pectinophora gossypiella*, and *Bemisia argentifolii* (Hagler et al., 1991, 1993, 1994) for use in studying egg and adult female predation in the field (Hagler et al., 1992; Hagler and Naranjo, 1994a,b). Our MAb library provided an avenue to qualitatively assess the impact of over a dozen predator species on populations of key insect pests; provided a quick, efficient, and cost effective technique for screening numerous predators in a conservation biological control program (Hagler and Naranjo, 1994a,b; Naranjo and Hagler, 1998); and provided a method to compare the efficacy of *in vitro*-reared predators with that of their wild counterparts in an augmentative biological control program (Hagler and Naranjo, 1996). We have optimized the use of pest-specific MAbs in an ELISA to assay over 1,000 predators per day.

Attempts to monitor GWSS populations and their natural enemies in Southern California are complicated by the presence of a native species of sharpshooter, the smoke tree sharpshooter, *Homolodisca lacerta*. The eggs of this species are virtually indistinguishable from those of *H. coagulata* with the naked eye. Thus it is difficult to separate the relative rates of predation and parasitism of GWSS and smoke tree sharpshooter in areas where these two species overlap. The similarity also prohibits positive identification of GWSS eggs intercepted during quarantine inspections of plant shipments. A pest-specific MAb can be used to accurately identify pests that are difficult to differentiate by the naked eye. For example, Greenstone (1995) developed an egg-specific MAb diagnostic test that differentiates *Heliothis virescens* from *H. zea*. Pest control advisors have used this MAb in a squashblot immunoassay to rapidly and positively screen field collected eggs. Early detection of *H. virescens* infestations is critical for effective and environmentally sound pest management. A MAb specific to GWSS egg would be an invaluable tool for early monitoring of pest infestation and decision-making in pesticide application. We propose to develop a GWSS egg-specific MAb that can be used in an ELISA to identify key predators of GWSS and differentiate GWSS eggs from taxonomically similar insect species.

Project Title :
Isolation and Characterization of GWSS Pathogenic Viruses

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Objectives of Proposed Research:
1. Isolate and characterize viruses pathogenic for the GWSS.
2. Establish continuous cell lines from embryonic GWSS tissues which support GWSS virus replication.
3. Develop nonhost production methods and increase GWSS virus efficacy by genetic modification.

Justification and Importance of Proposed Research:
The control of pest insects and insect-vectored diseases is vital for productive and profitable agriculture. However, in most agricultural situations there is a need for more selective, effective, and sustainable methods for crop and plant protection. Biological control offers such a method that is environmentally friendly. One biological control strategy that has recently been implemented against the GWSS in Southern California by UC Riverside and CDFA entomologists is the field release of a wasp that can parasitize the GWSS. Insect pathogens such as viruses which are often natural epizootic in the field offer another biological control strategy. In order to identify such epizootics, scientists have classically waited for an outbreak of disease and then tried to isolate the causative agent. Alternatively, one can take advantage of the fact that in any population there are multiple infective agents and look for these agents using biochemical and molecular methods. At present there are no known viral epizootics of the GWSS. In this proposal, we will isolate and characterize viral pathogens of the GWSS using traditional biochemical and molecular biological strategies, develop cell lines which support efficient replication of these viruses, and design nonhost production methods and attempt to increase the efficacy of these viruses. The GWSS viruses that are isolated and GWSS-derived cell lines that are established under this proposal will be made freely available to other researchers.

Project Title :
Potential of Conventional and Biorational Insecticides for GWSS Control

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Objectives of Proposed Research:
1. Evaluate Neem oil and IGRs for repellency, mortality, and other effects such as oviposition on GWSS and natural enemy populations.
2. Evaluate effect of all biorational approaches on the incidence and spread of Pierce's Disease.

Justification and Importance of Proposed Research:
The Glassy-winged sharpshooter (GWSS), Homalodesca coagulata has become a serious threat to California's grape industry because of its relatively high efficiency in transmitting the bacteria *Xylella fastidiosa* that causes Pierce's Disease (PD). The bacterium multiplies in the vine xylem tissue and ultimately limits water transport that results in the death of the vine. Historically, efforts to reduce insect vector populations with insecticides to prevent the occurrence of plant disease spread have not been very successful. More promising results have occurred with insect behavior modifiers such as repellents that prevent or limit the vector feeding or other activity.

Natural chemicals like leaf and seed extracts from the neem tree, *Azadirachta indica* A. Juss (Meliacae) are behavior-modifying substances. Insect growth regulators (IGR) that modify growth and development may also have a place in glassy-winged sharpshooter management. Also, incorporation of these biorationals in chemical control systems can lower selection pressure against GWSS by conventional insecticides and be an important part of resistance management.

Some of the products have biological properties such as repellency, feeding and oviposition deterrence, hormone like growth disrupting activity, and low mammalian toxicity. They are also less toxic to natural enemies of pests than synthetic insecticides.

Project Title :
Development of Trapping Systems to Trap the GWSS *Homalodisca coagulata* Adults and Nymphs in Grape

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Objectives of Proposed Research:
1. Determine the spectral sensitivity of the glassy-winged sharp shooter (GWSS) to both reflected and emitted light in order to choose the color providing the best reflectance for trap improvement and development.
2. Development of semi-selective intercept traps to monitor the Glassy-winged sharpshooters in grape and crops or ornamentals in close proximity to grape.
3. Relate trap catches to the number of sharpshooters in a grapevine or citrus tree and develop and test the effectiveness of sticky barriers to monitor GWSS nymphs.

Justification and Importance of Proposed Research:
The glassy-winged sharpshooter *Homalodisca coagulata* (GWSS) (Homoptera: Cicadellidae) was introduced into California in the late 1980s (Sorensen and Gill 1996, Phillips 1998). The glassy-winged sharp shooter is native to the southeastern United States (Young 1958) where it vectors the bacterium *Xylella fastidiosa* causing Pierce's disease (PD) in grape (Adlerz and Hopkins 1979), phoney peach disease in peach and nectarine (Turner and Pollard 1959, Mizell and French 1986), alfalfa dwarf, and several ornamentals and shade trees (Hopkins 1989). Since its introduction into California, it has become established in large numbers in certain areas. Pierce's disease has been a problem in California for more than 100 years, but the problems were occasional and isolated. GWSS is a more efficient vector of *X. fastidiosa* because it is a stronger flier than native California sharpshooters, and it can feed on the xylem of seemingly dormant woody stems. The wine industry in Temecula, CA has been seriously impacted by Pierce's disease losing about 30% of its vineyards to date making it the worst PD outbreak in California since the late 1800s. Strains of *X. fastidiosa* known to cause plant diseases in California include grape (PD), oleander (oleander leaf scorch), alfalfa (alfalfa dwarf) and almond (almond leaf scorch) (Purcell et al. 1999). The combination of PD and GWSS in California poses a serious threat to the wine and grape industries. One of the crucial components and cornerstones of an integrated pest management is the monitoring for the presence and density of a pest. Proper detection methods allow for optimum integration of biological, cultural, physical, chemical and regulatory measures to manage a pest. Yellow sticky traps have been used extensively in the southeastern U.S. for monitoring leafhoppers including GWSS in peach (Ball 1979, Yonce 1983) and citrus (Timmer et al. 1982). However, the reliability of current methods to detect the GWSS in California are questionable, and traps specifically designed for GWSS do not currently exist. To compound the situation, current methods are not standardized. For example, different sizes and shades of yellow sticky traps are being used in monitoring programs. Furthermore, the relationship of trap catches to actual populations of GWSS in grape or citrus are currently unknown.

Project Title:
Glassy-winged Sharpshooter Impact on Yield, Fruit Size, and Quality

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Objectives of Proposed Research:

1. Determine the impact of GWSS on fruit yield, size, fruit quality and size distribution when GWSS are controlled when compared to untreated blocks of Valencia oranges, 'Washington' navel oranges, and grapefruit.
2. Evaluate the effects of high GWSS populations on fruit quality (sugar/acid ratios, juice quality, peel thickness and firmness, susceptibility to post-harvest disorders) in Valencia oranges.
3. Evaluate the effects of large GWSS populations on water stress and nutrient loss.
4. Determine if Admire enhances fruit size, tree health and vigor.

Justification and Importance of Proposed Research:
Currently, management of GWSS in citrus is part of abatement programs assisting in the management of GWSS to limit the spread of Pierce's Disease in grapes. The advantages of managing GWSS on citrus in California (re: the impact on the citrus) are unknown. Additionally the availability of alternatives to conventional insecticides for management of the GWSS is currently limited.

Project Title :
Biocontrol of GWSS in California: One Cornerstone for the Foundation of an IPM Program

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Objectives of Proposed Research:
1. Exploit indigenous natural enemies of GWSS through improved understanding of their biology, ecology, phenology, and host plant preferences.
2. Increase the natural enemy fauna of GWSS by prospecting for additional biological control agents in the home range of GWSS (SE USA and NE Mexico) for release in California.
3. Investigate the feasibility and efficacy of augmentative releases of insectary reared GWSS parasites at times when natural enemy activity is low in the field.

Justification and Importance of Proposed Research:
We propose the development of a biological control program for the reduction of glassy-winged sharpshooter (GWSS) densities in California. Biological control of GWSS alone is unlikely to be the solution to the GWSS and Pierce's disease problem affecting gapes but will be an important cornerstone in an IPM program for this pest. A substantial reduction in vector numbers by natural enemies would have a significant impact on the viability and cost effectiveness of other control strategies that will need to be combined in an IPM program to simultaneously manage GWSS and Pierce's disease.

Project Title :
Mating behavior of the glassy-winged sharpshooter, *Homolodisca coagulata*

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Objectives of Proposed Research:
1. Determine the role of vibrational signals in mate recognition, attraction, courtship, and copulation.
2. Assess the feasibility of developing new or improved monitoring traps by using vibrational signals to attract adults.

Justification and Importance of Proposed Research:
Leafhoppers are among our most important agricultural pests. A tremendous amount of basic and applied research has been directed at their management. Most research, however, has focused at the population and community level. Little research has done on leafhopper mating systems. Yet, theoretical and some experimental research on leafhoppers and their relatives (planthoppers) clearly indicate that seasonal patterns of abundance and dispersal are intimately linked to a species mating system. Thus, determining rules that govern mating behavior may ultimately contribute an understanding of population and community level processes (Thornhill and Alcock 1983, Hunt and Nault 1991, Ott 1993). For example, laboratory studies of mate finding tactics in the leafhopper *Graminella nigrifrons*, a vector of maize chlorotic dwarf virus, led to accurate predictions about the effects of leafhopper gender and mating status on the spatial pattern of disease in the field (Hunt and Nault 199 1, Hunt et al. 1993).

Experiments proposed under objective one represent a logical starting point for elucidating the mating system of the glassy-winged sharpshooter. Although beyond the scope of this proposal, initiation of these studies may ultimately contribute to an understanding of local and regional dispersal, seasonal phenology, population dynamics, and disease epidemiology. Importantly, determining the repertoire of signals emitted during mating and their function will be critical to the success of experiments described under objective two.

Experiments proposed under objective two are designed to assess the feasibility of developing new or improved monitoring traps. Current methods for monitoring populations (yellow sticky cards and scouting for egg masses) have serious limitations. The attractiveness of sticky cards varies throughout the year; thus the number of individuals captured is not correlated to population density. Scouting for egg masses is labor intensive. To my knowledge there is no precedent for the use of mating signals to monitor leafhopper populations, although acoustic monitoring devices have been developed to detect infestations (e.g. grain samples and wood structures) (see Mankin et al. 2000, for references). If my experiments indicate that vibrational signals enhance the efficiency of current traps, it will be relatively easy to develop cost-effective prototype traps for further development and testing. Such traps would valuable to state and private efforts to detect new infestations and to monitor established populations.

Project Title :
Classical Biological Control of *Homalodisca coagulata*

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Objectives of Proposed Research:

The program will encompass the total array of activities available for classical biological control from:
1. Climate matching.
2. Taxonomic review.
3. Foreign exploration.
4. Quarantine evaluation.
5. Release and post-release evaluation and
6. We propose to support collections in South America, provide technical support for insect and host plant colonization, quarantine evaluation of parasitoids, and in release and evaluation in California.

Justification and Importance of Proposed Research:

The successful importation, release and establishment of new natural enemies of the glassy-winged sharpshooter (GWSS) would significantly reduce populations and lower the rate of transmission of Pierce's Disease.

We propose to engage ARS' South America Biological Control Laboratory, Hurlingham, Agrentina, in the collection of sharpshooter eggs and nymphs, colonize emerging parasitoids, and ship them to APHIS quarantine facility, Mission, TX where they will be colonized on GWSS and identified by Dr. Serquei Triapitsyn (UC-Riverside). Parasitoids will be evaluated for efficacy on GWSS and non-target hosts, where available. The most promising species will be transported and released at predetermined sites in California. Subsequently, GWSS will be monitored for parasitoid establishment, dispersal and effectiveness.

ARS' Systematic Entomology Laboratory, Beltsville, MD has initiated a taxonomic review of *Homalodisca* and related genera, and will collaboratively produce a map depicting sharpshooter distribution throughout South America. Climate matching is in progress, so collection sites will be identified.

Project Title :
Studies on Bacterial Canker and Almond Leaf Scorch

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Objectives of Proposed Research:
1. Determine timing during the growing season when almond trees are susceptible to infection by *Xylella fastidiosa*.
2. Inoculate shoots of peach/almond hybrids and other almond species to determine their relative resistance/susceptibility to *Xylella fastidiosa*.
3. Determine whether *Xylella fastidiosa*. can pass through a high-worked peach rootstock to infect individually grafted almond scions.

Project Title :
Production and Screening of *Xylella fastidiosa* Transposon Mutants and Microscopic Examination of *X. fastidiosa* -Resistant and Susceptible Vitus Germplasm

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Objectives of Proposed Research:
1. Produce and screen 5,000 random Tn5 mutants of *Xylella fastidiosa* for virulence in chardonnay grapevines and in vitro attachment to chitin and cellulose substrates.
2. Examine xylem morphology, rate of colonization and production of tyloses and pectic materials in *Xylella fastidiosa* -resistant and susceptible Vitis/Muscadinia grapes.

Justification and Importance of Proposed Research:
Our group has been addressing several research objectives in cooperation with the Long Tenn Research Project on Pierce's disease that has been funded by the American Vineyard Association, California Department of Food and Agriculture and the USDA- funded, Viticulture Consortium. One of our projects involved the development of a transformation and transposon mutagenesis system for the bacterium that causes Pierce's disease (PD), *X. fastidiosa.* I am very pleased to report that due to the efforts of the graduate student that has been working on this project, Ms. Magalie Guilhabert, we now have 135 Tn5 transposon mutants from her first attempt using a newly developed transposon mutagenesis system and have more recently produced 85 Tn5 mutants of the Temecula strain that is currently being sequenced in Brazil. . The kanamycin-resistant, mutants Tn5 were confirmed by Southern blot and more important the insertions are random throughout the genome which greatly facilitates the knocking out and identification of *X. fastidiosa* genes that may mitigate plant pathogenesis and insect transmission. At least 6 other labs around the world have been attempting to achieve this breakthrough in *X. fastidiosa* research. We are now requesting additional funds to produce and screen 5,000 Tn5 mutants, a number which should give us good coverage in our attempts to knock out nonvital *X. fastidiosa* genes that are probably involved with plant pathogenesis and/or insect transmission.

We are also requesting funds to support detailed microscopic studies on xylem morphology and relative rate of *X. fastidiosa* colonization in *X. fastidiosa*-resistant (Muscadinia hybrids) and *X. fastidiosa*-susceptible (Vitis *vinifera,* cultivar Chardonnay) grapes. Although regular microscopy can be used to better understand the mechanisms involved in this host/pathogen interaction, we are currently in the process of putting the green fluorescent protein (GFP) into *X. fastidiosa* cells using the transposon system developed in our lab. GFPtagged *X. fastidiosa* cells will be easily viewed inside grape xylem vessels using confocal, epifluroscent microscopy. This study will provide basic information on the mechanism(s) involved with arresting the colonization of grape by *X. fastidiosa,* and probably give some idea whether a few or many genes are involved in this resistance. Information on the basic morphology of xylem architecture in susceptible and resistance grape varieties is minimal. It will be important to determine whether resistant varieties differ in element size, the quantitative and temporal production of tyloses and gums in response to the presence of *X. fastidiosa* cells in the xylem. Such information will be of significant value in locating and developing PD-resistant grape cultivars and if morphological markers associated with *X. fastidiosa* resistance can be identified they will greatly facilitate the screening of progeny in Walker's breeding project. This study will not duplicate, but rather complement, the microscopy study of Purcell who is examining the colonization of *X. fastidiosa* in systemic, non-systemic and microsite infection plant hosts.

Project Title :
Biological, Cultural, and Chemical Management of Pierce's Disease

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Objectives of Proposed Research:
1. Understand how *Xylella fastidiosa* moves, and the patterns of its movement, in systemic (grape, blackberry) and non-systemic (willow) plant hosts using microscopy. (AHP, PCA, BCK)
2 Understand how temperature influences the movement and survival of *Xylella fastidiosa* and the incidence and/or severity of PD. (AHP)
3. Determine whether vegetation barriers between riparian areas and vineyards and/or insecticide-treated "trap crops" at the vineyard edge can reduce the incidence of PD. (AHP, EAW, BCK, MAW)
4. Develop transformation / transposon mutagenesis systems for *Xylella fastidiosa* using existing or novel bacterial transformation vectors. Use *Xylella fastidiosa* mutants to identify bacterial genes that mediate plant pathogenicity, movement, or insect attachment. (BCK)
5. Isolate and identify endophytic bacteria that systemically colonize grapevine. Develop methods to genetically transform grape endophytes to express anti- *Xylella fastidiosa* peptides. (BCK)

6a. Develop a genetic map to *Xylella fastidiosa* resistance using *V. vinifera* X (*V. rupestris* X *M. rotundifolia*) seedling populations and AFLP (amplified fragment length polymorphism) markers, identify resistance markers, and identify potential resistance genes. (MAW)

6b. Utilize DNA markers for resistance to rapidly introgress *Xylella fastidiosa* resistance into several *V. vinifera* winegrapes and/or utilize genetic engineering procedures (when available) to move above identified *Xylella fastidiosa* resistance genes into winegrapes. (MAW)

Obj 7 — Shared funding with CDFA 50/50

7a. Determine the resistance of 10 grape genotypes to PD after mechanical inoculation and natural infection with *Xylella fastidiosa*. Elucidate the xylem chemistry of these grape genotypes and statistically correlate both chemical profiles and specific molecular markers to PD resistance. (PCA, MAW)

7b. Determine the resistance of common host plants (willow, resistant; blackberry, susceptible) to *Xylella fastidiosa* and discern the relationship of specific chemical profiles to resistance. Utilize these techniques to examine resistance mechanisms of resistant seedlings identified in 6a. (PCA, AHP)

7c. Validate the influence of chemical profiles and specific chemical markers on the growth and survival of *Xylella fastidiosa* by tests in in-vitro culture. (PCA)

Justification and Importance of Proposed Research:
The systemic movement of *Xylella fastidiosa* within the plant xylem system is essential to this bacterium's ability to cause disease and probably for its indefinite survival in natural environments. Numerous microscopic studies of plants affected by diseases caused by *X. fastidiosa* (for example: Davis et al. 1978, 1980, Mollenhauer and Hopkins 1974, Lowe et al. 1976, Kitajima 1981) have revealed high concentrations of bacteria in some xylem cells, but it is notable that in all of these studies, adjacent xylem elements are often devoid of bacteria. The important basic question of how the bacteria move from cell to cell is still unanswered.

Our initial hypothesis is that bacterial multiplication is an important requisite for cell-to-cell movement. Do *X. fastidiosa* populations in nonsystemic hosts reach high or low densities within infected cells? Do *X. fastidiosa* populations in systemic hosts with low populations of *X. fastidiosa* (as determined by dilution plating) attain high populations in few cells or lower populations in many cells? We will examine the behavior of *X. fastidiosa* in nonsystemic hosts such as willow and mugwort and in plants with low, but systemic populations, such as blackberry. The occlusion of xylem cells

with *X. fastidiosa* in willow, for example, would illustrate that bacterial aggregates that completely fill xylem cells is not sufficient for systemic movement.

The *X. fastidiosa* oleander strain is not systemic in grape and vice versa (A.H. Purcell et al., unpublished data). The typical fate of *X. fastidiosa* in most woody plant species is to multiply without systemic movement (A.H. Purcell, unpublished). We will investigate if this is true for the oleander strain in grape and the grape strain in oleander. Both of these strains are systemic in their pathological hosts but not in the opposite host. We will seek to identify whether oleander strains multiply in grape using dilution plating on solid culture medium (PW) and confocal fluorescent microscopy.

A recent development that aids the study of bacterial movements in plants is the emergence of scanning confocal laser microscopy (SCLM). With SCLM, the specimen is scanned with a focused laser beam and the fluorescent signals are detected by a photomultiplier. Because only signals arising from the focused plane are detected, with SCLM, non-destructive optical sections of a specimen can be viewed with minimal out-of-focus fluorescence such as plant autofluorescence (White et al. 1987). The new techniques to study bacterial biofilms could provide valuable information on the distribution of *X. fastidiosa* within xylem tissue. The application of the SCLM coupled with image analysis techniques permits the study of living, fully hydrated microbial biofilms (Lawrence et al. 1991). Success in introducing novel genes into *X. fastidiosa* (Objective 4) to create new but grape-virulent strains with reporter gene constructs allowing easy *X. fastidiosa* detection in grape tissues would greatly facilitate studies of *X. fastidiosa* movement in plants. Such a system could enable *X. fastidiosa* detection with SCLM in plants without fixing, dehydrating, staining, or otherwise preparing plant specimens. Thus the same tissues could be examined repeatedly to follow *X. fastidiosa* movements, especially those events associated with the cell to cell movements that are critical to disease. If *X. fastidiosa* can be genetically engineered to express the green fluorescent protein (GFP) gene, the movement of bacteria could be followed through the plant similar to the methods used in studies on the movement of *Erwinia amylovora* in apple (Bogs et al. 1998). Our use of GFP-mutants for histological studies of movements or of biofilm formation will depend upon success of Objective 4. The GFP-mutants of *X. fastidiosa* will be introduced into plants by needle puncture (detailed in Hopkins 1980). Sections of tissue from the point of inoculation will be made at various intervals (3 days to 8 weeks) and examined using SCLM and SEM. Repeated experiments may use different intervals based on our initial results - beginning perhaps as soon as a few hours after inoculation, for example.

An understanding of biofilms may also help explain *X. fastidiosa* movement and pathogenicity. A biofilm is an aggregate of attached cells (Whiteley et al. 1997) produced when bacteria adhere to a surface, initiate glycocalyx (exopolysaccharide) production and form microcolonies (Costerton et al. 1995). *X. fastidiosa* appears to produce biofilms that are unique compared to other documented biofilms in that they are inhabited only by a single bacterial species and occur within plant xylem and insect guts. Others have speculated the matrix material surrounding aggregations of *X. fastidiosa* within plants improves the bacterium's extraction of nutrients and provide physical protection (Davis et al. 1980, Hopkins 1989). We suspect that biofilm formation is also critical to *X. fastidiosa*'s movement from cell to cell within plants and necessary for its survival within the vector foregut, from which it is transmitted to plants by insects. We examine *X. fastidiosa*'s occurrence within a spectrum of host plants — from those in which it multiplies rapidly but does not exhibit systemic spread (willow); to those in which it multiplies and moves but does not reach high enough levels to cause disease; to pathological hosts such as grape. We will also develop methods to produce and examine biofilms under in vitro conditions so as to be able to experimentally manipulate environmental conditions and determine their effects on biofilm formation. If we are successful in developing *X. fastidiosa* transformation protocols (Objective 4), we should be able to provide conclusive genetic evidence for the potential role of biofilms in plant pathogenesis or insect transmission by knocking out biofilm biosynthesis gene(s) using transposon mutagenesis.

Other Investigators: Alexander H Purcell (AHP), Edward A. Weber (EAW), M. Andrew Walker (MAW) and Peter C. Andersen (PCA)

Project Title :
The Development of Pierce's Disease in Xylem: The Roles of Vessel Cavitation, Cell Wall Metabolism and Vessel Occlusion

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Objectives of Proposed Research:
1. This proposal is directed toward discovering the plant responses to infection that are fundamental to the progression of Pierce's Disease (PD) in grapevine.
2. To describe the establishment of *Xylella fastidiosa* infection in grapevines and the subsequent progression of PD in terms of "indicators" that are based on alterations in plant water status and xylem function.
3. Use the appropriate indicators as well as measurements of physiological, biochemical and hormonal factors to assess the role in PD development of grapevine cell wall metabolism that is triggered by *Xylella*.

Justification and Importance of Proposed Research:
PD is devastating; often infected vines die in two years. Yet, other than quantifying low leaf water potentials in symptomatic leaves, previous work has resolved little about how the disease kills vines. A key component of this proposal is to establish for the first time a quantitative time course of bacterial growth, xylem occlusion, impaired water transport, and water stress in infected leaves. This information is essential for subsequent efforts to be directed at the specific pathology that gives rise to leaf and vine death in PD.

The rapid progression of PD implies that either the plant response to infection quickly becomes systemic or that the bacterium moves quickly into the many vessels that transport water in the grapevine. Both possibilities implicate plant cell wall metabolism in disease progression. Radial movement of the bacterium among vessels would require breakdown of pit pore membranes between adjacent vessels. Axial movement similarly would require breakdown of intervessel pit pores. Alternatively, vascular gels may be generated as part of the vine response to infection, a phenomenon with which we have considerable experience (VanderMolen et al., 1983, 1986). Thus, a second important component of the proposed work is the identification of the vessel occluding material and discovering whether bacterial entry is necessary for loss of vessel function.

Vine water relations and Pierce's Disease. Fundamental to combating a disease is understanding how a disease develops, especially the early events in the process. Unfortunately, most work on PD has utilized leaf scorch (i.e. tissue death) symptoms as the signal to collect data. We are unaware of any work that has followed changes in water transport and water status early in the infection process, prior to the appearance of these more "terminal" symptoms.

An important hypothesis of this proposal (tested in Objective 1) is that symptoms of water deficits (decreased water potential and water conductance) occur early in infection, well before visible symptoms of desiccation; and, may not develop strictly in proportion to the growth of the *X fastidiosa* population. We have considerable experience in evaluating water transport in grapevine (Schultz and Matthews, 1988, 1993). The low number of occluded vessels in symptomatic vines (Purcell and Hopkins, 1996) raises at least two possibilities: first, that symptoms develop prior to extensive vessel blockage with bacteria or vascular gels, probably due to cavitation and embolism. Zimmerman (1983) proposed that the loss of water transport in infected vessels is due to cavitation well before occlusions are detectable. The work described here will resolve whether infected vessels become dysfunctional as a consequence of cavitation or occlusion.

Project Title :
A Survey of Insect Vectors of Pierce's Disease (PD) and PD Infected Plants for the Presence of Bacteriophage that Infect *Xylella fastidiosa*

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Objectives of Proposed Research:
1. To screen wild Graphocephala atropunctata and Homalodisca coagulata and plants with PD for the presence of bacteriophage.
2. To test any/all acquired bacteriophage for it's/their ability to infect and destroy *Xylella fastidiosa*.

Justification and Importance of Proposed Research:
Pierce's Disease (PD) is an incurable disease of grapevine caused by strains of *Xylella faslidiosa.* The bacterium gains entrance into grapevine through the feeding activities of the blue-green sharpshooter, Graphocephala afropunclata (Purcell, A.H., 1975) and the glassy-winged sharpshooter, Homalodisca coagulata (Purcell et al. 1979). PD is endemic to California, however, with the recent detection of the glassy-winged sharpshooter (GWSS) in California, patterns of PD distribution are likely to change and host plant infection and/or associated plant death rates are likely to soar, threatening a variety of commodities other than grapes, including citrus and almond (Purcell, pers. commun.).

The serious nature of GWSS in California and associated PD concern mandates rapid pest management. A combination of approaches will likely need to be incorporated including traditional and new approaches. I propose to take the latter approach investigating the possible use bacteriophage therapy to control PD. Bacteriophage (phage) therapy is considered to be an unconventional pathogen countermeasure where viruses are used to kill specific bacteria. Recent successful endeavors using phage to control Laclococcus garvieaea infection in yellowtail (Nakai et al. 1999) and the discovery that the natural antibiotic in dog saliva is a bacteriophage (Matzinger and Amheiter, 2000) lend momentum toward the exploration and use of novel ways to control bacterial infections.

To determine if phage may be used for control of PD, specific phage must be sought in living systems that contain *Xylella fastidiosa* or at least are exposed to *X. fastidiosa.* Therefore, I propose to examine wild blue-green and glassy-winged sharpshooters and PD-infected plants for the presence of bacteriophage. I am confident that phage will be found because I have recently detected phage in a variety of insects that I have examined (Lauzori, unpubl.). Once phage are isolated then experiments can go forth to determine if phage therapeutics may have a place in PD management.

The potential benefits of finding phage against *X. fastidiosa* are numerous. The phage potentially could control PD alone, or serve as a delivery system for a variety of therapeutic products, such as protective antigens for immunization of plants and insects against *X. fastidiosa*. It should be noted that manufacture of phage is inexpensive.

Project Title :
Developing a Novel Detection and Monitoring System for the GWSS

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Objectives of Proposed Research:

1. The primary objective of this project is to develop attractants for detection and monitoring the glassy-winged sharpshooter, *Homalodisca coagulata*.
2. One approach (strategy I) is based on the extraction, isolation, identification and synthesis of leaf compounds from plants that attract sharpshooters and other leafboppers.
3. The other potential attractants will be screened by a binding assay with an olfactory protein, odourant-binding protein (OBP), involved in the filtering of chemical signals in the insect antennae. Initially, OBP(s) from the GWSS will be isolated, cloned and expressed in bacteria.
4. Screening of candidate attractants with a binding assays using the recombinant protein will save considerable amount of time with field tests. Compounds that do not bind to OBP(s) cannot be transported to the olfactory receptors of the GWSS and, consequently, do not need to be tested. By contrast, ability to bind suggests a potential role in the insect olfaction. Synthetic compounds originally identified in plants (strategy I) as well as candidate compounds that bind the OBP(s) of the GWSS will be tested in the field in order to evaluate their potential for monitoring population levels of the GWSS. Depending on the potency of the newly developed attractants, we would evaluate their feasibility for utilization in detection and monitoring programs.

Justification and Importance of Proposed Research:
Attractants for the GWSS are critically needed to detect early invasions of this vector. Once detected using current technologies, their population densities may be too high to achieve eradication or effective control. Pheromones and other semiochemicals are invaluable tools for quarantine and monitoring insect populations. Although it is unlikely that chemical communication is the major means of sex recruitment in the GWSS, they do utilize chemical signals to locate host plants. It may be possible to discover plant-derived or other attractants for the GWSS by screening candidate compounds in a trial-and-error fashion, but this type of approach is extremely time-consuming and may take decades to accomplish (see Leal, 1998). Screening compounds which are detected by the insect antennae and generate electrophysiological signals (strategy 1), or bind to odourant-birding proteir(s) 9nd are transported to the olfactory receptors (strategy 11) may allow us to reduce the discovery time for the GWSS attractants. Once available, attractants would more effectively allow us to determine the temporal and spatial distribution, and relative abundance of the GWSS.

Project Title :
Cold Storage of Parasitized and Unparasitized Eggs of Glassy-winged Sharpshooter, *Homalodisca coagulata*

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Objectives of Proposed Research:
1. Determine the cold tolerance of the egg parasitoid, *Gonatocerus ashmeadi*, within host eggs of Glassy-winged Sharpshooter (GWSS) under specific environmental and developmental parameters. Assess whether chilling has latent effects on the quality of the adult parasitoid.
2. Determine the most effective method for cold storage of unparasitized GWSS eggs by examining the post-storage acceptability by the parasitoid, parasite survival, reproduction and host seeking behavior.
3. Determine the efficacy of extending the shelf life of parasitized GWSS eggs by preconditioning the host and/or the parasitoid by altering environmental and nutritional standards prior to, or after cold storage.

Justification and Importance of Proposed Research:
The spread of Pierce's disease by the glassy-winged sharpshooter is a multi-billion dollar threat to California wine and almond industry and the current strategy to control this threat involves an integrated pest management approach. One aspect of the management scheme is to employ the release of natural enemies of the GWSS to lower populations. The use of natural enemies of the GWSS is especially appropriate because nonchemical methods of control are frequently the only means available to suppress insect pests across California's broad range of ecosystems.

The egg parasitoid, *G. ashmeadi, is* a mymarid wasp that accounts for 95% of the observed parasitism on the GWSS in California and research is being initiated to develop methods for rearing large numbers of this insect for release in areas where augmentation is needed or where other control measures cannot be used. In the absence of techniques for propagating G. *ashmeadi* via artificial means, rearing this insect in large numbers also requires that the GWSS or another acceptable host be cultured to provide the eggs for this obligate parasite. Protocols designed for efficient mass-rearing generally include techniques which enable the production managers to hold their insects for varying periods of time to synchronize various aspects of the rearing procedure and for distribution to the release site as needed. Having the capability to hold a particular life stage or stages in abeyance during mass rearing is especially important when synchronizing the life cycles of two insects such as in a parasite-host relationship. Placing insects at subambient temperatures as a means to increase their shelf-life or hold them for later use has proved to be a valuable tool when implementing an IPM program (Leopold 1998). Considerable research effort has been expended over the last 65 years on insect cold storage and the implementation of cold storage techniques for insects as aids to rearing coincides with the development of reliable mechanical refrigeration (King 1934, Schread & Garman 1934, Hanna 1935). To this day, cold storage techniques are used in many successful commercially and governmentally-operated insect rearing programs. For example, a Swiss firm uses cold storage extensively throughout their multi-step protocol to produce the wasp, *Trichogramma brassicae*, commercially for com borer control (Bigler 1994). Further, this cold storage research is still needed to develop marketable bio-control programs. C. Glenister, the past president of the Association of Natural Bio-Control Producers, has stated that "the lack of storage technology is a limiting factor in the commercial exploitation of mass-rearing predaceous pentatomids for field release in augmentative control programs" (1998). Thus, low temperature storage is an integral part of the process of mass-rearing insects for use in agricultural pest control programs. It is the practical application of information provided by researchers studying arthropod cryobiology, dormancy, host-prey interactions, and mass-rearing methods. Cold storage allows insectary managers to gain flexibility and enables them to supply a purely biological product on demand. It is evident that the effectiveness of any biological agent used for pest control purposes depends on being released at the proper time. Unforeseeable environmental influences such as those impacting on pest migration, population fluctuations, and crop growth amplifies the need for precise timing, especially when releases of insects are to be integrated into multi-disciplinary control programs.

Project Title :
The Role of Cell-Cell Signaling in Host Colonization by *Xylella fastidiosa*

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Objectives of Proposed Research:
1. Characterize cell-cell signaling factors in *Xylella fastidiosa.*
2. Determine role of signaling factors on virulence and transmissibility of *X. fastidiosa.*
3. Identify degraders of signaling factors of *X. fastidiosa.*
4. Identify inhibitory analogs of signaling factors of *X. fastidiosa.*
5. Evaluate disease management using signaling factor degrading organisms, enzymes, and inhibitory analogs.

Justification and Importance of Proposed Research:
Pierce's disease of grape, a chronic problem in the grape industry in California now promises to be a far more devastating disease due to the introduction of the glassy-winged sharpshooter which is a far more effective vector of the pathogen *Xylella fastidiosa.* The management of this disease is particularly problematic since vector control has not been an efficient means of control, and the nature of the colonization of grape vines by the pathogen limit the utility of bactericides in killing the pathogen. As will be developed below, *X. fastidiosa* has a rather unique means of colonizing plants and causing symptoms that make strategies of disease control based on other bacterial diseases ineffective.

X. fastidiosa causes disease by multiplying within, and thus blocking, xylem vessels. Cells of the pathogen attach to the walls of xylem vessels and subsequently produce large amounts of extracellular polysaccharide (EPS), which almost certainly plays a major role in the blockage of vessels. *X. fastidiosa* obtains nutrients via the passage of the dilute xylem sap over adhering cells. The colonization of xylem vessels is rather discontinuous. Where cells colonize a vessel, they usually develop into large masses that cause plugging, while nearby in the same or adjacent elements there often is no such colonization. After inoculation, the pathogen apparently moves in a rather random fashion into different vessels where local blockage occurs. In early phases of the disease, water can still move through the vines via a torturous path, but eventually as enough of the vessels are blocked, the flow of water is reduced to below the demand of the vines under hot dry weather, leading to symptoms of water stress. Thus, the disease is apparently largely a "plumbing problem" caused by blockage of the xylem by cells and their EPS.

X. fastidiosa also colonizes the most exterior parts of the pump mechanisms of sharpshooters, adhering to the walls of the chamber. As in grape, the pathogen forms discontinuous aggregates in the sharpshooter that are encased in EPS. Thus, the physiological state of cells in the sharpshooter aggregates is probably similar to those in EPS-enclosed masses in grape.

The sites of colonization by *X. fastidiosa* in grape and sharpshooters show great similarities to microbial biofilms that form in other aquatic systems. While bacteria are normally thought of as individuals that can move freely as single cells in their environment, it is now clear that most bacteria commonly adhere to surfaces in large groups, and that only a small portion of the population is sessile as "planktonic" cells at a given time. Biofilms of bacteria develop on solid surfaces that are exposed to a continuous flow of nutrients to form thick layers. These structures consist primarily of an EPS matrix in which the bacteria are embedded. The EPS matrix is generally considered to be important in cementing cells together in the biofilm structure. Cells in biofilms are inherently more resistant to many stresses such as antimicrobial compounds, viruses, and predators. The EPS matrix aids in the nutrition on the cells by accumulating various types of nutrients in a way analogous to an ion exchange column. Thus, cells in such aggregates are much more able to grow and survive than planktonic cells, which might be thought of as "scouts" for other colonization sites. The process of biofilm formation is thought to be primarily a stochastic process whereby planktonic cells adhere to an uncolonized site, and

through limited growth, form a small aggregate of cells. As the aggregate increases in size, the environment of the site becomes more favorable for further colonization by cells in the biofilm. It is not hard to imagine that cells of *X. fastidiosa* colonize plants in such a manner.

Cells in biofilms are physiologically very different from planktonic cells. While genetically identical, cells in biofilms exhibit different patterns of gene expression than planktonic cells of the same species. Cells in biofilms usually produce much more protective EPS than planktonic cells and express many additional traits, for example, defense against antibiotics, etc. As many as 50 genes are selectively expressed in such aggregates, making these cells very different in behavior compared to solitary cells.

Cells in biofilm aggregates communicate with each other to achieve coordinated gene expression in a way that mimics multicellular organisms. Most bacteria produce small molecular weight signaling molecules that enable cells to determine how many cells of their own type are nearby. Bacteria use similar cell density-dependent signaling mechanisms to coordinate control of a set of genes in response to the presence of a "quorum" of like bacteria. Typically, cells constitutively produce low amounts of one or more signaling molecules that can then be sensed by members of a given species. When there are few neighboring cells, the signaling molecules simply diffuse away from the cell and are diluted out. Bacteria interpret this to mean that it has few neighbors. In contrast, as the number of neighboring sibling cells increase, their own production of signaling molecules increases the local concentration of the signal. Furthermore, each cell interprets the presence of neighbors and acts (usually by the binding of the signal molecule to regulatory proteins in the cell) to coordinately express a large set of genes, often associated with virulence and/or tolerance of a particular environment. For example, the pathogen *Pseudomonas aeruginosa* expresses at least 35 genes, encoding traits such as EPS production, proteases, etc., solely in such a cell density-dependent fashion. A variety of different signaling molecules are employed by different bacterial genera, and some strains employ more than one signaling system. The best studied and perhaps the most common signaling system involves the production of *N*-acyl homoserine lactones (AHL). However, some bacteria produce butyrolactones, small peptides, 3-hydroxypalmitic acid methyl ester, and partially characterized lipid-like molecules. The production of these signaling molecules is usually very important in the behavior/virulence of the bacteria. The virulence of many pathogens is greatly reduced when the ability to produce signaling compounds is disrupted by mutation. For example, the plant pathogen *Erwinia stewartii*, which causes a wilt disease of corn produces an AHL that accumulates with cell numbers as does EPS by obstructing water flow in the xylem tissue. Mutants unable to produce AHL are unable to produce EPS and are non-pathogenic. Likewise, we have found that mutant strains of the plant pathogen *Pseudomonas syringae* that are blocked in AHL production are unable to survive on plant surfaces. The coordinate expression of traits in a cell-density dependent fashion is explained by the fact that cells would not benefit from or be in a position to produce traits such as EPS when in isolation in or on a plant. Hence, the expression of genes in solitary cells would be deleterious. Conversely, when as part of a group, the protection of traits such as EPS production would be mutually beneficial and hence cells express such traits only when in cell aggregates.

There is strong circumstantial evidence that *X. fastidiosa* exhibits cell-cell communication via the production of signaling molecules. While there have been no direct measures of signal production in *X. fastidiosa*, its very close phylogenetic relationship to plant pathogenic bacteria in the genus *Xanthomonas*, coupled with the complete genome sequence of strains of *X. fastidiosa* provide considerable insight into cell-density expression of virulence factors in this species. Strains of *Xanthomonas* species produce at least two different types of signaling molecules. Many strains of *X.c. campestris* produce a butyryolactone derivative. Most strains of this group also have been shown to produce a diffusible signal factor (DSF). The production of EPS and extracellular enzymes in *X.c. campestris* is strictly regulated both during growth in liquid media and during disease. A cluster of genes (called *rpf* for regulation of pathogenicity factors) regulate the synthesis of these virulence factors . The gene *rpf*B, which encodes a long chain fatty acyl CoA ligase together with *rpf*F is involved in regulation of EPS and other factors via the production of DSF, which is thought to be a lipid derivative. DSF is released from cells and is absolutely required for EPS production since *rpf*B or *rpf*F mutants are completely a virulent. Remarkable synteny exists between the regions of the chromosome of *X.c. campestris* that contains the *rpf* cluster and a region of the *X. fastidiosa* genome. Furthermore, the amino acid sequence similarities between all of the genes that are represented in both chromosomes are VERY high. The extremely high degree of sequence relatedness between gene products implicated in the synthesis and perception of DSF in *X.c. campestris* and predicted gene products from *X. fastidiosa* provide strong circumstantial evidence for the existence of a DSF regulatory system in *X. fastidiosa*. As noted above many members of the genus *Xanthomonas* also produce a butyrolactone derivative that may function in similar ways as AHLs in regulation of EPS production. Based on the high degree of sequence conservation of *X.*

fastidiosa, it is likely that this pathogen also produces this signaling molecule as well. In contrast, homology searches for AHL biosynthesis genes from a number of bacterial species did not reveal any significant regions of homology with the genome of *X. fastidiosa*, nor was there evidence of similarity of *X. fastidiosa* genes with genes involved in synthesis of other signaling molecules such as peptides or hydroxypalmitic acid methyl ester. Such genes have also not been found in *Xanthomonas* species. Thus, *X. fastidiosa* appears to have a cell-cell signaling system very analogous to that of its closest relatives in *Xanthomonas*.

The recognition that cell-cell communication is common and important in the biology of many pathogenic bacteria has led to new strategies of disease control based on disruption of cell signaling. As noted earlier, the formation of biofilms by many plant pathogens such as *X. fastidiosa* makes their control by bactericides and other chemicals difficult since the cells are both sheltered from the materials as well as in a physiological state that is resistant to their effects. Thus, new strategies of disease control based on disruption of cell signaling is an active area of research in both plant pathology as well as medicine since the potential effects of cell signaling disruption are great. Signaling can potentially be disrupted in several ways. Because signaling molecules bind to regulatory proteins in the cell and thereby affecting transcription of the numerous genes involved in biofilm/virulence, it has been shown that analogs of the signaling molecules can compete with the cognate signaling molecule and disrupt gene regulation. For example, naturally-occurring analogs of the cognate AHL of *Vibrio* sp. compete for binding to the regulator protein involved in cell-density dependent gene expression, thereby preventing activation of genes that are normally turned on at high cell densities. Because of the specificity with which such signaling molecules bind to regulatory proteins, as well as the fact that numerous related signaling molecules exist, the potential is great that the "wrong" AHL from one organism will be found to be a competitive inhibitor of gene activation by the signaling molecule produced by another. Potentially, many such analogs can be screened for such inhibitory effects on gene expression in pathogens. Studies of bacterial colonists of aquatic plants has revealed that many produce compounds that interfere with the signaling systems of other organisms that colonize these aquatic plants. The search for such naturally-occurring inhibitors of signaling has only recently been started, but based on early results as noted above, should be a VERY fruitful strategy of disease control. One of the objectives of this proposal is to identify bacteria that produce inhibitors of the signaling system of *X. fastidiosa*.

Microbes have also recently been found that degrade the signaling systems of other microbes as a means of inhibiting its proliferation in natural environments. A *Bacillus* sp. was found to produce enzymes that degraded the AHL of the plant pathogen *Erwinia carotovora*, and to greatly decrease the virulence of this pathogen when introduced into the pathogen. These workers found that as many as 5% of the field isolates of bacteria had some potential to degrade AHLs. This is not surprising since the signaling molecules represent both a source of nutrient for the degrading organisms, and the degradation of the signal molecules may act to help defend the inactivating strains from antibiotics and other compounds that are often produced by other bacteria under the control of signaling molecules. One of the objectives of this proposal is to search for organisms with the ability to degrade the signaling molecules made by *X. fastidiosa*. Given that the local concentration of *X. fastidiosa* in infected grapes is estimated to be as high as 10^{13} cells/mL it is clear that density-dependent traits, analogous to those expressed in other biofilm-producing organisms, probably play an important role in the life of *X. fastidiosa* in both grape and in sharpshooters. Because the concentration of signaling molecules is a key factor in determining virulence gene expression in pathogens, a strategy of disease control based on controlling the production of or eliminating the signaling molecules made by *X. fastidiosa* is very attractive and will be pursued here.

Project Title :
Role of *Xylella fastidiosa* Attachment on Pathogenicity

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Objectives of Proposed Research:
1. Determine the effects of targeted mutations of selected attachment genes (i.g. firA,pilH,pilS and related genes) on *Xylella. fastidiosa* attachment.
2. Determine differences in pathogenicity between *Xylella. fastidiosa* attachment-deficient mutants and wild type PD strains.
3. Identify specific plant chemicals, pH, and various compounds that either promote or inhibit *Xylella. fastidiosa* attachment in vitro and in plants.

Justification and Importance of Proposed Research:
Xylella fastidiosa is a gram negative bacterium which causes serious diseases of plants such as Pierce's Disease (PD), citrus variegated chlorosis (CVC), or almond leaf scorch and inhabits many other insect and plant host (Purcell 1977). The control of plant diseases caused by this bacterium will ultimately require treating plants with chemical or biological methods, or manipulating plants genetically. In this proposal, we propose to identify inhibitors to the attachment and coloniztion processes of *X. fastidiosa*. A striking feature of *X. fastidiosa* is its polar attachment via the production of fimbriae (Kitajima et al. 1975, Purcell et al. 1979, Davis et al. 1981, Backus 1985, Purcell and Suslow 1988, H. Feil unpublished data). This is an adhesion mechanism that appears to be unique to *X. fastidiosa* and clearly requires traits special to this organism. This suggests the existence of either compounds or conditions that would prevent *X. fastidiosa* to form this polar fimbriae bundle and to attach to its host. A method of controlling X. fastidiosa would be to target these special traits that allow *X. fastidiosa* to adhere to its host. By interfering with the binding of X. fastidiosa to its host, these inhibitors would reduce *X. fastidiosa* virulence and therefore prevent the bacterium from establishing and causing disease.

Adhesion is a well-known strategy for bacteria for access to nutrients, especially in oligotrophic water as illustrated in examples provided by Marshal (1996). It is a primary requirement for *X. fastidiosa*, as for most characterized bacteria that are pathogenic to plants or animals (Marshall 1996). In the case of plant pathogenic or symbiotic bacteria such as *Agroacterium tumefaciens* and *Rhizobium leguminosarum* respectively, attachment to a host plant has been shown to be an essential step in invasion (Romantschuk et al. 1994). *X. fastidiosa* were found to adhere to xylem bessels of PD-infected grapevines (Brlansky et al. 1983) and adhesion is likely to be important for the colonization of *X. fastidiosa* to grape.

Bacteria produce different polymers on their surface that can act cooperatively in the binding process (Fletcher and Marshall 1982). The 3 major polymers are extrapolysaccharides (EPS), proteins (e.g. outer membrane proteins, fimbriae, flagella, or enzymes), and lipopolysaccharides. Several of these polymers have been reported for *X. fastidiosa*. Hopkins (1989) reported that *X. fastidiosa* from aggregates in xylem vessels held together by extracellular strands of bacterial origin. Chagas et al. (1992) also described that the strain of *X. fastidiosa* causing CVC was embedded in a lucent matrix adhering to the inner surface of the cell wall elements by means of fibril-like structures on the external bacterial cell wall. Within the insect, *X. fastidiosa* also appeared to be embedded in a gum-like amtrix in the pump chambers of inoculative sharpshooters' heads (Brlansky et al. 1983, Purcell et al.1979). It has been hypothesized that EPS is important for *X. fastidiosa* binding by increasing the ability of the bacteria to attach (Hopkins 1989, Davis 1988). Some aspects of EPS production and its role in the aggregation of *X. fastidiosa* will be studied as part of a different project in our laboratory.

Project Title :
Spatial and Temporal Relations Between GWSS Survival and Movement, Xylem Flux Patterns and Xylem Chemistry in Different Host Plants

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Objectives of Proposed Research:
1. To quantify the dynamics of Glassy-winged sharpshooter (GWSS) population movement between different host plants and into vineyards. (Year 1-3)
2. To quantify xylem flux patterns and to measure xylem chemistry to determine potential correlation's with sharpshooter movement from surrounding alternate host plants into vineyards. (Years 1-2)
3. To quantify egg production by female sharpshooters and nymphal survival on different host plants, and to correlate demographic statistics with xylem flux and chemistry of host plants. (Years 2-3)
4. To quantify temporal and spatial adult production in the field and relate GWSS migration into vineyards from different host plants with xylem flux and xylem chemistry. (Years 2-3)
5. To identify host-plants that are significant sources of PD through GWSS migration into vineyards. (yr. 2-3).

Justification and Importance of Proposed Research:
Pierce's Disease (PD) is caused by a xylem limited bacteria *Xylella fastidiosa* (Davis et al., 1978). The disease is spreading very rapidly in Temecula Valley, California, and has become the most serious threat to grape production in Southern California. The recent rapid spread of the disease, is correlated with the increase in population density of the glassy-winged sharpshooter *Homalodisca coagulata* (Say), which was introduced into California around 1990 (Sorensen and Gill, 1996). The current epidemic of PD transmission is closely correlated to GWSS population movement (Blua et al. 1999). Strategies aiming to control the spread of PD must rely heavily on a thorough understanding of patterns of sharpshooter movement within vineyards and more importantly, GWSS movement from infected host plants other than grapes into vineyards. Research is being carried out to control the spread of the disease: 1) scouting techniques; 2) chemical treatments; 3) physical barriers, and 4) biological control measures are being tailored to target the GWSS. However, to determine the success or lack thereof for each of these techniques, reliable information is critically needed about patterns of GWSS movement and the relative contribution from host plants that serve as reservoirs for the disease. We propose to study water relations and xylem fluid chemistry in GWSS host plants, in order to identify cues used by this insect that may explain the spatial and temporal patterns of GWSS egg production, population growth, and adult movement throughout the year. Marking techniques currently under testing in our laboratory for use in the field will allow us to reliably track the spatial and temporal dynamics of GWSS populations. By measuring xylem water potential and xylem chemistry we seek to identify cues used by adult GWSS when making decisions about egg laying and movement. We are particularly interested in the behavioral ecology of GWSS in relation to host plant chemistry and interplant movement as it contributes directly to the spread of PD into vineyards. Many plant species are recognized as suitable food sources for GWSS. However, the rate of development and survivorship of immature sharpshooters to adulthood varies across plant species (Brodbeck et al. 1995). By identifying xylem characteristics that allow for such a development, we seek to correlate patterns of GWSS movement with those of xylem quality for sharpshooter development and spatial movement across host plant species through time. Lastly, we propose to relate the information gathered on host plant use by the GWSS, with information about presence/absence of the particular *X. fastidiosa* strain that causes PD in grapes. There are several different strains of the same bacteria, but only one is the causal agent of I'D in grapes (Banks et al. 1999). The correct identification of GWSS host-plants that are not only good hosts for GWSS development, but also reservoir of the PD strain of *X. fastidiosa* will allow for improved targeting of IPM measures proposed to control GWSS growth and movement and minimize the impact of I'D on the California grape industry.

Project Title :
Seasonal Changes in the GWSS's Age Structure, Abundance, Host Plant use and Dispersal

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Objectives of Proposed Research:
1. Develop and test florescent dust and immunoglobulin G markers and a trapping system that allows rapid assessment of marked versus non-marked dispersers entering or leaving vineyards.
2. Identify the host plants (species) from which the glassy-winged sharpshooter disperses to the grape vines in spring and early summer.
3. Identify the host plants to which the glassy-winged sharpshooters disperse to and from the grapevines in late summer and fall, i.e., identify the overwintering host plants and/or habitats.
4. Identify the relative contribution of individuals from each host plant type (e.g. citrus, riparian vegetation, ornamental plants) to the pool of dispersing glassy-winged sharpshooter adults and to estimate their densities.
5. Develop a sampling system to estimate the density of egg batches laid in leaves of host plants (citrus initially, later to include riparian and ornamental plants) and various other hosts plants (as they are identified)
6. Develop a sampling system to estimate the density of nymphs on citrus initally and on other host plants (as they become identified).
7. Develop mark-release-recapture methods that both mark groups of sharpshooters and that uniquely identifies, individual sharpshooter adults and use this technique to estimate the survival, longevity and density of adult sharpshooter populations.
8. With these markers, Determine the residency time, mortality, emigration, immigration and density of adult glassy-winged sharpshooters on a host plant type (initially citrus).
9. Using the florescent dust and/or Immunoglobulin G marking system, determine the percentage of the adult populations that disperses from citrus into the vineyards.

Justification and Importance of Proposed Research:
We propose to develop several marking techniques that can be used in mark-release-recapture studies of the glassy-winged sharpshooter. One group of mark-release-recapture studies seeks to track and monitor the movements of adult glassy-winged sharpshooters from their overwintering hosts into the grape vineyards, within and among the vines with a vineyard, and between vineyards and other host plant habitats, e.g., ornamental and wild host plants. We are especially interested in determining when the sharpshooters leave and seeks to estimate sharpshooter densities within a localized movements, their time of year, and the number of individuals involved.

We also propose to develop standard sampling techniques for glassy-winged sharpshooter eggs and nymphs (the sharpshooter's immature stages). Using these techniques we propose to estimate the egg and nymphal densities on citrus, a principal host plant for the glassy-winged sharpshooter. We will extend these sampling protocols to other host plants and host plant habitats as they are identified and as time permits. Using a mark-release-recapture method we will estimate the density of sharpshooters to determine the proportion of these stages that survive to become adults.

Finally, we propose to use several marking techniques under field conditions to determine how long the adult sharpshooters live, the number of adults present on a host (species) (e.g. citrus), and the numbers of adults present in various subpopulations, e.g., in vineyards, ornamental plants, riparian vegetation or a citrus groves.

Project Title :
Kern County Pilot Project

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Objectives of Proposed Research:
1. Control Glassy-winged sharpshooter populations during a period of time when their distribution is narrowly restricted, within an agriculturally diverse growing area.
2. Monitor and manage GWSS populations in a defined, somewhat isolated, production area in Kern Co.
3. Monitor the incidence and minimize the potential for spread of Pierce's Disease in the project area, and facilitate citrus and stonefruit commodity movement from Kern Co.
4. Use chemical and biorational insecticides in a judicious manner to minimize impacts on beneficial organisms.
5. Release and determine the efficacy and impact of natural enemies against GWSS, and their compatibility with novel chemistry management strategies developed from the project.

Justification and Importance of Proposed Research:
This project attempts to identify and test management strategies in a location where GWSS populations and the threat of PD spread are greatest. Several novel chemistry insecticides, with limited impacts on beneficial insects, have recently become available to growers. However, their efficacy against this emerging pest threat are largely unknown. This project will identify the efficacy of these novel chemistries against GWSS, attempting to match their properties to various environmentally sound management methods. Finally, their integration into long term sustainable management strategies, like biological control, will be explored.

Project Title :
Genetic Transformation to Improve the Pierce's Disease Resistance of Existing Grape Varieties

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Objectives of Proposed Research:
1. Further improve a genetic transformation protocol in our laboratory to routinely produce transgenic vines of important grape varieties, particularly Chardonnay.
2. Introduce into an existing variety a gene encoding an antimicrobial compound shown to be effective against the causal agent of Pierce's disease.
3. Regenerate transgenic vines and determine whether they exhibit improved tolerance to Pierce's disease.

Justification and Importance of Proposed Research:
We have been able to establish embryogenic lines suitable for *Agrobacterium tumefaciens* genetic transformation and to develop an efficient transformation and regeneration system. Basically, pro-embryogenic calli are obtained from immature anthers cultured in PIV medium (Franks et al. 1999) and maintained by transferring to fresh PT medium (Hanson et al. 1999). For inoculation, a dilute Agrobacterium culture is dropped onto individual clumps of calli and incubated for 48 h. Transformed cells are then selected in PT medium supplemented with 100 $mg.l^{-1}$ kanamycin and 300 $mg.l^{-1}$ cefotaxime. After 8-12 weeks putative transformed calli are transferred to WP medium (Lloyd and McCown 1980) supplemented with 100 $mg.L^{-1}$ glutamine, 100 $mg.l^{1}$ asparagine, 100 $mg.l^{1}$ arginine and 0.5 $mg.l^{1}$ (2.22 uM) BA to induce embryo germination.

After having successfully expressed GUS in plants of Thompson Seedless, we have focused on the transformation of cultivars Chardonnay and Thompson Seedless and the rootstock Saint George using constructs that may be relevant for PD resistance. They include a gene that codes for a pear polygalacturonase inhibitor protein (PGIP) and two types of fusions of the green fluorescence protein (GFP) with the amino and carboxy-terminal of a ribosome-inactivating protein (RIP) from *Trichosanthes kirilowii*. PGIPs are localized in plant cell walls and play an important role in prevention of the penetration of microorganisms in several species (Glinka and Protsenko 1998, Lang and Dornenburg 2000). Although the chemical composition of the substance that occludes xylem vessels in PD affected plants is not yet known, PGIP might inhibit the breakdown of plant or bacterial cells walls that contribute to the occlusion. The fusion of GFP to the RIP secretory sequences will permit determination of the level of accumulation of the protein in the xylem sap, providing useful information for future studies related to the delivery of anti-*Xylella* gene products into the xylem. The GUS gene is also present in all the constructs and all the genes are under the control of CaMV35S promoter.

Project Title :
Insect-Symbiotic Bacteria Inhibitory to *Xylella fastidiosa* in Sharpshooters

Principal Investigator(s):
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Objectives of Proposed Research:

1. We will identify insect associated bacteria in GWSS and related insects in Southern California or other areas where the vector insects are endemic. We will then attempt to culture these bacteria.
2. Those bacteria that can be cultured will be identified.
3. These will be evaluated further for the production of antibiotics inhibitory to *Xylella*, for potential hazards to plants animals and humans and for their potential for genetic manipulation through techniques for genetic transformation.
4. Those that can be genetically transformed will be investigated for their ability to express transaenes that produce substances that inhibit, attack, destroy, or prevent the transmission of *X. fastidiosa*.
5. Concurrently, we will evaluate various peptide antibiotics and bacterial extracts for activity against *X. fastidiosa*. Those peptide antibiotics demonstrating in effective inhibition of *X. fastidiosa* could be candidates for introduction and production in the GWSS-associated bacteria.

Justification and Importance of Proposed Research:
We will culture, identify, then select or genetically transform insect-associated bacteria, especially gut bacteria, from Glassy-winged Sharpshooter to produce substances that inhibit or kill *Xylella fastidiosa*. We intend to use bacterial-plasmids derived from gram negative bacteria and the novel himar transoposon/transposase mediated system derived from the mariner insect transposon.

Xyella fastidiosa is the etiological agent of Pierce's Disease, PD, an important disease of grapes in the US (Hopkins, 1994, Varela, 1997 #71). This disease limits viticulture in Florida and the rest of the southeastern US. (Adlerz and Hopkins, 1979). It was observed in the Temecula valley of California in 1997. Though this disease had been known in Southern California (California Vine Disease, Anaheim Disease) since the 1880's (Gardner and Hewitt, 1974), it had not been reported before in the Temecula valley wine grape area. The appearance of PD in Temecula coincided with increased populations of the Glassy-winged sharpshooter, *Homalodisca coagulata*, GWSS. Because of the mobility and vector capacity of this insect, PD has become a cause for great concern to the Wine industry in California.

We propose to develop methods for the genetic transformation and/or transposon-mutagenesis of GWSS associated bacteria with genes encoding factors that will inhibit or kill *X. fastidiosa.*

Project Title :
Keys to Management of GWSS: Interactions Between Host Plants, Malnutrition and Natural Enemies

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Objective of Proposed Research:
1. To determine the relationship of host plant xylem chemistry, and leaf morphology on host selection, feeding and ovipositional behavior of GWSS and its parasites.

Justification and Importance of Proposed Research:
GWSS oviposits in many plant species, yet the majority of GWSS egg masses tend to be concentrated on a few select host species that apparently offer the quality of xylem fluid (food) required for survival of nymphs. Food quality for nymphs appears to be an important factor affecting the population increase of GWSS. We concentrated on developing field methods and data towards determining the host selection behavior and use for oviposition of known host plants preferred for feeding by the adult GWSS. Experimental sites that offered mixed host plants were established in isolated islands in an open field and a large planting of crape myrtle. Plants on the islands and the surrounding crape myrtle were examined for the presence of GWSS life stages weekly. Results from both sites indicated a statistically significant preference of GWSS for ovipositing on holly plants over all other plant species. The second host most frequently used by GWSS for oviposition was Bradford pear. A few egg masses were also found on other hosts. *Pyracantha* was chosen as oviposition host only very early in the summer and in the fall. Parasites were able to utilize the GWSS eggs on all hosts. The data suggests that proximity of adult and ovipositional hosts may greatly increase exposure of adult hosts plants to GWSS. We also worked on standardizing and quantifying methodology for rearing GWSS. Strict attention must be given to lighting conditions especially from late to early season. When diapause occurs, diapause can be terminated if three weeks of short daylength are followed by long daylength (we employ 16:8 light:dark regime) *so long as* the proper ovipositional hosts are present.

We have now begun the second phase of this experiment, which is to assess the suitability to GWSS based on xylem chemistry of California-relevant host species. We have collected xylem chemistry data on the host plants used in the field plots and these are ready for analyses. We are currently investigating Chardonnay grapes, Navel oranges, Spanish Pink Lemon and Crape Myrtle. Data from these hosts will be compared to rates of development on soybean in order to assess the value of each of these species as developmental hosts for GWSS. Quantitative analysis is needed to prioritize the role of each potential host for implementation of GWSS control measures.

By exposing GWSS eggs to parasite adults, we determined the duration of the susceptibility of GWSS eggs to the parasites *Gonatocerus ashmeadi and Gonatocerus morrilli.* Parasitoids can successfully parasitize 100% of GWSS eggs for at least 7 days after oviposition. We also investigated the overwintering behavior of *Gonatocerus* sp. and GWSS. We determined for the first time in the U.S. that GWSS as eggs and *Gonatocerus* sp. within parasitized egg masses can overwinter at north Florida winter temperatures. Laboratory experiments showed that parasitoids fed honeydew provided from excised leaves with live whiteflies lived twice as long as those fed simple honey solution. Perhaps parasitoid abundance could be enhanced by providing alternative food sources.

Project Title :
Host Selection Behavior and Improved Detection For GWSS, *Homalodisca coagulata* (Say)

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Objectives of Proposed Research:
1. To improve and optimize a trapping method(s) (size, configuration, spectral reflectance pattern, field placement, etc.) to detect and monitor GWSS.
2. To determine the mechanisms used by GWSS in host plant finding and selection.

Justification and Importance of Proposed Research:
Sorenson and Gill (1996) reported the colonization and establishment in California of the leafhopper, *Homalodisca coagulata* (Say), the glassy-winged sharpshooter. GWSS is a major vector of Pierce's disease (*Xylella fastidiosa*) and other diseases caused by *X. fastidiosa*. This insect has the potential to enhance the spread of Pierce's disease to devastating levels throughout the grape-growing regions of California. Such xylem-feeding leafhoppers are the exclusive vectors of *X. fastidiosa*, a bacterium which is the causal agent not only of Pierce's disease, but also of almond leaf scorch, plum leafscald, citrus variegated chlorosis, oleander leafscorch and many other diseases. Both the leafhopper vectors and *X. fastidiosa* are obligate xylophages. There is no cure for any disease caused by *X. fastidiosa*, and in the southeastern United States where *X. fastidiosa* is endemic, there are no acceptable control measures for the vectors or pathogen.

For 18 years GWSS has been a primary focus of research by the Principal Investigators. Our research has focused on GWSS behavior and we have elucidated some of the major host plants used by the vector (Mizell and French 1987), diurnal patterns of movements within crops, frequency of host plant use, and the underlying plant chemical and physical factors that determine the behavior and performance of GWSS and other sharpshooter vectors (Andersen et al. 1989, 1992, Brodbeck, et al. 1990, 1993, 1995, 1996, 1999, 2001). In the course of these studies we have also developed data on GWSS behavior in response to visual and other stimuli (see preliminary data below) including traps. Dr. Mizell has also invented and implemented other novel and highly innovative trapping techniques in several commodities and used them to investigate the behavior of a myriad of insects including weevils (Coleoptera: Curculionidae, Mizell and Tedders 2001, Mizell and Tedders 1999, Stansly et al. 1997, Tedders et al. 1996), deer flies (Diptera: Tabanidae, see the web site: extlab7.entnem.ufl.edu/pestalert/, Mizell et al. 2001), stink bugs (Hemiptera: Pentatomidae, Mizell et al. 2001, Mizell and Tedders 1998, patent pending), and an exotic ladybird beetle (Mizell, current project).

Early detection of GWSS populations is the key to containment and reducing the spread of this exotic vector in CA. The commercially-available Pherocon AM trap presently used by CDFA is easy to store, transport and use. However, it was not developed or tested for monitoring GWSS. Therefore, this trap is not highly efficient and no one to date (to our knowledge) except the Principal Investigator (RFM) has compared its performance to other traps in the field (see below). Yellow traps actively attract GWSS (Table 1) in preference to traps with other spectral patterns of hue (color) and intensity (Figure 1), therefore, yellow traps do not function just as blunder traps. Evidence of active visual attraction by GWSS provided the impetus to further investigate and attempt to exploit GWSS visual behavior. Our preliminary field tests of other trap configurations strongly suggest that trap parameters can be changed to dramatically increase the ability to detect and monitor GWSS and perhaps other vectors with similar behavior. Increased trap efficiency should enable detection of GWSS at much lower population levels perhaps shortening the time to detection of new local introductions which will reduce or slow the spread of GWSS in CA.
The terminology used in research on color vision is complex and confusing (Allan and Stoffolano 1986). Color is defined as the spectral composition of visible radiant light. Hue is defined by the dominant wavelength, i.e. violet (380-450nm), green (490-560nm), yellow (560-590 nm) etc. Saturation is based on the spectral purity of reflected light. When white is added to color, the color becomes less saturated. Intensity (brightness) describes the amount of incident light reflected or

transmitted by an object. Adding white increases while black decreases intensity. The spatial distribution of photon flux provides information on shape, size, distance and motion. Visual patterns depend upon the nature of the viewed surface, the optical background, the illuminant and the viewer's angle and sensitivity (Prokopy and Owens 1983). The visual capabilities of invertebrate insect species may be quite different from vertebrates such as human beings. The various color receptor types can be divided into 3 large groups: UV, blue and green with maximum sensitivity at 350, 440 and 510nm respectively (Menzel 1979). It is advisable to use the term radiation instead of color, for instance, radiation with a wavelength of 585 nm instead of "yellow". This must be born in mind because colors can look identical (to the human eye) and yet have different spectral compositions (hue, intensity and saturation) (Mazokhin-Porshnyakov 1969). Many insects such as honey bees, ants, moths and wasps can distinguish hue "color" but many insects do not. Insects are highly sensitive to short wavelengths in the UV range (350-400nm) that vertebrates cannot perceive and are also capable of discriminating polarized light directly from the sun. Therefore, adequate characterization of the visual stimuli responded to by insects in behavioral bioassays requires measurement of the reflectance spectra at wavelengths from 310 -750 nm using special equipment - photometer.

Dependent upon species, insect detection and monitoring usually exploits the behavioral response of the target species to visual and odor cues singularly or in combination. Sex attractants and aggregation pheromones have been identified for many species of insects, particularly species in the higher evolved Orders of Lepidoptera and Coleoptera, and may be used in traps to enhance capture. Plant volatiles are also important in arthropod behavior and may be used by phytophagous insects in finding and identifying host plants and by predacious species in detecting prey infestations. The cotton bollweevil, *Anthonomis grandis*, one of the most researched insect pests, has one of the most sophisticated pheromones with 4 or more chemical components. The boll weevil trap uses visual cues (yellow-green color) provided by a trap that apparently mimics the host plant's reflectance pattern in combination with the pheromone as bait to attract and capture the species. Unfortunately, neither pheromones nor plant volatile use in mate or host finding have been documented for GWSS or any species of related leafhopper and it is unlikely such responses occur. Many leafhopper species do produce sound that functions in mate recognition or defense (Claridge 1985). Given their strong visual attraction to yellow traps and their feeding habits relative to other related plant feeders, it appears very unlikely that GWSS uses chemical attraction in host finding. Therefore, based on behavioral parsimony (use of evolved sensory modalities for multiple functions), it is logical to conclude that leafhopper vision-based behaviors used to respond to traps likely function naturally to respond to host plants.

Prokopy and Owens (1983) reviewed literature on the visual detection of plants by herbivorous insects. They indicated that individual plants or plant parts provide 3 principle properties that serve as visual cues to foraging insects: spectral quality, dimensions and pattern. Spectral reflectance-transmission curves of foliage under diffuse light conditions are remarkably consistent over a wide range of species. Intensity of reflected or transmitted light is a more variable foliage parameter than spectral composition: intensity changes more with plant stress, leaf maturity, nutritive condition, foliage density, angle of illumination and background. Plant spectral quality, particularly hue and intensity, appears to be the major stimulus eliciting herbivore landing on living plants (Prokopy and Owens 1983). Interestingly from a pest management point of view, the most attractive object to a herbivore may not be one that most precisely mimics the natural visual stimulus (host plants) but one that embodies "supernormal" stimuli (Staddon 1975, Prokopy and Owens 1983).

Preliminary tests on GWSS host response behavior indicate that the vector can discriminate visually between host plants and other objects and may be able to visually discriminate between hosts of different species and quality. Detailed knowledge and understanding of the behavioral response to host plants by vectors is important for many reasons related to the potential for vector management.

The particle film, Surround, an inert mineral kaolin, an nontoxic alternative to chemical pesticides, is currently being tested extensively against GWSS in CA and many other pests in orchard and vineyard crops (Glenn et al 1999). Surround may affect insect behavior by changing the plant's physical appearance and by acting as a barrier. Insect behavior is also affected directly by binding of the particles to insect body parts. Surround particles are similar to talc and application to plants turns them white in appearance. This perhaps changes the plant's pattern of recognition normally used by insects in responding visually to hosts. Surround offers a method to change the short-term appearance of a host plant without affecting plant chemistry for behavioral bioassays related to vision. In addition this proposal offers a simple methodology to examine possible mechanisms by which Surround affects GWSS response behavior.

Given the need to increase the efficiency of detection of GWSS and our preliminary data and knowledge of the behavior of GWSS and related species, we propose to develop, evaluate and optimize a practical detection and monitoring system for GWSS exploiting vision response. Concurrently, we will investigate the host selection behavior of GWSS, which is likely, the functional basis of the behaviors being exploited by traps. We will also use particle film as an experimental treatment to change the visual appearance of plants and at the same time investigate how particle film might affect GWSS behavior.

Project Title :
Sharpshooter-Associated Bacteria that may Inhibit Pierce's Disease

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Objectives of Proposed Research:
1. We will identify insect associated bacteria in GWSS and related insects in Southern California or other areas where the vector insects are endemic.
2. The bacteria that can be cultured will identified will be evaluated further for the production of antibiotics inhibitory to *Xylella*, for potential hazards to plants animals and humans and for their potential for genetic manipulation through techniques for genetic tgransformation. Those that can be genetically transformed will be investigated for their ability to express transgenes that produce substances that inhibit, attack, destroy, or prevent the transmission of *Xylella fastidiosa*.
3. We will evaluate various peptide antibiotics demonstrating effective inhibition of *Xylella fastidiosa* could be candidates for introduction and production in the GWSS-associated bacteria.

Justification and Importance of Proposed Research:
We will culture, identify, then select or genetically transform insect-associated bacteria, especially gut bacteria, from Glassy-winged Sharpshooter to produce substances that inhibit or kill *Xylella fastidiosa*. We intend to use bacterial-plasmids derived from gram negative bacteria and the novel himar transoposon/transposase mediated system derived from the mariner insect transposon.

Xyella fastidiosa is the etiological agent of Pierce's Disease, PD, an important disease of grapes in the US (Hopkins, 1994, Varela, 1997). This disease limits viticulture in Florida and the rest of the southeastern US. (Adlerz and Hopkins, 1979). It was observed in the Temecula valley of California in 1997. Though this disease had been known in Southern California (California Vine Disease, Anaheim Disease) since the 1880's (Gardner and Hewitt, 1974), it had not been reported before in the Temecula valley wine grape area. The appearance of PD in Temecula coincided with increased populations of the Glassy-winged sharpshooter, *Homalodisca coagulata*, GWSS. Because of the mobility and vector capacity of this insect, PD has become a cause for great concern to the Wine industry in California.

We propose to develop methods for the genetic transformation and/or transposon-mutagenesis of GWSS associated bacteria with genes encoding factors that will inhibit or kill *X. fastidiosa.*

Project Title :
Reproductive Biology and Physiology of the GWSS

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Objectives of Proposed Research:

1. To study the reproductive biology of the glassy-winged sharpshooter, *Homolodisca coagulata*.
2. To investigate the anatomy, histology and ultrastructures of the female and male reproductive organs and accessory glands.
3. To compare physiological differences in female and male reproductive cycles between the summer and overwintering populations. Differences in female reproduction will be compared by examining the number of oogenesis cycles (egg formation wid egg maturation), nurnbei of batches uf eggsthat females can lay, and hatching rates of eggs deposited on summer vs winter host plants. For the male insects, the number of spermatogenesis cycles (sperm formation and sperm maturation), and the number and "quality" of sperm will be compared. Histological and cytochemical methods as well as transmission electron microscopy (TEM) will be used to study oogenesis and spermatogenesis cycles.
4. To investigate the effect of photoperiod, temperature, nutrition, and hormones in triggering the onset and breaking of reproductive diapause in female insects, as expressed by levels of vitellogenesis (synthesis and deposition of female proteins into developing eggs during oogenesis). Vitellogenin and vitellin proteins will be isolated and characterized by using SDS-PAGE and other methods.

Justification and Importance of Proposed Research:
Little is known about the reproductive biology of the GWSS. Blua et al. (1999) documented 2 generations of GWSS per year in Southern California. Oviposition occurs in late winter to early spring, and again in mid-to-late summer. Adult GWSS live several months and lay small eggs side by side in groups of about 10, but ranging from one to 27 (Turner and Pollard, 1959). The sausage-shaped eggs are deposited in the leaf epidermis of host plants, and appear as greenish blisters. The nymphs moult 4 times to develop into winged adults in 10-12 weeks (Turner and Pollard, 1959). A series of studies conducted in Florida demonstrated that in the summer, when Prunus spp. served a s a marginal host, GWSS abundance was tightly coupled to fecundity rate (Anderson et al., 1997). Because high abundance of GWSS and high consumption of xylem fluid were correlated with high glutamate and asparagine concentrations in the xylem fluid of the host plants (Brodbeck, et al., 1996), the amide concentrations in host plants may potentially cause oviposition preference in the female GWSS (Anderson et al., 1997). These studies support field observations to date in California which clearly indicate a preference for different plant species at different times of the year, with choice appearing to be linked to vegetative flushes of the Preferred host plants. Besides the aforementioned aspects of reproductive biology, the reproductive physiology of GWSS remains completely unknown. Even the anatomy and histology of the reproductive system has yet to be described. Studying the reproductive biology and physiology of GWSS will contribute to better estimates of its reproductive potential. Our methods will allow us to determine the influence of seasonality and resource variables on GWSS fecundity, and to better understand factors that impact its reproduction. This knowledge is important in determining how GWSS might choose plant hosts in the landscape, which plants are particularly good developmental hosts and why they are good hosts, and how control measures might best be implemented based upon season and stage of reproductive development. Better knowledge of reproductive biology might also lead to better decision support including choices of chemicals or non-chemical approaches.

Project Title :
Epidemiology of Pierce's Disease in the Coachella Valley

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Objectives of Proposed Research:
1. Determine the incidence and distribution of Pierce's Disease (PD) in the Coachella Valley.
2. Determine the relationship of citrus, an excellent host for GWSS, to the distribution of PD in vineyards.
3. Determine the relationship of citrus to the abundance of GWSS in vineyards.
4. Describe the epidemiology of PD in the Coachella Valley.

Justification and Importance of Proposed Research:
The table grape industry in the Coachella Valley is represented by 14,400 acres of producing vines (California Dept. Food and Agric., 1999), which generated grapes valued at $131 million in 1998 (Jose Aguiar, Riverside County Farm Advisor, personal communication). In the past, Pierce's disease (PD), a disease caused by the xylem-limited bacteria, *Xylella fastidiosa* Wells et al., has occurred in the Valley, but incidence has been limited to fields bordering moist, weedy "riparian" areas. *Xylella fastidiosa* is vectored by sharpshooters, a group of insects in the family Cicadellidae, that transmit bacteria from infected to healthy plants. Similar to other parts of California (Purcell 1974, 1975), the primary vector of PD in the Coachella Valley likely involved the blue green sharpshooter, and until recently the low incidence of disease has been of little concern to grape growers.

In 1997, the situation in California changed drastically. That year, PD was documented in the wine-grape region of the Temecula Valley in southern California, and the Temecula growers have experienced devastating losses to PD. The observed increase in disease incidence is chronologically linked with observed increases in numbers of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Blua et al. 1999, Purcell and Saunders 1999).

The glassy-winged sharpshooter was identified in the Coachella Valley in the early 1990's (Blua et al. 1999), and the Riverside County Agricultural Commissioner, in cooperation with CDFA, has documented increases in this PD vector. The rapid losses caused by GWSS-vectored PD in Temecula suggest that areas where the GWSS becomes established experience rapid PD spread and vine decline. A PD survey conducted by the PI in September 2000 of 8 vineyards in Temecula found plant decline or death due to PD ranging from 51%-87% (Perring et al. submitted). The most plausible explanation for the swiftness and severity of the PD epidemic in Temecula is the unique epidemiology created when GWSS is introduced into an area with endemic PD sources (Purcell and Saunders 1999). When this occurred in Temecula, the epidemic mimicked other grape growing regions in the US. In the southeast, GWSS-transmitted PD is the major factor limiting grape production (Purcell 1981).

There are no apparent biological or climatological factors that will limit the spread of PD in grapes in the Coachella Valley. While research aimed at reducing GWSS numbers through biological and chemical control may assist in the short term, long-term management will require an understanding of how *X. fastidiosa* is spread. For example in Georgia, phony peach disease, which is caused by another strain of GWSS-transmitted *X. fastidiosa*, exhibits a gradient of infection within peach orchards that is related to local sources of disease (KenKnight 1961). If a similar relationship were defined for PD in grapes in the Coachella Valley, growers could target PD source areas for GWSS control, remove local sources, create spatial distances between their vineyards and the sources, or plant new vineyards distant from the sources. One may speculate that another strategy is to plant new vineyards upwind from bacterial sources, presumably because vectors are subject to being dispersed by wind. While this might seem intuitive, there are conflicting data regarding how disease gradients relate to wind direction. For the Georgia phony peach epidemic, Ken Knight (1961) demonstrated that local disease gradients generally followed prevailing winds. Our data from Temecula showed that PD gradients were not related to wind direction (Perring et al. submitted).

Our proposal is designed to document the current levels of PD in the Coachella Valley and to describe the seasonal cycle of the GWSS. This will allow us to identify characteristics of fields with high and low disease incidence for the purpose of designing strategies to minimize PD spread. A potential component in the epidemiology, and a question of the growers in the Valley is the role of citrus to the abundance of GWSS and the incidence of PD. Our proposal will evaluate the citrus-grape interface so that control practices in both of these crops are scientifically supported. This is important because so much of the citrus in the Coachella Valley is under extensive integrated pest management, and there is an economic and environmental cost to broad-scale GWSS insecticide application in citrus.

Assuming PD will increase in the Coachella Valley, the current levels of GWSS in the desert coupled with the low incidence of PD signal the initial stages of the epidemic. This is the key time to document the distribution of PD within vineyards to determine if there are "edge effects" similar to pre-GWSS PD distribution, or if the distribution is spread more evenly through vineyards. Through the time course of this project, our sampling strategies will enable us to document the spread of disease from infected "foci" within the field, and correlate this spread with distance from citrus. Rapid spread from isolated vines in grape fields, long distances from external vector and pathogen sources will indicate a secondary spread from infected vines. The absence of this type of spread (i.e. more random distribution through time) will suggest primary spread. Currently, the pattern of *X. fastidiosa* spread by GWSS in California is unknown. This fundamental information is crucial to developing long-term management strategies, either within a single vineyard or within an area-wide program.

Project Title :
Survey of Egg Parasitoids of GWSS in California

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Objectives of Proposed Research:
1. Survey and collect sharpshooter egg parasitoids in Central/North Coast and San Joaquin Valley, and identify parasitoids collected.

Justification and Importance of Proposed Research:
Biological control of GWSS would be considerably enhanced with a GWSS egg mass parasitoid that would be more effective in the early spring. Determining the existing parasitoid complex on California sharpshooters is a critical precursor to doing any additional foreign exploration for effective GWSS parasitoids. An initial survey for existing parasitoids could save considerable time and dollars by making any necessary future foreign exploration more efficient.

Project Title:
Timing and Duration of Fresh Glassy-winged Sharpshooter Egg Masses in Lemon Fruit Rinds; Impact on Fruit Harvest and Shipments

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Objectives of Proposed Research:
1. Determine what periods of the year the GWSS is laying its egg masses into lemon fruit rinds.
2. Determine when lemon fruit rinds are susceptible to GWSS egg mass deposition.
3. Determine viability of 1st instar GWSS nymphs and GWSS adults on the four main commercial species of harvested citrus fruit.

Justification and Importance of Proposed Research:
As the GWSS saga continues to unfold across the state, California's citrus industry should be prepared to meet any challenges that develop regarding the presence of GWSS egg masses in fruit rinds. Having the proposed information on hand will allow the industry to maintain its proactive stance in the face of developing issues regarding the GWSS. Loss of first market grade due to the presence of GWSS egg masses in the fruit rinds is of concern. The California citrus industry will be able to address questions regarding the viability of GWSS on harvested citrus fruit for export or transit within the state or U.S.

Project Title :
Xylella fastidiosa Bacterial Polysaccharides with a Potential Role in Pierce's Disease of Grapes

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Objectives of Proposed Research:
1. To continue our structurally characterize of *Xylella* LPS and EPS extracted from the xylem sap of PD infected and non-infected grapevines (using two varieties, Chardonnay and Cabernet).
2. To assess quantitative and structurally changes to *Xylella* LPS and EPS in xylem sap (using two varieties, Chardonnay and Cabernet) during the infective time course of Pierce's disease.

Justification and Importance of Proposed Research:
Xylella fastidiosa causes Pierce's disease that affects wine, table, and raisin grapes, the symptoms usually being leaf scorch, and drying and wilting of the fruit clusters. The causative agent, *X. fastidiosa*, is a gram-negative bacterium that infects the host plant xylem (water conducting elements of the plant). *Xylella* are injected into the plant xylem by a specific insect vector, the glassy-winged sharpshooter, which feeds on xylem sap and spreads the bacteria from diseased to healthy plants. Vines develop symptoms when the bacteria block the water conducting system of the plant and reduce the flow of water to affected leaves. Severely infected vines die. Pierce's disease has chronically attacked vineyards in northern California, costing growers $33 million from 1995 – 1997 alone, and in southern California's Temecula Valley $6 million in damage to vineyards has been recorded since 1997. Other than severe pruning early in the infection there is presently no effective treatment or prevention of Pierce's disease. Considering the economic importance of PD to the Californian wine industry there is still considerable disagreement on the mechanism of pathogenesis, and as yet very little is known about the bacterial physiology of *X. fastidiosa*. Since a clear understanding of the disease is likely to be necessary to its control this situation needs to be remedied. *X. fastidiosa* are nonflagellated and are limited *in planta* to the xylem . How they are transmitted through the infected plant is unknown. Dysfunction of the water-conducting system, and/or build-up of phytotoxins or plant growth regulators have been proposed as mechanisms of pathogenesis. Evidence supports the hypothesis that the disease is caused by water stress due to xylem occlusions, either bacterial aggregates, host gum, tyloses and/or *Xylella* exopolysaccharides. The time course for the appearance of disease symptoms is virtually unstudied, but electron microscope studies show that aggregates of bacteria become immobilized to xylem walls by extracellular strands that are most abundant at the end of the bacterial rods. The strands esemble bacterial polysaccharide fibers and it has been suggested that they may aid the xylella in binding nutrients or conserving and concentrating digestive enzymes released by the bacteria for action against the host tissue.

Project Title:
Pruning for Control of Pierce's Disease

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Objectives of Proposed Research:
1. Determine if the recovery from Pierce's disease by severe pruning of grapevines continues on the regenerated vine growth for more than one season.
2. Test the repeatability of severe pruning to regenerate healthy vines from grapevines with Pierce's disease.
3. Test a new disease severity rating system to guide pruning vines with Pierce's disease.

Justification and Importance of Proposed Research:
This is a new proposal for funding an additional two years continuation of a current UC IP M project. When we submitted our original proposal in 1998, we wanted to test the feasibility of severe pruning of Pierce's diseased vines to generate healthy vines. We realized that if our first results were negative, a third year's work would be pointless. Because our results so far have exceeded our expectations, we now seek additional funding to confirm that vines from which we apparently eliminated Pierce's disease (PD) continue remain be free of PD symptoms in following years. Our results from one plot suggest that this needs to be confirmed. Secondly, we want to test a new disease rating system for guiding pruning decisions. Finally, we want to try a modified pruning method for one of our three categories of disease severity. The last two objectives can only be started in fall 2000 and completed in fall 2001, thus we are asking for two years support.

There are currently no therapeutic methods for grapevines with Pierce's disease. Our field experiments from 1997 to 1999 showed that severe pruning of vines with PD had no PD symptoms in new vine growth in the following season. It remains to be determined whether apparently healthy vines regenerated by severe pruning will remain free of PD. If this practice continues to be useful for a number of cultivars and regions, it will speed the replacement of Pierce's diseased vines by preserving an established rootstock to support new vine growth.

Our studies are designed to provide useful information for growers even if our pruning experiments do not produce methods that eliminate the disease from infected vines for more than one season. Some growers are experimenting with various below or on the cordon pruning practices against PD and claim worthwhile successes but have not compared their results to negative controls. The money and labor spent for pruning to eliminate PD would be wasted if pruning is not effective. Finally, the results of our proposed experiments will provide new data on the distribution and overwinter survival of *Xylella fastidiosa* relative to PD symptoms. The success or failure of pruning provides information on how far *X. fastidiosa* can invade the trunk or roots of vines with various degrees of symptom severity.

Project Title :
Transmission of *Xylella fastidiosa* to Almonds by the GWSS

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Objectives of Proposed Research:
1. Determine the efficiencies of acquisition and inoculation of *X. fastidiosa* by the GWSS to almonds.
2. Quantify populations of *X. fastidiosa* in infected almonds in the field throughout a season.
3. Determine the ability of the GWSS to inoculate and acquire *X. fastidiosa* from mature (>2 years) woody tissues of almond.

Project Title :
Characterization and Studies on the Fundamental Mechanisms of *Xylella fastidiosa* Transmission to Grapevines by the GWSS

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Objectives of Proposed Research:
1. Characterize the transmission of *Xylella fastidiosa* to grapes by the glassy-winged sharpshooter (GWSS).
2. Develop in vitro assays to assess vector transmission of *Xylella fastidiosa*.
3. Test the possibility of biological control of *Xylella fastidiosa* transmission through competition for attachment site.

Justification and Importance of Proposed Research:
The introduction of the glassy-winged sharpshooter (GWSS) in California has increased the importance of Pierce's disease (PD), from a lethal disease limited to relatively few vineyards in coastal Valleys and the Central Valley to become a serious threat to several crops throughout the State. The threat is not limited to the wine and table grape industries. PD strains of *Xylella fastidiosa* can cause disease in almond and alfalfa, but other strains can affect peach, citrus and plum, as well as numerous tree species such as oak and elm (Purcell 1997). Massive efforts and investments have been devoted to study the pathogen and the GWSS (list of funded projects, annexed to this Call for Proposals), but much remains to be discovered about an essential step in this vector-borne disease: the transmission of the pathogen. *X. fastidiosa* is spread by xylem sap-feeding insects such as sharpshooters that pick up *X. fastidiosa* from an infected plant and later have the capacity to put it back into a healthy plant (Purcell and Hopkins 1996).

Different strategies to control the spread of *X. fastidiosa* by the GWSS have been proposed, but the long-term solution to the problem will be to protect crop plants from the pathogen and not its dissemination. This objective is not expected to be achieved in at least the next few years, so alternatives to manage PD spread are needed. Currently, insecticides are the main tactics being used, and improved biological control to reduce GWSS populations is also being pursued. But information is needed to relate the numbers of GWSS to disease spread in order to establish economic thresholds for vector control. A better understanding of the transmission process might also provide new ideas for limiting disease dissemination by reducing the efficiency of *X. fastidiosa* transmission from plant to plant.

Why characterize GWSS transmission of *X. fastidiosa*?
Some of the important characteristics of transmission of *X. fastidiosa* by GWSS are not known. GWSS transmits *X. fastidiosa* to grapes, but there have been so far few studies of its transmission efficiency to grape or almond (Purcell and Saunders 1999). It has been suggested that the rapid impact of the GWSS in Temecula Valley may have been due to GWSS' distinctive behavior of feeding on woody tissues and dormant plants rather than its abundance alone. Transmission to woody tissues could increase the rate of chronic infection by *X. fastidiosa* if summer infections of the bases of grape canes or older wood establish chronic infections. This hypothesis has yet to be tested. Currently, it is thought that summer infections of the canes by traditional vectors in California do not often survive through the winter, explaining the lack of evidence to date for vine-to-vine spread of *X. fastidiosa* in California vineyards (Purcell 1981, unpublished data). In addition, GWSS feeds on dormant grapevines, but it is not known if it can acquire *X. fastidiosa* from dormant vines or infect dormant vines. If GWSS can infect dormant vines or acquire *X. fastidiosa* from or transmit *X. fastidiosa* to dormant vines, this would extend the period during which vineyards should be protected from GWSS.

Insecticides have been widely used to reduce GWSS numbers, but there is little information on the time required for the vectors to acquire or inoculate *X. fastidiosa* into a grapevine or how the numbers and infectivity level of GWSS relate to

disease spread. Reducing the number of vectors does not necessarily reduce the amount of transmission of *X. fastidiosa* by a proportional amount (Purcell 1981). In theory, the likelihood of *X. fastidiosa* transmission depends directly not only on the number of vectors per plant (*n*), but equally on transmission efficiency (*E*), the percentage of vectors that are infective (*i*), and how much time is spent feeding on the plant or how vector activity is distributed over time (*t*) (Purcell 1981). Persistence of infectivity in adults has great epidemiological importance because GWSS can fly long distances and has a long life span. Without information on vector transmission efficiency and an understanding of how physical and biological factors affect transmission, it is difficult to estimate the impact of control strategies based on reducing the vector population or to establish the most critical times of year for lowering vector populations.

We intend to fill gaps in GWSS/PD research with detailed studies on GWSS transmission of *X. fastidiosa* by addressing the following questions: a) can GWSS inoculate *X. fastidiosa* into woody tissue or acquire *X. fastidiosa* from dormant vines? b) how long does it take for GWSS to acquire/inoculate *X. fastidiosa*? c) what part of the GWSS foregut is involved in vector transmission? d) are there alternative tactics to reduce transmission of *X. fastidiosa* by understanding its underlying mechanisms?

Why do we need to understand fundamental aspects of *X. fastidiosa* transmission?
Although understanding the characteristics of GWSS transmission of *X. fastidiosa* to grapevines will provide essential data to develop control strategies of PD, knowing the basic mechanisms of *X. fastidiosa* transmission will give us insights and maybe new opportunities to break down the dissemination cycle. Different approaches and techniques have to be used to study the transmission of *X. fastidiosa* by the GWSS, ranging from greenhouse and in vitro transmission experiments, to microscopy and the use of molecular techniques.

Transmission experiments require time, large numbers of test plants and insects. Although diagnostic methods based on polymerase chain reaction (PCR) and others have been developed (Minsavage et al. 1994; Pooler et al. 1997), no studies have established the relationship between test results for vector insects and vector transmission of the pathogen. Hill & Purcell (1995) demonstrated that population levels of *X. fastidiosa* assessed by culturing bacteria from the head of the blue-green sharpshooter (BGSS) did not correlate with transmission. BGSS with levels of *X. fastidiosa* that were below a detection threshold of 100 cells per insect transmitted *X. fastidiosa* about as well as did BGSS with much higher populations of cultivable *X. fastidiosa.* The saturation of transmission efficiency by small numbers of *X. fastidiosa* imply that the active region in the vector from which the bacterium is transmitted is small. Understanding the efficiency and limitations of methods to detect *X. fastidiosa* is important, because estimates of what percentage of GWSS are capable of transmitting *X. fastidiosa* can otherwise only be made by transmission assays, which require special facilities and 6-10 weeks of incubation period at suitable temperatures in a greenhouse.

The observations that (i) sharpshooter vectors stop transmitting *X. fastidiosa* after molting until they are again fed on an infected plant and (ii) there is no latent period between acquisition of *X. fastidiosa* from infected plants and its inoculation into healthy plants (Purcell and Finlay 1979) imply that the bacteria are transmitted from the vector's foregut. Although *X. fastidiosa* has been observed in the foregut of vectors (Purcell et al. 1979; Brlansky et al. 1983), the location of *X. fastidiosa* within the vector's foregut from which the bacterium is transmitted has not been proven. Moreover, previous studies of *X. fastidiosa* transmission mechanisms used the BGSS or other sharpshooters in the tribe Cicadellini. GWSS and other sharpshooters in the tribe Proconiini seem to transmit *X. fastidiosa* less efficiently than members of the Cicadellini. The two tribes of sharpshooters differ greatly in morphology and some behaviors, so it is possible that GWSS may differ significantly in behavior or morphology in ways that affect its transmission of *X. fastidiosa*. We should not take for granted that GWSS transmission of *X. fastidiosa* is exactly the same as for members of the tribe Cicadellini. The possible locations from which *X. fastidiosa* may be released to infect plants extend from the stylet tips to the entrance of the midgut. We will seek to determine the location(s) of *X. fastidiosa* within the foregut that are critical for transmission. We hypothesize that *X. fastidios* may be transmitted in a similar manner to non-persistent viruses (Gray and Banerjee 1999) but with *X. fastidiosa* persisting by multiplication. Our efforts to identify the site within the vector foregut will test this hypothesis.

Partly because we cannot observe the GWSS feeding activities within grapes, and to separate salivation from ingestion and other activities, a technique known as electronic penetration graphic (EPG) is applicable (Walker and Backus 2000). With this technique, it is possible to monitor the insect's feeding activities, while identifying several phases of feeding such as ingestion and salivation. This powerful method should allow us to identify critical phases of feeding during which

inoculation of *X. fastidiosa* occurs. This would allow us to determine how the types and number of probes by an infective vector relate to transmission or during which part of the feeding process inoculation occurs.

An in vitro assay for studies of the transmission of *X. fastidiosa* is essential to many related areas of research, such as tests of the transmissibility of mutants of *X. fastidiosa* and efficiency of transmission of different strains of *X. fastidiosa*. Such methods would allow experimental control over the acquisition of *X. fastidiosa* by the vector. Unfortunately, such a system has not been developed yet. Davis et al. (1978) tested the possibility of in vitro acquisition of *X. fastidiosa*, but the sharpshooters tested did not transmit. Purcell and Finlay (1979) used the same approach to study sharpshooter transmission of bacteria other than *Xylella*. Recently we attempted in vitro acquisition of *X. fastidiosa* with the BGSS and GWSS. Our data demonstrated that vectors acquired *X. fastidiosa* from cultured *X. fastidiosa* cells suspended in sterile xylem sap but did not inoculate it to grapes (Almeida and Purcell, unpublished). We believe that the planktonic cells did not attach to the foregut.

We are testing different substrates and *X. fastidiosa* cells collected from different sources for successful transmission. Success in these experiments would enable us to test specific mutants of *X. fastidiosa*, identifying genes required for the dissemination cycle to be completed. The development of an efficient in vitro system will enable experiments to determine what characteristics make *X. fastidiosa* vector transmissible. Mutants could be easily screened and the available genetic data be used to understand the basis transmission of *X. fastidiosa.* This approach has been applied to test the transmission of plant viruses by aphids (Atreya and Pirone 1993), whiteflies (Morin et al. 1999). Previous success on similar studies with other vector-pathogen interactions (for example, the mollicute bacterium *Spiroplasma citri* and its leafhopper vector *Circulifer tenellus* (Fletcher et al. 1996), potyvirus and aphid vectors (Atreya and Pirone 1993, Wang et al. 1996), demonstrate the usefulness of molecular techniques in vector transmission studies. Transformation mechanisms of *X. fastidiosa* are now being published by Bruce Kirkpatrick's lab at UC Davis (random insertion transposon mutagenesis) and Fundecitrus researchers in Brazil (homologous recombination). Therefore transformation of *X. fastidiosa* should not be a limitation for future studies (not in this proposal) that utilize in vitro feeding assays.

Understanding the transmission mechanisms of *X. fastidiosa* may also help develop biological control strategies for *X. fastidiosa* transmission. Microbes that occur on plant surfaces and can attach to the foregut surface of sharpshooters may compete with *X. fastidiosa* for a specific attachment site (or sites) in the GWSS foregut. This competition could exclude one of the microbes from this essential region in the vector's mouthparts, and the first microorganism to colonize it would in principle be the successful one. Bacterial competition for preferential sites on plants is an approach used to control bacterial plant diseases like fire blight (Johnson & Stockwell 1998). One of the benefits of this approach is that once the biological control agent attaches to the specific site required for *X. fastidiosa* transmission by the GWSS, the adults GWSS might be permanently unable to transmit the pathogen. Our preliminary experimental data (unpublished) suggests that some GWSS are not able to transmit *X. fastidiosa.* Specifically; GWSS' acquisition of *X. fastidiosa* does not approach (asymptotically) 100%, but rather 10-70%. In addition, different groups of field-collected GWSS transmitted at dramatically different rates under the same experimental conditions. We seek to confirm and understand this phenomenon, including the possibility that other microbes can compete with *X. fastidiosa* for an attachment site on the GWSS' foregut. We have isolated miscellaneous bacteria and yeast from the surface-sterilized heads of non-transmitting GWSS and from test plants fed upon by these insects in transmission tests. Our approach will be to look for reduced transmission after GWSS access to plants sprayed (or naturally infected) with different bacteria and fungi obtained from various sources (GWSS head, grapevine leaf surface and internal tissues).

Project Title :
Alternatives to Conventional Chemical Insecticides for Control of GWSS

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Objectives of Proposed Research:
1. The objectives of our research is to evaluate two new insecticidal materials; the biorational control agent (Sugar esters) and a repellents/protectant (particle film) against GWSS in the laboratory, greenhouse, and field.
2. In the field studies, the effects of these treatments on Pierces disease, plant growth, yield and grape quality will also be determined. First years studies will determine the efficacy of the sugar esters and particle film.
3. Second years studies will determine how these materials could be incorporated into IPM for GWSS in grape.

Justification and Importance of Proposed Research:
Glassy-winged Sharpshooter (GWSS) is a major pest of grape because if vectors a serioud disease in grape called Pierce's Disease. Efforts are being made to control GWSS in citrus to prevent movement to grape. If this effort fails, then control efforts would need to shift to GWSS in grape. Further, there is a need to use softer insecticidal materials in citrus to preserve the already established citrus IPM program. The insecticides that are currently being used to control GWSS are few and many researchers are currently evaluating other conventional chemical insecticides. There is also a need to identify alternatives to chemical control.

Project Title :
Impact of Layering Control Tactics on the Spread of Pierce's Disease by the GWSS

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Objectives of Proposed Research:

1. To evaluate, through Field experimentation, the simultaneous application of multiple chemical, physical and cultural control treatments for the management of Pierce's Disease in grapes under current densities of glassy-winged sharpshooters in the Temecula Valley of California.
2. To determine the ability of a variety of treatment and treatment combinations on 1) their ability to reduce glassy-winged sharpshooter density and feeding and 2) their ability to reduce the rate of spread of PD in newly planted vineyards. Treatment and treatment combinations to be evaluated are 1) full rate application of the neonciotinoid insecticide acetarniptrid, 2) full rate application of tetracycline, 3) full rate application of kaolin, 4) 8 m barrier screens, 5) acetamiprid + tetracycline, 6) acetarniprid + kaolin, 7) aretamiprid + barriers, 8) tetracycline + kaolin, 9) tetracycline +

Justification and Importance of Proposed Research:
Pierce's Disease (PD), a grapevine malady induced by the bacterium *Xylella fastidiosa*, is present in the United States in grape-growing regions where winters are mild. It is considered the principle factor limiting the grape industry in the southeastern United States. In the summer of 1997 an outbreak of PD was discovered for the first time in the Temecula Valley of California. Concomitant with this new PD epidemic in Temecula was a rapid increase in the range of a PD vector new to southern California, the glassy-winged sharpshooter (GWSS), Homalodisca coagulaicz. As result, an estimated 20-50% of Temecula's vineyards have been either infected with PD or completely destroyed by PD. The research proposed here will evaluate an overall GWSS vector control strategy that "layers" management tactics such that GWSS numbers theoretically approach a level that reduces PD transmission and occurrence to economically feasible levels. Using a standard field experimental approach, the following GWSS/PD control tactics will be evaluated singly and in all possible combinations: use of insecticides (acetamiprid), use of reflective films (kaolin), use of bacteric ides (tetracycline to prevent PD infection), use of large physical screen barriers (26 Ft in height).

Project Title :
Controlling the Spread of *Xylella fastidiosa* the Causal Agent of Oleander Leaf Scorch by Disrupting Vector Acquisition and Transmission

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Objectives of Proposed Research:
The goal of the research proposed herein is to determine if systemic insecticides can be part of an integrated pest management system to reduce the spread of *X. fastidiosa*, the causal agent of oleander leaf scorch, by sharpshooter vectors. Specific objectives are as follows.

1. To ascertain the degree to which a systemic insecticide affects the acquisition of *Xylella fastidiosa* by sharpshooters from diseased oleanders through time after plants are treated.
2. To characterize the relationship between time that sharpshooters are allowed access to diseased oleanders and the probability that they acquired *Xylella fastidiosa.*
3. To ascertain the degree to which a systemic insecticide affects transmission of *Xylella fastidiosa* to oleanders by pathogen-carrying sharpshooters through time after plants are treated.

Justification and Importance of Proposed Research:

Oleander Leaf Scorch (OLS) is a devastating disease that threatens to destroy statewide plantings of oleander, arguably the single most important ornamental scrub in California. The causal agent of this disease is the bacterium *Xylella fastidiosa*, and its vectors are the sharpshooters *Homalodisca coagulata* and *H. lacerta.*

Our recent study to develop an insecticide tactic to prevent OLS show that systemic insecticides have an outstanding potential to control disease spread by reducing vector population densities and by disrupting the pathogen-vector interaction that leads to successful pathogen acquisition and transmission. Systemic insecticides can be used with other control tactics, including biocontrol because they do not affect wasps that parasitize sharpshooter eggs. In addition, in a soil-applied formulation, they are especially safe for workers and the urban community where oleanders are used extensively.

Our field-based investigation will examine the effects of a soil-applied systemic insecticide on the ability of sharpshooters to acquire and transmit *X. fastidiosa,* and sharpshooter mortality through a six month period after a single insecticide treatment. Additional studies will document the seasonal population densities of sharpshooters to better implement plant protection tactics.

Project Title :
Developing an Integrated Pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula

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Objectives of Proposed Research:

1. POPULATION MONITORING: Determine the distribution and relative abundance of
   A. The glassy winged sharpshooter within and among the major cropping systems in the Temecula Valley.
   B. The sharpshooter egg parasitoids, (*Gonatocerus sp.),* within and among the major cropping systems of the Temecula Valley.
   C. The temporal and spatial occurrence of the Pierce's disease bacterium in the Temecula Valley in wild and cultivated hosts and in the GWSS.
2. BIOLOGICAL CONTROL: Evaluate sharpshooter egg parasitoids (*Gonatocerus ashmeadi* and other species as they become available) as a biological control agents to reduce and limit populations of glassy winged sharpshooter in citrus and grape cropping systems in Southern California.
   A. Determine the oviposition rate for the sharpshooter on a variety of host plants in a greenhouse.
   B. Develop rearing methodologies for GWSS egg parasitoids, including determining the viability of both parasitized and unparasitized eggs after periods of long-term storage under refrigeration (required for mass rearing of parasitoids).
   C. Construct a degree day model for development of both sharpshooters and parasitoids (required for mass rearing and release of parasitoids, also will allow predictions of periods of high and low population densities).
   D. Conduct preliminary field release studies and evaluate parasitoid longevity, reproduction, field persistence, dispersal, and impact on field populations of sharpshooter in both citrus and grapes.
   C. Conduct exploration for other parasitoids in the GWSS's native range (southeastern U.S. to south Texas and northern Mexico).
   3. USE OF INSECTICIDES TO CONTROL SHARPSHOOTERS AND LIMIT SPREAD OF DISEASE: Continue research evaluating pesticides to deter sharpshooters from feeding and/or rapidly kill them such that disease spread is decreased.
   A. Determine the degree to which neonicotinoids (e.g. imidacloprid) affect transmission of the PD organism to grapevine by pathogen-carrying sharpshooters through time after plants are treated.
   B. Determine the optimal deployment of neonicotinoids on grapevines to reduce vector pressure and disrupt transmission of the PD organism.
   C. Determine the impact of neonicotinoids in citrus on GWSS.
4. CHEMOTHERAPY: Develop a means of "curing" grapevines infected with the PD bacterium, or preventing the establishment of *X. fasditiosa* in grapevines.
   A. Determine the lowest concentrations of various agricultural formulations of zinc, manganese, copper and iron which inhibit the growth of *X. fastidiosa* in vitro.
   B. Determine the highest concentration of these materials that can be injected or applied to the soil or foliage without causing irreversible phytotoxicity.
   C. Determine what the resulting concentrations of these materials are in xylem sap collected from four widely used grape rootstocks grafted with Chardonnay scions.

Justification and Importance of Proposed Research:

In the 10 years since the GWSS was first identified in California, and in less than two years since PD was first discovered in Temecula, the malady has become a serious threat to wine-grape production in Temecula and potentially the entire state. A conservative estimate by John Moramarco (General Manager, Callaway Vineyards and Winery) indicates that PD has decimated approximately 10% of the total acreage of winegrapes in Temecula. Vineyard surveys by Blua et al.

indicate the devastation may be much worse (unpublished data). This past summer (1998) we observed the GWSS for the first time in citrus orchards that are commonplace in southern Kern County. If we extrapolate from the pattern observed in Temecula where citrus and grapes are the main crops, the potential devastation to the table-grape and raisin industry in California will be overwhelming. Also at risk in this area are almonds as almond leaf scorch is a disease induced by the PD bacterium (Davis et al. 1983).

For many years in California, PD has been prevalent in the north coastal, and to a lesser extent in the central-coastal, wine grape-growing areas where it is spread mainly by the blue-green sharpshooter. Because of the habitat preference of the blue-green sharpshooter, disease out-breaks are typically confined to the edge of vineyards adjacent to riparian areas (Purcell 1975). In the San Joaquin Valley, outbreaks of PD are associated with irrigated pastures or weedy fields bordering vineyards (Purcell and Frazier 1985). Again, disease spread has a strong "edge-effect" component. The pattern of disease spread observed in Temecula is different. First, because GWSS are produced mainly in citrus, disease outbreaks are related to the proximity of citrus. Second, the GWSS is a large sharpshooter and a strong flyer with a propensity to disperse far into a vineyard. In arrays of yellow sticky cards that we set in vineyards adjacent to citrus orchards, we see no appreciable difference in GWSS catches between 0, 10, 20, 30, and 40 meters from the edge of a vineyard (Blua et al., unpublished data). Disease mapping in Temecula vineyards in blocks that are 25 rows by 25 plants frequently shows no pattern of spread, with the entire block is showing strong symptoms throughout. In these mapping studies PD was confirmed by serological assays (Agri-Analysis, Davis, CA).

The research proposed here will provide an IPM strategy that potentially will limit the spread of Pierce's disease to vineyards. Theoretically this approach can be implemented quickly, yet also continuously be improved, pending the results of current and future research. This IPM strategy hopefully will be supplanted with a long-term strategy that involves resistant or tolerant cultivars. The research team assembled by this proposal represent researchers in departments of Entomology and Plant Pathology from University of California's Riverside, Berkeley and Davis campuses. Our collective experience with PD, vector-pathogen and plant-pathogen relations, diagnostics, and plant protection will insure that this mission-oriented research will progress rapidly, and provide a maximum impact on GWSS-spread PD in the Temecula valley, and outbreaks of PD and similar diseases that are likely to be discovered in other areas of California.

Project Title :
Economic Impact of Pierce's Disease on the California Grape Industry

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Objectives of Proposed Research:
1. This project aims to develop estimates of the economic impact of Pierce's Disease on the California grape industry.
2. The project will review both the current situation and provides estimates of future economic impacts if a new vector, the Glassy Winged Sharpshooter (GWSS) becomes established.

Justification and Importance of Proposed Research:

Pierce's Disease (PD) is not new to California. It was first observed and recorded in the 1880's when it was responsible for destroying more than 40,000 acres of grapevines in the Los Angeles basin. Localized infections of the disease have occurred in the Napa Valley since the 1880's. There have also been periodic epidemics over the last century where the disease has reached a higher incidence and become more widespread in the grape growing regions of the state. In the early 1990's, growers in Napa and Sonoma counties again began reporting symptoms of PD. The spread of PD into North Coast vineyards, while widespread, is mostly confined to riparian areas and near irrigated landscapes. The Blue-green Sharpshooter (BGSS) is the principal insect vector spreading the disease from the riparian habitats. Under BGSS, vine to vine spread is minimal, even though the disease is present in the vineyard. This is due to the nature of BGSS which does not travel far and has a limited ability to transmit the disease due to the small size of its mouth. In addition, much of PD infection is eliminated through the pruning process. Small vineyards planted next to BGSS habitat traditionally have had the highest risk due to infestations from the habitat. The disease basically has an edge effect of about 300 feet; hence, if the vineyard is 600x600 feet, then it is all edge. Since 1994, more than 1,000 acres of Napa and Sonoma county grapevines have been pulled and replanted (total 1999 bearing acreage equaled 66,700 acres) due to Pierce's disease with an estimated cost to growers of over $30 million in lost income, production, and replanting expense.

Up until the late 1990's, PD was known as a disease mostly prevalent in the North Coast grape growing areas. However, according to Bill Peacock, University of California Farm Advisor, Tulare county has battled PD since the 1930's. He claims the outbreak has been as severe as that in Napa, but the problem doesn't receive as much attention since it doesn't have the high profile that Napa does. The problem would be exacerbated with the introduction of a more efficient vector than the traditional Blue-green, Green, and Red-headed sharpshooters which are not aggressive in their travel and eating habits. Enter the Glassy-winged sharpshooter (GWSS) which recently became established in California and is a serious threat to vineyards since it moves faster and farther into vineyards than other species.

Since the early 1990's, GWSS has been seen in high numbers in citrus along the Southern California coast. During the past few years, it has become more abundant farther inland in Riverside and San Diego counties. In 1998 and 1999, high populations on citrus and adjacent vineyards were seen in southern Kern county. GWSS is expected to spread north into the citrus belt of the Central Valley and become a permanent resident of various habitats throughout northern California.

Project Title :
Surrogate Genetics for *Xylella fastidiosa:* Regulation of Exopolysaccharide and Type IV Pilus Gene Expression

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Objectives of Proposed Research:
1. To explore specificity determinants for transcription initiation in *Xylella fastidiosa*
2. To develop *Escherichia coli* as a surrogate host for the study of Xylella *fastidiosa* regulated gene expression.
3. Apply bioinfon-natics to evaluate transcription control signals in Xylella *fastidiosa* 9a5c.
4. Construct and characterize a *(D(gumB-IacZ)* operon fusion in *E coli.*
5. Determine the effect of *rpfGC* on *(D(gumB-lacZ)* expression in *E coli.*

Justification and Importance of Proposed Research:
Surrogate genetics. *Xylella fastidiosa* presents a formidable challenge to the molecular geneticist. There are no published methods available for the basic operations of genetic exchange, mutant isolation, and complementation. The slow generation time, poor plating efficiency and requirement for complex culture media are further complications. Despite these obstacles, several laboratories are actively engaged in developing genetic systems for *Xylella fastidiosa.* Nevertheless, it is a daunting task to create a new genetic system (Fink, 1988). The success of the model organisms (e.g., *Escherichia* coli and *Saccharomyces cerevisiae),* beyond their facile cultivation in the laboratory, stems from long-term investment by a large community of geneticists focused on a single strain, so that mutants and methods developed in one laboratory can be utilized by all.

Surrogate genetics (Maloy and Zahrt, 2000) provides a means to at least partially bypass these challenges. Here, one creates a hybrid organism, transplanting genes of interest from the poorly studied species (e.g., *Xylella fastidiosa)* into a well-studied surrogate host (e.g., *E. coli*). Given sufficiently related hosts, one expects the transplanted genes to function in the surrogate essentially as they do in the original. One may then exploit the advantageous proper-ties of the surrogate to perform a large number of experiments, making and discarding hypotheses to define various aspects of gene function. Once gene function in the surrogate has been thoroughly explored, one can perform a limited yet focused set of experiments, informed by the results from the surrogate, to examine function in the native host.

Project Title :
Chemical Control of GWSS: Establishment of Baseline Toxicity and Development of Monitoring Techniques for Detection of Early Resistance to Insecticides

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Objectives of Proposed Research:
1. Develop bioassay techniques to evaluate baseline toxicity of chemicals from major classes of insecticides against all life stages of glassy-winged sharpshooter GWSS;
2. Monitor all life stages of GWSS collected from insecticide-treated orchards and vineyards to determine changes in baseline susceptibilities towards insecticides;
3. Investigate the rate of evolution of resistance to a selected organophosphate (OP), pyrethroid and neonicotinoid in GWSS by artificial selection in the greenhouse;
4. Develop electrophoretic techniques to identify esterase profiles in individual GWSS of all life stages including eggs;
5. Develop a microplate assay to measure the levels of sensitivity of GWSS acetylcholinesterase (AChE) variants to inhibition by organophosphate OP insecticides commonly used for their control;
6. Monitor GWSS populations throughout California to determine the degree of phenotypic variation in esterase and AChE enzymes and how this relates to current pesticide practices.

Justification and Importance of Proposed Research:
In current efforts to halt the spread of Pierce's Disease (PD), insecticides hold much promise for the control of its primary vector, the glassy-winged sharpshooter (GWSS) *Homalodisca coagulata*. They represent an immediate remedial option against populations of GWSS, and their successful implementation in management schemes therefore requires that their efficacy be carefully evaluated and monitored to ensure maximum benefit. The use of systemic insecticides is a particularly attractive option for growers not only because of their lethal properties but also because of their ability to induce behavioral changes such as reduced feeding. The latter could have a serious impact on the spread of PD. Insecticide resistance poses the most serious threat to the long-term success of insecticides for controlling pests. Monitoring programs to detect resistant phenotypes as early as possible and to document their distribution should be key components of any resistance management strategy. Indeed, rather than waiting for resistance to happen, resistance management strategies should be in place as soon as insecticides are implemented as an integral part of the control strategy.

Our first priority is to assess the baseline toxicity of various insecticides against the GWSS with the aim of establishing the most effective products for use in management programs. The results of laboratory tests can offer immediate practical guidance to individual growers even before conducting expensive trials to determine the suitability of an insecticide. They also provide the most effective means of detecting changes in the susceptibility of insects arising from pesticide exposure. Changes in the toxicological response of a population to an insecticide is usually the first indication of resistance development; hence, the urgency for the development of bioassay tests and the derivation of toxicity data. We would then develop the project to address more fundamental questions concerning the likelihood of insecticide resistance development in the GWSS. We will approach the latter objective through extensive monitoring of populations collected from treated orchards and vineyards, as well as through long term greenhouse experiments in which insect populations will be subjected to insecticide exposure during each generation. Despite the difficulty some may have experienced in establishing a GWSS colony, we believe greater attention to plant selection and overall diligence would make it feasible. No scientific or experimental data is available which rules out the possibility of establishing a colony of GWSS under greenhouse conditions. Furthermore, there is no evidence to suggest that insect diapause is an impediment to the development of resistance, and it may be a convenient mechanism by which resistant insects can safely pass through unfavorable environmental conditions. Indeed, this will be the first study addressing this issue.

Is resistance a threat to the management of GWSS? Most models of resistance development assume that a certain level of selection occurs during each generation (Tabashnik, 1990). There are two reasons why resistance could have drastic consequences for control of GWSS. Firstly, the GWSS undergoes no more than two generations per year and could therefore be regarded as an unlikely candidate for the rapid build-up of resistant populations. To assume this without any actual experimental data is dangerous. Resistance develops as a result of a selection process in which susceptible individuals are removed from treated populations leaving behind a large proportion of resistant individuals which, in the absence of significant immigration of susceptibles, will form the main genetic pool of subsequent generations. Unless the populations are completely eliminated, the proportions of resistance genes will be higher in the next generation, even if there are lower overall numbers. The Colorado potato beetle, *Leptinotarsa decemlineata*, has an incredible capacity to evolve resistance despite having only two generations per year (Georghiou, 1986), but compensates for this by a high reproductive potential (May and Dobson, 1986). While the same may not be true for the GWSS, there is insufficient data available to make any broad presumptions regarding the potential for resistance development in this pest. And secondly, longer generation times also increase the risk of multiple exposures of populations to the same chemical, thereby enhancing the selective process further. Under these conditions, cross-resistance can become extremely critical.

We advocate using a combined toxicological and biochemical approach to studies of resistance. Knowledge of biochemical mechanisms conferring resistance has enabled researchers to develop sensitive assays for use in monitoring programs (Byrne et al., 1994). Although bioassays are absolutely essential to confirm the presence of resistance in a population, biochemical monitoring tools enable more accurate assessments of resistance gene frequencies. Because of this, important decisions concerning the most appropriate insecticides can be made while avoiding those most likely to be affected by cross-resistance. We therefore propose initiating studies on biochemical mechanisms of resistance, with particular attention to determining the phenotypic variation in esterases using polyacrylamide gel electrophoresis (PAGE). There are several reasons for studying esterases by this method. First, pyrethroid, OP and carbamate insecticides are esters and are therefore susceptible to attack by esterases. Separation of these enzymes on polyacrylamide gels enables direct comparisons between susceptible and resistant populations. Using this approach, both qualitative and quantitative changes in these enzymes have been attributed to resistance in a wide range of insect pests of agricultural, medical and veterinary importance (Byrne *et al.*, 2000). Monitoring esterase profiles of insects is a powerful tool for tracking the spread of resistant individuals. Second, esterases can be used to distinguish insect biotypes (Byrne and Devonshire, 1993). For example, the worldwide movement of whitefly biotypes and their subsequent establishment in new geographical locations was effectively monitored using PAGE of esterases. Most recently, the combined use of laboratory bioassays and PAGE techniques has confirmed biotype-specific resistance problems in both Spanish and Israeli populations of *Bemisia tabaci.* Third, esterase profiles can be used to distinguish between insect species (french-Constant et al., 1988). In California, the smoke tree sharpshooter coexists with the GWSS. The egg masses of these two species are not readily distinguishable from each other in the field. Esterase profiling would, therefore, provide an effective means of monitoring egg populations to establish the frequencies of the two species in different crop systems. This could have important implications for targeting chemical control measures against emerging immatures. Fourth, esterases offer a useful diagnostic of parasitism. Age-related expression of egg esterases would be disrupted by the developing parasitoid. The loss of host enzymes would be matched by the expression of unique parasitoid enzymes as they mature within the egg. This approach has been successfully applied to the detection of parasitoids in whitefly immatures. Fifth, the expression of esterase activity in GWSS eggs can be exploited as a marker of pesticide efficacy. Ovicidal activity can be implicated through the loss of expression of esterases, either due to the death of the egg or the inhibition of the esterases.

The development of biochemical monitoring techniques for studying potential resistance mechanisms, and implementing their use before widespread use of chemicals, would act as an early-warning system for detecting resistance problems. Changes in the frequencies of certain alleles can be the first indication that resistance is becoming a problem and that remedial action is required such as changing the insecticide class. The major advantage of this approach is that specific resistance mechanisms are monitored directly, thereby enabling rapid determination of changes. This pre-emptive approach to resistance management would be unique in its efforts to forestall the development of resistance in a crop where one resistance outbreak could prove economically disastrous.

There are no cases of resistance to insecticides in GWSS and as such no knowledge on its propensity to develop resistance. This is not surprising as it has not been targeted for control until its recent emergence as the primary vector of

PD in vines. Insecticide applications have thus far been limited, but as the problem progresses and the incidence of PD spreads, the use of insecticides is expected to increase, particularly in the absence of alternative control measures. Generally resistance evolves in almost all cases where insects have been subjected to selection pressure. Although the rate or degree of the occurrence of resistance to commonly used insecticides in natural populations of the GWSS cannot be predicted, now is the time to establish baseline toxicity to insecticides that will most likely be used against GWSS. Further, resistance monitoring techniques are essential to maintain an effective chemical management strategy.

Project Title :
Laboratory and Field Evaluations of Imidacloprid and Thiamethoxam against GWSS on Citrus and Grapes

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Objectives of Proposed Research:
1. Evaluate the distribution and titre of Admire and Platinum within citrus trees and grape vines over time.
2. Conduct bioassays of GWSS on field collected citrus tree and grape vine tissue, that will be evaluated for Admire and Platinum titre (concentration in the leaves, stems, etc).
3. Develop a bioassay for GWSS that can be used for baseline testing and toxicity determination in the field.
4. Study the behavior of GWSS adults and nymphs of citrus and vines treated with Admire.

Justification and Importance of Proposed Research:
The most immediate threat by GWSS to California agriculture as a whole is to vineyards. GWSS is a particular threat to vineyards due to its ability to spread Pierce's disease. But in addition to Pierce's disease, the bacterium *XyIella* causes almond leaf scorch, alfalfa dwarf, oleander leaf scorch, phoney peach and citrus variegated chlorosis. Genetic variants of *X. fastidiosa* are thought to be responsible for the myriad diseases that occur in a wide range of crop, ornamental and wild host plants. The potential spread of *Xylella* diseases by GWSS should be of concern to the agricultural industry, as should the high densities of GWSS that build on citrus during certain portions of the year. It is unknown what the costs are to citrus, grapes or other plant types from high numbers of feeding GWSS because the focus up to now has been on their involvement with the spread of Pierce's disease and the other *Xylella*-caused diseases. However, the high rate of feeding by even a single adult GWSS results in a large volume of xylem fluid removed from the host tree as evidenced by GWSS xylem stains on leaves. The cumulative impact of hundreds and even thousands of GWSS feeding on a single host tree or vine, not uncommon densities that have been observed in Riverside County, could negatively impact the vigor of a tree or vine over time, especially immature trees or vines. It is therefore important that all growers take seriously the threat to agriculture by GWSS as a direct pest and not be complacent about GWSS as some other commodity's problem.

One of the most encouraging developments in sucking insect management is the introduction and development of neonicotinoid insecticides such as Admire (imidacloprid) and Platinum (thiamethoxam). A single application of Admire') can offer protection to citrus trees or grape vines for 60 plus days under high sucking insect pressure. With Platinum nearing registration, information on the distribution within trees or vines and efficacy against GWSS also needs to be collected and incorporated into an effective management solution.

The availability of the neonicotinoid insecticides provides an important alternative to pyrethrold, carbamate, and organophosphate insecticides. Much prolonged control with Admire or Platinurn should result in fewer treatments than traditional insecticide sprays and less disruption to biocontrol efforts. As systemic insecticides, Admire and Platinum have much lower negative impact on the natural enemy complex of insect pests than sprayed contact insecticides. Only insects that feed on citrus or grapes would be exposed to the toxic effects of Admire or Platinum, leaving intact the complex of foraging natural enemies within an orchard or vineyard. Moreover, application of these systemic insecticides through existing drip or mini-sprinkler systems reduces applicator costs and the negative impression formed by the public over insecticide spraying.

The Principal Investigators are aware of the ongoing work of Drs. Beth Grafton-Cardwell and Phil Phillip on oranges and lemons. But we feel that our work will provide an essential understanding of the protection afforded by Admire and Platinum against GWSS, and will enable better decision-making by growers and PCAs in their efforts to suppress GWSS infestations. The research we are proposing is needed because of variables that impinge upon the amount of Admire or Platinum up taken by trees and vines including evapotranspiration demand, growth state (flushing or non-flushing), etc.

These variables can effect the distribution of systemic insecticides within the tree and vine tissues and interfere with the delivery of a lethal dose to the target insect or allow it to avoid those tissues. It is therefore important that the inherent limitations and advantages of any insecticide be well understood to allow for its maximum utility. Neonicotinoids such as Admire and Platinum are relatively safe selective non-toxic materials to humans that have to be evaluated so that they can be employed to their fullest potential by California's citrus and grape industries.

Project Title :
Area Wide Management of the GWSS in the Temecula Valley

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Objectives of Proposed Research:
1. This cooperative demonstration Glassy-winged sharpshooter (GWSS) Project proposes to examine the impact of an areawide GWSS management program on GWSS populations and Pierce's Disease (PD) incidence.
2. Determine the impact of the 2000 areawide management program on GWSS populations in citrus, grapes, and other plant hosts in the ecosystem in the 2001 season.
3. Determine the impact of the areawide program on GWSS adult oviposition, and nymphal development.
4. Determine the impact of the GWSS program on beneficial citrus insects, pest upsets and GWSS egg parasitism.
5. Evaluate the biological and economic effectiveness of an areawide insecticide program of GWSS.

Justification and Importance of Proposed Research:
GWSS is a particular threat to agriculture because of its ability to spread the bacterium *Xylella*. One of the most encouraging developments is the introduction of nicotinoids such as imidacloprid (Admire) for the control of sucking insects such as GWSS.

Because Admire is a systematic insecticide and is applied through mini-sprinklers and drip irrigation systems it has a much lower negative impression on the public's perception of contact sprays such as the organophosphates, carbamate and pyrethroid insecticides, and therefore it may be the best candidate for areawide suppression of GWSS.

The emergency treatment of 1300 acres of citrus in Temecula, CA with Admire (Imidacloprid) during April and May 2000 represented a pivotal shift toward an area-wide management of the glassy-winged sharpshooter (GWSS). Although table and wine grapes are the most vulnerable crops to GWSS as a vector of the bacterium *Xylella fastidiosa*, the causal agent of PD, other crops are being scrutinized for their contributions to GWSS population growth. Perhaps more than any other source, citrus is viewed as an important year long reproductive host of GWSS, but also one that concentrates GWSS populations over the winter months during the time that grapes and many ornamental hosts are dormant. In the 2000 season, the opportunity to treat the entire commercial plantings of citrus in the Temecula area with Admire was seized upon in an effort to destroy a substantial portion of the regional GWSS population.

Evaluations of the efficacy of the Admire treatments in Temecula citrus groves are still ongoing, but preliminary GWSS data collections indicate Admire and Lorsban are providing some measure of control.

Project Title :
The Genetics of Resistance to Pierce's Disease and Breeding Pierce's Disease Resistant Table and Raisin Grapes

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Objectives of Proposed Research:
1. Develop a genetic map to *Xylella fastidiosa* resistance using *Vitis vinifera x (V rupestris x M rotundifolia)* seedling populations and AFLP (amplified fragment length polymorphism) markers, identifying resistance markers, and possible identification of resistance genes.
2. Utilize DNA markers for resistance to rapidly introgress *Xylella fastidiosa* resistance into *V vinifera* cultivars and/or utilize genetic engineering procedures (when available) to move above identified *Xylella fastidiosa* resistance genes into *V vinifera* cultivars.
3. Develop PD resistant table and raisin grapes by crossing currently available forms of resistance with large berries and early seedless *V. vinifera* table and raisin grapes.

Justification and Importance of Proposed Research:
Renewed and intensified PD outbreaks in historic PD areas around the state and the introduction of GWSS into the southern San Joaquin Valley demonstrate the vulnerability of *V vinifera* table and raisin grape culture in California. All of California's commercially significant table and raisin gapes are susceptible to PD. No effective prevention or cure currently exists. Under severe PD pressure, such as the southeastern United States, culture of *V vinifera* grapes is not possible.

The breeding of new PD resistant table and raisin grapes will be much more direct than attempting to breed several PD-resistant wine grape cultivars. There are multiple sources of PD resistance that could be incorporated into *V vinifera* table and raisin grapes. It is likely that acceptable large berries seedless table grapes, and early thin-skinned raisin grapes can be produced in two generations of crosses from existing germplasm. In addition, these new resistant cultivars will also be selected to have very high levels of powdery mildew resistance. Unlike the wine industry where the need for "pure vinifera" cultivars is enforced by marketing and prejudice, new table grape cultivars are introduced frequently and, given adequate quality (seedlessness, color, season, flavor, texture), meet with ready consumer and industry acceptance. The overall objectives of this project are divided into two parts. The first two are directed at determining how resistance to *X. fastidiosa* is inherited, and at developing a genetic map to produce *X. fastidiosa* resistance markers for use in breeding and enable the discovery of *X. fastidiosa* resistance genes. The third objective focuses on breeding PD resistant table and raisin grapes utilizing high quality seedless table and raisin grapes from the Ramming program and *X. fastidiosa* resistance sources from the Walker program. Completion of these objectives is tied to the speed with which grape seedlings can be produced and evaluated.

The USDA/ARS Fresno grape breeding program has developed many important table (Flame Seedless 92, Crimson Seedless = 94 in production) and raisin grape cultivars (Fiesta). The Ramming program continues to release new high quality table and raisin grape cultivars (e.g. DOVine and Melissa) and has a number of advanced selections in various stages of testing for commercial production. Ramming's germplasm has the most advanced large-berried and seedless selections available, and is capable of rapidly producing high quality PD resistant cultivars.

I'D resistance exists in a number of Vitis species and in the related genus, *Muscadinia*. Resistant cultivars have been developed in public (Dunstan 1965, Loomis 1958, Mortensen 1977, 1983a, 1983b, Olmo 1986, Overcash 1981, 1982, among others) and private (Barrett, Bloodworth and others) breeding programs across the southeastern United States. These cultivars have high PD resistance, but relatively low fruit quality relative to V vinifera table and raisin grapes.

They must also resist downy and powdery mildew, black rot and anthracnose, which limit their ability to compete with *V. vinifera* table and raisin grapes. Anthracnose and black rot are very severe fruit diseases and have as great an effect on viticulture in the southeast as PD does. These diseases are not found in California, allowing breeders to incorporate more high quality *V vinifera* into their breeding efforts and enabling the production of much higher quality PD resistant cultivars in a shorter time span. The Walker lab has a wide range of PD resistant germplasm from the collections at the National Germplasm Repository, Davis; selections obtained from breeders in the southeastern U.S.; and from fertile *V rupestris* x *M rotundifolia* selections that allow utilization of the highest form of *X. fastidiosa* resistance, the muscadine grape *(M. rotundifolia*).

The Walker lab has completed the crosses necessary to fully examine the inheritance of *X. fastidiosa* resistance in *V rupestris x M. rotundifolia* selections. Seedlings from these crosses will be tested in spring 2001. Ramming and Walker have also completed 50 crosses (including 10 with seedless female parents) to introgress *X. fastidiosa* resistance into high quality table and raisin grape backgrounds. Future progress requires extra labor to establish and evaluate seedlings, establish and evaluate selections in the field for fruit quality, and complete the next round of crosses, which will be more dependent upon seedless parents and embryo rescue. The speed with which this work can be completed is dependent upon the research support devoted to it.

We have developed a unique collaboration between UC Davis and the USDA/ARS Fresno to address this important breeding effort. The Walker lab has developed rapid screening techniques for *X. fastidiosa* resistance (which it continues to improve), and has optimized the PCR detection of *X. fastidiosa.* They also have unique and very valuable *V rupestris* x *M rotundifolia* selections that offer the introduction of extremely high levels of *X. fastidiosa* resistance (8909-08 is among the best of these selections) into commercial grapes. The Ramming lab has outstanding sources of high quality large-berried seedless table and raisin grapes for crossing with *X. fastidiosa* resistance sources. They were instrumental in developing embryo rescue techniques (Emershad et al. 1989, Emershad and Ramming 1994), which although labor intensive, produce very high frequencies of seedless progeny. These techniques also allow the crossing of high quality seedless table and raisin grapes with pollen from *X. fastidiosa* resistant parents. Finally, both labs possess vineyard space and experience with evaluating progeny for resistance and quality.

APPENDIX

RESEARCH CATEGORIES AND PRIORITIES

Author	Project Title
Monitoring and Database Management - Total $1,123,478 (9.2%)	
Blackmer / Castle / Hagler / Naranjo/ Toscano	Sampling, Seasonal Abundance and Distribution of GWSS in Citrus and Grapes - *
Hix	Development of Trapping Systems to Trap the GWSS Homalodisca coagulata Adults and Nymphs in Grape
Hunt	Mating Behavior of the GWSS, *Homolodisca coagulata* - *
Leal / Zalom	Developing a Novel Detection and Monitoring System for the GWSS - *
Luvisi	Kern County Pilot Project
Mizell	Host Selection Behavior and Improved Detection For GWSS, *Homalodisca coagulata* (Say)
Redak	Developing an Integrated Pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula
Toscano / Redak / Hix / Blua	Area Wide Management of the GWSS in the Temecula Valley - *
Biology and Ecology of the Organisms - Total $1,351,191 (11.1%)	
Backus	Sharpshooter Feeding Behavior in Relation to Transmission of Pierce's Disease Bacterium -*
Cohen	Development of an Artificaial Diet for the GWSS -*
Daane	Biology and Ecology of GWSS in the San Joanquin Valley -*
Hix	Glassy-winged Sharpshooter Impact on Yield, Fruit Size, and Quality

Luck/Redak	Seasonal Changes in the GWSS Age Structure, Abundance, Host Plant use and Dispersal - *
Luck / Hoddle	Spatial and Temporal Relations Between GWSS Survival and Movement, Xylem Flux Patterns and Xylem Chemistry in Different Host Plants - *
Mizell	Keys to Management of GWSS: Interactions Between Host Plants, Malnutrition and Natural Enemies*
Peng/Zalom	Reproductive Biology and Physiology of the GWSS -*
Phillips	Timing and Duration of Fresh Glassy-winged Sharpshooter Egg Masses in Lemon Fruit Rinds; Impact on Fruit Harvest and Shipments - *
Purcell	Characterization and Studies on the Fundamental Mechanisms of *Xylella fastidiosa* Transmission to Grapevines by the GWSS - *
Biological Control of GWSS - Total $1,579,266 (13.0%)	
Hagler / Daane / Costa	A Monoclonal Antibody Specific to GWSS Egg Protein: A Tool for Predator Gut Analysis and Early Detection of Pest Infestation - *
Hammock / Kamita	Isolation and Characterization of GWSS Pathogenic Viruses
Hoddle / Redak / Luck / Granett	Biocontrol of GWSS in California: One Cornerstone for the Foundation of an IPM Program - *
Jones	Classical Biological Control of *Homalodisca coagulata*
Leopold	Cold Storage of Parasitized and Unparasitized Eggs of GWSS -*
Luvisi	Kern County Pilot Project
Phillips	Surveys for More Effective GWSS Parasitoids
Redak	Impact of Layering Control Tactics on the Spread of Pierce's Disease by the GWSS -*
Redak	Developing an Integrated pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula
Toscano / Redak / Hix / Blua	Area Wide Management of the GWSS in the Temecula Valley - *

Use of Pesticides and Alternative Treatments to Control GWSS/PD - Total $2,924,275 (24%)	
Blua/Walker	Impact of Sub-Lethal Doses of Neonicotinoids on GWSS Feeding and Transmission of Pierce's Disease
Grafton-Cardwell	Efficacy of Insecticides used for GWSS Control in Citrus
Grafton-Cardwell	Evaluation of Efficacy of Sevin Treatments in Porterville GWSS Infestation
Grafton-Cardwell	Screening Insecticides in Nursery Citrus for Efficacy Against Glassy-winged Sharpshooter
Henneberry/Akey/Toscano	Potential of Conventional and Biorational Insecticides for GWSS Control
Kirkpatrick/Purcell/Anderson/ Walker/Weber	Biological, Cultural, and Chemcial Management of Pierce's Disease -*
Luvisi	Kern County Pilot Project
Puterka	Alternatives to Conventional Chemical Insecticides for Control of GWSS
Redak	Impact of Layering Control Tactics on the Spread of Pierce's Disease by the GWSS -*
Redak	Developing an Integrated Pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula
Toscano	Chemical Control of GWSS: Establishment of Baseline Toxicity and Development of Monitoring Techniques for Detection of Early Resistance to Insecticides -*
Toscano/Castle	Laboratory and Field Evaluations of Imidacloprid and Thiamethozam against GWSS on Citrus and Grapes -*
Toscano / Redak / Hix / Blua	Area Wide Management of the GWSS in the Temecula Valley - *
Barriers & Trap Crops - Total $130,000 (1.1%)	
Kirkpatrick/Purcell/Anderson/ Walker/Weber	Biological, Cultural, and Chemical Management of Pierce's Disease -*

Luvisi	Kern County Pilot Project
Chemotherapy for PD in Grape Using Antibiotics and Other Treatments - $50,000 (0.4%)	
Kirkpatrick/Purcell/Anderson/ Walker/Weber	Biological, Cultural, and Chemical Management of Pierce's Disease -*
Redak	Developing an Integrated Pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula
Biological Control of PD - Total $1,024,347 (8.4%)	
Cooksey	Biological Control of Pierce's Disease with Non-pathogenic Strains of *Xylella fastidiosa* - *
Cooksey	Control of Pierce's Disease Through Degradation of Xanthan Gum - *
Lauzon	A Survey of Insect Vectors of Pierce's Disease (PD) and PD Infected Plants for the Presence of Bacteriophage that Infect *Xylella fastidiosa*
Miller / Peloquin / Lauzon / Lampe / Cooksey / Richards	Insect-Symbiotic Bacteria Inhibitory to *Xylella fastidiosa* in Sharpshooters - *
Peloquin	Sharpshooter-Associated Bacteria that may Inhibit Pierce's Disease
Stewart	Surrogate Genetics for *Xylella fastidiosa:* Regulation of Exopolysaccharide and Type IV Pilus Gene Expression - *
Epidemiology of PD - $827,258 (6.8%)	
Civerolo	Epidemiology of *Xylella fastidiosa* Diseases in California
Cooksey	Epidemiology of Pierce's Disease in Southern California: Identifying Inculum Sources and Transmission Pathways -*
Costa / Cooksey	Impact of Multiple Strain Infections of *Xylella fastidiosa* on Acquisition and Transmission By the GWSS - *
Gabriel	Role of Type I Secretion in Pierce's Disease -*
Kirkpatrick/Purcell/Anderson/ Walker/Weber	Biological, Cultural, and Chemical Management of Pierce's Disease -*

Perring	Epidemiology of Pierce's Disease in the Coachella Valley -*
Redak	Developing an Integrated Pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula
Movement/Spread/Monitoring Methods and Pathology of PD in Plants - Total $2,113,485 (17.4%)	
FAPESP / Civerolo	Sequence of *Xylella fastidiosa* Strain Causing Pierce's Disease of California Grapevine
FAPESP / Van Sluys	*Xyella fastidiosa* Genome Analysis - Almond and Oleander Comparison to Pierce's Disease Temecula and Citrus Strains
Gilchrist/Lincoln	Application of *Agrobacterium rhizogenes*-Mediated Transformation Strategies for a) Rapid High Through Put Screen for Genetic Resistance to Pierce's Disease in Grape that Maintains Clonal Integrity of the Recipient Host, and b) Rapid Screening for Virulence Determinants in *Xylella fastidiosa* - *
Kirkpatrick	Studies on Bacterial Canker and Almond Leaf Scorch - *
Kirkpatrick	Production and Screening of *Xylella fastidiosa* Transposon Mutants and Microscopic Examination of *Xf*-Resistant and Susceptible Vitus Germplasm - *
Kirkpatrick / Purcell / Anderson / Walker / Weber	Biological, Cultural, and Chemical Management of Pierce's Disease - *
Labavich/Matthews	The Development of Pierce's Disease in Xylem: The Roles of Vessel Cavitation, Cell Wall Metabolism and Vessel Occlusion - *
Lindow	The Role of Cell-Cell Signaling in Host Colonization by *Xylella fastidiosa*
Lindow	Role of *Xylella fastidiosa* Attachment on Pathogenicity -*
Miller / Peloquin / Lauzon / Lampe / Cooksey / Richards	Insect-Symbiotic Bacteria Inhibitory to *Xylella fastidiosa* in Sharpshooters - *
Price	Bacterial polysaccharides Expressed by Infective *Xylella fastidiosa* During Pierce's Disease
Purcell	Pruning for Control of Pierce's Disease
Purcell	Transmission of *Xylella fastidiosa* to Almonds by the GWSS -*

Redak	Controlling the Spread of *Xylella fastidiosa* the Causal Agent of Oleander Leaf Scorch by Disrupting Vector Acquisition & Transmission - *
Breeding - Cultivars of Grape Resistant to PD - $1,038,881 (8.5%)	
Adams	Identification of Molecular Markers in the Grapevine's Response to Infection by *Xylella fastidiosa*
Cook	Functional Genomics of the Grape-*Xylella* Interaction: Towards the Identification of Host Resistance Determinants
Cousins	Rootstock Variety Influence on Pierce's Disease Symptoms in Grafted Chardonnay (V*itis vinifera L.*) Grapevines
Hoddle/Redak/Luck/ Granett	Biocontrol of GWSS in California: One Cornerstone for the Foundation of an IPM Program -*
Kirkpatrick	Production and Screening of *Xylella fastidiosa* Transposon Mutants and Microscopic Examination of *Xf*-Resistant and Susceptible Vitus Germplasm - *
Kirkpatrick/Purcell/Anderson/ Walker/Weber	Biological, Cultural, and Chemical Management of Pierce's Disease -*
Meredith	Genetic Transformation to Improve the Pierce's Disease Resistance of Existing Grape Varieties-*
Walker/Ramming	The Genetics of Resistance to Pierce’s Disease and Breeding Pierce’s Disease Resistant Table and Raisin Grapes
Economic Analysis - $10,000 (0.1%)	
Siebert	Economic Impact Data Gathering for Pierce's Disease

* - Multi-Year Program

FY 2000-2001
FY 2001-2002

Funding Agency Key

Almond	Almond Board of California
AVF	American Vineyard Foundation
CA Citrus Nursery	California Citrus Nursery Advisory Board
Cal Trans	California Department of Transportation
CCGPRVE	California Competitive Grant Program for Research Viticulture/Enology
CDFA	California Department of Food and Agriculture
CDFA - 1232	California Department of Food and Agriculture - Funds From Assembly Bill 1232
Citrus Board	Citrus Research Board
Kern/Tulare	Kern/Tulare GWSS Task Force
Raisin	California Raisin Marketing Board
Riverside	County of Riverside
Table	California Table Grape Commission
UC - IPM	University of California Integrated Pest Management Project
UC - PD	University of California Pierce's Disease Grant Program
USDA	United States Department of Agriculture
USDA-APHIS	United States Department of Agriculture - Animal Plant Health Inspection Service
VC	Viticulture Consortium