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

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

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

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

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

OBJECTIVES

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

RESULTS

Objective 1

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

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

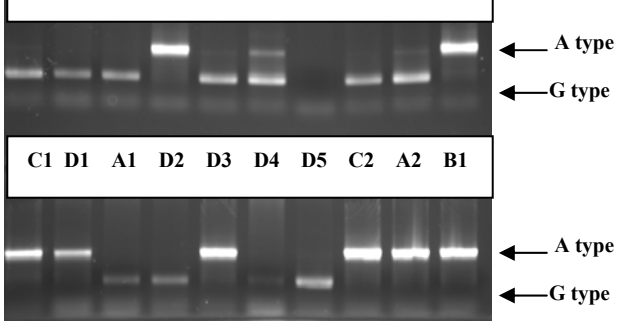

Figure 1. Polymerase chain reaction products from 18 *Xf* isolations collected in 2003 illustrating 2 genotypes (A = almond; G = grape).

Objective 2

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

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

CONCLUSIONS

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

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