

# 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 estimate the incidence of *Xf* detected from GWSS collected in different perennial cultivated and non-cultivated plant species. Based on results of seasonal plant utilization 2004-05, we conclude that host plant species significantly influences 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 orange, Spanish lemon, olive, avocado, plum, and pomegranate. Dispersing populations of adult GWSS were highest in citrus (lemon and navel) and pomegranate. Adult GWSS were also regularly collected from and observed feeding upon a wide range of non-crop weed species within and surrounding orchard crops. Overwintering adult GWSS were regularly collected in relatively low population densities on citrus (navel and lemon), pomegranate, avocado, plum, and non-crop annual weed species. Spatial patterns of adult GWSS capture within survey orchards varied among perennial crop species. Random distributions of adult GWSS were often observed in reproductive hosts including navel orange and Spanish lemon compared to population aggregates observed in avocado and olive. The presence of *Xf* in a subsample of GWSS collected among different perennial crops and on non-crop species was determined for collections in 2004 using PCR formats and the frequency of *Xf* detection in populations of GWSS varied among season in 2004.

## INTRODUCTION

The glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*) was introduced into southern California around 1990 and has continued to expand its range in the state (Varela et al. 2001). Populations of the GWSS are becoming widely distributed and the insect 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, Groves and Chen, 2003). Strains of *Xylella fastidiosa* (*Xf*) have a complex pathogenic relationship with a diverse host range including members of both monocots and dicots (Pooler and Hartung 1995). In addition, the genetic relationships associated with the ability to cause disease on a primary host and the ability to survive within reservoir hosts is not well understood (Hill and Purcell 1997, Purcell and Saunders 1999). Knowledge of the genetic diversity of strains that comprise the population of *Xf* in the central San Joaquin Valley (SJV) of California, 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. An accurate knowledge of GWSS host utilization in the central SJV, where they acquire the pathogen, when they move into susceptible crops, and when they spread the pathogen is critical to understanding and managing the spread of *Xf* diseases.

## OBJECTIVES

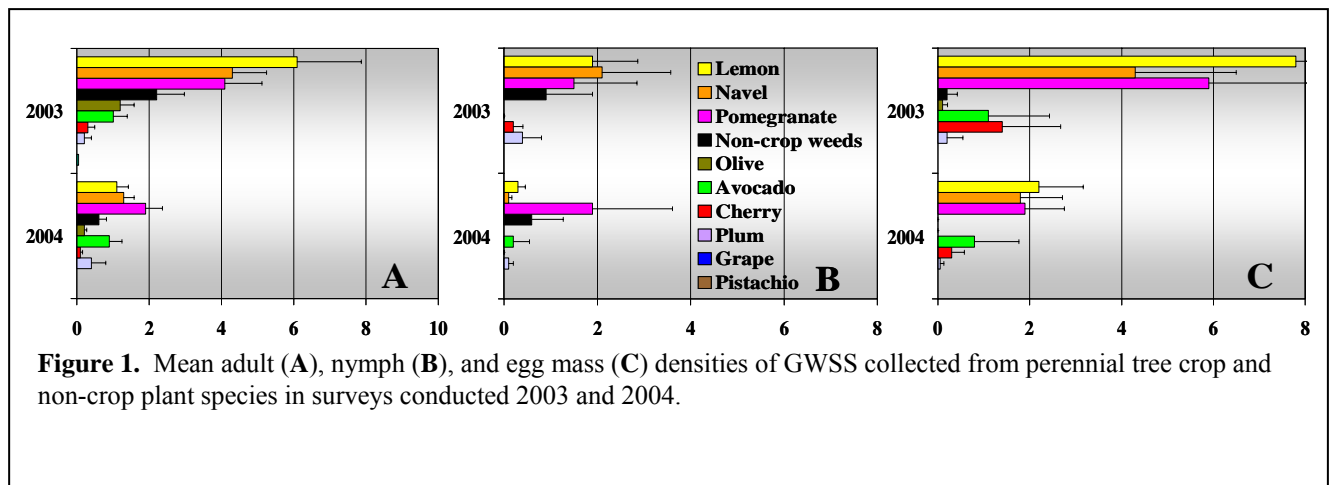
1. Identify and characterize the seasonal abundance of the primary vectors of *Xf* and seasonal patterns of insect dispersal.
2. Compare the incidence and genetic structure of *Xf* strains isolated from GWSS and other potential insect vector species collected from perennial, cultivated and non-cultivated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

## RESULTS

### Objective 1

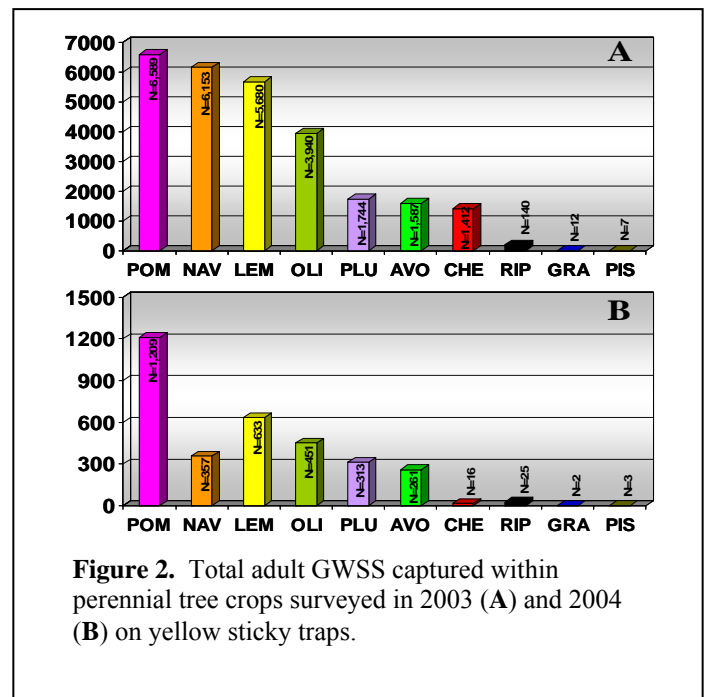
Seasonal host utilization patterns and dispersal of GWSS within and among a variety of perennial crop plant species was examined March 2003 to March 2005. Replicated experimental sites were located in GWSS-infested regions of Tulare County, California. Temporal and spatial patterns of crop utilization were monitored within perennial crop species including

citrus (navel orange and Spanish lemon), stonefruit (sweet cherry and plum), olive, pomegranate, and avocado at each of three, replicate locations for each crop type. Non-crop weed vegetation was monitored throughout the season at three experimental sites along with a single riparian habitat (Kaweah River). Throughout the sample interval, crops were sampled weekly for GWSS lifestages using a combination of yellow sticky traps, beat sampling, and timed visual counts. Beat-net counts, egg mass counts and visual inspections of all GWSS lifestages were used to estimate the seasonal patterns of host utilization within the various crop and non-crop species.



Results over both years of this study indicate that host plant species influences GWSS population biology. The greatest mean number of adult GWSS was collected from citrus (navel and lemon) and pomegranate (Figure 1). 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 monitored using yellow sticky traps suspended 2 m above the ground at each of 3 experimental locations for each crop sampled (Figure 2). Since March 2003, a total of 30,534 adult GWSS, 32 green sharpshooters (GSS, *Draeculacephala minerva*), and an additional 351 unidentified leafhopper species were captured on yellow sticky cards. In both years, the number of dispersing GWSS varied among crops species surveyed. Spatial patterns of GWSS capture, represented by plots of semivariance over distance, were dissimilar among crop species examined. For example, spatial dependence in GWSS capture was observed in pomegranate where the shape of the semivariogram were best fit by linear models with non-zero slopes. In contrast linear models with zero slopes best fit semivariance plots in navel orange in 2003. Specifically, partial variance in mean capture varied little among distances and transects within GWSS-reproductive citrus hosts compared to pomegranate where aggregations were detected along crop margins and mean capture rates declined with distance into fields away from citrus.



Throughout the winter periods (November-March) in 2003-04 and 2004-05, overwintering host utilization patterns of adult GWSS were monitored among the previously listed species. Overwintering adult GWSS were sampled monthly through this interval in perennial tree crops by beating/shaking all scaffolds over two, 80 ft<sup>2</sup> 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 were 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, 2003-04. 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 same interval,

respectively. Very few (N=68) adult GWSS were collected during the 2004-05 winter period among the species surveyed presumably as a result of the GWSS area-wide control program administered in Spring, 2004

## Objective 2

The presence of *Xf* in a subsample of vectors collected from different perennial crops and on non-crop species was completed for collections obtained in 2004. The bacterium was detected in populations of green sharpshooter (GSS, *Draeculacephala minerva*), watercress leafhopper (*Acinopterus angulatus*), and GWSS. Among a total of 452 adult *D. minerva* subjected to standard PCR detection with primers HL 5/6 (Francis et al. 2004), approximately 10% (N= 42) produced an amplicon size (221 bp) in gels. Among 96 adult *A. angulatus* tested to date, approximately 6% (N=6) produced a similar amplicon size indicative of infection by *Xf*. Among 731 adult GWSS tested in 2004, a similar amplicon was produced in 95 insects (13.0 %) collected among 7 perennial crop and non-crop habitats (Table 1). Averaging over plant species, the seasonal *Xf* detection frequency varied among the crops reported in 2004 with the highest detection occurring among overwintered, adult GWSS. Differences in *Xf* detection were not as apparent among the different plant species averaging over season ranging between

**Table 1.** Seasonal *Xf* detection summary among adult GWSS collected in different crop and non-crop habitats of Tulare County, 2004.

Season	AVO	LEM	NAV	Crop OLI	PLU	POM	RIP	TOTAL
Winter (Jan-Mar)	13.8% (N=65)	16.7% (N=78)	11.1% (N=91)	0.0% (N=23)	6.3% (N=16)	7.7% (N=33)	0.0% (N=3)	7.9% (N=309)
Spring (Apr-Jun)	33.3% (N=42)	30.2% (N=53)	38.1% (N=25)	42.9% (N=14)	45.5% (N=22)	32.5% (N=40)	22.2% (N=9)	35.0% (N=205)
Summer (Jul-Sep)	0.0% (N=51)	3.9% (N=66)	5.6% (N=18)	5.9% (N=17)	38.5% (N=13)	9.5% (N=21)	0.0% (N=12)	9.1% (N=198)
Winter (Oct-Dec)	0.0% (N=1)	0.0% (N=2)	0.0% (N=9)	0.0% (N=0)	0.0% (N=2)	0.0% (N=5)	0.0% (N=0)	0.0% (N=19)
TOTAL	11.8% (N=159)	12.7% (N=199)	13.7% (N=143)	12.2% (N=54)	22.6% (N=53)	12.4% (N=99)	5.6% (N=24)	13.0% (N=731)

5.6% – 22.6%. We are currently processing samples collected in 2003 from the same set of crops species and these results will be compared to those obtained in 2004. The diversity of amplified *Xf* will further be assessed using SSR markers deduced from the available genome sequences (Lin and Walker, 2004). Previous studies have demonstrated that these protocols generate sufficient polymorphisms within *Xf* to enable grouping of genotypes. Strain specific primers will also be used to investigate the pathotype profile of amplified products. Results from the 2004 season's research indicate substantial amounts of detectable *Xf* in GWSS populations, however further pathotype analyses are needed to differentiate the proportion of PD versus non-PD strains detected in potentially infectious vectors (Table 1). Finally, attempts will also be made to quantify *Xf* in selected insect vectors to identify the population dynamics of *Xf* within the vector populations. With recent improvements in technology, PCR-based techniques appear increasingly promising for bacterial pathogen detection in GWSS and other insect vector species. The bottleneck, however, lies in the preparation of inhibitor-free template DNA. We have recently developed a simple, sample preparation procedure for PCR amplification of *Xf* DNA. Adult insect heads were freeze-dried and used for PCR immediately. For PCR, the dried heads were pulverized and powder suspension used. Appropriate dilutions of powder suspension further minimized the effect of possible DNA polymerase inhibition. This recently developed PCR method will provide a more rapid and much less labor intensive platform for evaluating the infectious nature of potential vector species bypassing the laborious steps of whole-DNA extraction.

## CONCLUSIONS

Results obtained from our two year study have 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 and 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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