HOST PLANT PREFERENCE AND NATURAL INFECTIVITY OF INSECT VECTORS OF *XYLELLA FASTIDIOSA* ON COMMON WEEDS AND CROP PLANTS

Project Leader:
Kent Daane
Department of ESPM
University of California
Berkeley, CA 94702
daane@uckac.edu

Cooperators:
Christina Wistrom
Department of ESPM
University of California
Berkeley, CA 94720

Glenn Yokota
Department of ESPM
University of California
Berkeley, CA 94720

Rodrigo Almeida
Department of ESPM
University of California
Berkeley, CA 94720

Jennifer Hashim
Cooperative Extension
University of California
Bakersfield, CA 93307

Murray Pryor
Cooperative Extension
University of California
Bakersfield, CA 93307

Reporting Period: The results reported here are from work conducted October 2006 to July 2007.

ABSTRACT
Homalodisca vitripennis and *Spissistilus festinus* populations were surveyed bi-monthly in Kern County at sites with a variety of potential feeding and breeding hosts. Insects collected by sweeps and sticky traps were tested for *Xylella fastidiosa* (*Xf*) presence with vacuum-extraction and PCR. Comparisons of four techniques to detect *Xf* in insects are ongoing in greenhouse studies: live insect transmission to plants, vacuum-extraction PCR, culture of heads, and lyophilization with chloroform-phenol extraction. Assessment of each method's accuracy will improve comparison of research projects and field survey results. This project will provide information for control decisions by investigating the importance of vegetation management and targeted monitoring to reduce insect populations and inoculum potential.

INTRODUCTION
Despite an area-wide insecticide spray program, endemic glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, populations are still present near Bakersfield, especially in abandoned vineyards, and along roadsides and windbreaks. Recently, three cornered alfalfa hopper (TCAH), *Spissistilus festinus*, populations increased in the San Joaquin Valley. Histological studies show that TCAH normally feed in phloem tissue with occasional probes to xylem. The highly polyphagous feeding habits of TCAH may be of concern to researchers worried about the spread of *Xf* between and within crops like alfalfa, grapes and almonds. Common vineyard weeds and windbreak species are hosts of *Xf* (Costa et al. 2004, Wistrom and Purcell 2005, Shapland et al. 2006). While GWSS and TCAH feed on a wide range of plans, quantitative host preference data for only GWSS has been collected, and the studies focused mainly on citrus, grapes and ornamentals (Daane and Johnson 2005, Mizell et al. 2005).

Here, we investigated host plant preference and natural infectivity of GWSS and TCAH. While there is little data about the natural infectivity of GWSS in agricultural settings, between 10% and 20% of GWSS transmitted *Xf* in greenhouse tests (Almeida and Purcell 2003) and 1.25% of GWSS tested positive for *Xf* when collected from urban landscapes in Bakersfield (K. Daane, unpublished data). GWSS can be tested for *Xf* presence by vacuum-extraction and PCR. *Xf* transmission to grapes has only been correlated reliably with and bacterial presence in the precibarial region of sharpshooter mouthparts (Almeida and Purcell 2006). PCR-based vacuum-extraction (Bextine et al. 2005) of *Xf* in sharpshooter heads enabled more rapid, efficient, and convenient bacterial detection, compared to transmission tests (Purcell and Finlay 1980), or insect head culture (Hill and Purcell 1995). Lyophilization and maceration, followed by chloroform/phenol extraction, also reportedly sensitively detects *Xf* in sharpshooter heads. *Xf* transmission to grapevines is highly sensitive, so sharpshooter infectivity can be assessed when bacterial populations are below the detection thresholds of culture or PCR (Hill and Purcell 1995).

OBJECTIVES
1. Determine preference of insect vectors of *Xf* for common weeds known to host of *Xf* in the southern San Joaquin Valley.
2. Determine the proportion of collected insect vectors that carry *Xf*.
3. Compare the efficacy of *Xf* detection methods in insect vectors.

RESULTS

Five collection sites in Kern County were selected based on the presence of endemic GWSS populations and diverse crop plants. Three sites were located in or near vineyards, and two had Pierce's disease present. Each site was divided into at least seven areas, which were sampled twice a month for one year, beginning in October 2006. Only one of seven sites received a specific insecticide treatment for GWSS, where Admire (imidacloprid) was applied to eucalyptus, jojoba, and citrus.

To date, 350 GWSS and 301 TCAH total were collected from the five field sites. Populations of TCAH are reported because they were unexpectedly high throughout the year and occurred on the same hosts at roughly the same populations as GWSS. TCAH were the only other Cicadomorph species regularly observed. The largest average populations of TCAH were on alfalfa and of GWSS were on willow and eucalyptus (Figure 2). Four percent of GWSS and two percent of TCAH collected were nymphs. The numbers of insects collected varied by site and host (Figure 1). There was greater variability in insect numbers collected by sticky traps than sweeps.

While the initial plan was to look at vector populations primarily on weeds, very few weeds inside and adjacent to the study sites. Over the year, there were no weeds at all for 22% of samples taken, less than 10% weedy ground cover for 72% of samples, and only 6% of samples had more than 10% cover. No GWSS were collected on sweeps of those weeds in October, November, and May, and only TCAH were collected on white sweetclover (*Melilotus alba*) in May. There was no relationship between percent cover of weeds and the number of GWSS (one-way ANOVA, $P = 0.95$), but there may be some relationship between TCAH populations and weeds (one-way ANOVA, $P = 0.07$). Instead, GWSS and TCAH were consistently collected on perennial crop plants.

Host plant condition influenced both TCAH and GWSS preference. An average of 0.23 GWSS/100 sweeps were found on hosts with mature fruit, compared to 0.14 GWSS/100 sweeps on plants without fruit, and 0.04 GWSS/100 sweeps for plants with green fruit (one-way ANOVA, $P = 0.003$). A similar relationship was found between fruit maturity and TCAH preference (mature fruit = 0.64, no fruit = 0.35, and green fruit = 0.08 per 100 sweeps; one-way ANOVA, $P < 0.0001$). Both GWSS and TCAH preferred unpruned plants (0.128 GWSS, 0.323 TCAH per 100 sweeps) to recently-pruned plants (0.015 GWSS, and 0.12 TCAH per 100 sweeps; two-sample t-test, $P = 0.05$ for both comparisons). On average, 0.14 GWSS and 0.37 TCAH were collected per 100 sweeps on hosts with suckers or new growth, and 0.02 GWSS and 0.08 TCAH were collected on plants without (two-sample t-test, $P = 0.005$ for GWSS and $P = 0.0003$ for TCAH).

The highest populations of GWSS were collected in fall of 2006, decreasing from mid-December through early February. Populations remained very low through July 2007, although collections in August and September 2007 indicate that GWSS populations are rising, in the same locations and on the same hosts as they were initially collected in fall 2006. TCAH populations collected by sweeps were similar to GWSS populations, though TCAH increased continuously in fall 2006, whereas GWSS fluctuated somewhat in that time.

Both insect populations decreased following unusually cold temperatures from 12 to 23 January 2005. Nighttime low temperatures averaged 26.5ºC, compared to an estimated historic average of 37.4ºC, 11ºC below normal (CIMIS temperature data; Arvin Station; near sites 1 and 2). New growth on the evergreen plants surveyed was delayed and/or damaged by the cold temperatures, and citrus trees were pruned extensively to remove damaged shoots. Populations of TCAH and GWSS declined from a peak population in mid-December to the lowest populations eight weeks later in February. The decline from cold temperatures was long-lasting but not immediate. At site 2, locations 2 (citrus), 3 (jojoba/ eucalyptus), 5 (jojoba), 8 (citrus), and 10 (eucalyptus) were treated with systemic imidacloprid during the first week of March 2007. Prior to that GWSS catches increased at a higher rate on sticky traps compared to collections with sweep nets. Following the spray, GWSS populations decreased to their lowest levels, but TCAH populations took another two weeks to peak and then declined.

To date, no *Xf* was detected in any of the 635 adult GWSS or TCAH tested by vacuum-extraction and PCR. Pierce's disease symptoms were observed at sites 3 and 5 in fall 2007. Ivy and oleander samples with leaf scorch, collected at site 4 in spring 2006, were negative for *Xf*.

Comparisons between detection techniques are ongoing. In experiments conducted in fall 2006, only two of 121 GWSS tested transmitted *Xf* to grapes, none of the 45 insects tested by culture and one of 29 tested by vacuum-extraction PCR positive for *Xf*. An additional 88 insects (blue-green sharpshooter; BGSS) were tested so far this summer, with none of the 42 tested by vacuum-extraction PCR and three of nine tested by culture positive for *Xf*. Lyophilization-PCR and culture of plants inoculated by insects are ongoing, as are additional replications of this experiment.

DISCUSSION

The results reported here are still preliminary. The major host plants used by both GWSS and TCAH are perennial crops and windbreaks. There was little ground cover at any of the sites, and populations of TCAH or GWSS were not appreciably larger where it was present. Highest populations of TCAH and GWSS were on plants used as windbreaks: jojoba and eucalyptus. The weed control practiced by citrus growers for frost protection, combined with good sanitary practices and targeted irrigation resulted in few weeds at any of the study sites. Where there was ground cover, it was kept short through frequent mowing. When normal cultivation practices are followed, initial results suggest that it is unlikely that weeds or other groundcover play a role in hosting GWSS or TCAH, except weedy legumes, which may be breeding hosts of TCAH.

The results so far show that plant condition plays a role in TCAH or GWSS preference. Plants with suckers or lush growth were more attractive than plants that were recently pruned or had mature growth. This follows the idea that GWSS require very large volumes of xylem sap, and that host plant nutritional status is important for nymph development and survival (Andersen et al. 1989, Brodbeck et al. 2004). This would also show why plants with developing fruit were not as attractive to GWSS or TCAH; the green fruit may act as a nutrient and carbohydrate sink, decreasing levels of nutrients and sugar in the xylem and phloem tissues the insects feed upon. More data analysis and research into published literature is needed to speculate why plants with mature fruit were most attractive to GWSS and TCAH.

The cold snap in January also had unexpected results. GWSS and TCAH continued to be collected so the low temperatures did not kill the insects outright. Instead, GWSS populations began declining in December, and continued to decrease over the following two months. As the cold caused extensive damage to green, growing shoots preferred as feeding sources, perhaps there was reduced food available to the insects. In particular, nymphs would be affected by damage to feeding hosts since they are unable to move large distances to another host plant. This is suggested by the low GWSS populations lasting until late summer 2007. The usual springtime increase in GWSS populations was not observed in this study, suggesting that the cold had lasting impacts upon adult fecundity, nymph survival, and/or egg development.

The initial results from the field survey suggest that GWSS host plant use depends on the type and condition of host plants available as well as the time of year. Sweeps and visual inspection were different in the quantity of insects detected, although they roughly mirrored one another in monitoring trends in TCAH and GWSS populations. By detecting insects that are mobile, sticky traps may be helpful when GWSS are moving between hosts, for either nutritional or egglaying purposes, but underestimate GWSS populations when host plant conditions are attractive to GWSS, and thus remain on the plant for long periods of time.

Results from this project may help improve control decisions, by investigating the necessity of vegetation management or targeted insecticide sprays to reduce insect populations and inoculum potential, and providing some of the information required to develop a treatment threshold for GWSS populations in areas with endemic GWSS populations and Pierce's disease.

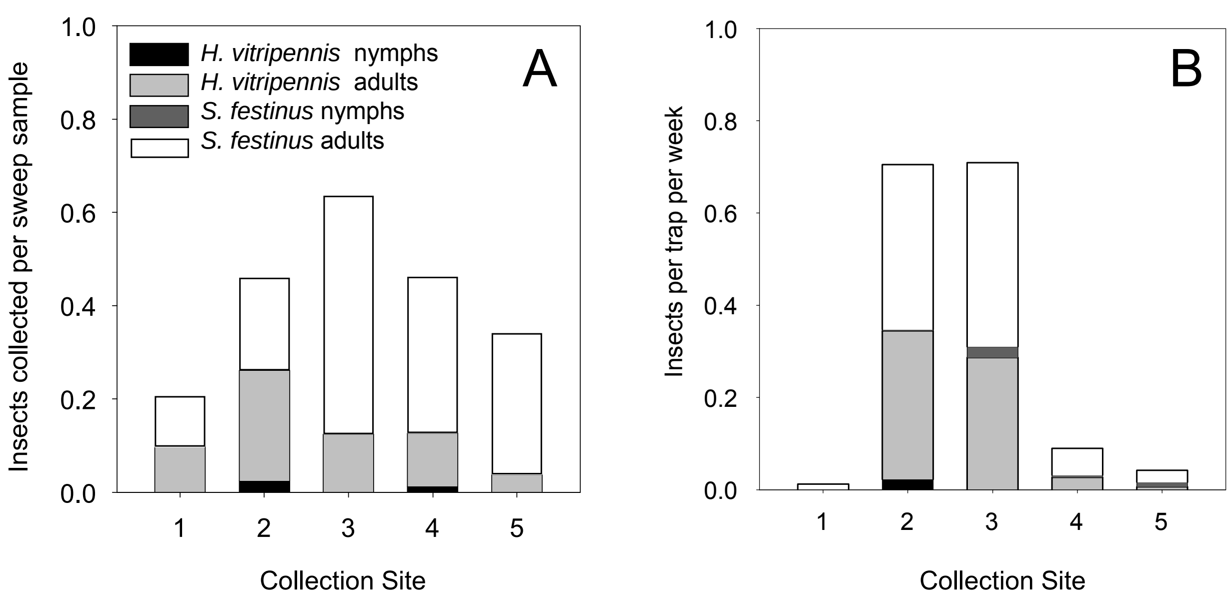

Figure 1. Average insect populations collected bi-monthly by A) 100 sweeps-beats or 30 second visual survey and B) sticky traps at field sites between July 2006 and July 2007.

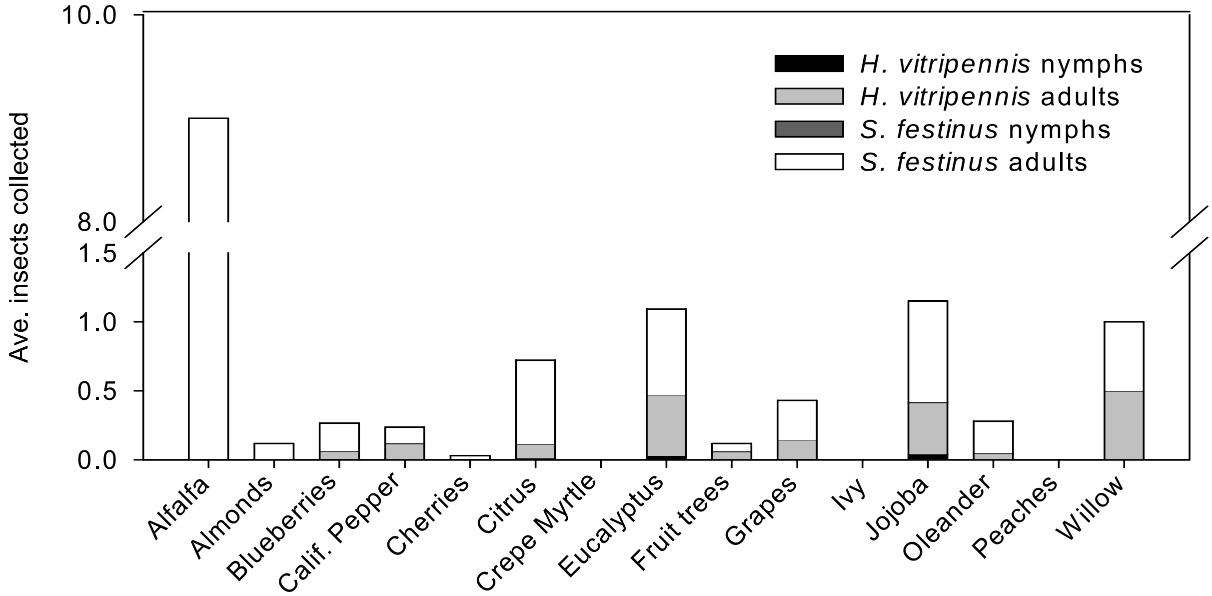

Figure 2. Average insect populations collected in 100 sweeps-beats or 30 second visual observation in bi-monthly surveys of plant hosts at all field sites between July 2006 and July 2007.

REFERENCES

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

Almeida, R.P.P and A.H. Purcell. 2006. Patterns of *Xylella fastidiosa* colonization of the precibarium of sharpshooter vectors relative to transmission to plants. Ann. Entomol. Soc. Am. 99: 884-890.

Bextine, B., M.J. Blua, D. Harshman and T.A. Miller. 2005. A SYBR green-based real-time PCR protocol and novel DNA extraction technique to detect *Xylella fastidiosa* in *H. coagulata*. J. Econ. Entomol. 98: 667-672.

Brodbeck, B.V., P.C. Andersen, R.F. Mizell, III, and S. Oden. 2004. Comparative nutrition and developmental biology of xylem feeding leafhoppers reared on four genotypes of *Glycine max*. Environ. Entomol. 33: 165-173.

Costa, H.S., E. Raetz, T.R. Pinckard, C.K. Gispert, R. Hernandez-Martinez, C.K. Dumenyo and D.A. Cooksey. 2004. Plant hosts of *Xylella fastidiosa* in and near southern California vineyards. Plant Dis. 88: 1255-1261.

Daane K.M. and M.W. Johnson. 2005. Biology and ecology of the glassy-winged sharpshooter in the San Joaquin valley. Proc. Pierce's Disease Research Symposium. Calif. Dept. Food and Agricul: 97-100.

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

Minsavage, G.V., C.M. Thompson, D.L. Hopkins, R. Leite and R.E. Stall. 1994. Development of a PCR protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathol. 84: 465-461.

Mizell, R.F., P.C. Andersen and P.A. Phillips. 2005. Pop. dynamics and interactions between GWSS and its host plants in response to CA phenology. Proc. Pierce's Dis. Res. Symposium. CDFA.: 117-121.

Mueller, A.J. and B.A. Dumas. 1987. Host plants of the three cornered alfalfa hopper (Hemiptera: Homoptera: Membracidae). Environ. Entomol. 16: 513-518.

Purcell, A.H. and A. Finlay. 1980. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathol. 69: 393-395.

Shapland, E.B., K.M. Daane, G.Y. Yokota, C. Wistrom, J.H. Connell, R. Duncan, and M. Viveros. 2006. Ground vegetation survey for *Xylella fastidiosa* in California almond orchards. Plant Dis. 90: 905-909.

Wistrom, C. and A.H. Purcell. The fate of *Xylella fastidiosa* in vineyards weed and other alternate hosts in California. Plant Dis. 89: 994-999.

FUNDING AGENCIES

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