XYLELLA FASTIDIOSA TRANSMISSION BY GLASSY-WINGED SHARPSHOOTERS AND SMOKETREE SHARPSHOOTERS FROM ALTERNATE HOSTS TO GRAPEVINES

Principal Investigator:
Thomas M. Perring
Department of Entomology
University of California
Riverside, CA 92521
thomas.perring@ucr.edu

Cooperators:
Tracy R. Pinckard
Department of Entomology
University of California
Riverside, CA 92521
tracy.pinckard@ucr.edu

Charles A. Farrar
Department of Entomology
University of California
Riverside, CA 92521
charles.farrar@ucr.edu

Reporting Period: The results reported here are from work conducted July 2007 to September 2008.

ABSTRACT

The goal of this project is to evaluate the importance of many common weed, agricultural, and cover crop plants that are found in close proximity to vineyards as sources of *Xylella fastidiosa* (*Xf*) from which glassy-winged sharpshooter (GWSS) and smoketree (STSS) sharpshooters can acquire and transmit *Xf* into grapevines. In our studies, we were unable to recover *Xf* from bell pepper, cotton, sunflower, horseweed, annual fescue 'Zorro', birdsfoot trefoil, or sudangrass plants. *Xf* was successfully isolated from needle-inoculated alfalfa, basil, lima bean, tomato, goosefoot, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand white clover, Hykon rose clover, cowpea, fava bean, field pea, meadow barley, and California red oats. We have determined that STSS can transmit *Xf* between fava bean plants, between alfalfa plants, and from alfalfa to grapevines. We were unable to detect successful transmission from fava bean-to-grapevine, tomato-to-tomato, tomato-to-grapevine, cowpea-to-cowpea, or cowpea-to-grapevine. All 36 STSS died on goosefoot before the end of the 48-hr acquisition access period, indicating that goosefoot is a poor host for STSS. Goosefoot also appears to be a poor host for *Xf*. We have confirmed successful transmission of *Xf* by GWSS for cowpea-to-cowpea and tomato-to-tomato tests (**Table 3**). GWSS transmission of *Xf* from cowpea-to-grapevine or tomato-to-grapevine could not be detected.

INTRODUCTION

Over 140 plants are known to host Pierce's disease (PD) strains of *Xylella fastidiosa (Xf)* (Costa et al. 2004, Freitag 1951, Raju et al. 1980, 1983, Shapland et al. 2006, Wistrom and Purcell 2005, http://www.cnr.berkeley.edu/xylella/temp/hosts.htm). Many of these plants are found in close proximity to vineyards, and some are even used as cover crops in vineyards (Statewide IPM Program 2007). While considerable research has identified *Xf* hosts, little work has been done to determine if sharpshooters can acquire the bacteria from these hosts and transmit it to grapevines. If this does not occur, then the alternate host is of little consequence in PD epidemiology. Conversely, plants that contribute inoculum for sharpshooter acquisition and transmission to grape should be removed if growers wish to reduce primary spread into their vineyards.

To successfully implement a program to remove pathogen sources, we first must identify those sources. The introduction into California of GWSS, an insect with a broad host range, theoretically increases the probability of disease spread from the these alternate host plants to grape. For this to occur, GWSS must feed on the infected plant in such a way to acquire *Xf* from plant, and successfully transmit the acquired pathogen to grapevines. While studies have shown mechanical and insect transmission to wide variety of alternate hosts (Freitag 1951, Purcell and Saunders 1999), they have demonstrated transmission from only a handful of alternate hosts to grapevines (Hill and Purcell 1995, 1997). We are unaware of research published on transmission of *Xf*, PD strain, from alternate hosts into grapevines using GWSS or STSS, a native California sharpshooter also found in grape growing regions, as the vector.

OBJECTIVES

Using GWSS and STSS vectors:

1. Evaluate the acquisition and transmission of *Xf* to grapevines from agricultural crop plants known to be PD hosts that are grown in the vicinity of vineyards.
2. Evaluate the acquisition and transmission of *Xf* to grapevines from weed plants known to be PD hosts that are grown in the vicinity of vineyards.
3. Evaluate the acquisition and transmission of *Xf* to grapevines from vineyard cover crop plants.

RESULTS AND DISCUSSION

Twenty-four of 42 proposed plant species have been needle-inoculated with *Xf* (**Table 1**). *Xf* does not appear to be able to survive in bell pepper, cotton, sunflower, horseweed, annual fescue 'Zorro,' birdsfoot trefoil, or sudangrass plants. A few positives were detected at two weeks post-inoculation with ELISA for bell pepper, cotton, and horseweed, but no plants tested positive by ELISA at four-weeks, nor were they positive by culture, indicating a possible transient infection or detection of dead *Xf* cells by early ELISA. Cultures were clean and negative for *Xf*. Sunflower tested positive by ELISA for all 20 plants, but the cultures were clean and negative. However, the sunflower also died very quickly, which may explain why it was not detected by culture. We will repeat needle inoculations for this plant species this winter. Final tests are pending for birdsfoot trefoil and annual fescue before we can comfortably say they are not hosts for *Xf*.

Xf was successfully isolated from needle-inoculated alfalfa, basil, lima bean, tomato, goosefoot, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand white clover, Hykon rose clover, cowpea, fava bean, field pea, meadow barley, and California red oats. We did not isolate *Xf* from basil until 16 weeks post-inoculation. All ELISA tests for Basil were positive, including for the negative controls, indicating that the commercial kit for *Xf* from Agdia, Inc., is not suitable for testing this plant species. The cultures for the negative controls were always negative, including at 16-weeks post-inoculation when the other cultures were obtained.

Table 1. ELISA and culture results for plant species needle-inoculated with *Xf*.

Type	Common Name	Scientific Name	ELISA +	Culture +	*Xf* Recovered?
Agriculture Crops	Alfalfa	*Medicago sativa*	20/20	14/20	Yes
	Basil	*Ocimum basilicum*	20/20*	10/20	Yes
	Bell Pepper	*Capsicum annuum*	5/20**	0/20	No
	Cotton, Upland	*Gossypium hirsutum*	2/15**	0/15	No
	Lima Bean, Fordhook 242	*Phaseolus lunatus*	2/18	1/18	Yes
	Tomato, Rutgers	*Solanum lycopersicum*	15/39	8/38	Yes
Weeds	Common Sunflower (commercial variety)	*Helianthus annuus*	20/20*	0/20	No
	Goosefoot	*Chenopodium sp.*	7/40***	5/33	Yes
	Horseweed	*Conyza Canadensis*	2/20**	0/20	No
	Spanish Broom	*Spartium junceum*	17/20	17/20	Yes
	Tree Tobacco	*Nicotiana sp.*	12/20**	2/20	Yes
Cover Crops	Annual Ryegrass	*Festuca sp.*	6/20	6/20	Yes
	Annual Fescue, Zorro	*Lolium multiflorum*	0/20	0/20	Final test pending
	Black Mustard	*Brassica nigra*	17/20	13/20	Yes
	Blando Brome	*Bromus hordeaceus*	16/20	13/20	Yes
	Birdsfoot Trefoil	*Lotus spp.*	10/20	0/20	Final test pending
	Clover, New Zealand White	*Trifolium repens*	15/20	2/20	Yes
	Clover, Hykon Rose	*Trifolium hirtum*	16/20	10/20	Yes
	Cowpea, California Blackeye	*Vigna unguiculata*	22/40	16/35	Yes
	Fava Bean, Windsor	*Vicia faba*	30/40	7/20****	Yes
	Field Pea, Miranda	*Pisum sativum*	14/39	3/11	Yes
	Meadow Barley	*Hordeum brachyantherum*	9/20	4/20	Yes
	Oat, California Red	*Avena sativa*	12/20	2/20	Yes
	Sudangrass	*Sorghum bicolor var. sudanense*	0/20	0/20	No

* False positives.
** Most or all positives in 2-week ELISA test; possible transient infection or dead cells detected.
*** Very slow-growing *Xf*, detected well after 4-weeks.
**** Fava bean contains many other microorganisms that contaminate and probably obscure positive culture results. Also, fava bean occasionally produces false positives by ELISA.

Insect transmissions have been done for both vectors on alfalfa, cowpea, fava bean, tomato, basil, and goosefoot (**Tables 2 and 3**). We confirmed successful transmission of *Xf* by STSS for fava bean-to-fava bean, alfalfa-to-alfalfa, and alfalfa-to-grapevine (**Table 2**). Therefore, STSS can transmit *Xf* between fava bean plants, between alfalfa plants, and from alfalfa to grapevines. We were unable to detect successful transmission from fava bean-to-grapevine, tomato-to-tomato, tomato-to-grapevine, cowpea-to-cowpea, or cowpea-to-grapevine. All 36 STSS died on goosefoot before the end of the 48-hr acquisition access period (AAP), indicating that goosefoot is a poor host for STSS. Goosefoot also appears to be a poor host for *Xf*, as few cultures were obtained from needle-inoculated plants, and all were extremely slow growing, except for one. Transmission tests using STSS on basil are underway at the time of this writing and results are not yet available.
We have confirmed successful transmission of *Xf* by GWSS via culture for cowpea-to-cowpea and tomato-to-tomato tests (**Table 3**). GWSS transmission of *Xf* from cowpea-to-grapevine or tomato-to-grapevine could not be detected. Transmission tests for GWSS on alfalfa are underway and the results are pending. Only four of 24 GWSS survived the 48-hr acquisition

access period (AAP) on goosefoot. The surviving four insects were placed on a clean grapevine test plant, although they appeared to be in the process of dying. Results for the single goosefoot-to-grapevine test will be available by the December meeting. Goosefoot appears to be a poor host for *Xf*, STSS, and GWSS, and is probably insignificant in PD epidemiology. Transmission tests for basil using GWSS have been completed, but results are not available at the time of this writing.

We had established clean, captive-reared GWSS and STSS for experiments. However, they were decimated by an infestation of the parasitoid wasp, *Gonatocerus ashmeadi* early this year. We determined the source of infestation, corrected it, and have been rebuilding the colonies since Spring 2008 by regularly collecting adults from the field for oviposition in captivity. Eggs produced are collected from field colonies and hatched in the laboratory, and then transferred to clean plants. We currently have a few hundred captive-reared nymphs of each species, and several mating pairs and gravid females still producing eggs. We resumed insect transmission experiments in early September 2008, and we are in the process of conducting transmission tests as the current captive-reared nymphs reach maturity. In the meanwhile, we have continued to grow, needle-inoculate, and evaluate potential PD hosts from the list.

Table 2. STSS transmission results for cowpea, fava bean, tomato, and alfalfa.

Transmission Test	ELISA +	Culture +	Successful Transmission?
Cowpea-to-Cowpea	5/5	0/5	Not detected
Cowpea-to-Grapevine	2/5	0/5	Not detected
Cowpea Group Grapevine-to-Grapevine Controls	3/6	0/6	Not detected
Fava Bean-to-Fava Bean	1/5	1/5	Yes
Fava Bean-to-Grapevine	4/5	0/5	Not detected
Tomato-to-Tomato	1/5	0/5	Not detected
Tomato-to-Grapevine	3/5	0/5	Not detected
Fava Bean/Tomato Group Grapevine-to-Grapevine Controls	2/4	0/4	Not detected
Alfalfa-to-Alfalfa	3/5	2/5	Yes
Alfalfa-to-Grapevine	2/5	2/5	Yes
Alfalfa Group Grapevine-to-Grapevine Controls	1/5	1/5	Yes
Goosefoot (none survived AAP)	0/0	0/0	No
Basil-to-Basil	Pending	Pending	Pending
Basil-to-Grapevine	Pending	Pending	Pending
Basil Group Grapevine-to-Grapevine Controls	Pending	Pending	Pending

Table 3. GWSS transmission results for cowpea, fava bean, tomato, goosefoot, and basil.

Transmission Test	ELISA +	Culture +	Successful Transmission?
Cowpea-to-Cowpea	4/5	2/5	Yes
Cowpea-to-Grapevine	3/5	0/5	Not detected
Cowpea Group Grapevine-to-Grapevine Controls	3/5	0/5	Not detected
Fava Bean-to-Fava Bean	2/5	0/5	Not detected
Fava Bean-to-Grapevine	1/5	0/5	Not detected
Tomato-to-Tomato	3/5	1/5	Yes
Tomato-to-Grapevine	2/5	0/5	Not detected
Fava Bean/Tomato Group Grapevine-to-Grapevine Controls	2/10	0/10	Not detected
Basil-to-Basil	Pending	Pending	Pending
Basil-to-Grapevine	Pending	Pending	Pending
Basil Group Grapevine-to-Grapevine Controls	Pending	Pending	Pending
Goosefoot-to-Grapevine	Pending	Pending	Pending
Alfalfa-to-Alfalfa	Pending	Pending	Pending
Alfalfa-to-Grapevine	Pending	Pending	Pending
Alfalfa Group Grapevine-to-Grapevine Controls	Pending	Pending	Pending

CONCLUSIONS

This project addresses the 2006 Scientific Summit category of *"Understanding transmission of the disease,"* and relates directly to the acquisition and transmission of *Xf* by GWSS. It also has relevance to several of the recommendations developed by the National Academy of Science, National Research Council (2004). First and foremost, the definition of the Category 1 research option is that it "holds a reasonable promise of generating successful tools for management of PD/GWSS, either in the short term or the long term." By determining the plants that truly contribute to primary spread by sharpshooters, we can give growers another strategy (i.e. removing those plants) in an effort to reduce bacterial inoculum around their vineyards. This proposal also meets the general criteria defined in the NRC report in recommendation 2.2, of "contributing to PD/GWSS management and its sustainability," and it applies specifically to recommendation 3.9 of examining plants "for effective transmission rates from host to grape."

Bell pepper, cotton, horseweed, and sudangrass did not sustain infection after needle-inoculation with *Xf*, indicating that these plants are very unlikely to harbor *Xf* infection in the field. This is especially good news about horseweed since it is an extremely common weed in vineyards and is reported as resistant to herbicides. It appears that *Xf* is also unable to infect annual fescue or birdsfoot trefoil via needle-inoculation, although results from the 16-week (final) test are still pending. Sunflower has been reported as a host by other researchers and we were surprised that we did not obtain any *Xf* isolates from it. We used a commercial variety, which we grow for our insect colonies, while obtaining seed from wild sunflower. We will test the wild sunflower along with the commercial variety again.

We obtained *Xf* isolates from alfalfa, basil, lima bean, tomato, goosefoot, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand white clover, Hykon rose clover, cowpea, fava bean, field pea, meadow barley, and California red oats. We recovered *Xf* from at least 50% of test plants for alfalfa, basil, Spanish broom, black mustard, Blando brome, and Hykon rose clover, indicating that these are likely hosts for *Xf* in the field. Although we obtained some isolates from lima bean, tree tobacco, annual ryegrass, New Zealand white clover, field pea, meadow barley, and California red oats, further testing is required to gain a better understanding of their potential as alternative hosts for *Xf* in the field, since needle-inoculation is a severe and unnatural form of infection that is unlikely to happen in the field. As in the case of the goosefoot, we found that we could obtain isolates from a needle-inoculated plant, but that it was a poor host overall for PD and both vectors tested. Therefore, goosefoot is unlikely to serve as a source or reservoir of *Xf* in the field. If these plants have natural defenses against acquiring or sustaining a *Xf* infection when needle-inoculated with millions of bacteria, it is likely that an infection by a vector transmitting far fewer bacterial cells would not be sustained either. However, there are insect-pathogen-plant interactions involved that must be tested before such a conclusion can be made definitively. Further studies mimicking more natural acquisition and transmission using insects should be done for a more complete understanding of the roles each plant and vector species might play in the field.

REFERENCES CITED

Costa, H. S., E. Raetz, and T. R. Pinckard. 2004. Plant hosts of *Xylella fastidiosa* in and near southern California vineyards. Plant Disease 88: 1255-1261.

Freitag, J. H. 1951. Host range of the Pierce's disease virus of grapes as determined by insect transmission. Phytopathology 41:920-932.

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

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

National Academy of Science, National Research Council. 2004. California agricultural research priorities: Pierce's disease. The National Academies Press, Washington, D.C.

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

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

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

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

Statewide, IPM Program. 2007. Agriculture and Natural Resources, University of California. (http://www.ipm.ucdavis.edu/PGM/r302700999.html)

Wistrom, C. and A. H. Purcell. 2005. The fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts in California. Plant Disease 89:994-999.

FUNDING AGENCIES

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