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

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Reporting Period: The results reported here are from work conducted July 2007 to September 2009.

ABSTRACT

This project is designed 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 (GWSS) and smoketree (STSS) sharpshooters can acquire and transmit *Xf* into grapevines. In our studies *Xf* was successfully isolated from needle-inoculated alfalfa, basil, lima bean, tomato, annual bluegrass, cheeseweed, wild-type sunflower, goosefoot, London rocket, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand White clover, Hykon Rose clover, cowpea, fava bean, Miranda field pea, meadow barley, California Red oats, and White sweetclover. We were unable to recover *Xf* from bell pepper, cotton, black nightshade, common groundsel, Evening Sun sunflower, horseweed, Zorro annual fescue, birdsfoot trefoil, or sudangrass plants. We have confirmed successful transmission of *Xf* by GWSS for alfalfa-to-alfalfa, alfalfa-to-grapevine, basil-to-basil, basil-to-grapevine, tomato-to-tomato, Blando brome-to-Blando brome, Blando brome-to-grapevine and cowpea-to-cowpea. GWSS transmission of *Xf* from tomato-to-grapevine, cowpea-to-grapevine, fava bean-to-fava bean, and fava bean-to-grapevine could not be confirmed with culturing. We have determined that STSS can transmit *Xf* between alfalfa plants, from alfalfa to grapevines, between Blando brome plants, from Blando brome to grapevines, and between fava bean plants. We were unable to confirm successful transmission by STSS from tomato-to-tomato, tomato-to-grapevine, cowpea-to-cowpea, or cowpea-to-grapevine, or from fava bean to grapevines. Goosefoot appears to be a poor host for GWSS, STSS, and *Xf*, as nearly all the vectors died before the end of the 48-hr acquisition access period. *Xf* isolates obtained from goosefoot were few and slow-growing.

LAYPERSON SUMMARY

Evaluating the potential of various common plant species found in and near vineyards to serve as reservoirs of Pierce's disease (PD), and the ability of glassy-winged (GWSS) and smoketree (STSS) sharpshooters to acquire and transmit PD from these alternative plant hosts, is fundamental to managing the primary spread of PD in California vineyards. Identifying the plants that contribute to primary spread enables growers to target these plants around their vineyards as a mechanism to reduce spread. Understanding how these two vectors contribute to primary and secondary spread can assist in the development of alternatives to the area-wide management program. To reduce primary spread, efforts must focus on reducing bacteria-carrying vectors from entering healthy vineyards through continued area-wide or local treatment programs outside the vineyard, barriers, trap crops, and/or removal of pathogen sources outside the vineyard.

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 the glassy-winged sharpshooter (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 a 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

<u>*Needle Inoculated Plants*</u>

Thirty-four plant species have been needle-inoculated with *Xf* (**Table 1**). *Xf* does not appear to be able to survive in bell pepper, cotton, black nightshade, common groundsel, Evening Sun sunflower, horseweed, Zorro annual fescue, birdsfoot trefoil, or sudangrass plants. A few positives were detected at two-weeks post-inoculation with ELISA for bell pepper, cotton, common groundsel, and horseweed, but no plants tested positive by ELISA at four weeks, nor were they positive by culturing. These results suggest a transient infection or detection of dead *Xf* cells by the early ELISA. Evening Sun Sunflower tested positive by ELISA for all 20 plants, but the cultures were clean and negative. However, the Evening Sun sunflower also died very quickly, which may explain why it was not detected by culture. Final results are pending for filaree, Shepherd's purse, and stinging nettle.

Xf was successfully isolated from needle-inoculated alfalfa, basil, lima bean, tomato, annual bluegrass, cheeseweed, wild-type sunflower, London rocket, goosefoot, Spanish broom, tree tobacco, annual ryegrass, black mustard, Blando brome, New Zealand White clover, Hykon Rose clover, cowpea, fava bean, Miranda field pea, meadow barley, California Red oats, and White sweetclover. We recovered one isolate of *Xf* for lima bean in the first needle-inoculation set, so we repeated this test. No isolates were recovered from a second needle-inoculated set, suggesting that lima bean is a poor host for *Xf*. We did not isolate *Xf* from basil until 16 weeks post-inoculation. All ELISA tests for Basil were positive, including those for the negative controls, indicating that the commercial kit for *Xf* from Agdia, Inc. is not reliable for testing this plant species. The cultures for the negative controls were always negative, including at 16-weeks post-inoculation when the positive cultures from other plants were obtained. We only recovered one isolate from annual bluegrass. Cultures from annual bluegrass and fava bean have been routinely heavily contaminated, regardless of plant age or inoculation status. Other microbes present in the plants may be obscuring the presence of *Xf* in those species. Healthy grapevines also were needle inoculated with every inoculation group as positive controls for each set.

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, Italian Large Leaf	*Ocimum basilicum*	20/20*	10/20	Yes
	Bell Pepper, Taurus	*Capsicum annuum*	5/20**	0/20	No
	Cotton, Upland	*Gossypium hirsutum*	2/15**	0/15	No
	Lima Bean, Fordhook 242	*Phaseolus lunatus*	2/38	1/38	Yes
	Tomato, Rutgers	*Solanum lycopersicum*	15/39	8/38	Yes
Weeds	Annual Bluegrass	*Poa annua*	8/20	1/20****	Yes
	Black Nightshade	*Solanum nigrum*	0/20	0/20	No
	Cheeseweed	*Malva parviflora*	7/20	16/20	Yes
	Common Groundsel	*Senecio vulgaris*	3/20**	0/20	No
	Common Sunflower, Evening Sun	*Helianthus annuus*	20/20*	0/20	No
	Common Sunflower, wild-type	*Helianthus annus*	19/20	7/20	Yes
	Filaree	*Erodium species*	Tests in Progress		
	Goosefoot	*Chenopodium species*	7/40***	5/33	Yes
	Horseweed	*Conyza Canadensis*	2/20**	0/20	No
	London Rocket	*Sisymbrium irio*	5/20	13/20	Yes
	Shepherd's Purse	*Capsell bursa-pastoris*	Tests in Progress		
	Spanish Broom	*Spartium junceum*	17/20	17/20	Yes
	Stinging Nettle	*Urtica species*	Tests in Progress		
	Tree Tobacco	*Nicotiana species*	12/20**	2/20	Yes

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

Type	Common Name	Scientific Name	ELISA +	Culture +	*Xf* Recovered?
Cover Crops	Annual Ryegrass	*Festuca species*	6/20	6/20	Yes
	Annual Fescue, Zorro	*Lolium multiflorum*	0/20	0/20	No
	Black Mustard	*Brassica nigra*	17/20	13/20	Yes
	Blando Brome	*Bromus hordeaceus*	16/20	13/20	Yes
	Birdsfoot Trefoil	*Lotus species*	10/20	0/20	No
	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
	Sweetclover, White	*Melilotus alba*	20/20	16/20	Yes

* 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.

<u>*Insect Transmission*</u>
Last year we lost our clean, captive-reared GWSS and STSS colonies to infestations of the parasitoid wasp, *Gonatocerus ashmeadi*. Several of the needle-inoculated plant species died before we were able to rebuild our colonies and perform transmission with them. Therefore, we re-grew and needle-inoculated new sets of those alternative host plants to use for transmission after the colonies sufficiently recovered. To date, transmission using both vector species has been completed for alfalfa, basil, tomato, annual bluegrass, cheeseweed, wild-type sunflower, goosefoot, London rocket, tree tobacco, annual ryegrass, Blando brome, cowpea, fava bean, California Red oats, and White sweetclover. Although transmission has been completed, we are still evaluating the test plants for basil, annual bluegrass, cheeseweed, wild-type sunflower, London rocket, annual ryegrass, California red oats, and White sweetclover, and final data are still pending. At the time of preparing this report, transmission was underway for Spanish broom, black mustard, New Zealand White clover, Hykon Rose clover, Miranda field pea, and meadow barley, with the final data expected to be available in four-eight weeks.

We have confirmed (by culture) successful transmission of *Xf* by GWSS for alfalfa-to-alfalfa, alfalfa-to-grapevine, basil-to-basil, basil-to-grapevine, tomato-to-tomato, Blando brome-to-Blando brome, Blando brome-to-grapevine and cowpea-to-cowpea (**Table 2**). GWSS transmission of *Xf* from tomato-to-grapevine, cowpea-to-grapevine, fava bean-to-fava bean, and fava bean-to-grapevine tested negative by culturing. 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.

We confirmed successful transmission of *Xf* by STSS for alfalfa-to-alfalfa, alfalfa-to-grapevine, Blando brome-to-Blando brome, Blando brome-to-grapevine and fava bean-to-fava bean (**Table 2**). We were unable to confirm successful transmission by STSS from 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.

Table 2. Results for transmission of *Xylella fastidiosa* by GWSS and STSS to date.

			GWSS		STSS	
Host Plant Type	PD Acquisition Host	PD Inoculation Host	ELISA +	Culture +	ELISA +	Culture +
Agriculture Crop	Alfalfa	Alfalfa	4/5	4/5	5/5	3/5
	Alfalfa	Grapevine	4/5	4/5	4/5	4/5
	Basil	Basil	9/9	9/9	Tests in Progress	
	Basil	Grapevine	8/9	8/9	Tests in Progress	
	Tomato, Rutgers	Tomato, Rutgers	3/5	1/5	1/5	0/5
	Tomato, Rutgers	Grapevine	2/5	0/5	3/5	0/5

Table 2. Results for transmission of *Xylella fastidiosa* by GWSS and STSS to date (continued).

			GWSS		STSS	
Host Plant Type	PD Acquisition Host	PD Inoculation Host	ELISA +	Culture +	ELISA +	Culture +
Weed	Annual Bluegrass	Annual Bluegrass	Tests in Progress		Tests in Progress	
	Annual Bluegrass	Grapevine	Tests in Progress		Tests in Progress	
	Cheeseweed	Cheeseweed	Tests in Progress		Tests in Progress	
	Cheeseweed	Grapevine	Tests in Progress		Tests in Progress	
	Common Sunflower, wild-type	Common Sunflower, wild-type	Tests in Progress		Tests in Progress	
	Common Sunflower, wild-type	Grapevine	Tests in Progress		Tests in Progress	
	Goosefoot	Goosefoot	0/0	0/0	0/0	0/0
	Goosefoot	Grapevine	0/1	0/1	0/0	0/0
	London Rocket	London Rocket	Tests in Progress		Tests in Progress	
	London Rocket	Grapevine	Tests in Progress		Tests in Progress	
	Spanish Broom	Spanish Broom	Tests in Progress		Tests in Progress	
	Spanish Broom	Grapevine	Tests in Progress		Tests in Progress	
	Tree Tobacco	Tree Tobacco	Tests in Progress		Tests in Progress	
	Tree Tobacco	Grapevine	Tests in Progress		Tests in Progress	
Cover Crop	Annual Ryegrass	Annual Ryegrass	Tests in Progress		Tests in Progress	
	Annual Ryegrass	Grapevine	Tests in Progress		Tests in Progress	
	Black Mustard	Black Mustard	Tests in Progress		Tests in Progress	
	Black Mustard	Grapevine	Tests in Progress		Tests in Progress	
	Blando Brome	Blando Brome	1/4	1/4	4/4	3/4
	Blando Brome	Grapevine	2/4	1/4	0/4	1/4
	Clover, New Zealand White	Clover, New Zealand White	Tests in Progress		Tests in Progress	
	Clover, New Zealand White	Grapevine	Tests in Progress		Tests in Progress	
	Clover, Hykon Rose	Clover, Hykon Rose	Tests in Progress		Tests in Progress	
	Clover, Hykon Rose	Grapevine	Tests in Progress		Tests in Progress	
	Cowpea, California Blackeye	Cowpea, California Blackeye	4/5	2/5	5/5	0/5
	Cowpea, California Blackeye	Grapevine	3/5	0/5	2/5	0/5
	Fava Bean, Windsor	Fava Bean, Windsor	2/5	0/5	1/5	1/5
	Fava Bean, Windsor	Grapevine	1/5	0/5	4/5	0/5
	Field Pea, Miranda	Field Pea, Miranda	Tests in Progress		Tests in Progress	
	Field Pea, Miranda	Grapevine	Tests in Progress		Tests in Progress	
	Meadow Barley	Meadow Barley	Tests in Progress		Tests in Progress	
	Meadow Barley	Grapevine	Tests in Progress		Tests in Progress	
	Oat, California Red	Oat, California Red	Tests in Progress		Tests in Progress	
	Oat, California Red	Grapevine	Tests in Progress		Tests in Progress	
	Sweetclover, White	Sweetclover, White	Tests in Progress		Tests in Progress	
	Sweetclover, White	Grapevine	Tests in Progress		Tests in Progress	

CONCLUSIONS

Bell pepper, cotton, black nightshade, common groundsel, Evening Sun sunflower, horseweed, Zorro annual fescue, birdsfoot trefoil, 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 particularly good news for horseweed since it is an extremely common weed in vineyards and is reported to be resistant to herbicides. In their PD management program, growers can choose to target weeds other than those identified here, knowing that these species do not sustain infection with *Xf*. In addition, growers can safely select

Zorro annual fescue, birdsfoot trefoil, and sudangrass as cover crops with confidence that their choice will not contribute to PD spread in their vineyards.

We recovered *Xf* from at least 50% of test plants for alfalfa, basil, cheeseweed, London rocket, Spanish broom, black mustard, Blando brome, Hykon Rose clover, and White sweetclover, indicating that these can serve as hosts for *Xf* in the field. We obtained isolates from three-46% of needle-inoculated plants for lima bean, tomato, wild-type sunflower, goosefoot, tree tobacco, annual ryegrass, New Zealand White clover, cowpea, fava bean, Miranda field pea, and meadow barley. The results from the transmission studies using these plants (pending) should provide 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 be sustained. 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.

Alfalfa and Blando brome are good hosts for *Xf*, GWSS, and STSS, indicating that they can serve as a reservoir of *Xf* and source of infection in the field for these vectors. Both GWSS and STSS successfully transmitted *Xf* between alfalfa plants, between Blando brome plants, from alfalfa into grapevines, and from Blando brome into grapevines. These two plant species (one a crop plant and the other a cover crop) should not be around or in vineyards where *Xf* or sharpshooters are present.

GWSS successfully transmitted *Xf* between basil plants, from basil to grapevines, between cowpea plants, between tomato plants, and from fava bean to grapevines, but not between fava bean plants, or from cowpea to grapevines. STSS also successfully transmitted between fava bean plants, but not from fava bean into grapevines, between cowpeas, or from cowpea into grapevines. It is possible that *Xf* isolates were obscured by other microbes present in the plants and on the media plates (cowpea and fava bean contain numerous other microbes that grow on media plates for PD), and positive transmission occurred, but was not detected. It also is possible that these plant species would not naturally serve as acquisition sources by these vectors, but because they were unnaturally needle-inoculated, some transmission did occur. Pending further results, careful consideration should be applied when using cowpea or fava bean as cover crops in vineyard areas with known *Xf* infection, or sharpshooter populations, since they can serve as sources. In the unlikely event that basil is grown near vineyards, it could may be a major contributor to the spread of PD, since both GWSS and STSS favor this host, and it could sustain high populations of vectors and harbor *Xf*. The final transmission results (pending) will provide a better understanding of which plant hosts, in combination with GWSS or STSS vectors, are more important in the epidemiology of this plant pathogen.

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FUNDING AGENCIES

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

ACKNOWLEDGEMENTS

We thank Richard Stouthamer and Robin Bromley for sequencing our *Xf* to verify the strain, Crystal May for tireless help in rearing our insect colonies, performing experiments, and collecting samples, Jung-Joon Park for assisting needle inoculations and Carmen Gispert for providing infected grapevines from the field as our source plants. Chuck Farrar was instrumental in helping us through the first year of this project. Sunridge Nursery donated non-infected grapevines for use as test plants, and we are grateful for their contribution.