CDFA PIERCE'S DISEASE & GLASSY-WINGED SHARPSHOOER BOARD PROGRESS REPORT (JULY 2007 – JUNE 2008)

A. Project title: *Xylella fastidiosa* Transmission by Glassy-Winged Sharpshooters and Smoke Tree Sharpshooters from Alternate Hosts to Grapevines

B. CDFA contract number: 07-0298

C. Time period covered by the progress report: July 2007 – June 2008

D. Principal Investigator and Cooperators:

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E. List of objectives and description of activities conducted to accomplish each objective:

All of the objectives are designed to evaluate glassy-winged sharpshooter and smoketree sharpshooter. The methodologies being used for each objective are similar, therefore they are combined and presented following the list of objectives.

1. Evaluate the acquisition and transmission of *X. fastidiosa* 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 *X. fastidiosa* 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 *X. fastidiosa* to grapevines from vineyard cover crop plants.

Here we describe our general methodology for each plant species evaluated. Twenty-five plants are grown from seed in a greenhouse for needle inoculations (pin-prick method). Twenty plants are inoculated with *X. fastidiosa* subsp. *fastidiosa*, (Temecula strain PD) suspended in succinate citrate phosphate (SCP) buffer, and 5 are inoculated with SCP buffer only to serve as negative controls. Plants then are tested by ELISA at 2, 4, and 16 weeks post-inoculation, and by culture at 4 and 16 weeks post-inoculation. Plants that test positive for *X. fastidiosa* infection at 4 weeks are considered hosts and subsequently are used for insect transmissions.

For each *X. fastidiosa* host, 5 infected plants are selected as acquisition hosts (the same 5 acquisition plants are used for each vector species). Transmission tests use one vector species at a time. Twelve insects (from our greenhouse reared clean colonies) are placed on each of the five acquisition plants for an acquisition access period (AAP) of 48-hrs. Plants are checked at 24-hrs to make sure vectors are alive and feeding on the plant. From each acquisition plant, 5 insects are transferred to a clean test plant of the same species as the acquisition plant, and 5 insects are transferred to a clean test grapevine for an inoculation access period (IAP) of 96-hrs. All IAP plants are checked at 24-hrs to be sure the vectors are alive and feeding on the inoculation host. The remaining 2 insects of the original 12 are labeled and frozen at -80˚C. Following the IAP, all insects are collected, labeled, and frozen at -80˚C for later evaluation using DNA purification and PCR methods for detection of *X. fastidiosa* in the insect heads.

Seeds have been acquired for all cover crop and agricultural species, except for Burr medic, which we have been unable to find in pure form. Seeds for weed species have been acquired for black mustard, annual bluegrass, black nightshade, cheeseweed, common groundsel, common sunflower, goosefoot, horseweed, London rocket, poison hemlock, shepherd's purse, Spanish broom, stinging nettle, and tree tobacco. Seeds of other weed species are still being collected.

We had established clean, captive-reared GWSS and STSS for experiments. However, they were decimated by an infestation of the parasitoid wasp, *Gonatocerus ashmeadi*. We determined the source of infestation, corrected it, and have been rebuilding the colonies since early 2008 by regularly collecting eggs, nymphs, and adults from the field for hatching, maturing or egg laying in captivity. Eggs produced are hatched in the laboratory and transferred to clean plants. We currently have a few hundred captive-reared nymphs of each species, and several hundred mating pairs and gravid females producing many more eggs everyday, thus we should be ready to resume insect transmission experiments in August 2008. Meanwhile, we have continued to grow, needle-inoculate, and evaluate potential PD hosts from the list.

Thirty-four plant species have been grown in the greenhouse from seed. Six of the weed species matured too quickly to be used in our studies and these will be repeated this autumn. Twenty-eight plant species have been grown and needle-inoculated with the Temecula PD strain of *X. fastidiosa*. We have completed insect transmission studies for buckwheat, cowpea, fava bean, and tomato last autumn. We have 2 sets of needle-inoculated buckwheat, cowpea, fava bean, and tomato because we decided to repeat insect transmission tests for these species. We also have 2 sets of needle-inoculated goosefoot and 'Miranda' field pea due to a very slow-growing infection and a rapid death, respectively.

F. Research accomplishments and results for each objective:

Twenty-eight plant species that have been needle-inoculated with PD (Table 1). PD does not appear to be able to survive in basil, bell pepper, cotton, sunflower, or horseweed. Basil and sunflower produced false positives using the ELISA kits we purchase from Agdia, Inc. All basil and sunflower produced strong positives when tested by ELISA, including the negative and non-inoculated controls. All plants were cultured for isolation of PD, but were clean and negative. A few positives were detected at 2-weeks post-inoculation with ELISA for bell pepper, cotton, and horseweed, but no plants tested positive by ELISA at 4-weeks, nor were they positive by culture,

indicating a possible transient infection or detection of dead PD cells by early ELISA. Cultures were clean and negative for PD.

PD was successfully isolated from lima bean, goosefoot, field pea, tomato, and tree tobacco in very few cultures, indicating that PD is able to survive in these plants after mechanical inoculation, but these appear to be poor hosts of PD and may not be substantial natural hosts in the field. Insect transmissions have been done for tomato and GWSS successfully transmitted PD from tomato to tomato in 1 confirmed test. No successful insect inoculations could be detected for GWSS tomato-to-grapevine nor for STSS tomato-to-tomato, nor STSS tomato-to-grapevine transmission tests (Tables 2 & 3).

Alfalfa is already a known host of PD, but insect transmissions using STSS have not been performed. Thus we have needle-inoculated alfalfa for use in insect transmission tests. Spanish broom, cowpea, buckwheat, fava bean, and cilantro appear to be good hosts for PD when needle-inoculated, as all have been confirmed by several successful isolations of PD. However, many cilantro died by 4-weeks post-inoculation. Insect transmission tests will further our understanding of them as reservoirs of PD in the field.

Vetch was needle-inoculated and tested positive by ELISA, but most plants were dead by 4-weeks post-inoculation. Only 2 were alive for culture. Of those 2, 1 had tested positive by ELISA for PD, but both cultures were clean and negative for PD. Vetch needle-inoculations will be repeated. These plants that die rapidly may not be significant hosts in the field.

Table 1: ELISA and culture results for plant species needle-inoculated with PD.

Type	Common Name	Scientific Name	ELISA +	Culture +	PD Host?
Agriculture Crops	Alfalfa	*Medicago sativa*	20/20	14/20	Yes
	Basil	*Ocimum basilicum*	20/20*	0/20	No
	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.*	6/20***	4/13	Yes
	Horseweed	*Conyza canadensis*	2/20**	0/20	No
	Spanish Broom	*Spartium junceum*	17/20	13/20	Yes
	Tree Tobacco	*Nicotiana sp.*	12/20**	2/20	Yes
Cover Crops	Alyssum	*Alyssum sp.*	10/20	7/20	Yes
	Annual Ryegrass	*Festuca sp.*	Tests in Progress		
	Annual Fescue, Zorro	*Lolium multiflorum*	Tests in Progress		
	Black Mustard	*Brassica nigra*	Tests in Progress		
	Blando Brome	*Bromus hordeaceus*	Tests in Progress		
	Birdsfoot Trefoil	*Lotus spp.*	Tests in Progress		
	Buckwheat	*Fagopyrum sp.*	19/30	16/30	Yes
	Cilantro	*Coriandrum sativum*	7/20	7/10	Yes
	Clover, New Zealand White	*Trifolium repens*	Tests in Progress		
	Clover, Hykon Rose	*Trifolium hirtum*	Tests in Progress		
	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*	Tests in Progress		
	Oat, California Red	*Avena sativa*	Tests in Progress		
	Sudangrass	*Sorghum bicolor var. sudanense*	Tests in Progress		
	Vetch, Cahaba White	*Vicia sativa*	2/25	0/2	Requires re-test

* False positives

** Most or all positives in 2-week ELISA test; possible transient infection or dead cells detected.

*** Very slow-growing PD, 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 transmission tests were completed for buckwheat, cowpea, fava bean, and tomato. Plants were tested at 4-weeks, 8-weeks, 12-weeks, 16-weeks, and as late as 6-months post-transmission. Several plants tested positive by ELISA but could not be confirmed by culture. All four plants work well with the ELISA kit, although fava bean will sometimes produce false positives. The only 2 successful PD isolations were for GWSS transmitting from cowpea-to-cowpea and from tomato-to-tomato, and for STSS transmitting from fava bean-to-fava bean. No other successful transmissions have been detected to date, including for grapevine-to-grapevine controls. It is possible that the infection will take a substantial amount of time to detect due to the few *X. fastidiosa* cells that are transmitted by insects at one time. However, we are using Redglobe grapevines, a very susceptible variety, as our controls. Most of the alternative host test plants have died, but we continue to keep and monitor the test grapevines. It appears that neither insect can successfully transmit PD between buckwheat plants, although it may be possible for both to transmit from buckwheat to grapevine.

Table 2: GWSS and STSS transmission results for buckwheat and cowpea.

Insect	Transmission Test	ELISA +	Culture +	Successful Transmission?
GWSS	Buckwheat-to-Buckwheat	0/3 (2 died quickly)	0/3	No
	Buckwheat-to-Grapevine	2/5	0/5	Not confirmed
	Cowpea-to-Cowpea	4/5	2/5	Yes
	Cowpea-to-Grapevine	3/5	0/5	Not confirmed
	Grapevine-to-Grapevine	3/5	0/5	Not confirmed
STSS	Buckwheat-to-Buckwheat	0/5	0/5	No
	Buckwheat-to-Grapevine	2/5	0/5	Not confirmed
	Cowpea-to-Cowpea	5/5	0/5	Not confirmed
	Cowpea-to-Grapevine	2/5	0/5	Not confirmed
	Grapevine-to-Grapevine	3/6	0/6	Not confirmed

Table 3: GWSS and STSS transmission results for fava bean and tomato.

Insect	Transmission Test	ELISA +	Culture +	Successful Transmission?
GWSS	Fava Bean-to-Fava Bean	2/5	0/5	Not confirmed
	Fava Bean-to-Grapevine	1/5	0/5	Not confirmed
	Tomato-to-Tomato	3/5	1/5	Yes
	Tomato-to-Grapevine	2/5	0/5	Not confirmed
	Grapevine-to-Grapevine	2/10	0/10	Not confirmed
STSS	Fava Bean-to-Fava Bean	1/5	1/5	Yes
	Fava Bean-to-Grapevine	4/5	0/5	Not confirmed
	Tomato-to-Tomato	1/5	0/5	Not confirmed
	Tomato-to-Grapevine	3/5	0/5	Not confirmed
	Grapevine-to-Grapevine	2/4	0/4	Not confirmed

G. Publications, reports, and presentations where the information generated from research was presented:

Publications

Perring, T.M., T.R. Pinckard, and C.A. Farrar. 2007. *Xylella fastidiosa* transmission by glassy-winged sharpshooters and smoketree sharpshooters from alternate hosts to grapevines. Pp. 268-270 *In* T. Esser (ed.) Proceedings, 2007 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Presentations

Perring, T.M., T.R. Pinckard, and C.A. Farrar. *Xylella fastidiosa* transmission by glassy-winged sharpshooters and smoke tree sharpshooters from alternate hosts to grapevines. Poster Presentation at the Pierce's Disease Research Symposium, Dec. 12-14, 2007, San Diego, CA.

H. Research relevance statement:

The goal of this project is to evaluate the importance of many common weed, agricultural, and cover crop plant species that are found in close proximity to vineyards as sources of *Xylella fastidiosa* from which glassy-winged (GWSS) and smoketree (STSS) sharpshooters can acquire and transmit Pierce's Disease (PD) into grapevines. Acquisition from non-grapevine hosts and subsequent transmission to grape is of fundamental importance to primary spread of *X. fastidiosa* in California vineyards. Identifying the plants that are contributing 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 areawide management program. To reduce primary spread, efforts must focus on reducing bacteria-carrying vectors from entering healthy vineyards through continued areawide or local treatment programs outside the vineyard, barriers, trap crops, and/or removal of pathogen sources outsides the vineyard.

I. Summary in lay terms of the specific accomplishments of the research project:

We have needle-inoculated 28 different weed, cover crop, and agricultural plant species with the Temecula strain of Pierce's Disease (PD) to evaluate their potential as reservoirs of disease in the field. Of those 28, 10 are in the process of being tested at the time of this report, 5 species do not appear to sustain infection, and the remaining 13 sustain some type of PD infection. Plants under current evaluation include several popular vineyard cover crops: annual ryegrass, annual fescue, black mustard, 'Blando brome', birdsfoot trefoil, New Zealand white clover, Hykon rose clover, meadow barley, California Red oats, and sudangrass. PD does not seem to survive in basil, bell pepper, cotton, horseweed, or sunflower (commercial variety), and therefore these plants probably do not serve as reservoirs of PD in the field. Lima bean, tomato, goosefoot, and tree tobacco can harbor PD infections, but appear to have some resistance, as relatively few plants became infected, or infections are weak or very slow growing. Alfalfa, Spanish broom, alyssum, cilantro, buckwheat, cowpea, and fava bean are all alternative hosts for PD. Field pea is also a confirmed host for PD, but it along with cilantro and vetch seem to die rapidly after infection, and therefore may not be important hosts in the field.

GWSS and STSS insect transmission tests have already been performed for buckwheat, cowpea, fava bean, and tomato. Neither insect species appear to be able to transmit PD between buckwheat plants. However, both species may be able to acquire PD from buckwheat, cowpea, fava bean, and tomato and transmit it to grapevines, but this is unconfirmed by isolation of viable pathogen cells (necessary since many tests will detect dead cells or produce false positives). It has been confirmed that GWSS can successfully transmit PD between cowpea plants and between tomato plants. It also has been confirmed that STSS can successfully transmit between fava bean plants.

J. Summary and status of intellectual property produced during this research project:
Aside from the published proceedings and the presentation at the CDFA PD conference, no intellectual property was produced as a result of this research project.