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

I. PROJECT TITLE: *Xylella fastidiosa* Transmission by Glassy-Winged Sharpshooters and Smoke Tree Sharpshooters from Alternate Hosts to Grapevines

II. PRINCIPAL INVESTIGATORS AND COOPERATORS

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III. 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.

For each plant species evaluated, 25 plants were grown from seed in a greenhouse for needle inoculations (pin-prick method). Twenty plants were inoculated with *X. fastidiosa* subsp. *fastidiosa*, (Temecula strain PD) suspended in succinate citrate phosphate (SCP) buffer, and 5 were inoculated with SCP buffer only to serve as negative controls. Plants then were tested by ELISA at 2, 4, and 16 weeks post-inoculation, and by culture at 4 and 16 weeks post-inoculation. Plants that tested positive for *X. fastidiosa* infection at 4 weeks were considered hosts and were subsequently used for insect transmissions.

For each *X. fastidiosa* host, 5 infected plants were selected as acquisition hosts (the same 5 acquisition plants were used for each vector species). Transmission tests used one vector species at a time. Twelve insects (from our greenhouse reared clean colonies) were placed on each of the five acquisition plants for an acquisition access period (AAP) of 48-hrs. Plants were checked at 24-hrs to make sure vectors were alive and feeding on the plant. From each acquisition plant, 5 insects were transferred to a clean test plant of the same species as the acquisition plant, and 5

insects were transferred to a clean test grapevine for an inoculation access period (IAP) of 96-hrs. All IAP plants were checked at 24-hrs to be sure the vectors were alive and feeding on the inoculation host. The remaining 2 insects of the original 12 were labeled and frozen at -80˚C. Following the IAP, all insects were 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.

IV: SUMMARY OF MAJOR RESEARCH ACCOMPLISHMENTS AND RESULTS FOR EACH OBJECTIVE

General Summary:
These studies generate a tremendous amount of data and we present detailed results for each plant below. Here we give an overall summary of our results to date. We could not detect infection in needle-inoculated bell pepper or goosefoot at 4-wks post-inoculation, however infection was detected in goosefoot at 16 wks post-inoculation. While evidence indicates that bell pepper is not a *X. fastidiosa* host, we are conducting further research on goosefoot to resolve its host status. Buckwheat, cowpea, fava bean, tomato, and field pea all sustained *X. fastidiosa* infection at 4-wks post-inoculation and bacterial isolates were obtained for each species, confirming infection. Field pea died right after the 4-wk tests and could not be used for insect transmission tests, therefore we are repeating the field pea inoculations for insect transmission tests.

Insect transmission tests have been completed using both GWSS and STSS for buckwheat, cowpea, fava bean, and tomato. Both GWSS and STSS successfully transmitted *X. fastidiosa* from cowpea, fava bean, and tomato back into their respective hosts. Neither vector transmitted bacteria from buckwheat to buckwheat. Both GWSS and STSS transmitted *X. fastidiosa* from all alternate hosts in this group, including buckwheat, to grapevine.

Objective 1:
Grapevine Controls: Grapevines, variety 'Redglobe', were donated as rooted cuttings from Sunridge Nursery, Inc, in Bakersfield, CA. Twenty grapevines were needle-inoculated as controls against which to test the other plant species. Five grapevines also were inoculated with SCP buffer to serve as negative controls. Infection was detected in 8 of 20 grapevines by ELISA at 2-wks and all of them ultimately tested positive (Table 1). Culture results showed 16/18 plants were positive at 4-wks with a total of 19/20 positives after 16 weeks (Table 2). Most of the infected grapevines died within 16 weeks post-inoculation. All five negative controls tested negative for all tests and survived.

Insect transmission from grapevine to grapevine were conducted and 2/10 plants have tested positive by ELISA (Table 3). While none of the grapevines have tested positive by culture (Table 4), we are continuing to monitor them by ELISA and culturing.

Bell Pepper: Five of twenty *X. fastidiosa*-inoculated bell pepper plants were positive by ELISA for the 2-weeks post-inoculation (Table 1). However, all subsequent ELISA tests and culture tests have been negative for *X. fastidiosa* (Tables 1 and 2). We believe that the initial 5 ELISA positives represented a transient infection and that *X. fastidiosa* does not survive in bell pepper. Plants never showed any symptoms and 19 of 20 survived until being disposed at 18 weeks post-

inoculation. The one plant that died was accidentally damaged in handling at 15-weeks post-inoculation. Evidence indicates that bell pepper is not a host of *X. fastidiosa*, and it was not used in insect transmission studies.

Tomato: One tomato plant died the day after inoculation, apparently because it did not survive the mechanical damage of the pin-prick method. A total of 11 of 19 inoculated plants tested positive by ELISA for *X. fastidiosa*, with maximum detection of infection occurring at 4-wks post-inoculation (Table 1). Three plants tested positive by culturing (Table 2). The cultures for tomato tended to be contaminated with other microorganisms despite control plates being clean. It appears there are other microorganisms within tomato plants that can contaminate PD3 plates and may obscure positive *X. fastidiosa* growth on the plates. Infection was detected by ELISA and culture through the 16-wk tests. Seventeen of 19 plants survived until disposal at 18-wks post-inoculation without any potential PD symptoms. All negative controls were negative for all tests and survived until disposal. Tomato plants are considered potential alternative hosts for *X. fastidiosa* and insect transmission studies were conducted.

GWSS successfully transmitted *X. fastidiosa* from infected tomato (needle-inoculated) to clean tomato plants for 3 of 5 test plants and from infected tomato to clean grapevine for 2 of 5 test plants (Table 3). One of 5 tomato to tomato test plants has tested positive by culturing, but no *X. fastidiosa*-positive cultures have been obtained for tomato to grape (Table 4). Evaluations for the 12-wk test for this transmission group are in progress at the time of this report. STSS successfully transmitted *X. fastidiosa* from infected tomato to clean tomato for 1 of 5 test plants, and from infected tomato to clean grapevine for 3 of 5 plants (Table 5). Positive *X. fastidiosa*-isolates have not yet been obtained for this group (Table 6). No tomato or grapevine test or control plants show symptoms at this time.

Presently we are growing alfalfa, cotton, and lima bean and they are scheduled for needle inoculation as follows: lima bean, Feb. 25-29, 2008; cotton, Mar. 3-7, 2008; and alfalfa, March 10-14, 2008. Insect transmission experiments will begin at 5-6 weeks post-inoculation for plant species that are shown to be hosts for *X. fastidiosa* at 4 weeks post-inoculation. All seed for selected agricultural crop species has been obtained.

Objective 2:

Goosefoot: No infection was detected in 20 goosefoot test plants at 2-wks post-inoculation and 1 tested positive by ELISA at 4-wks (Table 1), but this could not be confirmed by culture (Table 2). Based on these results, goosefoot was considered to not be a host for *X. fastidiosa*, thus we did not conduct insect transmissions. However, infection was detected in 6 of 20 test plants at 16-wks and confirmed by culture in 4 of the test plants (Tables 1 and 2). All negative goosefoot controls were negative for all tests and survived until the end of the study. Infection developed slowly in this species thus we need to evaluating its potential as a reservoir of *X. fastidiosa*. At present, we are growing more goosefoot for a second inoculation set that may be used for insect transmission tests.

We are currently growing black nightshade, cheeseweed, common groundsel, common sunflower, horseweed, london rocket, shepherd's purse, spanish broom, stinging nettle, and tree tobacco. Common groundsel, common sunflower, and stinging nettle are scheduled for needle

inoculation as follows: common groundsel, Mar. 3-7, 2008, common sunflower, Feb. 25-29, 2008, and stinging nettle, Mar. 10-14, 2008. The other species listed above have germinated, but are not large enough for inoculation yet. However, we expect to inoculate all of the above listed species within the next 6 weeks. Seed has been obtained for 12 of the 21 weed species being evaluated. Seed collection for weed species is ongoing as it becomes available in the field

Objective 3:

Buckwheat: Five of 18 test plants tested positive for *X. fastidiosa* by ELISA at 2-wks, and 12 of 18 were positive at 4-wks (Table 1). *Xylella fastidiosa* isolates were obtained for 9 of 13 cultures (Table 2), confirming viable infection. Maximum detection of *X. fastidiosa* occurred at 4-wks, but most plants were dead by 16-wks. Buckwheat showed some necrosis at the leaf margins, but the negative controls showed the same pattern, so these were dismissed as either side effects of the SCP buffer or a normal trait of buckwheat. All negative controls were negative for all tests and survived until disposal.

Neither GWSS nor STSS successfully transmitted *X. fastidiosa* from buckwheat-to-buckwheat, but both vectors successfully transmitted *X. fastidiosa* from buckwheat-to-grapevine as tested by ELISA (Table 3 and 5). Therefore, both vectors are able to acquire *X. fastidiosa* from buckwheat, but we are unsure as to why they did not transmit from buckwheat-to-buckwheat. We have not yet obtained any *X. fastidiosa* cultures from these transmissions (Tables 4 and 6).

Cowpea: A total of 9/20 needle-inoculated cowpea plants tested positive by ELISA, with maximum detection occurring at 4-wks (Table 1). Six isolates of *X. fastidiosa* were obtained from 15 cultures, confirming infection in most of the plants (Table 2). Cultures tended to be contaminated, although control cultures were not, indicating other microorganisms present in cowpea that may obscure detection of *X. fastidiosa* isolates. All negative control plants were negative for all tests and survived until disposal. Some plants displayed yellowing lower leaves, while the negative controls did not, but we are unsure if this was due to normal necrosis of older leaves or a symptom of *X. fastidiosa* infection in cowpea.

Both GWSS and STSS successfully transmitted *X. fastidiosa* from cowpea-to-cowpea and from cowpea-to-grapevine at determined by ELISA (Table 3 and 5). GWSS transmitted *X. fastidiosa* from cowpea into 4 of 5 cowpea test plants, and 3 of 5 grapevine test plants. Two isolates of *X. fastidiosa* transmitted by GWSS into cowpea have been obtained, but none have been obtained yet for *X. fastidiosa* vectored by GWSS from cowpea-to-grapevine (Table 4). STSS transmitted *X. fastidiosa* from cowpea into 5 of 5 cowpea test plants, and 2 of 5 grapevine test plants as determined by ELISA (Table 5). No isolates of *X. fastidiosa* vectored by STSS from cowpea have been obtained yet (Table 6).

Fava Bean: Nineteen of 20 test plants were positive by ELISA (Table 1), but only 1 isolate was obtained (Table 2). All cultures from fava bean were contaminated, although control cultures were not, indicating the presence of other microorganisms in fava bean plants that may obscure detection of *X. fastidiosa*. Maximum detection of *X. fastidiosa* by ELISA occurred at 4-wks with 18 of 20 plants testing positive, with most plants dead by 16-wks. All negative controls tested negative by all tests and survived until disposal. No PD-like symptoms were observed.

Both GWSS and STSS successfully transmitted *X. fastidiosa* from fava bean to fava bean, and from fava bean to grapevine. GWSS infected 2 of 5 fava bean plants and 1 of 5 grapevines by 8-wks post-inoculation (Table 3). No isolates of *X. fastidiosa* have been obtained yet from these (Table 4). STSS infected 1 of 5 fava bean plants and 4 of 5 grapevines to date (Table 5). One isolate of *X. fastidiosa* vectored by STSS from fava bean to grapevine has been obtained (Table 6). Twelve week tests for this set are in progress.

We have completed needle inoculations for field pea, but those plants died before insect transmissions could be performed. We are currently growing field pea again and it is scheduled for needle inoculation the week of Feb. 25-29, 2008. We also are growing black mustard, cilantro, and 'Cahaba' vetch. The vetch is scheduled for needle inoculation the week of Feb. 25-29, 2008, and the other two are germinated and should be ready for needle inoculation within the next 4 weeks. Seed has been obtained for 13 of the 15 species selected for evaluation. Seed for the other two species will be purchased from a commercial grower in March 2008

Table 1: ELISA Test Results for Needle Inoculations of Potential Alternative *X. fastidiosa* Plant Hosts

Plant Name	Plant Type	+ELISA/ Total tested at 2-wks	+ELISA/ Total tested at 4-wks	+ELISA/ Total tested at 16-wks	Total + by ELISA/Total tested	Total Percent Infected (%)
Bell Pepper	Agricultural Crop	5/20	0/20	0/19	0/20*	0.0*
Tomato	Agricultural Crop	5/19	7/19	3/17	11/19	57.9
Goosefoot	Weed Species	0/20	1/20	5/13	6/20	30.0
Buckwheat	Cover Crop	5/18	12/18	0/1	12/18	66.7
Cowpea	Cover Crop	4/20	7/20	0/7	9/20	45.0
Fava Bean	Cover Crop	10/20	18/20	1/3	19/20	95.0
Field Pea	Cover Crop	4/20	10/20	Dead, N/A	13/20	65.0
Grapevine	Control	8/20	17/20	7/7	20/20	100.0

* Infection in bell pepper was not confirmed by culture or subsequent ELISA tests, so we determined that the 5 positive results at 2-wks indicated a transient infection, and that bell pepper is not a host for PD.

Table 2: Culture Test Results for Needle Inoculations of Potential Alternative *X. fastidiosa* Plant Hosts

Plant Name	Plant Type	+Culture/ Total tested at 2-wks	+Culture/ Total tested at 4-wks	+Culture/ Total tested at 16-wks	Total + by Culture/Total tested	Total Percent Cultured (%)
Bell Pepper	Agricultural Crop	Not done, N/A	0/5	0/19	0/20	0.0
Tomato	Agricultural Crop	Not done	2/9	1/17	3/18	16.7
Goosefoot	Weed Species	Not done	0/1	4/13	4/13	30.8
Buckwheat	Cover Crop	Not done	9/13	Dead, N/A	9/13	69.2
Cowpea	Cover Crop	Not done	6/9	0/8	6/15	40.0
Fava Bean	Cover Crop	Not done	1/19**	0/3	1/19	5.3**
Field Pea	Cover Crop	Not done	3/11	Dead, N/A	3/11	27.3
Grapevine	Control	Not done	16/18	7/7	19/20	95.0

** Cultures from fava bean were always contaminated, although control cultures were not, indicating other microorganisms present in fava bean probably obscuring detection of PD on the plates.

Table 3: ELISA Test Results for Transmission Plants Using GWSS Vectors

Acquisition Plant	Inoculation Plant	+ELISA/ Total tested at 4-wks	+ELISA/ Total tested at 8-wks	+ELISA/ Total tested at 12-wks	Total +ELISA/ Total tested
Buckwheat	Buckwheat	0/3	0/3	Dead, N/A	0/3
Buckwheat	Grapevine	0/5	0/5	2/5	2/5
Cowpea	Cowpea	3/5	3/5	1/3	4/5
Cowpea	Grapevine	2/5	0/5	1/5	3/5
Grapevine	Grapevine	0/4	2/4	2/4	3/5
Fava Bean	Fava Bean	0/5	2/2	Pending***	2/5
Fava Bean	Grapevine	2/5	1/5	Pending***	1/5
Tomato	Tomato	0/5	3/5	Pending***	3/5
Tomato	Grapevine	1/5	2/5	Pending***	2/5
Grapevine	Grapevine	1/10	2/10	Pending***	2/10

*** 12-wk tests for this group are in progress at the time of this report.

Table 4: Culture Test Results for Transmission Plants Using GWSS Vectors

Acquisition Plant	Inoculation Plant	+Culture/ Total tested at 4-wks	+Culture/ Total tested at 8-wks	+Culture/ Total tested at 12-wks	Total +Culture/ Total tested
Buckwheat	Buckwheat	0/3	0/3	Dead, N/A	0/3
Buckwheat	Grapevine	0/5	0/5	0/5	0/5
Cowpea	Cowpea	0/5	0/5	2/5	2/5
Cowpea	Grapevine	0/5	0/5	0/5	0/5
Grapevine	Grapevine	0/5	0/5	0/5	0/5
Fava Bean	Fava Bean	0/5	0/5	Pending***	0/5
Fava Bean	Grapevine	0/5	0/5	Pending***	0/5
Tomato	Tomato	0/5	1/5	Pending***	1/5
Tomato	Grapevine	0/5	0/5	Pending***	0/5
Grapevine	Grapevine	0/10	0/10	Pending***	0/10

*** 12-wk tests for this group are in progress at the time of this report.

Table 5: ELISA Test Results for Transmission Plants Using STSS Vectors

Acquisition Plant	Inoculation Plant	+ELISA/ Total tested at 4-wks	+ELISA/ Total tested at 8-wks	+ELISA/ Total tested at 12-wks	Total +ELISA/ Total tested
Buckwheat	Buckwheat	0/5	0/5	Dead, N/A	0/5
Buckwheat	Grapevine	0/5	1/5	1/5	2/5
Cowpea	Cowpea	5/5	2/5	2/5	5/5
Cowpea	Grapevine	1/5	0/5	1/5	2/5
Grapevine	Grapevine	0/6	0/6	3/6	3/6
Fava Bean	Fava Bean	0/5	1/1	Pending***	1/5
Fava Bean	Grapevine	2/5	4/5	Pending***	4/5
Tomato	Tomato	0/5	1/4	Pending***	1/5
Tomato	Grapevine	1/5	3/5	Pending***	3/5
Grapevine	Grapevine	1/4	2/4	Pending***	2/4

*** 12-wk tests for this group are in progress at the time of this report.

Table 6: Culture Test Results for Transmission Plants Using STSS Vectors

Acquisition Plant	Inoculation Plant	+Culture/ Total tested at 4-wks	+Culture/ Total tested at 8-wks	+Culture/ Total tested at 12-wks	Total +Cultrue/ Total tested
Buckwheat	Buckwheat	0/5	0/5	Dead, N/A	0/5
Buckwheat	Grapevine	0/5	0/5	0/5	0/5
Cowpea	Cowpea	0/5	0/5	0/5	0/5
Cowpea	Grapevine	0/5	0/5	0/5	0/5
Grapevine	Grapevine	0/6	0/6	0/6	0/6
Fava Bean	Fava Bean	0/5	1/5	Pending***	1/5
Fava Bean	Grapevine	0/5	0/5	Pending***	0/5
Tomato	Tomato	0/5	0/5	Pending***	0/5
Tomato	Grapevine	0/5	0/5	Pending***	0/5
Grapevine	Grapevine	0/4	0/4	Pending***	0/4

*** 12-wk tests for this group are in progress at the time of this report.

V. PUBLICATIONS OR REPORTS RESULTING FROM THE PROJECT

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.

VI. PRESENTATIONS ON RESEARCH

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.

VII. RESEARCH RELEVANCE STATEMENT

This project addresses the 2006 Scientific Summit category of "*Understanding transmission of the disease*," and relates directly to the acquisition and transmission of *X. fastidiosa* 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."

VIII. LAY SUMMARY OF CURRENT YEAR'S RESULTS

We artificially inoculated 7 plant species that are found in or near grape vineyards, plus grapevine controls, with a PD strain of *Xylella fastidiosa* to determine if they can be infected with PD, thereby providing a potential reservoir of the disease in or near a vineyard. The 7 species of plants included other agricultural plants (bell pepper and tomato), weed species (goosefoot), and cover crops (buckwheat, cowpea, fava bean, field pea). Plants that tested

positive for infection at 4-wks after artificial inoculation were considered hosts of *X. fastidiosa* and these were used in insect transmission tests. We found that bell pepper is not a host for *X. fastidiosa*, therefore it could be planted near vineyards without fear of harboring *X. fastidiosa*. Infection could not be detected in goosefoot until after 8-wks post-inoculation and we are continuing work with this species. Field pea sustained infections of *X. fastidiosa* at 4-wks, but the plants did not survive much longer and could not be used in insect transmission tests, so we are repeating this species this year. Buckwheat is a host for *X. fastidiosa*, and GWSS and STSS acquired *X. fastidiosa* from buckwheat and transmitted it into grapevines. Tomato, cowpea, and fava bean all sustained infections of *X. fastidiosa*. Both sharpshooters transmitted *X. fastidiosa* from infected tomato to tomato and grapevine, from infected cowpea into cowpea and grapevine, and from infected fava bean into fava bean and grapevine. Therefore, these 3 alternative hosts can serve as reservoirs of *X. fastidiosa* from which an insect vector can acquire and infect nearby grapevines. Cowpea and fava bean are sometimes used as cover crops in vineyards and our data suggest caution in doing so for areas with PD outbreaks and/or populations of GWSS or STSS vectors.

IX. STATUS OF FUNDS

According to records through December 2007, the project had utilized $71,756 or 36% of the 2 year allocation for this project. Given that just 25% of the project time frame was completed, the PI will make adjustments to the spending on this project to insure financial health through the 2 year time frame.

X. SUMMARY AND STATUS OF INTELLECTUAL PROPERTY PRODUCED DURING THIS RESEARCH PROJECT

No intellectual property was produced as a result of this research project.