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CDFA PIERCE'S DISEASE & GLASSY-WINGED SHARPSHOOTER BOARD PROGRESS REPORT (JULY 2007 – FEBRUARY 2009)

Project Title:
Xylella fastidiosa Transmission by Glassy-Winged Sharpshooters and Smoke Tree Sharpshooters from Alternate Hosts to Grapevines

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Time Period Covered by Report: July 2007-February 2009

Objectives, Activities, Progress and Findings:

The objectives of the project are:

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.

Plant Selection and Propagation
Cover crop, weed, and other agricultural plant species were selected from the Statewide UCIPM website that are found growing in or near California grape vineyards. Several plants also were chosen from the literature, as previously found to be PD hosts by inoculation through needle or other vectors, but not glassy-winged sharpshooter (GWSS) or smoketree sharpshooter (STSS). Plants were propagated in greenhouses from seed bought from commercial suppliers or collected in the field (weeds), except for grapevine cuttings, which were donated by Sunridge Nurseries, Inc., Bakersfield, CA. Forty-nine to 56 plants were grown from seed for each species. Donated grapevine cuttings were potted in 1-gal pots and kept in a greenhouse.

Progress update: Seed has been obtained for all plant species, except burr medic, annual bursage, chickweed, cocklebur, morninglory, bindweed, and speedwell, which we have been unable to purchase or find in the field. Currently we are growing stinging nettle, shepherd's purse, filaree, and prickly lettuce for inoculation. Common groundsel, cheeseweed, London rocket, sweetclover, wildtype sunflower, and annual bluegrass have been grown and inoculated, and we are in the process of testing for PD host status.

Mechanical Inoculations

Redglobe grapevine cuttings infected with a wildtype PD were obtained from the Bakersfield area. The PD was isolated and identified as *Xylella fastidiosa* subspecies *fastidiosa* (Temecula strain PD) using MLST for all grapevines (Scally et al. 2005, Schuenzel et al. 2005). For each inoculation event, samples were isolated from several grapevines onto PWG media. At 7-d old, bacterial isolates from several source plants were combined and suspended in cold succinate citrate phosphate (SCP) buffer for inoculation. Subsamples of inoculum were serially diluted and CFU's counted in a dissecting microscope to estimate concentrations.

Twenty plants of each PD host species were randomly selected and inoculated at the 2nd and 3rd internodes by puncturing 1-5 holes in the stem through a 20µl drop of inoculation suspension using a #0 insect pin. Plants were observed for uptake of inoculum. Five plants of each species were inoculated with SCP only buffer to serve as negative controls. Potted Redglobe grapevines also were inoculated with PD and with SCP only buffer to serve as controls for each inoculation group. Grasses were inoculated differently with 1 drop at the base of a single plant, just above the roots.

Leaf samples were taken from the 4th node at 2-wks post-inoculation, and the 5th node at 4-wks post-inoculation and processed by ELISA and culture. Plants confirmed positive for PD by culture at 4 weeks or later were considered hosts for PD and used in insect transmission tests.

Progress update: We have completed tests to determine PD host status for 25 species of plants. Seventeen species have been determined to sustain PD infection from mechanical inoculation and 8 species do not sustain PD infection from mechanical inoculation.

Determining PD Host Status

Two 3cm samples of petiole, leaf blade, and/or stem were cut from plant samples collected for 2, 4, and 16 weeks post-inoculation tests. Samples were surface-sterilized in a series of 5 x 30sec sterile baths as follows: 20% bleach (1:5 dilution of 5.25% hypochlorite solution), 95% ethyl alcohol, then 3 sterile deionized water rinses. Surface sterilized samples then were finely chopped (roughly 1-3mm thick disks or pieces) in 600µl sterile SCP buffer using sterile forceps and scalpels and allowed to set for 10-15min. Two hundred microliters of buffer were pipetted from the chopped mash onto each of 2 PD3 media plates. The plates were wrapped with Parafilm and, after allowing the sample to settle into the media for about 30 min, they were incubated, inverted at room temperature in a dark drawer for 10-30 days. Plates were checked for the presence of PD at 10, 20, and 30 days. DNA was purified from isolated PD after 10 days or later, and any samples that appeared to be possible PD were frozen at -80ºC for PCR verification.

ELISA tests were made using a commercial kit from Agdia, Inc. following the kit instructions with the following variations: 500µl of general extraction buffer was added to each of the leftover chopped samples from plating, and allowed to set for 10min; then 100µl of each sample was loaded into a well on the ELISA plate. (Both the ELISA and culture test were made from the same sample.)

Progress Update: We have isolates from all positive PD plates in cold storage. Currently we are processing some questionable, but potential PD isolates from insect transmission plants.

Insect Rearing

For rearing *Homalodisca vitripennis* (GWSS) and *Homalodisca lacerta* (STSS), nymphs and adults are collected locally from the UCR campus using insect nets and separated by species in the laboratory. Leaves with eggs also are collected from plants on campus and hatched in the laboratory in Petri dishes. Field collected nymphs and adults are placed into a large colony on clean basil, sunflower, cowpea, corn, chrysanthemum, bell pepper, orange, and lemon plants in the greenhouse for oviposition. Eggs are collected weekly from the colonies and hatched separately. Hatched nymphs are placed into a new, clean colony with the same plant species and allowed to reach maturity for use in transmission experiments. Eggs from the field and hatched in Petri dishes in the laboratory also are placed in the clean colony for use in experiments. In the clean colonies, eggs are left on plants in the colony and nymphs are allowed to hatch naturally into the colony (self-producing).

Progress update: Both GWSS & STSS colonies became infested with parasitoid wasps in the summer and fall of 2008, which crashed both colonies, despite our best attempts to save them. We have rebuilt the colonies during the previous few months and again have thriving, reproducing colonies for both species.

Insect Transmission Tests

For each PD plant host species, 5 infected plants were selected as acquisition hosts. Twelve insects from our greenhouse reared clean colonies were placed on each of the five acquisition plants plus 1-2 infected grapevines (controls) 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. Insects on infected grapevine controls were similarly transferred onto non-infected grapevines. 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.

Progress update: We have completed insect transmission tests for both GWSS and STSS for alfalfa, brome, cowpea, fava bean, goosefoot, and tomato. Transmission tests using GWSS on basil have been completed, but the STSS colony completely crashed at that time, and we were unable to complete tests on basil using STSS. Due to the colonies crashing, we were unable to complete insect transmission tests for all PD plant hosts tested to date this winter. Now that our

colonies are thriving, we are planting seeds and inoculating plants of those species already determined to be PD hosts, but still require insect transmission tests. Those plants include: basil (for STSS only), lima bean, Spanish broom, tree tobacco, annual ryegrass, black mustard, 'New Zealand White' clover, 'Hykon Rose' clover, 'Miranda' field pea, meadow barley, and 'California Red' oats. The next round of insect transmission is scheduled to begin the first week in April.

Results and Discussion

Twenty-five plant species have been inoculated and tested to determine host potential for PD via mechanical inoculations. PD isolates have been isolated from 17 species, but some appear to be better hosts for PD than others. We recovered PD isolates from 50% or more of the test plants for 6 species: alfalfa, basil, black mustard, 'Blando' brome, and Hykon Rose clover. These may be very good hosts for PD in the field and should probably be controlled around vineyards where PD and/or GWSS or STSS are present.

We recovered PD isolates in 20-50% of test plants for 7 species: 'Rutgers' tomato, goosefoot, annual ryegrass, 'California Blackeye' cowpea, 'Miranda' field pea, 'Windsor' fava bean, and meadow barley. These are probably also good reservoirs of PD in the field, except for goosefoot, which appears to be a very poor host for either GWSS or STSS. We only recovered isolates for 1-2 test plants for lima bean, tree tobacco, 'New Zealand White' clover, and 'California Red' oats. Therefore, some plants appear to be better hosts for PD than others tested, and although isolates were obtained from a few of some species by mechanical inoculation, they may not be significant PD hosts in the field.

No isolates were obtained from 8 species tested: 'Taurus' sweet bell pepper, 'Upland' cotton, black nightshade, 'Evening Sun' sunflower, horseweed, 'Zorro' annual fescue, birdsfoot trefoil, and sudangrass. Plants from which we could not obtain any isolates may have defenses against PD infection and may be useful in disease and pest management practices (this requires further investigation). We were unable to obtain any isolates from horseweed, which is especially good news, since it so prevalent in vineyards and resistant to control measures.

We have grown and inoculated annual bluegrass, cheeseweed, common groundsel, London rocket, and wild sunflower, and are currently evaluating their potential as PD hosts. Stinging nettle, shepherd's purse, prickly lettuce, and filaree, are being grown and will be inoculated with PD at the end of March.

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*	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	Annual Bluegrass	*Poa annua*	Tests in Progress		
	Black Nightshade	*Solanum nigrum*	0/20	0/20	No
	Cheeseweed	*Malva parviflora*	Tests in Progress		
	Common Groundsel	*Senecio vulgaris*	Tests in Progress		
	Common Sunflower ('Evening Sun' variety)	*Helianthus annuus*	20/20*	0/20	No
	Common Sunflower, wild-type	*Helianthus annuus*	Tests in Progress		
	Goosefoot	*Chenopodium sp.*	6/20***	4/13	Yes
	Horseweed	*Conyza canadensis*	2/20**	0/20	No
	London Rocket	*Sisymbrium irio*	Tests in Progress		
	Spanish Broom	*Spartium junceum*	17/20	13/20	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	No
	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	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	*Medicago spp.*	Tests in Progress		

* 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 have been completed for both GWSS and STSS for alfalfa, brome, cowpea, fava bean, and tomato. GWSS successfully transmitted PD from alfalfa to alfalfa and from alfalfa to grapevine, from basil to basil and from basil to grapevine, from brome to brome and from brome to grapevine, from cowpea to cowpea, and from tomato to tomato. GWSS appears to be a very good vector of PD between alfalfa plants, from alfalfa to grapevines, and from basil to grapevines, but did not transmit PD as well from cowpea, fava bean or brome. We were unable to obtain PD isolates for GWSS transmission from cowpea to grapevine, fava bean to fava bean, fava bean to grapevine, tomato to grapevine, or goosefoot to grapevine. At the same time, we were unable to recover PD from grapevine to grapevine transmission controls for cowpea, fava bean, and tomato groups, indicating that there may have been something unusual about those cohorts of insects and/or plants. Therefore, we feel theses tests need to be repeated.

STSS successfully transmitted PD from fava bean to fava bean, from alfalfa to alfalfa, and from alfalfa to grapevine, indicating it is a good PD vector in the presence of alfalfa and grapevines. STSS did not successfully transmit from fava bean to grapevine, from tomato to tomato, from tomato to grapevine, from cowpea to cowpea, from cowpea to grapevine, from brome to brome, or from brome to grapevine. The grapevine to grapevine control plants did not test positive for cowpea, fava bean, or tomato groups either, which may indicate a problem with that cohort of plants or insects. Again, we will repeat these tests. Three of 4 of the brome group grapevine controls were positive for PD, so the negative transmission results for STSS on brome indicate that STSS cannot transmit PD between brome plants or from brome to grapevines.

We attempted to complete transmission tests for goosefoot, but both insect species died in less than 12hrs during acquisition, indicating that goosefoot is a poor host for both insect species and is unlikely to serve as a PD host where those insects are major vectors. Only 1 GWSS survived from goosefoot to grapevine and it has repeatedly tested negative for PD. Also, PD isolates were obtained for less than 50% of mechanically inoculated test goosefoot plants, indicating that it may not be a good host for PD in the field at all.

Due the untimely demise of our colonies late last summer, and/or the short life of some of the plant species, we were unable to complete transmission tests for lima bean, Spanish broom, tree tobacco, annual ryegrass, black mustard, ‘New Zealand White’ clover, ‘Hykon Rose’ clover, ‘Miranda’ field pea, meadow barley, or ‘California Red’ oats for either insect species, or for STSS on basil, over the winter. Therefore, we are re-growing these plants for inoculation and transmission. As soon as they test positive for PD by culture, we will complete transmission tests for these species. ‘Miranda’ field peas are being grown and inoculated at the time of this writing and transmission tests are scheduled to occur the first week of April.

Table 2: GWSS and STSS transmission results.

Insect	Transmission Test	ELISA +	Culture +	Transmission Confirmed?
GWSS	Alfalfa-to-Alfalfa	4/5	4/5	Yes
	Alfalfa-to-Grapevine	4/5	4/5	Yes
	Alfalfa Grapevine-to-Grapevine Controls	2/2	2/2	Yes
	Basil-to-Basil	9/9*	2/9	Yes
	Basil-to-Grapevine	6/9	7/9	Yes
	Basil/Goosefoot Grapevine-to-Grapevine Controls	3/6	3/6	Yes
	Brome-to-Brome	1/4	1/4	Yes
	Brome-to-Grapevine	0/4	1/4	Yes
	Brome Group Grapevine-to-Grapevine Controls	3/4	3/4	Yes
	Cowpea-to-Cowpea	4/5	2/5	Yes
	Cowpea-to-Grapevine	3/5	0/5	No
	Cowpea Group Grapevine-to-Grapevine	3/5	0/5	No
	Fava Bean-to-Fava Bean	2/5	0/5	No
	Fava Bean-to-Grapevine	1/5	0/5	No
	Tomato-to-Tomato	3/5	1/5	Yes
	Tomato-to-Grapevine	2/5	0/5	No
	Fava Bean/Tomato Group Grapevine-to-Grapevine Controls	2/10	0/10	No
	Goosefoot	0/0	0/0	No
	Goosefoot-to-Grapevine	0/1	0/1	No
STSS	Alfalfa-to-Alfalfa	5/5	3/5	Yes
	Alfalfa-to-Grapevine	4/5	4/5	Yes
	Alfalfa Grapevine-to-Grapevine Controls	1/3	1/3	Yes
	Brome-to-Brome	4/4	0/4	No
	Brome-to-Grapevine	0/4	0/4	No
	Brome Group Grapevine-to-Grapevine Controls	3/4	4/4	Yes
	Cowpea-to-Cowpea	5/5	0/5	No
	Cowpea-to-Grapevine	2/5	0/5	No
	Cowpea Group Grapevine-to-Grapevine	3/6	0/6	No
	Fava Bean-to-Fava Bean	1/5	1/5	Yes
	Fava Bean-to-Grapevine	4/5	0/5	No
	Tomato-to-Tomato	1/5	0/5	No
	Tomato-to-Grapevine	3/5	0/5	No
	Fava Bean/Tomato Group Grapevine-to-Grapevine Controls	2/4	0/4	No
	Goosefoot	0/0	0/0	No

Intellectual Property Issues:
No intellectual property has been produced as a result of this research project.

Appropriate References:

Scally, M., E. L. Schuenzel, R. Stouthamer, and L. Nunney 2005. Multilocus sequence type system for the plant pathogen *Xylella fastidiosa* and relative contributions of recombination and point mutation to clonal diversity. Appl. Environ. Microbiol. 71: 8491-8499.

Schuenzel, E., M. Scally, R. Stouthamer, and L. Nunney. 2005. A multigene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen *Xylella fastidiosa.* Appl. Environ. Microbiol. 71: 3832-3839.

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 grapevine. Pp. 268-270 in Esser, T. (ed.) Proceedings, 2007 Pierce's disease research symposium. California Department of Food and Agriculture, Sacramento, CA.

Perring, T.M., T.R. Pinckard, and C.A. Farrar. 2008. *Xylella fastidiosa* transmission by glassy-winged sharpshooters and smoketree sharpshooters from alternate hosts to grapevine. Pp. 231-234 in Esser, T. (ed.) Proceedings, 2008 Pierce's disease research symposium. California Department of Food and Agriculture, Sacramento, CA.

Research Relevance Statement:

Evaluating the potential of various common plant species found in and near vineyards to serve as reservoirs of PD, and the ability of GWSS and STSS to acquire and transmit PD from these alternative plant hosts, is fundamental to understanding 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 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 outside the vineyard.