Final Report for CDFA contract number 07-0298

Xylella fastidiosa transmission by glassy-winged sharpshooter and smoketree sharpshooter from alternate hosts to grapevines.

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Introduction:

Over 140 plants are known to host Pierce's Disease (PD) strains of *Xylella fastidiosa* (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 used as cover crops in vineyards (Statewide IPM Program 2007). While considerable research has identified *X. fastidiosa* 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 grapevines should be removed if growers wish to reduce primary spread of *Xylella fastidiosa* into their vineyards.

To successfully implement a program to remove *X. fastidiosa* sources, we first must identify those sources. The introduction into California of GWSS, an insect with a broad plant host range, theoretically increases the probability of disease spread from these alternate host plants to grapevines. For this to occur, GWSS must feed on infected plants in such a way as to acquire *X. fastidiosa* from them 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 host plants to grapevines (Hill and Purcell 1995, 1997). We are unaware of research published on transmission of *X. fastidiosa,* PD strain, from alternate plant hosts into grapevines using GWSS and STSS, a native California sharpshooter also found in grape growing regions, as the vectors.

Objectives, Activities, Progress and Findings:

Using GWSS and STSS as vectors, the objectives of this project were:

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.

Methods and Materials:

Plant Selection and Propagation

Common weeds and cover crops found in or near California grape vineyards were selected using the Statewide IPM Program (2007). This list of plants was cross-referenced with previously published inoculation studies and field surveys of *X. fastidiosa* and we selected plants that developed systemic infections in 50% or more of the plants inoculated by Wistrom and Purcell (2005), and plants identified as *X. fastidiosa* hosts in almond orchards (Shapland et al. 2006) and in vineyard areas of southern California (Costa et al. 2004). Additional plants were chosen because they are commonly found in or around vineyards, but they have not been previously inoculated with *Xylella fastidiosa*, or used in transmission studies for GWSS and STSS. Finally several plants were chosen from the literature, as previously found to be *X. fastidiosa* hosts by inoculation through needle or other vectors, but not GWSS or STSS. This resulted in the identification of 43 plant species at the beginning of the study.

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 plants were grown from seed for each species in a climate-controlled greenhouse. Donated grapevine cuttings were potted in 1-gal pots and kept in a climate-controlled greenhouse.

Needle Inoculation of Plants

Redglobe grapevine cuttings, infected with a wild-type PD strain of *X. fastidiosa*, were obtained from the Bakersfield area. The bacteria was isolated and identified as *X. 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, bacteria isolates from several source plants were combined and suspended in cold succinate citrate phosphate (SCP) buffer for inoculation. Subsamples of inoculum were serially diluted, plated in volumes of 20µl onto PWG media, and CFU's were counted in a dissecting microscope 7 days later to estimate concentrations.

A minimum of 20 individual plants (more in some cases) of each *X. fastidiosa* host species were selected randomly 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 additional plants of each species were inoculated with SCP-only buffer to serve as negative controls. Potted Redglobe variety grapevines also were inoculated with *X. fastidiosa* and with SCP-only buffer to serve as controls for each inoculation group. Grasses and shepherd's purse plants were inoculated differently with 1 drop at the base of a single plant, just above the roots.

Determining *X. fastidiosa* Host Status

From each putative *X. fastidiosa* host, leaf samples were taken from the 4th node at 2-wks post-inoculation, and the 5th node at 4-wks post-inoculation, or the nearest available leaf above the desired node. After the first 4-wks, plants were sampled at 4-week intervals until a positive was obtained or until 16 weeks post-inoculation.

Plant samples were analyzed by ELISA and by culturing. For the ELISA tests we used 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.) For culturing, two 3cm subsamples of petiole, leaf blade, and/or stem (depending on plant morphology) were cut from plant samples collected. Subsamples 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 sit for 10-15min. Two hundred microliters of buffer were pipetted from the chopped mash onto 2 PD3 media plates. The plates were wrapped with Parafilm and, after allowing the sample to settle into the media for about 30 min, were incubated, inverted at room temperature in a drawer (dark) for 10-30 days. Plates were checked for the presence of *X. fastidiosa* at 10, 20, and 30 days. Representative samples from each plant species or transmission test were frozen at -80 ºC and processed using PCR for *X. fastidiosa* confirmation using PD specific primers (Hernandez-Martinez et al. 2006). Plants confirmed positive for *X. fastidiosa* by culture were considered hosts for PD and used in subsequent insect transmission tests.

Insect Rearing

Homalodisca coagulata (GWSS) and *Homalodisca lacerta* (STSS) nymphs and adults were collected locally from the UCR campus using insect nets. Leaves with eggs were collected from plants on campus and hatched in the laboratory in Petri dishes. Field collected nymphs and adults were placed into the greenhouse in cages containing basil, sunflower, corn, chrysanthemum, bell pepper, orange, and lemon plants. In adult cages, eggs were collected weekly from the colonies and hatched separately. Hatched nymphs were 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 were placed in the clean colony for use in experiments. In the clean colonies, eggs were left on plants in the colony and nymphs were allowed to hatch naturally into the colony (self-producing).

Insect Transmission Tests

For each plant identified as a *X. fastidiosa* host in the inoculation experiments above, 5 infected plants were selected as acquisition hosts. Twelve insects from our 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 plants. 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 single infected grapevine controls were similarly transferred onto 2 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.

For plant species where *X. fastidiosa* was recovered from less than 5 needle-inoculated plants, we performed the transmission test repeatedly on the infected source plants until at least 4 test plants of each (alternate host and grapevine) for each insect species were exposed to putatively-infected insects.

Results and Discussion:

From the initial list of 43 plant species, we were unable to obtain seed for burr medic, annual bursage, chickweed, cocklebur, morninglory, bindweed, and speedwell, so these were not tested. We collected seed for poison hemlock, and prickly lettuce, but germination was extremely low to 0%, so these plants were not evaluated. We also had germination and inoculation problems with filaree, shepherd's purse, and stinging nettle.

Needle-Inoculated Plants

Thirty-one plant species were inoculated and tested to determine their host potential for *X. fastidiosa* via mechanical inoculations (Table 1). *X. fastidiosa* does not appear to be able to survive in the following needle-inoculated plants: bell pepper, cotton, black nightshade, common groundsel, 'Evening Star' sunflower, horseweed, annual fescue, birdsfoot trefoil, or sudangrass. Among these plants, some had a few positives that were detected at 2-weeks post-inoculation, but none tested positive at 4-weeks post-inoculation, nor were positive cultures obtained. These results may have been due to transient infections, detection of dead *X. fastidiosa* cells by ELISA, or false ELISA positives due to cross reactions between plant chemicals and the ELISA kit. For example, 'evening star' sunflower plants tested 20/20 positives using ELISA, but culture tests were clean and negative.

X. fastidiosa was successfully isolated from 22 species, but some appear to be better hosts for *X. fastidiosa* than others (Table 1). For the agricultural crops, alfalfa, basil, lima bean, and tomato showed infections of 70%, 50%, 2.6%, and 21%, respectively. *X. fastidiosa* hosts among the weed species included annual bluegrass (5%), cheeseweed (80%), wild sunflower (35%), goosefoot (15%), London rocket (65%), Spanish broom (85%), and tree tobacco (10%). For the

cover crop plants, *X. fastidiosa* was recovered from annual ryegrass (30%), black mustard (65%), 'Blando' brome (65%), 'Hykon Rose' clover (50%), 'New Zealand White' clover (10%), cowpea (46%), fava bean (35%), field pea (27%), meadow barley (20%), California red oat (10%), and white sweet clover (80%). While results suggest a number of these plants are good hosts for *X. fastidiosa*, since needle-inoculation is a severe and unnatural form of infection, they may or may not be natural hosts for *X. fastidiosa* under natural conditions.

We were only able to obtain 1 positive isolate out of 20 needle-inoculated plants for annual bluegrass, possibly due to high contamination of our cultures. Multiple tests including culture controls indicate that this was due to other microbes within the plant and could not be avoided. We had similar contamination problems with cultures of lima bean, annual bluegrass, cowpea, and fava bean. Positive cultures for these species may have been obscured by the other microbes.

Lima bean, tree tobacco, and goosefoot appear to be poor hosts for *X. fastidiosa*. An additional test performed for lima bean resulted in cleaner, negative cultures. Tree tobacco and goosefoot cultures were clean, with few isolates obtained. Despite obtaining relatively few isolates for New Zealand white clover and California red oats, they appear to be potentially good sources for *X. fastidiosa*, as both survived well after infection, and the cultures from each were highly populated.

Table 1: ELISA and culture results for plant species needle-inoculated with *X. fastidiosa*.

Type	Common Name	Scientific Name	ELISA +	Culture +	*X.f.* host?
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' variety)	*Helianthus annuus*	20/20*	0/20	No
	Common Sunflower, wild-type	*Helianthus annuus*	19/20	7/20	Yes
	Goosefoot	*Chenopodium album*	7/40	5/33***	Yes
	Horseweed	*Conyza canadensis*	2/20**	0/20	No
	London Rocket	*Sisymbrium irio*	5/20	13/20	Yes
	Spanish Broom	*Spartium junceum*	17/20	17/20	Yes
	Tree Tobacco	*Nicotiana species*	12/20**	2/20	Yes

* False positives
** Most or all positives in 2-week ELISA test; possible transient infection or dead cells detected.
*** Very slow-growing *X. fastidiosa*, detected well after 4-weeks.
**** Contains many other microorganisms that contaminate and probably obscure positive culture results.

Table 1: ELISA and culture results for plant species needle-inoculated with *X. fastidiosa* (cont.)

Type	Common Name	Scientific Name	ELISA +	Culture +	*X. f.* host?
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
	Brome, Blando	*Bromus hordeaceus*	16/20	13/20	Yes
	Birdsfoot Trefoil	*Lotus species*	10/20	0/20	No
	Clover, Hykon Rose	*Trifolium hirtum*	16/20	10/20	Yes
	Clover, New Zealand White	*Trifolium repens*	15/20	2/20	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
	Sweet clover, White	*Medicago species*	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 *X. fastidiosa*, detected well after 4-weeks.
**** Contains many other microorganisms that contaminate and obscure positive culture results.

Insect Transmission

Glassy-winged Sharpshooter (GWSS)
Transmission was conducted only on plant species from which we recovered *X. fastidiosa* in the PD3 cultures. Thus, there was no testing on bell pepper, cotton, black nightshade, common groundsel, 'Evening Star' sunflower, horseweed, annual fescue, birdsfoot trefoil, or sudangrass (Table 1). Transmission tests were not performed with lima bean, because the one plant from which we recovered *X. fastidiosa* died quickly after the test. The repeated needle-inoculation of lima bean produced no *X. fastidiosa* isolates. We attempted to perform transmission trials on goosefoot, but all the STSS died less than 24-hrs into the AAP, and all but one GWSS died during the same time period. We transferred the 1 surviving GWSS to a grapevine, but we could not detect any *X. fastidiosa* in that grapevine 6 months post-IAP. Thus even though goosefoot can serve as a weak host for *X. fastidiosa* it is a poor host for both GWSS and STSS.

GWSS successfully transmitted *X. fastidiosa* from the alternate host species back into the same alternate host species for the following: alfalfa, basil, tomato, brome, cowpea, sweet clover, and cheeseweed and it successfully transmitted from the alternate host species into grapevines for alfalfa, basil, brome, field pea, California red oat, sweet clover, annual bluegrass, cheeseweed, common sunflower, London rocket, and tree tobacco (Table 2). Combined, these results implicate 5 plants (alfalfa, basil, brome, sweet clover and cheeseweed) as reservoirs and sources of *X. fastidiosa* in vineyards where these plants and GWSS occurs.

Interestingly, GWSS successfully transmitted *X. fastidiosa* from the following alternate hosts into grapevine, but did not transmit from these hosts to the same host: field pea, California red oat, annual bluegrass (results may be obscured by contamination as discussed before), common sunflower, London rocket, and tree tobacco (Table 2). GWSS's failure to horizontally transmit *X. fastidiosa* between plants of the same species suggests that these plants may not naturally be infected by GWSS in the field (in our studies, the source plants were needle inoculated). These plants may have natural defenses against infection at the lower titers an insect would transmit as compared to needle-inoculation. There also may be other biological or physical barriers in the insect-plant-pathogen interface that prevents GWSS from infecting these hosts. It also is possible that the titers were so low by GWSS infection, or that infection was not sufficiently widespread throughout the plant for us to detect the bacteria in our tests. However, if these hosts are infected by GWSS or another vector or inoculation method, then GWSS could acquire *X. fastidiosa* from these hosts and transmit it to grapevines (Table 2).

There were 2 species, tomato and cowpea, in which GWSS transmitted *X. fastidiosa* between plants of the same alternate host species, but they did not transmit from those alternate hosts into grapevine. Again, this may be due to unknown biological or physical barriers in the insect-plant-pathogen interface. Another possibility is that the titers were so low in these alternate host species that transmission could not be detected. It would be interesting to investigate further why GWSS cannot transmit *X. fastidiosa* from these hosts into grapevine.

For 5 of the plants species tested, there was no transmission between the plants of the same species nor was there transmission from the alternate host to grapes (Table 2). These plants included annual ryegrass, New Zealand White clover, Hykon Rose clover, fava bean, goosefoot, and Spanish broom.

Smoketree sharpshooter (STSS)

STSS transmitted *X. fastidiosa* between the alternate host plants and from these same alternate hosts into grapevine for 5 species: alfalfa, basil, Brome, Hykon Rose clover, and Spanish broom. These plant species could serve as *X. fastidiosa* reservoirs and sources from which STSS could acquire the bacteria and transmit it into grapes. It would be prudent not to have them adjacent to or in vineyards where STSS is present. In situations where these plants already occur, careful management should be applied to controlling vector populations and removing infected grapevines as soon as possible.

For many of the plant species tested, STSS acquired from the alternate hosts and transmitted into grapevines, but did not transmit from one alternate host to another. Plants in this group were: annual ryegrass (results may be due to culture contamination in the plates), field pea, California

Red oats, sweet clover, annual bluegrass, cheeseweed, common sunflower, London rocket, and tree tobacco. Although it appears STSS cannot transmit into these alternate hosts, if they are infected by another vector, then STSS can acquire and transmit *X. fastidiosa* from them into grapevines.

STSS did not successfully transmit *X. fastidiosa* between plants of the same alternate hosts or from these hosts into grapevines for tomato, black mustard, New Zealand white clover, cowpea, meadow barley or goosefoot. These plants appear to be poor natural hosts for *X .fastidiosa* or there may be biological or physical barriers in the insect-plant-pathogen interface that prevent transmission from or to these plants. Again, the titers may be so low as to be undetected or unavailable for acquisition.

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

Crop Plants

Insect	Transmission Test	ELISA +	Culture +	Transmission Confirmed?
GWSS	Alfalfa-to-Alfalfa	4/5	4/5	Yes
	Alfalfa-to-Grapevine	4/5	4/5	Yes
	Basil-to-Basil	9/9	9/9	Yes
	Basil-to-Grapevine	8/9	8/9	Yes
	Tomato-to-Tomato	3/5	1/5	Yes
	Tomato-to-Grapevine	2/5	0/5	No
STSS	Alfalfa-to-Alfalfa	5/5	3/5	Yes
	Alfalfa-to-Grapevine	4/5	4/5	Yes
	Basil-to-Basil	5/5	3/15	Yes
	Basil-to-Grapevine	1/5	9/15	Yes
	Tomato-to-Tomato	1/5	0/5	No
	Tomato-to-Grapevine	3/5	0/5	No

Table 2: Results for transmission of *Xylella fastidiosa* by GWSS and STSS (cont.)

Cover Crop Plants

Insect	Transmission Test	ELISA +	Culture +	Transmission Confirmed?
GWSS	Annual Rygrass to Rygrass	0/5	0/5	No
	Annual Rygrass to Grapevine	3/5	0/5	No
	Brome-to-Brome	1/4	1/4	Yes
	Brome-to-Grapevine	2/4	1/4	Yes
	Clover (New Zealand White) to Clover	3/5	0/5	No
	Clover (NZW) to grapevine	3/5	0/5	No
	Clover (Hykon Rose) to Clover	0/5	0/5	No
	Clover (HR) to Grapevine	4/5	0/5	No
	Cowpea-to-Cowpea	4/5	2/5	Yes
	Cowpea-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
	Field Pea to Field Pea	2/5	0/5	No
	Field Pea to Grapevine	0/7	5/7	Yes
	Oat, California Red to Oat	0/5	0/5	No
	Oat to Grapevine	3/5	3/5	Yes
	Sweetclover to Sweetclover	2/5	1/5	Yes
	Sweetclover to Grapevine	0/5	5/5	Yes

Table 2: Results for transmission of *Xylella fastidiosa* by GWSS and STSS (cont.)

Cover Crop Plants

Insect	Transmission Test	ELISA +	Culture +	Transmission Confirmed?
STSS	Annual Rygrass to Rygrass	3/5	0/5	No
	Annual Rygrass to Grapevine	2/4	4/4	Yes
	Black Mustard to Mustard	0/4	0/4	No
	Black Mustard to Grapevine	1/4	0/4	No
	Brome-to-Brome	4/4	3/4	Yes
	Brome-to-Grapevine	0/4	1/4	Yes
	Clover (New Zealand White) to Clover	2/5	0/5	No
	Clover (NZW) to grapevine	4/5	0/5	No
	Clover (Hykon Rose) to Clover	3/5	2/5	Yes
	Clover (HR) to Grapevine	4/5	3/5	Yes
	Cowpea-to-Cowpea	5/5	0/5	No
	Cowpea-to-Grapevine	2/5	0/5	No
	Fava Bean-to-Fava Bean	1/5	1/5	Yes
	Fava Bean-to-Grapevine	4/5	0/5	No
	Field Pea to Field Pea	0/5	0/5	No
	Field Pea to Grapevine	3/5	2/5	Yes
	Meadow Barley to Barley	0/5	0/5	No
	Meadow Barley to Grapevine	1/5	0/5	No
	Oat, California Red to Oat	3/5	0/5	No
	Oat to Grapevine	0/5	5/5	Yes
	Sweetclover to Sweetclover	2/5	0/5	No
	Sweetclover to Grapevine	1/5	5/5	Yes

Weed Plants

Insect	Transmission Test	ELISA +	Culture +	Transmission Confirmed?
GWSS	Annual Bluegrass to Bluegrass	3/4	0/4	No
	Bluegrass to Grapevine	0/3	3/3	Yes
	Cheeseweed to Cheeseweed	1/5	1/5	Yes
	Cheeseweed to Grapevine	2/5	1/5	Yes
	Common Sunflower to Sunflower	5/5	0/5	No
	Sunflower to Grapevine	3/5	5/5	Yes
	Goosefoot	0/0	0/0	No
	Goosefoot-to-Grapevine	0/1	0/1	No
	London Rocket to London Rocket	4/4	0/4	No
	London Rocket to Grapevine	0/4	4/4	Yes
	Spanish Broom to Spanish Broom	0/5	0/5	No
	Spanish Broom to Grapevine	2/5	0/5	No
	Tree Tobacco to Tree Tobacco	0/5	0/5	No
	Tree Tobacco to Grapevine	1/5	5/5	Yes
STSS	Annual Bluegrass to Bluegrass	3/4	0/4	No
	Bluegrass to Grapevine	2/4	4/4	Yes
	Cheeseweed to Cheeseweed	1/5	0/5	No
	Cheeseweed to Grapevine	0/5	5/5	Yes
	Common Sunflower to Sunflower	5/5	0/5	No
	Sunflower to Grapevine	1/5	4/5	Yes
	Goosefoot	0/0	0/0	No
	Goosefoot-to-Grapevine	0/0	0/0	No
	London Rocket to London Rocket	1/4	0/4	No
	London Rocket to Grapevine	0/4	3/4	Yes
	Spanish Broom to Spanish Broom	0/5	1/5	Yes
	Spanish Broom to Grapevine	3/5	1/5	Yes
	Tree Tobacco to Tree Tobacco	0/5	0/5	No
	Tree Tobacco to Grapevine	0/5	5/5	Yes

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

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Contribution to Solving the PD Problem in California:

Understanding the *X. fastidiosa* host status of common plant species found in and near vineyards and the ability of common vectors to acquire and transmit *X. fastidiosa* from these alternative plant hosts, is fundamental to predicting and managing *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.

Xylella fastidiosa was successfully isolated from 22 species, but some appear to be better hosts for *X. fastidiosa* than others. Of these 22 species, our studies found that sharpshooters were able to transmit *X. fastidiosa* from 7 alternate hosts to themselves and also to grape plants (Table 3). Growers should be informed of these 7 plants (alfalfa, basil, brome, sweet clover, cheeseweed, hykon rose clover, and Spanish broom), and they should reduce the abundance of these plants in and around their vineyards. Should this not be feasible, then emphasis should be placed on vigilant monitoring of GWSS and STSS and aggressive vector control should be practiced.

Table 3. Plant species to which GWSS and STSS transmitted *X. fastidiosa*

GWSS		STSS	
Transmitted to Self	Transmitted to Grape	Transmitted to Self	Transmitted to Grape
Alfalfa	Alfalfa	Alfalfa	Alfalfa
Basil	Basil	Basil	Basil
Tomato	Brome	Brome	Annual Ryegrass
Brome	Field Pea	Hykon Rose	Brome
Cowpea	Calif. Red Oat	Spanish Broom	Hykon Rose
Sweet Clover	Sweet Clover	Fava Bean	Field Pea
Cheeseweed	Annual Bluegrass		California Red Oats
	Cheeseweed		Sweet Clover
	Common Sunflower		Annual Bluegrass
	London Rocket		Cheeseweed
	Tree Tobacco		Common Sunflower
			London Rocket
			Spanish Broom
			Tree Tobacco