Evaluation of Olive as a Host of *Xylella fastidiosa* and Associated Sharpshooter Vectors

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Abstract

Krugner, R., Sisterson, M. S., Chen, J., Stenger, D. C., and Johnson, M. W. 2014. Evaluation of olive as a host of *Xylella fastidiosa* and associated sharpshooter vectors. Plant Dis. 98:1186-1193.

Olive (*Olea europaea*) trees exhibiting leaf scorch or branch dieback symptoms in California were surveyed for the xylem-limited, fastidious bacterium *Xylella fastidiosa*. Only approximately 17% of diseased trees tested positive for *X. fastidiosa* by polymerase chain reaction, and disease symptoms could not be attributed to *X. fastidiosa* infection of olive in greenhouse pathogenicity assays. Six strains of *X. fastidiosa* were isolated from olive in Southern California. Molecular assays identified strains recovered from olive as belonging to *X. fastidiosa* subsp. *multiplex*. Pathogenicity testing of olive strains on grapevine and almond confirmed that *X. fastidiosa* strains isolated from olive yield disease phenotypes on almond and grapevine typical of those expected for subsp. *multiplex.* Mechanical inoculation of *X. fastidiosa* olive strains to olive resulted in infection at low efficiency but infections remained asymptomatic and tended to be self-limiting. Vector transmission assays demonstrated that glassy-winged sharpshooter (*Homalodisca vitripennis*) could transmit strains of both subspp. *multiplex* and *fastidiosa* to olive at low efficiency. Insect trapping data indicated that two vectors of *X. fastidiosa*, glassy-winged sharpshooter and green sharpshooter (*Draeculacephala minerva*), were active in olive orchards. Collectively, the data indicate that *X. fastidiosa* did not cause olive leaf scorch or branch dieback but olive may contribute to the epidemiology of *X. fastidiosa*-elicited diseases in California. Olive may serve as an alternative, albeit suboptimal, host of *X. fastidiosa*. Olive also may be a refuge where sharpshooter vectors evade intensive areawide insecticide treatment of citrus, the primary control method used in California to limit glassy-winged sharpshooter populations and, indirectly, epidemics of Pierce's disease of grapevine.

Diseases caused by the xylem-limited, fastidious bacterium *Xylella fastidiosa* have been a problem in California for more than 100 years, with grapevine (20,32), almond (28,41,42), and alfalfa (43,50) being the most affected crops. *X. fastidiosa* is widely distributed in the Americas, causing vascular occlusion disease in some host species while other host species remain asymptomatic (22,26,38). Individual strains of *X. fastidiosa* differ in host range and may be classified into subspecies by multilocus sequence typing (MLST) (40,53). Strains of *X. fastidiosa* subsp. *fastidiosa* cause Pierce's disease of grapevine (14) and are capable of causing disease in other hosts, including almond (6). *X. fastidiosa* subsp. *multiplex* strains do not cause disease in grapevine but are commonly isolated from almond expressing leaf scorch disease (6,7,15,28), and subsp. *multiplex* strains also cause disease in numerous perennial crop and landscape plants, including peach (13,51), plum (37), purple-leafed plum, and sweetgum (18). In South America, citrus variegated chlorosis and coffee leaf scorch are caused by strains of subsp. *pauca* (1,5,17). Oleander leaf scorch (8,36) is caused by strains belonging to a distinct clade referred to as subsp. *sandyi*.

X. fastidiosa is transmitted by xylem-sap-feeding leafhoppers (28,33). Historically, the key native vectors in California were the blue-green sharpshooter (*Graphocephala atropunctata* Signoret) in Napa Valley (35) and green sharpshooter (*Draeculacephala minerva* Ball) in the San Joaquin Valley (12,34,43). In the late 1980s, the glassy-winged sharpshooter (*Homalodisca vitripennis* (Germar)) was introduced to California (4,45,46) and is now well established in Southern California and the southern San Joaquin Valley. Glassy-winged sharpshooter, a known vector of *X. fastidiosa* (2), is polyphagous (47) and highly mobile (3,10,25). Establishment of glassy-winged sharpshooter in citrus was a primary factor in recent epidemics of Pierce's disease in Southern California (31) and the southern San Joaquin Valley (29,30,44).

California is the sole producer of olive (*Olea europaea* L.) in the United States, with approximately 17,800 ha planted and a production value estimated at $130 million per annum (49). Although most commercial production is located in the San Joaquin and Sacramento Valleys, olive trees are commonly planted throughout California as ornamental trees in urban and rural areas. In recent years, leaf scorch and branch dieback symptoms in olive became a concern to growers, homeowners, and landscape managers in California. Such symptoms are typical of those caused by *X. fastidiosa,* prompting us to investigate the role of *X. fastidiosa* in olive leaf scorch or branch dieback disease. Recently, multiple fungal species have been isolated from symptomatic trees in California, with pathogenicity of some fungal isolates demonstrated by experimental inoculation of olive (48). However, those experiments did not address pathogenicity of *X. fastidiosa* to olive. Furthermore, limited information is available concerning the role of olive as a source of *X. fastidiosa* (9,19) and sharpshooter vectors (11). Here,

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Accepted for publication 20 March 2014.

http://dx.doi.org/10.1094/PDIS-01-14-0014-RE
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we describe studies designed to evaluate (i) prevalence of *X. fastidiosa* infection of olive in California, (ii) molecular typing of *X. fastidiosa* strains isolated from olive, (iii) pathogenicity of *X. fastidiosa* strains from olive, (iv) transmission of *X. fastidiosa* to olive by glassy-winged sharpshooter, and (v) activity of known insect vectors of *X. fastidiosa* in olive orchards.

Materials and Methods

Sampling olive for *X. fastidiosa*. Sampling sites in urban areas and commercial orchards were determined by visual inspection to identify trees displaying leaf scorch or branch dieback symptoms. Symptoms were documented by photography. From October 2008 to September 2012, tissue samples were collected from 198 symptomatic olive trees found in Southern California (i.e., ornamental trees in San Diego, Orange, Riverside, Los Angeles, and Ventura Counties), the San Joaquin Valley (i.e., trees in commercial orchards in Kern, Tulare, and Fresno Counties), and the Sacramento Valley (i.e., ornamental trees in Yolo County). Samples were transported to the laboratory and screened for presence of *X. fastidiosa* by polymerase chain reaction (PCR) using primers RST31 and RST33, as described (27), and by culturing on PW medium (13). Axenic cultures recovered from olive and validated as *X. fastidiosa* by molecular methods (see below) were added to the United States Department of Agriculture–Agricultural Research Service San Joaquin Valley Agricultural Sciences Center *X. fastidiosa* collection stored at –80°C.

Molecular typing of *X. fastidiosa* strains. For molecular identification, genomic DNA was extracted (6) from 7- to 14-day-old cultures. Preliminary identification of subspecies affiliation was accomplished using the four-primer PCR assay (6), in which amplicon profile of subsp. *fastidiosa* may be distinguished from that of subsp. *multiplex*. Known strains of subsp. *fastidiosa* (Temecula) and *multiplex* (Dixon) were used as standards. Further characterization was accomplished using MLST (40). Genomic DNA of select olive-infecting strains isolated in this study (LM10, RH1, and Fillmore) and the RC75 strain isolated by others (kindly provided by A. Purcell) was used as a template for PCR amplification of *pilU* and seven housekeeping genes (*cysG*, *gltT*, *holC*, *malF*, *leuA*, *nuoL*, and *petC*). Amplified products were cloned into pGEMT-easy. A consensus sequence was determined for each amplicon based on sequences of three independent clones. For MLST, consensus sequences of all eight amplicons were concatenated into a single sequence (7,480 nucleotides) for each strain. Concatenated sequences of *X. fastidiosa* strains isolated from olive were aligned with concatenated sequences of select reference strains available in GenBank. The resulting multiple alignment was used as input data to generate a neighbor-joining tree based on 1,000 bootstrap replications. Nodes with bootstrap support of less than 50% were collapsed to polytomies. Concatenated sequences for *X. fastidiosa* subsp. *pauca* strain 9a5c was used as an outgroup to root the tree.

Mechanical inoculation of *X. fastidiosa* to olive. To determine whether strains of *X. fastidiosa* isolated from olive persist and cause disease in olive, six strains of *X. fastidiosa* isolated from olive were grown in pure culture and mechanically inoculated (21) to 1-year-old (approximately 30 cm in height) greenhouse-reared olive plants. Inocula were prepared from 7- to 10-day-old cultures as a turbid cell suspension of approximately 10^8 cells/ml in water. A small drop of inoculum (7 µl) (or water, for negative controls) was placed at three different locations of the main stem (bottom, middle, and top); the stem was pierced with a needle through each drop. In total, 30 plants (*O. europaea* L.) of each olive cultivar ('Mission', 'Manzanillo', 'Sevillano', 'Arbequina', 'Arbosana', 'Koroneiki', and 'Barouni') were inoculated with *X. fastidiosa* strain RH1 a total of four times between March and September 2009. In October 2009, a second group of 30 plants (Manzanillo) was inoculated with *X. fastidiosa* strain Fillmore. A third group of plants (Arbequina) was inoculated with strains RH1 (2 plants), LM10 (14 plants), and Fillmore (3 plants) in June 2011. A fourth group of plants (Arbequina) was inoculated with RH1 (5 plants), LM10, (10 plants), Fillmore (5 plants), and Oceanside (5 plants) in October 2012. Each group of inoculated plants included mock-inoculated plants as controls. All test plants were kept in an insect-free greenhouse and monitored over a period of 1 year for symptom development and presence of *X. fastidiosa* by PCR (using primers RST31 and RST33) and culturing.

Mechanical inoculation of *X. fastidiosa* to grapevine and almond. Pathogenicity tests in almond and grapevine were conducted to complement MLST typing. Strains identified as belonging to subsp. *fastidiosa* were expected to cause disease in grapevine and almond, whereas strains identified as subsp. *multiplex* were expected to cause disease in almond but not grapevine. *X. fastidiosa* strains isolated from olive in this study (RH1, LM10, Fillmore, and Oceanside) were mechanically inoculated to grapevine (*Vitis vinifera* L. 'Chardonnay'), almond (*Prunus dulcis* (Mill.) D.A. Webb 'Sonora'), and olive using the same inocula described above (i.e., fourth group of olive plants inoculated in October 2012). As positive controls, strains Temecula and Stag's Leap (subsp. *fastidiosa*) were inoculated to grapevine and strains M12 and Dixon (subsp. *multiplex*) were inoculated to almond. Strain M23 (subsp. *fastidiosa*) was inoculated to both grapevine and almond. Inocula were prepared and delivered to test plants as described above, except that test plants were inoculated only on a single date. As a negative control, groups of 2 to 10 plants per host species were mock inoculated with water. All test plants were kept in an insect-free screenhouse and monitored for 1 year for symptom development and presence of *X. fastidiosa* by PCR and culturing.

Glassy-winged sharpshooter transmission assays. Transmission assays were conducted to confirm that glassy-winged sharpshooter is a vector of *X. fastidiosa* strains isolated from olive. Laboratory colonies of glassy-winged sharpshooter were established and maintained as described by Krugner (23). Plants infected by mechanical inoculation (described above) were used as acquisition source plants. Source plants for reference strains Temecula and Stag's Leap (subsp. *fastidiosa*) were infected grapevines (Chardonnay). Source plants for reference strains Dixon and M12 (subsp. *multiplex*) were infected almond plants (Sonora). Almond was used as a source plant for strain M23 (subsp. *fastidiosa*). Source plants used for *X. fastidiosa* strains isolated from olive in this study were almond (strains RH1 and Fillmore) or Arbequina olive (strain LM10). Source plants for *X. fastidiosa* olive strain Oceanside were not available, because no mechanically inoculated plants became infected. All source plants were verified as infected by PCR and culturing. Colony-reared adult glassy-winged sharpshooters were given a 96-h acquisition access period (AAP) on source plants. At the end of the AAP, insects were transferred in groups of 10 to test plants (Arbequina olive grown in 3.8-liter pots) for a 96-h inoculation access period (IAP). Following the IAP, insects were removed; test plants were treated with a foliar application of insecticidal soap (Safer Brand Insect Killing Soap; Woodstream Corp.) and the systemic insecticide imidacloprid (Admire Pro; Bayer CropSciences) as a soil drench. To verify that colony insects were not contaminated with *X. fastidiosa,* groups of 10 adults not given an AAP on source plants were caged on test plants for a 96-h IAP. To verify that test plants were not contaminated with *X. fastidiosa* from the source nursery, test plants not exposed to glassy-winged sharpshooters also were maintained in the same insect-free greenhouse as inoculated test plants. Inoculated and control test plants were maintained at 23 to 27°C and 22 to 25% relative humidity under natural light during summer and supplemented with artificial light during early spring, late fall, and winter. Plants were assayed for presence of *X. fastidiosa* at 12 and 24 weeks post IAP using PCR and culturing methods described above. On each sampling date, one leaf with petiole intact was collected from the bottom, middle, and top of each plant and subjected to analysis.

Insect vector activity in olive orchards. To document activity of insect vectors of *X. fastidiosa* in olive orchards, yellow card sticky traps (14 by 22.9 cm; Seabright Laboratories) were moni-

Fig. 1. A, Branch dieback and **B,** leaf scorch symptoms observed in olive trees sampled for *Xylella fastidiosa* in California. Note that *X. fastidiosa* was cultured from both samples shown but correlation of *X. fastidiosa* with symptoms in olive was low.

tored in two olive orchards. Both orchards were located in Fresno County, CA. Site A was planted in 2003 and consisted of Arbequina, Arbosana, and Koroneiki. Site B was planted in 2009 and consisted of Arbequina and Arbosana. Site A was located within a known glassy-winged sharpshooter-infested zone. Site B was located outside the glassy-winged sharpshooter-infested zone, near permanent pastures known to harbor populations of green sharpshooters (43). Traps were placed at canopy height and replaced biweekly. At time of collection, the number of glassy-winged sharpshooters and green sharpshooters on each trap were recorded. Trapping at site A was initiated in March 2010 and ended in September 2013. Trapping at site B was initiated in March 2011 and ended August 2013. Traps at site A were placed along five transects, with a total of 26 traps placed in the orchard. At site B, 14 traps were placed around the perimeter of the orchard. Traps were removed from both sites in late October through November of each year during harvest. No insecticides were applied to either orchard during the study period. Herbicides and fungicides were applied in accordance with standard grower practices.

Results

Prevalence of *X. fastidiosa* in olive in California. Samples were collected from olive trees displaying branch dieback (Fig. 1A) or leaf scorch (Fig. 1B) symptoms. Symptoms observed on ornamental trees in urban areas in southern California were similar to symptoms observed on trees in commercial orchards in the San Joaquin Valley and Yolo County. In total, samples were collected from 198 olive trees, with *X. fastidiosa* detected by PCR in 16.6% of samples (Table 1). Prevalence of *X. fastidiosa* was greater in Southern California than in the San Joaquin Valley or Yolo County. Specifically, 38.5% (30 of 78) of symptomatic trees sampled in Southern California tested positive for *X. fastidiosa* by PCR, whereas only 2.5% (3 of 121) of symptomatic trees sampled in the San Joaquin Valley and Yolo County tested positive for *X. fastidiosa* by PCR (Table 1).

In total, six strains of *X. fastidiosa* were isolated from olive trees at several locations in Southern California: La Mirada (strains LM10 and LM14), Rolling Hills (strains RH1 and RH2), Fillmore (strain Fillmore), and Oceanside (strain Oceanside) (Table 1). The

Table 1. Survey of olive for *Xylella fastidiosa* in California

Collection date	County	Location	Samples positive by PCR[a]	Number of *X. fastidiosa* strains isolated
May 2009	Yolo	Davis	0/4	0
June 2010	Fresno	Fresno	0/10	0
July 2011		Fresno	0/20	0
July 2011		Fresno	0/6	0
July 2012		Clovis	0/26	0
August 2008	Tulare	Porterville	0/3	0
August 2008		Terra Bella	0/2	0
August 2009		Ducor (site 1)	0/10	0
August 2009		Ducor (site 2)	3/10	0
June 2011		Woodlake	0/3	0
June 2011		Lemon Cove	0/4	0
June 2011		Lindsay	0/16	0
August 2008	Kern	Bakersfield	0/7	0
August 2009	Ventura	Piru	2/5	0
August 2009		Fillmore	1/7	1
August 2009		Ventura	3/3	0
October 2008	Los Angeles	Rolling Hills	0/5	1
April 2009		Rolling Hills	3/3	1
March 2009		Rancho Bernardo	3/3	0
May 2011		La Mirada	6/17	2
May 2009	Orange	Newport Beach	2/8	0
May 2009		Costa Mesa	1/7	0
May 2009	Riverside	Riverside	0/9	0
August 2009		Riverside	5/6	0
May 2009	San Diego	Carlsbad	3/3	0
October 2012		Oceanside	1/1	1

[a] Numerator denotes number of plants positive by polymerase chain reaction (PCR) and denominator denotes number of plants tested.

Fillmore strain was isolated from an olive tree present in a single row located between two mature citrus orchards; all other olive-infecting strains were isolated from ornamental trees located in urban settings. Each culture was verified as *X. fastidiosa* based on PCR (with primers RST31 and RST33) using genomic DNA extracted from cultures. Attempts to isolate strains of *X. fastidiosa* from olive trees in the San Joaquin Valley or Yolo County were unsuccessful.

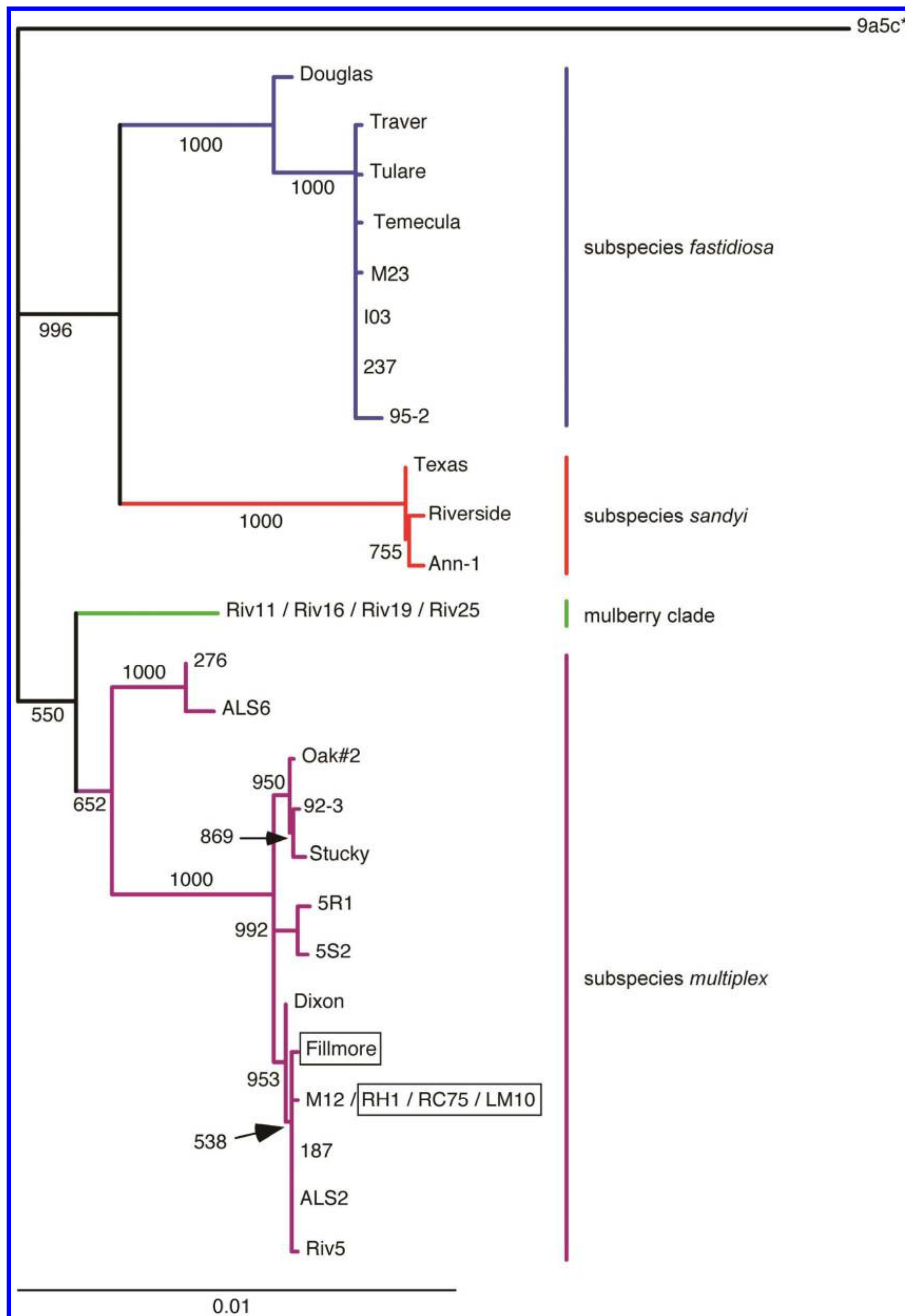

Fig. 2. *Xylella fastidiosa* strains isolated from olive in California belong to subspecies *multiplex*. Presented is a neighbor-joining tree showing relationships of *X. fastidiosa* strains from olive (RH1, LM10, Fillmore, and RC75; designated with boxes) with characterized reference strains of *X. fastidiosa*. Input data were a multiple alignment of concatenated sequences (7,480 nucleotides) for seven housekeeping genes and *pilU*. Multiple taxa appearing on a single branch were 100% identical in sequence. Colored lines denote individual clades, some of which have been assigned as subspecies. Bootstrap values (based on 1,000 replications) are shown adjacent to nodes; *X. fastidiosa* subsp. *pauca* strain 9a5c is designated with an asterisk and was used as an outgroup to root the tree. Scale bar at bottom left indicates a genetic distance of 0.01.

***X. fastidiosa* strains from olive belong to subsp. *multiplex*.** Preliminary typing using the four-primer assay (6) indicated that the six *X. fastidiosa* strains isolated from olive in this study were indistinguishable from reference strains of subsp. *multiplex* and distinct from reference strains of subsp. *fastidiosa* (*data not shown*). MLST was performed on a subset of strains isolated from olive (LM10, RH1, and Fillmore) in this study and strain RC75 (provided by A. Purcell), also isolated from olive in California. Sequences determined in this study were deposited in GenBank as accessions KF954183 to KF954214. Alignment of concatenated sequences for the eight loci examined revealed that LM10, RH1, and RC75 were 100% identical to each other and to subsp. *multiplex* reference strain M12; the Fillmore strain differed from M12 at only 2 base positions (99.97% identity). In the neighbor-joining tree (Fig. 2) based on an alignment of concatenated sequences, the four olive-infecting strains clustered with known strains of subsp. *multiplex* in a clade distinct from subsp. *fastidiosa*, subsp. *sandyi*, and a clade containing strains from mulberry.

***X. fastidiosa* strains from olive yield disease phenotypes on almond and grapevine typical of those expected for subsp. *multiplex*.** Inoculation of almond with *X. fastidiosa* strains isolated from olive or reference strains of subsp. *multiplex* resulted in a high correlation among symptoms (leaf scorch), detection by PCR, and isolation of the bacterium by culturing (Table 2). In contrast to reference strains of subsp. *fastidiosa*, inoculation of grapevine with *X. fastidiosa* strains from olive did not produce symptoms. Furthermore, all grapevines inoculated with *X. fastidiosa* olive strains were negative by PCR and did not yield live cultures. Collectively, pathogenicity tests using grapevine and almond as test hosts supported placement of *X. fastidiosa* strains isolated from olive in subsp. *multiplex.*

Table 2. Mechanical inoculation of *Xylella fastidiosa* strains to three test species[a]

Strain (subspecies), assay	Grapevine	Almond	Olive
Temecula (*fastidiosa*)			
Symptoms	1/5	NT	NT
PCR	1/5	NT	NT
Culture	1/5	NT	NT
Stag's Leap (*fastidiosa*)			
Symptoms	9/20	NT	NT
PCR	7/20	NT	NT
Culture	7/20	NT	NT
M23 (*fastidiosa*)			
Symptoms	12/20	3/10	NT
PCR	8/20	3/10	NT
Culture	9/20	3/10	NT
Dixon (*multiplex*)			
Symptoms	NT	8/10	NT
PCR	NT	8/10	NT
Culture	NT	8/10	NT
M12 (*multiplex*)			
Symptoms	NT	12/14	NT
PCR	NT	9/14	NT
Culture	NT	9/14	NT
LM10 (*multiplex*)			
Symptoms	0/5	1/9	3/24[b]
PCR	0/5	1/9	2/24[c]
Culture	0/5	1/9	0/24
RH1 (*multiplex*)			
Symptoms	0/5	7/14	15/217[b]
PCR	0/5	6/14	6/217[c]
Culture	0/5	6/14	0/217
Fillmore (*multiplex*)			
Symptoms	0/8	3/8	0/38
PCR	0/8	1/8	0/38
Culture	0/8	1/8	0/5
Oceanside (*multiplex*)			
Symptoms	0/1	0/4	0/5
PCR	0/1	0/4	0/5
Culture	0/1	0/4	0/5

[a] Numerator denotes number of plants with symptoms or positive for *X. fastidiosa* by polymerase chain reaction (PCR) or culturing; denominator denotes number of plants tested; NT = not tested.
[b] Expressing leaf scorch or branch dieback symptoms but negative for *X. fastidiosa* by PCR.
[c] Positive for *X. fastidiosa* by PCR but asymptomatic.

***X. fastidiosa* infection of olive does not correlate with disease symptoms.** Field surveys resulted in detection of *X. fastidiosa* from approximately 17% of symptomatic trees tested. In greenhouse studies (Table 2), only 8 of 284 olive trees inoculated with *X. fastidiosa* strains RH1, Fillmore, LM10, or Oceanside tested positive by PCR for *X. fastidiosa.* However, no olive test plants positive for *X. fastidiosa* by PCR developed symptoms. Leaf scorch symptoms were observed on 22 olive test plants: 18 test trees inoculated with *X. fastidiosa* (but PCR-negative) and 4 test trees mock inoculated with water. Even after multiple attempts, *X. fastidiosa* was not detected by PCR or cultured from any olive test plant displaying symptoms. Collectively, the results indicate that leaf scorch or branch dieback symptoms of olive were not well correlated with *X. fastidiosa* infection in the field and were not reproducible by inoculation of *X. fastidiosa* to olive under controlled conditions in the laboratory.

***X. fastidiosa* infection of olive may be transient rather than chronic.** Among test plants inoculated with *X. fastidiosa* olive strain RH1, two, one, one, and two test plants of Arbequina, Arbosana, Mission, and Barouni, respectively, tested positive for presence of *X. fastidiosa* via PCR conducted at 12 weeks post inoculation. However, no test plants inoculated with strain RH1 tested positive for the bacterium at 24 weeks and 1 year after inoculation. In addition, none of the plants inoculated with the Fillmore strain tested positive for the bacterium via PCR at 12 weeks, 24 weeks, and 1 year after inoculation. Two test plants (Arbequina) inoculated with strain LM10 tested positive by PCR for *X. fastidiosa* at 12 weeks, 24 weeks, and 1 year after inoculation. Despite repeated detection by PCR of the LM10 strain over a 1-year period, both infected test plants did not express branch dieback or leaf scorch symptoms. No other olive test plants were positive for *X. fastidiosa* via PCR. All attempts to reisolate *X. fastidiosa* from inoculated olive test plants failed. Collectively, these results suggest that *X. fastidiosa* infection of olive may be self-limiting, such that chronic infection may be uncommon.

Glassy-winged sharpshooter transmits *X. fastidiosa* to olive at low efficiency. On average, 9.6 (range 6 to 10) insects per plant were alive at the end of the 96-h IAP on olive test plants. At 24 weeks after the IAP, branch dieback symptoms were observed in nine test plants, including one control plant that was not exposed to insects. However, all symptomatic test plants were negative for presence of *X. fastidiosa* via PCR. At 24 weeks after the IAP, *X. fastidiosa* was detected in a total of 6 of 145 test plants (Table 3). Among *X. fastidiosa*-positive test plants, one had been exposed to insects that acquired strain RH1, three plants had been exposed to insects that acquired the subsp. *multiplex* reference strain Dixon, and two plants had been exposed to insects that acquired the subsp. *fastidiosa* reference strain M23. No other asymptomatic plant tested positive for presence of *X. fastidiosa* via PCR and all attempts to reisolate the bacterium from glassy-winged sharpshooter-inoculated olive test plants failed.

Table 3. Glassy-winged sharpshooter transmission assays of *Xylella fastidiosa* strains to olive test plants

Strain (subspecies)	Acquisition source	Test plant PCR[a]
Temecula (*fastidiosa*)	Grapevine	0/11
Stag's Leap (*fastidiosa*)	Grapevine	0/25
M23 (*fastidiosa*)	Almond	2/26
Dixon (*multiplex*)	Almond	3/26
M12 (*multiplex*)	Almond	0/27
LM10 (*multiplex*)	Olive	0/4
RH1 (*multiplex*)	Almond	1/26

[a] Numerator denotes number of plants positive for *X. fastidiosa* by polymerase chain reaction (PCR) and denominator denotes number of plants tested.

Sharpshooter vectors are active in olive orchards. Yellow sticky trap counts revealed presence and activity of glassy-winged sharpshooter and green sharpshooter in two olive orchards located in Fresno County. Although glassy-winged sharpshooters were common at site A (Fig. 3A), only three green sharpshooters were caught (2 June 2010, 13 December 2010, and 20 June 2011) during the 4-year sampling period. Therefore, only data for glassy-winged sharpshooters are presented in Figure 3A. At site A, glassy-winged sharpshooters were common between June and August, and peaked with an average (± standard error of the mean) of 0.08 ± 0.02 (n = 29), 0.10 ± 0.03 (n = 55), 0.03 ± 0.01 (n = 10), and 0.03 ± 0.01 (n = 11) adults per trap per day in 2010, 2011, 2012, and 2013, respectively. Site B was not located inside a known glassy-winged sharpshooter-infested zone and, as expected, no glassy-winged sharpshooters were observed. Site B was located adjacent to a permanent pasture known to harbor green sharpshooters. The number of green sharpshooters caught at site B was low, with no clear seasonal patterns (Fig. 3B), a result similar to that reported for green sharpshooter movement into almond orchards and vineyards (12). In total, 17, 12, and 1 green sharpshooter were caught on sticky traps in 2011, 2012, and 2013, respectively. Green sharpshooters prefer grasses over woody perennials (34). Accordingly, movement of green sharpshooters into olive is likely transient and may have been due to proximity of a permanent pasture serving as a green sharpshooter source habitat.

Discussion

The role of *X. fastidiosa* in etiology of olive leaf scorch and branch dieback in California. Correlation of *X. fastidiosa* infection with leaf scorch or branch dieback symptoms of olive was poor in both the field survey and greenhouse pathogenicity or vector transmission assays. These observations indicated that *X. fastidiosa* strains reported here did not cause leaf scorch or branch dieback disease in olive maintained under the described conditions. Furthermore, in greenhouse assays, there was no overlap in olive test plants expressing symptoms with olive test plants in which presence of the pathogen was detected by PCR (Table 2). Several explanations for the presence of symptoms in test plants (including mock-inoculated plants) lacking detectable *X. fastidiosa* are plausible: some nursery-grown test plants may have been infected with a pathogen capable of causing disease in olive (48), or symptoms observed in some test plants were due to abiotic stress (drought) during the extended post inoculation incubation period. Such confounding issues are not unknown in studies of pathogens (such as *X. fastidiosa*) of perennial hosts that have a long incubation period and cause symptoms by interruption of xylem sap flow.

Under greenhouse conditions, establishment and multiplication of *X. fastidiosa* strains in olive by mechanical inoculation methods or insect transmission occurred at low frequency. By comparison, inoculation of grape or almond with reference strains and select olive-infecting strains resulted in a much higher correlation of symptom expression, pathogen detection by PCR, and recovery by culturing. These observations indicate that the strains of *X. fastidiosa* isolated from olive behaved as expected for subsp. *multiplex* genotypes. Therefore, limited infection rates, lack of persistence of the pathogen over time, and absence of disease symptoms in olive may be considered the result of pathogen–host interactions specific to olive. Because some olive plants were inoculated with the same inocula used to infect almond, low infectivity and lack of disease symptoms in olive cannot be explained as a general loss of pathogenicity in culture. Thus, olive may be considered a host in which *X. fastidiosa* acts similar to an endosymbiont, as has been shown for common riparian plants (35) and *Arabidopsis thaliana* (38), in which infected plants remain asymptomatic and bacterial populations are limited. However, additional data on the fate of *X. fastidiosa* in olive trees under field conditions over a period of years are needed to address this hypothesis.

Three species of fungi were identified as causal agents of olive twig and branch dieback in California (48). However, nothing is known about the causal agent of leaf scorching symptoms. Because *X. fastidiosa* was found in trees showing twig and branch dieback, leaf scorching, or both symptoms, further studies are needed to evaluate whether co-infection by fungal pathogens and *X. fastidiosa* alters symptom expression.

The role of olive in epidemiology of diseases caused by *X. fastidiosa*. Although *X. fastidiosa* did not cause olive leaf scorch or branch dieback disease, olive may, under certain circumstances, serve as a reservoir for *X. fastidiosa*. In Southern California, *X. fastidiosa* infection of olive was common (Table 1), such that olive may contribute to incidence of *X. fastidiosa* in ornamental and landscape perennials susceptible to infection by strains of subsp. *multiplex*. Higher incidence of *X. fastidiosa* in olive in Southern California may be attributed to high population levels of the glassy-winged sharpshooter, which has a restricted range in the San Joaquin Valley. In the San Joaquin Valley, where most almond production occurs, infection of olive by *X. fastidiosa* was uncommon (Table 1). This observation suggests a limited contribution of olive as a source of *X. fastidiosa* inocula for the expansive almond industry in the San Joaquin Valley, even though strains of *X. fastidiosa* isolated from olive can experimentally cause almond leaf scorch disease (Table 2).

The glassy-winged sharpshooter can reproduce (R. Krugner, *unpublished*) and overwinter (11) on olive. Although population densities of glassy-winged sharpshooter can be considerably variable among host plant species (52), population dynamics of glassy-winged sharpshooter observed in olive was similar to those reported in other hosts and locations in California. In general, there

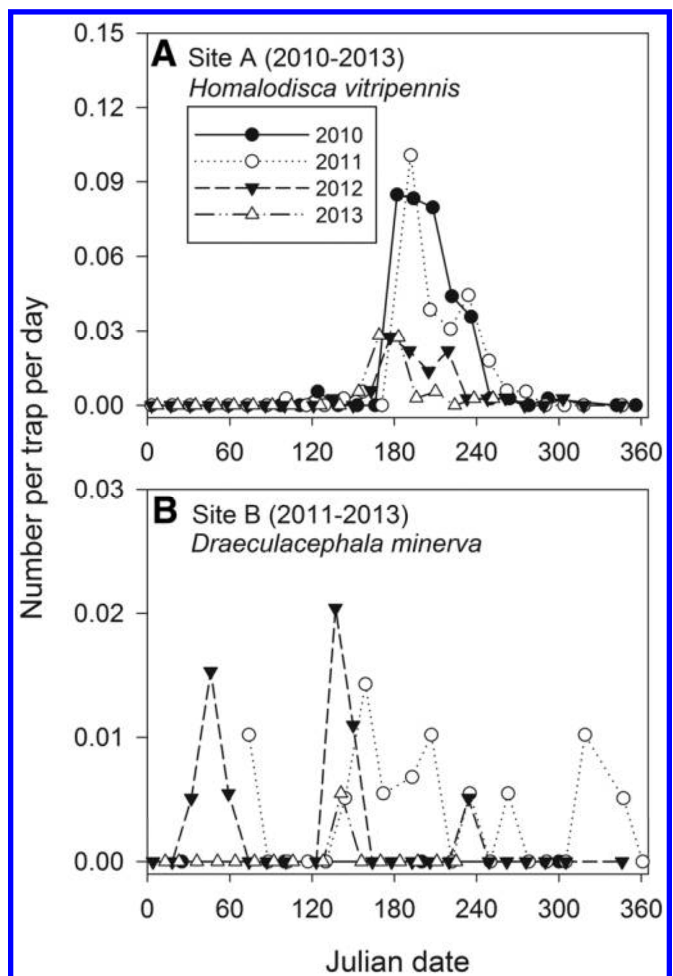

Fig. 3. Sharpshooter vector activity in olive orchards. **A,** Number of glassy-winged sharpshooters captured per trap per day from 2010 to 2013 at site A. Three green sharpshooters also were captured at site A during the trapping period (2 June 2010, 13 December 2010, and 20 June 2011). **B,** Number of green sharpshooters captured per trap per day from 2011 to 2013 at site B. No glassy-winged sharpshooters were caught at site B.

is an increase in adult sharpshooter levels from late June to a peak in mid- to late July consistent with the appearance of first-generation adult emergence (24). Furthermore, trapping data from Fresno County (Fig. 3A) clearly indicate that glassy-winged sharpshooters are active in olive plantings. Thus, olive may contribute indirectly to the epidemiology of Pierce's disease by providing a refuge for glassy-winged sharpshooters. In contrast, green sharpshooter movement into olive was transient (Fig. 3B), suggesting that olive is unlikely to serve as a source of green sharpshooter.

California represents a mosaic of urban areas and regions of intensive, diverse agriculture in which *X. fastidiosa,* numerous hosts, and associated sharpshooter vectors are widely distributed, complicating management of vector populations and sources of inocula. Under such conditions, potential benefits of vector or pathogen control in olive are difficult to predict with respect to reduced incidence of disease and increase in crop yield or quality. Elsewhere, the situation may be different. In 2013, *X. fastidiosa* infection of olive in Italy was reported (39) but details on pathogen genotype, prevalence, and means of spread are limited. Because the European Union (16) considers *X. fastidiosa* an exotic, high risk pathogen, there is intense interest in olive as a host of *X. fastidiosa* and the role of olive in epidemiology of diseases caused by *X. fastidiosa*.

Acknowledgments

This research was supported by a California Olive Committee grant awarded to R. Krugner and by the United States Department of Agriculture–Agricultural Research Service appropriated project 5302-22000-010-00D. We thank T. de la Torre, M. Venegaz, and A. J. Salyers for assisting in plant and insect maintenance; G. Phillips for maintaining bacterial cultures and assisting isolation; P. Dwyer for collection of field trapping data; and Duarte nursery for donating grapevines.

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