Phenology of *Xylella fastidiosa* and Its Vector Around California Almond Nurseries: An Assessment of Plant Vulnerability to Almond Leaf Scorch Disease

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Abstract

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Management of almond leaf scorch disease requires knowledge of all possible infection pathways. The disease is caused by the xylem-limited bacterium *Xylella fastidiosa*, which is transmitted by several species of sharpshooters. The objectives of this research were to elucidate the fate of bacteria in planta after inoculations in almond nursery plants and to determine patterns of insect vector population dynamics and temporal distribution of *X. fastidiosa*–infected plants relative to host plant assemblages in habitats surrounding commercial nurseries. In an experimental nursery, disease incidence was markedly affected by rootstock type. Prior to bud grafting, 'Nemaguard' rootstock seedlings were not susceptible to bacterial infection. After bud grafting with a susceptible scion ('Sonora'), scions were susceptible to infection regardless of rootstock genotype. Surveys near commercial nurseries revealed that only habitats with permanent grass cover sustained vector populations throughout the season. A total of 87 plant samples tested positive for *X. fastidiosa* (6.3%) using enzyme-linked immunosorbent assay (ELISA), with a higher number of *X. fastidiosa*–infected plants found in weedy alfalfa fields than in other habitat types. Among plant species infected by *X. fastidiosa*, 33% were winter annuals, 45% were biennials or perennials, and 22% were summer annuals. Collectively, these findings identified a potential pathway for *X. fastidiosa* infection of almonds in nursery situations.

Almond leaf scorch disease (ALSD) is caused by the xylem-limited bacterium *Xylella fastidiosa* Wells et al. (13,35), which also attacks other economically important *Prunus* crops, such as peach (10,52) and plum (41). ALSD is found throughout almond production areas of California, with incidence in affected orchards ranging from 0.08 to 17% depending on region and cultivar (5,9). ALSD strains of *X. fastidiosa* are transmitted by several species of sharpshooters and spittlebugs (35,36), but *Draeculacephala minerva* Ball (Hemiptera: Cicadellidae) is perhaps the only species that plays a role in pathogen spread to almond in California, as the distribution of *D. minerva* overlaps with almond production regions.

Effective management of a disease requires knowledge of all infection pathways. Proximity of susceptible crops to insect vector habitats is known to affect incidence of Pierce's disease in vineyards (19), which is also caused by *X. fastidiosa* (11). In almond orchards, in contrast, the random distribution of symptomatic trees and the absence of distinct disease gradients associated with adjacent vector habitat (20,36) demonstrate that the relationships among proximity to vector habitat, the distribution of vectors in the orchard, and disease incidence are not as clear. Nonetheless, it is known that *D. minerva* moves between almond orchards and adjacent pastures and alfalfa fields (30), and the pathogen is present in *D. minerva* and in vegetation both in and around almond orchards (9). Clearly, this species is an important vector of *X. fastidiosa* strains causing ALSD, and it may be responsible for some level of primary pathogen spread into orchards. Alternatively, another route of primary pathogen spread could be from infected nursery stock at the time of orchard establishment. Infection may occur in nurseries, either by the use of infected budwood or transmission of the pathogen by insect vectors from surrounding vegetation into the nursery. Work by Hutchins et al. (28), Mircetich et al. (35), and Boyhan et al. (3) showed that *X. fastidiosa* can be transmitted by grafting in peach, almond, and plum, respectively. To our knowledge, primary spread of *X. fastidiosa* through the planting of infected almond nursery stock has not been considered.

Field production of almond nursery stock from seed to plant commercialization in California takes approximately 13 months. Nursery trees are cultivated in high-density, open-field plots (12,000 plants/ha) and thus are potentially susceptible to insect vector colonization and pathogen infection. Rootstock seeds and clonal cuttings are planted in late fall; seedling emergence occurs from February to March. Scions are propagated on actively growing rootstocks through bud-grafting during May and June, and then the grafted trees are pulled from the field soil the following winter. Because of the small tree size associated with the proximity to vector habitats, immigrating insect vectors carrying *X. fastidiosa* may visit a greater number of trees in the nursery than in a mature orchard.

In this study, we asked whether peaks in insect vector activity and abundance and *X. fastidiosa* incidence in vegetation found near almond nurseries are synchronized with periods of almond nursery plant susceptibility to infection. The primary objectives of this research were (i) to elucidate the fate of *X. fastidiosa* inoculations to young almond trees at the nursery stage to identify periods of plant resistance and susceptibility, (ii) to determine persistence of *X. fastidiosa* infection in nursery stock, and (iii) to evaluate the potential of infected nursery stock to develop ALSD after they were moved for orchard establishment. To evaluate the risk of infection of nursery stock from outside sources, a fourth objective in this study was to quantify vector populations and pathogen infection in host plant assemblages in habitats surrounding commercial nursery growing grounds. We tested the hypotheses that (i) establishment and multiplication of *X. fastidiosa* in nursery stock is seasonally affected by rootstock type; and (ii) natural vegetation in

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and around nursery plots include hosts for both *X. fastidiosa* and insect vectors. Combined, the experiments of this study provide further insight into the epidemiology and management of ALSD.

Materials and Methods

Establishment of almond nursery stock. An experimental almond nursery was established at the USDA-ARS research center in Parlier, CA, to evaluate the temporal susceptibility of nursery stock to pathogen infection during the production phase. In late November 2007 and 2008, approximately 270 seeds each of peach 'Nemaguard' (ALSD-resistant; 32) and almond 'Nonpareil' (ALSD-susceptible) were planted 15 cm apart in rows within large field cages (3 m width × 1.8 m height × 11 m length), and used as rootstocks for almond scion 'Sonora' (ALSD-susceptible). 'Nemaguard' is the most common *Prunus* rootstock used in California for both stone fruits and almonds (4), and 'Sonora' is a widely planted scion. 'Nonpareil' is not used commercially as a rootstock, but was used in this study to represent a *X. fastidiosa*–susceptible almond rootstock. 'Sonora' budsticks were harvested from a young orchard from which ALSD was absent. Emerged rootstock seedlings (planted in fall 2007 and 2008) were grown until mid-June 2008 and 2009, respectively. In mid-June, rootstock seedlings were budgrafted with 'Sonora' scions at approximately 15 cm above ground level. Two weeks after bud grafting, rootstocks were pruned to just above the bud union. Any remaining rootstock buds located below the bud union were removed to avoid growth of side branches. Scion branches were not pruned during the study.

Inoculation of almond nursery plants. Establishment and persistence of *X. fastidiosa* in experimental nursery trees were evaluated by mechanical inoculations or insect-transmission assays. Mechanical inoculations were conducted as described by Fritschi et al. (17) using the Dixon strain of *X. fastidiosa* subsp. *multiplex*. Inoculum was prepared from 7- to 10-day-old cultures as a turbid cell suspension of approximately 10^8 cells/ml (32). A small drop of inoculum (7 µl) (or water, for the negative controls) was placed on three different locations of the plant main stem (bottom, middle, and top), and the stem was pierced with a needle underneath each drop. Almond plants used as an inoculum source for insect vectors (source plants) were also inoculated in this way, using the same Dixon strain. Presence of *X. fastidiosa* in symptomatic source plants was confirmed by recovery of the pathogen (12) and polymerase chain reaction (PCR; 15) prior to transmission tests. For insect transmission assays, field-collected adult *D. minerva* were caged on source plants using a mesh screen bag and allowed a 96-h acquisition access period (AAP) on the infected almonds. At the end of the AAP, insects were transferred to test plants in groups of 10 into a two-piece (front and back), rectangular plastic cage (10 cm length × 12 cm width × 16 cm height) with mesh screen on the front and back walls. Front and back pieces of plastic cage were attached to the top 15 cm of the plant using rubber bands. Cotton was used to seal the gap between the cage and plant stem. The plant and cage assemblage was staked to provide structural support and prevent damage to the plant. Insects were allowed a 72-h inoculation access period (IAP) on test plants.

Concomitantly with insect transmission assays, a second set of test plants were mechanically inoculated with the same strain of *X. fastidiosa* (Dixon) used in the insect transmission assays, and a third set of plants were mechanically inoculated with water only. Inoculations to three sets of healthy test plants were made at six different times of the season: March, April, and May (i.e., inoculations to rootstocks during growth phase); and July, September, and October (i.e., inoculations to established scions). Each of the mechanical inoculation treatments (pathogen and water) was replicated 15 times per inoculation date, whereas insect inoculations were replicated 5 to 15 times depending on insect availability. The experiment was repeated twice (2008 and 2009 seasons). Trees were kept in field cages until January of the following year, and then were transplanted into an orchard at a spacing of 2.5 × 4.6 m between trees and rows, respectively. The fate of bacteria in planta after inoculations was evaluated for 2 years, based on symptom development, culturing, and PCR. In brief, DNA was purified using the Q1 Aquick Gel Extraction Kit according to the manufacturer's instructions (Qiagen, Inc., Valencia, CA), and PCR was conducted as described by Francis et al. (15). Presence of ALSD in plants was determined by the combination of symptom expression (i.e., marginal scorching on leaves) and identity confirmation of *X. fastidiosa* via PCR on bacteria cultured from plants. Symptom development was evaluated for 5 months in 30-day intervals starting in late June of each year. Culturing of *X. fastidiosa* from plants was conducted as described above.

Monitoring of insect vector population dynamics. The population dynamics of *X. fastidiosa* vectors in vegetation located in and around commercial almond nurseries was monitored from October 2007 to October 2008. During this period, sharpshooter activity was monitored using yellow sticky traps (15 cm width × 30 cm height) systematically placed around the perimeter of five almond nursery blocks: two in Merced County and three in Stanislaus County, CA. These particular blocks were selected as experimental sites based on the presence of sharpshooters within naturally occurring vegetation surrounding these fields, as identified through a preliminary study of a total of seven sites, which were surveyed from summer to winter in 2007. Seven common vegetation types (habitats) located adjacent to nursery blocks (10 to 15 m) were selected for sampling: irrigated pasture, drainage ditch, alfalfa field, weedy alfalfa field, noncultivated perimeter, orchard floor, and cover crop. Two parallel rows of traps, located on the edge of the nursery blocks and on the edge of the surrounding vegetation, were used to monitor sharpshooter movement into the crop. The distance between the edge of nursery blocks and surrounding vegetation varied from 10 to 15 m, and the distance between traps within the perimeter of the crop and surrounding vegetation was ca. 25 m. To avoid a potential interference in sharpshooter attraction between traps, rows were offset by ca. 12 m. Sticky traps were collected and replaced biweekly. Throughout the trapping period, insect population densities in surrounding vegetation were monitored by collecting sweep net samples every 6 weeks. A sample consisted of 50 standard sweeps in a 20-m linear transect. A total of 10 samples were collected from each nursery at every sampling date. Samples were placed individually in plastic bags and transported to the laboratory for analysis.

Incidence of *X. fastidiosa* and plant species composition in vector habitats. Sites for sampling were selected near the almond nursery growing grounds identified above. At each site, the relative cover and abundance of plant species were measured in 10 systematically placed transects in the habitats described above. Linear transects were placed parallel to the nursery blocks at 11 to 16 m from the edge of the crop. Plant species composition within linear transects (10 m long × 0.3 m wide) was measured every six weeks from February to November 2008 by recording species richness (i.e., number of plant species per transect) and species diversity (Simpson's Index of Diversity) (47). Voucher plant specimens were collected and dry-pressed for identification. At each sampling date, leaf samples (three to four leaves per plant) were collected from the most abundant plant species present within each transect and tested for presence of *X. fastidiosa*. The number of samples per plant species was proportional to the abundance of the species in the transect. Samples were collected from the bottom, middle, and top part of the plant, and placed in plastic bags. Five samples were collected from each transect and transported to the laboratory in a cooled container for analysis. Plant samples were tested for presence of *X. fastidiosa* using an enzyme-linked immunosorbent assay (ELISA) kit (Agdia Inc., Elkhart, IN) according to the manufacturer's instructions. At each sampling date, additional leaf samples were collected from plants located in areas of the USDA-ARS research center in Parlier with no history of presence of *X. fastidiosa* or insect vectors. These plant species representatives were used as negative controls for each plant species tested. Briefly, leaf petioles and midvein were macerated in plastic sample bags with 1 ml of extraction buffer and transferred to 96-well plates. Plates were read using a Multiskan MCC/340 microplate reader (Thermo

Fisher Scientific Inc., Pittsburgh, PA). A test was considered positive for *X. fastidiosa* if the average absorbance reading of two replicated wells was greater than two times the average absorbance reading of the negative controls.

Results

Inoculation of almond nursery plants. After inoculations, none of the plants exhibited ALSD symptoms while growing in the experimental nursery, but plants did exhibit symptoms after they were transplanted to the orchard. In both repetitions of the study, ALSD symptoms in inoculated plants in the orchard were first seen from middle to late summer (i.e., first leaf). There was no clear correlation between inoculation date and first appearance of symptoms. The presence or absence of *X. fastidiosa* in symptomatic and asymptomatic plants was confirmed by culturing and PCR assays. None of the control plants showed ALSD symptoms or tested positive for *X. fastidiosa*. Disease incidence was markedly affected by inoculation method (mechanical versus insect vector), rootstock type, and inoculation season (Fig. 1). None of the 114 plants used in insect transmission assays in 2008 became infected, whereas 10 of 52 plants tested in 2009 were infected. Attempts to infect 'Nemaguard' rootstock plants before bud grafting (i.e., March through May) by needle inoculation failed in 2008 (0/39 plants) and 2009 (0/44 plants), whereas *X. fastidiosa* was successfully established in 'Nonpareil' rootstock plants in both years. That is, after bud grafting inoculated 'Nonpareil' rootstock plants with healthy 'Sonora' scions (ALSD-susceptible), *X. fastidiosa* established in the rootstock plants moved to the scion and caused disease. Conversely, unsuccessful establishment of bacteria in the 'Nemaguard' rootstock plants resulted in no disease development on the scion. Inoculations to the scion (July through October) resulted in disease development regardless of the rootstock type (Fig. 1).

Monitoring of insect vector population dynamics. A total of 22 species of Cicadomorphs were collected in sweep net samples. Of these, *D. minerva* was the only known vector of *X. fastidiosa* captured. Other relevant insect vectors of plant pathogens captured included the Cicadellids *Colladonus montanus* (van Duzee), *Acinopterus angulatus* Lawson, and *Euscelidius variegatus* (Kirschbaum), which are known to transmit a phytoplasma associated with Western X-disease of stone fruits in North America (51). The numbers of *D. minerva* adults captured were highest (F = 405.50; df = 1, 33; $P < 0.001$) on the edges of irrigated pastures, followed by drainage ditches and edges of weedy alfalfa fields (Fig. 2A). Insect population densities in weedy alfalfa fields were approximately threefold higher than in relatively weed-free alfalfa fields.

Season-long sampling revealed a significant curvilinear relationship (F = 96.97; df = 1, 48; $P < 0.0001$; R^2 = 0.814) between the numbers of *D. minerva* and the percentage of cover by grass species in sampled habitats (Fig. 3), such that the numbers of insects caught in the samples increased with increasing grass cover. Although some habitats referred to here as noncultivated perimeter, orchard floor, and cover crop had a relatively high percentage of grass cover during winter and spring months, only habitats with permanent grass cover (i.e., irrigated pastures and drainage ditches) were shown to sustain robust *D. minerva* populations throughout the season.

Insect catch data from yellow sticky traps, when pooled across all habitats, showed five peaks of *D. minerva* adult activity throughout the sampling period: 8 November 2007, 15 February, 11 August, 5 September, and 16 October 2008 (Fig. 4B). Traps located on the edge of surrounding habitats consistently captured more *D. minerva* adults than traps located on the edge of nursery stock growing grounds. Despite the reduced insect activity from mid-March to early May, trap catches within nursery stock grounds indicated that *D. minerva* adults were actively moving between the surrounding vegetation and the nursery crop.

Incidence of *X. fastidiosa* and plant species composition in vector habitats. From February to November 2008, 102 plant species were identified and 1,387 samples were collected. A total of 87 samples tested positive for *X. fastidiosa* (6.3%) (subspecies

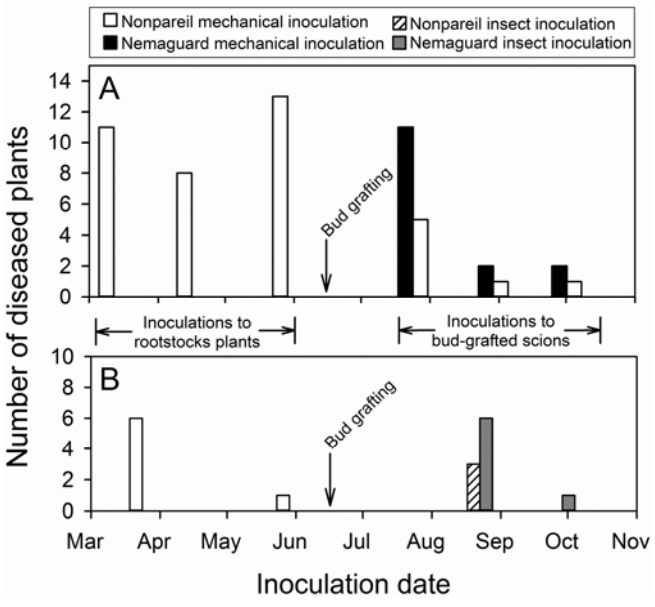

Fig. 1. Total numbers of plants with almond leaf scorch disease following six different inoculation periods during the nursery phases in **A,** 2008, and **B,** 2009. From March through May of both study years, inoculations were made to rootstock plants ('Nemaguard' [ALSD-resistant] and 'Nonpareil' [ALSD-susceptible]) prior to bud-grafting with the scion ('Sonora' [ALSD-susceptible]). From July through October, inoculations were made to established 'Sonora' scions.

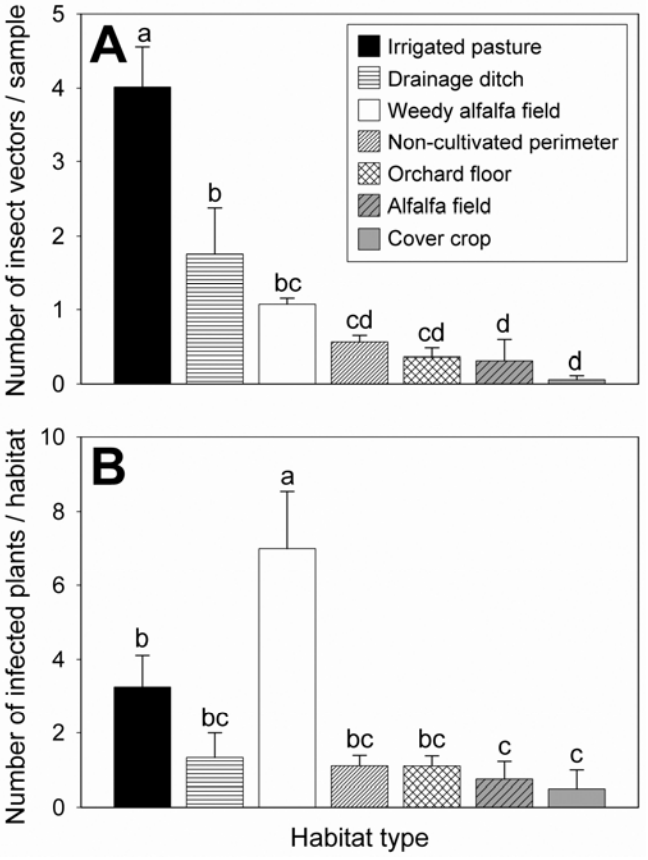

Fig. 2. A, Mean (±SEM) numbers of *Draeculacephala minerva* adults in sweep net samples collected from vegetation in habitats surrounding almond nursery grounds, and **B,** mean number of *Xylella fastidiosa*–infected plants per habitat. Bars representative of habitat type having the same letter above them do not differ significantly ($P < 0.05$) according to a Tukey's HSD test.

not determined) with a higher (F = 5.024; df = 1, 32; P < 0.0001) number of *X. fastidiosa*–infected plants found in weedy alfalfa fields than in the other habitat types (Fig. 2B). In weedy alfalfa fields, a total of five out of 32 alfalfa plants tested positive for *X. fastidiosa*, whereas 16 out of 53 samples of other plant species tested positive. In contrast, samples collected from relatively weed-free alfalfa fields showed that only three out of 136 alfalfa plants tested positive for *X. fastidiosa*, whereas none of the samples (n = 9) from other plant species were positive. In irrigated pastures, a total of 11 of 37 grass samples and 15 out of 182 samples of nongrass plants were positive for *X. fastidiosa*.

Measurements of plant species richness and species diversity showed that alfalfa fields and drainage ditches were the least and the most rich and diverse habitats, respectively. The mean (±SEM) plant species richness in alfalfa fields and drainage ditches was 1.133 ± 0.133 and 5.778 ± 1.806 species per transect per sampling period, respectively. Species diversity (Simpson's Index of Diversity) in alfalfa fields and drainage ditches was 0.004 ± 0.004 and 0.569 ± 0.161 per transect per sampling period, respectively. Values of species richness and diversity for irrigated pastures and weedy alfalfa fields were intermediate among the habitats. On average (±SEM), plant species richness in irrigated pastures and weedy alfalfa fields was 3.62 ± 0.73 and 4.22 ± 1.13 species per linear transect per sampling period, respectively. Species diversity values in irrigated pastures and weedy alfalfa fields were 0.225 ± 0.081 and 0.287 ± 0.082 per linear transect per sampling period, respectively.

Although measurements of plant species richness and diversity within habitats did not markedly vary throughout the sampling period, plant species composition in habitats changed according to plant species' life cycle (e.g., annual versus perennial) and seasonality (e.g., winter versus summer). Among the 40 plant species that tested positive for *X. fastidiosa*, about one-third were winter annuals, one-third were biennials or perennials, and one-third were summer annuals that accounted for about 33.3, 44.8, and 21.8% of all *X. fastidiosa*–positive plants, respectively (Table 1). Although the majority of the *X. fastidiosa*–positive plant species reported here had been reported as hosts in previous surveys, a total of 19 new plant species are reported here as potential hosts of *X. fastidiosa*. These included seven species that a congener had been previously reported as a host and the first report of 12 new plant genus and species (Table 1). Among the six sampling dates, *X. fastidiosa* detection was highest (F = 27.215; df = 5, 1289; P < 0.0001) during the month of February, followed by July. There were no significant differences in proportion of infected plants among the other sampling dates (Fig. 4A).

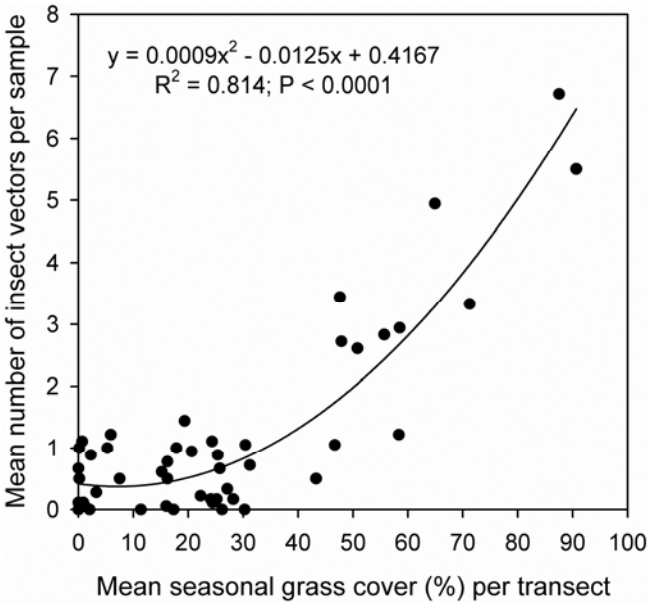

Fig. 3. Relationship between the mean (±SEM) numbers of *Draeculacephala minerva* adults captured in sweep net sampling and the mean seasonal grass cover on habitats surrounding almond nursery grounds.

Discussion

The goal of this study was to investigate the potential role of infected nursery stock in contributing to ALSD occurrence in commercial almond orchards. Surveys conducted in vegetation found near commercial nursery growing grounds revealed that vector population densities and incidence of *X. fastidiosa* are highly dependent on vegetation type. As both vector and pathogen were found in close proximity to almond nurseries, spread of *X. fastidiosa* into nurseries is considered plausible.

X. fastidiosa was detected in cultivated and natural vegetation near five nursery grounds located 20 to 75 km apart. In the past 60+ years, numerous studies have demonstrated the importance of noncrop plants species as potential sources of *X. fastidiosa* (1,8,16,21–24,27,33,34,38–40,46,49,53). Although most *X. fastidiosa* host plant species documented here have been reported as hosts in other surveys, 19 new plant hosts were identified.

Genetically diverse strains of *X. fastidiosa* isolated from multiple host plant species in California have been divided into two subspecies groups (44): *multiplex* and *fastidiosa*; and one genetically distinct clade (Oleander) (45). Both subspecies, *multiplex* and *fastidiosa*, are known to infect almonds (44). ELISA conducted here for detection of *X. fastidiosa* does not provide genotypic identification or determination of pathogenicity to other crops such as grapes and alfalfa. Therefore, a case-by-case, in-depth phylogenetic evaluation of strain identity found in each locale is necessary before implementing measures to remove infected plants. Moreover, only plants that support systemic bacterial movement and high bacterial population, and are feeding hosts for insect vectors, can serve as sources of inoculum (53). For instance, surveys reported here showed that *D. minerva* population densities and frequency of *X. fastidiosa* infection in habitats did not correlate. Despite higher numbers of vectors in irrigated pastures compared to weedy alfalfa fields, fewer plants tested positive for *X. fastidiosa* in pastures than in weedy alfalfa fields. One hypothesis to explain this contrast is that grasses in pastures are excellent

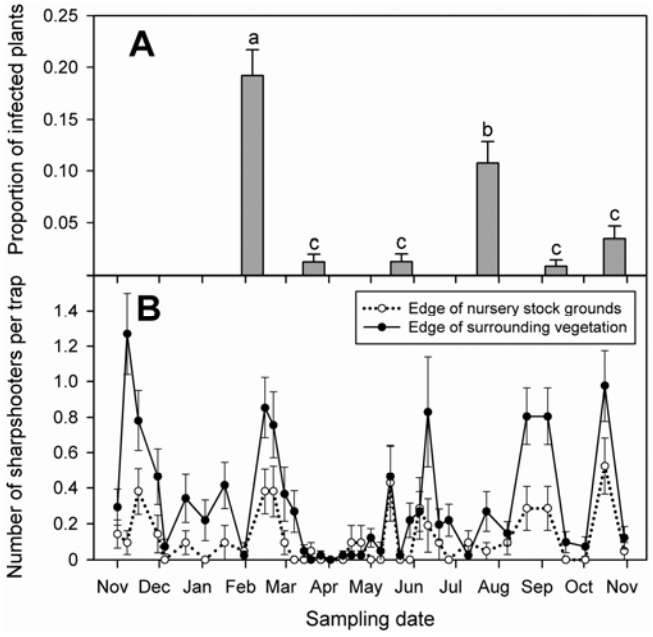

Fig. 4. A, Mean (±SEM) proportion of *Xylella fastidiosa*–infected plants found in habitats surrounding almond nursery grounds in six sampling dates during the season, and **B,** mean (±SEM) numbers of *Draeculacephala minerva* adults captured in yellow sticky traps placed between nursery growing grounds and surrounding vegetation. Bars representative of sampling dates having the same letter above them do not differ significantly (P < 0.05) according to a Tukey's HSD test.

hosts for the vector but not the bacterium, whereas alfalfa plants are excellent hosts for the bacterium, but not the vector. Therefore, plants other than the crop that are present in weedy pastures and weedy alfalfa fields may mediate survival and persistence of bacteria and insect vectors in these habitats, respectively.

D. minerva is well known to be abundant in irrigated pastures, stream banks, and weedy alfalfa fields with perennial grass cover (9,37,49); proximity of such habitats to almond orchards with high incidence of ALSD has been documented (36). However, the role of *D. minerva* in the primary spread of *X. fastidiosa* in almond orchards was not well understood until recently. Surveys conducted in orchards in California's Sacramento and San Joaquin valleys showed a genetic link between strains causing ALSD and those carried by *D. minerva* collected in ground vegetation found in and near orchards, which makes *D. minerva* a strong candidate for primary spread of *X. fastidiosa* from alternative hosts to the crop (9,46). Results from surveys reported here, such as high vector abundance and presence of *X. fastidiosa* in host plants located adjacent to the crop, are in agreement with findings from previous investigations. However, this study is the first to establish the presence of vectors and *X. fastidiosa* specifically with almond nurseries.

Geographic separation from commercial orchards and production of nursery stock and budwood sources in protected structures are key biosecurity measures that have been established in the Brazilian citrus nursery industry to prevent movement of pests and pathogens, including several vector-borne pathogens such as *X. fastidiosa*. In São Paulo State, Brazil, analysis of spatial distribution patterns of citrus variegated chlorosis (CVC), caused by *X. fastidiosa* subsp. *pauca* (7), revealed that the pathogen was distributed in the orchard due to planting of infected nursery stock (42). In the CVC pathosystem, the Cicadellid *Bucephalogonia xanthophis* (Berg) was found to be the most abundant vector in citrus nursery plants (43) and the most efficient vector of *X. fastidiosa* to citrus (29). State and industry self-imposed regulations for mandating the use of insect-proof structures for citrus nursery production significantly reduced the losses caused by CVC (18).

Differences in insect vector behavioral ecology and biology may explain the lower incidence of ALSD in California compared to that of CVC in São Paulo. Habitats preferred by *D. minerva* are limited to areas with permanent grass cover, and this insect species rarely moves into orchards to feed on almond plants. In contrast, young citrus plants appear to be preferred feeding (and perhaps reproductive) hosts for *B. xanthophis* (54). Invasion, establishment, and spread of glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Hemiptera: Cicadellidae), in California changed the epidemiology of xylellae diseases. GWSS is a highly mobile (2) polyphagous sharpshooter with over 100 known hosts (26,50).

Table 1. Total number of infected plants over the number of sampled individuals on each collection date and their temporal distribution in habitats surrounding five nursery stock blocks

		Sampling date					
Plant species	**Plant family**	**2/6/08**	**3/21/08**	**5/23/08**	**7/24/08**	**9/11/08**	**10/23/08**
Bromus diandrus Roth[a]	Poaceae	1/1	np[b]	np	np	np	np
Avena fatua L.	Poaceae	4/4	np	np	np	np	np
Hordeum murinum L. subsp. *murinum*	Poaceae	2/5	0/3	np	np	np	np
Erodium botrys (Cav.) Bertol.[a]	Geraniaceae	1/4	0/4	np	np	np	np
Lolium perenne L.[a]	Poaceae	5/5	–[c]	np	np	np	np
Capsella bursa-pastoris (L.) Medik.[d]	Brassicaceae	1/4	0/3	0/2	np	np	np
Poa annua L.	Poaceae	3/6	–	–	np	np	np
Stellaria media (L.) Vill.	Caryophyllaceae	1/10	0/2	–	np	np	np
Senecio vulgaris L.	Asteraceae	2/5	1/8	0/1	np	np	np
Ranunculus repens L.[d]	Ranunculaceae	1/2	0/2	–	–	np	np
Cyperus eragrostis Lam.	Cyperaceae	1/1	0/1	–	–	np	np
Geranium dissectum L.[d]	Geraniaceae	0/3	0/3	–	1/1	np	np
Medicago polymorpha L.	Fabaceae	2/5	0/2	–	–	–	np
Lactuca serriola L.	Asteraceae	–	–	0/6	2/6	0/3	np
Verbena litoralis Kunth[d]	Verbenaceae	0/3	1/2	0/3	–	0/3	np
Silybum marianum (L.) Gaertn.[d]	Asteraceae	1/6	0/3	0/1	0/1	–	0/1
Erodium moschatum (L.)	Geraniaceae	1/22	0/13	0/1	–	0/1	–
Ludwigia grandiflora (Michx.)[d]	Onagraceae	0/3	1/12	–	–	0/6	–
Marrubium vulgare L.[d]	Lamiaceae	3/6	0/4	0/1	0/2	0/1	0/2
Medicago sativa L.	Fabaceae	7/26	0/23	1/30	0/26	0/31	0/32
Cynodon dactylon (L.) Pers.	Poaceae	–	–	2/9	1/2	1/7	0/4
Sonchus oleraceus L.	Asteraceae	1/2	0/12	0/8	1/5	0/1	0/2
Malva parviflora L.	Malvanaceae	7/29	0/17	0/10	2/6	0/11	0/16
Conyza canadensis (L.) Cronquist	Asteraceae	–	–	0/10	1/9	0/8	0/6
Rumex crispus L.	Polygonaceae	3/14	0/7	0/9	–	0/1	3/5
Coronopus didymus (L.) Smith[d]	Brassicaceae	1/10	–	0/1	0/2	–	1/1
Plantago lanceolata L.[d]	Plantaginaceae	0/2	0/1	0/2	0/3	0/3	1/3
Datura wrightii Regel	Solanaceae	np	0/8	0/25	4/18	0/24	0/34
Prunus dulcis (Mill.) D.A. Webb	Rosaceae	np	0/12	0/14	2/20	0/33	0/35
Vitis spp.	Vitaceae	np	0/2	0/2	1/2	0/2	0/2
Convolvulus arvensis L.	Convolvulaceae	np	np	0/6	2/10	0/3	0/2
Salsola tragus L.[d]	Chenopodiaceae	np	np	0/2	1/2	0/1	0/2
Eriochloa contracta Hitchc.[a]	Poaceae	np	np	np	1/1	–	np
Echinochloa crus-galli (L.) P. Beauv.	Poaceae	np	np	np	1/4	0/2	0/2
Polygonum arenastrum Jord.[a]	Polygonaceae	np	np	np	1/5	0/1	0/3
Polygonum lapathifolium L.[a]	Polygonaceae	np	np	np	1/5	–	0/3
Agrostis gigantea Roth[b]	Poaceae	np	np	np	0/1	–	1/5
Carex L.[d]	Cyperaceae	np	np	np	–	0/1	1/1
Xanthium spinosum L.[a]	Asteraceae	np	np	np	0/1	0/13	1/5
Portulaca oleracea L.	Portulaceae	np	np	np	2/8	np	np

[a] Congener species previously reported as a host.
[b] np = Plant species not present in or near line transects.
[c] – = No plant samples collected.
[d] First report of *Xylella fastidiosa* detection in plant genus and species.

Analysis of the potential geographic range of GWSS indicates that most habitats in California are suitable for their proliferation (25), including northern portions of the San Joaquin Valley (SJV) where most almond nurseries are located. The California Department of Food and Agriculture–Pierce's Disease Control Program is directed at limiting northward spread of GWSS from southern portions of the SJV (6). Therefore, discontinuation of the GWSS eradication program may lead to a range expansion of GWSS into almond nursery production regions in the northern SJV.

Differences in almond rootstock susceptibility to *X. fastidiosa* can be an important factor in nursery plant protection against bacterial infection, at least for the first part of the growing season (plant emergence to bud grafting). Use of 'Nemaguard', a resistant peach rootstock, prevented symptom development and detectable infection until periods prior to bud grafting, whereas use of 'Nonpareil', a factitious susceptible almond rootstock, made plants vulnerable to *X. fastidiosa* infection during that same period. Although the majority of bearing almond trees in California are grown on peach rootstocks (e.g., 'Nemaguard' and 'Lovell'), new rootstocks are becoming available, including several peach × almond (PEAL) hybrids (14) with unknown susceptibility/resistance to *X. fastidiosa*. Recently, Ledbetter and Rogers (32) examined ALSD development in almond, peach, and a prospective PEAL hybrid rootstock and found resistance to *X. fastidiosa* in the PEAL hybrid. Therefore, further assays on commercially available rootstocks are needed to elucidate the fate of *X. fastidiosa* inoculation and disease development.

Proximity of *X. fastidiosa* and insect vectors to commercial almond nurseries in California was demonstrated, providing evidence for *X. fastidiosa* infection of nursery stock. However, as ALSD incidence in California is typically low in almond orchards, primary spread via infected nursery stock also must be low under the current ecological conditions. During the nursery phase, experimental plants did not show symptoms of ALSD, which makes it impossible to use symptom expression for roguing prior to commercialization. Moreover, screening the large numbers of plants cultivated by commercial nurseries (12,000 plants/ha) for presence of *X. fastidiosa* using assays such as culturing, PCR, or ELISA is impractical, laborious, and could result in the addition of unnecessary production costs. Therefore, removal and replacement of diseased plants soon after orchard establishment may be the most cost effective practice for both almond growers and almond nursery stock producers. However, should conditions change (e.g., increase in GWSS populations), higher rates of *X. fastidiosa* infection of nursery stock may warrant attention.

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