Infection of Blueberry Cultivar ‘Emerald’ with a California Pierce’s Disease Strain of *Xylella fastidiosa* and Acquisition by Glassy-Winged Sharpshooter

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Abstract

Bacterial leaf scorch disease caused by *Xylella fastidiosa* occurs in southern highbush blueberry varieties in the southeastern United States. Susceptibility to *X. fastidiosa* varies by blueberry cultivar, and these interactions are often strain-specific. *Xylella fastidiosa* subsp. *fastidiosa* is the causal agent of Pierce’s disease in grapevines, and it has been problematic in the San Joaquin Valley of California since the introduction of the glassy-winged sharpshooter (*Homalodisca vitripennis*). The glassy-winged sharpshooter is known to feed on blueberry, a crop that is expanding in the San Joaquin Valley. Currently, little is known about the potential for the spread of *X. fastidiosa* between grape and blueberry in this region. The ability of a Pierce’s disease strain of *X. fastidiosa* from the San Joaquin Valley to cause

disease in southern highbush blueberry and the potential for the glassy-winged sharpshooter to transmit *X. fastidiosa* between blueberry and grapevine were investigated. Experimental inoculations showed that the *X. fastidiosa* subsp. *fastidiosa* strain Bakersfield-1 can cause disease in blueberry cv. Emerald, and that the glassy-winged sharpshooter can acquire *X. fastidiosa* from artificially inoculated blueberry plants under laboratory conditions. Understanding the possibility for *X. fastidiosa* strains from the San Joaquin Valley to infect multiple crops grown in proximity is important for area-wide pest and disease management.

Keywords: fruit, prokaryotes, small fruits

Bacterial leaf scorch disease caused by *Xylella fastidiosa* commonly occurs in southern highbush and rabbit eye blueberry varieties in the southeastern United States (Chang et al. 2009). This disease is characterized by marginal leaf scorch symptoms that progress to leaf necrosis, defoliation, loss of fruit yield, and death of the plants (Chang et al. 2009). The first strains of *X. fastidiosa* isolated from blueberry in this region were classified as *X. fastidiosa* subsp. *multiplex*, the genetic group of *Xylella* strains known to cause disease in almond, peach, oak, and sycamore (Hopkins and Purcell 2002). Further genetic characterization of blueberry-infecting strains revealed genetic differences from previously described *X. fastidiosa* subsp. *multiplex* strains (Nunney et al. 2014; Van Horn et al. 2017), which were potentially the result of horizontal gene transfer and recombination between *X. fastidiosa* subsp. *fastidiosa* and subsp. *multiplex* (Nunney et al. 2014). Additionally, some strains of *X. fastidiosa* subsp. *fastidiosa*, the causal agent of Pierce’s disease of grapevine, have been reported to cause disease of blueberry under greenhouse conditions (Oliver et al. 2015). Susceptibility to bacterial leaf scorch varies by blueberry cultivar, and these interactions can be strain-specific (Holland et al. 2014; Oliver et al. 2015). Since the first report in Georgia, bacterial leaf scorch of blueberry has been identified in Florida, Kentucky, and Louisiana (Chang et al. 2009; Ferguson and Clark 2017; Harmon and Hopkins 2009; Leonberger and Gauthier 2015).

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Spread of *X. fastidiosa* is reliant on transmission by xylem-feeding insect vectors (Krugner et al. 2019), and the glassy-winged sharpshooter (*Homalodiscus vitripennis*) has been implicated in outbreaks of bacterial leaf scorch in blueberry in the southern United States (Chang et al. 2009; Harmon and Hopkins 2009; Tertuliano et al. 2012). In the San Joaquin Valley of California, Pierce’s disease caused by *X. fastidiosa* subsp. *fastidiosa* has been problematic over the past 20 years since the introduction of the glassy-winged sharpshooter to southern California. Additionally, in the San Joaquin Valley, it is common to find multiple crops grown in close proximity that are confirmed or potential hosts for *X. fastidiosa* and glassy-winged sharpshooter, thus complicating disease management. Area-wide pest control programs have been in place since 2001 to manage glassy-winged sharpshooter populations, particularly in vineyards (Pierce’s disease) and citrus orchards (overwintering host for glassy-winged sharpshooter) (Haviland and Stone-Smith 2016; Perring et al. 2001). During the early 2000s, vector populations in Kern County, California, were successfully controlled by area-wide insecticide treatments; however, resurgent glassy-winged sharpshooter populations in 2012 to 2015 led to increased Pierce’s disease incidence and greater potential for the spread of *X. fastidiosa* in the area (Haviland and Stone-Smith 2016). The blueberry cultivars typically planted in the San Joaquin Valley are southern highbush varieties, and many of these are known to be susceptible to *X. fastidiosa* (Holland et al. 2014; Tertuliano et al. 2012), thereby highlighting the need for more information regarding the potential risk of bacterial leaf scorch disease in California.

The goals of this study were to evaluate a Pierce’s disease-causing *X. fastidiosa* strain for the ability to induce bacterial leaf scorch symptoms in blueberry and the potential of the glassy-winged sharpshooter to act as a vector for bacterial leaf scorch-associated strains of *X. fastidiosa* in the San Joaquin Valley of California. Understanding the likelihood of *X. fastidiosa* transmission from blueberry to grape, and vice versa, is important for evaluating risks associated with expanding blueberry acreage into areas where Pierce’s disease strains of *X. fastidiosa* are prevalent.

Materials and Methods

Plant material and experimental inoculations. Five southern highbush blueberry cultivars (*Vaccinium* sp. Hybrids ‘Emerald’,

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‘Star’, ‘Jewel’, ‘Misty’, and ‘O’Neal’) were screened for susceptibility to a *X. fastidiosa* strain (Bakersfield-1) isolated from grapevines (Burbank and Ortega 2018) exhibiting Pierce’s disease symptoms in Kern County, CA. Two-year-old blueberry plants in 3.8-liter pots were obtained from a commercial nursery and grown in a greenhouse with supplemental light (16 h light/8 h dark). Ten plants per cultivar were artificially inoculated by needle prick on three separate stems with a total of 60 μ l of cell suspension ($\approx 10^8$ CFU/ml in 1 $\times$ phosphate-buffered saline [PBS]), and five additional plants per cultivar were inoculated with buffer alone to serve as negative controls. Plants were evaluated for symptom development over the course of 4 months. At the end of the evaluation period, stem and leaf samples were collected for bacterial isolation and testing by quantitative PCR (qPCR). Cultivars that had detectable levels (PCR and culture positive) of *X. fastidiosa* colonization (‘Emerald’ and ‘O’Neal’) were included in a second round of inoculations with 15 plants per cultivar inoculated in the same manner.

Evaluation of infection status. During the 4-month evaluation period after inoculation, symptom severity was evaluated using the 0 to 7 rating scale developed by Oliver et al. (2015): 0 = asymptomatic and 7 = severe disease (defoliation). The 5-cm stem sections were collected for bacterial isolation from a different branch than the one inoculated. Stem sections were surface-sterilized by successive washes in 70% ethanol and 10% sodium hypochlorite (Fisher Scientific, Waltham, MA), followed by two washes with sterile dH₂O. Then, stems were crushed in 2 ml 1 $\times$ PBS, serially diluted, plated on PD3 medium, and incubated at 28°C. After 10 days of incubation, colonies were confirmed as *X. fastidiosa* by PCR using species-specific primers (Hernandez-Martinez et al. 2006).

Plants were also independently sampled and tested for the presence and quantity of *X. fastidiosa* via qPCR. Leaf midribs and petioles from five leaves from each plant were excised and combined. Samples were lyophilized and ground mechanically for DNA extraction. Each sample was mixed with 1 ml of lysis buffer consisting of 50 mM EDTA, 100 mM Tris-HCl (pH 8), 0.25 mg/ml proteinase K, 3.5% SDS, 10 mg/ml sodium bisulfite, and 2.5% polyvinylpyrrolidone. After 15 min of incubation at 65°C, samples were centrifuged at 14,000 rpm for 5 min; the upper phase was removed and mixed with 200 μ l of 7.5 M ammonium acetate. Samples were incubated on ice for 15 min and centrifuged at 14,000 rpm for 3 min; the supernatant was removed and placed in a new tube. Nucleic acid was then precipitated with an equal volume of isopropanol and washed twice with 70% ethanol. The DNA pellet was resuspended in 50 μ l TE buffer for storage and diluted 1:10 in sterile dH₂O for qPCR analysis. Then, 5 μ l of total DNA was used as the qPCR template. A qPCR was performed using Applied Biosystems Fast SYBR Green Master Mix (Fisher Scientific) and *X. fastidiosa*-specific primers targeting the 16S rRNA sequence (Burbank and Stenger 2016). Quantification was based on a standard curve consisting of DNA extracted from known cell concentrations of *X. fastidiosa* (10^2 to 10^7 CFU/ml) combined with DNA extracted from uninoculated blueberry plants (1:2 ratio of bacterial:plant DNA). A C_t value of ≤ 30 cycles was considered PCR-positive. The quantity of *X. fastidiosa* (CFU) was normalized to the total DNA concentration.

Insect transmission experiments. Glassy-winged sharpshooter eggs were collected from *Photinia* sp. in Kern County, CA, in Apr. 2018. Nymphs were reared in a containment laboratory on a mixture of cowpea, sunflower, and sorghum. On each of two test dates, a subset of insects was taken directly from the colony and screened with qPCR to ensure that reared insects did not carry *X. fastidiosa* (10 on the first test date; 9 on the second test date). Subsequently, adult glassy-winged sharpshooters were caged on three *X. fastidiosa*-positive (confirmed by qPCR and culturing) blueberry plants (cv. Emerald) for a 7-day acquisition access period (AAP; 50 on the first test date; 36 on the second test date). A subset of insects (10 on the first test date; 9 on the second test date) was taken directly from blueberry after the AAP and evaluated with qPCR to confirm acquisition. The remaining insects were transferred to 2-year-old potted grapevines (cv. Chardonnay) pruned to a single cane for a 4-day inoculation access period (IAP). To confirm that the insects fed on grapevine, they

were held in individual feeding sachets attached to grapevine stems that allowed for the collection of excreta (Sisterson 2014), with each grapevine receiving three insects. At the end of the experiment, all glassy-winged sharpshooters were tested individually by qPCR for the presence of *X. fastidiosa*. All transmission experiments were conducted in a growth chamber at 27°C and 14 h light/10 h dark. For qPCR detection of *X. fastidiosa* in insects, DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Redwood City, CA), and qPCR was performed following the protocol of Rapicavoli et al. (2015). After insects were removed, grapevines were treated with systemic insecticide and moved to a greenhouse for disease development observations. Acquisition and transmission experiments were repeated independently. The first test date used 36 insects caged on 12 grapevines, and the second test date used 24 insects caged on eight grapevines, for a total of 60 insects and 20 grapevines (three insects on each plant). Grapevines were held for 6 months and tested with qPCR as described at 12, 16, 20, and 24 weeks after inoculation.

Monte Carlo simulations of transmission experiments. To complete Monte Carlo simulations, a program was written in C++ (Visual C++; Microsoft, Redmond, WA) that simulated the transmission experiment. Each run of the program created 20 plants, with each plant receiving three insects for a total of 60 insects. As each insect was assigned to a plant, a random number between 0 and 1 was drawn; if the random number was less than the observed probability of acquisition (0.38), then the insect was assumed to have acquired *X. fastidiosa*. Subsequently, each insect that acquired *X. fastidiosa* was provided an opportunity to inoculate the plant it was placed on by drawing a second random number between 0 and 1. If the random draw was less than a specified inoculation probability, then the plant became infected. For simulations, the probability of inoculation varied between 0 and 0.20 in increments of 0.0005. For each specified inoculation probability, 1000 simulations were completed and the percentage of simulations that resulted in not observing transmission was recorded.

Isolation and characterization of *Xylella fastidiosa* Bakersfield-1. Strain Bakersfield-1 isolated from grapevine (cv. Flame Seedless) in Kern County, CA, showed symptoms of Pierce’s disease. This strain was previously identified as belonging to subspecies *fastidiosa* based on PCR and multi-locus sequence analysis, but genetic identity was not resolved beyond the subspecies level (Burbank and Ortega 2018). For genomic DNA extraction, *X. fastidiosa* Bakersfield-1 was grown for 5 days on PD3 agar plates at 28°C. Cells were harvested in 1 $\times$ PBS, and 1 ml of cell suspension adjusted to approximately OD₆₀₀ = 0.5 was used for DNA extraction with a DNeasy Blood & Tissue kit (Qiagen) following the manufacturer’s protocol for Gram-negative bacteria. After cell lysis and prior to column purification, DNA samples were treated with 20 μ l of RNase A (20 mg/ml) for 10 min at room temperature. DNA samples were eluted in 100 μ l of sterile dH₂O and concentrated to 30 μ l in a Speedvac concentrator (Eppendorf, Hauppauge, NY). The DNA quantity was evaluated using a Quant-IT dsDNA fluorescent quantification kit (Life Technologies, Carlsbad, CA), with fluorescence measurements performed on an Infinite M100 Pro plate reader (Tecan, San Jose, CA). The DNA quality was evaluated by 1% agarose gel electrophoresis and stained with ethidium bromide. Samples were stored at –20°C.

Bakersfield-1 genome sequence and bioinformatic analysis. A Bakersfield-1 sequencing library was prepared using the 1D Native barcoding genomic DNA kit from Oxford Nanopore Technologies (Oxford, UK) and 1 μ g of input DNA. The library was sequenced with a MinION device (Oxford Nanopore Technologies) and base-calling was performed using Guppy software v3.0.3 (Oxford Nanopore Technologies). The complete genome of Bakersfield-1 was assembled using Canu v1.8 (Koren et al. 2017), polished with Nanopolish v0.11.0 (Loman et al. 2015), and annotated with RASTtk (Brettin et al. 2015) (NCBI Bioproject #PRJNA545724). The full-length nucleotide sequences for *leuA*, *petC*, *malF*, *cysG*, *holC*, *nuoL*, and *gltT* were extracted from genome sequences of Bakersfield-1 and 32 additional *Xylella fastidiosa* strains. Sequences were aligned with PRANK (Löytynoja and Goldman 2010) and concatenated with

FASconCAT-G (Kück and Longo 2014), and a maximum-likelihood phylogeny was generated using Mega X (Kumar et al. 2018). The sequence type was determined using the *Xylella fastidiosa* MLST website (<https://pubmlst.org/xfastidiosa/>) at the University of Oxford (Jolley et al. 2004; Scally et al. 2005). Genome and plasmid alignments were generated by Nucmer v4.0.0 (Kurtz et al. 2004), and synteny plots were visualized with a custom R script (Supplemental Fig. S1).

Insecticide application records and glassy-winged sharpshooter trap counts. To document glassy-winged sharpshooter activity near blueberry fields in California, trapping data from the California Department of Food and Agriculture (CDFA) were requested for the period from 2014 to 2018. Specifically, the CDFA maintains a series of sticky traps throughout California to monitor glassy-winged sharpshooter populations. Trapping data were requested from two blueberry fields located in an area with a history of increased glassy-winged sharpshooter populations and a history of Pierce's disease (Haviland and Stone-Smith 2016). Blueberry site A was 9 ha with two sticky traps maintained on the perimeter, whereas blueberry site B was 64 ha with six sticky traps maintained on the perimeter. Traps were replaced approximately every 2 weeks year-round.

To assess the risk of glassy-winged sharpshooters establishing in blueberry fields, publicly available pesticide use reports for all commercial blueberry acreage in Kern County, CA, were obtained from the Kern County Agriculture and Measurement Standards department (<http://www.kernag.com/ep/permit-use/permit-use.asp>) for the years 2014 to 2018. The frequency of applications of insecticide classes documented to kill glassy-winged sharpshooters (carbamates, neonicotinoids, organophosphates, pyrethroids) (Grafton-Cardwell et al. 2003; Prabhaker et al. 2006) as well as insecticide classes that are likely to kill glassy-winged sharpshooters based on the mode of action (butenolides and ryanoids) were quantified. Insecticides with limited activity against glassy-winged sharpshooters and short residual periods such as pyrethrins (Hix 2002) were excluded. Each insecticide record was associated with a unique site identification number that reports the area treated and total area planted. The insecticide records were used to estimate the percentage of blueberry hectares that received at least one application of a compound known to kill glassy-winged sharpshooters.

Results

***Xylella fastidiosa* Bakersfield-1 infects blueberry cv. Emerald.** Of the five blueberry cultivars tested, only 'Emerald' developed significant levels of infection with *X. fastidiosa*, as indicated by symptom development, detection of *X. fastidiosa* by qPCR, and recovery of live *X. fastidiosa* from 'Emerald' stem tissue (Table 1). All other cultivars tested had only one or two plants that tested positive for *X. fastidiosa* by qPCR; no bacteria were isolated from the point of inoculation, and scorching symptoms were absent. During the second round of inoculations, 'Emerald' plants again developed moderate leaf scorch symptoms (Figs. 1 and 2) with systemic *X. fastidiosa* colonization (Fig. 3). Cultivar 'O'Neal' was included in second-round inoculations as a less susceptible control. Seven 'O'Neal' plants (out of 15) showed minor leaf scorch symptoms (Figs. 1 and 2), and low levels of *X. fastidiosa* colonization (Fig. 3).

Table 1. First-year cultivar screen of blueberry plants 20 weeks after inoculation with *X. fastidiosa* Bakersfield-1

Blueberry cultivar (<i>Vaccinium</i> sp.)	qPCR-positive plants after 20 wk ^a	Culture-positive plants
Star	2/10	0/10
Emerald	5/10	5/10
Jewel	2/10	0/10
Misty	1/10	0/10
O'Neal	2/10	1/10

^a Plant samples with a C_t value <30 cycles were considered positive for *X. fastidiosa*. Negative control plants (mock inoculated) were included in qPCR testing and did not show any amplification.

Glassy-winged sharpshooters can acquire *X. fastidiosa* from blueberry. Testing of a subset of insects immediately after the 7-day AAP on blueberry indicated that 58% (11 out of 19 over two test dates) tested positive for *X. fastidiosa* (Fig. 4A). All insects (19 over two test dates) tested directly from the source colony were negative for *X. fastidiosa* (Fig. 4A). After the AAP on blueberry, a total of 60 insects were confined on grapevines (3 insects per vine) for a 4-day IAP in individual feeding sachets that allowed for measurement of excreta production. Of the 60 insects moved to grapevine, 92% survived to the end of the 4-day IAP. Feeding on grapevine was confirmed, with insects producing an average ($\pm$ SE)

Fig. 1. Disease rating of blueberry cultivars 'Emerald' and 'O'Neal' at 4 months after inoculation with *Xylella fastidiosa* strain Bakersfield-1. Leaf scorch symptoms of blueberry cultivars 'Emerald' (Em) and 'O'Neal' (ON) were evaluated at 4 months after inoculation with *X. fastidiosa* strain Bakersfield-1. Disease ratings were assigned using the 0 to 7 rating scale developed by Oliver et al. (2015), where 0 = asymptomatic and 7 = severe disease (i.e., defoliation). Fifteen plants of each cultivar were needle-inoculated with *X. fastidiosa* strain Bakersfield-1. Cultivar 'Emerald' plants inoculated with *X. fastidiosa* strain Bakersfield-1 showed significant disease development (Tukey means comparison test, $P < 0.001$), whereas the mean disease rating of cultivar 'O'Neal' plants inoculated with strain Bakersfield-1 was not significantly different from that of mock-inoculated control plants, despite showing mild scorch symptoms.

Fig. 2. Leaf scorch symptoms of blueberry inoculated with *Xylella fastidiosa* strain Bakersfield-1. Representative leaf scorch symptoms of blueberry cultivars 'Emerald' (Em) and 'O'Neal' (ON) inoculated with *X. fastidiosa* strain Bakersfield-1 (Xf) compared with mock-inoculated (-) control plants. Photos were taken at 4 months after inoculation. Top and bottom panels show leaves from different plants.

of 5.3 ± 0.6 ml of excreta over the 4-day IAP. At the end of the transmission experiments, 38% (21 out of 55 over both test dates and excluding insects that died during the IAP) of insects tested positive for *X. fastidiosa* by qPCR. However, after being held in the greenhouse for 6 months, none of the 20 grapevines tested positive for *X. fastidiosa*, and no visible Pierce's disease symptoms were observed.

Because transmission of *X. fastidiosa* by the glassy-winged sharpshooter is considered inefficient compared with other vector species (Almeida and Purcell 2003), it is possible that transmission would not be observed if probabilities of acquisition and/or inoculation are low. Based on qPCR data, we concluded that 38% of insects provided with an IAP had acquired *X. fastidiosa*. To estimate a range for the probability of inoculation over the 4-day IAP without observing transmission, a Monte Carlo simulation method was used (Manly 2001).

Based on the simulations, for an experiment using 20 plants and a 0.38 chance of acquiring *X. fastidiosa* during the AAP, the probability of not observing transmission decreased as the probability of inoculation over the 4-day IAP increased (Fig. 4B). With an inoculation probability of 0.13 during the IAP, there was a 5% chance of not observing transmission. Therefore, it is reasonable to estimate that the probability of inoculation resulting in infection over a 4-day IAP is within the range of 0 and 0.13.

Genetic characterization of *X. fastidiosa* subsp. *fastidiosa* strain Bakersfield-1. The genome sequence was assembled into a single ≈ 2.5 -Mb circular chromosome and one ≈ 38 -kb circular plasmid, with ≈ 133 -times average coverage. A multi-locus sequence typing analysis using the scheme described previously by Scally et al. (2005) classified Bakersfield-1 as ST1, and a maximum-likelihood phylogeny generated from concatenated full-length housekeeping genes grouped strain Bakersfield-1 with typical *X. fastidiosa* subsp. *fastidiosa* strains originating from California (e.g., M23 and Temecula-1) (Supplemental Fig. S2). The Bakersfield-1 chromosome was colinear with that of *X. fastidiosa* subsp. *fastidiosa* strains M23 (Fig. 5A) and Temecula-1 (Supplemental Fig. S3). The 38-kb plasmid had high nucleotide sequence identity (99.99%) to and was colinear with pXFAS01 from M23 (Fig. 5B); this plasmid is absent from Temecula-1.

Glassy-winged sharpshooters are infrequently found in blueberries planted near vineyards. Trap catches of glassy-winged sharpshooters on the perimeter of blueberry fields were low in 2014, peaked in 2015, and declined to low levels in 2016 (Fig. 6A). In 2017 and 2018, only a single glassy-winged sharpshooter was caught.

Fig. 3. Quantification of *Xylella fastidiosa* populations in infected blueberry plants by quantitative PCR (q-PCR). Leaf midribs and petioles from five leaves on each plant were excised and combined for DNA extraction. Quantification of bacterial CFU is based on a standard curve consisting of DNA extracted from known cell concentrations of *X. fastidiosa* (10^2 to 10^7 CFU/ml) combined with DNA extracted from uninoculated blueberry plants (1:2 ratio of bacterial:plant DNA). Ct values of ≤ 30 cycles that had a single melt curve peak were included in quantification. *Significant differences based on an analysis of variance (ANOVA) followed by a Tukey means comparison test ($P < 0.001$).

Blueberry site A was managed as three blocks. Two of the three management blocks at blueberry site A were not treated with a compound in an insecticide class known to affect glassy-winged sharpshooters between 2014 and 2018. However, one block at blueberry site A was treated with applications of a neonicotinoid, a pyrethroid, and an organophosphate in 2016. No insecticide treatments affecting glassy-winged sharpshooters were reported at blueberry site B between 2014 and 2018. Across Kern County, blueberry fields were treated with insecticides from several classes likely to affect glassy-winged sharpshooters, including butenolides, carbamates, neonicotinoids, organophosphates, pyrethroids, and ryanoids. Across years, the percentage of blueberry production in Kern County reporting at least one treatment affecting glassy-winged sharpshooters ranged between 32 and 66%, with higher insecticide use in 2014 through 2016 than in 2017 and 2018 (Fig. 6B). Therefore, many blueberry fields in Kern County did not receive treatments that limited glassy-winged sharpshooter establishment during years of high local abundance.

Discussion

Blueberry acreage in California has more than doubled in the past 10 years, producing more than \$34 million of fresh market

Fig. 4. Glassy-winged sharpshooter acquisition of *Xylella fastidiosa* from blueberry. **A**, Percentage ($\pm$ SE) of glassy-winged sharpshooters testing positive for *X. fastidiosa* prior to the acquisition access period (AAP) on blueberry, directly after the AAP on blueberry, and after the inoculation access period (IAP) on grapevine. Date 1 included 10 insects tested before the AAP, 10 insects tested after the AAP, and 35 insects tested after the IAP (insects that died during the IAP were excluded). Date 2 included 9 insects tested before the AAP, 9 insects tested after the AAP, and 20 tested after the IAP (insects that died during the IAP were excluded). **B**, Results of Monte Carlo simulations used to estimate the probability of not observing transmission assuming a probability of acquisition during the AAP of 0.38. Each point represents the proportion of 1000 simulations in which transmission was not observed. The dashed line indicates the 5% value for not observing transmission during an experiment that used 20 plants, with each plant receiving 3 insects.

blueberries in 2017 (NASS 2019). Although bacterial leaf scorch disease caused by *X. fastidiosa* has not been reported in blueberries in California to date, this pathogen and its insect vectors are present in some areas where blueberries are grown (Haviland and Stone-Smith 2016), including the area surrounding the fields studied (Stenger et al. 2019). In the southeastern United States, bacterial leaf scorch of blueberry is generally associated with strains of *X. fastidiosa* classified as subspecies *multiplex*, rather than the strains of

subspecies *fastidiosa*, which commonly cause Pierce's disease (Chang et al. 2009). However, some strains of *X. fastidiosa* subspecies *fastidiosa* are also able to infect blueberry following artificial inoculation (Oliver et al. 2015), and there is increasing evidence to suggest that the *X. fastidiosa* host range can be highly variable among strains within the same subspecies (Nunney et al. 2019). Strain Bakersfield-1, isolated from grapevine in the San Joaquin Valley and characterized as subspecies *fastidiosa*, contains a plasmid with

Fig. 5. *Xylella fastidiosa* Bakersfield-1 is genetically similar to strain M23. Syntenic plots of *X. fastidiosa* subsp. *fastidiosa* strains Bakersfield-1 and M23. Top, Nucmer alignment comparing chromosomes of M23 (x-axis) to Bakersfield-1 (y-axis). Bottom, Nucmer alignment comparing pXFAS01 from M23 (x-axis) to the pXFAS01 homolog present in Bakersfield-1 (y-axis). Nucleotide positions are indicated on both axes in base pairs.

high sequence similarity to a previously characterized plasmid (pXFAS01) from strain M23, which was originally isolated from almond in central California and also infects grapevine (Burbank and Van Horn 2017; Chen et al. 2005; Chen et al. 2010; Rogers and Stenger 2012) (Fig. 5). The presence of this plasmid and high sequence identity suggest that among the well-described *X. fastidiosa* subsp. *fastidiosa* strains isolated in California, Bakersfield-1 appears to be the most genetically similar to M23, a strain that was previously not known to infect blueberry.

Strain Bakersfield-1 was able to infect southern high bush blueberry, specifically cv. Emerald, which is known to be resistant to other strains of *X. fastidiosa* (Brannen et al. 2008; Tertuliano et al. 2012). This highlights the importance of understanding varietal and strain differences in evaluating susceptibility to *X. fastidiosa* and risk to new crops. However, the other four cultivars tested in this study did not appear to be susceptible to *X. fastidiosa* Bakersfield-1, and disease development in 'Emerald' was only moderate compared with bacterial leaf scorch caused by *X. fastidiosa* strains belonging to subspecies *multiplex* (Chang et al. 2009; Holland et al. 2014). In a field setting, the risk of transmission from blueberry to grape and vice versa would be dependent on cultivar susceptibility (of both crops) to the *X. fastidiosa* strains present in the area and vector population levels. The probability of transmission from grape to blueberry was not directly measured in this study, but given the low efficiency of glassy-winged sharpshooter transmission in other plant species (Almeida and Purcell 2003), it is likely to be low in blueberry as well,

making the probability of transmission largely dependent on the vector population and plant susceptibility. Therefore, based on the current information, it is likely that the risk of disease in blueberries from *X. fastidiosa* strains causing Pierce's disease in grapevines in the San Joaquin Valley is relatively low unless more susceptible cultivars are planted or *X. fastidiosa* strains that more aggressively colonize blueberry are introduced.

A related consideration is whether moderately infected blueberries are a potential inoculum source of *X. fastidiosa* relevant to Pierce's disease in grapevine. Although typically considered to be field-resistant to *X. fastidiosa*, blueberry cv. Emerald was shown to be a preferred feeding host for glassy-winged sharpshooters during choice tests with other blueberry cultivars (Tertuliano et al. 2012). Under laboratory conditions, glassy-winged sharpshooter acquired *X. fastidiosa* from artificially inoculated 'Emerald' blueberry plants during 7 days of feeding (Fig. 4A). Although 38% of the glassy-winged sharpshooters used in the transmission experiment tested positive for *X. fastidiosa* after the IAP, no transmission to grapevine was observed. Because transmission efficiency of glassy-winged sharpshooters is known to be low compared with other vectors of *X. fastidiosa*, (Almeida and Purcell 2003; Daugherty and Almeida 2009), it is possible that transmission would have occurred at a measurable rate if more vectors were evaluated. The Monte Carlo simulations indicated that the probability of inoculation during the 4-day IAP is likely to be between 0 and 0.13 (Fig. 4B). Because the probability of inoculation may be low, the lack of transmission in these experiments is not conclusive regarding the ability of glassy-winged sharpshooters to transmit *X. fastidiosa* between blueberry and grapevine.

Bacterial populations in the plant must reach a certain threshold level before acquisition occurs, which would be influenced by host susceptibility (Hill and Purcell 1997). Once infections have progressed to the point suitable for acquisition, rates of transmission would become dependent on the number of vectors present. During years with high glassy-winged sharpshooter populations in Kern County vineyards, the glassy-winged sharpshooters were caught on traps located on the perimeters of blueberry fields (Fig. 6). The presence of glassy-winged sharpshooters near blueberry fields is dependent on glassy-winged sharpshooter abundance in habitats surrounding blueberry, because insects move out from areas of high population density by random dispersal (Krugner et al. 2012). However, when glassy-winged sharpshooter populations were controlled by area-wide treatments primarily applied to citrus, trap counts were low or zero in blueberry, even though these fields were generally not treated with insecticides targeting glassy-winged sharpshooters (Fig. 6B). This suggested that blueberry is not a preferred feeding host of glassy-winged sharpshooters in this area where several other crops are present, including grapevine and citrus. Several other blueberry cultivars tested in this study did not become infected from artificial inoculation of an *X. fastidiosa* strain representative of Pierce's disease strains in Kern County. As long as disease is managed in nearby vineyards through insecticide treatments and rogueing, there is probably a limited risk of spread of *X. fastidiosa* to blueberry or of blueberry acting as a reservoir of *X. fastidiosa* for further spread to grape. However, if new strains of *X. fastidiosa* were introduced, or if more susceptible blueberry cultivars were planted, then the risk of bacterial leaf scorch in blueberry in California would need to be re-evaluated.

Fig. 6. Glassy-winged sharpshooter abundance and insecticides applied to blueberry fields. **A**, Mean ($\pm$ SE) number of glassy-winged sharpshooters caught per year on each trap on the perimeter of blueberry fields A and B that were in an area with a history of increased glassy-winged sharpshooter abundance. **B**, Percentage of blueberry hectares in Kern County reporting at least one application of insecticide classes that are likely to kill glassy-winged sharpshooters.

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