Seasonal Population Dynamics of *Draeculacephala minerva* (Hemiptera: Cicadellidae) and Transmission of *Xylella fastidiosa*

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ABSTRACT The grass sharpshooter, *Draeculacephala minerva* Ball (Hemiptera: Cicadellidae), is a very common and often abundant grass-feeding leafhopper in California. Its population dynamics and ability to transmit *Xylella fastidiosa* were monitored over a 2-yr period in California's San Joaquin Valley. Collections of individuals from natural populations in irrigated pastures and alfalfa, *Medicago savita* L. fields adjacent to *X. fastidiosa*-infected almond (*Prunus* spp.) orchards indicated the occurrence of three discrete generations per year that peaked during the summer. Population densities varied significantly among experimental field survey sites. Insects captured on intercepting mesh traps, yellow sticky cards, and UV-light traps indicated local movement of these insects into and surrounding *X. fastidiosa*-infected, almond orchards. Local movement and seasonal transmission of *X. fastidiosa* from infected almonds to *Catharanthus roseus* (L.) G. Don indicated that this insect may be partly responsible for the slow spread of almond leaf scorch now recently observed in California's San Joaquin Valley.

KEY WORDS population dynamics, transmission efficiency, grass sharpshooter, almond leaf scorch, Pierce's disease (PD) of grapevines

Important diseases caused by the xylem-limited bacterium *Xylella fastidiosa* include Pierce's disease (PD) (in grapevines), almond leaf scorch (ALS), citrus variegated chlorosis, oleander leaf scorch, alfalfa dwarf, phony peach disease, and periwinkle wilt (Turner 1959, Moller et al. 1974, Davis et al. 1978, McCoy et al. 1978, Rossetti 1991, Purcell et al. 1999). In many of these plant hosts, characteristic symptoms include visible leaf scorching due to an accumulation of bacterial colonies and gums resulting in significant vessel occlusion (Stevenson et al. 2004). In almonds, there is reportedly decreased productivity, scaffold dieback, and ultimately death of ALS-affected trees (Mircetich et al. 1976). The discovery of ALS in the SJV has not raised widespread concern among almond growers because the primary vectors seem to be native sharpshooters that do not move far from preferred feeding and reproductive hosts (Purcell 1980, Purcell and Frazier 1985). Thus, the rate of disease spread is believed to be low. However, the arrival of the highly mobile, and rapidly dispersing glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), into California has significantly increased the threat of pathogen spread among a variety of important crops, including almonds (Almeida and Purcell 2003).

Little is known about the epidemiology of almond leaf scorch disease in California. In studies investigating PD, outbreaks were detected in fields near permanent pastures, alfalfa, *Medicago sativa* L., fields or permanent irrigation ditches (Purcell and Frazier 1985). The regular occurrence of *X. fastidiosa*-infectious leafhoppers within these habitats led to the conclusion that two native sharpshooters were responsible for the spread of this disease: the grass sharpshooter, *Draeculacephala minerva* Ball (Hemiptera: Cicadellidae), and *Xyphon fulgida* (Nottingham) (Frazier 1944). However, neither of these two sharpshooter species are frequently found on grape. These insects only occasionally use grapes as feeding hosts. Apparently, a similar situation occurs in almonds (*Prunus* spp.). In California surveys of almond leaf scorch, the grass sharpshooter was the only known insect vector detected in areas with high incidence of ALS (Purcell 1980). Contrary to PD in vineyards, the absence of gradients of almond leaf scorch near the breeding habitats suggests that factors other than the presence of vectors play a significant role in the spread of ALS (Purcell 1980). Such factors might include differential host responses to infection by *X. fastidiosa.* As a self-infertile, open-pollinated crop, almond orchards are composed of two or more cultivars grown in adjacent rows for optimal pollination efficiency. Recent investigations have revealed that certain almond cultivars (e.g., 'Peerless', 'Sonora', and 'Nonpareil') are more suscepti-

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ble to *X. fastidiosa* infection than others with respect to symptom development and bacterial populations (Teviotdale and Connell 2003).

Grass sharpshooters are common in California's coastal valleys as well as in irrigated portions of the Central Valley (Purcell and Frazier 1985). This insect has a wide host range, but it is especially abundant in grasses and sedges in moist habitats, with high populations frequently encountered in Bermuda grass, *Cynodon dactylon* (L.) (Hewitt et al.1942). Although no mass movement (e.g., dispersal or immigration) of this species has been reported previously, it is generally held that grass sharpshooter populations remain localized, and display minimal dispersal, with their greatest activity occurring during early evening (Winkler et al.1949). Moreover, Gibson (1915) observed that only specific environmental conditions supported flight and that strong winds suppressed flight activity.

In the San Joaquin Valley, three discrete generations of the grass sharpshooter per year have been reported (Winkler et al. 1949, Purcell and Frazier 1985). Eggs of the first generation are deposited in late February. The second and third generations occur in early May and mid-July, respectively (Winkler et al. 1949). In southern Arizona, Gibson (1915) observed six generations within field cages that corresponded closely to field observations. Purcell and Frazier (1985) estimated a developmental threshold temperature of 13.5°C for the first generation of eggs by using Gibson's data, but the data were inadequate to estimate other developmental thresholds and rates. The total nymphal period varied from 20 to 51 d depending upon season (Gibson 1915, Hewitt et al. 1942, Winkler et al. 1949).

D. minerva has been documented as a competent vector of *X. fastidiosa* strains that cause PD (Hewitt et al. 1946) and ALS (Mircetich et al. 1976). Successful transmission using insect vectors has been confirmed from naturally infected almonds to almond seedlings and grapevines in greenhouse studies (Mircetich et al. 1976) and from grapes to field-grown almond trees. The inability of this vector species to transmit the bacterium to their typical feeding hosts, such as Bermuda grass [*Cynodon dactylon* (L.) Pers.] and watergrass [*Echinochloa crus-galli* (L.) Beauv.], is poorly understood (Hill and Purcell 1997). These plants are very poor hosts of *X. fastidiosa* and that is probably why no transmission events have been observed.

In this study, we investigated the population dynamics of the grass sharpshooter and its ability to transmit *X. fastidiosa* in California's San Joaquin Valley. Our primary objectives were to 1) characterize the seasonal population dynamics and abundance of grass sharpshooter; 2) determine the time of the year when this species disperses into almond orchards; and 3) compare the transmission efficiency of grass sharpshooter versus glassy-winged sharpshooter from field-grown cuttings of infected almond trees to a susceptible host plant species, Madacasgar periwinkle [*Catharanthus roseus* (L.) G. Don].

Materials and Methods

Seasonal Population Dynamics of Grass Sharpshooter in Pastures and Alfalfa Fields. Standard sweep net collections (described below) were conducted from January 2004 through December 2006 in irrigated grass pastures, alfalfa fields, and almond orchard floor vegetation to measure the relative abundance of different stadia of grass sharpshooter in these habitats. Other insects were also captured, including other cicadellids and the threecornered alfalfa hopper, *Spissistilus festinus* (Say). No cercopid species were founded during the period of observation. Irrigated pastures and alfalfa fields selected for this study were located adjacent to orchards with a documented incidence of ALS (Groves et al. 2005). Two experimental sites were selected in Fresno County, site 1 (36° 49′20.48″ N, 119° 44′01.27″ W) and site w (36° 46′35.91″ N, 119° 36′28.53″ W) and one in Kern County, site 3 (35° 24′ 13.61″ N, 119° 17′39.23″ W). Both alfalfa fields in Fresno County were established in 2001 with the *M. sativa*'WL 525 HQ'. In Kern County, the *M. sativa*'WL 625′ was established in 2002, and all three alfalfa field sites were flood irrigated. All three forage pastures monitored in these experiments were colocated adjacent to the described alfalfa pastures, and they were similarly flood irrigated. Pastures were established before 1991 (>11 yr old), and they were composed of a mixture of *C. dactylon*, *E. crus-galli*, white clover (*Trifolium repens* L.), tall fescue (*Festuca arundinaceae* Schreb.), smooth crabgrass [*Digitaria ischemum* (Schreb.) Schreb. ex Muhl.], orchardgrass (*Dactylis glomerata* L.), and perennial ryegrass (*Lolium perenne* L.).

Populations of grass sharpshooter present within vegetation on the floor of almond orchards were monitored at both locations in Fresno and at the single location in Kern County. At Site one in Fresno County, the orchard was 14 yr old and consisted of four almond cultivars—*Prunus amygdalus* [Mill.] D.A. Webb (Rosaceae) 'Sonora', 'Ne-plus', 'Butte', and 'Mission'—alternately planted 9.1 m between rows and 7.9 m within rows. The orchard at site 2 in Fresno County was planted in 1988 and consisted of Sonora, 'Carmel', and 'Nonpareil' almonds alternately planted 6.7 m between rows and 7.9 m within rows. The Kern County orchard at site 3 was planted in 1995 and consisted of Sonora, 'Fritz', and Nonpareil almonds alternately planted 7.3 m between rows and 6.1 m within rows.

A standard sweep sample unit was composed of 50 sweeps with a 38-cm-diameter circular net. Ten sweep net samples, ≈10 m apart, were collected while walking along two linear transects (five samples per transect) parallel to the direction of irrigation floodwater in each of the alfalfa, pasture, and almond floor habitats. We hypothesized that the soil moisture gradient, as determined by distance to the irrigation head, might influence grass sharpshooter population densities within pastures. Each sweep sample unit (plant samples, insects, and debris) was placed into a cooled ice chest and taken to the laboratory where samples were frozen until all potential insect vectors were

collected and counted at a later date. Collected vector specimens were held at −20°C for further analysis. Voucher specimens of sap-feeders were submitted to the Plant Pests Diagnostic Laboratory, California Department of Food and Agriculture (CDFA), Sacramento, CA, and to the Systematic Entomology Laboratory, U.S. Department of Agriculture, Agriculture Research Service, Beltsville, MD.

Seasonal Movement of Grass Sharpshooter into Almond Orchards. Three different trapping systems were evaluated to measure the relative movement of grass sharpshooter into almond orchards and along their perimeters including suspended mesh traps (described below), yellow sticky cards, and UV (UV) light traps during a similar 2-yr interval from January 2004 to December 2006. Suspended mesh traps consisted of fiberglass screen panels (2.25 by 0.90 m, effective sticky area 3.75 m^2) covered by a commercial glue (Stickem Special, Seabright Laboratories., Emeryville, CA). Six mesh traps per location were attached to plastic frames, inspected every 2 wk, and replaced after 4–6 wk. Suspended mesh traps were placed uniformly around the perimeter of each survey orchard to measure insect activity at the orchard interface. Yellow traps consisted of sticky cards (27.5 by 22.5 cm, effective sticky area 372 cm^2) (Seabright Laboratories) fastened to poles at 1.5 m in height, and these were systematically distributed around the orchard block perimeter (on the block edge) and within the orchard to detect insect movement from the edges. Twenty-four to 28 cards per location were checked and replaced every 2 wk. Two UV light traps (Bioquip, Rancho Dominguez, CA) were placed along a single orchard edge of each of three almond orchards and spaced ≈80 m apart. Traps contained a commercial insecticide (active ingredient, 2,2-dichlorovinyl dimethyl phosphate, 18.6%, Spectrum, Ft. Lauderdale, FL). Each trap was operated daily for 3 h at dawn (0500–0800 hours) and 3 h at dusk (1800–2100 hours). Light traps at each location were inspected every 2 wk over a 2-yr period for sharpshooters, spittlebugs, and other potential insect vectors.

Transmission Efficiency. *Insects.* A *X. fastidiosa*-free colony of grass sharpshooter was maintained at the USDA–ARS San Joaquin Valley Agricultural Sciences Center at Parlier, CA. Founding adults were collected from an irrigated pasture in Fresno County in early 2004 and allowed to oviposit on Bermuda grass grown under greenhouse conditions. Plants were removed from oviposition cages, and young nymphs were subsequently transferred to ironweed, *Vernonia noveboracensis* (L.) Michx., a reported nonhost of *X. fastidiosa* (Marucci et al. 2003). Adults of this generation were transferred to healthy Bermuda grass and several generations were maintained under greenhouse conditions held at 24°C and a photoperiod of 16:8 (L:D) h (illuminated by overhead high-pressure, sodium vapor lights) and tested regularly for presence of *X. fastidiosa.* Adult glassy-winged sharpshooters were also used to measure *X. fastidiosa* transmission from ALS-affected almond trees. Insects were provided from a *X. fastidiosa*-free colony maintained at the CDFA Arvin Field Station, Arvin, CA.

Acquisition Access Period (AAP). In total, five almond trees (Sonora) showing visible symptoms of almond leaf scorch from a 2003 survey and documented as *X. fastidiosa*-infected using a combination of double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) (Agdia Inc., Elkhart, IN), and conventional polymerase chain reaction (PCR) (Groves at al. 2006) were used as acquisition sources for transmission experiments. Monthly from March to October in 2004 and March to September in 2005, two 20-cm stem sections were removed from each of two scaffolds per tree. On each of two scaffolds, a single 20-cm stem section was cut from the basal portion near the main trunk, whereas the remaining section was cut from the distal portion near the outermost, tree canopy. Once cut, each green stem section containing foliage was immediately immersed into water and placed into water-filled, 30-cm^3 floral water pics. Upon return to the laboratory, stem sections with foliage were then enclosed within 605 cm^3, clear, plastic corsage boxes with two ventilation windows covered by a fiberglass screen. Groups of five adult grass sharpshooter and glassywinged sharpshooter were confined in each corsage box and allowed to feed freely for an AAP of 3 d.

Inoculation Access Period (IAP). After the 3 d AAP, each group of five adults exposed to ALS-affected almond stem sections were then separated and individually transferred to healthy, 2-mo old Madagascar periwinkle (*C. roseus*'Cooler series, coconut'). Madagascar periwinkle were germinated from seed (Park Seed Wholesale, Greenwood, SC) and maintained under greenhouse conditions (24°C and a photoperiod of 16:8 [L:D]). For each date, 40 glassywinged sharpshooters and 40–60 grass sharpshooters were allowed to feed on periwinkles for an IAP of 4 d. After this period, insects were collected and individually held in 95% ethanol at −20°C for later analysis. After the 4 d IAP, periwinkle plants were held under similar greenhouse conditions for up to 16 wk postinoculation.

Xylella fastidiosa *Detection.* The presence of *X. fastidiosa* in Madagascar periwinkle indicator plants was confirmed 8 and 16 wk postinoculation by using a combination of DAS-ELISA and PCR (Francis et al. 2006). DAS-ELISA was conducted according to instructions provided by the manufacturer of the test kit (Agdia Inc.). Briefly, 50–75 mg of leaf petiole tissue was homogenized in 1× phosphate-buffered saline by using a Homex six tissue extractor (BioReba Inc., Reinach, Switzerland). One hundred microliters of this solution was dispensed into replicate wells on microtiter plates. All plates included positive and negative controls. ELISA results were recorded with a Multiskan MCC/340 microplate reader (ThermoLabsystems Corp., Vantaa, Finland) at a wavelength of 490 nm. Plants were scored positive for *X. fastidiosa* if the optical density (OD) of test wells was greater than that of the mean OD of noninoculated, healthy control plants of the same plant variety plus three standard deviations.

PCR assays were performed on all samples testing positive for the presence of *X. fastidiosa* using DAS-ELISA. Briefly, PCR assays generated a 221-bp amplicon representative of *X. fastidiosa* (Francis et al. 2006). DNA templates were prepared by initially pulverizing plant tissues and subjecting the sample to a modified CTAB miniprepartion, extraction procedure (Zhang et al. 1998). PCR was then carried out in a 25-μl reaction volume containing 1 μl of cell suspension ($\approx$10–25 ng of genomic DNA template) in 1$\times$ reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$) with the addition of 0.2 mM dNTPs, 1 U of *Taq*DNA polymerase (TaKaRa taq Hot Start Version, Takara Bio Inc., Shiga, Japan), and 0.2 μM each of forward and reverse primers. DNA amplification was carried out in a thermocycler (model PTC-100, MJ Research, Watertown, MA) according to the published protocol specified by Francis et al. (2006).

Statistical Analysis. Insect population counts were analyzed by habitat and monitoring method with analysis of variance (ANOVA) followed by least significant difference (LSD) tests using SAS, version 9.1 (PROC MIXED, SAS Institute 2002–2003). Before analysis, data were log transformed to normalize variance. Comparisons of mean *X. fastidiosa* transmission efficiency among months and between insect vector species were carried out after an initial arcsine, square root data transformation. Significant differences among means were determined by ANOVA (PROC MIXED) by using REPEATED options statement. All means presented in tables and figures were back-transformed.

Results

Sweep collections in pastures and alfalfa fields adjacent to almond orchards with previous records of ALS indicated that grass sharpshooter was the most predominant of the known insect vectors among other cicadellids and cercopids collected in this study. Over the three habitats sampled using sweep net samples, in total 26,239 adult grass sharpshooters and no adult (or immature) glassy-winged sharpshooters were collected. The number of grass sharpshooters on almond floor were so low that the analysis is meaningless The largest proportion were swept in irrigated, permanent pasture habitats, with a total 25,040 (95.4%) adult grass sharpshooters followed by irrigated forage alfalfa where 1,222 (4.5%) adult grass sharpshooters were collected.

Over time, a significant habitat main effect (F = 95.98; df = 2, 339; $P < 0.0001$) was observed for the mean number of adult grass sharpshooters collected during the study with far greater numbers collected in permanent pasture in comparison to either alfalfa or weedy habitats on the orchard floor. We next analyzed insect densities within each habitat type separately along transects parallel to the direction of irrigation. Among the weed species constituting the vegetation on almond orchard floors, the numbers of grass sharpshooters were not influenced by irrigation gradient

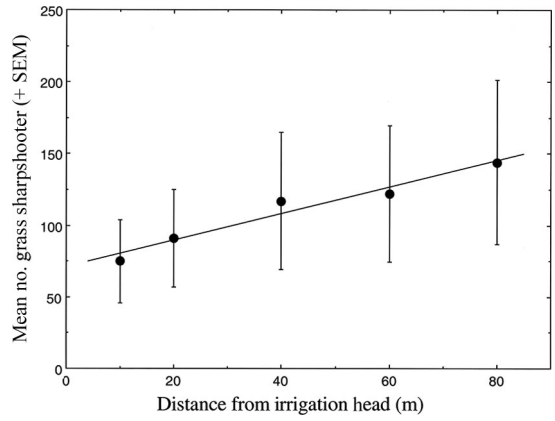

Fig. 1. Mean numbers ($\pm$SEM) of adult grass sharpshooter collected at different sections of permanent, irrigated pasture at site 1 in Fresno County. Numbers were highly correlated (R^2 = 0.9494) with distance from the irrigation head at this experimental site.

(F = 0.40; df = 4,461, P = 0.8058). In contrast, a notable trend in increasing grass sharpshooters populations distant to the irrigation head was observed in both alfalfa (F = 2.74; df = 4, 484; P = 0.0284) and permanent pastures (F = 2.8; df = 4, 1041; P = 0.0249). As an example, populations of adult grass sharpshooters at site 1 had the greatest overall correlation (R^2 = 0.9494) between the number of insects captured with sweep nets and the distance from the irrigation head (Fig. 1).

A proportion of the grass sharpshooters population breeding in pastures and alfalfa fields may have dispersed into adjacent almond orchards even in the absence of weeds on the orchard floor (Fig. 2). Peak population densities of adult grass sharpshooters, as measured by sweep net sampling in 2004, occurred in late May, early July, and again in late September at two of the three experimental sites. In 2005, three peak periods of adult capture were again observed at each of the three permanent pasture sites in both Fresno and Kern counties. Peak captures of adult grass sharpshooters occurred in May, July, and they were somewhat protracted throughout the fall, peaking in September and October. Population densities of this insect varied significantly among the test locations (F = 8.91; df = 2, 482; $P < 0.0001$), with highest total populations recorded at site 1 where nearly 20-fold higher populations were collected compared with either pasture site. Temporal patterns of grass sharpshooters captured in alfalfa were not significantly (F = 0.92; df = 4, 688; P = 0.3094) different among the test locations sampled, and no discernible patterns in insect generations were evident from these collections.

Insects moved into almond orchards and along their perimeters at different times of the year. Peaks of insect dispersal mirrored the peaks of natural populations within irrigated pastures (Fig. 3A and B). Two "abnormal" peaks were detected in both Fresno County locations, sites 1 and 2, during the winter. Reports of weather conditions (data not shown) in-

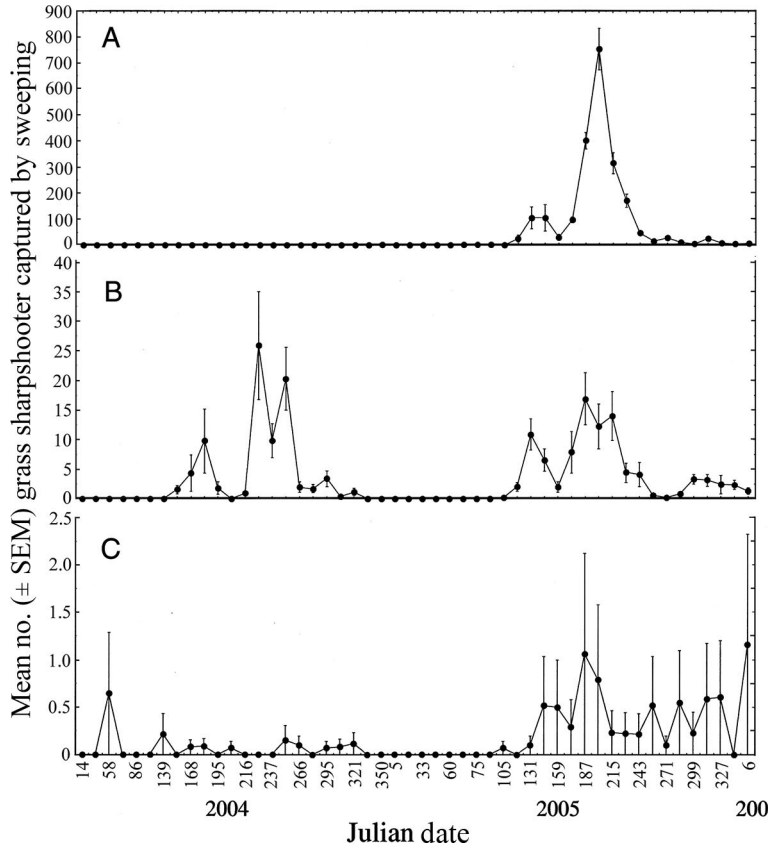

Fig. 2. Mean numbers (±SEM) of adult grass sharpshooter captured with sweep nets in three irrigated, permanent pastures adjacent to site 1 (Fresno County) (A), site 2 (Fresno County) (B), and site 3 (Kern County) (C) during the 2-yr interval from January 2004 to January 2006.

dicated that those peaks followed rainfall in the area (≈15 mm/day). Captures higher or equal to one individual per trap composed 82.2% of total observations (325 of 395 d) at site 1, 41% at site 2 (237 of 577 d), and 54% at site 3 (315 of 577 d). Analysis of the cumulative proportion of insects moving into the almond orchards (Fig. 4) revealed a significant date × location interaction ($F = 2.09$, df = 10, 50; $P = 0.0026$) during the spring period of 2004 and again during both the spring ($F = 2.09$, df = 12, 60; $P = 0.0026$) and fall period ($F = 2.09$, df = 7, 35; $P = 0.0026$) of 2005. Specifically, temporal patterns of adult grass sharpshooter movement in the late spring and early summer differed significantly between experimental sites in both years of the study. However, the midpoint of adult grass sharpshooter movement, estimated from logit-transformed cumulative proportions captured, did not differ among experimental sites in 2004 or 2005 with an estimated average midpoint of Julian day 192 (11 July) and day 169 (18 June), respectively. Between years of the study, the estimated midpoints varied by just over 3 wk (23 d). During fall 2004 and 2005, adult grass sharpshooters dispersal occurred over a more discrete interval of time. The greatest proportion of adult grass sharpshooter movement at the site 1 location in Fresno County extended over a 2-wk period between 1 and 21 December. In 2005, the patterns of adult grass sharpshooter capture again varied among locations ($F = 2.09$, df = 60, 30; $P = 0.0026$), with the greatest difference occurring between Fresno and Kern counties. Temporal patterns of grass sharpshooter capture were very similar between sites 1 and 2 in Fresno County with an estimated midpoint of adult capture occurring on Julian day 331 (23 November). In contrast, the peak period of grass sharpshooter capture at site 3 (Kern County) was protracted over a longer interval, and an estimated midpoint of adult capture (25 October) occurred nearly 1 mo earlier than at the Fresno County sites.

Results from the light trap collections revealed only very small peaks, or periods of capture, during the 2004 sampling year. In contrast, comparatively high rates of capture, and presumably dispersal, occurred in 2005 among all of the three experimental sites (Fig. 5). In 2005, peak capture at site 1 (Fresno County) occurred on Julian day 201 (20 July), whereas the remaining two test locations reported peak periods of collection 15 and 30 d later for site 2 (Fresno County) and site 3 (Kern County), respectively. Moreover, the magnitude of the peak at site 1 was nearly 10- and 5-fold

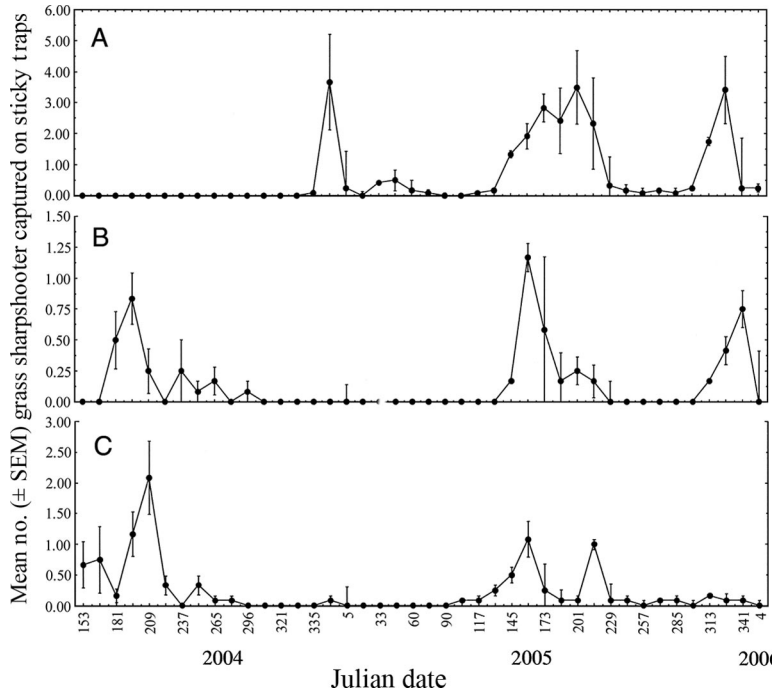

Fig. 3. Mean numbers (±SEM) of adult grass sharpshooter captured on suspended mesh traps in three locations: site 1 (Fresno County) (A), site 2 (Fresno County) (B), and site 3 (Kern County) (C) during the 2-yr interval from January 2004 to January 2006.

higher than the peaks observed at sites 2 and 3, respectively. Very few insects were captured on the series of yellow sticky cards deployed within and surrounding the experimental orchards. Over the course of the entire study, 33 adult grass sharpshooters in total were captured on 2,059 yellow sticky traps placed along orchard perimeters as well as along linear transects within test orchards over the 2-yr interval. Specifically 14, 14, and 5 adult insects in total were collected at sites 1–3, respectively. As a result of these

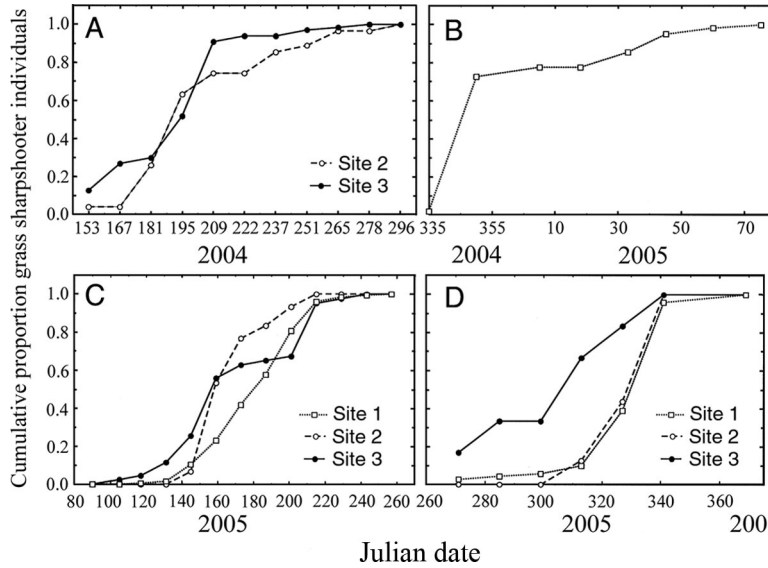

Fig. 4. Mean cumulative proportion of adult grass sharpshooter captured on suspended mesh traps placed along the perimeter of almond leaf scorch affected orchards during: summer 2004 (A), fall 2004–2005 (B), summer 2005 (C), and fall 2005 (D) at the three test locations: site 1 (Fresno County) (A), site 2 (Fresno County) (B), and site 3 (Kern County) (C).

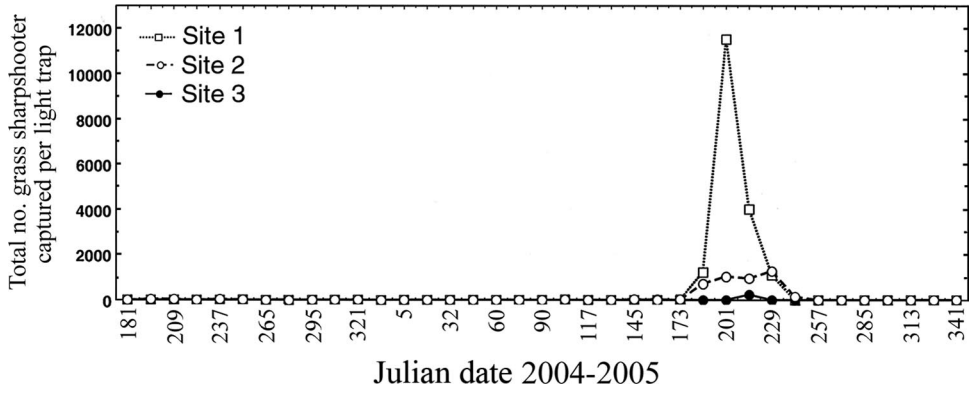

Fig. 5. Total number of grass sharpshooter captured on UV-light traps at the three experimental orchards perimeters: site 1 (Fresno County) (A), site 2 (Fresno County) (B), and site 3 (Kern County) (C) during the 2-yr interval from January 2004 to January 2006.

very low totals, no analyses were performed on these data, and no observable trends in the timing or location of capture were evident (data not shown).

Seasonal differences in transmission efficiency of the grass sharpshooters and glassy-winged sharpshooters were detected using *X. fastidiosa*-infected almond trees as an acquisition source for the bacterial pathogen (Fig. 6). Both insect species used in our experiments were able to transmit *X. fastidiosa* from mature, symptomatic almond trees to Madagascar periwinkle beginning at the time of symptom onset (June) until leaf senescence (October–November). Estimated rates of transmission efficiency varied significantly over time ($F = 5.75$; df = 9, 81; $P < 0.001$) in our study with peak transmission efficiency of *X. fastidiosa* observed in the mid-season (July) followed by declining rates of transmission later in the growing season. No significant time × vector species interaction was observed ($F = 0.63$; df = 7, 81; $P = 0.7291$), and the mean percentage of transmission efficiency was observed to be

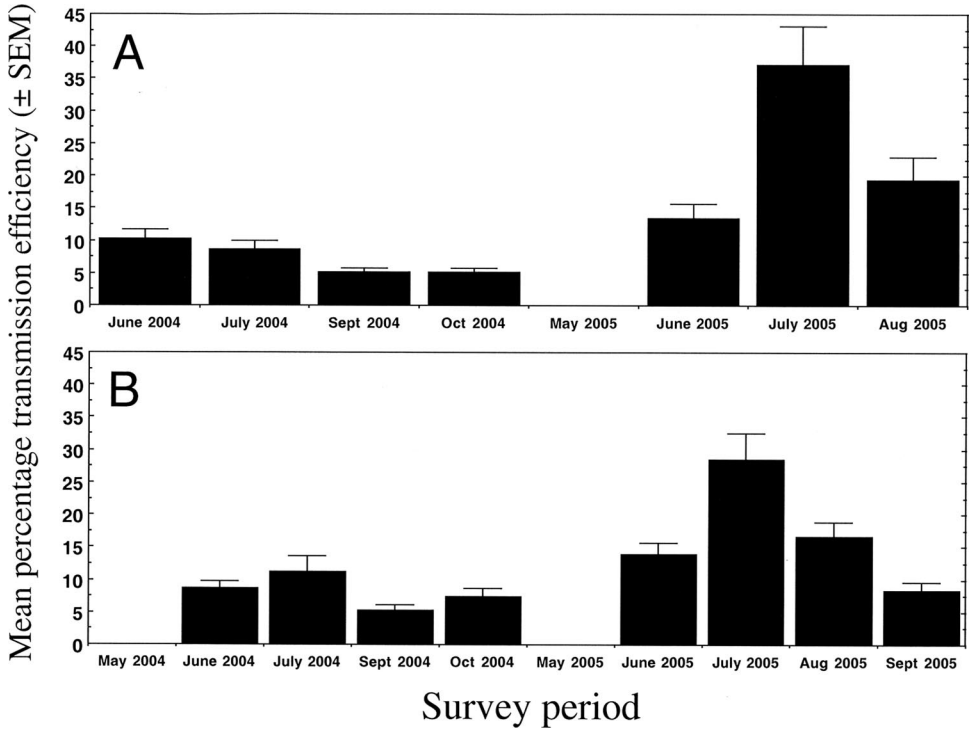

Fig. 6. Mean monthly transmission efficiency (percentage ± SEM) of *X. fastidiosa* by *D. minerva* (A) and *H. vitripennis* (B), when provided a 3-d acquisition access period on ALS-affected, mature almond trees and a 4-d inoculation access period to healthy *C. roseus*.

equal for both insect vector species ($F = 1.38$; df = 1, 81; $P = 0.2475$) averaging 9.9 and 9.5% efficiency for *D. minerva* and *H. vitripennis*, respectively.

Discussion

Previous reports have hypothesized that grass sharpshooter may not be the primary vector of almond leaf scorch in California (Purcell and Frazier 1985). Several factors should be considered when screening for a primary vector: relative abundance of the insect vector, potential of target crop infection, proportion of infectious insects in natural habitats, and efficiency of transmission. Our investigation demonstrated that grass sharpshooter is the most abundant insect vector in pastures neighboring almond orchards. Adult grass sharpshooters also move into almond orchards and along their perimeters throughout the growing season. A proportion of dispersing grass sharpshooters are potentially infectious vectors carrying strains of *X. fastidiosa* (J.C.C.-L. et al., unpublished data), and they transmit *X. fastidiosa* strains as efficiently as *H. vitripennis.* Three of these factors fluctuated over the season: the natural populations of insects within their breeding habitats, their movement patterns into almond orchards, and their transmission efficiency of *X. fastidiosa* when using almond trees as an infection source. This seasonality may be explained in part by the interactive effects of weather conditions, fluctuations in the quality of the food source, availability of more suitable feeding sites, and seasonality of bacterial population *in planta.*

Previous studies identified rainfall as a significant potential environmental factor that has the most pronounced influence on PD spread. The more pronounced peaks of grass sharpshooter dispersal from their breeding habitats were shortly after a heavy rain in the areas of this study. As expected from previous reports (Gibson 1915, Purcell and Frazier 1985), large grass sharpshooter populations were found breeding on irrigated pastures, especially Bermuda grass. In contrast, weedy alfalfa fields supported modest numbers of this insect compared with areas of irrigated, forage grasses. The ephemeral presence of ground vegetation in almond orchards apparently did not permit the establishment of a resident grass sharpshooter population in the orchards and likely would not afford the bacterium, *X. fastidiosa*, a long-term refuge. However, captures of these insects on sticky mesh traps indicated that they are frequent visitors to almond orchards even when suitable ground vegetation on the orchard floor is often absent.

Over the two-year survey period, grass sharpshooter populations fluctuated over time. Food quality seems to be a factor that triggers the fluctuations of these insects. It seems that xylem sap nutrient content, especially ratios of certain essential amino acids, is a factor that influences fluctuations of xylem-feeding sharpshooters, including *H. vitripennis* (Andersen et al. 2005, Bi et al. 2005). The extent to which plant xylem chemistry in these irrigated pastures influences grass sharpshooter preference or population performance is not well understood and deserves further investigation.

During the course of this study, large field densities of grass sharpshooters were often associated with areas of standing water. Daane et al. (1995) documented a similar pattern for leafhopper *Erythroneura variabilis* Beamer, a phloem-feeder, where the highest recorded population densities occurred in areas with reduced drainage and a higher incidence of competing weed vegetation. Preferred on-plant feeding sites of grass sharpshooter are restricted to tender or succulent parts of the plant (Gibson 1915) in contrast to glassy-winged sharpshooter, which will feed on woody stems and dormant tissues (Almeida and Purcell 2003). Temporal presence of these succulent tissues may also be a factor that influences grass sharpshooter population dynamics in these irrigated forage pasture habitats.

The overall goal of this project was to increase our understanding of the epidemiology of ALS in the central and southern San Joaquin Valley of California where ALS has recently emerged as a serious threat. A primary focus of this research has been to accurately identify the natural vectors of *X. fastidiosa*-ALS strains associated with ALS. An accurate knowledge of the vector species that transmit *X. fastidiosa* in the central and southern San Joaquin Valley, where they acquire the pathogen, when they move into orchards, and when they spread the pathogen to almonds is critical to understanding the epidemiology of this disease.

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