Oviposition and Flight Activity of the Blue-Green Sharpshooter (Hemiptera: Cicadellidae) on Southern California Wild Grape and First Report of Associated Egg Parasitoids

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ABSTRACT Oviposition of *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), the blue-green sharpshooter, was assessed on wild grape, *Vitis girdiana* Munson, in southern California. Female blue-green sharpshooter preferred to oviposit into new growth, primarily succulent young grape tendrils and canes. The midrib and petioles of small grape leaves also were used for oviposition, but to a lesser extent. Mature canes, and medium- and large-sized leaves were not used for oviposition. Two parasitoids, *Gonatocerus latipennis* Girault and *Polynema* sp. (both Hymenoptera: Mymaridae) were reared from field collected blue-green sharpshooter eggs. *Polynema* sp. and *G. latipennis* constitute the first documented egg parasitoids of blue-green sharpshooter. Deployment of sentinel plants and reciprocal tests were implemented and further confirmed the parasitization of blue-green sharpshooter eggs by these two parasitoid species. Yellow sticky card trap catch of adult blue-green sharpshooter was significantly correlated to combined blue-green sharpshooter nymphal and *Polynema* sp. emergence in 2004, but not in 2005. A degree-day model was developed for onset of blue-green sharpshooter flight activity and emergence in the field.

KEY WORDS *Gonatocerus latipennis*, leafhopper biology, phenology, *Polynema* sp., *Vitis girdiana*

The native blue-green sharpshooter, *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae: Cicadellinae), has been a pest of California-grown grapes for nearly a century because of its excellent transmission efficiency of *Xylella fastidiosa* Wells et al., the xylem-limited bacterium that causes Pierce's disease, a severe malady of commercially grown grapes (Hewitt et al. 1942, Hill and Purcell 1995). Numerous auchenorrhynchans are capable of transmitting *X. fastidiosa;* however, the blue-green sharpshooter is the principal native vector occupying the Pacific coastal fog belt, an important area for premium wine grape growing regions in California (Hewitt et al. 1942, Freitag and Frazier 1954, Purcell 1975).

The blue-green sharpshooter's range is limited to moist, humid environments such as riparian or coastal habitats (Winkler 1949), but it also occurs in such habitat as far east as Arizona where conditions are typically more arid than similar areas in California (DeLong and Severin 1949). Within such favorable environments, blue-green sharpshooter is highly polyphagous; capable of feeding and reproducing on a variety of native annuals and perennials as well as many exotic ornamental plants (Severin 1949, Winkler 1949). Purcell (1976) identified the most common host plants for blue-green sharpshooter in northern California riparian areas as blackberry (*Rubus* spp.), elderberry (*Sambucus* spp.), wild grape (*Vitis californica* Bentham), mugwort (*Artemesia douglasiana* Besser), and nettle (*Urtica* spp.).

Oviposition by females of the blue-green sharpshooter, as reported by Severin (1949), constituted single eggs deposited into petioles of "grape cuttings." However, a clear definition of a grape cutting was not provided. Therefore, it is unclear whether these cuttings were the succulent terminal ends of the grape canes, or whether they consisted of cane, tendrils, petioles, and leaves. Occurrence of adults and nymphs on grape and other plants have been reported to be most concentrated on succulent new terminal growth (Winkler 1949, DeLong and Severin 1949, Purcell 1976: quantified in Purcell 1975). Although it might be presumed as likely that oviposition also favors the new terminal growth, this has never been quantified. In northern California, the blue-green sharpshooter is univoltine (Severin 1949), but little is known of this sharpshooter's phenology in southern California, where this study was conducted. This type of information is particularly important, because grape vine susceptibility to *X. fastidiosa* is thought to be the reason for the destruction of the southern California wine industry in the 1880s (Pierce 1892, Gardner and Hewitt 1974). The blue-green sharpshooter was named as one of the key native vectors responsible for the transmission of this bacterium in the southern California disaster, then referred to as the California vine disease

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or Anaheim disease (Pierce 1892, Winkler 1949, Gardner and Hewitt 1974).

The economic importance of this grape pest has led to research devoted to epidemiologically related issues pertaining to the acquisition and spread of *X. fastidiosa* in vineyards (Purcell 1975, 1979, 1981). Despite the importance of blue-green sharpshooter to the grape industry in California, very little basic biological and ecological knowledge for blue-green sharpshooter exists. Important shortcomings such as within-plant oviposition preferences, phenology in southern California, and identification of the native parasitoid fauna have been identified as major deficiencies in understanding the autecology of this insect. The objectives of this study were to determine the within-plant oviposition of blue-green sharpshooter on wild grape, identify associated egg parasitoids, and ascertain whether yellow sticky card captures could be correlated to population phenology in the field in southern California.

Materials and Methods

Field Sites. Two sites west of Temecula, Riverside Co., CA (a major wine grape growing region in southern California) in the Sandia Creek area were used for all studies conducted on blue-green sharpshooter and its natural enemies. Both sites were bordered by a creek with year-round surface water. Site 1 was in a shaded ravine, ≈30 m in depth (hereafter referred to as creek site, 33° 29.468′ N, 117° 14.897′ W; elevation 299 m). The site 2 was 3.9 km southwest of the creek site along a roadside (hereafter referred to as road site, 33° 28.234 N, 117° 16.111 W; elevation 161 m). Both sites could be classified as a riparian oak woodland plant community. Wild grape, *Vitis girdiana* Munson, and poison oak, *Toxicodendron diversilobum* (Torr. & Gray) Greene, are the dominant understory vegetation, and oak (*Quercus* sp.), sycamore (*Platanus racemosa* Nutt.), and willow (*Salix* sp.) are the dominant overstory species at the study sites. Other understory plant species at both sites included mugwort (*Artemesia douglasiana* Besser), stinging nettle (*Urtica* sp.), mint (*Mentha* sp.), cocklebur (*Xanthium strumarium* L.), and blackberry (*Rubus ursinus* Cham. & Schlecht). All of these understory plant species are known to be feeding or oviposition hosts for blue-green sharpshooter in northern California (Purcell 1976). Initial observations of blue-green sharpshooter at these field sites revealed the greatest visual counts of adult feeding and copulation, nymphal feeding, and exuviae on wild grape. Therefore, *V. girdiana* was selected as the native host plant for pursuing the objectives of this study.

Insect Identification. Cicadellids were sent to Dr. S. L. Winterton at California Department of Food and Agriculture (Sacramento, CA) for identification. Mymarid parasitoids were identified by Dr. S. V. Triapitsyn at Department of Entomology, University of California (Riverside, CA). Voucher specimens of cicadellids and mymarids were deposited at the Entomology Research Museum, University of California, Riverside. Voucher specimen identifiers are as follows: *Graphocephala atropunctata* female, #UCRC ENT 136624, and male, #UCRC ENT 136623; *Carneocephala triguttata* Nottingham (Hemiptera: Cicadellidae: Cicadellinae) male, #UCRC ENT 110578; *Gonatocerus latipennis* Girault (Hymenoptera: Mymaridae) female, #UCRC ENT 136625, and male, #UCRC ENT 136626; and *Polynema* sp. (Hymenoptera: Mymaridae) female, #UCRC ENT 136627, and male, #UCRC ENT 136628.

Determination of Blue-Green Sharpshooter Oviposition on Wild Grape. To determine the within-plant oviposition of blue-green sharpshooter on *V. girdiana*, a survey was conducted by collecting plant material from the creek site in August 2003 and 2004. On 5 August 2003, harvested material from wild grape was deposited into plastic bags and transported in a cooler to the laboratory. The field collected material consisted of 50 canes (25 cm in length from apical tip); 50 tendrils (16.9 ± 2.8 cm); and 100 large (16.4 ± 1.8 cm), 100 medium (11.8 ± 0.5 cm), and 100 small (8.1 ± 2.3 cm) grape leaves (measured along the longest axis ± SD) with petioles still attached. The tendrils and small leaves with petioles came from the 50 canes, whereas large and medium leaves were randomly collected throughout the field site. Subsequent to collection, each of the 50 canes were cut into thirds (upper, middle, and lower), and five cane portions of the same third were combined and placed into dual 40-dram plastic ventilated vials to keep moist (see Irvin and Hoddle 2005 for description of dual rearing vials). Tendrils were divided into groups of 10 and placed into five separate 150- by 15-mm glass petri dishes. Large and medium leaves were placed on water-saturated foam pads within metal trays. Fifty of each leaf size were placed top side up and 50 top side down. Trays with the same size of leaves and same side exposed then were placed into 55- by 45- by 45-cm clear acrylic cages. Small leaves were placed in 100- by 15-mm petri dishes, 50 top side up, and 50 top side down. Petioles were excised from all leaves and placed into 12 150- by 15-mm petri dishes. Each dish contained 25 petioles, all of which were from the same size leaf. All petri dishes were provisioned with two moistened filter papers and sealed with a strip of paraffin wax. The plant material in all cages and petri dishes were examined daily for emergence of blue-green sharpshooter nymphs and parasitoids until 2 September 2003.

Ten entire grape canes were sampled on 14 August 2003 to account for possible oviposition substrates not sampled in the 5 August survey described above. These entire canes also were cut into thirds (apical, middle, and basal; not to be confused with the apical 25 cm of cane described above that was cut into upper, middle, and lower sections). Each third was placed into a water-filled 473-ml jar leaving 26.5 ± 5.6 cm of cane exposed for emergence. Canes and jars then were placed into three separate clear acrylic cages according to their cane section. These were examined daily for blue-green sharpshooter nymphal and parasitoid emergence until 10 September 2003. Because

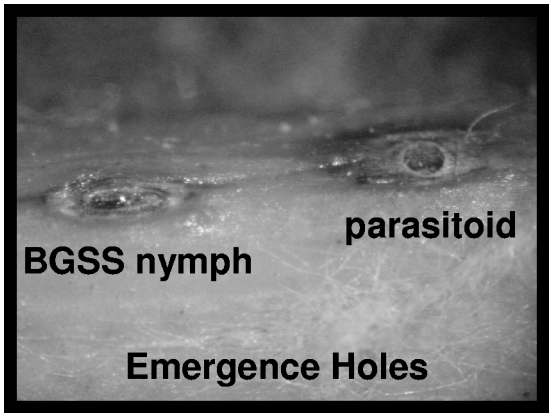

Fig. 1. Blue-green sharpshooter nymphal and parasitoid recent emergence holes in wild grape cane.

few insects emerged from these cane sections, plant material was examined under a Leica MZ12 stereomicroscope at 20× zoom (Leica Microsystems Inc., Bannockburn, IL) for recent (approximately ≤2 mo old) emergence holes from blue-green sharpshooter nymphs and parasitoids. Recent emergence holes were defined as those that were green and moist in appearance with very little browning of the plant tissue. Recent blue-green sharpshooter nymph emergence holes were elliptical, whereas parasitoid holes were circular (Fig. 1). Older emergence holes were defined as those that were completely brown and hardened, or occurred as an obvious ovate scar.

On 6 August 2004, 16 entire canes were sampled following the procedure outlined in the previous paragraph. These were examined daily for blue-green sharpshooter nymphal and parasitoid emergence until 7 October 2004. Average cane length in 2004 was longer (164.8 ± 27.5 cm) than that of canes used in 2003 (109 ± 16.9 cm). Canes were cut into thirds (apical, middle, and basal) and put into water filled jars leaving 44.9 ± 9.2 cm exposed for emergence of blue-green sharpshooter nymphs and any parasitoids. Despite differences in overall cane length, growth habit and overall appearance were similar. Therefore, each cane section was assumed to be representative of comparable growth for each year.

To combine emergence data from the 10 and 16 entire canes harvested on 14 August 2003 and 6 August 2004, respectively, the average number of recent blue-green sharpshooter nymphal and parasitoid emergence holes per centimeter of each cane section was calculated. The number of recent emergence holes from the cane substrate type per individual cane section was divided by the length in centimeters for the same individual cane section (i.e., if 21 emergence holes were counted on the cane substrate type on the apical section of one cane, then 21 was divided by the length [25 cm] of that particular apical cane section to give 0.84 holes per cm of apical cane section for that cane substrate sample). The number of emergence holes per centimeter of each individual cane section was determined for each substrate type. The resulting emergence holes per centimeter of cane were then summed for each cane section (apical, middle, and basal) by substrate type and divided by the sample size ($n = 26$).

Confirmation of Reared Parasitoids with Blue-Green Sharpshooter Eggs. To confirm host association of the emerged parasitoids from the oviposition studies above, sentinel plants with blue-green sharpshooter eggs were deployed at the study sites. Three sweet basil, one chrysanthemum, and two wild grape plants were exposed to a blue-green sharpshooter laboratory colony (≈150 female blue-green sharpshooter, maintained on sweet basil in a temperature controlled greenhouse at 26.7 ± 0.6°C and a photoperiod of 16:8 [L:D] h) for 3 d to allow for oviposition. Plants were then removed from the colony and transported to the creek site on 30 August 2003 to allow for parasitization of the blue-green sharpshooter eggs. These sentinel plants were removed from the field site on 2 September 2003 and visually inspected for all larval and adult insects, which then were removed before placing plants into separate clear acrylic cages in the laboratory (27.6 ± 0.4°C and photoperiod of 16:8 [L:D] h). Plants were observed daily and numbers of emerging blue-green sharpshooter nymphs and parasitoids were recorded until 14 October 2003.

On 1 August 2004, the above-mentioned sentinel plant procedure was repeated with two wild grape and four sweet basil plants. Because oviposition on chrysanthemum was very low in 2003, this plant was not used in 2004. Deployed plants were left for 3 d at the study site before being returned to the laboratory where they were observed daily for emergence of blue-green sharpshooter nymphs and parasitoids until 10 October 2004.

To further confirm the association of emerged egg parasitoids from sentinel plants with blue-green sharpshooter, reciprocal tests were performed to ensure that reared parasitoids from sentinel plants did not emerge from eggs laid by other insects during field deployment. Emerged male and female parasitoids (1 d old and fed honey water) from the 2004 sentinel plant study were released into cages containing a sweet basil plant with <2-d-old blue-green sharpshooter eggs and allowed 2 d to parasitize oviposited eggs. These plants were then held at 27.6 ± 0.4°C and photoperiod of 16:8 (L:D) h for 30 d for parasitoid emergence.

Blue-Green Sharpshooter and *Polynema* sp. Emergence and Flight Activity. Temperature, relative humidity, and light intensity were recorded every 0.5 h at both sites by using Hobo H8 Pro Series data loggers (Onset Computer Corporation, Bourne, MA) from 9 January 2004 to 23 December 2005. Daily average temperature and relative humidity, and daily maximum light intensity readings were calculated. Daily precipitation and days with missing Hobo data were retrieved from the CIMIS (2005) weather station #62 for Temecula, CA. Creek and road site daily maximum and minimum temperatures were combined and averaged over the previous 2-wk sampling intervals to be

consistent with trap and plant sampling protocols described below. Correspondingly, precipitation was summed over the same 2-wk sample periods. This process generated 25 observations for 2004 and 26 observations for 2005.

In total, 12 yellow double-sided sticky traps (11- by 15-cm) were placed at the two field sites (six at each site) described above to monitor blue-green sharpshooter adult and parasitoid flight activity. Traps were deployed on 9 January 2004 and collected and replaced with new traps every 2 wk for 2 yr, ending on 6 January 2006. At the creek site, traps were placed along a transect 1.2 m from, and parallel to, the creek at a height of 1.4 m. Sticky traps were attached with binder clips to the horizontal arm of a T shaped wooden stake. Three of these wooden stakes were deployed at each study site. Sticky traps were set up in pairs on the horizontal arm 22 cm apart, and the distance between wooden stakes with traps was 7.8 m. At the road site, sticky traps were placed on stakes along a transect 3.1 m from, and parallel to, the road at a height of 1.1 m. Sticky traps were set up in pairs on stakes 22 cm apart with 14.8 m between stakes. Sticky traps and Hobo weather data for the 21 January 2005 sample period were destroyed by flooding at the creek study site, which washed away equipment. The light intensity data logger was recovered, and no data were lost. A new weather data logger was redeployed on 4 February 2005. Sticky traps were redeployed on 9 March 2005 after storm and flood activity ceased.

When wild grape had ≥30 cm of cane for collection (16 April 2004 and 15 April 2005) at study sites, 12 apical 30-cm cane sections were harvested every 2 wk from each site for a total of 24 canes per sample date. Tendrils were excised from cut canes and individually placed into 100- by 15-mm petri dishes provisioned with two moistened filter papers and sealed with a strip of paraffin. Cane sections devoid of leaves, petioles, and tendrils were placed into dual 50 dram vials with 25 cm of cane above water to allow for emergence. Harvested plant material was checked daily for emergences of blue-green sharpshooter nymphs and parasitoids. All emergences were recorded by the sample period from which it was collected, not the actual emergence date in the laboratory. Therefore, blue-green sharpshooter nymph and parasitoid emergences reflect blue-green sharpshooter oviposition in the field before the sample date. This was done to avoid any discrepancies between laboratory temperatures and temperatures in the field. In 2005, no grape canes could be collected from the creek site during the sample periods of 10 June to 5 August because of extensive grape dieback associated with powdery mildew outbreak. In 2004 and 2005, wild grape cane sampling ceased with plant senescence in November.

Statistical Analyses. Blue-green sharpshooter nymphal and parasitoid emergence data from entire wild grape canes collected on 14 August 2003 and 6 August 2004 were not normally distributed and required a nonparametric analysis. A nonparametric one-way analysis of variance (ANOVA) was performed on the ranked average emergence per centimeter by substrate type between cane sections and within cane sections by using the Kruskal–Wallis test at the 0.05 level of significance (PROC NONPAR1WAY, SAS Institute 1999). There was no difference between years, so data were pooled for 2003 and 2004. Average ranks were used in the case of ties (Conover 1999). The H_0 investigated was that the distribution of ranks for each blue-green sharpshooter oviposition substrate type would be similar between cane sections. The second H_0 evaluated was that the distribution of ranks between substrate types would be similar within cane section. If the null hypothesis was rejected, then multiple comparisons were performed and two means were considered significantly different if the inequality was satisfied (Conover 1999). All means and standard errors presented from this entire cane study are from nonranked data.

For the blue-green sharpshooter and *Polynema* sp. flight activity and emergence study, the correlation between blue-green sharpshooter adult trap catch, and combined blue-green sharpshooter nymph and *Polynema* sp. emergence data for each year was determined with a correlation analysis (PROC CORR, SAS Institute 1999). A similar correlation analysis was performed for *Polynema* sp. adult trap catch and *Polynema* sp. emergence for each year. The data set used for these correlation analyses matched the period of grape cane collection for each year. This used 16 sample periods each for 2004 and 2005.

Feil et al. (2000) found a significant correlation between temperature and early spring trap catch of the blue-green sharpshooter in northern California when temperatures were ≥14.5°C. Degree-days (DD) were calculated by adding the daily maximum [T_{max}] and minimum temperatures [T_{min}], dividing this sum by 2, and then subtracting 14.5 (the minimum threshold for flight activity):

$$DD = ((T_{max} + T_{min})/2) - 14.5$$

Although a minimum flight activity threshold has been developed for blue-green sharpshooter, a minimum oviposition temperature threshold has not yet been determined for either the blue-green sharpshooter or its associated egg parasitoids. Because oviposition thresholds are variable for other leafhoppers and their egg parasitoids (Sher and Shields 1991, Martinson and Dennehy 1995, Agboka et al. 2004, Tokuda and Matsumura 2005), we used the 14.5°C flight activity threshold, developed by Feil et al. (2000), for both blue-green sharpshooter and egg parasitoid cumulative trap catch and biweekly emergence data as the activity threshold temperature in our DD calculations. The cumulative DD, blue-green sharpshooter adult sticky card trap catch, and combined blue-green sharpshooter nymph and *Polynema* sp. emergence data were calculated for each year beginning 1 January and ending 24 and 23 December in 2004 and 2005, respectively, then plotted by sample period to correspond with sticky card trap and emergence data. The cumulative DD, parasitoid sticky card trap catch, and parasitoid emergence data were calculated for the

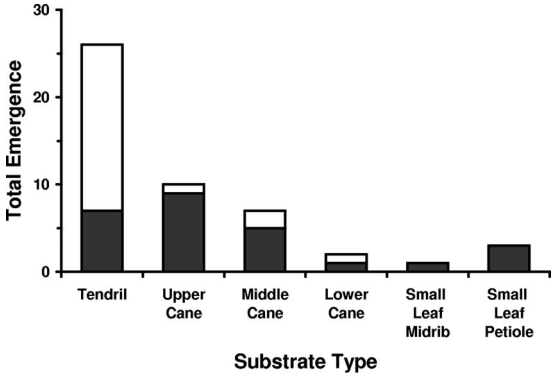

Fig. 2. Total blue-green sharpshooter nymphal (solid bars) and parasitoid (open bars) emergence per substrate type sampled from the terminal 25 cm of wild grape canes: for small leaf midrib and small leaf petiole, $n = 100$; for all others, $n = 50$.

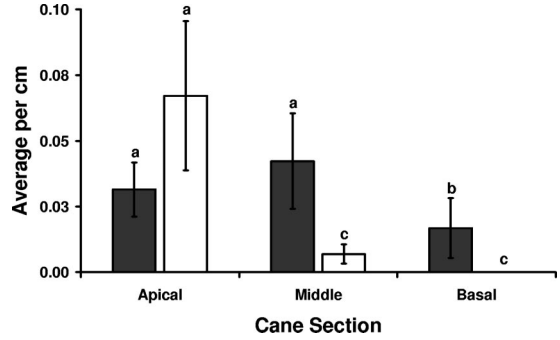

Fig. 3. Average combined emergence of blue-green sharpshooter nymphs and *Polynema* sp. per centimeter of cane section by substrate type from 26 wild grape canes sampled in 2003 and 2004. Closed bars represent tendrils and open bars represent canes. Cane sections of the same substrate type with the same letter are not significantly different ($P > 0.05$). Substrate types from the same cane section with the same letter are not significantly different ($P > 0.05$), Kruskal–Wallis test.

same time periods in 2004 and 2005 and plotted by sample period.

Results

Determination of Blue-Green Sharpshooter Oviposition on Wild Grape. In total, 26 *G. atropunctata*, 18 *Polynema* sp. (*Polynema ?eutettixi* Girault or a new species near it; S. V. Triapitsyn, personal communication) and five *G. latipennis* parasitoids emerged from plant material collected from the study site on 5 August 2003 (Fig. 2). No insects emerged from the upper or lower surfaces of large or medium leaves or their respective petioles and are consequently excluded from the figure. The largest blue-green sharpshooter nymph emergence occurred in the upper third of the cane, with fewer nymphs emerging from tendrils and middle and lower cane sections. A small number of blue-green sharpshooter nymphs emerged from small leaves and their petioles. For all parasitoid species, emergence was greatest from grape tendrils (Fig. 2).

A summary of entire cane emergence data for 2003 and 2004 can be found in Table 1. The majority of emergence holes for blue-green sharpshooter nymphs and parasitoids were on the apical cane section or on tendrils occurring along the length of entire canes; no emergence holes were counted from basal cane sections in either year (Fig. 3).

For the nonparametric analysis of the tendril substrate type, the average number of combined recent blue-green sharpshooter nymphal and parasitoid emergence holes per centimeter was significantly different ($\chi^2 = 6.4$, df = 2, $P = 0.04$) between the cane sections. The average number of recent blue-green sharpshooter nymphal and parasitoid emergence holes were similar ($P > 0.05$) between apical and middle tendrils and both were greater ($t = 2.26, 2.25$, df = 75, $P = 0.03, 0.03$) than basal tendrils (Fig. 3).

For cane substrate type, the average number of combined recent blue-green sharpshooter nymphal and parasitoid emergence holes per cm were significantly different ($\chi^2 = 13.1$, df = 2, $P = 0.002$) between cane sections. For these cane sections, the middle and basal section had similar average emergence holes ($P > 0.05$), and both were significantly different ($t = 2.17, 3.91$, df = 75, $P = 0.03, 0.0002$) from the apical

Table 1. Emergence of blue-green sharpshooter nymphs and parasitoids as measured by recent emergence holes from entire canes sampled 14 August 2003 ($n = 10$ canes) and 6 August 2004 ($n = 16$ canes)

Cane section	Substrate type	2003		2004		Total
		Nymph	Parasitoid	Nymph	Parasitoid	
Apical	Tendril	1	5	6	20	32
	Cane	16	21	2	10	49
	Leaf petiole	1	0	1	0	2
	Leaf midrib	0	0	0	0	0
Middle	Tendril	8	5	5	21	39
	Cane	0	0	3	5	8
	Leaf petiole	0	0	2	9	11
	Leaf midrib	0	0	1	0	1
Basal	Tendril	2	5	2	5	14
	Cane	0	0	0	0	0
	Leaf petiole	0	1	1	2	4
	Leaf midrib	0	0	0	0	0
Total		28	37	23	72	160

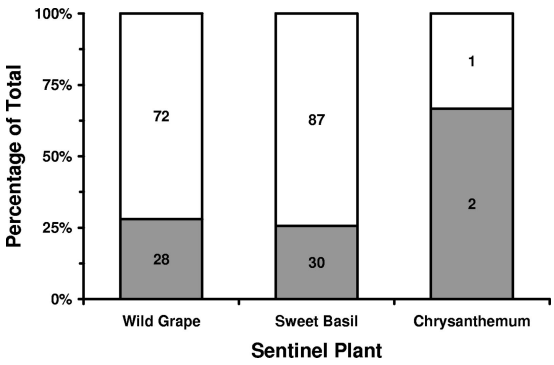

Fig. 4. Percentage of total number of blue-green sharpshooter nymph (closed bars) and *Polynema* sp. (open bars) adult emergence from three species ($n = 4$ grape, $n = 7$ basil, and $n = 1$ chrysanthemum) of sentinel plants. Numbers within bars are the total number of emergences.

cane section, which had the greater average blue-green sharpshooter nymphal and parasitoid emergence holes (Fig. 3).

The average number of combined recent blue-green sharpshooter nymphal and parasitoid emergence holes per centimeter between tendril and cane substrate types within apical cane section was not significantly different ($P > 0.05$), but it did differ significantly ($\chi^2 = 5.1$, df = 1, $P = 0.02$) between tendril and cane substrate types within the middle and basal cane sections ($\chi^2 = 4.2$, df = 1, $P = 0.04$). In these latter sections, a greater average emergence was observed from tendrils rather than canes (Fig. 3).

Egg Parasitoid Confirmation. In 2003, 55 blue-green sharpshooter nymphs and 142 *Polynema* sp. (54 males, 88 females) emerged from the six sentinel plants. In 2004, five blue-green sharpshooter and 18 *Polynema* sp. (two males, 16 females) emerged from the six sentinel plants. These data were combined across both years to give a total of 60 blue-green sharpshooter nymphs and 160 *Polynema* sp. (56 males, 104 females; Fig. 4) reared from sentinel plants. Emerged nymphs were reared to adults to confirm species identity as blue-green sharpshooter. Parasitization rates of blue-green sharpshooter eggs on deployed sentinel plants by *Polynema* sp. ranged from 72% on wild grape to 74% on sweet basil and 33% on chrysanthemum over a 3-d exposure in the field. No *G. latipennis* were recovered from the sentinel plants. In the reciprocal test, where blue-green sharpshooter eggs were exposed to *Polynema* sp. that emerged from sentinel plants, a total of 11 *Polynema* sp. were recovered from the sweet basil. This confirmed parasitization of blue-green sharpshooter eggs by this native parasitoid.

Blue-Green Sharpshooter and *Polynema* sp. Emergence and Flight Activity. The 2-yr average ± SD daily relative humidity was 78.3 ± 12.9% for the road site and 78.5 ± 16.2% for the creek site. The 2-yr average ± SD logarithmic daily maximum light intensity for the road site was 3.5 ± 0.5 Lum/m^2 and for the creek site was 2.8 ± 0.3 Lum/m^2, indicating the creek site received less sunlight.

Two-week average maximum and minimum temperatures followed a yearly cyclical pattern with mean minimum temperatures reaching 2.6°C in January and February 2004 and 3.2°C in December 2005 to a mean maximum of 32.0°C in July 2004 and 31.3°C in July 2005 (Fig. 5A). Daily maximum temperatures did not drop below 15.8°C in 2004 (February) but did drop to a low of 13.0°C in January 2005. Two-wk cumulative precipitation showed a distinct seasonal pattern with a rainy season and a dry season in southern California. The rainy season occurred from November to May and the dry season occurred from June to November. In early January 2005, southern California experienced its second wettest rainy season on record (NOAA 2006). As a result, road and creek sites had severe flooding. At the flood peak, the road site was submerged with ≈15 cm of water, whereas the creek site ravine filled with an excess of 3 m of rushing water for nearly 24 h. The road site experienced minimal damage when flood waters receded, whereas the creek site architecture was permanently altered. Nearly all of the blue-green sharpshooter-inhabited areas of the creek site were covered with silt and denuded of vegetation by flood water.

Throughout the 2 yr of survey data, there was continuous blue-green sharpshooter flight activity at the monitored sites. In total. 3,681 and 1,579 blue-green sharpshooter adults were trapped in 2004 and 2005, respectively. Trap catch was 57% lower in 2005, probably because of flood damage over winter. There were three distinct peaks of blue-green sharpshooter flight activity for 2004 and two peaks for 2005. Peak I (Fig. 5B) corresponded with high daily maximum temperatures (27.3°C) for the 19 March 2004 sample period. This peak is lacking in 2005. Peak trap catch (peak II, Fig. 5B) of blue-green sharpshooter adults occurred during the sample period of 25 June 2004 and 11 July 2005, respectively. A smaller peak III is observed 15 October 2004 and 25 November 2005, respectively. Peak III in 2005 occurred four sample periods later than that observed in 2004.

Trap catch data indicate one distinct peak each year for *Polynema* sp., but similar to blue-green sharpshooter, there were continual background catches of this parasitoid except in periods of heavy precipitation (i.e., 5 March 2004 and 4 February to 4 March 2005) (Fig. 5B). In total, 854 and 322 *Polynema* sp. adults were trapped in 2004 and 2005, respectively. Trap catch was 62% lower in 2005. Peak trap catch of *Polynema* sp. adults occurred two sampling intervals later than the blue-green sharpshooter peak II during the sample period 23 July 2004, and one sampling interval later during the sample period 22 July in 2005 (Fig. 5B). In 2004, there was a single continuous period of nymphal and parasitoid emergence from 28 May to 3 September 2004 (Fig. 5C). In 2005, blue-green sharpshooter and parasitoid emergence spanned the period from 13 June to 14 October 2005. The combined peak emergence of blue-green sharpshooter nymphs and parasitoids occurred on 9 July 2004 and 24 June in 2005 (Fig. 5C).

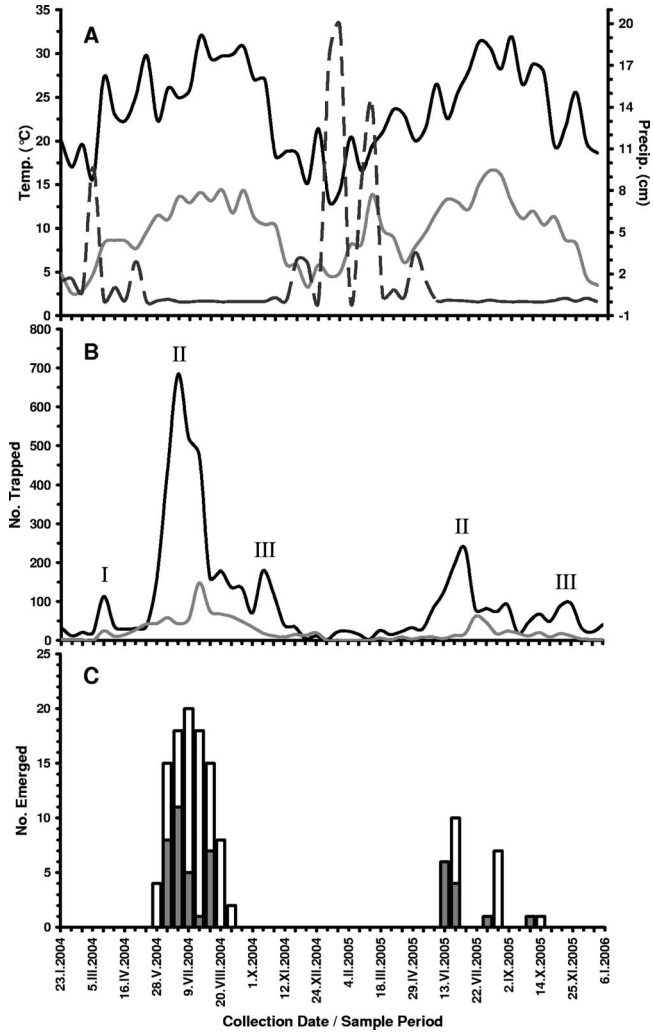

Fig. 5. (A) Two-week average maximum (solid black line) and minimum (solid light gray line) temperature (°C) on left axis, and 2-wk cumulative precipitation (hashed line) on right axis in 2004 and 2005. (B) Total number of 2-wk blue-green sharpshooter (solid line) and *Polynema* sp. (gray line) adult trap catch in 2004 and 2005. Roman numerals describe peaks mentioned in text. (C) Blue-green sharpshooter nymph (closed bars) and *Polynema* sp. (open bars) adult emergence from field collected grape samples in 2004 and 2005.

A significant correlation ($P < 0.0001, R = 0.88$) was detected between adult blue-green sharpshooter trap catch and the combined nymphal and parasitoid emergence data over the 16 sample periods in 2004. This correlation indicates that variation in emergence data explains 77% of the observed variation in trap data for 2004. A nonsignificant correlation was observed for the 16 sample periods analyzed in 2005 ($P = 0.08, R = 0.45$). The maximum blue-green sharpshooter trap catch occurred in the same sample period as the maximum blue-green sharpshooter nymph emergence for 2004 but not in 2005 (Fig. 5C).

A significant correlation ($P = 0.0002$, $R = 0.80$) existed between adult *Polynema* sp. trap catch and *Polynema* sp. emergence data over the 16 sample periods in 2004, but this was not significant for the 16 sample periods analyzed in 2005 ($P = 0.78, R = -0.08$). This correlation indicates that variation in *Polynema* sp. emergence data explains 64% of the observed variation in *Polynema* sp. trap data for 2004. The maximum *Polynema* sp. trap catch occurred in the same sample period as the maximum *Polynema* sp. emergence in 2004 but not in 2005 (Fig. 5C).

The cumulative DD, trap catch, and emergence data for blue-green sharpshooter (Fig. 6A and B) and *Polynema* sp. (Fig. 7A and B) in 2004 and 2005 are similar in pattern. In 2004 and 2005, the total cumulative DD were 862 and 884, respectively. The cumulative DD follow a sigmoid-shaped curve for both years; characterized by a slow increase until day 121 (73 DD) and day 133 (88 DD), followed by a rapid increase until day 289 (852 DD) and day 287 (840 DD) in 2004 and 2005 respectively, after which day the accumulation of DD plateaus for the remainder of the

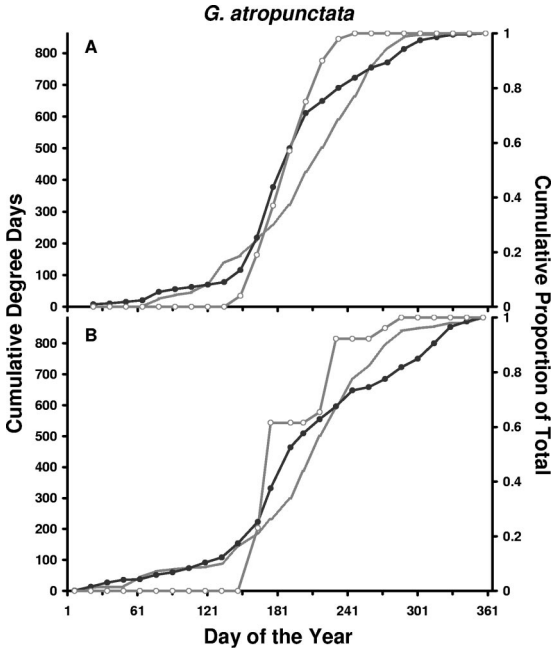

Fig. 6. Cumulative DD (hashed line, left axis), cumulative proportion of total *G. atropunctata* trap catch (closed circles, right axis), and cumulative proportion of total *G. atropunctata* and *Polynema* sp. emergence (open circles, right axis) in 2004 (A) and 2005 (B) by day of the year.

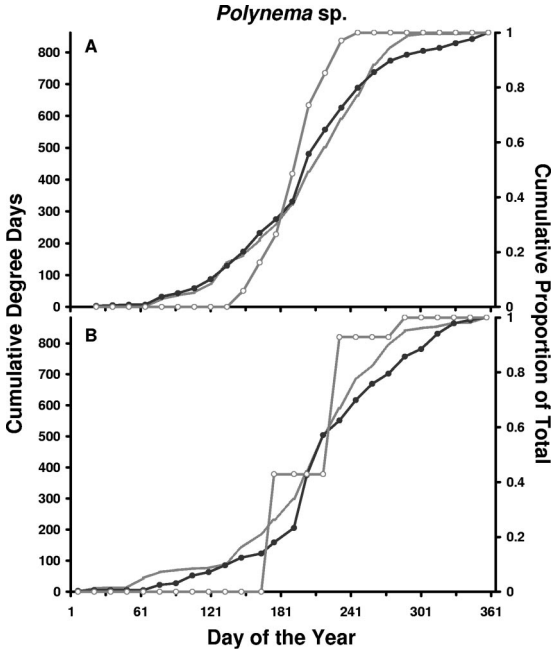

Fig. 7. Cumulative DD (hashed line, left axis), cumulative proportion of total *Polynema* sp. trap catch (closed circles, right axis), and cumulative proportion of total *Polynema* sp. emergence (open circles, right axis) in 2004 (A) and 2005 (B) by day of the year.

year. The blue-green sharpshooter cumulative trap catch rapidly increased at 162 DD and 142 DD in 2004 and 2005, respectively (Fig. 6A and B). Based on daily temperatures in the field over the period of rapid increase, this equates to a 4.7-d average temperature accumulation difference between years for the onset of blue-green sharpshooter trap catch. The combined blue-green sharpshooter and *Polynema* sp. emergence rapidly increased at 162 and 187 DD (only 4.6 d average temperature accumulation difference for the onset of emergence) in 2004 and 2005, respectively (Fig. 6A and B). For both years, the cumulative *Polynema* sp. trap catch closely follows the cumulative DD with a short period of rapid increase occurring at 425 and 388 DD in 2004 and 2005, respectively (Fig. 7A and B). Over the short period of rapid increase this equates to a 5.6 d average temperature accumulation difference between years. Cumulative *Polynema* sp. emergence data rapidly increased at 162 and 232 DD (a 12.1-d average temperature accumulation difference for the onset of emergence) for 2004 and 2005 (Fig. 7A and B).

Discussion

Although blue-green sharpshooter is the most efficient known native vector of *X. fastidiosa* in California (Hewitt et al. 1942, Freitag and Frazier 1954, Purcell 1975), only a minor body of literature exists on the fundamental aspects of its biology and ecology, despite it being a serious pest of California grape production for >100 yr (Winkler 1949, Gardner and Hewitt 1974). Two mymarid egg parasitoids, *G. latipennis* and *Polynema* sp., were reared from blue-green sharpshooter eggs from field-collected plant material. This is the first documented association of these native California parasitoids with their host. The association of *Polynema* sp. with blue-green sharpshooter then was further confirmed through sentinel plant and reciprocal tests on three host plants used by blue-green sharpshooter; sweet basil, wild grape, and chrysanthemum. Because this parasitoid can locate host eggs on various plants, including those not native to blue-green sharpshooter habitat (sweet basil and chrysanthemum), this suggests that the search behavior of *Polynema* sp. may be adapted to host-mediated odor cues or that all plants in suitable blue-green sharpshooter habitat are searched randomly, or perhaps, some combination of these two factors influences *Polynema* sp. searching activity.

Work presented here confirms the discovery of Severin (1949) that blue-green sharpshooter eggs are laid singly and embedded beneath the epidermis of grape "cuttings." However, we found within plant oviposition sites used by blue-green sharpshooter females were not just limited to leaf petioles. Preferred oviposition sites were in new growth, consisting primarily of the terminal 25 cm of succulent canes of *V. girdiana* as well as tendrils along the entire length of the grape cane, with petioles and leaf midribs being used for oviposition to a much lesser extent. Within the terminal 25 cm of cane, blue-green sharpshooter seems to

oviposit in the apical most third, with correspondingly fewer emergences recorded from the middle and lower thirds of this section. Grape tendrils were most frequently used for oviposition irrespective of their location on the cane. Older (large and medium) leaves and respective petioles were not used as oviposition substrates, but younger (small) leaf midribs and petioles were, thus confirming what we found in the entire cane study, and supporting the earlier findings of Severin (1949). These oviposition preferences differ markedly from other cicadellid pests of grape, specifically the western grape leafhopper, *Erythroneura elegantula* Osborn, and *Erythroneura variabilis* Beamer (Hemiptera: Cicadellidae: Eurythroneurini). Both of these species oviposit into epidermal leaf tissue on the undersides of grape leaves (UC-IPM 2005).

In most places on the plant where blue-green sharpshooter eggs were found, *Polynema* sp. was always associated with this cicadellid. The total *Polynema* sp. trap catch was 22% of total blue-green sharpshooter trap catch, parasitization of sentinel plants ranged from 33 to 74%, and overall percentage of parasitization from 2-wk sample emergences was 65%. This indicates *Polynema* sp. may be an important biological control agent, and perhaps the key parasitoid species, for the blue-green sharpshooter in natural riparian habitats of southern California. It is unclear whether *Polynema* sp. uses alternative cicadellid hosts in southern California. However, during the course of sticky card monitoring at both field sites, *Carneocephala triguttata* was trapped during nonpeak blue-green sharpshooter flight activity periods (550 and 141 in winter of 2004 and 2005, respectively; E.A.B., unpublished data), and this species may be an alternate host during periods of low blue-green sharpshooter egg abundance in riparian habitats.

Blue-green sharpshooter adults and nymphs were observed feeding on mugwort, stinging nettle, blackberry, cocklebur, and mint at both study sites. Interestingly, few or no caste exuviae, or mating blue-green sharpshooter adults, were found on these plants suggesting that they may not be preferred oviposition hosts in the habitat sampled, especially if wild grape is abundant. These host plant records for blue-green sharpshooter have been reported previously for northern California (Severin 1949, Winkler 1949, Purcell 1976), and these observations are now confirmed for the first time for blue-green sharpshooter populations in southern California. In 2004 and 2005, trap catch and emergence data confirm that blue-green sharpshooter is univoltine in southern California. Also, peak trap catch occurred at the same time as peak emergence for blue-green sharpshooter and for *Polynema* sp. in 2004. Therefore peak trap catch for blue-green sharpshooter in southern California can be directly linked to peak oviposition activity by this cicadellid on wild *V. girdiana.*

Yellow sticky card trap catch data in 2004 correlated significantly with observed blue-green sharpshooter oviposition and emergence at the two study sites in southern California. Heavy winter rains in 2005 probably promoted the powdery mildew epidemic that caused the subsequent dieback of wild grape at the creek site. Almost 100% grape vine mortality prevented collection of canes for survey work and most probably had an adverse effect on oviposition by blue-green sharpshooter females on wild grapes in the general vicinity of the study sites. Consequently, fewer cane samples may have been responsible for a lack of a significant correlation between emergence and trap catch data in 2005. Furthermore, flight activity in 2005 was approximately one-half of that observed in 2004. Again, this was most likely attributed to disruption of the overwintering population by above normal precipitation, widespread flooding and catastrophic habitat disruption, and a virulent powdery mildew epidemic. However, future sampling of vagile blue-green sharpshooter adults using double-sided yellow sticky traps may be directly correlated with the phenological fluctuations of the blue-green sharpshooter in riparian oak woodland communities dominated by a wild grape understory. Although the use of yellow sticky card traps have been recommended for monitoring blue-green sharpshooter migration from wilderness areas into commercial grape vineyards in northern California (UC-IPM 2005), the information gained in this study indicate this trapping method is also useful in monitoring flight activity of this pest species in southern California.

At the two sites used in this study, it seems that 2-wk average maximum temperatures $\geq$22.5°C are required for blue-green sharpshooter peak flight activities. This peak activity threshold of 22.5°C helps explain sticky card capture for the first peak in 2004, when flight activity increased despite a paucity of oviposition hosts at the study sites. Sticky card capture for the third peak in 2005 was later than that observed in 2004 and may be explained by the sharp decrease in maximum daily temperature (from 27.8°C to 19.5°C) followed by an increase in maximum daily temperature to 25.5°C when flight activity was noticed. Overall, it seems that the first and second peaks in 2004, and the second peak in 2005, are most likely overwintering blue-green sharpshooter adults, whereas the third peak in 2004 and 2005 is most likely the F1 generation produced by surviving overwintering adults. The third peak in 2004 and 2005 may not be representative of the true F1 population density, because winter temperatures declined rapidly and remained relatively low for the remainder of the year. Peak flight activity of *Polynema* sp. monitored with yellow sticky traps indicated a lag of 2 to 4 wk behind blue-green sharpshooter peak trap catch and oviposition. This observed phenology may suggest that blue-green sharpshooter is the major host of *Polynema* sp.

Total cumulative DD above 14.5°C was similar between years even though 2005 winter was the second wettest winter in southern California recorded history. Because daily average temperatures in southern California rarely dropped below 14.5°C, it is not possible to confirm this minimum temperature threshold for flight activity reported by Feil et al. (2000). Despite the differences in environmental conditions between years, the onset of blue-green sharpshooter peak trap

catch began at a similar DD, indicating that the 14.5°C minimum flight threshold temperature (Feil et al. 2000) is adequate in predicting flight activity and oviposition in southern California. Blue-green sharpshooter trap catch was significantly correlated to blue-green sharpshooter oviposition, which also could be predicted by the 14.5°C minimum threshold. This DD model predicted onset of blue-green sharpshooter flight activity and oviposition to within five field days between years. Cumulative *Polynema* sp. trap catch closely followed the DD model indicating this species' flight activity had a strong dependency on temperature throughout the 2-yr study period.

Although these studies were conducted on wild grape, the information acquired from 2 yr of survey work may be of use for improving integrated pest management programs for managing blue-green sharpshooter on grapes in California. Purcell and McBride (1999) showed that blue-green sharpshooter flight activity could be dramatically suppressed through alteration of habitat vegetation. It is not known to what extent this strategy is being used in grape growing areas of California for blue-green sharpshooter control. This tactic uses replacement vegetation that is unsuitable for blue-green sharpshooter oviposition, which is intentionally planted to replace favored host plants in habitats used by blue-green sharpshooter (Purcell and McBride 1999). This vegetation management approach would seem disruptive of the ecology of fragile native riparian habitats.

If vegetation management is a control option that can be pursued responsibly then it may be worthwhile to consider plant management strategies that enhance and preserve resident native parasitoid spp. as a part of a conservation biological control program. The caveat of this approach is not to increase the blue-green sharpshooter populations and thus increase the likelihood of PD transmission to grape crops. More work is needed to develop vegetation management strategies to enhance *Polynema* sp. and *G. latipennis* but not simultaneously improving habitat for blue-green sharpshooter and Pierce's disease. Perhaps through a better understanding of the oviposition preferences of blue-green sharpshooter, its natural enemy complex, and factors affecting pest flight activity, ecologically sound and sustainable management programs, such as conservation biological control, can be developed to suppress populations of this native pest species that threaten commercial agricultural enterprises, especially vineyards adjacent to riparian areas.

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