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Research Article

Reduced Susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to Commonly Applied Insecticides

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Abstract

Pest management for the glassy-winged sharpshooter, *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae), in Kern County, California relies on the application of insecticides. These treatments have contributed to low *H. vitripennis* field counts since applications were initiated in 2001. However, densities have been high in recent years despite continued management, prompting efforts to evaluate the susceptibility of current populations to insecticides. *H. vitripennis* adults were subjected to bioassays with five commonly applied insecticides, and the results were compared to baseline toxicities determined in 2002. Two neonicotinoids, imidacloprid and thiamethoxam, were evaluated using systemic uptake bioassays. Contact toxicities of the neonicotinoid acetamiprid and pyrethroids bifenthrin and fenpropathrin were estimated using leaf dip bioassays. Dose-mortality responses were analyzed by probit analysis. For each compound, there was no significant difference in annual LC_{50} values determined over 2 yr. Compared to baseline toxicities, acetamiprid and bifenthrin were found to be significantly less toxic to *H. vitripennis*. The LC_{50} values of these two compounds increased sevenfold and 152-fold, respectively. Tests with the neonicotinoids revealed a trend of decreasing susceptibility levels within each season followed by reversion back to early season LC_{50} estimates in the following year. In addition, data showed seasonal and site variation in susceptibility to imidacloprid, possibly due to differential applications in nearby fields.

Key words: glassy-winged sharpshooter, resistance, neonicotinoid, pyrethroid, bioassay

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae), is a major pest of economically important perennial crops and a vector of the plant pathogenic bacterium *Xylella fastidiosa* (Wells et al. 1987). The native range of *H. vitripennis* is the southeastern United States and eastern Mexico, but it was introduced in the 1980s to California, where it established as a pest with economic significance (Sorensen and Gill 1996, Blua et al. 1999). Its importance primarily lies in its ability to transmit the causal agent of Pierce's disease (PD) of grapes, *X. fastidiosa*, a xylem-limited, Gram-negative bacterium with several different strains and many hosts, including wine and table grapes (Davis et al. 1978, Wells et al. 1987, Almeida and Purcell 2003). In California, *H. vitripennis* transmission of *X. fastidiosa* threatens the over $5 billion grape production industry as well as production values of over $7 billion in almonds, citrus, stone fruits, and shade trees combined (CDFA PDCP 2016).

In agricultural areas of California, where citrus is the preferred overwinter and reproductive host of *H. vitripennis* (Blua et al. 2001), pest management within orchards is central to the control of *H. vitripennis* and PD. Vineyards in close proximity to citrus orchards are at high risk of PD outbreaks (Perring et al. 2001; Park et al. 2006, 2011). Unlike the southern California citrus growing regions, which have relied on biological control as a central component of citrus management, pest management within the San Joaquin Valley, including Kern County, has relied principally on insecticide applications (Grafton-Cardwell 2000, Morse et al. 2006, Sisterson et al. 2008). Under the administration of the United States Department of Agriculture (USDA) in concert with the California Department of Food and Agriculture (CDFA) and county departments of agriculture, the Glassy-winged Sharpshooter Area Wide Management Program has been successful in reducing GWSS numbers since applications were initiated in 2001. However, from 2012 to 2015, *H. vitripennis* densities in portions of Kern Co. have been at levels similar to those before the establishment of the program (Haviland 2015).

Persistent use of chemical insecticides as a primary means of pest management has long been recognized as a likely route to the development of resistance. In a previous study, *H. vitripennis* baseline susceptibilities to 10 pesticides were established (Prabhaker et al. 2006). Three different regional populations of *H. vitripennis*, including the Kern Co. population, were bioassayed in 2001 and 2002 to determine their pesticide susceptibility levels at that time. Subsequent bioassays compared to these baseline toxicities could demonstrate any shifts in toxicity to *H. vitripennis*, which could be used to facilitate pesticide use decisions for GWSS management. The purpose of this study was to determine if *H. vitripennis* has become less susceptible to five insecticides commonly applied for pest management in Kern Co.

Materials and Methods

H. vitripennis Field Collections

Collections of *H. vitripennis* adults were made using 15-inch sweep nets on citrus and grapes and bucket samples on citrus (Castle et al. 2005). In the field, insects were transferred to 60 × 60 × 60 cm insect rearing tents (BugDorm Store, MegaView Science Co., Ltd., Taiwan) containing basil (*Ocimum basilicum* L. variety Genovese; Johnny's Selected Seed, Fairfield, ME) for interim feeding and transportation to the University of California (UC) Cooperative Extension Kern County station (2015 and 2016 collections) or to UC Riverside (2017 collections) for bioassay. Bioassays that were performed on the collections transported to the UC Cooperative Extension station were prepared immediately upon arrival, while those transported to UC Riverside were maintained at 23 ± 2°C and a photoperiod of 12:12 (L:D) h for 24 h after collection. To minimize control mortality in 2017, only those insects that were actively feeding on basil after 24 h were chosen for bioassays.

To evaluate the susceptibility of *H. vitripennis* to various insecticides in Kern Co., field collections of *H. vitripennis* were made in different locations throughout the county in the summer and early fall of three consecutive years, 2015–2017. In 2015, *H. vitripennis* was collected from organic citrus groves in the Edison, CA area on three dates in July and August. In September and October, collections were made on three dates from organic citrus located on General Beale Road (GBR). In 2016, with lower *H. vitripennis* numbers throughout Kern Co., collections were made on four dates in July, August, and October. In July and August, *H. vitripennis* was collected from table grapes in the GBR area, while collections in October were from organic navel oranges in the same area.

In 2017, *H. vitripennis* were collected from organic citrus at two sites in the Edison area and two sites north of Bakersfield, CA along Highway 65 on four dates from July to October. These sites were selected at varying distances from imidacloprid-treated host sites to determine if local application had an impact on susceptibility to this insecticide (Fig. 1). Pesticide treatment records from 1 January to 9 October 2017 and GWSS trap counts were obtained from the CDFA GWSS and PD Control Program and uploaded to a Geographic Information System (ArcGIS, ESRI, Redlands, CA) containing land use data. Geo-referencing treatment records and area-wide trapping information informed the selection of two sites within 0.5 miles of an early season (April, May) imidacloprid application and two sites with no early season imidacloprid applications within at least 1.5 miles (Fig. 1). Each collection site was in a citrus orchard and had relatively high trap counts in June (most sites with over 200 adults per trap) when collections began. Two sites were located in the Edison region, W Edison (near early imidacloprid application) and E Edison (not near application), and two sites were off of Highway 65, N Hwy 65 (near application) and S Hwy 65 (not near application). *H. vitripennis* were collected from these four sites and monitored for imidacloprid susceptibility throughout the 2017 season.

Insecticides

The insecticide susceptibility of *H. vitripennis* was tested on five products comprising two insecticide classes including both contact and systemic activity (Table 1). All insecticides were tested on *H. vitripennis* adults. In early 2015 bioassays, differing concentrations of the various chemicals were tested to determine the appropriate range of experimental doses. Following this procedure, we identified at least five concentrations of each pesticide, which were prepared by serial dilution with DI water, and controls were tested with DI water. Bioassays were conducted using all five products on at least three different dates in 2015 and at least one date in 2016 due to considerably lower *H. vitripennis* densities throughout Kern Co. than in 2015. In anticipation of low population levels in 2017, and because of its widespread and frequent use in citrus groves and vineyards, only imidacloprid was used in bioassays. Focus on this one insecticide allowed for its use as a standard for comparing insecticide susceptibilities of *H. vitripennis* collected from sites with unique patterns of local imidacloprid applications over the season (Fig. 1) as well as evaluating susceptibility as the season progressed.

Bioassay Techniques

Systemic Insecticides

Susceptibility to the systemic neonicotinoid pesticides imidacloprid and thiamethoxam was evaluated using uptake bioassays adapted for excised citrus leaves (Prabhaker et al. 2005, Prabhaker et al. 2006). Non-treated grapefruit (*Citrus x paradisi* Macfadyen) terminal leaves with 2- to 3-inch stems were excised, washed with DI water, and air-dried. Leaf stems were placed in 3-inch aquatubes (Syndicate Sales Inc., Kokomo, IN) with lids cut to hold the stems. Each aquatube contained 9 ml of insecticide serial dilution or water, and uptake of these materials was allowed for 24 h. A minimum of five replicates were performed for each bioassay. After 24 h of uptake, five *H. vitripennis* adults were briefly anesthetized (<10 s) with CO_2 and transferred to 7-cm^2 clip cages. The clip cages were attached to the leaves with *H. vitripennis* exposed to the abaxial surface for 24 h with uptake of chemicals continuing through the exposure period. After this period, the number of dead GWSS were counted in each clip cage; immobile insects were counted as dead. Bioassays were maintained at 23 ± 2°C and a photoperiod of 12:12 (L:D) h.

Foliar Insecticides

Leaf dip bioassays were performed following the procedures of Prabhaker et al. (2006) for the foliar insecticides acetamiprid, bifenthrin, and fenpropathrin. Briefly, terminal leaves of non-treated grapefruit trees were excised and washed as previously mentioned and immersed in insecticide serial dilutions and water controls for 30 s. Dipped leaves were air-dried for 1 h, and the leaf stems were placed through the lids in 3-inch aquatubes filled with water to maintain leaf freshness and encourage feeding on the leaf. A minimum of five replicates were performed for each bioassay. Clip cages were attached to the abaxial surface of the leaf, and five adult *H. vitripennis* were placed in each cage for exposure to treatments. Mortalities were recorded after a 24-h exposure period as previously described.

Data Analysis

Probit analysis using POLO software was used to estimate LC_{50} values, 95% fiducial limits (FL), slopes of the regression lines, and

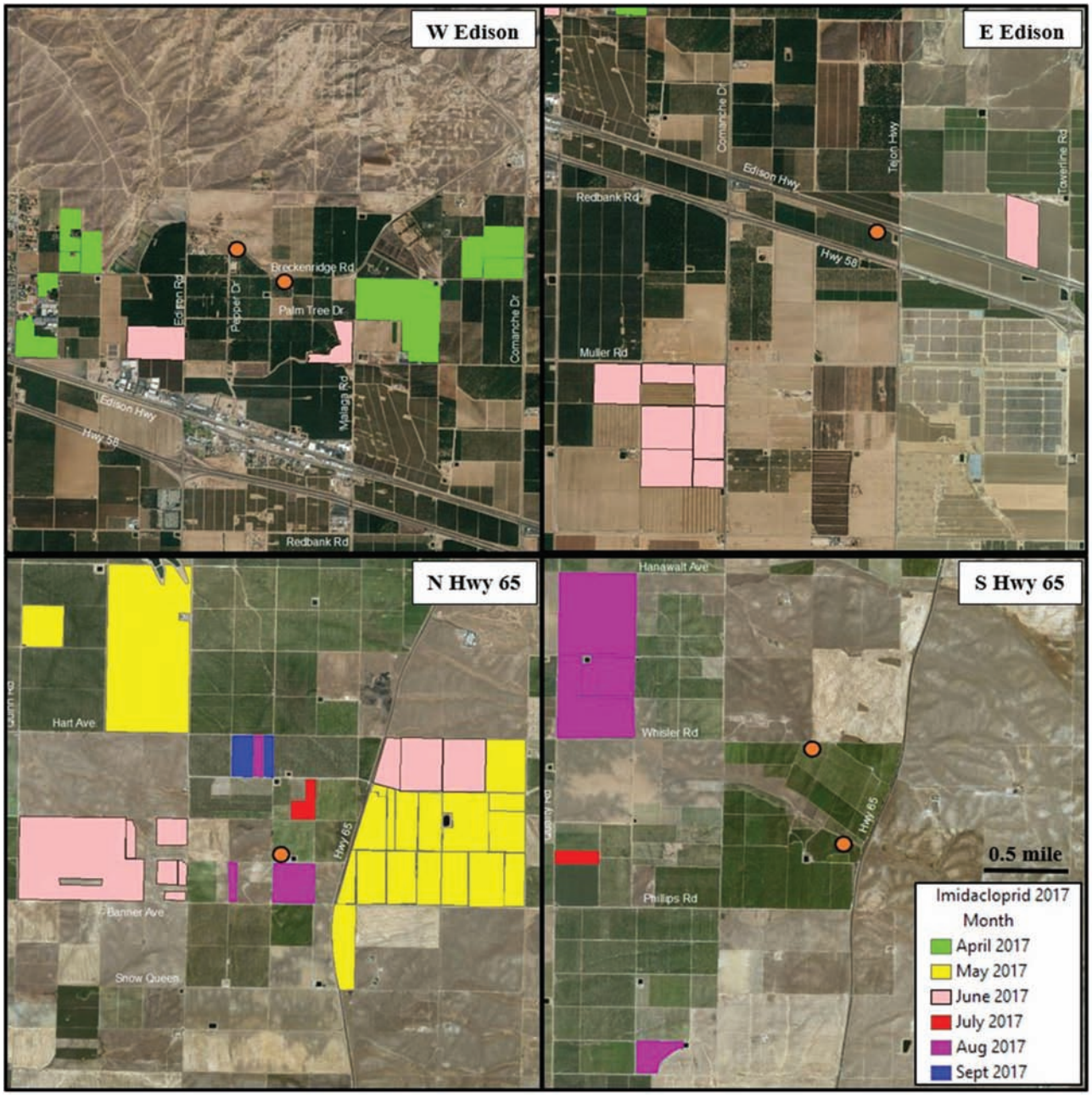

Fig. 1. Locations of *Homalodisca vitripennis* collections in 2017. Orange dots represent the exact collection sites. In the upper left quadrant is site W Edison; upper right is E Edison; lower left is N Hwy 65; and lower right is S Hwy 65. Each quadrant contains the approximately 3 mi² region surrounding each site. The legend indicates the months in which imidacloprid applications were made to field near the collection sites.

Table 1. Insecticides tested against *Homalodisca vitripennis* populations in Kern County, California in 2015–2017

Insecticide class	Compound	Product name	Manufacturer
Neonicotinoid	Imidacloprid	Admire Pro	Bayer, Research Triangle Park, NC
	Thiamethoxam	Platinum 75 SG	Syngenta, Greensboro, NC
	Acetamiprid	Assail 70 WP	UPI, King of Prussia, PA
Pyrethroid	Bifenthrin	Capture 2 EC	FMC, Philadelphia, PA
	Fenpropathrin	Danitol 2.4 EC	Valent USA, Walnut Creek, CA

chi-square (χ^2) goodness of fit tests (Russell et al. 1977, Roberston et al. 1980). Estimates were reported for the individual bioassays performed for each compound tested per collection date. Annual LC_{50} estimates were calculated by combining all bioassays per compound performed in the same year. Overall LC_{50} estimates were calculated for each compound by combining all bioassays performed over the years when the annual LC_{50} values were not statistically different. In the 2017 imidacloprid bioassays, individual LC_{50} estimates were

calculated on populations collected from different sites in the same day. All bioassays from a given day were combined to determine the total LC_{50} estimate, and all bioassay results from 2017 were combined to calculate an annual LC_{50}. Datasets demonstrating high variability in bioassay response were analyzed with PoloSuite probit analysis to obtain approximations of LC_{50} values along with 95% FL and regression slopes (Robertson et al. 2007). Significance was attributed to differences in LC_{50} values which had no overlap in the 95% FL. When 95% FL were indeterminable, 90% FL were reported.

Additionally, for data collected on imidacloprid in 2017, a generalized linear mixed model (GLMM) with a binary response (dead/alive) and a binomial distribution (glmer function in R 3.4.3, R Core Team 2017) was applied to test the effects of sampling dates, locations, and imidacloprid concentrations on mortality of the insects. In the model, date, location, and concentration were considered as fixed effects and clip cage was treated as a random effect. Subsequently, the glht function was used to conduct Tukey pair-wise comparisons of mortality among different sampling dates ($P < 0.05$). In a similar manner, mortality data at the N and S Hwy 65 locations were fit to a GLMM with a binomial distribution. Again, date and concentration were considered as fixed effects, and clip cage was considered as a random effect. The GLMM was followed by Tukey's test to compare the mean mortality rates among different sampling dates ($P < 0.05$).

Results

Toxicities of Systemic Insecticides

Imidacloprid

H. vitripennis susceptibility to imidacloprid was tested over 3 yr, 2015–2017 (Table 2). Annual LC_{50} totals were similar over the 3 yr, ranging from 2.51 to 3.43 µg/ml. Each year, tests performed in July demonstrated lower LC_{50} values than those performed in October, with a general trend of increasing LC_{50} values over the testing season. In 2015, LC_{50} values increased 28-fold from early July (LC_{50} = 0.38 µg/ml) to late October (LC_{50} = 10.65 µg/ml) with no overlap in 95% FL, indicating a significant difference. The high χ^2 determined for the 22 July bioassay indicates significant variability in GWSS responses to the various doses. In addition, when combining the bioassays across the 2015 season, some of which had significantly different LC_{50} values, the resulting annual χ^2 indicates significant variability in dose responses over the season. In 2016, there was a threefold increase from early (LC_{50} = 4.00 µg/ml) to late (LC_{50} = 12.58 µg/ml) season; these values were not significantly different. In 2017, an overall 4.5-fold increase was observed from the total July LC_{50} value (1.91 µg/ml) to the total October LC_{50} (8.71 µg/ml), and these values were not significantly different based on the overlapping 95% FL. Though LC_{50} values were relatively high at the end of each season, susceptibility increased (LC_{50} values decreased) by June/July of the following year, suggesting that the seasonal

Table 2. Toxicities of imidacloprid to *Homalodisca vitripennis* determined in uptake bioassays in multiple locations in Kern County, CA

Year	Date	Location	*n*	LC_{50} µg/ml (95% FL)	Slope ± SE	χ^2 (df)
2015	9 July	Edison	226	0.38 (0.16–0.76)	0.96 ± 0.15	4.24 (6)
	22 July	Edison	175	[a]1.92 (0.10–955.01[b])	1.28 ± 0.18	22.07 (3)
	3 Aug.	Edison	320	3.98 (2.32–6.21)	1.10 ± 0.14	2.30 (4)
	16 Sept.	GBR	150	7.03 (2.73–23.30)	0.63 ± 0.11	0.10 (3)
	7 Oct.	GBR	150	3.41 (0.90–10.79)	0.64 ± 0.14	1.86 (3)
	21 Oct.	GBR	150	10.65 (5.27–22.68)	1.10 ± 0.19	2.48 (3)
	Annual	Kern Co.	1,171	2.51 (0.98–5.29)	0.77 ± 0.06	53.68 (13)
2016	27 July	GBR	150	4.00 (1.21–15.03)	1.18 ± 0.19	3.33 (3)
	16 Aug.	GBR	124	[a]0.05 (0.000–1.73[b])	0.55 ± 0.17	5.44 (2)
	4 Oct.	GBR	151	9.46 (2.75–30.51)	0.72 ± 0.17	2.30 (3)
	19 Oct.	GBR	150	12.58 (5.48–36.43)	0.78 ± 0.13	0.86 (3)
	Annual	Kern Co.	575	3.43 (0.61–17.76)	0.74 ± 0.07	10.02 (3)
2017	24 July	E Edison	270	4.01 (0.63–11.31)	1.26 ± 0.23	3.15 (3)
		W Edison	140	[a]0.38 (0.02–12.49)	0.88 ± 0.13	9.12 (3)
		S Hwy 65	150	0.80 (0.13–2.07)	1.29 ± 0.36	2.46 (3)
		N Hwy 65	150	1.79 (0.54–3.98)	1.50 ± 0.37	1.73 (3)
		Total	710	1.91 (0.57–4.21)	1.29 ± 0.15	4.80 (3)
	8 Aug.	E Edison	238	1.27 (0.26–4.73)	0.95 ± 0.12	4.71 (3)
		W Edison	50	[a]1.12 (0.03–22.72)	0.90 ± 0.20	3.57 (3)
		S Hwy 65	237	0.56 (0.09–2.09)	1.11 ± 0.15	5.48 (3)
		N Hwy 65	59	[a]0.13 (0.08–0.18)	1.37 ± 0.58	0.09 (3)
		Total	584	0.70 (0.09–3.07)	0.99 ± 0.08	14.84 (3)
	12 Sept.	S Hwy 65	150	[a]8.99 (1.00–47.78[b])	1.15 ± 0.25	6.48 (3)
		N Hwy 65	150	51.53 (21.33–204.99)	1.02 ± 0.27	2.50 (3)
		Total	300	22.12 (12.05–40.89)	1.07 ± 0.19	0.79 (3)
	9 Oct.	S Hwy 65	504	8.71 (2.93–27.28)	0.89 ± 0.09	5.62 (3)
	Annual	Kern Co.	2,098	2.90 (1.05–6.45)	0.88 ± 0.05	11.59 (3)
Overall: 2015–2017		Kern Co.	3,844	2.91 (1.93–4.21)	0.82 ± 0.04	47.27 (15)
2001		Kern Co.	312	1.27 (0.68–2.54)	1.1 ± 0.30	6.24 (4)
2002		Kern Co.	295	0.36 (0.09–0.51)	1.2 ± 0.35	4.76 (4)

Toxicity levels in 2001 and 2002 from Prabhaker et al. 2006 are included for comparison.

[a]LC_{50} determined by probit analysis using PoloSuite because of high variability in dose responses.

[b]90% FL reported in place of indeterminable 95% FL.

decrease in imidacloprid toxicity to *H. vitripennis*, whether significant or not, did not continue into the next year. In both 2016 and 2017, annual LC_{50} estimates had high χ^2 values, indicating that when combining the bioassays within each year, there was significant variation in the dose responses.

Considering specific 2017 sites independently, the observed seasonal increase was higher and significant at two particular sites, N Hwy 65 and S Hwy 65. At N Hwy 65, the LC_{50} declined slightly from 1.79 µg/ml in July to 0.13 µg/ml in August, but then increased significantly to 51.53 µg/ml in September, a 29-fold change from July to September. LC_{50} values for *H. vitripennis* collected from S Hwy 65 had similar trends, decreasing slightly from 0.80 µg/ml in July to 0.56 µg/ml in August before a significant increase to 8.71 µg/ml in October, an 11-fold change from July to October (Table 2). Bioassays on GWSS collected at the East and West Edison locations had low LC_{50} values in July (4.01 and 0.38 µg/ml, respectively), and the subsequent collections in August were not significantly different from the July values (1.27 and 1.12 µg/ml, respectively). Unfortunately, there were insufficient GWSS numbers present at these two sites in September, so we were unable to determine if the trends that were present in the Hwy 65 sites existed in the Edison locations.

Further analyses using the GLMM showed that the fixed effects of date, location and concentration were significantly different ($P < 0.01$). Mean comparison showed that combined mortality from all sites decreased significantly over the season, from a high of 50.5% on 24 July to 23.7% and 29.6% on 12 September and 9 October, respectively (Table 3). The same trend was observed when mortality was compared between different dates within the Hwy 65 sites. At S Hwy 65, the highest mortality was observed on 24 July, and it decreased significantly over the season. The lowest mortality at this location was 27.3% on 12 September, which was not significantly different from 9 October (29.6%). At N Hwy 65, the mortality was 53.3 and 62.1% in the months of July and August, respectively. While the mortality did not change significantly from July to August, it was significantly lower later in September (20.0%) (Table 3).

Thiamethoxam

The toxicity of another systemic neonicotinoid, thiamethoxam, to *H. vitripennis* was tested by uptake bioassays. Over the 2 yr of testing, LC_{50} values ranged from 0.13 to 2.96 µg/ml, with annual estimates of 0.74 µg/ml for 2015 and 1.48 µg/ml for 2016 (Table 4). As seen in the imidacloprid bioassays, susceptibility to thiamethoxam decreased over each season. These results varied throughout the season more than with imidacloprid, but in both years, there was an increase in LC_{50} values from July to October. In 2015, this increase was ninefold from early July (LC_{50} = 0.13 µg/ml) to late October (LC_{50} = 1.20 µg/ml) with a small overlap in 95% FL (0.548–0.551 µg/ml). In 2016, there was a fourfold increase from late July (LC_{50} = 0.53 µg/ml) to late October (LC_{50} = 2.06 µg/ml) and these differences were statistically different. Also similar to the imidacloprid results, the increase observed over the 2015 season did not carry into 2016. The susceptibility was nearly the same in late July of 2016 (LC_{50} = 0.53 µg/ml) as it was in late July of 2015 (LC_{50} = 0.52 µg/ml), and the annual LC_{50} estimates were not significantly different. For both test years, the individual bioassays had low χ^2 values, indicating that dose-response variation was not significant in the individual bioassays. The χ^2 values for the annual and overall LC_{50} estimates were significant, reflecting the variation among the seasonal bioassays.

Toxicities of Foliar Insecticides

Acetamiprid

Susceptibility to acetamiprid, a neonicotinoid with contact activity, was tested on three dates each in 2015 and 2016. In both years, the annual LC_{50} were determined to be 2.88 µg/ml and 0.94 µg/ml, respectively; these were not significantly different (Table 5). Susceptibility significantly declined 14-fold from July (LC_{50} = 0.74 µg/ml) to late October of 2015 (LC_{50} = 10.41 µg/ml), but it remained at a relatively constant level throughout the 2016 season. *H. vitripennis* were more susceptible to acetamiprid early in the second year of testing than they were at the end of the first year, suggesting that susceptibility tends to decrease over a season but then is higher early the following year. All combined LC_{50} estimates (annual and overall) and all individual bioassays but one (27 July 2016) showed nonsignificant χ^2 values, indicating that variation in dose response among the bioassays was relatively low.

Bifenthrin

Annual susceptibilities to bifenthrin were statistically similar with overlapping FL in 2015 and 2016 at 0.54 µg/ml and 1.03 µg/ml, respectively (Table 6). In 2015, susceptibility varied and did not follow a consistent pattern over the season. However, over the entire season in both years, late-season susceptibility was numerically lower than early season, but these shifts were not significant. The χ^2 values in all but one test (4 October 2016) were nonsignificant.

Fenpropathrin

Another pyrethroid, fenpropathrin, was tested on five collection dates in 2015 but could be tested on only one date in 2016 (Table 7). The toxicity of fenpropathrin ranged from 0.05 µg/ml (95% FL: 0.02–0.11 µg/ml) to 3.12 µg/ml (95% FL: 0.82–7.90 µg/ml) in 2015. The LC_{50} values tended to be higher in the GBR population than in the Edison population, though there was overlap in some of the 95% FL, and the GBR population was tested later in the season. In 2016, toxicity from the single sampling date in the GBR region was not significantly different from the other GBR collections nor the 2015 annual estimate. Variation in dose response was low for both individual and combined bioassays, as indicated by the nonsignificant χ^2 values.

Table 3. Imidacloprid-induced mortality of *Homalodisca vitripennis* collected in 2017 at different locations in Kern County, CA analyzed by a GLMM

Year	Date	Combined mortality (%)	S Hwy 65 mortality (%)	N Hwy 65 mortality (%)
2017	24 July	50.5 (147)a	61.3 (30)a	53.3 (30)a
	8 Aug.	46.4 (120)b	47.5 (48)b	62.1 (12)a
	12 Sept.	23.7 (60)c	27.3 (30)c	20.0 (30)b
	9 Oct.	29.6 (101)c	29.6 (101)c	—

Values within the same column followed by the same letter are not significantly different, Tukey's test ($P < 0.05$). The number of replicates (clip cages containing five insects) on each date are given in parentheses.

Table 4. Toxicities of thiamethoxam to *Homalodisca vitripennis* determined in uptake bioassays in multiple locations in Kern County, CA

Year	Date	Location	*n*	LC_{50} µg/ml (95% FL)	Slope ± SE	χ^2 (df)
2015	9 July	Edison	175	0.13 (0.06–0.55)	1.42 ± 0.20	7.57 (4)
	22 July	Edison	150	0.52[a] (0.000–2.38)	1.81 ± 0.47	3.97 (3)
	16 Sept.	GBR	150	1.74 (0.85–3.55)	0.98 ± 0.14	1.94 (3)
	7 Oct.	GBR	150	2.03 (0.82–4.16)	1.18 ± 0.23	1.21 (3)
	21 Oct.	GBR	150	1.20 (0.55–2.60)	0.85 ± 0.12	1.83 (3)
	Annual	Kern Co.	775	0.74 (0.35–1.50)	0.93 ± 0.07	15.53 (6)
2016	27 July	GBR	143	0.53[a] (0.49–0.56)	2.45 ± 0.51	0.02 (3)
	16 Aug.	GBR	125	2.96[a] (0.000–139.02)	0.75 ± 0.15	2.16 (2)
	4 Oct.	GBR	145	1.30[a] (0.000–244.92)	1.37 ± 0.22	4.03 (2)
	19 Oct.	GBR	150	2.06 (0.95–4.61)	0.84 ± 0.13	1.71 (3)
	Annual	Kern Co.	563	1.48 (0.35–4.94)	1.02 ± 0.08	11.33 (3)
Overall: 2015–2016		Kern Co.	1,338	1.03 (0.54–1.87)	0.97 ± 0.05	20.67 (6)

[a]LC_{50} determined by probit analysis using PoloSuite because of high variability in dose responses.

Table 5. Toxicities of acetamiprid to *Homalodisca vitripennis* determined in leaf dip bioassays in multiple locations in Kern County, CA

Year	Date	Location	*n*	LC_{50} µg/ml (95% FL)	Slope ± SE	χ^2 (df)
2015	9 July	Edison	150	0.74 (0.38–1.44)	1.10 ± 0.16	1.81 (3)
	7 Oct.	GBR	150	3.19 (0.23–68.59)	0.59 ± 0.11	4.20 (3)
	21 Oct.	GBR	150	10.41 (5.18–22.64)	1.03 ± 0.17	1.36 (3)
	Annual	Kern Co.	450	2.88 (1.06–8.13)	0.77 ± 0.07	4.41 (3)
2016	27 July	GBR	150	0.40[a] (0.006–46.89)	0.97 ± 0.14	10.78 (3)
	4 Oct.	GBR	150	1.76 (0.66–5.15)	0.59 ± 0.10	0.66 (3)
	19 Oct.	GBR	150	0.42 (0.02–3.03)	0.30 ± 0.09	1.90 (3)
	Annual	Kern Co.	450	0.94 (0.15–3.59)	0.59 ± 0.07	4.23 (3)
Overall: 2015–2016		Kern Co.	900	1.78 (1.11–2.75)	0.67 ± 0.05	2.36 (3)
2001		Kern Co.	315	0.44 (0.18–0.56)	2.0 ± 0.14	4.85 (4)
2002		Kern Co.	320	0.08 (0.02–0.14)	1.4 ± 0.11	3.87 (3)

Toxicity levels in 2001 and 2002 from Prabhaker et al. 2006 are included for comparison.
[a]LC_{50} determined by probit analysis using PoloSuite because of high variability in dose responses.

Table 6. Toxicities of bifenthrin to *Homalodisca vitripennis* determined in leaf dip bioassays in multiple locations in Kern County, CA

Year	Date	Location	*n*	LC_{50} µg/ml (95% FL)	Slope ± SE	χ^2 (df)
2015	9 July	Edison	151	0.43[a] (0.000–2.33)	1.57 ± 0.38	4.53 (3)
	22 July	Edison	150	0.16 (0.05–0.40)	0.83 ± 0.14	2.69 (3)
	16 Sept.	GBR	145	2.36[a] (0.004–11.48)	1.26 ± 0.30	3.91 (3)
	7 Oct.	GBR	150	0.18 (0.04–0.55)	0.59 ± 0.11	0.69 (3)
	21 Oct.	GBR	150	3.04 (1.23–8.23)	0.69 ± 0.11	2.94 (3)
	Annual	Kern Co.	746	0.54 (0.21–1.15)	0.74 ± 0.06	3.15 (3)
2016	27 July	GBR	152	0.70 (0.38–1.28)	1.30 ± 0.20	0.43 (3)
	4 Oct.	GBR	150	1.54[a] (0.006–12,862.05)	0.97 ± 0.14	12.29 (3)
	Annual	Kern Co.	302	1.03 (0.29–3.72)	1.09 ± 0.11	6.73 (3)
Overall: 2015–2016		Kern Co.	1,048	0.67 (0.30–1.29)	0.82 ± 0.06	4.00 (3)
2001		Kern Co.	312	0.0005 (0.0002–0.0038)	1.4 ± 0.24	3.76 (4)
2002		Kern Co.	320	0.0126 (0.0085–0.0347)	1.7 ± 0.32	2.88 (4)
2003		Kern Co.	285	0.0001 (0.00009–0.0004)	2.9 ± 0.27	2.64 (4)

Toxicity levels in 2001–2003 from Prabhaker et al. 2006 are included for comparison.
[a]LC_{50} determined by probit analysis using PoloSuite because of high variability in dose responses.

Discussion

H. vitripennis populations in Kern County, California have been under constant monitoring by the CDFA Glassy-winged Sharpshooter Area Wide Management Program since 2001. Shortly after the program's initiation, *H. vitripennis* numbers in the region remained low (less than 40,000 trapped per year) through 2011 (Haviland 2015). In 2012, populations rose sharply, remaining high through 2013 and 2014 relative to previous counts, then spiked again in 2015 (nearly 200,000 trapped). The unprecedented numbers at this time led to the working hypothesis that *H. vitripennis* was developing resistance to commonly applied chemical pesticides in Kern County.

Table 7. Toxicities of fenpropathrin to *Homalodisca vitripennis* determined in leaf dip bioassays in multiple locations in Kern County, CA

Year	Date	Location	*n*	LC_{50} µg/ml (95% FL)	Slope ± SE	χ^2 (df)
2015	9 July	Edison	150	0.19 (0.02–1.45)	0.87 ± 0.14	5.53 (3)
	22 July	Edison	150	0.05 (0.02–0.11)	0.79 ± 0.15	2.39 (3)
	16 Sept.	GBR	145	3.12 (0.82–7.90)	0.87 ± 0.20	0.12 (3)
	7 Oct.	GBR	140	0.30[a] (0.001–5.94[b])	0.41 ± 0.11	5.09 (3)
	21 Oct.	GBR	150	0.57 (0.02–7.07)	0.66 ± 0.11	5.34 (3)
	Annual	Kern Co.	735	0.33 (0.19–0.54)	0.60 ± 0.05	3.46 (4)
2016	27 July	GBR	150	0.80 (0.32–1.70)	1.13 ± 0.20	1.13 (3)
Overall: 2015–2016		Kern Co.	885	0.40 (0.19–0.77)	0.66 ± 0.05	4.45 (4)
2001		Kern Co.	306	0.064 (0.045–0.205)	1.2 ± 0.21	5.82 (4)
2002		Kern Co.	215	0.020 (0.007–0.060)	1.1 ± 0.25	4.76 (4)

Toxicity levels in 2001 and 2002 from Prabhaker et al. 2006 are included for comparison.

[a]LC_{50} determined by probit analysis using PoloSuite because of high variability in dose responses.

[b]90% FL reported in place of indeterminable 95% FL.

In 2001 and 2002, Prabhaker et al. (2006) developed and conducted bioassays on *H. vitripennis* which established the baseline susceptibility levels of different populations of the pest, including those in Kern County, to various insecticides. Determination of the current *H. vitripennis* susceptibility levels could indicate if resistance to these insecticides was occurring. After the sudden upsurge followed by sustained high *H. vitripennis* population densities in Kern County, this study aimed to determine if pesticide resistance could be a factor contributing to the pest's increase within recent years.

Five of the 10 previously tested insecticides were chosen for susceptibility monitoring within this study. Each of these chemical compounds, comprising two classes of insecticides, were applied to known *H. vitripennis* host crops, including both citrus and grapes, within Kern County between 2001 and 2017 (CDFA PDCP 2016). The baseline susceptibility LC_{50} values established previously in Prabhaker et al. (2006) were compared to current LC_{50} values using similar bioassay methodology.

Uptake bioassays were performed to test *H. vitripennis* susceptibility to the neonicotinoids imidacloprid and thiamethoxam. The annual LC_{50} values determined for imidacloprid in 2015, 2016, and 2017 ranged from 2.51 to 3.43 µg/ml (Table 2). These values were not significantly different; therefore all bioassays performed during this time were combined to determine an overall current susceptibility level of 2.91 µg/ml with 95% FL of 1.93–4.21 µg/ml. The average LC_{50} value between 2001 and 2002 was 0.82 µg/ml. From the previous study to the present one, there has been a 3.5-fold decrease in susceptibility of *H. vitripennis* to imidacloprid. When taking previously determined 95% FL into consideration for determining significant differences, the 2001 95% FL (0.68–2.54 µg/ml) overlaps with the current 95% FL. Therefore, the 3.5-fold susceptibility decrease is not significant.

It is important to consider one difference between the techniques used in the previous bioassay (Prabhaker et al. 2006) and those used in the present study. Previously, the excised leaves were removed from the pesticide solutions after 24 h of uptake and placed in water during the 24 h GWSS exposure period. However, in the current study, leaves remained in the chemical solution for the 24 h exposure period. This difference could have resulted in higher mortality rates in the current bioassays due to more chemical exposure than in the previous study. If so, the current uptake bioassays overestimated the relative toxicity and had the leaves been removed from the additional chemical uptake; we might expect to have higher survival, concluding a less susceptible population now versus 2001–2002.

For thiamethoxam, which can be applied as a systemic or a foliar insecticide, the overall 2015–2016 50% lethal concentration was 1.03 µg/ml (95% FL: 0.54–1.87 µg/ml) (Table 4). Thiamethoxam was tested in this study by uptake bioassay as a systemic pesticide, as this has been the typical mode of application of this chemical for pest control on citrus and grapes in Kern County. When this compound was tested on *H. vitripennis* previously, it was bioassayed as a foliar insecticide, which was its primary mode of application in 2001 and 2002. Therefore, the current susceptibility levels cannot be compared to those determined previously. Instead, this is the first study to test the systemic toxicity of thiamethoxam to *H. vitripennis*. The levels determined herein establish the baseline toxicity of this chemical to GWSSs in Kern County.

The contact toxicities of the remaining three compounds, acetamiprid, bifenthrin, and fenpropathrin, were tested by leaf dip bioassay. In the previous study by Prabhaker et al. (2006), two methods of testing foliar insecticides, leaf dip and Petri dish bioassays, were compared. Results showed that there were no consistent significant differences in the LC_{50} values obtained by one method versus the other. Therefore, comparison of the results of current leaf dip bioassays to the results obtained previously by Petri dish bioassay, as was the case for bifenthrin and fenpropathrin, should not introduce any bias for or against changes in susceptibility. Leaf dip bioassays were completed for acetamiprid and bifenthrin in the previous study.

The average baseline susceptibility of *H. vitripennis* to acetamiprid in 2001 and 2002 was 0.26 µg/ml. The overall toxicity of acetamiprid determined in this study was 1.78 µg/ml (95% FL: 1.11–2.75 µg/ml) (Table 5). This change in susceptibility represents a sevenfold decrease. When comparing the current 95% FL range to the previous 95% FL range (2001: 0.18–0.56; 2002: 0.02–0.14), the difference in susceptibility from then to now is significant. Although only sevenfold, this increase in the LC_{50} values represents a significant shift in *H. vitripennis* susceptibility to acetamiprid, indicating that resistance could be developing.

Bioassay of the two pyrethroid insecticides showed a significant shift in susceptibility to bifenthrin and nonsignificant changes in the susceptibility to fenpropathrin. In previous studies, bifenthrin was tested for 3 yr (2001–2003) with variability among the 3 yr (Prabhaker et al. 2006). The 2015–2016 tests showed little variation, with an overall LC_{50} value of 0.67 µg/ml (95% FL: 0.30–1.29 µg/ml) (Table 6). The shift from an average LC_{50} value of 0.0044 µg/ml in 2001–2003 to the current LC_{50} value, with no overlap in 95% FL, represents a significant 152-fold decrease in susceptibility.

Approximately 150 times the original baseline amount of bifenthrin is now required for 50% mortality, indicating that *H. vitripennis* has developed resistance to this compound. Fenpropathrin was tested in 2001 and 2002 with an average LC_{50} of 0.042 µg/ml and overlapping 95% FL ranging from 0.007 to 0.205 µg/ml. The current overall LC_{50} of 0.40 µg/ml (95% FL: 0.19–0.77 µg/ml, Table 7) is 9.5-fold higher, and the 95% FL overlap, but slightly. This suggests that susceptibility to this compound may be shifting.

In some of the 2015 collections and to a lesser degree in 2016, there appeared to be seasonal decreases in susceptibility. This was the case for the neonicotinoids imidacloprid, thiamethoxam, and acetamiprid in which later season bioassays (September or October) tended to show lower susceptibility than early season bioassays (June or July) with some significantly so (i.e., imidacloprid in 2015, thiamethoxam in 2016 and very near significant in 2015, and acetamiprid in 2015) (Tables 2, 4, and 5). Except for one bioassay in 2015 with imidacloprid (22 July), all imidacloprid and thiamethoxam bioassays had nonsignificant individual test χ^2 values but significant χ^2 values for combined annual and overall LC_{50} estimates. These analyses suggest a seasonal shift from higher to lower susceptibility to these compounds.

To take a closer look at this trend, four different sites in Kern County were chosen for monthly monitoring using imidacloprid in 2017, and the 2017 county spray records were parsed for imidacloprid applications within a 1.5-mile radius around each site. Two of the four chosen sites were sampled monthly from early (July) to late (September/October) in the season. Unfortunately, sharpshooter numbers were too low for bioassays in September and October at the Edison sites. The other sites (S Hwy 65 and N Hwy 65) provided sufficient GWSS for late season bioassays which demonstrated significant increases in LC_{50} values from the earliest sampling to the latest sampling (Table 2). Analysis of mortality responses using a GLMM corroborated the significant decrease in susceptibility from early to late season at the N and S Hwy 65 locations (Table 3). By the end of the season, both sites had imidacloprid applied to fields within 1.5 miles, but the timing and proximity of these applications were different between the sites (Fig. 1). At N Hwy 65, imidacloprid was applied not only to several fields, but to fields very close, even adjacent, to our collection site. In addition, imidacloprid was applied early and often throughout the collection period. In comparison, the applications at S Hwy 54 were at greater distances from our collection sites, they were infrequent, and they were later in the season than the applications in the N Hwy 65 area. Thus, it is reasonable to expect that the *H. vitripennis* at N Hwy 65 were exposed to more imidacloprid than those at S Hwy 65, which may explain why N Hwy 65 had a more dramatic LC_{50} increase (29-fold) than did S Hwy 65 (11-fold). Unfortunately, the amount of imidacloprid or any other pest management treatment that test insects were exposed to could not be controlled for this study. As such, uncontrolled environmental or behavioral factors could have contributed to the seasonal reduction in susceptibility. With the understanding that persistent, prolonged insecticide exposure leads to the development of resistance aside, pairing site-specific susceptibility levels with geographic models of seasonal spray records and *H. vitripennis* population dynamics could illuminate factors contributing to resistance development.

Continuous monitoring of the susceptibilities of key agricultural pests to commonly applied pesticides within a given region could inform changes in insecticide use which help prevent the development of resistance. Critical to this work is the establishment of baseline toxicities, and we provide the baseline data for thiamethoxam applied as a systemic material. For our other comparisons, we were fortunate to have *H. vitripennis* susceptibilities to ten insecticides that were collected some 15 yr prior to the present study (Prabhaker et al. 2006). In our study, the subsequent re-testing of these compounds demonstrated that *H. vitripennis* has developed significantly lower susceptibility to two compounds, acetamiprid and bifenthrin. While the reduction in susceptibility to acetamiprid was moderate at sevenfold, the susceptibility reduction over 15 yr to bifenthrin was high at 152-fold. Understanding shifts in insecticide susceptibility could help minimize further resistance development and be useful to growers in selecting the most effective chemicals in their pest management programs.

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