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Influence of Insecticides on Oviposition Behavior of Western Tarnished Plant Bug on Strawberry

Shimat Villanassery Joseph[1, 2, *], Mark Bolda[3]

[1]University of California Cooperative Extension, Salinas, USA
[2]Department of Entomology, University of Georgia, Griffin, USA
[3]University of California Cooperative Extension, Watsonville, USA

Email address:
svjoseph@uga.edu (S. V. Joseph)
[*]Corresponding author

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Abstract: Western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is a serious insect pest of strawberry in California. Several effective insecticides are applied to manage *L. hesperus* in strawberry. These insecticides can induce behavioral changes to *L. hesperus* oviposition such as deterrence and avoidance which are poorly understood in strawberry production. This information can improve integrated *L. hesperus* management. The objectives of this study were to determine oviposition behavior of adult *L. hesperus* under 1) no-choice experiment with no insecticide-treated strawberry plant, 2) no-choice experiment with sulfoxaflor, flonicamid and novaluron-treated strawberry plant; and 3) choice experiment with non-treated and sulfoxaflor, flonicamid and novaluron-treated strawberry leaf petioles in semi-field settings. When the distribution of *L. hesperus* eggs within the non-treated strawberry plants were evaluated at upper, middle and lower strata of the plant as well as at various leaf parts, eggs were uniformly distributed along all three strata of the plant and most of the eggs were found on leaf petiole than on leaf blade, mid-rib or veins. In no-choice experiment, number of the eggs laid by the *L. hesperus* was significantly lower in the sulfoxaflor-treated than in the non-treated plants. In the choice experiment, number of eggs was significantly greater on non-treated petioles than insecticide- treated when the insecticide was novaluron. There was no difference in *L. hesperus* egg density between sulfoxaflor or flonicamid-treated and non-treated petioles.

Keywords: Strawberry, Lygus Bug, Sulfoxaflor, Flonicamid, Novaluron, Insecticide

1. Introduction

Western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae) is a serious insect pest of strawberry (*Fragaria* × *ananasa* Duchesne) in California [1, 2]. The value of strawberry crop production in California in 2016 was estimated to be ~$1.83 billion USD [3], reaching ~$685 million USD in Monterey County alone, California [4]. Using piercing and sucking mouthparts, nymphs and adults of *L. hesperus* feed on the developing embryos of the young fruits and disrupt the normal fruit development resulting in distorted or cat-faced fruits [5]. Cat-faced fruits are rarely harvested or marketed [2].

The nymphs and adults of *L. hesperus* are polyphagous as they feed, develop and oviposit on several host plant species, including strawberry [2]. In central coast of California, the *L. hesperus* population builds up in weed hosts such as wild radish (*Raphanus raphanistrum* L.), common groundsel (*Senecio vulgaris* L.), lupines (*Lupinus* spp.), and mustards *(Brassica* spp.) from April to May and regularly invades strawberry fields [6]. Similarly, second-year strawberry fields or organically managed fields can also serve as food source for the early development of *L. hesperus* populations. *L. hesperus* leave these weeds when flowers dry up or weeds senesce. The invading female *L. hesperus* oviposits on strawberry plants upon arrival. Little is known on the oviposition behavior of female *L. hesperus* within a strawberry plant.

Oviposition behavior of related species, *Lygus rugulipennis* Poppius, has been described [7]. It involves probing of the plant surface for a suitable location using the tip of the labium and stylets. Once a site is marked for oviposition, an egg is

inserted into the plant tissue. Female *L. hesperus* deposits eggs in the plant tissue with the operculum exposed [8]. The location of oviposition can be influenced by tenderness [9], host maturity [10] of the plant tissue, and the presence of trichomes [11]. It was also shown that crop variety, plant height [12] and insecticide residues on the plant surface [13] affect oviposition behavior.

In California, *L. hesperus* management primarily involves the use of insecticides [14-17]. Several insecticides with diverse modes of action are recommended for *L. hesperus* control [6]. Most of the recommended insecticides target the nervous system such as pyrethroids (e.g., bifenthrin), neonicotinoids (e.g., acetamiprid), organophosphates (e.g., malathion) while other new and effective insecticides with distinctly different modes of action such as sulfoxaflor, flonicamid and novaluron have been explored [15-17]. Sulfoxaflor insecticide, a neurotoxin is classified within sulfoxamines (Insecticide Resistance Action Committee [IRAC] group 4c [18]) and is translaminar, systemic and effective against *L. hesperus* [15, 16]. Although sulfoxaflor is not registered for use on strawberry in California to date, the U.S. Environmental Protection Agency has established the tolerance for sulfoxaflor on strawberry [19]. Flonicamid is classified within IRAC group 29 and belongs to pyridinecarboxamide chemical class. Although the exact target site of flonicamid is unknown, it causes feeding cessation in insects once ingested. Novaluron is classified in IRAC group 15 and belongs to benzoylurea class of chemistry affecting biosynthesis of chitin in insects [18, 20-22]. Insecticides can affect the normal physiological functions of hormone or nervous system, which can alter insect behavior including levels of oviposition [23, 24]. Also, it is unclear if the deposited insecticide residues on the foliage alter the oviposition behavior and induce behavioral resistance such as avoidance of treated surface for oviposition in the strawberry plant. To date, there is no documentation on oviposition behavior of *L. hesperus* on strawberry plants in the central coast of California and if insecticide residue alters such behavior. Thus, the major objectives of this study were to determine 1) the distribution of *L. hesperus* eggs within non-treated strawberry plant, and 2) the effects of deposited insecticide residues on oviposition behavior of *L. hesperus*. This information can be used to refine the *L. hesperus* management tactics such as insecticide coverage, placement and application timing.

2. Materials and Methods

2.1. General Methods

In 2013 and 2014, experiments were conducted in the shade house and greenhouse of University of California Cooperative Extension station in Salinas, California. During early spring, strawberry seedlings 'Albion' and 'San Andreas' were obtained from the commercial nursery and was temporarily stored at 4°C. The seedlings were transplanted into 3.7 L pots within two weeks using potting medium (SunGro sunshine mix, aggregate 4, Agawam, Massachusetts) in April 15, 2014 and April 4, 2015. The potted plants were fertilized every month using 20-20-20 NPK fertilizer (Scotts Miracle-Gro, Marysville, Ohio). Plants were maintained in a shade house that allows ~75% light and were irrigated at 2-d interval. The runner shoots were removed at monthly interval to stimulate plant growth. The plants were retained to flower, but fruits were removed as fruits were not uniformly present on all the potted plants. Same sized plants were used for each type of experiments.

Experiments were conducted from the first week of July to the last week of August in both years, which is the peak strawberry production period in the central coast of California. For various experiments, the *L. hesperus* adults were field-collected from mustard weeds (e.g., shortpod mustard and Malva weed) using sweeping net in Chualar, California surrounding the organic strawberry and vegetable fields. The assumption was that the collected *L. hesperus* adults had minimal exposure to insecticides. *L. hesperus* populations develop on weed hosts possibly they undergo at least one generation before they move into strawberry fields. Movement into strawberry field is related to inferior nutritional value of the weed hosts. The *L. hesperus* adults were sorted from the sweep samples and were maintained for 24 h on green beans pods (*Phaseolus vulgaris* L.) until used for various experiments. The adult *L. hesperus* individuals were not sorted by gender because the assumption was that males and females would be present in equal proportions in the landscape [25]. Field-collected adults were used for various experiments over laboratory raised adults as these adults naturally attack the strawberry plants in the field, although they can be at different physiological status, age, mated or virgin. Adult *L. hesperus* individuals were exposed to caged plants in various experiments for 14 d so that adults would have sufficient time to oviposit on the plant tissue. Adults *L. hesperus* were used only once and not re-used in additional experiments.

The first experiment focused on oviposition behavior of *L. hesperus* on non-treated strawberry plant. The second experiment examined the pattern on egg laying when exposed to insecticide-treated plants. Both these experiments were conducted in no-choice set-up. The third experiment was conducted with a choice of insecticide-treated and non-treated leaf petioles.

2.2. No-Choice Experiment

2.2.1. Non-Treated Experiment

This experiment was conducted in the shade house outdoors. Five random *L. hesperus* adults were caged on a potted 'Albion' strawberry plant by enclosing within a bug dorm cage (Bioquip products, cat # 1466AV, Rancho Dominguez, California) for 14 d. After 14 d of exposure, strawberry plant was cut at the crown area and removed. Then the plant was cut into upper, middle and bottom strata. Each stratum had leaves, petioles and flowers, whereas the bottom stratum also had the crown. The plant parts such as leaves, petioles and flowers within each stratum were thoroughly examined for *L.*

hesperus eggs. Because very few numbers of flower were found in each stratum and number of eggs from these flowers were almost zero, eggs from this structure were not included in the analysis. The experimental potted-plants were arranged on a shade house bench with 22 plant replicates.

2.2.2. Insecticide Experiment

The strawberry variety and set-up for this experiment were same as non-treated, no-choice experiment (as previously described). Insecticides used in the experiments were selected based on efficacy, recent registration or potential for registration on strawberry and unique modes of action relative to the older insecticide chemistries which primarily targeted nervous system (e.g., pyrethroids or neonicotinoids). The insecticides used in the experiments were: 1) sulfoxaflor at 328.7 mL (formulation) per ha; 2) novaluron at 730.7 mL (formulation) per ha; and 3) flonicamid at 198.9 g (formulation) per ha (Table 1). Thus, the treatments were: 1) sulfoxaflor, 2) novaluron, 3) flonicamid and 4) non-treated control. The concentrations of formulations were 880.1, 1955.6, and 532.3 ppm. The experiment was arranged in a randomized complete block design with 16 replications. The insecticides were applied using CO_2 powered single boom (one nozzle) handheld sprayer on the potted strawberry plants (plant material only) with two passes from the either sides of the plant. The entire pot and the surface of the soil media were covered using a black plastic bag to ensure that only the plant parts received the insecticide. The water volume used for the applications was at 373.5 L per ha at 30 psi. An adjuvant, Dyne-Amic® was added at 0.25% v/v to all of the insecticide treatments. The applied insecticides were allowed to dry for 2 h before introduction of *L. hesperus* adults. The treated plants were exposed to five random *L. hesperus* adults per plant. Each plant was enclosed in bug dorm cage for 14 d. After 14 d, plants were cut into upper, middle and lower strata and number of *L. hesperus* eggs on the leaf structures such as leaf blade, midrib, veins, and petiole were quantified after careful examination under a dissecting microscope.

Table 1. *Details of the insecticide products used in various experiments.*

Brand name	Active ingredient (%)	Company	Location
Sequoia®	Sulfoxaflor (21.8)	Dow AgroScience	Indianapolis, Indiana, USA
Rimon® 0.83EC	Novaluron (9.3%)	Arysta LifeScience North America LLC	Cary, North Carolina, USA
Beleaf® 50 SG	Flonicamid (50%)	FMC Corporation	Philadelphia, Pennsylvania, USA
Dyne-Amic®	Methyl esters of C16–C18 fatty acids, polyalkyleneoxide modified polydimethylsiloxane, and alkylphenol ethoxylate (99%)	Helena Chemical Company	Collierville, Tennessee, USA

2.3. Choice Experiment

Strawberry leaf petioles were used as oviposition substrates in this experiment because adult *L. hesperus* laid most of their eggs on petioles. Petioles (10 cm long) of 'San Andreas' strawberry plants were prepared after cutting them from the plant base and removing the leaflets. The choice assay was build using clear 0.381-mm Dura-Lar film (Grafix, Maple Heights, Ohio). A 29.5 × 22.5-cm rectangular section of Dura-Lar was rolled from end-to-end and the 1.5 cm overlaid strip of film was connected using masking tape to form a cylinder (22.5-cm height and 9-cm diameter). On top of the cylinder, no-see-um mesh fabric (cat# 7250NSW, Rancho Dominguez, California) was glued using glue gun forming a cage and the bottom of the cylinder had 2-cm thick floral form. The experiment was performed in greenhouse at ~22°C. The insecticides included in the choice assay were 1) sulfoxaflor, 2) novaluron, and 3) flonicamid. The treatment for this experiment was combination of an insecticide- and non-treated petioles within each cylindrical cage assay. The treatments were: 1) nontreated-nontreated, 2) sulfoxaflor-nontreated, 3) novaluron-nontreated, and 4) flonicamid-nontreated. Six petioles were used with a cylindrical cage assay where three of them were insecticide-treated and the other three were non-treated. Insecticide solutions were prepared in 151.2 L per ha water volume. For insecticide-treated petioles, the petioles were dipped in the insecticide solution for 5 s and dried on a paper towel for 30 min. Once dried, these six petioles (three insecticide treated and three non-treated petioles) were vertically erected on floral form of the choice assay. Petioles were ~2.5 cm apart within the floral form. Five *L. hesperus* adults were randomly picked and introduced into a cylindrical cage assay. These cylindrical assays were placed on trays with a pool of water to avoid drying out the floral form. After exposing strawberry plants to *L. hesperus* adults for 14 d, petioles were thoroughly examined for *L. hesperus* eggs under a dissecting microscope. The experiment was arranged on randomized complete block design and replicated for a total of 20 times per insecticide treatment combination except non-treated treatment (all six petiole untreated) which was replicated 15 times. The experiment was conducted in two sets with 10 replicates each time for all the treatments. There was only five replications for non-treated choice treatment in the second set. After 14 d of exposure, the petioles were thoroughly evaluated for the presence of *L. hesperus* eggs. Data were combined for analysis purpose.

2.4. Statistical Analysis

All data analyses were conducted using SAS software [26]. For the egg data from no choice non-treated experiment, analysis of variance of the number of *L. hesperus* eggs was conducted in PROC GLIMMIX with log link function and distribution as negative binomial. The variables included were treatment (location of eggs: leaves versus petiole), strata (upper, middle and bottom) and treatment × strata interaction as fixed effects. For the no-choice experiment with insecticide treatment, analyses of variance of the number of *L. hesperus*

eggs were conducted in PROC GLIMMIX with log link function and distribution as negative binomial. The variables included were treatment (insecticides), strata (upper, middle and bottom) and treatment × strata interaction as fixed effects. For both no-choice experiments, least squares means were separated by pairwise t test ($p < 0.05$) after back-transformation. For choice insecticide experiment, the data were log-transformed (ln [x + 1]) and student's t-test analysis was performed on the number of eggs in the choice tests after log-transformation using PROC TTEST procedure.

3. Results

3.1. No-Choice Experiment

3.1.1. Non-Treated Experiment

In the non-treated experiment, treatment (leaf or petiole) was significantly different for *L. hesperus* eggs (treatment: $F_{1, 126} = 22.4$, $p < 0.001$) whereas, strata ($F_{2, 126} = 2.0$, $p = 0.140$) and treatment × strata interaction ($F_{2, 126} = 0.7$, $p = 0.508$) were not significantly different. The number of *L. hesperus* eggs (> 95%) was significantly more on the leaf petiole of the strawberry plant than on other parts of the leaf (Figure. 1).

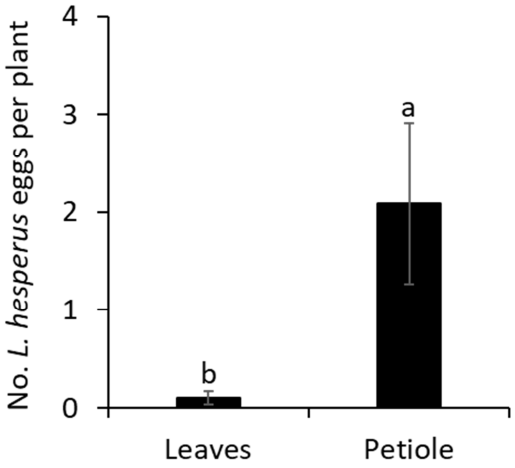

Figure 1. *(A) Least squares mean (±SE) number of L. hesperus eggs laid on the leaves and petiole of non-treated strawberry plant when they were exposed to five L. hesperus adults for 14 d. Bars with the same letters are not significantly different (pairwise t test, p = 0.05).*

3.1.2. Insecticide Experiment

In the no-choice insecticide experiment, treatment (insecticides) was significantly different for *L. hesperus* eggs (treatment: $F_{3, 220} = 4.8$, $p = 0.003$) whereas, strata ($F_{2, 220} = 2.8$, $p = 0.064$) and treatment × strata interaction ($F_{6, 220} = 1.8$, $p = 0.109$) were not significantly different. A significantly lower number of eggs was found in sulfoxaflor treatment than non-treated or flonicamid or novaluron treatments ($F_{3, 44} = 3.2$, $p = 0.015$, Figure 2). There was no significant difference in number of eggs among flonicamid, novaluron and non-treated treatments.

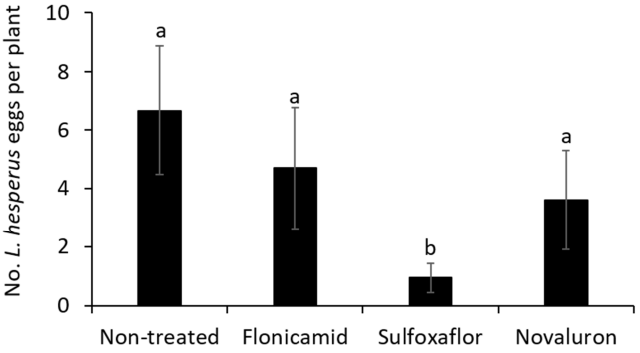

Figure 2. *Least squares mean (±SE) number of L. hesperus eggs found on strawberry plant when the plants were treated with insecticides and were exposed to five L. hesperus adults in the no-choice experiment for 14 d. Bars with the same letters are not significantly different (pairwise t test, p = 0.05).*

3.2. Choice Experiment

The number of *L. hesperus* eggs found on non-treated petioles was not significantly different (Figure 3A). When compared between novaluron-treated and non-treated petioles, a significantly more number of *L. hesperus* eggs was found on the non-treated petioles than on novaluron-treated petioles (Figure 3D). There was no significant difference between flonicamid- or sulfoxaflor-treated petioles, and non-treated petioles (Figures 3B and C).

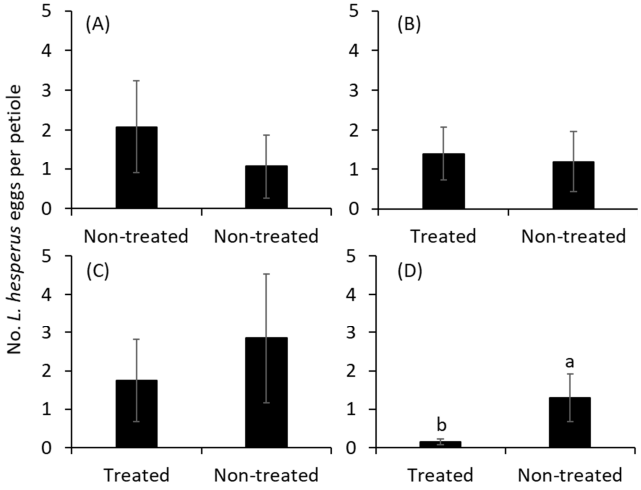

Figure 3. *Mean (±SE) number of L. hesperus eggs found on strawberry leaf petiole when three petioles were not-treated (water) and other three petioles were treated with (A) water, (B) flonicamid, (C) sulfoxaflor and (D) novaluron, and were exposed to five L. hesperus adults for 14 d in choice experiment (student's t test, p = 0.05). Not significant data have no letters.*

4. Discussion

In the current study, *L. hesperus* laid most of the eggs (> 90%) on strawberry leaf petioles rather than on other leaf parts such as midrib or veins or leaflets which is consistent with a previous potted study in strawberry [27]. Other plant structures such as inflorescence, flowers and fruits were not exposed to *L. hesperus* adults in the current study. Previously study showed that 43% of the total *L. hesperus* eggs were laid on strawberry leaf structures mostly on petiole and the rest of

the eggs (57%) on the inflorescence structures [27]. We focused our study on leaf structures for consistency, as structures related to inflorescence were not uniformly present on the potted strawberry plants. In cotton (Gossypium *hirsutum* L.), Balachandran et al. (2014) studied oviposition pattern in both potted and field-grown cotton plants and found that > 70% of the *L. hesperus* eggs were on the leaf petiole. Similarly, other studies in cotton reported up to 97% [11], and 87% [28] *L. hesperus* oviposition occurred on leaf petiole.

Lygus hesperus eggs were uniformly distributed across the strata of the strawberry plant which suggests that *L. hesperus* has no specific preference in egg laying at any particular region of the plant. In cotton, however, more number of *L. hesperus* eggs were found on the upper stratum of the plant than on other strata [13]. The height of strawberry plant is ~ 30 cm whereas, a cotton plant can reach up to 180 cm. Nevertheless, understanding the normal *L. hesperus* oviposition pattern on a non-treated strawberry plant is essential to record any change on oviposition pattern when the adult *L. hesperus* are exposed to insecticide-treated plants. Besides plant height, previous reports suggest that tissue hardiness and tenderness, and host maturity can also play critical role in *L. hesperus* oviposition in cotton [9-10, 29]. These factors have not yet been studied in strawberry setting as strawberry plant constantly produce new shoots from the crown as long as the runner shoots are routinely removed [2].

In no-choice scenario, when the strawberry plants treated with insecticides with varied modes of action, the number of eggs found on the plant leaf structures was reduced in plants when treated with sulfoxaflor compared to non-treated plants. However, in the choice scenario, a similar number of *L. hesperus* eggs were recovered from petioles treated with sulfoxaflor and non-treated. This suggests that sulfoxaflor residues, when present, can suppress *L. hesperus* oviposition but it did not show any signs for avoidance. Sulfoxaflor targets the nervous system of the insect, which is a same target site for organophosphate, acephate. Balachandran et al. (2014) has previously shown that acephate will suppress oviposition of *L. hesperus* on treated cotton plant. This suggests that sulfoxaflor is not only effective in reducing nymphs and adults of *L. hesperus* [16, 17], it is also effective in reducing oviposition. This information is valuable as sulfoxaflor can be used when adult activity is detected in scouting.

With novaluron residues on strawberry plant, however, number of eggs on leaf petiole was similar compared to non-treated control in no-choice scenario but in the choice scenario, number of eggs was greater on non-treated petioles than on novaluron-treated petioles. This suggests that novaluron residues can alter oviposition behavior of *L. hesperus* as they have the tendency to avoid and lay eggs where the novaluron residues are minimum. Similarly, in cotton, more number of *L. hesperus* eggs was found in a region of the plant where residues of a pyrethroid (zeta-cypermethrin) and an organophosphate (acephate) insecticide were minimum [13] which indicate that the normal oviposition behavior of *L. hesperus* can be altered in the presence of insecticide residues. Novaluron is an insect growth regulator and a contact insecticide. It is not effective in causing adult *L. hesperus* mortality on contact, but its residues are effective against nymphs by affecting their growth and development [16].

Similar to previous study [13], the current study also finds that flonicamid did not elicit any changes to oviposition behavior of *L. hesperus* as number of eggs was similar on flonicamid-treated and non-treated petioles. Perhaps, because flonicamid affects insect survival when they feed and ingest the insecticide residue in the plant tissue rather than mere direct contact.

Overall, number of eggs recovered from the plants was low compared to previous studies [for e.g., 27], although the exact reason is unknown. The field-collected adults could be at varied physiological status such as age, reproductive maturity, fat body reserves and metabolic rates. We opted to use the field-collected adults over laboratory-raised adults for the various experiments because they are naturally occurring, exposed the environmental factors such as fluctuating temperature and relative humidity, food source, and varied fat body reserves. Most importantly, these adults invade strawberry fields, and oviposit, and the nymphs emerging from these eggs causes economic damage. Various experiments were conducted at shade house and greenhouse conditions and eggs recovered from the plants were similar.

5. Conclusions

This study shows that insecticides such as sulfoxaflor and novaluron can interfere with the normal egg laying behavior of the *L. hesperus*. Sulfoxaflor tend to be suppressing the *L. hesperus* oviposition. The results suggest that insecticide coverage is essential to minimize behaviorally induced resistance in the *L. hesperus* in strawberry. Future studies should focus on understanding oviposition pattern of *L. hesperus* when the *L. hesperus* adults move to the strawberry fields from other hosts, and this information may be used to streamline the control sprays using sulfoxaflor.

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