Characterization of *Lygus hesperus* (Hemiptera: Miridae) Feeding and Oviposition Injury on Celery Seedlings

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ABSTRACT

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The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), can be a serious pest of celery, *Apium graveolens* dulce (Mill.) Pers. in the Central Coast region of California. Little is known about injury symptoms caused by *L. hesperus* adults on celery. When celery seedlings were exposed to *L. hesperus* adults, their crown area was found destroyed with necrotic dead tissue. Destroyed crowns of dead seedlings were observed when seedlings were exposed to five or more *L. hesperus* adults for ≥24 h but was not observed on seedlings exposed ≤12 h. In addition, elongated lesions were noticed on the celery petiole and were significantly greater when exposed to five or more *L. hesperus* adults for as little as 4 h. Number of elongated lesion injuries and number of eggs recovered from elongated lesions was correlated ($r = 0.66$; $P < 0.001$). Data provide key diagnostic symptoms of *L. hesperus* feeding and ovipositional injury on celery seedlings. Also, this study expands our understandings on the effects of *L. hesperus* adult density and duration of exposure which reinforces the need for regular scouting, and immediate management decisions and action to reduce losses from *L. hesperus* injury in both celery seedlings and mature plants in greenhouse and field settings, respectively.

INTRODUCTION

The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is a sporadic but serious pest of celery, *Apium graveolens* dulce (Mill.) Pers. in the Central Coast region of California. Celery was valued $180 million USD in Monterey County, CA, alone (Monterey County Crop Report 2014). *Lygus hesperus* attacks young celery plants in greenhouses and mature plants in production fields, causing severe economic losses amounting to more than a million U.S. dollars (S. V. Joseph, *unpublished data*).

Injury caused by *L. hesperus* on celery seedlings and mature plants is not completely understood, although feeding injury on seedlings of other vegetables has been superficially documented (Varis 1978; Fye 1984; Jacobson 2002). Red to brown elongated lesions are suspected to be *L. hesperus* feeding injuries on both young seedlings and mature plants. Also, in the greenhouse, celery seedlings suffer severe stunting or poor plant growth, often suspected to be attributable to *L. hesperus* feeding injury. The mouthparts of *L. hesperus*, often described as piercing-sucking, consists of four stylets. *Lygus hesperus* use these stylets to probe host plants and feed on the plant fluids. When *L. hesperus* feeds, it inserts the stylets into the ruptured site. Once inserted, the stylets hardly move but facilitate a steady flow of saliva concentrated with enzymes which preorally digest the meristematic tissue and the slurry of digested tissue is ingested (Miles 1972; Backus et al. 2005). This type of feeding is called the “macerate-and-flush” method and the resulting injury causes necrotic tissue on the plant feeding site. Because *L. hesperus* does not secrete gelling saliva and leaves no feeding sheath behind within the affected tissue after stylets are removed, it becomes difficult to validate if the injury on celery was indeed caused by *L. hesperus* feeding.

During early spring in California’s Central Coast region, *L. hesperus* develops on weed hosts such as wild radish (*Raphanus raphanistrum* L.), common groundsel (*Senecio vulgaris* L.), lupines (*Lupinus* spp.), milk thistle [*Silybum marianum* (L.) Gaertn.]. and mustards (*Brassica* spp.) surrounding the production fields, ditches, and roadways (Zalom et al. 2012). Similarly, alfalfa or bean fields also serve as hosts for *L. hesperus* in California’s Central Coast (Swezey et al. 2013). As weed hosts senesce, *L. hesperus* adults tend to leave them, seeking food, water, and shelter elsewhere including seedlings in greenhouses and mature plants in fields. The invading female *L. hesperus* settle on celery plants to feed and oviposit (Hill 1933; Boivin et al. 1991). *Lygus hesperus* females lay 161 eggs (mean) at 26.7°C (Mueller and Stern 1973). Management of *L. hesperus* on celery primarily involves use of chemical insecticides (Hill 1932), particularly pyrethroids, carbamates, and organophosphates (Godfrey and Trumble 2008). Thus, proper early diagnosis of *L. hesperus* feeding injury is critical in determining the need of insecticide use and application timing for its control. The major objectives of this study were to: (i) characterize the injury caused by *L. hesperus* on celery seedlings; and (ii) assess the effects of *L. hesperus* density and exposure time on injury development on celery.

EXPERIMENTS AND STATISTICAL ANALYSIS

Experiments were conducted at the University of California Cooperative Extension (UCCE) Monterey County greenhouse in Salinas, CA, in 2015. Celery seeds were planted and raised in

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trays in a commercial greenhouse in Salinas and were transferred to a UCCE Monterey greenhouse when seedlings were eight weeks old. Seedlings were transplanted to 3.78-liter pots filled with potting media (Sunshine aggregate plus mix 4, Sun Gro, Agawam, MA). The transplants were irrigated on a 2-day schedule and because the plants were maintained for short intervals, fertilizer was not added. *Lygus hesperus* adults were field-collected within 24-h of each experiment from weed hosts surrounding strawberry and vegetable fields in Salinas and Chualar, CA. Cages were build using clear 0.381-mm Dura-Lar film (Grafix, Maple Heights, OH). 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 high and 9-cm in diameter). On top of the cylinder, no-see-ums mesh fabric (cat# 7250NSW, Rancho Dominguez, CA) was glued using a glue gun to form a cage and the bottom of the cylinder was left open. *Lygus hesperus* adults were field collected and maintained in a collapsible mesh cage (catalog#1466AV, Bioquip, Rancho Dominguez, CA). Adults were extracted from field collection cages using an aspirator and introduced on single celery plants caged with cylindrical cages. The 2.5-cm bottom edge of the cylindrical cage was inserted into the soil media to secure and seal. *Lygus hesperus* adults were caged at densities of 0, 1, 5, and 10 adults (male or female) per pot. The ratio of the male or females in the sampled populations was approximately 50:50 based on preliminary survey. The caged celery seedlings were exposed to *L. hesperus* for 4 h, 12 h, 24 h, and 7 days. The experiment for each exposure time (4 h, 12 h, 24 h, and 7 days) was conducted individually. However, the 7-day exposure experiment was conducted with all the *L. hesperus* densities (0, 5, and 10 adults) except the treatment of one adult per cage. For 12-h, 24-h, and 7-day exposure experiments, single plant pots were replicated 10 times and arranged in a randomized pattern on greenhouse benches. The 4-h exposure experiment was replicated seven times. After the exposure period, transplants were uprooted, placed in plastic bags and stored at 4°C for evaluation. All evaluations were completed within 7 days after the termination of exposure periods. All the *L. hesperus* adults were recovered alive at the end of the experiment. Transplants were examined for *L. hesperus* injury types (feeding or oviposition injury) under a 10× dissecting microscope. Once the whole transplant was assessed, the petioles were individually removed from the crown area and evaluated for *L. hesperus* injury and presence of eggs.

Categorical data, number of seedlings dead, and destroyed crown per *L. hesperus* adult densities for each exposure time were analyzed using a nominal logistic regression (JMP 12.01, SAS Institute Inc., Cary, NC). Because experiments were conducted by individual exposure interval at a given time, the data were neither combined nor analyzed using a model where *L. hesperus* density and exposure interval served as factors. When there was a significant overall treatment effect (*L. hesperus* adult densities) in each exposure time, *L. hesperus* density treatments were compared by examining the odds ratio between two treatments,

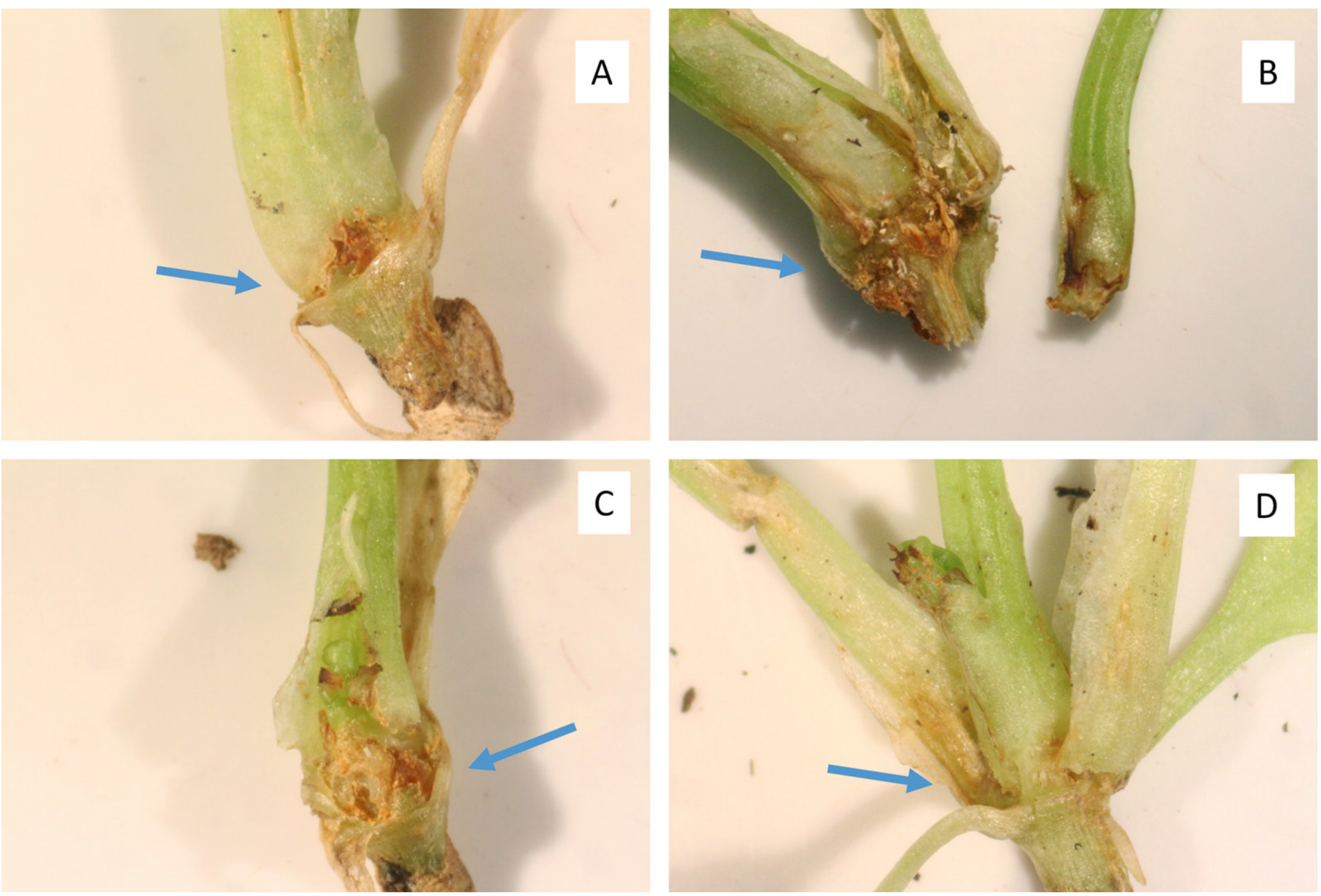

FIGURE 1

(**A-D**) *Lygus hesperus* adult(s) feeding injury at the crown area of the seedlings. The arrows indicate dead necrotic tissue that developed after *L. hesperus* feeding.

i.e., probabilities of finding destroyed crown were compared between two *L. hesperus* density treatments by examining a chi-square value of odds ratio. ANOVA was performed on the number of elongated lesions on the celery petiole for each density using the PROC GLM procedure in SAS (2012, SAS Institute Inc., Cary, NC), and means were separated using the Tukey's HSD method ($P < 0.05$). The null hypothesis was that the variance was equal among *L. hesperus* density for a given exposure time ($P < 0.05$). Means and standard error for the variables were calculated using PROC MEANS procedure in SAS. Correlations (Pearson's correlation) between number of elongated lesions and number of eggs recovered from the elongated lesion were assessed using the PROC CORR procedure in SAS.

EFFECTS OF ADULT DENSITY AND TIME OF EXPOSURE ON INJURY

The two major injury types noticed with *L. hesperus* adult exposure to celery seedlings were necrotic dead tissues at the crown region (Fig. 1A-D) and elongated lesions on the petiole (Fig 2C and D). Dead necrotic tissue or dead seedlings was not observed when exposed to various *L. hesperus* densities for 4 and 12 h (Table 1). However, when exposed for 24 h, significant greater percentage of dead and destroyed crown with necrotic tissue was recorded with five and 10 adults than with zero and one adult treatments. At 7 days exposure, 100% of the seedlings died when exposed to 10 *L. hesperus* adults. The number of seedlings with destroyed crowns was significantly greater when exposed to five adults compared with unexposed seedlings. Percentage of dead seedlings when exposed to five adults was significantly lower than when exposed to 10 adults. Thirty percent of the seedlings died when exposed to one adult for 7 days, which was lower than those with exposure to five adults for the same interval. None of the seedlings died when they were not exposed to *L. hesperus* for 7 days.

The elongated lesions were significantly greater in five and 10 *L. hesperus* adult treatments than one and no adults at 4, 12, and 24 h (Table 2). There was no injury detected on petioles that did not receive any *L. hesperus* adults. After a 7-day exposure, significantly greater number of elongated lesions was found on petioles with five adults than with no adult. Number of injury lesions and number of eggs recovered from the lesion showed a significant correlation ($r = 0.66$; $P < 0.001$).

IMPLICATIONS OF THE RESULTS ON PEST MANAGEMENT

Lygus hesperus feeding injury as dead necrotic tissue was found at the crown area of the celery seedling. Results show that the incidence of injury in the crown area increases with the number of *L. hesperus* adults and longer intervals of exposure (24 h and 7 days). This suggests that if *L. hesperus* invade celery plants in the greenhouse and remain for more than 24 h, extensive feeding injury at the crown area can be detected. It is unclear why feeding by *L. hesperus* adults was observed at the crown area of the celery seedlings. One possibility is that they use the cracks and crevices in the soil to hide and move from the soil directly to

FIGURE 2

(**A**) *Lygus hesperus* eggs laid on the petiole (operculum 2 to 2.5 mm wide), (**B**) disintegration of tissue at the site of *L. hesperus* oviposition, and (**C-D**) elongated lesion (0.5 to 2 cm long) developed on the petiole after oviposition.

TABLE 1
Percentage of destroyed crown and dead celery seedlings by *L. hesperus* adult feeding at various adult densities and exposure periods.

No. of *L. hesperus* adults	Exposure time							
	4 h		12 h		24 h		168 h (7 days)	
	Destroyed crown	Dead plants	Destroyed crown	Dead plants	Destroyed crown	Dead plants	Destroyed crown	Dead plants
0	0 A	0 A	0 A	0 A	0 B	0 B	0 B	0 C
1	0 A	0 A	0 A	0 A	0 B	0 B	—	—
5	0 A	0 A	0 A	0 A	60 A	10 A	85.7 A	30 B
10	0 A	0 A	0 A	0 A	80 A	70 A	—	100 A
χ^2 (df)	0.0 (3)	0.0 (3)	0.0 (3)	0.0 (3)	23.5 (3)	21.0 (3)	10.9 (1)	28.8 (2)
P	1.000	1.000	1.000	1.000	<0.001	<0.001	<0.001	<0.001

Symbols with similar case letters within column are not significantly different (comparing χ^2 of odds ratio, α = 0.05).

feed at the crown area. Another possibility is that *L. hesperus* adults prefer feeding on meristematic tissue in the crown or growing areas where most of the photosynthates are translocated and concentrated (Tingey and Pillemer 1977; Young 1986). In contrast, other studies suggest that *L. hesperus* adults also feed on other plant structures, preferring the vegetative structures of the plants over reproductive structures such as seeds (Snodgrass 1998) or meristematic tissues such as apical buds of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], causing bud abortion in nursery trees (Schowalter et al. 1986; South 1991), and on cotton floral buds (*Gossypium hirsutum* L.) causing necrotic dead tissue (Mauney and Henneberry 1979).

Another type of injury found on the celery is elongated lesions on the petiole ranging from 0.5 cm to 1 cm. It is likely that elongated lesions are related to *L. hesperus* oviposition as there was a correlation between numbers of eggs recovered from these lesions. *Lygus hesperus* females thrust their eggs into the plant tissue which might provide protection from predators and parasitoids and help reduce desiccation (Wheeler 2001). Only the operculum is exposed from the celery tissue (Fig. 2A). Also, *L. hesperus* tend to lay eggs in an aggregated pattern (Fig. 2B) and it appears that the tissue breaks down, creating an elongated lesion on the petioles (Figs. 2C and D). In addition to vascular bundles that run along the petiole, celery petioles have collenchyma tissue, elongated cells that store water (Leroux 2012). The cell wall of collenchyma cells has irregular thickness (Leroux 2012) and *L. hesperus* prefers tender areas for oviposition (Alvarado-Rodriguez et al. 1986) which suggests that these cells are sensitive to ovipositional injury resulting on elongated lesions. It is also likely that when a female *L. hesperus* initiate oviposition on petiole, it lays all the eggs in an aggregated manner on a given site rather than moving around and depositing eggs at various sites on the petiole. This oviposition pattern further contributes to development of an elongated lesion on petiole. Moreover, there were more elongated lesions when higher numbers of adults were exposed for a shorter interval (<12 h). This suggests that if females move into the greenhouse or field, they can quickly oviposit and trigger elongated lesions on the petiole.

TABLE 2
Mean (± SE) number of elongated lesions recorded on celery seedlings at various *L. hesperus* adult densities and exposure periods.

No. of *L. hesperus* adults	Exposure time			
	4 h	12 h	24 h	168 h (7 days)
0	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 C	0.0 ± 0.0 B
1	4.3 ± 1.9 B	3.3 ± 0.6 B	2.2 ± 0.6 B	—
5	15.8 ± 1.9 A	11.3 ± 1.4 A	7.9 ± 0.5 A	13.1 ± 3.7 A
10	18.7 ± 1.7 A	12.8 ± 2.1 A	7.8 ± 0.6 A	—
F (df1,df2)	28.1 (3,18)	25.7 (3, 27)	61.6 (3, 21)	12.5 (1,6)
P	< 0.001	< 0.001	< 0.001	0.012

Means followed by same letters within the column are not significantly different (Tukey's HSD Test, $P < 0.05$). Non-transformed data are presented.

This result provides insights for the management of *L. hesperus* in the greenhouse just as newly arrived *L. hesperus* females can oviposit and feed on the celery petioles. *Lygus hesperus* feeding on the crown of the celery seedling causes stunting. The trays with stunted celery seedlings are discarded. Depending on the extend of *L. hesperus* infestation in the greenhouse, the damage can result in a serious loss. *Lygus hesperus* oviposition on petioles can develop into elongated lesions. In the field, the economic loss related to elongated lesions is driven by market price. When market value is high, those affected wrapping petioles are trimmed off during harvest and the rest of the petioles is marketed, which causes considerable reduction in the yield. However, if these elongated lesions are extensive in the field or detected in the innermost petioles of the celery, the entire celery crop is rejected and not harvested regardless of market price. The losses to *L. hesperus* injury can easily surpass a million dollars, if 13 to 15 celery fields (each field ~6 ha) are rejected before harvest. Results indicate that monitoring greenhouses or fields is critical to reducing establishment of *L. hesperus* population by timely management. Feeding injury can develop if the plants are not managed after detection of incoming adults in the greenhouse and field.

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