

Effects of plants and supplemental prey on establishment of *Dicyphus hesperus* (Hemiptera: Miridae)

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Abstract

Dicyphus hesperus Knight (Hemiptera: Miridae) is an omnivorous natural enemy used to control insect pests in greenhouse structures. The establishment and population development of predators in a banker plant system must be understood prior to their deployment in a pest management program. Banker plants are non-crop plants that are infested with a supplemental food source, which provides a suitable environment for predators to establish their population. The objective of this research was to investigate the effect of mullein (*Verbascum thapsus* L.; Scrophulariaceae) as banker plants and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as supplemental food on the development of *D. hesperus* in a tomato greenhouse production system. The nymphal development duration did not differ between mullein (22.5 d) and tomato (24.5 d) with *E. kuehniella* eggs but was significantly longer on mullein (25.8 d) without *E. kuehniella* eggs, whereas nymphs did not complete their life cycle on tomato without *E. kuehniella* eggs. Adult longevity and percentage survival to adulthood was 30.4 d and 91.9% on mullein with *E. kuehniella* eggs, and 26.9 d and 87.5% on tomato with *E. kuehniella* eggs, but were much reduced on mullein without *E. kuehniella* eggs (22.44 d and 75%). The population growth of the predator varied in response to host plant and presence of supplemental food source. In greenhouse experiments, the population of *D. hesperus* showed a greater increase on tomato provided with *E. kuehniella* eggs as a supplement food source than on mullein with supplemental food. We conclude that there was no benefit in our experiments to using mullein as a banker plant rather than using the commercial crop, tomato, supplemented with *E. kuehniella* eggs.

Key Words: predator; conservation biological control; banker plants; *Ephestia kuehniella*

Resumen

Dicyphus hesperus Knight (Hemiptera: Miridae) es un enemigo natural omnívoro utilizado para controlar las plagas de insectos en los invernaderos. El establecimiento y el desarrollo de la población de depredadores en un sistema de plantas-banco deben entenderse antes de su despliegue en un programa de manejo de plagas. Las plantas-banco son plantas no cultivadas que están infestadas con una fuente de alimento suplementaria, que proporciona un ambiente adecuado para que los depredadores establezcan su población. El objetivo de esta investigación fue investigar el efecto de gordolobo (*Verbascum thapsus* L.; Scrophulariaceae) como plantas-banco y huevos de *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) como alimento suplementario sobre el desarrollo de *D. hesperus* en un sistema de producción de invernadero de tomate. La duración del desarrollo de la ninfa no difirió entre gordolobo (22.5 d) y tomate (24.5 d) con huevos de *E. kuehniella*, pero fue significativamente mayor en gordolobo (25.8 d) sin huevos de *E. kuehniella*, mientras que las ninfas no completaron su ciclo de vida en tomate sin huevos de *E. kuehniella*. La longevidad de los adultos y el porcentaje de sobrevivencia hasta la edad adulta fue de 30.4 d y 91.9% en gordolobo con huevos de *E. kuehniella*, y 26.9 d y 87.5% en tomate con huevos de *E. kuehniella*, pero se redujeron mucho más en gordolobo sin huevos de *E. kuehniella* (22.44 d y 75%). El crecimiento de la población del depredador varió en respuesta a la planta hospedera y la presencia de una fuente de alimento suplementario. En experimentos en el invernadero, la población de *D. hesperus* mostró un mayor aumento en el tomate provisto con huevos de *E. kuehniella* como fuente de alimento suplementario que en el gordolobo con alimento suplementario. Llegamos a la conclusión de que no hubo ningún beneficio en nuestros experimentos al usar el gordolobo como una planta banco en lugar de usar el cultivo comercial, tomate, suplementado con huevos de *E. kuehniella*.

Palabras Clave: depredador; control biológico de conservación; plantas-banco; *Ephestia kuehniella*

Dicyphus hesperus Knight (Hemiptera: Miridae) is a widely distributed zoophytophagous true bug native to North America (Henry & Wheeler 1988). In a previous study conducted in British Columbia, Canada, *D. hesperus* completed its development on the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), and two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), and has reduced pest numbers (McGregor et al. 1999). *Dicyphus hesperus* has contributed to the reduction of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in previous studies (Calvo et al. 2016). Methods for establishing populations of biocontrol agents in the

greenhouse with a limited number of releases can increase the viability of biocontrol as a management option for crop pests (Huang et al. 2011). The use of an alternative host plant other than the commercial crop is often necessary to help to preserve the predator and maintain their population density (Sanchez et al. 2003). Banker plants are non-crop plants that act as long-lasting rearing units for natural enemies. Banker plants typically are infested with herbivores or provisioned with supplemental food (Stacey 1977; Hansen 1983). The main purpose of banker plants is to maintain, and hopefully increase, the predator population even if the pest is not present (Bennison 1992; Frank 2010).

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In previous studies, mullein, *Verbascum thapsus* L. (Scrophulariaceae), was used as an alternative (i.e., banker) host to evaluate the development response of *D. hesperus* on tomato plants. Whitefly populations were successfully controlled on tomato by *D. hesperus* in greenhouses with and without mullein plants; however, the abundance of predators was greater on the tomato plants with mullein plants than on tomato plants alone (Sanchez et al. 2003). The reproductive rate and longevity of *D. hesperus* was higher on mullein plants provided with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as supplemental food sources than on mullein plants with no supplemental food at 20 °C and under a 16:8 (L:D) photoperiod (Sanchez et al. 2004). *Ephestia kuehniella* eggs are produced by commercial insectaries and are used frequently as a dietary supplement to maintain certain predators, such as *D. hesperus* and *M. caliginosus* (Gillespie & McGregor 2000; Callebaut et al. 2004).

Based on the previous studies referenced above, we chose to investigate mullein as an alternative (i.e., banker) host plant for the establishment of *D. hesperus* in tomato crops under typical Florida greenhouse growing conditions, which experience higher temperatures than in British Columbia, Canada, where mullein was previously tested for predator culture (Sanchez et al. 2003). We compared the establishment of *D. hesperus* on tomato and mullein under controlled growth chamber and greenhouse conditions. For the practical and effective use of *D. hesperus*, the predator must remain reproductively active throughout the cropping season. Thus, the main objective of this research was to evaluate the establishment of *D. hesperus* on different plants with and without supplemental food sources. We evaluated (1) the survival and development of *D. hesperus* on mullein and tomato, with and without *E. kuehniella* eggs as supplemental food, under controlled conditions, and (2) the population development of *D. hesperus* under commercial greenhouse conditions on mullein with and without *E. kuehniella* eggs and on tomato with *E. kuehniella* eggs.

Materials and Methods

DICYPHUS HESPERUS COLONY ESTABLISHMENT AND MAINTENANCE

Tomato plants (variety 'Lanai') were grown from seed in 20 cm diam pots with Fafard 2 soil potting mix (BWI Apopka, Plymouth, Florida, USA) in a greenhouse. Plants were watered as required and fertilized with Jacks 20:20:20 All Purpose nutrient mix (Hummert International Company, Earth City, Missouri, USA) (10 g for 3 L) in 125 mL water every wk. Mullein seedlings (*Verbascum thapsus*) were purchased from Gloeckner and Company (Harrison, New York, USA) and were transplanted into 10-cm-diam pots in a greenhouse at the University of Florida/IFAS Gulf Coast Research and Education Center, Wimauma, Florida, USA. Each mullein plant was provided with 20:20:20 water-soluble nutrient (10 g for 3 L) in 125 mL water every 15 d.

A colony of *D. hesperus*, established from individuals originally purchased from Beneficial Insectary (Redding, California, USA), was reared in a growth room (24–28 °C, 48–55% RH, and 12:12 h [L:D] photoperiod) on 6-wk-old mullein plants contained in a Bug Dorm (60 × 60 × 60 cm, MegaView Science Co. Ltd., Taichung, Taiwan). *Ephestia kuehniella* eggs, purchased from Beneficial Insectary, were provided attached to a 2 cm × 7.5 cm adhesive section of a yellow Post-It Note (3M Corporation, St. Paul, Minnesota, USA). Approximately 0.25 g of eggs (0.1 g *Ephestia* per 100 *D. hesperus*) was provided to each Bug Dorm every wk as a protein source. HOBO data loggers (Onset Technology Company, Pocasset, Massachusetts, USA) were set up to record temperature and relative humidity.

LIFE CYCLE OF DICYPHUS HESPERUS ON TOMATO AND MULLEIN WITH AND WITHOUT EPHESTIA EGGS UNDER GROWTH ROOM CONDITIONS

The development time of *D. hesperus* from first instar nymph to adult, survival of nymphs to adulthood, and adult longevity were evaluated on mullein and tomato plants with and without eggs of *E. kuehniella* as a dietary supplement. The 4 treatments evaluated were (1) mullein with *E. kuehniella* eggs, (2) mullein without *E. kuehniella*, (3) tomato with *E. kuehniella*, and (4) tomato without *E. kuehniella*.

In order to produce individual *D. hesperus* first instar nymphs to track under the 4 treatments, 5 plants each of tomato and mullein about 4 wk old were kept separately in individual cages (35 × 35 × 60 cm) (Bio-Quip®, Compton, California, USA) in a growth room (24–28 °C, 48–55%, and 12:12 h [L:D] photoperiod). Five pairs of male and female *D. hesperus* that were adults for 1 wk were released into each cage. Each cage was provided with 0.25 g *E. kuehniella* eggs on 2-cm adhesive strips every week. When a first instar nymph appeared on a mullein or tomato plant, it was removed and placed into a translucent round plastic box (5 cm high × 13 cm diam) containing either a new tomato or mullein leaf, with or without *E. kuehniella* eggs, depending on the treatment. First instar nymphs produced by adults reared on tomato were used in the tomato treatments, and nymphs produced by adults reared on mullein were used in the mullein treatments. Leaf petioles were covered with wet cotton to maintain turgidity. Eight replicates (first instar nymphs) were selected for each treatment. The trial was repeated 3 times for a total of 24 replicates for each of the 4 treatments. The 4 rearing substrate treatments were arranged in a randomized complete block design in a growth room under the same conditions as described above. Nymphs were observed every d and moved to a new leaf every 3 to 4 d. The date of molting from 1 nymphal instar to the next was recorded according to the change in size and the presence of cast skin. Dates of molts were recorded until individuals reached the adult stage to calculate the d spent in each instar stage, and d required to complete development from nymph to adult under the 4 treatments. In order to determine treatment effects on adult longevity, adults were kept on the same treatment until they died.

POPULATION DEVELOPMENT OF DICYPHUS HESPERUS ON MULLEIN WITH AND WITHOUT EPHESTIA EGGS AND ON TOMATO WITH EPHESTIA EGGS UNDER GREENHOUSE CONDITIONS

The population development of *D. hesperus* on 3 different combinations of plant and supplemental food was studied in the greenhouse at the University of Florida in Gainesville, Florida, USA. It was evaluated in a greenhouse under 3 different treatments: mullein with and without *E. kuehniella* eggs, and tomato with *E. kuehniella* eggs. In the experiment reported above, *D. hesperus* completed its life cycle on these 3 diets, but not on tomato without a supplemental protein source. Therefore, tomato without *E. kuehniella* eggs was not evaluated in this trial.

On 14 Aug 2017, 60 mullein plants and 30 tomato plants, 6 to 8 wk old, were established individually in cages (35 × 35 × 60 cm) (Bio-Quip®, Compton, California, USA) for the following treatments: (1) mullein with *Ephestia* eggs, (2) mullein without *Ephestia* eggs, and (3) tomato plants with *Ephestia* eggs. Each treatment was replicated 6 times. Five pairs of 1-wk-old *D. hesperus* males and females were introduced into each cage, and treatments were arranged in a randomized complete block design with 6 blocks. After 3 wk, 1 plant was

randomly selected from each of the 3 treatments in each of the 6 blocks. All nymphs and adults were aspirated from cages and counted. Additional plants were selected from each treatment in each block on the sixth, ninth, twelfth, and fifteenth wk after introduction of the mated adult *D. hesperus*. Fresh 6-wk-old plants were placed in each cage on wk 12 without removing the original plant, which was beginning to senesce.

STATISTICAL ANALYSIS

Life history parameters of *D. hesperus* reared under 4 different treatments were compared with PROC GLIMMIX, and TUKEY-HSD was used for mean separation (SAS 2001). Nymphal survival was analyzed by using PROC LOGISTIC (SAS 2015). Trial was set as a random factor in the analysis.

The population development of *D. hesperus* in the Gainesville greenhouse experiment was compared among the 3 treatments with ANOVA using PROC MIXED in SAS (SAS 2001). We did independent comparisons among treatment wk by wk for 15 wk where treatment and sample date were factors. Population trends were plotted over 15 wk. Mean separation was done by Tukey-Kramer in SAS.

Results

LIFE CYCLE OF *DICYPHUS HESPERUS* ON TOMATO AND ON MULLEIN WITH AND WITHOUT *EPHESTIA* EGGS UNDER CONTROLLED CONDITIONS

There were significant differences in total nymphal development time among the 3 treatments that supported complete nymphal development: mullein with and without *E. kuehniella* eggs and tomato with *E. kuehniella* eggs ($F = 10.44$; $df = 2,57$; $P < 0.0001$) (Fig. 1). The nymphal development times of *D. hesperus* reared on mullein with *E. kuehniella* eggs and tomato with *E. kuehniella* eggs were not significantly different, but were significantly faster than on mullein without *E. kuehniella* eggs. *Dicyphus hesperus* died before reaching the third instar on tomato without *E. kuehniella* eggs (Fig. 1). The duration of the first instar did not differ with or without *E. kuehniella* eggs on tomato treatments, but was significantly shorter on mullein

with *E. kuehniella* than without ($F = 5.20$; $df = 3,90$; $P = 0.0023$). The duration of the second instars did not differ among treatments ($F = 1.92$; $df = 3,76$; $P = 0.133$). The third instar development time was significantly shorter when reared on mullein with *E. kuehniella* eggs compared to mullein without *E. kuehniella* eggs and tomato with *E. kuehniella* eggs ($F = 8.06$; $df = 2,56$; $P = 0.0008$), and the development of fourth instar also differed among treatments ($F = 23.50$; $df = 2,56$; $P < 0.0001$) (Fig. 1).

Survivorship of nymphs varied among the 4 treatments. None of the nymphs survived to adulthood on tomato without *E. kuehniella* eggs (Fig. 2). The percentage of *D. hesperus* surviving on mullein and on tomato with *E. kuehniella* eggs was not significantly different, but was significantly different than the percentage surviving on mullein without *E. kuehniella* eggs ($F = 1.31$; $df = 2,56$; $P = 0.5262$). Adult longevity of *D. hesperus* reared on mullein with and without *E. kuehniella* eggs and tomato with *E. kuehniella* eggs differed significantly, and was longer on mullein with *E. kuehniella* eggs than on tomato with *E. kuehniella* eggs; both were longer than on mullein without *E. kuehniella* eggs ($F = 39.75$; $df = 2,57$; $P < 0.0001$) (Fig. 2).

POPULATION DEVELOPMENT OF *DICYPHUS HESPERUS* ON MULLEIN WITH AND WITHOUT *EPHESTIA* EGGS AND ON TOMATO WITH *EPHESTIA* EGGS UNDER GREENHOUSE CONDITIONS

The population growth of *D. hesperus* was significantly different on the 3 treatments ($F = 8.24$; $df = 2,64$; $P = 0.0007$), and the averaged totals of all instars of *D. hesperus* collected each sample period were calculated (Fig. 3). Populations increased 5-fold on tomato with *E. kuehniella* eggs, and 2.5-fold on mullein with *E. kuehniella* eggs, but did not increase on mullein without *E. kuehniella* eggs by the end of 15 wk. The population increased gradually on tomato provided with supplemental food of *E. kuehniella* eggs. The population remained constant on mullein with *E. kuehniella* eggs for a while, then increased after the ninth wk, whereas on mullein without *E. kuehniella* eggs, the predator maintained its initial population density. HOBO data loggers were set up to record the temperature, which averaged 25.3 °C, ranging from 16.2 to 34.8 °C during the 15-wk experiment, which is the preferred temperature for *D. hesperus* growth.

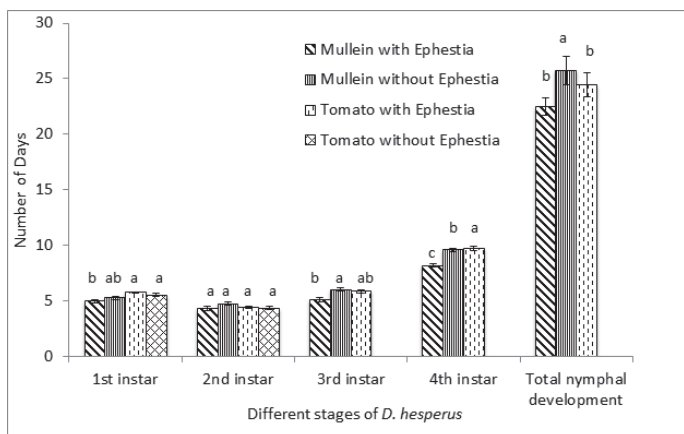


Fig 1. Average number of d spent in each instar and total nymphal development time of *Dicyphus hesperus* in 4 treatments: mullein plant with *Ephestia* eggs, mullein plant without *Ephestia* eggs, tomato plant with *Ephestia* eggs, and tomato plant without *Ephestia* eggs conducted in the growth room. Data shown are means \pm SE; different letters indicate significant differences within instar and total nymphal development time of *D. hesperus* (Tukey's, $\alpha < 0.05$).

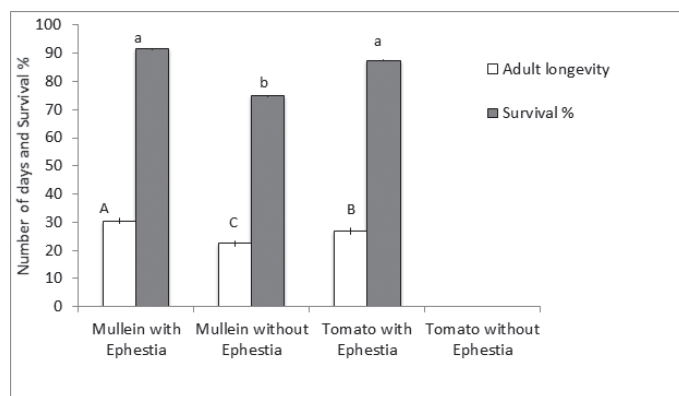


Fig 2. Adult longevity of *Dicyphus hesperus* reared from first instar nymphs on 4 treatments. Experiment conducted in the growth room of the University of Florida/IFAS Gulf Coast Research and Education Center, Wimauma, Florida, USA. Data shown are means \pm SE; capital letters indicate significant differences within treatments for adult longevity, and lowercase letters indicate significant differences for survival of *D. hesperus* (Tukey's, $\alpha < 0.05$).

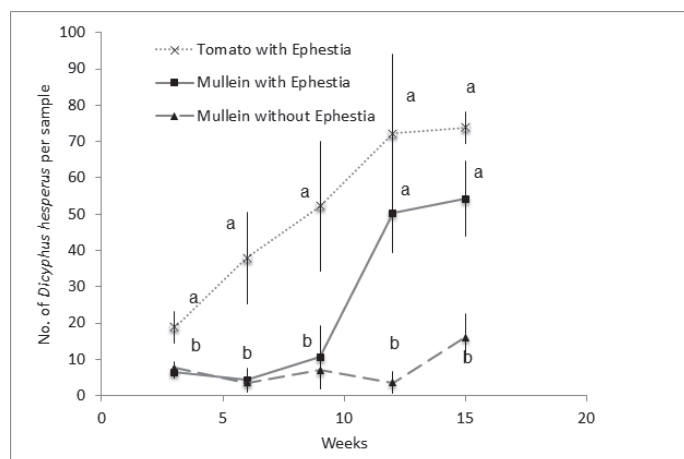


Fig 3. Average number of *Dicyphus hesperus* per sample on each treatment (tomato with *Ephestia* eggs, mullein with and without *Ephestia* eggs) over 15 wk. The experiment was completed in the greenhouse at the University of Florida in Gainesville, Florida, USA, and data shown are means \pm SE. Different letters describe significant differences in population size of *D. hesperus* on 3 different treatments each wk.

Discussion

The introduction of supplement food source had a significant impact on population development of *D. hesperus* on both the main crop (tomato) and the banker plant (mullein). From the first development experiment conducted under controlled growth room conditions, we found that *D. hesperus* can survive on mullein and tomato with supplement food and mullein without supplement food. Thus, we only studied the dynamics of *D. hesperus* on these 3 treatments in the greenhouse. Tomato plant with *E. kuehniella* eggs was the best combination for establishment of *D. hesperus*. Mullein with *E. kuehniella* eggs also showed population growth, but *D. hesperus* on mullein without *E. kuehniella* eggs could maintain their population only. Adult longevity was longer on mullein and tomato with a supplemental food source, which clearly indicates that *D. hesperus* needs supplemental food to enhance reproduction (McGregor et al. 2000). Plants are primarily a water source for *D. hesperus* (Gillespie & McGregor 2000). Mullein can maintain the predator population, but will not be an option to increase the population of predators without a supplemental protein source.

The life cycle period of *D. hesperus* was shorter on mullein with *E. kuehniella* eggs than on tomato with *E. kuehniella* eggs, but the population growth rate in the greenhouse was twice as high on tomato with *E. kuehniella* eggs as on mullein with *E. kuehniella* eggs. This indicates that the fecundity could be higher on tomato (i.e., 63 eggs in 10 wk) than on mullein (i.e., 49 eggs in 10 wk) (Sanchez et al. 2003). According to previous studies, *D. hesperus* required 8 wk to reduce the whitefly population by 88% (Calvo et al. 2016). In this study we found that *D. hesperus* survived for 15 wk in all 3 treatments, but the population density was greater in tomato and mullein with supplement source. From this study we conclude that *D. hesperus* does not need alternative host plants as long as tomato plants have supplemental food sources, such as *E. kuehniella* eggs, but in the absence of supplement food they cannot increase their population. The presence of prey or supplement source on tomato is important to establish predator population in the greenhouse. The presence of *Nicotiana tabacum* L. (Solanaceae) as a banker plant for *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), and *Sesamum indicum* L. (Pedaliaceae) for *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) favored the early establishment of predator in greenhouse (Arnó et al. 2000; Biondi et al. 2016). Mullein is suitable

refuge for *D. hesperus*, and when tomato crops are not available, it can enhance the early establishment of the predator. It is important to learn more about predator establishment on different plant species. The banker plant system has potential to improve biological control efficiency. Further research is needed to integrate adoption of a biological control method with a banker plant system.

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