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SHORT COMMUNICATION

Impact of odor signals on *Cycloneda sanguinea* (Coleoptera: Coccinellidae) searching behavior

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Abstract

G. Heit, G. Cohen, and G. Mareggiani. 2008. Impact of odor signals on *Cycloneda sanguinea* (Coleoptera: Coccinellidae) searching behavior. Cien. Inv. Agr. 35(2):167-172. Volatile infochemicals generated by aphids, their host plants, or from the interaction between host plant and herbivore (herbivore-induced volatile plant compounds) are among the environmental stimuli used by predators to locate prey. The objective of this study was to evaluate the olfactometric response of the predator *Cycloneda sanguinea* (Coleoptera: Coccinellidae) to volatile infochemicals associated with the tomato plant, *Lycopersicon esculentum* Mill. (Solanaceae), and the aphid prey *Myzus persicae* Sulzer (Hemiptera: Aphididae). Predator behavior was evaluated in an airflow olfactometer at 24 ± 2ºC with 65 ± 10% relative humidity and a 16 h photoperiod. Latency time and the number of visits to the active chamber where the odor source was located were recorded. The odor sources studied were: a. thirty healthy aphids; b. uninfested tomato leaves; c. infested tomato with 30 healthy aphids; d. infested tomato leaves with 30 stressed aphids; e. thirty stressed aphids. *C. sanguinea* adults were strongly attracted to the odors of infested tomato leaves, but for the other odor sources, they did not visit active chambers more often than inactive chambers. *C. sanguinea* took less time to find active chambers with tomato leaves infested with healthy aphids than to find chambers with aphids or tomato leaves alone. These results suggest that *C. sanguinea* uses herbivore-induced volatile plant compounds as reliable indicators of herbivore infestation to locate prey.

Key words: *Cycloneda sanguinea,* infochemicals, *Lycopersicon esculentum, Myzus persicae,* olfactometer.

Introduction

Coccinellids are polyphagous opportunists that prey on a wide range of food sources. Despite their considerable polyphagy, they are very selective as far as essential food is concerned (Soares *et al*., 2004). When aphids dominate their menu, it can be expected that their foraging behavior will be adaptive and complex. The use of chemical information from lower trophic levels by the predators of herbivores is presumed to adaptively increase predator efficiency by reducing searching time and increasing attack rates on prey (Ninkovic *et al*. 2001).

Responses to host and plant-derived infochemicals and their use in host location have been extensively documented in insect parasitoids (Turlings *et al*., 2004, 1991; Dicke and Van Loon, 2000; Turlings and Benrey 1998; Tumlinson *et al*. 1993; Vet and Dicke 1992). Although less attention has been paid to the use of infochemicals by generalist insect predators, there is scattered evidence that some prey-associated infochemicals modify coccinellid foraging behaviour, even in the presence of herbivore-induced volatile plant compounds (Acar *et al*., 200; Al Abassi, 2000; Mondor and Roitberg, 2000; Dicke 1999; Takabayashi and Dicke, 1996).

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The objective of this study was to evaluate the olfactometric response of the predator *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae) to volatile infochemicals associated with the prey *Myzus persicae* Sulzer (Hemiptera: Aphididae) and one of its host plant *Lycopersicon esculentum* Mill. (Solanaceae).

Materials and methods

Insect rearing

The mass rearing of *C. sanguinea*, a South American native species, was initiated with adult coccinellids collected in the field. Green peach aphids, *M. persicae*, were used to rear *C. sanguinea* in standardized environmental conditions (24 ± 2°C, 65 ± 10% relative humidity, and a 16 h photoperiod). The host plant used was *L. esculentum* cv. Platense.

Predator behavior

Predator behavior was evaluated in an airflow olfactometer under the standardized temperature and humidity conditions described above. Light was provided by a 60 Watt daylight lamp during the entire bioassay. The olfactometer consisted of a circular central arena with four arms at 90 degrees, each one with a rectangular chamber at the end (Ciarla *et al.*, 2005). An adult ladybeetle, starved for 24 h, was introduced into the central arena. An odor source was randomly placed in one of the four chambers (active chambers), while the other three chambers were empty (inactive chambers). Six to seven expanded leaves cut from tomato were used in each bioassay. Infested leaves were obtained from tomato plants infested with *M. persicae* nymphs prior to the bioassay.

Treatments and evaluation

The odor sources evaluated were: a. thirty non-stressed aphids; b. uninfested tomato leaves (3 g) obtained from the apical branch; c. tomato leaves (3 g) obtained from an apical branch infested with 30 non-stressed aphids; d. tomato leaves (3 g) from the apical branch infested with 30 stressed aphids; e. thirty stressed aphids. Aphids were stressed by disturbance with a N° 2 paintbrush for 1 min before starting each trial. Thirty aphids per chamber were used to allow comparisons with Acar *et al.* (2001), who estimated that the amount of (E)-β-farnesene excreted by thirty aphids is approximately 1 μl.

The number of visits to each chamber and the latency time (time spent by each predator to make a chamber selection) (Buchwald and Breed, 2005) were recorded during a 60 min period (n = 50 for each treatment). Coccinellids that did not choose a chamber within 60 min were considered to be “non-responders”. Plant material and insects were used only once. The olfactometer was cleaned with 96% ethanol and allowed to dry before each predator was introduced into the chamber.

Design and statistical analysis

Treatments were distributed according to a complete randomized design with 50 replicates of each treatment. A G^2 test was used to test the null hypotheses of randomized chamber selection (the probability of selection of the active chamber was 0.25 for each treatment). Unplanned tests of homogeneity were performed to identify the causes for rejection of the null hypothesis (Sokal and Rohlf, 1995). Latency time was analyzed through a non-parametric analysis of variance according to Kruskal-Wallis’s test (Zar, 1999). Nemenyi’s test was used to compare latency time medians ($\alpha = 0.05$) (Zar, 1999).

Results and discussion

The total number of visits of *C. sanguinea* adults to the active and inactive chambers is shown in Figure 1. The total G^2 was highly significant ($G^2_{4,0.05} = 11.77$, $p = 0.0082$), indicating that adult ladybeetles did not visit the chambers randomly. Unplanned tests of homogeneity showed that *C. sanguinea* adults were strongly attracted to the volatile compounds produced by infested tomato leaves ($p < 0.0001$) and visited the active chamber more frequently than the inactive ones. Ladybeetles did not visit the active chamber more often than the inactive ones when the other four odor sources were offered ($p > 0.05$).

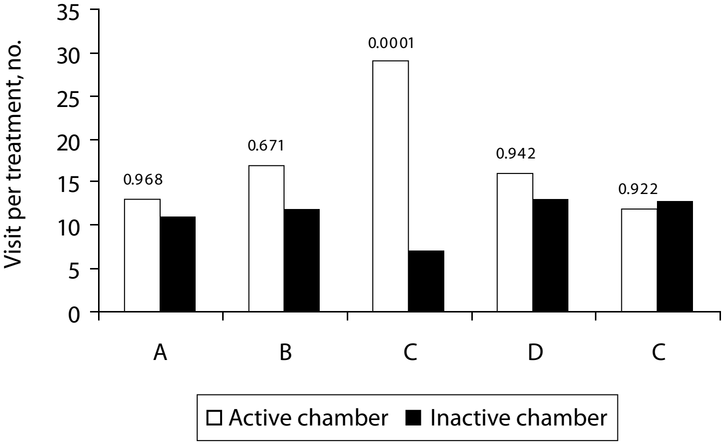

Figure 1. Effects of odors on *Cycloneda sanguinea* adults as determined by the number of visits to chambers containing the following odor sources: A. non-stressed aphids; B. uninfested tomato leaves; C. infested tomato leaves with non-stressed aphids; D. infested tomato leaves with stressed aphids; E. stressed aphids. Aphids were stressed by disturbing them with a N° 2 paintbrush for 1 min before starting each trial. Numbers above each bar indicate p values for unplanned tests of homogeneity. The constant critical value used was $\chi^2_{0.05,4} = 9.488$.

The difference between the number of responders and non-responders in each treatment was not significant with respect to the odor sources evaluated ($G^2_{4,0.05} = 3.25$, p = 0.5162) (Table 1). On the basis of a Kruskal-Wallis test, *C. sanguinea* did not spend equal amounts of time to select the active chamber in the five treatments considered ($H_{4,86,0.05} = 14.36$, p = 0.0062) (Figure 2). Similarly, based on Nemenyi's test, latency time was significantly ($p < 0.05$) lower when the odor sources were tomato leaves infested with 30 non-stressed aphids or 30 stressed aphids. In the former case, the number of visits to the active chamber was also significantly different from the rest of the treatments (Figure 1). These results indicate that the interaction between tomato plants and *M. persicae* had a significant effect on predator searching behavior ($p < 0.0001$), but only when non-stressed aphids were present on plants.

Our results are supported by Obata (1997), who used choice tests to discover that the ladybeetle *Harmonia axyridis* was more attracted to the odor of aphid-infested leaves than to the odor of uninfested ones. Ninkovic *et al.* (2001) showed that *Coccinella septempunctata* adults were significantly attracted to volatile compounds emitted from barley plants infested with aphids, but not from healthy plants or from undisturbed aphids. However, the results of this study differ from the findings of Acar *et*

Table 1. Effects of odor signals on the numbers of responding and non-responding *Cycloneda sanguinea* adults.

Treatments	Responders[1]	Non-responders[1]
Healthy aphids (n = 30)	46	8
Uninfested tomato leaves	52	7
Infested tomato leaves with 30 healthy aphids	51	6
Infested tomato leaves with 30 stressed aphids	55	4
Stressed aphids (n = 30)	50	10
Chi Squared = 3.25, p = 0.5162		

[1] Responders and non-responders were coccinellids that chose or did not choose a chamber within 60 min, respectively. A G^2 test was used to test homogeneity of proportions (gl = 4, p = 0.05).

al. (2001) and Mondor and Roitberg (2000), who showed that the coccinellids *Hippodamia convergens* and *H. axyridis* were attracted to the odors of undisturbed aphids. Moreover, in this study, there was some indication that coccinellids were attracted to stressed aphids more than to non-stressed aphids (Figure 2).

The difference between the attraction of adult coccinellids to infested and uninfested leaves could be attributed to qualitative differences in the volatile compound blend emitted in both cases. Two classes of induced volatile compounds (IVCs) have been described in plants (Dudareva *et al.*, 2006). One IVC class is released from mechanically damaged leaves immediately (0-5 min) after damage and mainly consists of C6 green volatile compounds, whereas a second class of compounds (mainly terpenes) is also synthesized after damage, but is not released until a few hours after the initial injury (Holopainen, 2004).

Some authors have studied the impact of aphid-generated semiochemicals, such as alarm pheromone, on coccinellid searching behavior. Along these lines, Nakamuta (1991) found that (E)-β-farnesene, a key component of most aphid alarm pheromones, did not elicit searching behavior in *C. septempunctata*. Moreover, Mondor and Roitberg (2000) showed that *H. axyridis* was not attracted to cornicle droplets containing aphid alarm pheromone. It would not likely be an advantage for the predator to spend too much time searching in areas where aphid alarm pheromone is present (Kunert *et al.*, 2005).

Al Abassi *et al.* (2000), demonstrated that (E)-β-farnesene affects *Coccinella septumpunctata* behavior, but that its effects are mitigated by b-caryophyllene, which inhibits the neuronal response induced by (E)-β-farnesene. Although adult coccinellids display electrophysiological responses to aphid alarm pheromone, under our experimental conditions, not only aphid alarm pheromone, but also a complex mixture of host plant and herbivore odors, were present. These additional compounds might have functioned as modifiers of coccinellid response.

The results of our olfactometric bioassays suggest that *C. sanguinea* searching behavior is modified in the presence of different sets of volatile compounds that might act as reliable indicators of herbivore infestation.

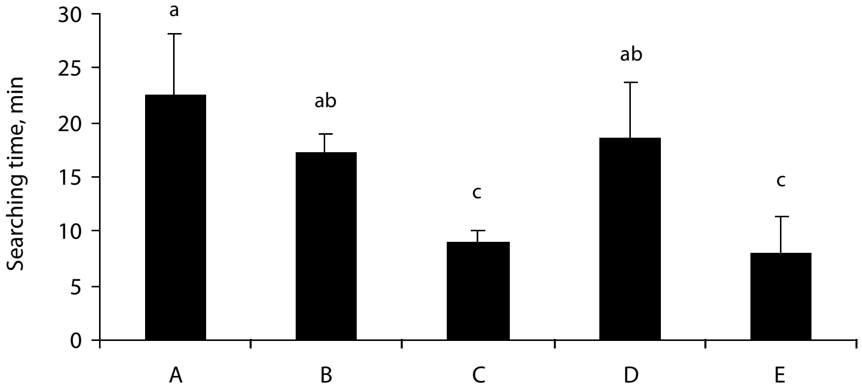

Figure 2. Mean latency time required by *Cycloneda sanguinea* adults to select the active chamber. A. non-stressed aphids; B. uninfested tomato leaves; C. infested tomato leaves with non-stressed aphids; D. infested tomato leaves with stressed aphids; E. stressed aphids. Bars (± standard error) with different letters above them are statistically different according to Nemenyi's test (p = 0.05).

Resumen

Los infoquímicos volátiles emitidos por áfidos, sus plantas hospederas o de la interacción entre sus hospederos y las plantas (volátiles inducidos por herbivoría) son uno de los estímulos ambientales usados por los enemigos naturales durante la búsqueda de presas. El objetivo de este estudio fue evaluar la respuesta olfatométrica del depredador *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae) hacia los infoquímicos asociados con *Lycopersicon esculentum* Mill. (Solanaceae) y *Myzus persicae* Sulzer (Hemiptera: Aphididae) como presa. El comportamiento del depredador se evaluó en un olfatómetro de aire forzado, a 24 ± 2ºC, 65 ± 10% de humedad relativa, y 16 h de fotoperiodo, determinando el tiempo de latencia y el número de visitas a la cámara activa donde se ubicó la fuente de volátiles. De acuerdo con el tratamiento, las fuentes de volátiles fueron: a. 30 áfidos no estresados, b. Hojas de tomate sanas, c. Hojas de tomate infestadas con 30 áfidos no estresados, d. Hojas de tomate infestadas con 30 áfidos estresados, e. 30 áfidos estresados. De acuerdo con los resultados obtenidos, *C. sanguinea* fue fuertemente atraída por los volátiles de las hojas de tomate infestadas, pero no visitó la cámara activa más frecuentemente que las cámaras pasivas (vacías) en los restantes tratamientos considerados. Fue menor el tiempo requerido por *C. sanguinea* para seleccionar la cámara activa con hojas de tomate con áfidos no estresados, que en los tratamientos con áfidos solos u hojas de tomates libres de áfidos. Los resultados de los ensayos olfatométricos sugieren que *C. sanguinea* utilizaría los volatiles inducidos por herbivoría para localizar sus presas como indicadores confiables de infestación del herbívoro.

Palabras clave: *Cycloneda sanguinea,* infoquímicos, *Lycopersicon esculentum, Myzus persicae,* olfatómetro.

Acknowledgments

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