Responses of the predatory species, *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae), to the volatiles from its prey, *Aphis craccivora* Koch. and *Vicia faba* plant

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Abstract

**Background:** Plants after infestation emit specific volatiles, which can influence foraging behavior of insect predators of herbivores. This work aimed to evaluate the responses of the predatory species, *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae), adults to volatiles emitted from its cowpea aphid prey, *Aphis craccivora* (Koch.), and from *Vicia faba* plant to improve the efficiency of searching for its prey. An olfactometer bioassay was used for this investigation.

**Results:** The results revealed that both male and female of *C. undecimpunctata* significantly used olfactory cues from infested plant + aphids, but the mated females exhibited a complete response (47.50%). The mated male was attracted to aphids (30.0%) more than other tested predatory types, indicating the capacity to exploit volatiles induced by aphids.

**Conclusions:** The results supported that *C. undecimpunctata* adult uses plant volatiles induced by *A. craccivora* infestation, which might act as reliable indicators to find its prey.

**Keywords:** *Coccinella undecimpunctata*, *Aphis craccivora*, *Vicia faba*, Olfactometer, Free choice, Semiochemicals

Background

The faba bean plant (*Vicia faba* L.) is an essential crop in several regions of worldwide, including Egypt. The cowpea aphid, *Aphis craccivora* (Koch.) (Homoptera: Aphididae), is one of the serious aphids’ species of this crop (Salman et al. 2007). Aphid’s population can become very abundant over time, and space damaging the plants directly by sucking sap and indirectly through transmitting viruses (Dedryver et al. 2010). Beetles of the family Coccinellidae (Coleoptera), including *Coccinella undecimpunctata*, are considered potential predators of aphids, including *A. craccivora* (Jabbar et al. 2020). Plants produce several secondary volatile compounds that are important in insect-plant interaction. These volatiles can be attractant for the insects for feeding, mating, and oviposition (Norkute et al. 2020). Recently, several investigators found that many plants attacked by herbivore insects indirectly defend themselves by emitting volatiles, which adaptively increases natural enemies’ efficiency by reducing searching time and increasing attack rates on their hosts (Ninkovic et al. 2001). However, very little attention has been dedicated to the role of semiochemicals emitted by legume plants and aphids on natural enemies’ searching behavior, especially the predators. Therefore, this work aimed to evaluate the role of volatiles emitted from *A. craccivora* aphid and *V. faba* plant in increasing the efficiency of *C. undecimpunctata* adults on searching capacity.
Methods

Insect colonies

The aphid *A. craccivora* colonies were collected from the Experimental Farm, Faculty of Agriculture. Then, they were maintained in a growth chamber at (25 ± 2 °C, 65 ± 5% RH, and a 16-h photoperiod) on bean plants grown in 15-cm-diameter plastic pots, using a fritted clay and peat moss medium. When plant stems “reached” 8 mm diameter, they were placed into double-walled delicate nylon-mesh cages (40 × 34 × 50 cm), and aphids were released and allowed to settle down on the plants and multiply. Plants that became severely necrotic from aphid feeding were replaced with fresh ones. The ladybeetle, *C. undecimpunctata*, was collected from the wheat plants located at the same farm, maintained in a growth chamber (25 ± 2 °C, 65 ± 5% RH, and a 16-h photoperiod) into double-walled delicate nylon-mesh cages (40 × 34 × 50 cm). The beetle’s colonies were feed on the wheat aphid, *Schizaphis graminum* (Hemiptera: Aphididae), and reared on wheat (*Triticum aestivum*) seedlings. For all experiments, *C. undecimpunctata* adults were famished for 24 h before beginning any investigation.

Olfactometer bioassays

Behavioral responses of *C. undecimpunctata* adults to volatiles from different sources were evaluated in a 4-arm olfactometer (Model QT-WII01), a custom-made by Channel Tech Co., Ltd., Beijing, China, under the laboratory conditions described above. A 60-W lamp was provided during the entire bioassay. The olfactometer’s four arms were connected to a pump that maintained continual pure air in the four arms and at meanwhile, vacuuming out by central suction the odors emitted by the treatments. The four airflow meters were connected by the four arms to ensure that the control and odorant airstreams are equal in each arm. Both sexes were used in separate experiments to test the effect of different odor sources. An individual ladybeetle adult (virgin male or virgin female or mated male or mated female) was placed on the surface of the central arena. Each odor source was placed randomly in one of the three chambers (three active chambers), and the 4th chamber was empty (inactive chamber). The odor sources evaluated were: un-infested bean seedling, 20 individuals of *A. craccivora* alone, combination ‘infested bean seedling + 20 individuals of aphids.’ Each adult was used only once. After 10 observations, the total number of individual visits in the arms was represented as one replicate. Eight replicates were used for each experiment.

Statistical analysis

The data from olfactometer experiments were statistically evaluated by analyzing variance two-way ANOVA, followed by Fisher’s LSD test. The significance level was set to 0.05.

Results

In the experiments of the present study, individuals of *C. undecimpunctata* responded to the olfactometer’s different odor sources with partly evident differences. The two-way analysis of variance provided information regarding the interaction between predator type and attraction types. The analysis indicated a significant difference in attraction types (*F* = 79.63; *df* = 3; *P* < .001), and interaction between predator types and attraction types (*F* = 4.71; *df* = 9; *P* < .001), while difference among predator types was non-significant (*F* = 0.13; *df* = 3; *P* = 0.13).

Olfactory bioassay on different predator types (virgin and mated males and females) of *C. undecimpunctata* adults searching behavior showed that the odor of “infested bean plant + aphids” was significantly more attractive (*P* < 0.001) to the ladybird beetle than the other odors from attraction types “un-infested bean plant or aphids” (Table 1). Differences between males and females of *C. undecimpunctata* in the olfactory response to “infested bean plant + aphids” odor were very remarkable. The percentage of the positive reaction from mated females to the odor source “infested bean plant + aphids” was significant higher (*F* = 3.62; *df* = 3,7; *P* = 0.03) than the percentage of response of tested virgin and mated males. Virgin males and females were response a stronger to volatiles emitted from un-infested bean plant (*F* = 9.47; *df* = 3,7; *P* < 0.001) than mated males and females (Table 1). Non-significant difference was found among the tested virgin and mated males and females to the chamber without an odor source (*F* = 1.00; *df* = 3,7; *P* = 0.41). The statistical analysis of attraction types revealed that volatiles emitted from “infested bean plant + aphids” had a significant influence on all predator’s types compared with other attraction types (*P* < 0.001).

Means followed by the same capital letter per row and the same small letter per column do not differ by the Fisher LSD test at 5% probability.

There was a clear tendency between both sexes, but non-significant difference was observed between the percentage of virgin and mated males (Fig. 1) in response to the odor sources “infested bean plant + aphids” (*F* = 0.02; *df* = 1,7; *P* = 0.83). The highest percentage of positive response in the case of mated males was obtained by the odor emitted by aphids (*F* = 14.91; *df* = 1,7; *P* = 0.006) and by un-infested bean plant (*F* = 58.33; *df* = 1, 7; *P* = < 0.001) compared with virgin males.

In the case of virgin and mated females (Fig. 2), no evident tendencies were observed in response “aphids,” and so the percentage responding between the odor source
aphids were non-significant different (\(F = 0.47; \text{df} = 1, 7; P = 0.52\)). In fact, more percentage of mated ladybeetle females responded to the odor source “infested bean plant + aphids” (\(F = 14.54; \text{df} = 1, 7; P = 0.007\)) compared with virgin female. On the other hand, the virgin ladybeetle females showed significant difference in attraction by the odor source “un-infested bean plant” (\(F = 21.00; \text{df} = 1, 7; P = 0.003\)). However, these experiments also showed clearly that the combination of infested bean plant with aphids was the most attractive odor source for adults of \(C.\ undecimpunctata\) tested in the olfactometer.

### Discussion

In response to herbivore damage, numerous plants produce a specific blend of volatile chemicals, which can interfere with the foraging behavior of adult predators of herbivores (Dicke et al. 2003), including ladybeetle foraging behavior (Pettersson et al. 2008). In the experiments with \(C.\ undecimpunctata\), results showed that most of the positive responses appeared with odor source from “infested bean plant + aphids,” while an odor source from un-infested bean plant or aphids alone had a lower response percentage (Table 1). Several investigators supported the results. Bahlai et al. (2008) found that volatiles related to damage caused by prey feeding on an insect host and prey waste products may play a widely important role in prey-finding than volatiles associated with prey items alone. Ninkovic et al. (2001) showed that infested barley plants with \(Rhopalosiphum padi\) L. (Hemiptera: Aphididae) aphids significantly attracted more \(C.\ septempunctata\) adults than

![Fig. 1 Percentage of response of the virgin and mated males of \(Coccinella undecimpunctata\) to different odor sources, tested in a 4-arm olfactometer (single individual experiment)](image-url)

### Table 1 Percentage of response ± SE of virgin and mated male and female of \(Coccinella undecimpunctata\) to different odor sources, tested in a 4-arm olfactometer (single individual experiment)

| Predator type | No. | No. of non-response | % Response | Un-infested bean plant | Aphids | Infested bean plant with aphids | Empty chamber | \(P\) value |
|---------------|-----|---------------------|------------|------------------------|--------|-----------------------------|--------------|------------|
| Virgin male   | 80  | 15                  | 25.00 ± 1.89 a (AB) | 21.25 ± 3.50 b (B) | 31.25 ± 2.26 b (A) | 3.75 ± 1.83 a (C) | < 0.001 |
| Virgin female | 80  | 16                  | 21.25 ± 1.25 a (B) | 17.50 ± 2.50 b (B) | 36.25 ± 4.20 ab (A) | 7.50 ± 2.50 a (B) | < 0.001 |
| Mated male    | 80  | 14                  | 12.50 ± 2.50 b (B) | 30.00 ± 2.67 a (A) | 32.50 ± 5.90 b (A) | 7.50 ± 2.50 a (B) | < 0.001 |
| Mated female  | 80  | 12                  | 13.75 ± 1.83 b (B) | 20.00 ± 2.67 b (B) | 47.50 ± 3.13 a (A) | 7.50 ± 1.82 a (C) | < 0.001 |

\(P\) value

- L.S.D (A) 4.12
- L.S.D (B) 4.12
- L.S.D (A*B) 8.25
healthy plants or undisturbed aphids alone. Norkute et al. (2020) also found that the *C. septempunctata* adults were significantly more attracted to volatile emitted from aphid-infested barley plants than un-infested plants, and the females were more prone than males to utilize olfactory cues when searching for their prey. The adult of Asian lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) preferred aphid-infested plants over un-infested plants or aphids alone (Xiu et al. 2019). In a Y-tube olfactometer assay, the male and female of *H. axyridis* were showed attracting to *Sophora japonica* flowers to a similar extent (Chun-li et al. 2018). The Y-tube olfactometer bioassays revealed that *H. axyridis* and *Oenopia conglobata* (Coleoptera: Coccinellidae) species used of information from a plant-prey (*Vicia faba*-Aphis fabae) system, but *H. axyridis* exhibited a more complete response (Rondoni et al. 2017). Experienced *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) beetles were more attracted toward cues associated with aphid-infested plants compared with naive beetles (Wang et al. 2015). Adults of *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) were most attracted to the odors from infested tomato leaves + healthy aphids, *Myzus persicae* Sulzer (Hemiptera: Aphididae) than odor sources from healthy aphids or un-infested tomato leaves or infested tomato leaves + stressed aphids or stressed aphids (Heit et al. 2008). The present study showed that mated females of *C. undecimpunctata* had significantly higher than virgin females in response to the odor source “infested bean plant + aphids” (Fig. 2). This response might be due to females being more attracted than males to odor sources the need to provide a high quality food for egg production (Norkute et al. 2020). Ladybeetle adult usually orients its movement towards prey using olfactory cues. As shown in Table 1, non-significant difference was observed in the response between individuals of virgin male and female, and between mated male and female to un-infested bean plant. This response is possible because the host’s secondary metabolism could have induced the volatiles after aphid attacks to attract both sexes of natural enemies as a defense (Francis et al. 2004). The most electro-physiologically active compounds of volatile released from *V. faba* and attract different species of aphids were identified as (Z)-3-hexen-1-ol, 1-hexanol, (E)-2-hexenal, (R)-(−)-linalool, (E)-caryophyllene, and (E)-β-farnesene (Webster et al. 2008). The semiochemicals associated with different species of aphids were identified. α-pinene, β-pinene, Z,E-nepetalactone and (−)-β-caryophyllene, and (E)-β-farnesene were the most compounds found (Leroy et al. 2012). These compounds were potential attractants for *H. axyridis* adults (Leroy et al. 2012; Gencer et al. 2019).

**Conclusions**

The results of this study suggest that *C. undecimpunctata* searching behavior is based on the use of different sets of volatile compounds from many sources that might act as reliable indicators for the presence of its prey. Further investigations are needed to identify the volatile compounds emitted from the infested bean plant.
and aphid, which attract the C. *undecimpunctata* adults. Additionally, to evaluate the regulatory effects on the ladybeetles by these compounds, a new study that monitors the ladybeetle populations under field conditions when using the potential semiochemicals combinations should be conducted.

**Abbreviations**

*C*: Celsius; *cm*: Centimeter; *h*: hour; *var.*: Variety

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I am the only author of this manuscript. So I am responsible for all the steps. The author read and approved the final manuscript.

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