


ORIGINAL ARTICLE

Aphids-induced plant volatiles affect diel foraging behavior of a ladybird beetle *Coccinella septempunctata*Milda Norkute¹, Ulf Olsson² and Velemir Ninkovic¹ ¹Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden and ²Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract The ladybird beetle *Coccinella septempunctata* (L.) is an important biocontrol agent of pests such as various aphid species. Despite being one of the most studied coccinellid species, many aspects of its foraging behavior are still not completely understood. This study focuses on the diel foraging behavior of *C. septempunctata*, investigating their olfactory orientation toward aphid-infested plants, walking activity on plants and on the soil, and feeding rates. In the scotophase the ladybird beetles were significantly more attracted to the odor of aphid-infested plants, on which they also showed considerably higher walking activity than on uninfested controls. Females were more prone to utilize olfactory cues when searching for prey and fed at higher rates than males; this shows that they are better adapted to nocturnal activity, as they require higher food intake. *Coccinella septempunctata* have the same feeding rate during the scotophase as in the photophase. Our study shows that *C. septempunctata* has the potential to forage in the scotophase if prey is abundant. The results support the hypothesis that volatiles of aphid-infested plants can attract or arrest foraging adult ladybird beetles, even in the darkness, which makes a considerable contribution to efficient prey search and enhances feeding capacity.

Key words aphids; C7; feeding; ladybirds; photophase; scotophase

Introduction

The ladybird beetle *Coccinella septempunctata* (L.) is one of the most widespread aphidophagous coccinellids (Hodek & Michaud, 2008) and has proved to be an effective biocontrol agent for selected aphid species such as *Macrosiphum euphorbiae*, *Myzus persicae nicotianae*, etc. (Deligeorgidis *et al.*, 2005; Katsarou *et al.*, 2005; Obrycki *et al.*, 2009). Although it is one of the most studied coccinellid species, various aspects of its ecology and behavior are not yet completely understood (Hodek & Michaud, 2008). For example, most observations on ladybird beetle feeding activity have been made in daytime

conditions (Honek, 1985; Takahashi, 1993; Elliott *et al.*, 2000), overlooking night feeding activity.

While some sources have reported that ladybird beetles are diurnal insects (Nakamuta, 1987; Elliott *et al.*, 2000), there are potential advantages for ladybird beetles that forage during the night. Firstly, foraging at night could be a temporal antipredator trade-off, reducing the probability of encountering with diurnal insect-feeding predators. Antipredator trade-offs are common in many animal taxa and in many different contexts (Lima, 1998). Despite having very few natural enemies, adult *C. septempunctata* can be preyed on by birds and be parasitized by several species of parasitoid wasps, the most studied of which is *Dinocampus coccinellae* (Ceryngier *et al.*, 2012).

Second, ladybird beetles can allocate a greater proportion of daytime to different activities, such as mating, searching for oviposition, sites and migration. Minoretti and Weisser (2000) found that the ladybird beetles spend a considerable proportion of their time engaging in activities other than feeding or searching for prey. Females

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have to spend a large amount of time locating sites suitable for oviposition in order to increase the chance of survival for their progeny (Evans, 2003; Weber & Lundgren, 2009), while males spend a lot of time searching for mates (Honek, 1985).

Furthermore, night feeding could compensate when foraging at daytime is unfavorable due to environmental conditions. There is evidence that ladybird beetle activity is reduced with increasing wind velocity (Takahashi, 1993; Elliott *et al.*, 2000). Moderate to strong winds form during periods of atmospheric instability occurring from mid-morning to late afternoon because of solar radiation, which makes night feeding more advantageous. Changes in both foraging and mating behaviors are common among insects as a response to changing barometric pressure (Pellegrino *et al.*, 2013).

Coccinella septempunctata is a typical oligophagous feeder. Its diet is mostly restricted to aphids, with supplementary consumption of other insects, pollen, and fungal spores (Hodek, 1973; Triltsch, 1997; Evans *et al.*, 1999; Ricci *et al.*, 2005; Radonic *et al.*, 2018). Focusing on a small range of prey requires more time to identify said prey. In order to be more efficient in locating their prey, oligophagous predators use a range of visual, olfactory, and other clues (Obata, 1986; Radonic *et al.*, 2018). Ladybird beetles are known to use visual cues, such as plant color (Lorenzetti *et al.*, 1997) or color contrast (Harmon *et al.*, 1998). They have also been found to utilize olfactory cues, which attracts them toward plants infested with aphids (Ninkovic *et al.*, 2001), and sometimes to particular aphid species (Sengonca & Liu, 1994).

The present study seeks to contribute to a better understanding of the diurnal feeding behavior of ladybird beetles in a controlled laboratory environment. We have examined (i) whether ladybird beetles are foraging in scotophase, (ii) whether olfactory cues are exploited equally to locate the prey in scotophase and photophase, (iii) if the feeding capacity of starved ladybird beetles in scotophase differs from photophase, and (iv) what role gender plays in these foraging mechanisms of a ladybird beetle.

Materials and methods

Insects

Individuals of the ladybird beetle *Coccinella septempunctata* (L.) were collected from natural habitats close to Uppsala, Sweden (59°47'00.0"N, 17°39'00.0"E) and a breeding population was maintained in the laboratory. The adult ladybird beetles used in the experiments were older than 7 d and had been reared in a mixed culture

of both sexes in climate chambers at a constant temperature of 22 °C, humidity of 60% and photoperiod of L12 : D12. One climate chamber was set to have a light photoperiod from 08:00 to 20:00, and the second one from 20:00 to 08:00. The provided diet consisted of bird-cherry oat aphid *Rhopalosiphum padi* (L.) on oat (*Avena sativa*, L., cv. Belinda); pea aphid *Acyrtosiphon pisum* (Harris) on peas (*Vicia faba*, L. cv. Sutton dwarf) and flowering oilseed rape (*Brassica napus* L. cv. Mosaic) as source of pollen. For each experiment, ladybird beetles were randomly collected from culture and separated by sex according to Baugaard (1980). Prior to the experiments, the ladybird beetles were starved for 24 h. *Rhopalosiphum padi*, used as prey species in the experiments, was reared on oat and kept in another greenhouse chamber under the same regime as the ladybird beetles.

Diel food searching behavior

The effects of food availability on time budget and diel behavior were examined by video recording ladybird beetles walking on both aphid-infested and healthy plants in light and dark photic environments.

We used barley plants *Hordeum vulgare* L. cultivar Shandy in the experiments to avoid the influence of associative learning directed to the plant volatiles. Barley plants were grown in plastic pots (90 × 90 × 70 mm) potted with soil (Special Hasselfors garden, Hasselfors, Sweden) and sown 10 plants in each pot. Plants were grown in a growth chamber at 20 ± 1 °C, with a L16 : D8 light cycle and light intensity at 150 μmol/m²/s (Hortilux Schröder, HPS 400W, The Netherlands). At the early two-leaf stage (7 d after planting), 20 aphids per plant were placed carefully on the soil, from where they climbed and settled on the barley plants. The aphids used to infest the plants were wingless, second- to third-instar individuals. The plants were left for the aphids to settle and feed for 2 d before they were used in the experiments. One half of the pots with plants were infested with aphids while the uninfested plants were used as controls.

The recording arena consisted of two white cardboard boxes, each of size 120 × 120 × 250 mm, attached sideways to one another. The front of each box had a 200 × 115 mm opening through which the video recording was made. To avoid the influence of odors entering from the neighboring box, the top of each box was connected to a vacuum pump by a tube with air suction at 250 mL/min.

A pot with barley plants was placed in each of the boxes, one containing aphid-infested plants and another uninfested (control). The position of the pots with infested and uninfested barley was altered randomly

for each filmed trial. The inner walls of the pots were coated with a layer of Fluon (Fluortek TP50) to prevent the ladybird beetles from climbing out. The Fluon is inert and appears to be nontoxic (Sherwood, 1955) and widely used in insect studies (e.g., Carter & Dixon, 1982; Armbrrecht *et al.*, 2007). The camera was positioned so that the openings of both boxes were visible from an elevated angle, to ensure high visibility of ladybird beetle activity close to the barley seedlings. The visibility near the front edge of the pot was limited.

A single adult ladybird beetle was placed on the soil in each pot in front of the plants. The recordings were carried out simultaneously in two rooms, one completely dark and one with a light intensity of $60 \mu\text{mol}/\text{m}^2/\text{s}$. The temperature in the recording rooms was maintained at $22 \pm 2 \text{ }^\circ\text{C}$ and humidity at 40%–60%. The ladybird beetles were filmed for 12 h, from 08:00 until 20:00. The camera trials were repeated for 10 d, using only male or female insects each time. The daytime activity of the ladybird beetles was recorded using a Sony Handycam HDR-XR550VE camera (60 frames per second, resolution 1920×1080), while night-time activity was recorded using an infrared FLIR SC7000 camera (2 frames per second, resolution 640×512).

Video recordings were analyzed using the Observer XT10 software at 12 \times speed. We recorded the total number of time the ladybird beetles engaged in the following behaviors: (1) walking on a plant, (2) walking on soil, (3) sitting on a plant, (4) sitting on soil, and (5) out of vision. The percentage of time dedicated for each behavior is presented in the pie charts (Fig. S1). The length of time that each insect performed each of the five behaviors was analyzed using General Linear Mixed models (see, e.g., Olsson, 2011). The Mixed procedure from the SAS (2014) package was used. The model included presence/absence of aphids, time of day (day or night), sex, and interactions among these, as fixed factors, and used individual as a random factor. Separate analyses were performed for each of five ladybird beetle behaviors. Residuals were checked for normality and homoscedasticity using diagnostic plots. No apparent deviations from the assumptions were detected. Pairwise comparisons were adjusted for multiplicity using the Tukey–Kramer test.

Olfactory response

The behavioral effect of the infested and healthy plants on coccinellid behavior in scotophase and photophase was tested with a two-arm olfactometer, as previously described by Ninkovic *et al.* (2001).

The set-up consisted of an olfactometer with two extended arms. The air was drawn from the center of the arena through the tubes, creating an airflow with a flow rate of 180 mL/min. Each arm was connected to Perspex cages of $120 \times 90 \times 400 \text{ mm}$, with a hole of 80 mm diameter in one side to allow air to enter. One pot with aphid-infested plants was placed in a cage, while a pot with healthy plants was placed in a cage connected to opposite arms of the olfactometer. Teflon tubing was used to connect the cages. The plants were not visible to the ladybird beetles. Five set-ups were used simultaneously, with the positions of the treatment arms alternating randomly. To account for any positional bias, the position of the aphid-infested and uninfested plants was switched between the left and right arms of each olfactometer.

An adult ladybird beetle was placed in the central area of each olfactometer. After an acclimatization period of 10 min, the position of each individual insect was recorded 10 times every 2 min. The cumulative number of visits in the arm zones after ten observations was regarded as one replicate (Ninkovic *et al.*, 2001). If an insect was motionless during three consecutive observations, we considered it to be unresponsive, discarded the replicate, and started a new one with a fresh insect. The olfactometer was cleaned with 70% alcohol after each trial to remove any chemical odors released from the previously tested ladybird beetle. The experiment was carried out in both light and dark photic environments, with the same light arrangement as in the previous experiment. The position of ladybird beetles in the dark was recorded with the aid of red LED light from a Black Diamond Spot headlamp.

The mean number of visits in each olfactometer arm (containing odor of infested or uninfested plants) was compared using a nonparametric Wilcoxon Matched Pairs test. The test was performed separately for each photic environment and gender. The analysis was performed on SPSS statistical software.

Feeding rate

An experiment was carried out to investigate ladybird beetle feeding rate under dark and light photic conditions. Twenty ladybird beetle adults were selected randomly from the colony and divided into two groups of 10 individuals, consisting of females and males in each: one group was those fed only during the photophase and the other group was those fed only during the scotophase. The bottoms of Petri dishes of 10 cm in diameter were covered with filter paper, on which 80 nymphs and apterous adult aphids were placed. At the beginning of the light and dark photoperiod, a single ladybird beetle was placed in

Table 1 Summarized results from Glimmix procedure on total walking times on plant and on the soil.

	Num DF	Den DF	Walking on plant		Walking on soil	
			<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
Presence of aphids	1	32	1.23	0.2762	7.16	0.0117
Gender	1	32	0.01	0.9190	0.52	0.4773
Gender × presence of aphids	1	32	2.14	0.1534	0.05	0.8226
Photoperiod	1	32	32.04	<0.0001	32.60	<0.0001
Photoperiod × presence of aphids	1	32	11.60	0.0018	3.38	0.0754
Gender × photoperiod	1	32	3.41	0.0743	3.74	0.0619

each Petri dish and allowed to feed for the following 12 h. The Petri dish was then covered with parafilm with small perforations to allow gaseous exchange. Insects had access to water by means of a cotton wool plug connected to the water in a small container placed on the filter paper. After 12 h of feeding, the ladybird beetles were transferred to a new Petri dish and the number of aphids consumed was counted. No aphids were provided for the following 12 h, to restrict ladybird beetle feeding activity only to photo/scotophase. This feeding regime was maintained for five consecutive days. Throughout the experiment, Petri dishes with insects were kept in a climate chamber with an L12:D12 light cycle, photophase from 08:00 to 20:00 and scotophase from 20:00 to 08:00. A constant temperature of 15 °C and humidity of 80% was maintained in the chambers.

The data comprised repeated-measures data for the same individuals over 5 d. Therefore, mixed statistical models were used (Littell *et al.*, 2006). The response variable is a count: the number of aphids eaten, out of 80. To account for over dispersion, Generalized Linear Mixed Model (Glimmix) with negative binomial error distribution was used (SAS Institute Inc., 2014). The fixed part of the model included photoperiod (day or night), sex, time (5 d) and two-way interactions between these, and used individual as a random factor. Residuals were checked for normality and homoscedasticity using diagnostic plots. No apparent deviations from the assumptions were detected. Pairwise comparisons were adjusted for multiplicity using a Tukey test.

Results

Diel food-searching behavior

The results from the Glimmix analysis showed that ladybird beetle walking activities on plants and on the soil were affected by photoperiod (Table 1). The total time spent walking on plants was significantly greater in the

photophase (115.75 min) than the scotophase (56.4 min) during the 12 h filming trial ($P < 0.0001$) (Table 1). The interaction between the presence of aphids and photoperiod was also significant ($P = 0.0018$) (Table 1). In the scotophase, the total walking duration of ladybird beetles was significantly greater on aphid-infested plants (80.00 min) than on aphid-free plants (28.01 min) ($P = 0.0178$ Tukey–Kramer); in the photophase, the times spent walking on aphid-infested plants (103.01 min) and aphid-free plants (104.09 min) were similar ($P = 0.3663$ Tukey–Kramer) (Table 1; Fig. 1). Furthermore, ladybird beetles walked longer on the aphid-free plants in the light period (104.09 min) than in the dark period (28.01 min) ($P < 0.0001$ Tukey–Kramer). In the presence of aphids, however, walking activity during the photophase (103.01 min) and the scotophase (80.00 min) did not differ significantly ($P = 0.4090$ Tukey–Kramer) (Fig. 1). Glimmix analysis showed a trend of interacting effects of photic environment and walking activity of males and females ($P = 0.0743$). In the photophase females (105.0 min) spent slightly less time walking on plants compared to males (126.5 min), while in the scotophase females (62.7 min) walked more on the plants compared to males (41.5 min).

Mixed-model analysis showed that walking activity on the soil was significantly affected by both presence of aphids and photoperiod (Table 1). The ladybird beetles spent almost twice as long walking on the soil when aphids were absent (128.02 min) than when aphids were present (75.67 min) ($P = 0.0117$). Furthermore, they walked more often on the soil in the photophase (158.57 min) than in the scotophase (43.51 min) ($P < 0.0001$). The interaction between the presence of aphids and photoperiod was almost significant ($P = 0.0754$). In the scotophase, the time spent walking on the soil with aphid-infested plants (34 min) was similar to that with aphid-free plants (52 min) ($P = 0.923$ Tukey–Kramer), while in the photophase the total walking duration of ladybird beetles was significantly greater with aphid-free plants (204 min) than with aphid-infested plants (113 min) ($P = 0.0143$

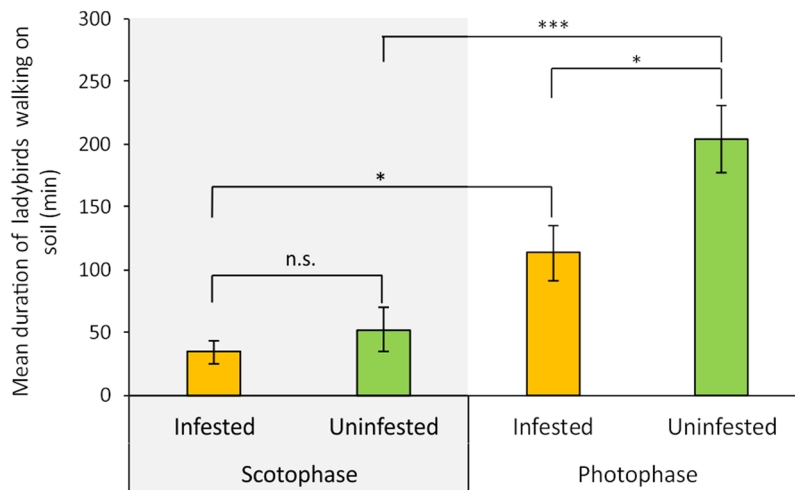


Fig. 1 Walking time of *Coccinella septempunctata* adults on infested and uninfested plants in light and dark photoperiods; mean \pm SE. Significant differences in walking behavior are indicated (Tukey–Kramer, * $P < 0.05$, *** $P < 0.001$ and n.s. referring to nonsignificant).



Fig. 2 Walking time of *Coccinella septempunctata* adults on soil with infested and uninfested plants in light and dark photoperiods; mean \pm SE. Significant differences in walking behavior are indicated (Tukey–Kramer, * $P < 0.05$, *** $P < 0.001$ and n.s. referring to nonsignificant).

Tukey–Kramer) (Fig. 2). Furthermore, ladybird beetles walked longer on the soil with the aphid-infested plants in the photophase and longer with the aphid-free plants in the scotophase ($P = 0.046$; $P = 0.0002$ Tukey–Kramer). The interaction between the gender and photoperiod was almost significant ($P = 0.0619$). In the photophase females (185.3 min) spent more time walking on the soil compared to males (131.9 min), while in the scotophase the trend was opposite for females (31.4 min) and males (57.0 min).

According to the Glimmix analysis ladybird beetle resting activities were significantly affected by photoperiod

on the plants ($P < 0.0001$) but not on the soil (Table S1; Fig. S2).

Olfactory responses

The olfactory bioassay on ladybird beetle searching behavior showed that the odor of aphid-infested barley plants was significantly more attractive to *C. septempunctata* adults than the odor of uninfested plants, in almost all tested combinations (Fig. 3). During the photophase, the odor of infected plants had a significant

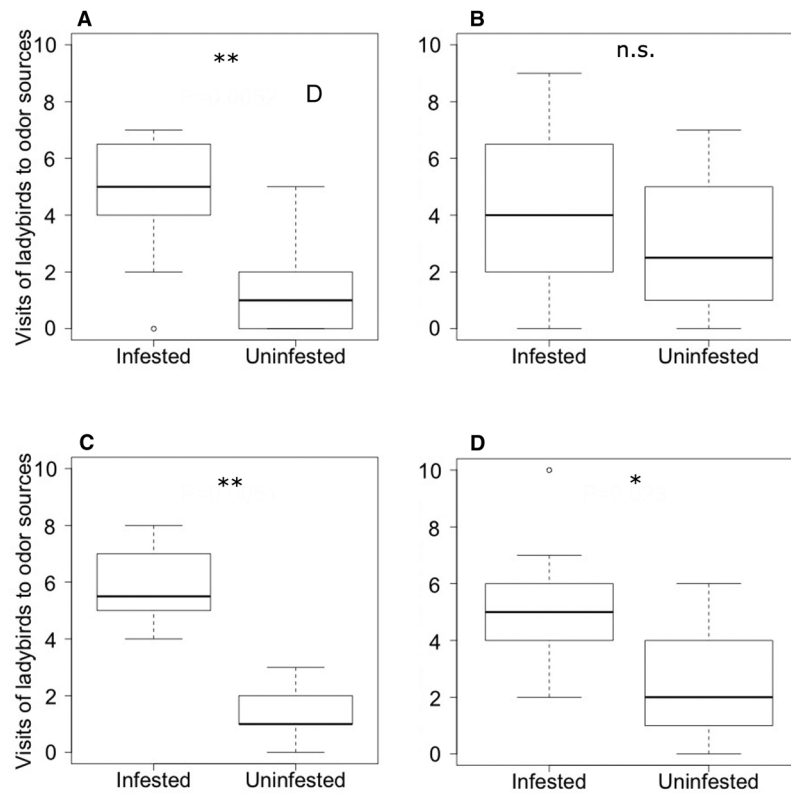


Fig. 3 Frequency of ladybird beetle visits in the olfactometer arms with odor of infested and uninfested plants by (A) females in the photophase, (B) males in the photophase, (C) females in the scotophase, and (D) males in the scotophase. Significant differences in visiting frequency are indicated (Wilcoxon Matched pairs, $*P < 0.05$, $**P < 0.01$, and n.s. referring to nonsignificant). Boxplot indicates the median number of visits in each olfactory arm (horizontal line), interquartile (box), and range (vertical dashed lines).

arresting/attracting effect on females (Fig. 3A) plants ($n = 25$, $Z = 1.53$, $P = 0.005$), but this effect was not observed in males ($n = 13$, $Z = 25.5$, $P = 0.125$) (Fig. 3B). However, during the scotophase both females ($n = 10$, $Z = 2.8$, $P = 0.005$) and males ($n = 15$, $Z = 2.27$, $P = 0.023$) significantly preferred the odor of aphid-infested barley plants over the odor of uninfested plants (Fig. 3C,D).

Feeding rate

The GLIMIX procedure revealed that starved ladybird beetles consume the same amount of aphids in the dark as in daylight. The effect of photic environment on aphid consumption was not significant between day and night (Table 2). Ladybird beetles had the same feeding rate in the photophase (44.34 aphids on average consumed) and the scotophase (41.32 aphids on average consumed) over the 12 h period ($P = 0.46$) (Fig. 4A). Females consumed significantly more aphids than males ($P = 0.0071$) (Fig. 4B). Aphid consumption rate decreased over the 5 d experimental trial, for both sexes and during both day and

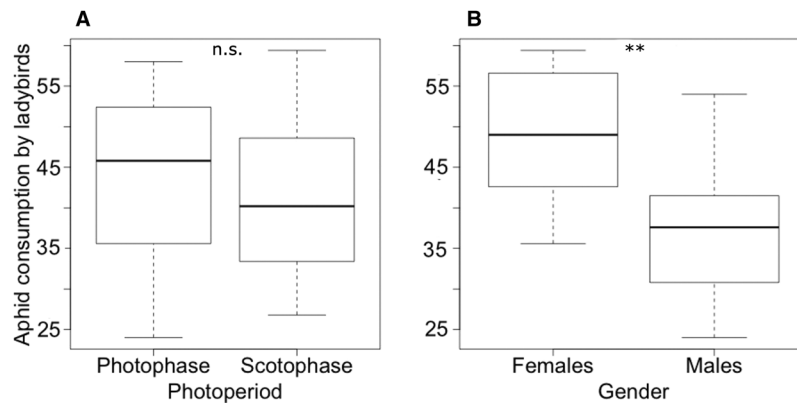
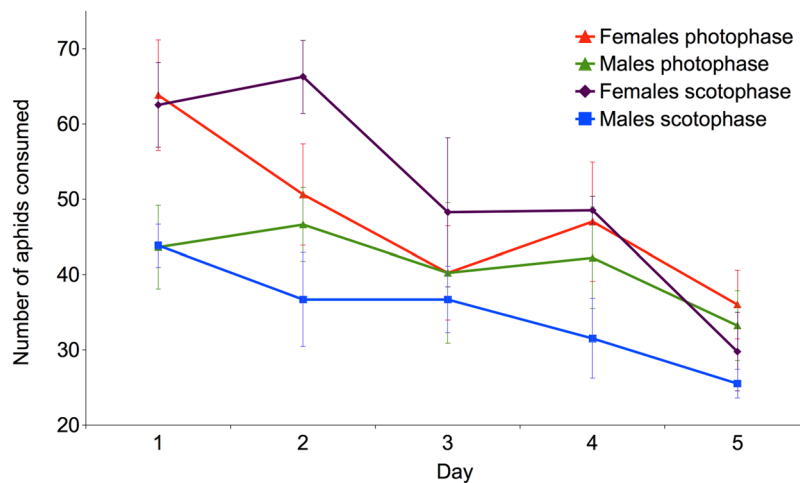
night ($P < 0.0001$) after a starvation period of 24 h. Aphid consumption was highest on the first day (52.50 aphids) and lowest on the last day (30.90 aphids) (Fig. 5).

Discussion

Our results show that *Coccinella septempunctata* exhibit distinct prey search and feeding activity in the scotophase, as long as prey is available. Ladybird beetles are capable of feeding in complete darkness at the same rate as in daylight, even though they cannot see the prey. In the darkness the both sexes of ladybird beetles showed significant attraction toward the odor of aphid-infested plants, indicating that olfactory cues are exploited in prey detection. Interestingly, in the photophase females continued to be attracted to odor of aphids-infested plants, while males did not. Females exhibited higher feeding rates both in the photophase and the scotophase. Our results demonstrate that ladybird beetles continue their foraging activity during darkness using volatiles of aphid-infested plants in

Table 2 Summarized results from GLIMIX procedure on ladybird beetles feeding rate of aphids during scotophase and photophase.

	Feeding rate			
	Num DF	Den DF	F value	P value
Photoperiod	1	16	0.57	0.4629
Gender	1	16	9.53	0.0071
Gender × photoperiod	1	16	1.66	0.2165
Day	4	68	8.11	<0.0001
Photoperiod × day	4	68	0.57	0.6842
Gender × photoperiod	4	68	0.79	0.5339

**Fig. 4** The number of aphids consumed by ladybird beetles shown within the photoperiods (A) and by gender (B). Significant differences in aphid consumption are indicated (Glimmix, $**P < 0.01$, and n.s. referring to nonsignificant). Boxplot indicates the median (horizontal line), interquartile (box), and range (vertical dashed lines).**Fig. 5** Diurnal aphid consumption by female and male *Coccinella septempunctata* during 5 d experimental trial; mean \pm SE, $n = 20$.

location and detection of prey, which can result in the same feeding rate as during the photophase.

Visual cues play an important role for ladybird beetles in daytime activities, such as migration or aphid detection (Stubbs, 1980; Nakamura, 1984; Harmon *et al.*, 1998).

These cues cannot be utilized overnight, so the foraging behavior of ladybirds in the scotophase is likely determined by the use of olfactory cues. This is well illustrated by the results from our olfactory bioassays, as both males and females demonstrated olfactory orientation toward

aphid-infested plants in the scotophase (Fig. 3). There is evidence that aphid-induced plant volatiles can be attractant stimuli for adult coccinellids (Han & Chen, 2000; Schaller & Nentwig, 2000; Ninkovic *et al.*, 2001; Zhu & Park, 2005; Girling & Hassall, 2008). No previous studies have shown ladybird beetle attraction to volatiles of aphid-infested plants in the scotophase. Volatiles of aphid-infested plants allow the predator to detect attracted plants, but not the aphid individuals. By visual cues ladybird beetles perceived aphids only at short range, a few millimeter distance, during foraging on the infected plants (Stubs, 1980; Nakamuta, 1984). The first encounter with the prey is a very important moment in ladybird beetle foraging activity (Hodek & Evans, 2012). There is evidence that an encounter with prey acts as a stimulus for a switch in searching behavior from extensive to intensive (Nakamuta, 1982; Nakamuta, 1985). The strong attraction for ladybird beetles to odor from aphid-infested plants compared to aphid-free ones (Fig. 3) indicates that prey searching behavior may be also triggered by the presence of olfactory cues even before the first encounter with an aphid. The pattern in the search for prey is more or less dependent on the presence of feeding opportunities, rather than being rigidly linked to circadian activity (Curio, 1976).

Daytime may be exploited to migrate in search of better patches, as this requires the use of visual cues to assess habitat quality (Seagraves, 2009). *Coccinella septempunctata* does not fly in the dark (Elliot *et al.*, 2000). In our filmed trials, we observed that most of the walking in the scotophase was restricted to the aphid-infested plants (Fig. 1). However, if ladybird beetles had no encounter with prey and were not exposed to volatiles of aphid-infested plants, they quit searching (Supplementary material—video). In the photophase, we observed a significant increase in walking on the soil, particularly when aphids were not present on the plants, suggesting the walking activity is driven by the need to search for patches with aphid colonies.

It has been shown that ladybird beetles are capable of feeding in the scotophase (Nakamuta, 1987; Harmon *et al.*, 1998), but these studies recorded lower aphid consumption rate in the dark compared to the light. We found that starved ladybird beetles are capable of feeding in the scotophase at the same rate as in the photophase (Fig. 4) and they exhibit the highest feeding potential after a starvation period (Fig. 5). Females tended to be more active than males in nocturnal foraging. In the scotophase, they were more attracted to aphid-infested plants (Fig. 3) and had higher feeding rates in general (Fig. 4). Females might be more active nocturnal foragers than males because of their greater energy requirements,

for egg production and oviposition. There is evidence that coccinellid adult females are usually more voracious than males (Evans, 2003; Khan, 2003; Omkar & Pervez, 2004; Rauf *et al.*, 2013). *Coccinella septempunctata* females prefer to oviposit at the end of the scotophase and in the early photophase hours (Omkar *et al.*, 2010), implying that female ladybird beetles have the need to forage and feed even in the dark. In our experiments, males did not show strong olfactory orientation toward aphid-infested plants during the photophase. Honek (1985) observed that walking males frequently touched aphids without attacking or eating them, suggesting that the locomotory activity of males can often have motivations other than searching for food, including searching for mates.

All of our experiments were conducted in fully controlled environments, which limits the application of our results in predicting ladybird beetle behavior in more natural environments. There is evidence that *C. septempunctata* is temperature-dependent (Hodek, 1973; Honek, 1985; Elliot *et al.*, 2000; Ashraf *et al.*, 2010; Khan & Khan, 2010), exhibiting increased activity with increasing temperature. Therefore, it is likely that results in natural conditions would exhibit lower activity and feeding rates in the scotophase, when it is normally colder than in the photophase. On the other hand, naturally occurring temperature fluctuations could promote feeding rate (Hodek, 1973), resulting in higher demand for food in both the photophase and the scotophase. There is still much to understand about ladybird beetle foraging activity in the scotophase. It would be interesting to observe diurnal ladybird beetle activity in more natural settings, including naturally occurring temperature fluctuations and interactions with conspecifics.

The results of this study contribute to an improved understanding about ladybird beetle feeding behavior in a dark photic environment. Our results suggest that *C. septempunctata*, especially the females, are capable of remaining active in searching for and consuming prey, and utilize olfactory cues in the scotophase if the beetle is starved and if prey is available. Nocturnal foraging could work as a temporal compensatory activity to maintain or increase ladybird beetle feeding capacity over a season. All ladybird beetle activity that we observed in the scotophase was related to search for prey. This leads us to conclude that starving *C. septempunctata* could maintain a continuous diurnal feeding pattern.

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Disclosure

The authors declare that they have no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 The percentage of time dedicated by ladybird beetle adults for five different behaviors (walking on a plant, walking on soil, sitting on a plant, sitting on soil and out of vision): (A) in the photophase on aphid infested plants, (B) in the photophase on healthy plants, (C) in the scotophase on aphid infested plants, and (D) in the scotophase on healthy plants. Light area indicates photophase and shaded area indicates scotophase.

Fig. S2 Resting time of *Coccinella septempunctata* adults on soil and plants in light and dark photoperiods; mean \pm SE.

Table S1 Summarized results from GLIMIX procedure on total sitting times on plant and on the soil.

Video Ladybug behaviour during darkness with and without aphids present.