ASSESSMENT OF PATCH QUALITY BY APHIDOPHAGOUS LADYBIRDS: LABORATORY STUDY ON THE MINIMUM DENSITY OF APHIDS REQUIRED FOR OVIPOSITION

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ABSTRACT

Many studies indicate that there is a density of aphids below which ladybirds are unlikely to lay eggs. This is adaptive as theory indicates that a certain minimum population density of aphids is required if hatchling larvae are to survive. The responses of gravid females of the two spot ladybird, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), recorded over a period of an hour, to colonies of 5 and 50 pea aphids on bean plants and similar plants each previously infested with the same number of aphids for 48 hours were determined. Proportionally more of the ladybirds on plants with 50 aphids or that were previously infested with the same number of aphids for 48 hours laid eggs and larger clusters of eggs, and were less active than those on plants that were infested with or had previously been infested with five aphids. That is, gravid females showed similar oviposition and activity responses to aphid abundance and different levels of honeydew contamination. This indicates that honeydew contamination may be an important cue used by ladybirds when locating and assessing the abundance of prey in aphid colonies.

Keywords: *Adalia bipunctata*, Aphididae, arrestant, Coccinellidae, cues for oviposition, honeydew, minimum aphid density, patch quality, predator

Introduction

Experimental and field studies indicate that there is a density of aphids below which ladybirds are unlikely to lay eggs (Dixon 1959, 2000; Wratten 1973; Honěk 1978; Mills 1979). In addition, in the field two spot ladybirds [*Adalia bipunctata* (L)] tend to lay their eggs before aphid populations peak in abundance (Hemptinne et al. 1990, 1992; Dixon 2000). That is, there is a window in the development of a patch of aphids when ladybirds are most likely to lay eggs. The closing of the window appears to be initiated by adult ladybirds' response mainly to the tracks left by conspecific larvae, which contain a species specific oviposition-deterring pheromone, but also to the odor and tracks of intraguild predators (Růžicka 1997, 2001, 2003, 2006; Hemptinne et al. 1992; Doumbia et al. 1998; Hemptinne et al. 2001; Fréchette et al. 2003a,b; Oliver et al. 2006; Michaud and Jyoti 2007; Sarmento et al. 2007). Although, there are good theoretical grounds and empirical data for a minimum threshold the mechanism by which ladybirds determine aphid density has not been studied. It seems unlikely that ladybirds determine aphid density in terms of numbers of aphids per unit area. Early studies on oviposition in ladybirds indicate that it is associated with the level of satiation of the females. When feeding where aphids are abundant they are more likely to be satiated and less active, and therefore likely to remain in the area and lay eggs (Banks 1954; Dixon 1959). However, there are a few studies that indicate that aphid honeydew can act as an arrestant for ladybird larvae (Carter and Dixon 1984) and a cue for oviposition for adult ladybirds (Evans and Dixon 1986), and therefore adult lady-birds might use the degree of honeydew contamination as a surrogate cue of local aphid abundance. Whether they use one cue or several cues or even a hierarchy of cues when searching for suitable places to lay their eggs can only be resolved experimentally.

The objective of this study is to determine whether gravid ladybirds respond differently to aphid colonies of different sizes and the role of honeydew contamination in their assessment of aphid abundance.

Material and Methods

Ladybirds

Larvae and adults of the two spot ladybird, *A. bipunctata,* were reared in a glasshouse at 20–22 °C and a photoperiod of L : D of 16 : 08 h. They were fed on pea aphids, *Acyrthosiphon pisum* (Harris), which were reared on broad beans plants (cv. *Aquadulce claudia*).

Female ladybirds of similar size and age were selected and kept under standard conditions in order to minimize variation between individuals. Couples (one male and one female) were identified by allowing them to mate. Thereafter, each couple was kept in a 9 cm Petri-dish along with a piece of corrugated paper and an excess of a mixture of instars of aphids. The Petri dish, corrugated paper and aphids were changed daily. Females used in the experiments had laid one cluster of eggs per day over the previous 5 days. Prior to these experiments other female ladybirds were monitored for five days in order to determine when during the day they were most likely to lay eggs.

Bean plants

The cultures of pea aphid, *A. pisum*, were reared on broad bean (cv. *Aquadulce claudia*) plants planted in plastic trays (42 × 24 × 8 cm) and kept in a separate chamber of a glasshouse (area 26 m^2) at 20–22 °C and a photoperiod of L : D of 16 : 08 h. For the first and second experiments, seeds of broad bean were individually germinated in pots, 11 cm in diameter by 9.5 cm tall. When the young plants reached a height of 15 cm and had 6 leaves they were used in the experiments. The plants were divided into four groups. In the first group the plants were each infested with 5 aphids and then left for 48 hours. Those in the second group were infested with 50 aphids and similarly left for 48 hours. Those in the third group were infested with 5 aphids for 48 hours and then prior to the experiment the aphids were removed, but not the exuviae and honeydew. Those in the fourth group were infested with 50 aphids for 48 hours and then treated similarly to the previous group. Prior to their use in experiments the plants in all four groups were checked twice per day, once in the morning and once in the evening and on each occasion new born nymphs in excess of the initial 5 or 50 aphids, respectively, were removed. The young aphids that were removed were taken from the periphery of the colonies in order to avoid disturbance. The plants were kept in a glasshouse under the same conditions as the stock culture.

Experiments

Oviposition response of gravid female ladybirds to different numbers of aphids per plant.

One gravid female ladybird was released at the base of a bean plant with a colony of either 5 or 50 aphids. Then over a period of 1 hour it was recorded whether and when the ladybird laid eggs, the number of eggs laid, the time spent walking or resting and whether it flew from the plant.

Oviposition response of gravid female ladybirds to different levels of aphid contamination.

One gravid female ladybird was released at the base of a bean plant that had been previously infested with either 5 or 50 aphids for 48 hours. Then over a period of 1 hour the following were recorded: whether and when the ladybird laid eggs, the number of eggs laid, the time spent walking or resting and whether it flew from the plant.

The above two experiments were performed in a chamber (area 13 m^2) of a glasshouse between 9.00 am to 2.00 pm, which was the period preliminary observations indicated the ladybirds were most likely to lay eggs.

Statistical analysis

Fisher's Exact Test was used to compare ratios. Means of the times spent walking and resting, and the number of eggs laid were compared using a Student's t-test.

Results

Oviposition response of gravid female ladybirds to different numbers of aphids per plant

A greater proportion of the gravid female ladybirds released on the plants infested with 50 aphids laid eggs than of those released on plants infested with 5 aphids (Table 1). Although proportionally more ladybirds flew from the plants with only 5 aphids than those with 50 aphids the difference was not significant (Table 1), possibly because few beetles were used in this experiment. In addition, the females that remained for the whole hour on plants infested with 50 aphids spent significantly less time walking and more time resting than those on plants infested with 5 aphids (Table 3). Although it was not possible to measure the speed of movement of the ladybirds on the plants the impression was that they moved much more slowly on plants infested with 50 aphids.

Table 1 The number of female *Adalia bipunctata* that remained or flew, the average time they remained before flying, the number that laid eggs and their average clutch size, when placed on plants infested with either 5 or 50 aphids and observed for a period of 1 hour (Total number of ladybirds used in each case 9).

Plants with	Remained Flew		Flew after (min)	Laid eggs	Average clutch size
	Number		Mean ± SE	Number	Mean ± SE
5 aphids	5	4	38.75 ± 8.88	0	0
50 aphids	9	0		6	11.33 ± 2.85
	P = 0.08 Fisher's Exact Test χ² = 7.14			P < 0.01 Fisher's Exact Test χ² = 12	

Table 2 The number of female *Adalia bipunctata* that remained or flew, the average time they remained before flying, the number that laid eggs and their average clutch size, when placed on plants previously infested with either 5 or 50 aphids and observed for a period of 1 hour (Total number of ladybirds used in each case 20).

Plants previously infested with	Remained Flew		Flew after (min)	Laid eggs	Average clutch size
	Number		Mean ± SE	Number	Mean ± SE
5 aphids	9	11	34.91 ± 3.04	3	2.67 ± 0.33
50 aphids	12	8	33.50 ± 4.48	11	8.37 ± 0.72
	P = 0.5 Fisher's Exact Test χ² = 0.9		P = 0.50 t = 0.69; d.f. = 14	P < 0.05 Fisher's Exact Test χ² = 7.03	P < 0.001 t = 3.99; d.f. = 38

Table 3 Time spent walking and resting by the females of *Adalia bipunctata* that did not fly from the plants infested with either 5 or 50 aphids.

Plants infested with	Walking (min)	Resting (min)
	Mean ± SE	Mean ± SE
5 aphids	44.33 ± 2.58 (n = 5)	15.67 ± 2.57 (n = 5)
50 aphids	16.00 ± 2.83 (n = 9)	44.00 ± 2.83 (n = 9)
	P < 0.001	P < 0.001

Oviposition response of gravid female ladybirds to different levels of aphid contamination

Proportionally more of the gravid female ladybirds on the plants previously infested with 50 aphids laid eggs and larger clutches of eggs (Table 2) than those on plants previously infested with 5 aphids. Although proportionally fewer ladybirds flew from plants previously infested with 50 than 5 aphids the difference, as in the previous experiment, was insignificant (Table 2). The females that remained for the whole hour on the plants previously infested with 5 aphids spent significantly more time walking and less time resting than those on plants previously infested with 50 aphids (Table 4).

Table 4 Time spent walking and resting by the females of *Adalia bipunctata* that did not fly from the plants previously infested with either 5 or 50 aphids.

Plants previously infested with	Walking (min)	Resting (min)
	Mean ± SE	Mean ± SE
5 aphids	34.10 ± 2.54 (n = 5)	25.90 ± 2.54 (n = 5)
50 aphids	26.85 ± 2.79 (n = 6)	34.15 ± 2.81 (n = 6)
	P < 0.05	P < 0.05

Discussion

Less progress has been made in understanding the foraging behavior of insect predators than of parasitoids. This is mainly because studies on insect predators and ladybirds in particular, have focused mainly on the searching behavior of the larvae. There are few studies on the searching behavior of adult predators, which select the patches of prey their larvae are dependent on for their development (Ferran and Dixon 1993). For aphidophagous ladybirds there is a poor understanding of the cues adults use to locate their preferred habitats and prey, and the priorities for partitioning of resources between investing in egg laying and foraging (Dixon 2000). There are several theoretical and laboratory studies on where and when gravid ladybirds should lay eggs. These indicate that it is most advantageous if they lay their eggs early in the development of a patch of prey and cease laying eggs as soon as conspecific and heterospecific larvae hatch and begin foraging in these patches. That is, there is an "egg window" early in the development of a patch of prey and the ability of ladybirds to synchronize the laying of eggs with this window is likely to be important in determining their fitness. Too early and their larvae will be unable to find sufficient prey per unit time to survive and too late and their eggs are either likely to be eaten by conspecific larvae or there is insufficient time before the aphids become too scarce to sustain ladybird larval development. Considerable progress has been made in identifying the cues associated with the closing of the egg window. These appear to be mainly chemicals in the tracks left by conspecific larvae (Oliver et al. 2006; Klewer et al. 2007; Magro et al. 2007 and references there in). Although there are several field and laboratory studies that indicate that a certain minimum population density of aphids is required for ladybirds to start laying eggs there have previously been no studies on how they assess aphid abundance. This is surprising as prey abundance is important in determining the survival of their offspring.

The result reported here indicate that proportionally more gravid females of *A. bipunctata* laid eggs and larger clutches of eggs on plants infested with 50 than with 5 aphids. Although more ladybirds flew within the hour of observation from plants infested with 5 than 50 aphids, it was not significant, but those on plants infested with 50 aphids did spend significantly more time resting and less time walking than on plants infested with 5 aphids. That is, as previously reported, this ladybird shows a density dependent oviposition and activity response to aphid density. The objective of the second experiment was to determine whether the physical presence of aphids was necessary for these responses. The results were similar to those obtained in the first experiment in that even in the absence of aphids proportionally more of the ladybirds on plants previously infested with 50 aphids laid eggs and larger clutches of eggs, and spent more time resting and less time walking than on plants previously infested with 5 aphids. That is, the overall behavior of the adults in the two experiments in terms of egg laying and activity was similar.

The early studies on ladybirds indicated that the laying of eggs close to aphid colonies was possibly simply a consequence of the nutritional status of the females. As they tended to be satiated they spend less time moving and when they did move they tended to remain in the area and in addition their satiated state made it more likely they would oviposit (Banks 1954; Dixon 1959). Subsequent studies indicated that honeydew could act as an arrestant and stimulus for oviposition (Carter and Dixon 1984; Evans and Dixon 1986). However, the fact that ladybirds will apparently lay eggs both in the field and laboratory in the absence of aphids or cues associated with their presence has tended to obscure the issue (Sakuratani and Nakamura 1997; Hodek and Honěk 1996; Oliver et al. 2006). If ladybirds cannot resorb mature eggs then gravid females will eventually lay eggs whether aphids are present or not. Therefore, the occasional laying of eggs in the absence of aphids does not necessarily invalidate what appears to be indicated by several previous studies and that reported here – ladybirds can utilize cues associated with the presence of aphids when ovipositing and as a consequence lay their eggs close to aphid colonies, the most commonly observed situation in the field (Dixon 1959). Accepting this it is now important to determine whether cues other than the contamination associated with aphids can act as an arrestant and stimulate oviposition. In particular, does the physical presence of aphids play an additional role in ladybird oviposition?

Parallel studies on other aphidophaga, in particular hoverflies, indicate that the adults of these predators also use aphid associated cues when foraging for oviposition sites and assessing patch quality. As with ladybirds honey-

dew also appears to act as an arrestant for gravid hoverflies (Dixon 1959; Itô and Iwao 1977; Sanders 1979; Sutherland et al. 2001) although features in addition to larval tracks (Hemptinne et al. 1993), such as the presence of winged aphids may be used to indicate patch quality (Kan 1988). Thus, although the details differ it is likely that all aphidophaga and other insect predators utilize similar cues associated with prey abundance when ovipositing.

Acknowledgements

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