

OVIPOSITION BEHAVIOUR OF THE TWO-SPOT LADYBIRD BEETLE *Adalia bipunctata* (L.) (COLEOPTERA: COCCINELLIDAE) ON PLANTS WITH CONSPECIFIC LARVAL TRACKS.

B. FRÉCHETTE, C. ALAUZET & J.-L. HEMPTINNE

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There are many laboratory studies on the oviposition-detering effect of conspecific larval tracks on the two-spot ladybird beetle, *Adalia bipunctata* (L.). However, this phenomenon has not been studied in the field. In near natural field conditions *A. bipunctata* foraging on aphid infested plants, with or without conspecific larval tracks, were reluctant to lay eggs on and quickly left the plants with conspecific larval tracks compared to the control plants. The results of this study indicate that *A. bipunctata* reacts to conspecific larval tracks on plants in near natural conditions, as they do in the laboratory.

Bruno Fréchette (e-mail : frechette_bruno@yahoo.ca), Claude Alauzet, Université Paul Sabatier. Laboratoire d'écologie terrestre. UMR 5552. 118 route de Narbonne (4 R 3). FR-31062 Toulouse cedex 04. France & Jean-Louis Hemptinne, Ecole Nationale de Formation Agronomique. Laboratoire d'Agro-écologie 000271JE1. B.P. 87. FR-31326 Castanet-Tolosan cedex. France.

INTRODUCTION

Aphidophagous ladybird beetles seeking oviposition sites have to move between patches of aphids, evaluate patch quality and decide whether to feed and/or lay eggs. Patch quality is important since it determines the probability of survival of their progeny (KINDLMANN & DIXON 1993, 1999).

Ladybirds should avoid ovipositing in patches of aphids that are already exploited by conspecific larvae. Laying eggs in such patches will greatly increase the risk of cannibalism (MILLS 1982), which is an important mortality factor in natural population of several species of ladybird beetles (OSAWA 1989, 1993; HIRONORI & KATSUHIRO 1997).

Many field observations indicate that ladybird eggs are often laid near aphid colonies that are in an early phase of development (BANKS 1955; HEMPTINNE et al. 1992). These and other

observations led KINDLMANN & DIXON (1993, 1999) to propose that there is in the development of aphid patches a time, "egg window", when it is most advantageous for ladybirds to lay their eggs. This stresses the importance of the age of an aphid colony in determining its suitability for oviposition. Since ladybird larvae and aphid colonies have similar development times, the theory stipulates that ladybird should lay a limited number of eggs early in the development of aphid colonies. If eggs are laid later the aphid colony might disperse and disappear before the ladybird larvae complete their development. In the absence of food the risk of cannibalism greatly increases (AGARWALA & DIXON 1992).

Females of the two-spot ladybird beetle, *Adalia bipunctata* (L.), do not use the age structure of aphid colonies to assess their quality as oviposition sites (HEMPTINNE et al. 2000). However, in Petri dishes they refrain from ovipositing when confined with conspecific

larvae or adults (HEMPTINNE et al. 1992), or on substrates contaminated with conspecific larval tracks (DOUMBIA et al. 1998).

The oviposition-detering effect of larvae on the reproductive behaviour of aphidophagous was first described for *Adalia bipunctata* L. by HEMPTINNE et al. (1992) and that it was their larval tracks for *Chrysopa oculata* Say by RŮŽIČKA (1994). Subsequently the same phenomenon was recorded for many species of chrysopids (RŮŽIČKA 1996, 1997a, 1997b, 1998), ladybirds (RŮŽIČKA 1997b, 2001; YASUDA et al. 2000; HEMPTINNE et al. 2001) and the cecidomyiid fly *Aphidoletes aphidimyza* (Rondani) (RŮŽIČKA & HAVELKA 1998).

The experiments on the reproductive behaviour of the two-spot ladybird beetle *A. bipunctata* were done in the laboratory. The goal of this study was to determine the oviposition response of *A. bipunctata* in near natural conditions on broad bean plants contaminated with larval tracks.

YASUDA et al. (2000) counted the number of eggs laid by *Harmonia axyridis* Pallas on plants previously walked on by larvae of *H. axyridis* and *Coccinella septempunctata* L. This study was done in cages and the behaviour of the females was not observed. HEMPTINNE et al. (1992) observed that *A. bipunctata* confined in Petri dishes with conspecific larvae were more active than control beetles. Therefore, we also recorded the behaviour of the females.

MATERIALS & METHODS

Ladybirds

The eggs of the two-spot ladybird, *A. bipunctata*, were obtained from a stock culture. Larvae were reared at $20 \pm 1^\circ\text{C}$, LD 16:8, and fed an excess of pea aphids, *Acyrtosiphon pisum* Harris. Adults were isolated within 24 hours of their emergence from pupa. When their integuments had hardened, their sex was determined and they were kept in male female pairs. Every day, these pairs of adults were put in clean Petri dishes with a piece of corrugated paper and an excess of pea aphids;

any eggs laid were counted and removed daily. Ladybirds selected for the experiment were between 10-20 days old and had laid at least one egg batch in the last 5 consecutive days.

Bean plants

Broad-bean plants, *Vicia faba* L., used in the experiment were about 15 cm high and had 6 leaves. Experimental plants were those on which 5 fourth instar larvae had walked for 45 minutes 16 hours before the beginning of the experiment. Both control and experimental plants were then infested with about 100 aphids and left for 15 hours for the aphids to settle.

Experiments

Experiments were performed outdoors in the shade between 10:00 am and 1:00 pm in June and July 2001 and 2002. One female was released at the base of a bean plant and (1) whether or not it laid eggs, (2) the time spent *Walking*, *Resting*, *Eating* and *Ovipositing* and (3) its residence time were recorded. Whether the beetles walked or flew off the plants was also recorded. Mean temperature at the beginning of the experiment was $22.3 \pm 0.3^\circ\text{C}$. There were 40 replicates for each treatment.

Statistical analysis

Differences in proportions were compared using Pearson tests. Percentages were arcsin transformed before analysis and then compared using a t-test or a Wilcoxon test if the distribution of the results was not normal according to the Shapiro-Wilk test. All analysis were made using Jmp In® (SALL et al. 2001). For the residence times, the values for experimental and control beans were sorted into three categories: *short* (0-60 min), *medium* (60-120 min) or *long residence times* (more than 120 min). The distributions of frequencies were compared using a Log Likelihood Ratio test computed by hand following ZAR (1996).

RESULTS

Oviposition

A significantly greater proportion of females laid eggs on control than on experimental plants (Fig. 1; Pearson: $\chi^2=11.114$; $df=1$; $p=0.0009$). Only one female (2.5%) laid eggs on an experimental plant.

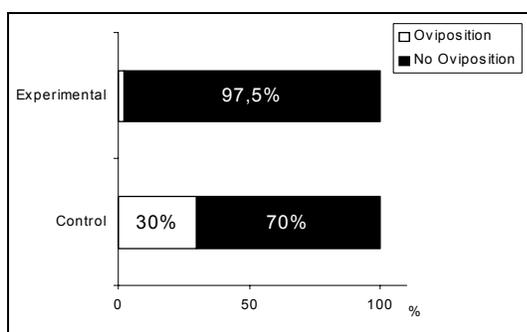


Fig. 1. The percentage of *A. bipunctata* females that laid eggs on control and experimental plants.

Residence time

Ladybirds tended to stay for a shorter period of time (0-60 min) on experimental plants than on control plants (Figure 2; Log Likelihood Ratio: $G=8.48$; $df=2$; $p<0.05$).

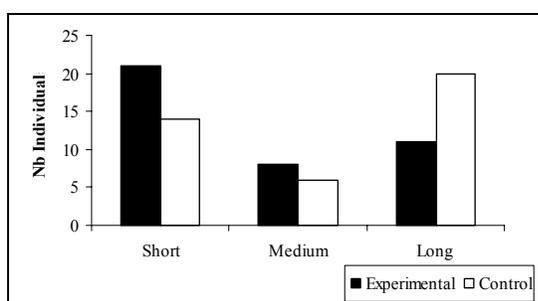


Fig. 2. The number of *A. bipunctata* females that had short, medium, and long residence times on control and experimental plants.

Behaviour

Only the behaviour of beetles that stayed at least 30min on the plants ($n=30$ for control, $n=26$ for experimental) was analysed. Other than in the

percentage of the time allocated to *Oviposition* (Wilcoxon: $\chi^2=7.9929$; $df=1$; $p=0.0047$), there were no significant differences in that allocated to *Walking* (t-test: $F=0.9087$; $df=1$; $p=0.3447$), *Resting* (t-test: $F=0.0060$; $df=1$; $p=0.9387$) or *Eating* (t-test: $F=0.0991$; $df=1$; $p=0.7541$) (Fig. 3). In both treatments, a similar percentage of beetles (12.5%) left the plants without eating, while 87.5% ate at least one aphid. Of the females that laid eggs and completed oviposition before the experiment ended, 80% (8 out of 10) ate at least 1 aphid before leaving the plant.

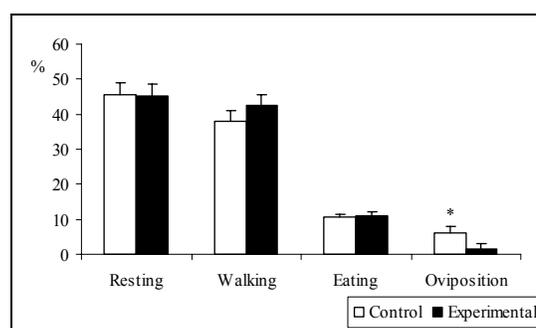


Fig. 3. The percentage of time spent in different activities by *A. bipunctata* females on control and experimental plants. Significant difference ($p<0.05$) is indicated by an asterisk (*).

Way of leaving the plants

Of the ladybirds that left plants before the end of 3 hours ($n=24$ for control plants, $n=31$ for experimental plants) most left by flying (70.8% for control plants, 77.4% for experimental plants), and the rest by walking (29.2% for control plants, 22.6% for experimental plants). The differences in percentages between treatments were not significant (Pearson: $\chi^2=0.309$; $df=1$; $p=0.5782$).

However, temperature had a significant effect on the tendency to fly (Pearson: $\chi^2=8.744$; $df=1$; $p=0.0031$). For both treatments combined, the percentage flying was significantly higher at $\geq 21^\circ\text{C}$ (83.7%, $n=43$) than at $< 21^\circ\text{C}$ (41.7%, $n=12$).

DISCUSSION

This study provides strong evidence that the effects of the pheromone in larval tracks on the

oviposition behaviour of *A. bipunctata* observed in the laboratory are also relevant to natural conditions. *A. bipunctata* females leave earlier and are reluctant to lay eggs on plants on which conspecific larvae have previously walked, even if food is abundant.

However, the agitated behaviour observed by HEMPTINNE et al. (1992) was not observed in this study. Females spend the same percentage of time walking, resting and eating on both control and experimental plants. Moreover, even though females tended to leave experimental plants earlier than control plants, the same percentage (87.5%) ate at least one aphid before leaving in both treatments. This indicates that females may refrain from ovipositing on aphid infested bean plants with larval tracks, but will stay on these plants and feed when hungry. That is, the only difference in behaviour was the acceptance or rejection of the plant as an oviposition site.

Even though the results clearly demonstrate that larval tracks deter oviposition, the low percentage of females that laid eggs on control plants (30%) is worrying. The reason may be that the ladybirds used in the experiment were not all equally 'ready' to oviposit within the 3 hour observation period. It is possible that female foraging behaviour depends on some intrinsic conditions, such as their egg load (MIKENBERG et al. 1992). A female that is not ready to oviposit may respond differently to environmental cues than one that is about to lay eggs. In addition, a female that takes a long time to find a 'suitable' site for oviposition may accept a site that she would have normally rejected as in HEMPTINNE et al. (1992). There is need for a better understanding of the physiological ecology of ladybirds.

A better knowledge of a ladybird's oviposition cycle could lead an improvement in experimental design and clearer results. Working only with beetles ready to, but not desperate to oviposit may result in a greater percentage ovipositing.

The response of adult ladybirds to larval tracks could help to explain how ladybirds distribute themselves in the environment. It may account for why even in patches of high aphid density there are often few ladybird eggs. For example, there is not a density dependent increase in oviposition by *A. bipunctata* in response to an

increase in abundance of its aphid prey on lime trees in the field (WRATTEN 1973). When cereal aphid density is augmented in wheat fields there is an augmentation in the adult numbers of both *Hippodamia convergens* Guérin-Méneville and *C. septempunctata*, but egg density is very low and about the same in both augmented and control patches of prey (ELLIOTT & KIECKHEFER 2000).

The effect of larval tracks on the distribution of aphidophagous ladybirds could affect their potential as biological control agents. By only laying a few eggs early in the development of an aphid colony (KINDLMANN & DIXON 1993), ladybirds are unable to mark aggregative response to patches of prey, a characteristic feature of effective biological control agent (BEDDINGTON et al. 1978).

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