EFFECTS OF CONSPECIFIC AND HETEROSPECIFIC LARVAL TRACKS ON MOBILITY AND SEARCHING PATTERNS OF *Cycloneda limbifer* SAY (COLEOPTERA: COCCINELLIDAE) FEMALES

Z. RŮŽIČKA & R. ZEMEK

RŮŽIČKA, Z. & R. ZEMEK 2003. Effects of conspecific and heterospecific larval tracks on mobility and searching patterns of *Cycloneda limbifer* Say (Coleoptera: Coccinellidae) females. Pp. 85-93 *in* A.O. SOARES, M.A. VENTURA, V. GARCIA & J.-L. HEMPTINNE (Eds) 2003. Proceedings of the 8th International Symposium on Ecology of Aphidophaga: Biology, Ecology and Behaviour of Aphidophagous Insects. *Arquipélago*. Life and Marine Sciences. Supplement 5: x + 112 pp.

EthoVision, a computerised video tracking system was used to monitor the behaviour of females of an aphidophagous coccinellid *Cycloneda limbifer* Say. Time spent on, and distance walked within 30 minutes, were recorded on simultaneously provided clean substrates and substrates with fresh tracks of conspecific or heterospecific first instar larvae. Females spent longer and walked a greater distance on substrates with fresh tracks of conspecific larvae than on clean substrates. In contrast, females of C. *limbifer* spent less time and walked a shorter distance on substrates with fresh tracks of larvae of the coccinellid *Ceratomegilla* (*Semiadalia*) *undecimnotata* (Schneider) than on clean substrates. During the middle period (10-20 minutes) of the tests, the speed of movement of *C. limbifer* females was significantly lower on substrates with conspecific larval tracks, and significantly higher on substrates with tracks of *C. undecimnotata* larvae than on clean substrates. Previous results show a strong intra- and interspecific oviposition-deterring effect of fresh larval tracks of these coccinellid species on *C. limbifer*. Results presented paradoxically indicate that the tracks of conspecific larvae significantly increase the time *C. limbifer* spent on a substrate, while those of *C. undecimnotata* larvae decrease it.

Zdenik Růžička (e-mail: ruzicka@entu.cas.cz*) & R. Zemek, Institute of Entomology, Academy of Sciences, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic.*

INTRODUCTION

Mechanisms regulating complex behaviour in insects are often found after laborious and time consuming experiments. The incidental discovery of the oviposition-deterring effect of the tracks of an aphidophagous insect (RŮŽIČKA 1994) is an exception. It came after a lot of effort had been devoted to investigating spacing in different predators (SOLOMON 1949, 1964; KUCHLEIN 1966; MARKS 1977; HEMPTINNE & DIXON 1991; FERRAN & DIXON 1993).

Tracks of conspecific larvae deter females of many aphidophagous coccinellids from ovipositing (RŮŽIČKA 1997b; DOUMBIA et al. 1998; YASUDA et al. 2000; RŮŽIČKA 2001). Interspecific oviposition-deterring effects of larval tracks in coccinellids (RŮŽIČKA 2001) are less frequently recorded than in chrysopids (RŮŽIČKA 1998). Intra- and interspecific oviposition-deterring effects of the tracks of first instar larvae were especially strong between the coccinellids *C. limbifer* and *C. undecimnotata*. The intensity of the oviposition-deterring effects of fresh conspecific tracks and tracks of *C. undecimnotata* larvae on *C. limbifer* were similar, but the density of faecal spots on substrates with tracks of C. *undecimnotata* larvae was significantly lower than on clean substrates, that on substrates with fresh conspecific tracks was slightly higher, though not significantly so, than on clean substrates (RŮŽIČKA 2001). This indicates that fresh conspecific tracks only deter *C. limbifer* females from ovipositing, but heterospecific tracks deter them from ovipositing and searching.

In contrast, females of the coccinellid *Harmonia axyridis* Pallas laid fewer eggs and were observed less frequently on plants with tracks of conspecific larvae than with tracks of *Coccinella septempunctata* larvae (YASUDA et al. 2000). The authors suggested that the less time spent on plants contaminated with conspecific than on those with heterospecific tracks may account for why fewer eggs were laid on plants with conspecific tracks.

Advanced technologies offer reliable ways of monitoring searching behaviour in insects. The new modes of digital data collection provide highly accurate information on insect mobility, which is less dependent on the skill and or time available to researchers. In this study, computerised video monitoring of coccinellid females enabled a more detailed analysis of the effects of oviposition-deterring larval tracks.

In order to find out whether repellent effects are associated with the oviposition-deterring effects of larval tracks, the searching behaviour of single *C. limbifer* females was compared on simultaneously provided clean substrates and substrates with either conspecific or *C. undecimnotata* larval tracks. The mobility of single females was recorded by EthoVision, a computer-aided automatic video tracking system, which enables continuous monitoring of small objects within selected areas of an arena.

MATERIALS AND METHODS

Insects

Experiments were done using Cycloneda limbifer Casey (origin Cuba 1996) and Ceratomegilla undecimnotata (Schneider) [=Semiadalia undecimnotata (Schneider)] (origin North Bohemia, Czech Republic). Females used in experiments were 10-25 days old. The laboratory culture of C. limbifer was reared on Aphis fabae Scopoli, and that of C. undecimnotata on Acyrthosiphon pisum Harris. Cultures of both aphids were maintained on horse bean, Vicia faba L.

Experimental design

The effect of larval tracks on the behaviour of females of the aphidophagous ladybird *C. limbifer* was studied in experiments similar in design to those used previously (RŮŽIČKA 1997b, 2001). The behaviour of a female was monitored on a clean substrate and an adjacent substrate with larval tracks within a circular arena (Fig. 1). The arena was the bottom of glass Petri dish, 18.5 cm in diameter. The rim of the dish was painted with Fluon (polytetrafluorethylene), which prevented the coccinellid from leaving the arena. The test substrates consisted of strips (40 x 200 mm) of transparent plastic sheet transversally folded every 10 mm, which resulted in a total length of 130 mm. The transparency of the material enabled females to be monitored on both the upper and lower surfaces of each strip. The strips were placed parallel and 2.5 cm apart, each inside a 53x145mm rectangle substrate zone, within the monitored circular arena. They were fixed to the bottom of the Petri dish with narrow strips of clear adhesive tape. The substrate zones covered 57% of the area of the circular arena.

In order to ensure recording of females present also on the margin of substrates, the substrate zones around the folded strips were slightly larger then the substrates. The positions of a female on the test substrates and outside substrate zones were recorded for 30 minutes with a computerised video tracking system. The monitoring started three minutes after the female was carefully placed in the centre of the arena. In order to avoid effects of bias the substrates with larval tracks were placed alternately in the left and right rectangles. In addition, blank tests with two clean substrates were carried out to reveal whether females had a preference for one of the two rectangles, which would indicate a bias due to, e.g. temperature, light intensity or geomagnetism. The behaviour of each female was monitored first in a blank test and then, after 5-10 minutes, in a choice test. This was replicated 14 times. No female laid eggs during the tests.

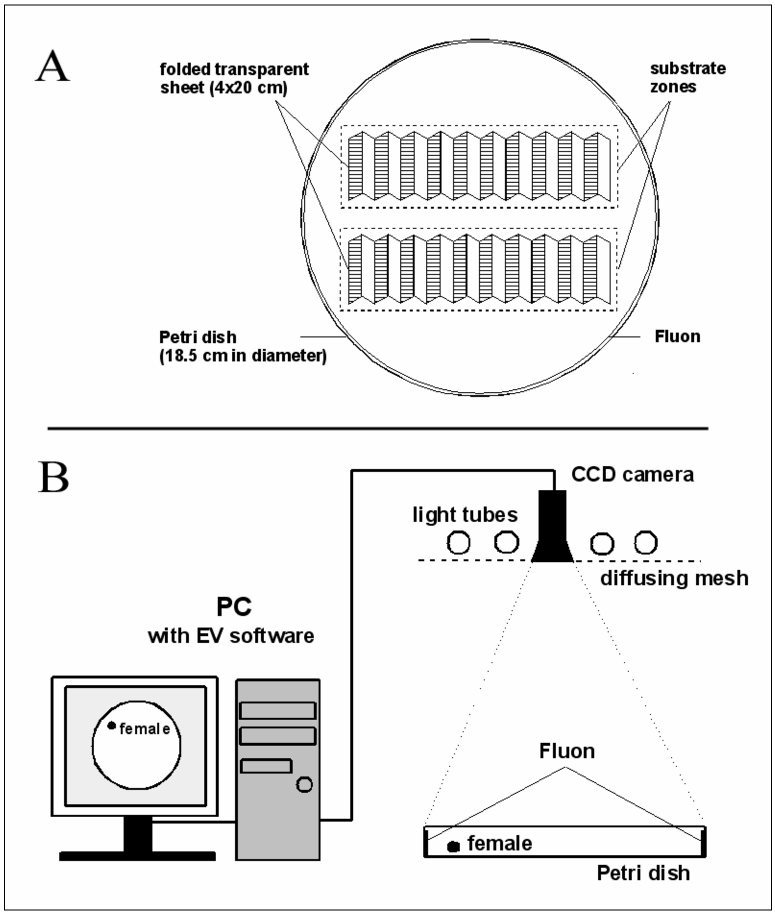

Fig. 1. Diagram of the arena (A) with two substrates and the monitoring apparatus (B). Fluon (polytetrafluorethylene) painted on inner wall of Petri dish.

Contamination with larval tracks

A pair of plastic strips was exposed to 40 unfed first instar conspecific or heterospecific larvae for 4 hours in a Petri dish, 18.5 cm in diameter. The inner rim of the dish was painted with Fluon to keep larvae in the dish. Strips were used for experiments within 6 hours of exposure to larvae.

Video tracking

The experiments were carried out in a dark room (2.6H2.7H2.7 m) illuminated from above by a series of eight 150 cm long fluorescent tubes (Narva LS 65 W-1 coolwhite). Diffused lighting was achieved by placing a thin white cloth under the lamps. The light intensity at floor level in the arena was 800 Lux. Temperature in the room during the experiments was kept at 26 1 ∀C. A Petri dish with a beetle in it was placed on a white sheet of paper. White was used to ensured a high contrast between the background and the beetle, necessary for detection by the computer. The experimental arena was scanned by a colour CCD camera equipped with a zoom lens and fixed to the ceiling above the centre of the arena. The composite video signal from the camera was fed into a computerised video tracking system placed outside the room. The recording system consisted of a video monitor (Sony), a computer (486DX2, 66 Mhz) with a frame grabber (Targa Plus, TrueVision) and EthoVision software (Noldus Information Technology, 1997). The location of a beetle in the arena was determined automatically by the software using a grey scaling method of object detection. The co-ordinates of the centre of the animal's body were calculated using a spatial resolution of 254H238 pixels. Tracking was done 10 times per second which was the highest possible sample rate (BELL 1991) taking into consideration the speed of the processor and storage capacity of the computer. Nevertheless, this gave an accurate representation of the track.

Data analysis

The digitised paths of individual beetles were used to calculate distances walked and time spent on each of the two test substrates and on the surrounding glass. For details of the algorithms used in the data analysis see Noldus Information Technology (1997). Female behaviour on both substrates was compared in three subsequent periods: 0-10, 10-20 and 20-30 minutes. Differences in the time spent on the two substrates were tested using non parametric Wilcoxon signed paired sample test (SIEGEL & CASTELLAN 1988). The same test was used to compare distance walked and speed of movement on the substrates.

RESULTS

Blank tests

The effect of bias on individual females of *C. limbifer* walking on two clean substrates was not significant. The results for females on the two clean substrates, in the first choice test with conspecific (values in parenthesis) and the second, with heterospecific larval tracks (values in brackets), revealed no significant bias in the time spent on substrates in the left and right positions (P=0.5830), [P=0.8077]. The values for the periods 0-10, 10-20 and 20-30 minutes were (P=0.3258), [P=0.3910]; (P=0.9515), [P=0.3575] and (P=1), [P=0.1937]. Total distances walked by emales in the blank tests on the right and left substrates were similar (P=0.7148), [P=0.7148]. Records of the tracks of the most representative female, i.e. the one with the ratio of the distances walked on the two substrates nearest to the average value, in each blank test is illustrated in Fig. 2. The distances walked during the three subsequent periods of 10 minutes were similar (P=1), [P=0.5416]; (P=0.1040), [P=0.6698] and (P=0.7148), [P=0.5416] (Fig. 3 and 4). The average speed of females on clean substrates in the left and right positions when associated with conspecific larval tracks was 6.7 mm/sec and 6.0 mm/sec. The average speed of females on clean substrates in the left and right positions when associated with tracks of heterospecific larvae was 5.6 mm/sec and 5.1 mm/sec. In both blank tests, the speed of walking on clean substrates, in the right and in the left position, did not differ either in the whole test (P=0.3258), [P=0.5416], or in three consequent periods (P=0.3590), [P=0.8311]; (P=0.9700), [P=0.5771], and (P=0.1940), [P=0.1040] (Fig. 5).

During the blank tests, females of *C. limbifer* spent (21 %) and [18 %] of the total time on the glass bottom of the Petri dish outside substrate zones. The average distances (0∀SE) walked on the glass were (527∀92 cm) and [392∀88 cm], i.e. (40 %) and [34 %] of the total distance walked in the monitored area. The average speed of females on the glass away from the substrates was (13.8 mm/sec) and [12.6 mm/sec].

Choice experiments with fresh tracks of conspecific larvae

The total residential time and the distance walked on substrates with fresh tracks of unfed conspecific first instars were significantly longer than on clean substrates (P=0.0134 and P=0.0203). The average speed of females was 10.9 mm/sec on the clean substrate and 8.9 mm/sec on the substrate with tracks. The average speed on both substrates in the test did not differ significantly (P=0.1531).

Females of *C. limbifer* spent significantly longer on substrates with tracks than on clean substrates only during the first and the second period of 10 minutes (P=0.0353 and P=0.0203). In the last period, the difference in favour of the substrate with tracks was not significant (P=0.5830) (Fig. 3). Also distances walked on substrates with larval tracks were significantly longer than on clean substrates in the first and second period (P=0.0245 and P=0.0419), but not significantly so in the last period (P=1). Walking speed of females on substrates with tracks was significantly lower than on clean substrates only in the middle period, i.e. between 10-20 minutes (P=0.0250), but not in the first and the last periods (P=0.6770), and (P=0.6360), (Fig. 5). A record of the tracks of the most representative female is illustrated in Fig. 2.

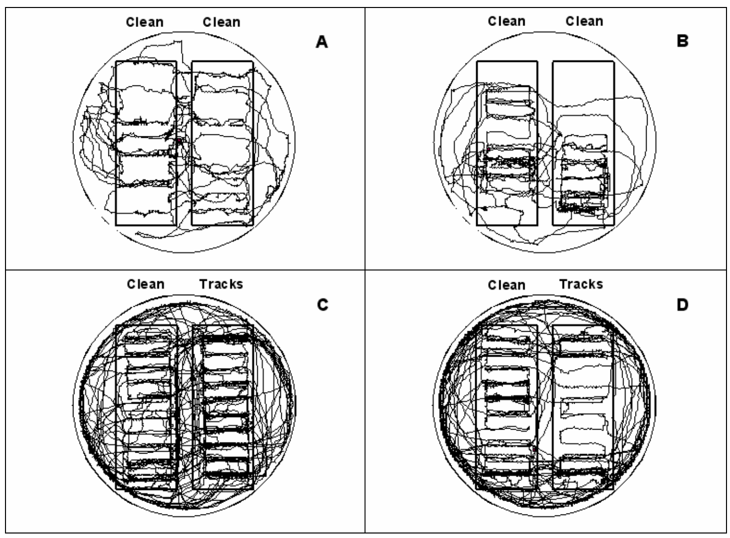

Fig. 2. Tracks of average *Cycloneda limbifer* females in blank tests (A and B) and in choice tests with conspecific (C) and *Ceratomegilla undecimnotata* (D) larval tracks.

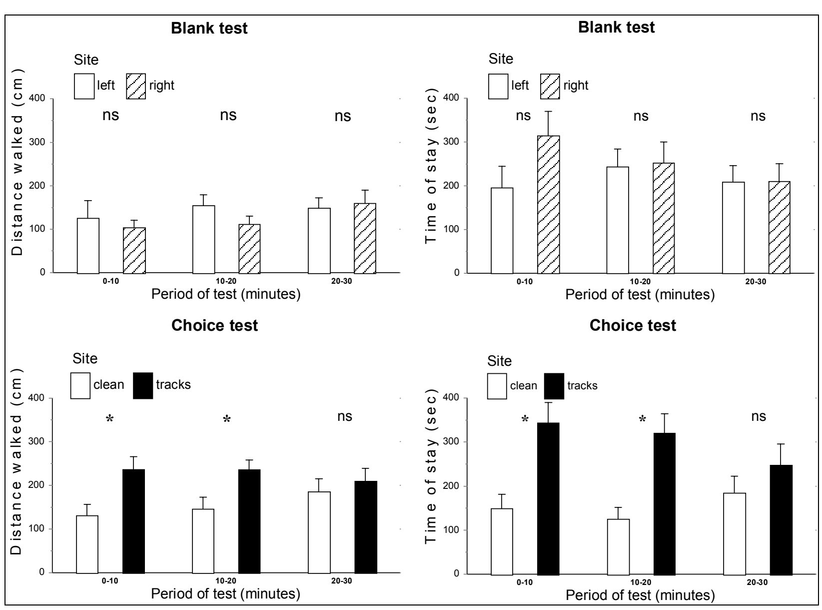

Fig. 3. Effects of conspecific larval tracks on the behaviour of females of *Cycloneda limbifer*. Results (mean ∀ SE) both for the behaviour on two clean substrates in blank tests and on one clean and one contaminated substrate in subsequent choice tests. Wilcoxon paired sample test (two-tailed P value), * = P<0.05, ns = not significantly different (P∃0.05).

During the tests, females of *C. limbifer* spent 24 percent of the time on the glass outside substrate zones. The average distance walked by a female on the glass was 863∀158 cm (43% of the total distance walked in monitored area). The average speed of females on glass outside substrate zones was 19.8 mm/sec.

Choice experiments with tracks of C. undecimnotata larvae

The total residential time and the distance walked on substrates with fresh tracks of unfed heterospecific first instars were significantly shorter than on clean substrates [P=0.0023 and P=0.0134]. The average speed of females during the choice test was 7.5 mm/sec on the clean substrate and 9.2 mm/sec on the substrate with tracks. The speed on substrates with tracks was significantly higher than on clean substrates [P=0.0107].

C. limbifer spent significantly less time on substrates with fresh tracks of unfed first instars of *C. undecimnotata* than on clean substrates in the second period (10-20 minutes) of the test [P=0.0040]. Differences in the first and the last period of 10 minutes were not significant [P=0.1189 and P=0.2166] (Fig. 4). The distances walked on clean substrates were significantly longer than on substrates with tracks of heterospecific larvae during the second period (10-20 minutes) of the test [P=0.0295], but not in the first and the last period [P=0.0906 and P=0.1726] (Fig. 4). Walking speed of females on substrates with tracks was also significantly higher than on clean substrates only in the middle period of the test [P=0.0005], but not in the first (0-10 minutes) and the last (20-30 minutes) periods [P=0.4631 and P=0.583] (Fig. 5). A record of the track of the most representative female is illustrated in Fig. 2.

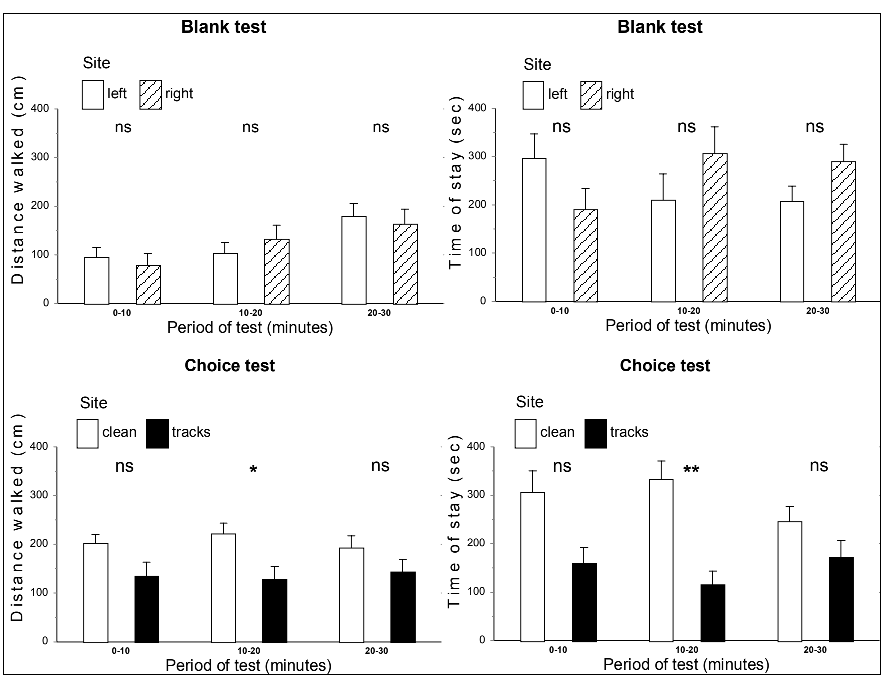

Fig. 4. Effects of larval tracks of *Ceratomegilla undecimnotata* on the mobility of *Cycloneda limbifer*. Results are for the behaviour (mean ∀ SE) on two clean substrates in blank tests and on one clean and one contaminated substrate in subsequent choice tests. Wilcoxon paired sample test (two-tailed P value), ** = P<0.01, * = P<0.05, ns = not significantly different (P∃0.05).

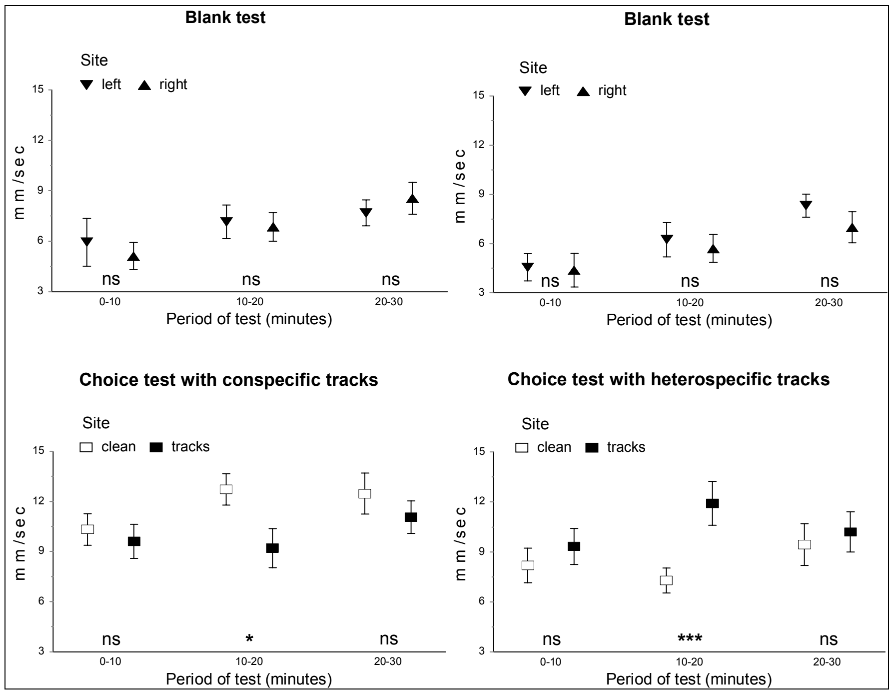

Fig. 5. The speed of movement (mean ∀ SE) of *Cycloneda limbifer* females on two clean substrates in blank tests and on a clean substrate and substrate with tracks of conspecific or *Ceratomegilla undecimnotata* larvae in subsequent choice tests. Wilcoxon paired sample test (two-tailed P value), *** = P<0.001, * = P<0.05, ns = not significantly different (P∃0.05).

During the tests, females of *C. limbifer* spent 26 percent of the time on the glass outside substrate zones. The average distance (0∀SE) walked by a female on the glass was 937∀150 cm, (48 % of the total distance walked in monitored area). The average speed of females on the glass was 19.1 mm/sec.

DISCUSSION

Adult coccinellids usually do not stay on plants very long. If not laying eggs, they walk or fly away after several minutes, exceptionally after hours. Therefore, the presence of larval tracks is most likely to change the searching behaviour of females soon after their arrival on a plant. Automatic monitoring of females of *C. limbifer* provided considerably more information on the effects on their behaviour of substrates with larval tracks than did faecal spot densities left by females in choice tests designed to study these effects over 20 hours (RŮŽIČKA 2001).

The current results confirm a former assumption that the higher densities of faecal spots left by females on clean substrates than on those with larval tracks of *C. undecimnotata* indicate a repellent effect of contaminated substrates (RŮŽIČKA 2001). Residential time and total distance walked by *C. limbifer* females on clean substrates were significantly longer than on substrates with heterospecific tracks. This effect was strongest during the middle period (10-20 minutes) of the test. Also, the speed of females was higher on contaminated than on clean substrates at this time.

The analysis revealed that the fresh tracks of conspecific larvae affect the searching behaviour of *C. limbifer* females. In the first 20 minutes of the test, residential time and the distance walked were significantly higher on substrates with conspecific tracks than on clean substrates. Both parameters were also significantly lower on clean substrates over the whole 30 minutes of this test. This effect of larval tracks was not evident in the previous study (RŮŽIČKA 2001). In addition, the speed of females was higher on contaminated than on clean substrates in the middle period. This effect of fresh conspecific tracks was surprising, because the oviposition-deterring effect of conspecific larval tracks is at least as strong as the oviposition- deterring effect of heterospecific tracks (RŮŽIČKA 2001). This is the first report that larval tracks can increase the time of stay and decrease the speed of search of conspecific females in an insect predator. Fresh tracks of conspecific first instar larvae may indicate, at least in some coccinellid species, that prey might be present, even though the tracks indicate it is an unsuitable site for oviposition.

In the absence of aphids, the effect of conspecific tracks on *C. limbifer* declined after 20 minutes. Results confirm that the strongest effects of larval tracks on female mobility can be expected shortly after their arrival on a plant. BÄNSCH (1966) observed that adult coccinellids search model plants without aphids for 23 minutes.

Larvae of the pyralid *Ephestia kuehniella* Zeller secrete an oviposition-deterring pheromone from salivary glands and contaminate their food with the secretion (CORBET 1971). While low numbers of larvae attract conspecific females to lay eggs, high numbers deter them (CORBET 1973). Females of another species, *Plodia interpunctella* (Hübner), lay significantly more eggs in sites contaminated with 1 or 5 larvae than on clean sites, but lay significantly fewer eggs on sites contaminated with 10 larvae (PHILLIPS & STRAND 1994). A similar response is not observed in aphidophagous predators. The oviposition-deterring effect of larval tracks steadily increases with increase in density of tracks (RŮŽIČKA 1997a; DOUMBIA et al. 1998) as well as size of larvae (RŮŽIČKA 1997b).

Chemical analysis of the larval tracks of the coccinellid *Adalia bipunctata* (L.) revealed a wide spectrum of semiochemicals, mainly hydrocarbons (HEMPTINNE et al. 2001), however, effects of individual compounds or sets of compounds on conspecific females are unknown. The strong intraspecific oviposition-deterring effect of conspecific larval tracks (RŮŽIČKA 2001) and their effects on the foraging of *C. limbifer* may either be stimulated by the same or different substances present in the tracks.

This study showed that the fresh tracks of *C. undecimnotata* larvae and those of conspecific larvae affect the searching of *C. limbifer* females differently. This was unexpected because the oviposition-deterring effects of fresh conspecific and heterospecific larval tracks were almost identical (RŮŽIČKA 2001).

The difference in the effect of fresh tracks of conspecific and heterospecific first instar larvae on the searching behaviour of females is here reported in aphid predators for the first time. The different behaviour of females on substrates with conspecific tracks can have an adaptive significance. Fresh larval tracks may stimulate females to search sites more thoroughly, because the food is likely to be present. The prolongation of the search on sites with tracks may also give a better assessment of the ratio of prey to conspecific competitors. This may enable females to more effectively asses site quality.

ACKNOWLEDGEMENTS

This research was supported by the grant of the Grant Agency of the Czech Republic, No. 206/00/0809, from the Entomology Institute project Z5007907 (Acad. Sci. CR) and the grant project S5007102 (Grant Agency Acad. Sci. CR). We thank M. Červenská and R. Guttwirthová for their assistance with experiments and cultures of insects.

REFERENCES

BÄNSCH, R. 1966. On prey seeking behaviour of aphidophagous insects. Pp. 123-128 *in*: I. HODEK (Ed.). *Ecology of Aphidophagous Insects*. Prague: Academia.

BELL, W.J. 1991. *Searching Behaviour: The Behavioural Ecology of Finding Resources*. London: Chapman & Hall.

CORBET, S.A. 1971. Mandibular gland secretion of larvae of the flour moth, *Anagasta kuehniella*, contains an epideictic pheromone and elicits oviposition movements in a hymenopteran parasite. *Nature* 232: 481-484.

CORBET, S.A. 1973. Oviposition pheromone in larval mandibular glands of *Ephestia kuehniella*. *Nature* 243: 537-538.

DOUMBIA, M., J.-L. HEMPTINNE & A.F.G. DIXON 1998. Assessment of patch quality by ladybirds: role of larval tracks. *Oecologia* 113: 197-202.

FERRAN, A. & A.F.G. Dixon 1993. Foraging behaviour in ladybird larvae (Coleoptera: Coccinellidae). *European Journal of Entomology* 90: 383-402.

HEMPTINNE, J.-L. & A.F.G. DIXON 1991. Why ladybirds have generally been so ineffective in biological control? Pp. 149-157 *in* L. POLGÁR, R.J. CHAMBERS, A.F.G. DIXON & I. HODEK (Eds). *Behaviour and Impact of Aphidophaga*. The Hague: SPB Academic Publishing.

HEMPTINNE, J.-L., G. LOGNAY, M. DOUMBIA & A.F.G. DIXON 2001. Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Chemoecology* 11: 43-47.

KUCHLEIN, J.H. 1966. Some aspects of the prey-predator relation. Pp. 237-242 *in* I. HODEK (Ed). *Ecology of Aphidophagous Insects*. Prague: Academia.

MARKS, R.J. 1977. Laboratory studies of plant searching behaviour by *Coccinella septempunctata* L. larvae. *Bulletin of Entomological Research* 67: 235-241.

Noldus Information Technology 1997. *EthoVision: Video Tracking, Motion Analysis & Behavior Recognition System*. Reference Manual, Version 1.90., Wageningen.

PHILLIPS, T.W. & M.R. STRAND 1994. Larval secretion and food odors affect orientation in female *Plodia interpunctella. Entomologia Experimentalis et Applicata* 71: 185-192.

RŮŽIČKA, Z. 1994. Oviposition-deterring pheromone in *Chrysopa oculata* (Neuroptera: Chrysopidae). *European Journal of Entomology* 91: 361-370.

RŮŽIČKA, Z. 1997a. Persistence of the oviposition-deterring pheromone in *Chrysopa oculata* (Neur.: Chrysopidae). *Entomophaga* 42: 109-114.

RŮŽIČKA, Z. 1997b. Recognition of oviposition-deterring allomones by aphidophagous predators (Neuroptera: Chrysopidae, Coleoptera: Coccinellidae). *European Journal of Entomology* 94: 431-434.

RŮŽIČKA, Z. 1998. Further evidence of oviposition-deterring allomone in chrysopids (Neuroptera: Chrysopidae). *European Journal of Entomology* 95: 35-39.

RŮŽIČKA, Z. 2001. Oviposition responses of aphidophagous coccinellids to tracks of coccinellid (Coccinellidae) and chrysopid (Chrysopidae) larvae. *European Journal of Entomology* 98: 183-188.

SIEGEL, S. & N.J. CASTELLAN JR. 1988. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Company.

SOLOMON, M.E. 1949. The natural control of animal populations. *Journal of Animal Ecology* 18: 1-35.

SOLOMON, M.E. 1964. Analysis and Processes Involved in the Natural Control of Insects. Pp. 1-54 *in* J.B. CRAGG (Ed). *Advances in Ecological Research 2*. London and New York Academic Press.

YASUDA, H., T. Takagi & K. Kogi 2000. Effects of conspecific and heterospecific larval tracks on the oviposition behaviour of the predatory ladybird *Harmonia axyridis* (Coleoptera, Coccinellidae). *European Journal of Entomology* 97: 551-553.

Accepted 31 May 2003.