



## The effect of aphid prey quality on searching behaviour of *Adalia bipunctata* and its susceptibility to insecticides

P. Kalushkov

Institute of Zoology, Bulgarian Academy of Sciences, Blvd. Tzar Osvoboditel 1, 1000 Sofia, Bulgaria

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### Abstract

After consumption of one individual of a suitable prey, such as *Phorodon humuli* (Schrank) (Aphididae), *Adalia bipunctata* L. (Coleoptera, Coccinellidae) adults changed from extensive to intensive searching behaviour. However, after the consumption of one individual of three other aphid species: *Aphis fabae* Scop., *Aphis craccivora* Koch or *Aphis spiraephaga* Müller, which are unsuitable prey, this coccinellid remained immobile for 12–76 min and did not switch to intensive search after moving off. Adult *A. bipunctata* fed an unsuitable prey, *A. fabae*, for 2 weeks were 2–4 times more susceptible to pyrethroid insecticides (permethrin, deltamethrin, fenpropathrin, esfenvalerate, alfa-cypermethrin and bioresmethrin) and insect growth regulators (diflubenzuron, triflumuron, fenoxycarb, flufenoxuron, teflubenzuron) than those fed a suitable prey, *P. humuli*.

The searching behaviour of coccinellids could thus be another criterion for determining the suitability of aphids as prey. The fact that the nutritional quality of aphids can affect the susceptibility of predatory coccinellids to insecticides should be borne in mind in integrated pest management programs.

### Introduction

After consuming a suitable prey item aphidophagous predators switch from extensive to intensive searching behaviour (Banks, 1957). Intensive searching behaviour has also been called 'area-concentrated searching behaviour' (Banks, 1957; Dixon, 1959; Curio, 1976; Nakamuta, 1982, 1985), 'area-restricted searching behaviour' (Carter & Dixon, 1982) and 'success motivated search' (Vinson, 1977). Most aphid species live in colonies, and therefore it is likely that another aphid will be close to where the first aphid is captured (Dixon & Wratten, 1971). Thus coccinellids tend to be in the extensive search mode when moving between prey patches and change to intensive search on catching a suitable prey item. Switching to intensive search seems to be learned as it is not shown by newly emerged adults (Ettifouri & Ferran, 1992). Foraging behaviour is affected by abiotic and biotic factors and the physiological state of the predator (Ferran & Dixon, 1993).

The hop aphid, *Phorodon humuli* and *Aphis fabae* reared on *Philadelphus coronarius* are suitable prey for *Adalia bipunctata* (Mills, 1981; Kalushkov, 1994; Hodek & Honek, 1996) However, *Aphis craccivora*, reared on *Vicia faba* is unsuitable prey for *Harmonia axyridis*, *Semiadalia undecimnotata* and in some cases for *Coccinella septempunctata* (Hodek, 1960, Okamoto, 1966), both *A. fabae* and *A. craccivora* cultured on *Vicia faba* are unsuitable prey for *A. bipunctata* (Kalushkov, 1994) and *A. spiraephaga* is unsuitable for *A. bipunctata* (Kalushkov, 1998).

Conflicting results were obtained when studying the effect of insecticides on *A. bipunctata*. The coccinellids were reared under identical conditions, apart from being fed different species of aphid.

The research reported here was carried out to determine whether the species of aphid prey fed to *Adalia bipunctata* may affect its searching behaviour and susceptibility to insecticides.

## Materials and methods

**Insects, plants.** Adults of the ladybird beetle *Adalia bipunctata* were collected from lime trees (*Tilia sp.*) in Sofia during May and reared in the laboratory on the lime aphid, *Eucallipterus tiliae* L. taken every day from the same trees. The experiments on searching behaviour were carried out one week after the adults were collected from the field.

For the toxicological experiments, pupae of *A. bipunctata* were collected from the same lime trees. On emergence the adults were separated into two groups: one group was fed *A. fabae* and the other *P. humuli*. The susceptibility of these coccinellids to insecticides was studied after they had been reared on these diets for 2 weeks.

The adult *A. bipunctata* used in the experiments on searching behaviour were fed apterous females of the hop aphid, *P. humuli*, *A. spiraephaga*, the insecticide susceptible black bean aphid, *A. fabae*., or the cowpea aphid, *A. craccivora*. *A. fabae* and *A. craccivora* were reared on broad bean (*Vicia faba* L.) grown in soft wood sawdust inside nylon isolators.

The aphids and coccinellids were reared under the same temperature conditions ( $25 \pm 1$  °C during the day and at  $20 \pm 1$  °C during the night) and photophase (16 h).

**Insecticides.** The pyrethroid insecticides: Ambush 25 EC (25% permethrin), Decis 2.5 EC (2.5% deltamethrin), Danitol 10 EC (10% fenpropathrin), Sumialfa 5 EC (5% esfenvalerate), Vaztac 10 EC (10% alfa-cypermethrin), Isathrin 100 EC (10% bioresmethrin) and the insect growth regulators: Dimilin 25 DP (25% diflubenzuron), Alsystin 25 WP (25% triflumuron), Insegar 25 WP (25% fenoxycarb), Cascade 5 EC (5% flufenoxuron) and Nomolt 15 EC (15% teflubenzuron) were used.

**Searching behaviour.** The searching behaviour of the adult coccinellids was recorded by an apparatus, similar to that described by Nakamuta (1982). It consisted of a gray vinyl-chloride cylinder (30 cm diameter and 5 cm height) resting on white paper placed on an horizontal acrylic plate. The top of the cylinder was covered with a transparent acrylic plate, on which a sheet of cellophane was laid. The beetles were starved for 24 h before the experiment. This period was sufficient to elicit searching behaviour. A beetle was released into the observation arena and was habituated to the surroundings for 3 min. After that, the

searching path of the beetle was traced on the sheet of cellophane with a felt pen for 3 min. Every 15 s the track was marked, so that the average walking speed could be computed. After 3 min an apterous adult aphid was placed in front of the beetle using forceps and the tracing of its track was continued after the beetle consumed the aphid. The temperature in the arena was  $25 \pm 1$  °C and observations were conducted during the light phase when the predator was active. An ipsometer was used to measure the length of the trace.

**Toxicological experiments.** Insecticides were sprayed onto aphids in a sedimentation tower by a standard method (Anonymous, 1979). Bean leaves with long stalks were placed with their lower side upper most at the bottom of the sedimentation tower and sprayed with an insecticide emulsion. In the controls distilled water was applied. The insecticides were applied at  $24 \pm 2$  °C. After 5 min petioles of the leaves were inserted into vials with water, a fluon coated plexite cylinder was fixed on each leaf and 2 h after spraying approximately 20–30 aphids were placed in each cylinder. The same equipment was used for the direct spraying of adults of *A. bipunctata*. A Petri dish, 9 cm in diameter, coated on the inside with fluon and holding 10–15 coccinellids, was placed at the bottom of the sedimentation tower. The dishes were covered with wire mesh to prevent the beetles from escaping. After 5 min of sedimentation, beetles were transferred into tubes and fed aphids. A series of at least 5 concentrations of pyrethroid insecticide emulsified in distilled water were applied. Of the insect growth regulators only the concentrations recommended for application in the field were used.

**Assessment of results.** The searching behaviour results were analysed by the LSD test (least significant difference test,  $\alpha = 0.05$ ). In the toxicological experiments, mortality was recorded after 48 h for aphids and after 72 h for adult ladybirds. Probit analysis of Finney, after correction for mortality according to Abbott, was used in evaluating the results obtained with the pyrethroid insecticides, and the Fisher exact test was used in evaluating the mortality obtained with the insect growth regulators ( $\alpha = 0.05$ ).

Table 1. Searching behaviour shown by 24 h-starved *Adalia bipunctata* adults after the capture of individuals of different aphids. Figures in the same column followed by the same letter are not significantly different (LSD test)

Prey	n	Time spent eating (min)	Speed of walking (cm/15 s)		No of change of direction in 15 s		Duration of intensive search (s)
			Before consumption	After consumption	Before consumption	After consumption	
<i>Phorodon humuli</i>	20	5.6 ± 0.9 a (4–7)		4.8 ± 1.4 a		6.8 ± 3.1 a	16.8 ± 4.7 a (9–27)
<i>Aphis fabae</i>	20	34 ± 16 b (12–58)	14.2 ± 2.6	12.6 ± 3.4 b	1.9 ± 1.2	1.4 ± 0.9 b	0 b
<i>Aphis craccivora</i>	22	38 ± 16 b (16–68)		15.2 ± 3.1 b		1.6 ± 1.1 b	0 b
<i>Aphis spiraephaga</i>	30	44 ± 16 b (18–76)		14.8 ± 4.6 b		2.3 ± 1.3 b	0 b

## Results

**Searching behaviour.** *A. bipunctata* showed three types of searching behaviour. After 24 h starvation, beetles tended to move around the perimeter of the area (extensive search) and their average speed was  $14.2 \pm 2.6$  cm/15 s (Table 1).

After consuming individuals of the different aphid species the searching behaviour of adult coccinellids differed significantly ( $F = 121$ ,  $df = 9$ ,  $P < 0.001$ ). After being fed *P. humuli*, beetles tended to search intensively, within the area around where the prey was captured, and their speed of movement decreased to a level significantly lower than that before the prey capture (4.8 cm/15 s). The mean duration of intensive search was 16.8 s and then the intensive search changed to extensive search. Prior to prey capture adult coccinellids rarely changed direction, but after prey consumption they turned more frequently (Table 1). After eating one individual of *A. fabae*, *A. craccivora* or *A. spiraephaga*, coccinellids remained immobile on the place where the prey was captured for 12–76 min and some tried to clean their mouth parts with their legs. After this the ladybirds started to search extensively and their speed of movement was similar to that before the capture of prey.

**Searching behaviour.** Ten minutes after being offered *A. fabae* ( $n = 10$ ) or *A. craccivora* ( $n = 10$ ), beetles did not respond to the offer of more suitable prey namely *P. humuli*.

*A. bipunctata* took 4–7 min to eat an *P. humuli* adult and 12–76 min to eat adults of *A. fabae*, *A. craccivora* or *A. spiraephaga*. In general adult coccinellids did not completely consume *A. fabae*, *A. craccivora* or

*A. spiraephaga* and in many cases they started to move after 12–76 min with the prey still in their mouth.

**Susceptibility to pyrethroids.** For all six pyrethroid insecticides the  $LC_{50}$ - and  $LC_{90}$ -values for *A. bipunctata* adults fed *P. humuli* were higher than those fed *A. fabae*. For *A. bipunctata* adults fed *P. humuli*, the  $LC_{50}$  value was 2–4 times higher and the  $LC_{90}$  value was 2–3 times higher than that for adult coccinellids fed *A. fabae* (Table 2).

When bioresmethrin and fenpropathrin were used *A. bipunctata* adults exhibited higher susceptibility than the insecticide susceptible strain of *A. fabae*. The  $LC_{50}$  and  $LC_{90}$  values for *P. humuli* were higher probably due the insecticide resistance of the field strain used.

**Susceptibility to insect growth regulators.** In all experiments with insect growth regulators, mortality of *A. bipunctata* adults fed on *A. fabae* was twice of those fed on *P. humuli* (Table 3).

## Discussion

It has been shown here that food can significantly influence searching behaviour and susceptibility to insecticides in *A. bipunctata*.

Among European coccinellid species, *A. bipunctata* is highly susceptible to pesticides (Kalushkov, 1982; Zeleny et al., 1988) but when fed *A. fabae* reared on bean, this species showed even higher susceptibility.

Blackman (1965, 1967) tested the suitability of nine aphid species for *A. bipunctata* and found *Aphis fabae* reared on *Vicia faba* to be unsuitable food

Table 2. Toxicity of six pyrethroid insecticides on *Phorodon humuli*, *Aphis fabae* and *Adalia bipunctata* adults reared on these two species of aphid

Species	<i>n</i>	LC <sub>50</sub> (in%)	95% probability limits	LC <sub>90</sub> (in%)
Permethrin				
<i>A. fabae</i>	45	0.0005	0.0005–0.0007	0.0015
<i>P. humuli</i>	60	0.0007	0.0006–0.0008	0.002
<i>A. bipunctata</i> <sup>a</sup>	30	0.01	0.01–0.02	0.03
<i>A. bipunctata</i> <sup>b</sup>	30	0.03	0.02–0.03	0.07
Deltamethrin				
<i>A. fabae</i>	52	0.00002	0.00001–0.000025	0.00004
<i>P. humuli</i>	58	0.00002	0.00001–0.00003	0.00006
<i>A. bipunctata</i> <sup>a</sup>	30	0.0002	0.0001–0.00025	0.0005
<i>A. bipunctata</i> <sup>b</sup>	30	0.0004	0.0003–0.0005	0.001
Bioresmethrin				
<i>A. fabae</i>	45	0.005	0.004–0.008	0.03
<i>P. humuli</i>	60	0.01	0.01–0.03	0.125
<i>A. bipunctata</i> <sup>a</sup>	30	0.0015	0.001–0.004	0.01
<i>A. bipunctata</i> <sup>b</sup>	30	0.006	0.005–0.008	0.03
Fenprothrin				
<i>A. fabae</i>	57	0.0008	0.0006–0.0009	0.004
<i>P. humuli</i>	60	0.001	0.0008–0.00125	0.004
<i>A. bipunctata</i> <sup>a</sup>	30	0.0006	0.0005–0.0007	0.002
<i>A. bipunctata</i> <sup>b</sup>	30	0.002	0.001–0.003	0.006
Esfenvalerate				
<i>A. fabae</i>	48	0.0001	0.00008–0.000125	0.0005
<i>P. humuli</i>	55	0.0001	0.00008–0.000125	0.0006
<i>A. bipunctata</i> <sup>a</sup>	30	0.0002	0.0001–0.0003	0.0007
<i>A. bipunctata</i> <sup>b</sup>	30	0.0005	0.0004–0.0007	0.002
Alfa-cypermethrin				
<i>A. fabae</i>	45	0.00005	0.00004–0.00006	0.0006
<i>P. humuli</i>	52	0.00006	0.00005–0.00007	0.000125
<i>A. bipunctata</i> <sup>a</sup>	30	0.00025	0.0002–0.0004	0.0008
<i>A. bipunctata</i> <sup>b</sup>	30	0.0005	0.0003–0.0007	0.002

<sup>a</sup>*A. bipunctata* reared on *A. fabae*.

<sup>b</sup>*A. bipunctata* reared on *P. humuli*.

for this coccinellid. Similarly *A. fabae* has been reported as unsuitable food for *A. bipunctata* by Hariri (1966), and as an inadequate prey (p. 88) or toxic prey (p. 91) for this coccinellid by Majerus (1994). In mass-rearing, all larvae of *A. bipunctata* fed *A. fabae* reared on *V. faba*, died (Zeleny, pers. commun.).

*A. spiraephaga* is an introduced aphid in Central Europe. Three coccinellid species occur in the field

in association with *A. spiraephaga* in the Czech Republic: *A. bipunctata*, *Coccinella septempunctata* and *Anatis ocellata* (Starý, 1995). Although *A. bipunctata* is reported as a natural enemy of *A. spiraephaga*, laboratory experiments indicate that this aphid is very unsuitable prey for *A. bipunctata* (Kalushkov, 1998). All 1st instar larvae of *A. bipunctata* fed *A. spiraephaga* died.

Table 3. Toxicity of five insect growth regulators for *Adalia bipunctata* adults reared on *Phorodon humuli* and *Aphis fabae*. Figures in the same row followed by the different letters are significantly different (F-test)

Insecticides (concentration)	Mortality of <i>Adalia bipunctata</i> (%) when ( $n = 30$ ) reared on	
	<i>P. humuli</i>	<i>A. fabae</i>
Dimilin (0.05%)	33 ± 15 a	67 ± 23 b
Alsystin (0.05%)	30 ± 10 a	57 ± 15 b
Insegar (0.05%)	30 ± 17 a	70 ± 20 b
Nomolt (0.05%)	43 ± 15 a	83 ± 21 b
Cascade (0.1%)	40 ± 17 a	77 ± 23 b

The allelochemical substances ingested by the aphids from their host plants, are thought to be causal agents of the detrimental effect that those aphids have on ladybirds (Hodek, 1962, 1993). Probably *A. fabae*, *A. craccivora* and *A. spiraephaga* also ingest substances from their host plants that make them unsuitable for *A. bipunctata*. When reared on *P. humuli*, the longevity of *A. bipunctata* adults was 55–73 days and when reared on *A. fabae* or *A. fabae* + *A. craccivora* the longevity was 20–32 days (Kalushkov, 1994). It is not surprising that adult coccinellids fed unsuitable prey (*A. fabae*) for 2 weeks are more susceptible to pyrethroid insecticides and insect growth regulators than insects reared on suitable food (*P. humuli*), which presumably is a consequence of their low vitality.

Nakamuta (1982) reported that the duration of the period of intensive search by *C. septempunctata* adults following the consumption of one *Myzus persicae* adult was 35 s. The period of intensive search was greatly increased after the consumption of two or four prey individuals. As shown here *A. bipunctata* usually left the vicinity after 9–27 s following the consumption of *P. humuli*. Searching behaviour is determined by the nature of the prey most recently consumed (Nakamuta, 1985). Carter & Dixon (1982) assumed that the most important factor affecting intensive search is the hunger level. The present experiments indicate that the quality of prey also affects searching behaviour.

The intensive search observed after consumption of suitable prey is likely to rise the beetle's probability of encountering another aphid. Consequently a switch from linear to concentrated search induced by prey consumption is advantageous to the beetle (Nakamuta, 1982).

Putman (1957) and Lucas et al. (1997) found that coccinellid adults that consumed less attractive prey (mites) did not switch from extensive to intensive search behaviour. However, the behaviour of ladybird beetles after consuming toxic or unsuitable aphids has not been recorded previously. The absence of intensive search after consuming unsuitable prey may have a similarly adaptive role as the intensive search observed after the consumption of suitable prey. The searching behaviour of coccinellids could thus be another way of determining the suitability of aphid prey, and used in addition to completion of larval development, fecundity, survival etc. Aphid food can affect the susceptibility of coccinellids to insecticides and this factor has to be considered when including the use of coccinellid beetles as predators.

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