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The Effect of Rearing the Ladybird *Harmonia axyridis* on *Ephestia kuehniella* Eggs on the Response of Its Larvae to Aphid Tracks

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Larvae of Harmonia axyridis *Pallas (Col., Coccinellidae) exhibited two walking patterns during prey search. Extensive search occurred when searching for prey patches and was characterized by long linear paths and a fast speed. Intensive search, which appeared after the ingestion of a prey in a patch, resulted from a lowering of the linear speed and an increase in the number of stops and angular speed. When larvae reared on the aphid* Acyrthosiphum pisum *Harris (Hom., Aphidae) crossed an artificial substratum previously contaminated by this prey, they changed their path direction and adopted intensive search. They probably perceived aphid odor tracks and consequently modified their walking pattern. This gustatory capacity probably allowed very mobile larvae to locate prey patches more rapidly and improve encounter with preys in every patch.* H. axyridis *larvae reared on a substitute prey, the eggs of* Ephestia kuehniella *Zeller (Lep., Pyralidae), for more than a hundred generations, also changed their path orientation but retained extensive search. The weak response of these larvae to aphid tracks may have resulted from either a decrease in their sensitivity to gustatory aphid stimuli or their difficulty in associating aphid odor with aphid presence. These larvae needed more time and more preliminary encounters than larvae reared on aphids before catching prey.*

KEY WORDS: *Harmonia axyridis*; Coccinellidae; aphid; *Acyrthosiphon pisum*; unnatural prey; *Ephestia kuehniella*; mass rearing; locomotor behavior; extensive search; intensive search.

INTRODUCTION

Predators probably forage at three levels of prey density, namely, prey, prey patches, and habitat (Hassell and Southwood, 1978; Ferran and Dixon, 1993).

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This classification provides a framework for discussing the foraging behavior of ladybirds. Movement between patches within habitat is referred to as extensive search (Bond, 1980) or ranging (Dusenberry, 1989) and movement between prey within patches, intensive search or area-restricted search (Curio, 1976). Extensive search is characterized by relatively linear and fast movement and intensive search by slow sinuous movement interrupted by frequent stops. Intensive search is considered adaptive, as it increases the probability of a predator encountering further prey (Dixon, 1959; Carter and Dixon, 1982). Intensive search is characteristic of predators that have just ingested prey and is gradually replaced by extensive search if no further prey is ingested (Jander, 1975). The linear movment characteristic of extensive search increases the probability of the predator leaving an area (Bell, 1991).

Since it was imported from China in 1982, the polyphagous coccinellid *Harmonia axyridis* Pallas has been reared in our laboratory on the pea aphid *Acyrthosiphon pisum* Harris (Aphidae), and from 1986, a subculture has been reared for over ca. 100 generations on the eggs of the flour moth *Ephestia kuehniella* Zell (Pyralidae) killed by exposure to UV radiation (Daumal *et al.*, 1975). When the larvae reared on moth eggs are fed aphids, they show weaker intensive search than larvae reared on aphids (Ettifouri and Ferran, 1993). This reduced locomotor responsiveness may result from a modification of the predator's capacity to recognize visual and/or olfactory stimuli associated with aphid prey.

Several authors have demonstrated that adults orientate visually to host plants and prey (Allen *et al.*, 1970; Colburn and Asquith, 1970; Heidari and Copland; 1982; Kesten, 1969; Lambin *et al.*, 1996; Said *et al.*, 1985; Nakamuta, 1985; Obata, 1986). Little is known about the olfactory cues perceived by coccinellids (Obata, 1986; Heidari and Copland 1982). There is no evidence to indicate that larvae use visual or olfactory cues when searching for prey (Storch, 1976; Stubb, 1980). Hattingh and Samways (1995) think that the presence of prey is only perceived after physical contact. Therefore, the selection of aphid patches by coccinellids is considered to be mainly carried out by egg-laying females which have appropriate sensory abilities (Blackman, 1967; Hodek, 1973, Kindlmann and Dixon, 1993).

The maxillary palps of coccinellids, particularly the upper surface of the last segment, are covered by gustatory sensillae (Barbier *et al.*, 1989). Since larvae and adults frequently touch the substrate with these appendages, gustation (contact chemoreception) may be involved in prey detection.

This paper addresses four questions: (1) Do *H. axyridis* larvae fed on aphid change their motor pattern equally after encountering aphid tracks as after ingesting one aphid? (2) Do the larvae reared on *E. kuehniella* eggs present the same motor pattern change as larvae reared on aphids? (3) Does the intensity of this motor change depend on contact duration with aphid tracks? and (4) Does the continuous rearing of larvae with this unnatural prey eggs modify their predatory efficiency?

MATERIALS AND METHODS

H. axyridis and its prey were reared in the laboratory. Larvae reared on aphids or *E. kuehniella* eggs are referred to as Ap and Ek larvae, respectively.

Experiments were carried out with 48-h-old fourth-instar larvae, weighing from 20 to 25 mg. Prior to experiment, larvae were isolated in 2 × 3-cm plastic vials without food for 12 h.

Each larva was filmed on a circular white arena (height of walls, 40 cm; diameter, 50 cm), the floor of which was covered with a sheet of white paper (Fig. 1A). The experiments were done in a dark room at 22C ± 2°C, 70 to 80% RH, and 16L:8D light cycle.

A camera whose field of view covered the floor of the arena, a tape recorder, and a computer programmed for picture analysis were used to calculate automatically the coordinates of the larvae every 0.04 s and to derive from such data the path parameters. A software allowed the distinction of paths in or out of the aphid contaminated area for individual larvae (Fig. 1B). For coordinate computation, the area was divided into 512 lines and columns, the path was automatically smoothed (average of five successive points), and all coordinates (25/s) were used.

First Experiment. To compare the locomotory behavior of Ap and Ek larvae in the presence of aphids, each larva was kept in the center of the arena under a plastic vial for 1 min, then released, and its movement was filmed for 2 min (extensive search). Then each larva was placed in the center of the arena and supplied with one aphid (weight: 1.0 ± 0.2 mg per aphid), and after finishing feeding and resuming activity it was filmed for 3 min (intensive search).

Second Experiment. Comparison of locomotory behavior of Ap and Ek larvae on a substrate contaminated with aphid tracks, comprised two successive steps, namely, the obtention of aphid contaminated substrate and the recording of larval paths in an arena. A ring of green paper (internal diameter: 18 cm) was laid on a white paper sheet whose diameter was the same as the bottom of the arena (Fig. 1A). A quantity of aphids (3.0 ± 0.1 g per sheet) was released in the open central area of the white sheet, which was covered with a petri dish whose vertical walls had been previously coated with Fluon (Whitford, England) to prevent aphids climbing off the circular white sheet. The green paper was used only to protect the peripheral area of the white paper from aphid contamination. Four hours later, the green ring was removed, aphids, exuviae, and all solid particles were carefully swept off from the aphid contaminated area, and the white sheet was put on the floor of the arena. Each larva was released on the peripheral area 10 cm from the edge of the aphid contaminated area and

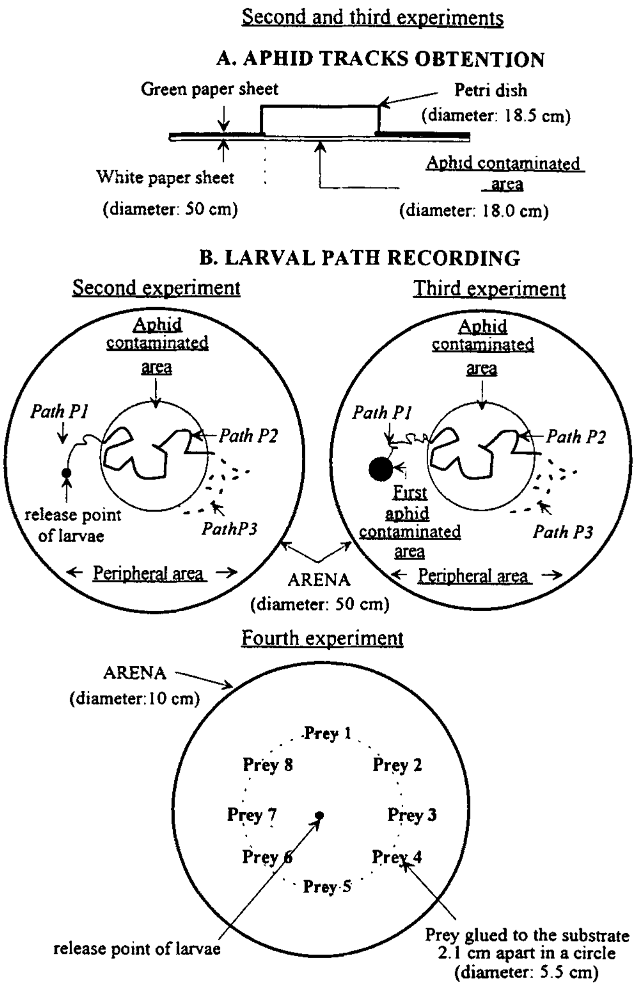

Fig. 1. Devices used to study the perception of an aphid-contaminated area by *Harmonia axyridis* larvae and to quantify their prey search behavior.

after 1 min had elapsed its movement was filmed (Fig. 1B). Each path was divided into three lengths corresponding to movements either on the aphid contaminated area (P2) or on the peripheral area before (P1) and after (P3) passage through the aphid contaminated area.

Third Experiment. To determine the effect of contact duration with an aphid contaminated area, experimental procedures were as described in Experiment 1 except that larvae were kept first for 1 min on an aphid contaminated ara (diameter: 2 cm) under a plastic vial (see black area; Fig. 1B). This contaminated paper was located 10 cm from the edge of the aphid contaminated area and removed as soon as the larva left it.

Fourth Experiment. To determine capture efficiency of Ap and Ek larvae, each larva was released in the center of a petri dish (diameter: 10 cm), the floor of which was a white paper to which eight prey items, either alive aphids or small groups of *E. kuehniella* eggs, had been previously glued (Fig. 1B). The prey items were arranged in a circle (diameter: 5.5 cm) with a prey item every 2.1 cm. Egg and aphid size were ca. 1 mm. Observations ceased as soon as larva caught a prey or 5 min after the release of a larva if no prey was captured.

Statistical Analysis

In the three first experiments the following parameters were measured: the number of stops per second, the linear speed (mm/s), excluding time spent stationary, and the angular speed (deg/s) computed from successive positions of the center of each ladybird.

Data were analyzed by means of nonparametric tests, Mann–Whitney *U* test for independent data (comparisons of the locomotory behavior of Ap and Ek larvae and of larvae after encountering prey or the aphid contaminated substrate), and Wilcoxon test for matched data (comparison of the successive path of each larva). Data were presented as averages and their corresponding confidence intervals ($P < 0.05$) and statistical tests were on the absolute values. Since the number of larvae measured in each of these experiments was greater than 30, the acceptance threshold was 1.96 (i.e., $P = 0.05$).

The larvae tracks obtained in the second experiment (total number: 88) were cross-tabulated according to five visually estimated variables (Table III, Fig. 2): the first variable (Var 1), the presence (Pre) or absence (Abs) of a change in path direction when larvae crossed the aphid contaminated area; the second variable (Var 2), whether a larva crossed the aphid contaminated area one (One) or several (Sev) times; the third variable (Var 3), whether the path length in the aphid contaminated area was either linear (Lino,), sinuous (Sino), or sinuous and looping (Slo) (Fig. 2); the fourth variable (Var 4), the corresponding path lengths in the peripheral area were referred to as Linp, Sinp, and Slp; and the fifth variable, (Var 5), trophic lines (Ap and Ek). This method allowed the construction of a matrix contingency table in which the values represented the number of larvae in each combination of state variables (Table III). The numbers in italics in the diagonal blocks are the sums of larvae belonging to the same state variable horizontally for each state variable in a column. In Var 2 (horizontally), for example, there was a total of 32 larvae (= 19 + 13) with or without a change of direction that crossed the aphid contaminated area once (Var 2, State 1), and 54 + 2 = 56 of those that crossed this area several

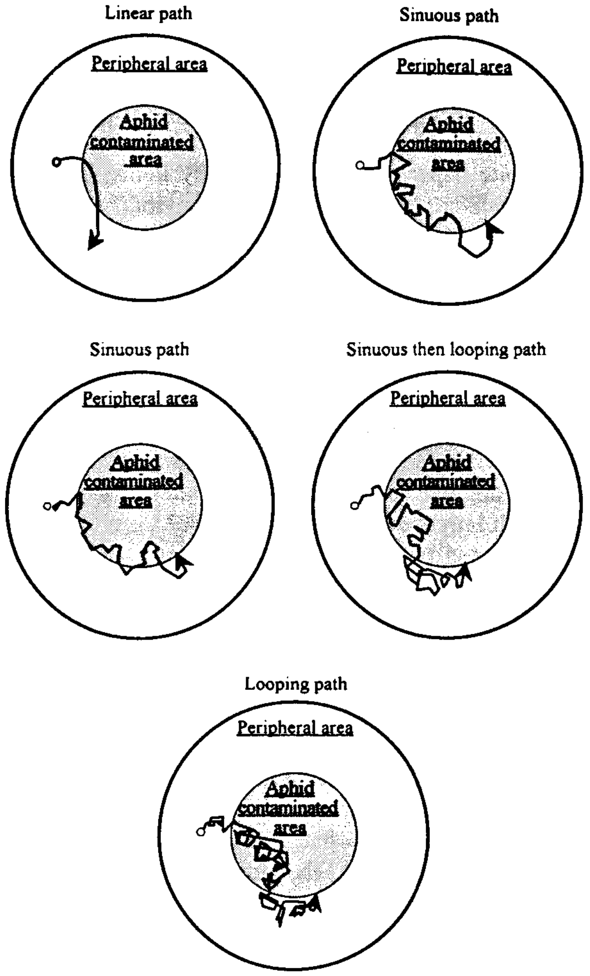

Fig. 2. Types of paths recorded in *Harmonia axyridis* larvae reared with the aphid *Acyrthosiphon pisum* or the flour moth *Ephestia kuehniella*. (°) Release point of larvae.

times (Var 2, state Sev). Numbers that are not in italics, represent the number of larvae in the states of two consecutive variables.

In the fourth experiment, the frequency of encounters followed by feeding, the number of encounters with prey, and the time that elapsed from the release of larva to the beginning of feeding were subjected to χ^2 and F tests, respectively.

RESULTS

First Experiment: The Transition from Extensive to Intensive Search in the Presence of a Prey

Whatever prey the larvae were reared on, the ingestion of one aphid induced a significant increase in the number of stops and angular speed and a significant decrease in the linear speed in Ap and Ek larvae (Table I). However, Ek larvae showed faster and less sinuous movements interrupted by fewer stops. That is, the intensive search pattern depended on larval rearing conditions as shown by Ettifouri and Ferran (1993). In this experiment, extensive search before prey encounter was also significantly different except for stops.

Second Experiment: The Transition from Extensive to Intensive Search in Areas Contaminated by Aphids

The percentage of larvae that reached the aphid contaminated area was 45.9 and 45.8% for Ap and Ek larvae, respectively.

Comparison with before feeding paths (first experiment) indicated that the initial movement of Ap larvae in the peripheral area (P1) was an extensive search

Table I. First Experiment: Variation of Path Parameters Recorded Before and After Feeding from *Harmonia axyridis* Larvae Reared on Either the Aphid *Acyrthosiphon pisum* or the Flour moth *Ephestia kuehniella*[a]

	Feeding		Comparison, before vs after (Wilcoxon)
Path parameter	Before	After	
A. Larvae fed on the aphid *Acyrthosiphon pisum* (Ap)			
Number of stops (/s)	0.7 ± 0.2	2.4 ± 0.6	5.1 S*** ↑
Linear speed (mm/s)	13.8 ± 1.1	10.8 ± 0.8	5.1 S*** ↓
Angular speed (°/s)	15.1 ± 1.4	26.3 ± 3.7	5.1 S*** ↑
B. Larvae fed on the flour moth *Ephestia kuehniella* (Ek)			
Number of stops (/s)	0.5 ± 0.2	1.8 ± 0.5	4.6 S*** ↑
Linear speed (mm/s)	15.9 ± 1.5	12.9 ± 1.1	4.9 S*** ↓
Angular speed (°/s)	12.8 ± 1.8	21.6 ± 3.5	4.6 S*** ↑
Ap vs Ek larvae comparisons (Mann–Whitney)			
Number of stops (/s)	1.93 NS	2.2 S* ↓	
Linear speed (mm/s)	3.51 S***	2.6 S** ↑	
Angular speed (°/s)	2.90 S***	2.6 S** ↓	

[a] S*, S**, and S***, significant differences at $P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively. Average ± confidence interval at $P < 0.05$ ($n = 40$ Ap or Ek larvae).

Table II. Second Experiment: Variation of Path Parameters Recorded Before (P1), in (P2), and After (P3) the Aphid-Contaminated Area from *Harmonia axyridis* Larval Population Reared on (A) the Aphid *Acyrthosiphon pisum* and (B) the Flour Moth *Ephestia kuehniella*[a]

	Path			Comparison		P1 vs P3
Path parameter	P1	P2	P3	P1 vs P2	P2 vs P3	
	A. Larvae fed on the aphid *Acyrthosiphon pisum* (Ap)					
Number of stops (/s)	1.1 ± 0.4	2.6 ± 0.7	1.7 ± 0.6	3.2 S** ↑	2.8 S* ↓	2.0 S* ↓
Linear speed (mm/s)	12.4 ± 0.9	10.9 ± 0.9	12.5 ± 1.2	2.5 S* ↓	2.6 S** ↑	0.01 NS
Angular speed (°/s)	16.5 ± 2.4	25.7 ± 2.7	20.6 ± 3.6	3.0 S** ↑	2.9 S** ↓	1.9 S* ↑
	B. Larvae fed on the flour moth *Ephestia kuehniella* (Ek)					
Number of stops (/s)	1.1 ± 0.3	0.9 ± 0.4	0.9 ± 0.5	1.4 NS	0.7 NS	1.1 NS
Linear speed (mm/s)	15.1 ± 1.0	17.6 ± 1.3	17.1 ± 1.0	2.2 S* ↑	0.1 NS	2.8 S** ↑
Angular speed (°/s)	12.5 ± 2.4	11.7 ± 2.9	12.2 ± 2.0	1.4 NS	0.3 NS	1.0 NS

[a] Wilcoxon test: S* and S**, significant differences at $P < 0.05$ and $P < 0.01$, respectively. Average confidence interval at $P < 0.05$ ($n = 44$ Ap or Ek larvae).

mode (compared using Mann–Whitney test: number of stops, $P = 0.22$; linear speed, $P = 0.34$; angular speed, $P = 0.34$) (Table IIA). In the aphid-contaminated area (P2), larvae adopted the intensive search mode. Of the two groups of larvae, 70.5 and 68.5%, respectively, showed an increase in the number of stops and an increase in the angular speed, and 63.6% of them, a decrease in linear speed. This movement was not significantly different from that observed after feeding (compared using Mann–Whitney test; number of stops, $P = 0.95$; linear speed, $P = 0.69$; angular speed, $P = 0.37$). This change in the pattern movement on the aphid-contaminated area (P2) indicates that larvae of *H. axyridis* can detect aphid tracks. In the peripheral area (P3), the larvae switched from intensive search to extensive search; the number of stops (70.5% of the larvae) and the angular speed (68.2%) decreased and the linear (68.2%) speed increased (Table IIA). That is, the intensive search induced by aphid contamination was restricted to the aphid-contaminated area. However, extensive search modes in (P1) and (P3) were different. In (P3), larva paths are characterized by more stops and a higher angular speed.

For the Ek larvae, the path parameters were not significantly different in the P1, P2, and P3 phases except for the linear speed, which increased slightly

Table III. Second Experiment: Distribution of *Harmonia axyridis* Larvae (Both Trophic Lines) in a Matrix Whose Lines and Columns Were Variables and State Variables Defined from Their Path in the Aphid-Contaminated and Peripheral Areas (for Further Explanation see Text)

	Var 1		Var 2		Var 3			Var 4		
	ABS	PRE	ONE	SEV	LINO	SINO	SLO	LINP	SINP	SLP
Var 1										
ABS	*15*									
PRE	*0*	*73*								
Var 2										
ONE	13	19	*32*							
SEV	2	54	*0*	*56*						
Var 3										
LINO	9	10	14	5	*19*					
SINO	6	32	18	20	*0*	*38*				
SLO	0	31	0	31	*0*	*0*	*31*			
Var 4										
LINP	9	14	18	5	9	13	1	*23*		
SINP	2	14	7	9	4	11	1	*0*	*16*	
SLP	4	45	7	42	6	14	29	*0*	*0*	*49*
Var 5										
Ap	4	40	13	31	5	19	20	*12*	*7*	*25*
Ek	11	33	19	25	14	19	11	*11*	*9*	*24*

($P < 0.05$) in the aphid-contaminated area. The increase in linear speed in the aphid-contaminated area suggested that larvae were repelled by aphid tracks.

In the cross table, the first diagonal blocks showed that 83% of larvae (73 of 88) changed their path direction when crossing the aphid-contaminated area (Table III). The behavior of the Ap and Ek larvae classified Var1 (change or not in path direction when larvae crossed aphid-contaminated area) in column–Var5 (larva lines) in line in the contingency table did not differ significantly ($\chi^2 = 2.9$, $P = 0.08$). Most Ap (40 of 44) and Ek (33 of 44) larvae responded to aphid contamination by changing their searching direction. Larval behavior classified Var2 (number of crossing the aphid-contaminated area)–Var5 did not differ significantly ($\chi^2 = 1.2$, $P = 0.27$) even though the frequency of larvae crossing several times into the aphid-contaminated area seemed higher in the Ap (31 of 44) than in the Ek (25 of 44). The analysis of Var3 (shape of the paths in the aphid contaminated area)–Var5 showed that the paths of the Ek larvae were more frequently linear (14 of 44) than those of Ap larvae (5 of 44), whereas looping paths were less numerous, 25% (11 of 44) and 45.4% (20 of 44) respectively ($\chi^2 = 6.9$, $P = 0.03$). The simple paths of these larvae may be linked with their faster movement. In the peripheral area (P3), the path characteristics seemed to be independent of larva line [comparison Var4 (shape of the paths in P3)–Var5, $\chi^2 = 0.31$, $P = 0.86$].

Larvae that did not show a direction change (Var1–Var1, 15 larvae) generally visited the aphid-contaminated area only once (13; 86.7%). All those (32) that searched the contaminated area once pursued linear (14; 43.7%) or sinuous (18; 56.3%) paths within this area. Most of the larvae that changed their search orientation (73) visited the aphid-contaminated area several times (54; 74.0%) and all of them (56) pursued mainly sinuous (20; 35.7%) or looping (31; 55.4%) paths. Larvae that showed linear (19) or sinuous (38) paths in the aphid-contaminated area adopted one of the three patterns in the peripheral area, whereas larvae that showed looping paths (31) adopted the same pattern of search in the peripheral area (29; 93.5%). The classification divided the larvae into two groups. Larvae of the first group (17.0%) responded slightly to aphid contamination, areas. Such a low response to prey was observed in the coccinellid *Semiadalia undecimpunctata* Schneider, where 11.3% of the larvae continued extensive search after prey feeding (Ferran *et al.*, 1994). Larvae in the second group (83.0%) responded strongly to aphid contamination, with change of direction, several searches of the aphid contaminated area, and search paths that were equally sinuous and looping in both areas.

Third Experiment: The Transition from Extensive to Intensive Search Relative to the Time Spent in the Aphid-Contaminated Area

In this experiment, 44.1% of Ap and 39.8% of Ek larvae reached the aphid contaminated area.

When Ap larvae visited the peripheral area (P1) after searching the first aphid-contaminated area, they adopted an intensive search pattern (Table IVA, Fig. 1B). The number of stops (Mann–Whitney test, $P = 0.83$) and the linear speed (Mann–Whitney test, $P = 0.75$) were not significantly different from those observed after feeding on an aphid, whereas the angular speed was significantly higher (Mann–Whitney test, $P = 0.003$). This search pattern persisted in (P2) and out of the aphid-contaminated area (P3). These results confirmed that Ap larvae showed a longer response to aphid contamination and suggested that the persistence of larval response to these stimuli may depend on contact duration with aphid contamination.

After leaving the first aphid-contaminated area, Ek larvae maintained the same extensive search pattern in the three areas (P1, P2, P3) (Table IVB). Compared with before feeding larvae (Table IB), these larvae showed no difference in number of stops (Mann–Whitney test, $P = 0.85$), linear speed (Mann–Whitney test, $P = 0.11$), and angular speed (Mann–Whitney test, $P = 0.65$).

Table IV. Third Experiment: Variation of Path Parameters Recorded Berfore (P1), in (P2), and After (P3) the Aphid-Contaminated Area from *Harmonia axyridis* Larva Reared on (A) the Aphid *Acyrthosiphon pisum* and (B) the Flour Moth *Ephestia kuehniella*[a]

	Path			Comparison		
Path parameter	P1	P2	P3	P1 vs P2	P2 vs P3	P1 vs P3
	A. Larvae fed on the aphid *Acyrthosiphon pisum* (Ap)					
Number of stops (/s)	2.3 ± 0.6	2.4 ± 0.7	1.9 ± 0.8	0.2 NS	0.8 NS	0.8 NS
Linear speed (mm/s)	10.3 ± 0.9	10.7 ± 1.1	11.9 ± 1.9	0.6 NS	1.8 NS	1.2 NS
Angular speed (°/s)	33.8 ± 4.5	31.3 ± 5.3	25.5 ± 5.8	1.1 NS	0.9 NS	0.9 NS
	B. Larvae fed on the flour moth *Ephestia kuehniella* (Ek)					
Number of stops (/s)	0.8 ± 0.3	0.6 ± 0.3	0.9 ± 0.4	1.3 NS	1.4 NS	1.5 NS
Linear speed (mm/s)	17.4 ± 1.2	16.0 ± 1.5	16.5 ± 1.4	1.1 NS	1.2 NS	0.6 NS
Angular speed (°/s)	12.7 ± 2.8	11.7 ± 3.1	12.4 ± 2.3	0.9 NS	0.5 NS	0.6 NS

[a] Wilcoxon test; NS, no significant difference. Average ± confidence interval at $P < 0.05$ ($n = 41$ Ap or Ek larvae).

Fourth Experiment: The Effect of Rearing Conditions on the Larval Capture Efficiency

Regardless of whether the larvae were offered aphids or *E. kuehniella* eggs, the rate of prey capture was lower for Elk larvae than Ap larvae ($\chi^2 = 34.0$, $P = 0.001$) (Table V). When Ap larvae searched for *E. kuehniella* eggs, the proportion of the larvae that encountered and then caught prey was similar to that of Ap larvae searching for aphids ($\chi^2 = 3.1$, $P = 0.21$). In contrast, Ek larvae encountered and captured significantly fewer *E. kuehniella* eggs than aphids ($\chi^2 = 10.9$, $P = 0.004$),

The mean number of encounters and the time of prey capture depended on larval rearing diets (Table VI). Ek larvae encountered more aphids before feeding than Ap larvae ($F = 10.3$, $P < 0.001$). The number of contacts in the other combinations between Ap and Ek larvae and offered preys was not significantly different. Ek larvae spent a longer time searching for aphids than Ap larvae ($F = 9.8$, $P < 0.002$). Compared to Ap larvae, the number of contacts before aphid capture in Ek larvae was approximately doubled, whereas the time they spent on searching aphid was increased threefold.

Table V. Fourth Experiment: Foraging Behavior in *Harmonia axyridis* Larvae Reared on Either the Aphid *Acyrthosiphon pisum* or the Flour Moth *Ephestia kuehniella* When They Searched for These Prey—Encounter and Prey Capture Frequency

		Frequency of larvae (%)		
Prey offered	Number of larvae	No contact preys	Contact unsuccessful prey capture	Contact, successful prey capture
A. Larvae fed on the aphid *Acyrthosiphon pisum* (Ap)				
A. pisum	33	0	6.3	93.7
E. kuehniella	36	8.3	8.4	83.3
B. Larvae fed on the flour moth *Ephestia kuehniella* (Ek)				
A. pisum	45	0	33.3	66.7
E. kuehniella	71	21.1	25.3	53.6

Table VI. Fourth Experiment: Foraging Behavior in *Harmonia axyridis* Larvae Reared on Either the Aphid *Acyrthosiphon pisum* or the Flour Moth *Ephestia kuehniella* When They Searched for These Preys—Number of Contacts with Prey and Search Time in Larvae with Successful Prey Capture[a]

	Prey offered	
Criterion	*A. pisum*	*E. kuehniella*
A. Larvae fed on the aphid *Acyrthosiphon pisum* (Ap)		
Number of contacts with prey	1.7 ± 0.4	1.7 ± 0.5
Search time (s)	25.6 ± 9.5	51.1 ± 15.2
Conacts/search time (/s)	0.007	0.033
B. Larvae fed on the flour moth *Ephestia kuehniella* (Ek)		
Number of contacts with prey	3.1 ± 0.2	2.1 ± 0.6
Search time (s)	70.0 ± 19.4	87.3 ± 14.9
Contacts/search time (/s)	0.044	0.024

[a] Mean ± confidence interval at $P < 0.05$.

Whatever the coccinellid population, larvae spent a longer searching for Ek eggs than for aphids ($F = 4.6$, $P = 0.002$). The encounter rate was twice as fast for aphids as for eggs in both Ap end Ek larvae.

DISCUSSION

When *Harmonia axyridis* larvae reared on the aphid *Acyrthosiphon pisum* walked on a substrate previously contaminated by this aphid, they changed their path orientation and switched from extensive search to intensive search. Most of the larvae followed sinuous and/or looping tracks on the aphid contaminated area. Eighteen percent of the larvae previously fed aphids either abruptly turned back into the contaminated area each time they encountered its boundary or zizagged in and out of the aphid-contaminated area several times. Such behavior has also been shown by the parasitoid, *Nemeritis canescens* (Waage, 1978). After leaving the aphid-contaminated area, extensive search replaced intensive search again but the time spent in intensive search depended on the time spent by larvae on aphid-contaminated area.

This change in locomotor behavior showed that *H. axyridis* larvae perceived the previous presence of aphids, probably by means of sensillae located on the maxillary palps (Barbier *et al.*, 1989). Since less than half of the larvae released 10 cm from the edge of the aphid-contaminated area reached this area, it is likely that perception of the aphid-contaminated area is by means of gustatory rather than olfactory sensillae. For these larvae, aphid contamination not only acted as an arrestant, as does honeydew for *Coccinella septempunctata* L. (Carter and Dixon, 1984), but also improved the predatory efficiency by inducing intensive search mode in prey patches.

In predators, transition from extensive to intensive search is an accurate tool to study search behavior and its variations according to environmental conditions. Prey ingestion, starvation (Carter and Dixon, 1982; Ferran and Dixon, 1993), and aphid contamination induce intensive search. However, the specific characteristics of the search pattern adopted depend on environmental factors (Ferran *et al*, 1994).

H. axyridis larvae reared on the eggs of the flour moth, *E. kuehniella*, for ca. 100 generations did not respond when they entered the aphid-contaminated area. They maintained an extensive search mode with mainly linear or sinuous paths. Nevertheless, since most larvae showed a change in path direction and increased their linear speed when they entered the aphid-contaminated area, they appeared to perceive aphid contamination. These observations may be correlated with a decrease in their predatory efficiency. Compared to larvae reared on aphids, larvae raised on eggs needed more encounters with prey and took longer to capture and feed on prey. To explain these behavioral changes, it is assumed that the continuous rearing of *H. axyridis* on *E. kuehniella* eggs resulted in conditioning to this unnatural prey. The larvae either could have lost a part of their sensitivity to aphid gustatory stimuli (they smelled aphid tracks for the first time), could be repelled by aphid smell, or could have become unable to associated aphid odor with aphid presence. These behavioral changes did not result from a trophic selection pressure through generations because they are easily reverted. In experiments conducted in rose gardens, Ek larvae successfully controlled the rose bush aphid *Macrosiphon rosae* (Ferran *et al.*, 1996). Successive encounters with prey probably allowed them to recover their specific behavioral abilities. The biological performances of Ek larvae and adults (Schanderl *et al.*,

1988) also show that these behavioral changes cannot be explained by a tolerance to *E. kuehniella* eggs. In the environment, *H. axyridis* fed on many aphids species. It is suggested that this conditioning may also occur when larvae changes natural prey.

Compared with larvae reared on aphids, the ones reared on *E. kuehniella* eggs had difficulty detecting aphids, and their capture efficiency was reduced. This atypical behavior did not preclude the success of mass rearing operations on strictly *E. kuehniella* eggs over ca. 100 generations. Despite the low attractiveness of these eggs, survey, and weight gain of larvae, fecundity and fertility of females continuously fed on this unnatural prey are similar to those of larvae and females fed on aphids (Schanderl *et al.*, 1988). Although the effects of conditioning of larvae on *E. kuehniella* eggs was temporary, it may decrease their efficiency as biological agents. The release of larvae that are irresponsive to the presence of aphids may explain why a large proportion of larvae is lost within the first hours following their release on plants even if they are heavily infested with aphids (Ferran *et al.* 1996). This loss can be reduced by exposing larvae prior to release to aphids or aphid tracks.

Aphids laid cornicle secretions, and honeydew on plants. Cornicle secretions consist of a more or less specific mixture of tryglycerides and the volatile alarm pheromone, mainly (*E*)-β-farnesene (Greenway and Griffith, 1973; Hedin *et al.*, 1991; Nault and Bower, 1974; Pickett and Griffith, 1980).). This secretion is considered to have a defensive function either through its direct physical effect on natural enemies (Nault and Phelan, 1984; Dixon and Stewart, 1975) or by including the flight of conspecifics (Nault and Montgomery, 1979). Honeydew, an anal excretion, is a mixture of sugars, amino acids, salts, and probably volatile components (Bouchard and Cloutier, 1985). The response of ladybeetles to cornicule secretions and honeydew is yet unclear. Nakamuta (1991) has shown that *Coccinella septempunctata bruckii* Mulsant does not respond to synthetic (*E*)-β-farnesene. In *Coccinella septempunctata* L., honeydew is an arrestant stimulus for searching larvae (Carter and Dixon, 1984) and is implicated as an oviposition stimulus for females (Evans and Dixon, 1986).

In aphidophagous coccinellids, females laid their eggs next to aphid patches but the efficiency of this behavior depends on environmental conditions (Ferran *et al.*, 1989, 1991). In contrast to adults, larvae must feed in the environment where they were born. Their mobility (Ferran *et al.*, 1991) and their ability to see artificial targets and dummies (Lambin *et al.*, 1996) and to perceive gustatory aphid stimuli contributed to their fitness by increasing the production of adults with optimal reproductive capacities.

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