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INFLUENCE OF LARVAL REARING DIET ON THE INTENSIVE SEARCHING BEHAVIOUR OF *HARMONIA AXYRIDIS* [*COL. : COCCINELLIDAE*] LARVAE

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The capture of prey by last instar larvae of the aphidophagous coccinellid *Harmonia axyridis* Pallas (Col., *Coccinellidae*) modified larval movement so that extensive search was replaced by intensive search. The continuous rearing of this species on eggs of *Ephestia kühniella* Zell. (Lep., *Pyralidae*) or on the aphid *Acyrthosiphon pisum* Harris (Homopt., *Aphidae*) led to conditioning. Only larvae that ate the same prey as they were reared on, adopted intensive searching movements after feeding. Using larvae reared on substitute prey in biological control systems may decrease the efficiency of the released predators.

KEY-WORDS : rearing, feeding behavior, food substitute, conditioning, intensive search.

In insects, learning of cues from food, oviposition sites or micro-habitat is a very frequent process associated with a selective behavioral reponse (Thorpe, 1956). Learning, the subsequent conditioning, improves the efficiency of resource search and in general, favours the adaptation of these animals to fast changes in environmental conditions (Drost *et al.*, 1988).

Many phytophagous insects detect olfactory and visual cues from plants. Such cues enable the insects to modify their subsequent feeding preferences (Visser, 1986). Olfactory and visual cues associated with hosts, or their micro-habitats, have also been well-documented for parasitoids (Vinson, 1976). In contrast, the olfactory, tactile and gustatory stimuli involved in prey capture by coccinellids are not completely known (Storch, 1976 ; Stubb, 1980 ; Obata, 1986 ; Nakamuta, 1984, 1985).

Generally, it is assumed that conditioning has occurred if the test coccinellids subsequently modify their feeding preferences. For example, once the coccinellid *Stethorus punctum* LeConte (*Coccinellidae*) had fed on *Tetranychus urticae* Koch it was found to prefer this prey species (Houck, 1986).

In general, coccinellids use two patterns of movement to explore their environment, the extensive search which involves a fast linear movement between prey patches ; and the intensive search which involves slow and sinuous movements. The latter movements are induced by the perception of prey cues (Bell, 1990), and are considered adaptative as they increase the probability of predators encountering further food (Bell, 1985 ; Murakami & Tsubaki, 1984).

Until now, the sequence of extensive search followed by feeding and intensive search seemed stereotyped in insects. Recent work (Delcomyn & Cocatre-Zielgen, 1988 ; Ferran

& Ettifouri, in press; Ettifouri & Ferran, in press) has shown however that feeding is sometimes followed by extensive search or by patterns of intensive search different of those expected.

Using two populations of *H. axyridis* reared in the laboratory on either the aphid *A. pisum* or eggs of the flour moth *E. kühniella* it was possible to study the effects of diet on the searching behaviour of larvae of *H. axyridis*.

MATERIALS AND METHODS

Since it was brought from China in 1982, *H. axyridis* has been reared in our laboratory on the aphid *A. pisum*. From 1986, a part of the population was reared on eggs of *E. kühniella* that had been killed by exposure to U.V. radiation (Daumal *et al.*, 1975).

Last instar larvae, weighing 15 mg (± 2 mg), were used from each population and were starved for 3 hours prior to testing. Each test larva was placed in the centre of a white arena (height: 40 cm, diameter: 50 cm) and filmed until it reached the wall of the arena. The larva was again placed intro the centre of this arena where either one living aphid (weight: 1.00 ± 0.20 mg), glued to the bottom with Super glu 3 or a group of 35 to 40 *E. kühniella* eggs concentrated in a drop of water had been previously introduced. The total weight of the eggs was approximately equal to that of the aphid. Once the larva had fed, it was filmed in the manner described previously. The experiment was carried out at 22 °C (± 1 °C), 70 % to 80 % of relative humidity, and under artificial lighting ($2 \cdot 10^3$ lux).

For each coccinellid population, 20 larvae were provided with the food they had been reared on previously, 20 other larvae received the opposite food. The four arrangements between the two variables being studied (prey used for rearing, prey offered alone) resulted in 160 paths, half of which were recorded before feeding started. To help in the presentation of the results, each arrangement was considered as a separate population designated by the successive letters A for aphid and E for eggs. The first indicated the food used during rearing, and the second the food offered in the test. For instance, the AE population contained larvae reared initially on aphids and supplied subsequently with eggs.

A camera whose field covered the area of the arena, a tape recorder, and a computer equipped with a card for picture analysis and special software (Coulon & Charras, 1983; Clement & Luciani, 1985) were used to calculate automatically the coordinates of the larvae every 0.04 second (25 pictures per second) and to derive from such data the path parameters. For coordinate computation, the screen of the filmed area contained 512 lines and columns, the path were smoothed. All coordinate pairs (25 per second) were recorded.

Each path was characterized using four parameters: the number of stops (per second), the linear speed without stops (in mm/s), the angular speed (in degrees/s) and the straightness rate obtained using the distance between the beginning and the end of the path, and the actual distance travelled by larvae.

Data were submitted to two kinds of statistical analysis. The first was the usual comparison of path paramater means. The second was a classification of all the paths to describe the observed movement patterns and to compare the locomotory activity of the larvae. All paths (160) characterized by the four previous parameters (without transformation) were analysed using a principal component analysis (Benzecri, 1979) and a hierarchical classification (Lerman, 1981). By sectioning the graphic representation of this hierarchy at the one-choice level, it was possible to identify a number of path groups (i.e. movement patterns characterized by different averages for each parameter). Using the hierarchical classification, each path was classified into one of three groups and the frequency of the different kinds of larvae (AA, AE, EE, EA) in each group was computed.

RESULTS

VARIATIONS OF PATH PARAMETERS

Before feeding, test larvae from the A and E populations differed only in the number of stops they made. In the A population, larvae stopped less frequently (table 1). The mean path parameters computed for all larvae during the extensive search were number of stops : 0.44 ± 0.02 (/s), linear speed : 12.9 ± 0.4 (mm/s), angular speed : 16.6 ± 1.0 (°/s) and straightness rate : 0.33 ± 0.02.

TABLE 1

Movement data on last instar larvae of Harmonia axyridis. *Variation of path parameters (recorded before feeding) from the two larval populations reared in the laboratory or on either the aphid* Acyrthosiphon pisum *or on the flour moth*

Path parameters	Rearing with Aphids	 Eggs	Values of t student
Number of larvae	40	40	
Number of stops (/s)	0.33 ± 0.09[a]	0.56 ± 0.58	S (2.1)*
Linear speed (mm/s)	13.3 ± 1.4	12.7 ± 0.6	NS (1.7)
Angular speed (degree/s)	16.9 ± 1.2	16.4 ± 1.6	NS (0.2)
Straightness rate	0.36 ± 0.03	0.31 ± 0.04	NS (0.2)

[a] confidence interval at $p < 0.05$, S : significant difference.

After feeding, the larval paths of the A population differed according to the prey offered (table 2-1, comparison between columns). Compared to the AE larvae, the AA larval paths have more numerous stops, a higher angular speed, a lower linear speed and a lower straightness ratio. The searching by the AA larvae was more intensive than by the AE larvae. The movement pattern of the AE larvae after feeding also differed from extensive search in number of stops (t Student = 2.10), linear speed (t = 3.36) and angular speed (t = 2.25). For similar reasons, EE larvae searched more intensively than EA larvae (table 2-2).

The movement patterns of AA and EE larvae differed only slightly (table 2, comparison between lines). The number of stops and the angular speed were higher, however, in AA larvae. In contrast, the path parameters of AE and EA larvae were similar.

Therefore, when larvae of *H. axyridis* ate the same prey as the one used during their rearing, they adopted a form of intensive search which differed according to the nature of the prey. However, after feeding on a different prey, the movement pattern changed and was intermediate between the previous intensive search and the extensive search.

The path variations can be grouped into four patterns : the extensive search (before feeding), the intensive search (AA larvae), and two other forms of "intensive search" characterized by either EE larvae, or AE, EA larvae. These results question the usual sequence of extensive searching and feeding followed by intensive searching.

TABLE 2

Movement data on last instar larvae of Harmonia axyridis. *Variation of path parameters (recorded after feeding) according to the food provided to the larvae during routine rearing and the subsequent behavioural tests*

1. *Coccinellid population fed on the aphid* Acyrthosiphon pisum *(A).*

	Offered prey		Comparison between columns
Path parameters	Aphids (AA)	Eggs (AE)	
Number of stops	4.4 ± 0.5[a]	1.4 ± 0.4	S (9.2)[b]
Linear speed (mm/s)	7.4 ± 0.3	9.7 ± 0.4	S (8.5)
Angular speed (degree/s)	61.3 ± 9.3	24.1 ± 2.6	S (7.4)
Straightness rate	0.07 ± 0.01	0.30 ± 0.06	S(6.7)

2. *Coccinellid population fed on eggs of flour moth (E).*

	Offered prey		Comparison between columns
Path parameters	Eggs (EE)	Aphids (EA)	
Number of stops (/s)	3.2 ± 0.6	1.5 ± 0.4	S (4.6)
Linear speed (mm/s)	7.6 ± 0.5	9.6 ± 0.8	S (4.5)
Angular speed (degree/s)	39.9 ± 7.2	26.5 ± 4.7	S (3.1)
Straightness rate	0.06 ± 0.01	0.28 ± 0.04	S (5.7)
Comparison between lines			
Number of stops (/s)	S (3.1)	NS (0.5)	
Linear speed (mm/s)	NS (0.6)	NS (0.3)	
Angular speed (degree/s)	S (3.5)	NS (0.8)	
Straightness rate	NS (0.2)	NS (1.3)	

[a] : interval of confidence at $p < 0.05$, b : t Student value, S : significant difference.

LARVAL LOCOMOTORY PATTERNS

In the principal component analysis, the first two axes explained 97 % of the total variation. The four parameters were linked in pairs, the number of stops with angular speed, and the linear speed with the straightness rate.

Figure 1 shows the results of the principal components analysis and the hierarchical classification. The section of this graph higher than 0.6 showed two path groups, corresponding to traditional extensive and intensive searching. Each of the previous groups was divided into two sub-groups at quite high levels of 0.3 and 0.55. The explanation of path parameter variations is obtained by divisions at levels lower than 0.6.

The section of the graph at the level 0.4 produced three groups. The first group, G1, was characterized by extensive search in which the number of stops / angular speed were both minimal, and the linear speed / the straightness rate were both maximal (table 3). The group with the opposite behaviour, G3, defined intensive search. The second intermediate group, G2, exhibited paths that were linked to intensive search.

The division at level 0.2 indicated four groups. The first two were types of extensive search. They result from the difference in the number of stops made by the larvae in the A

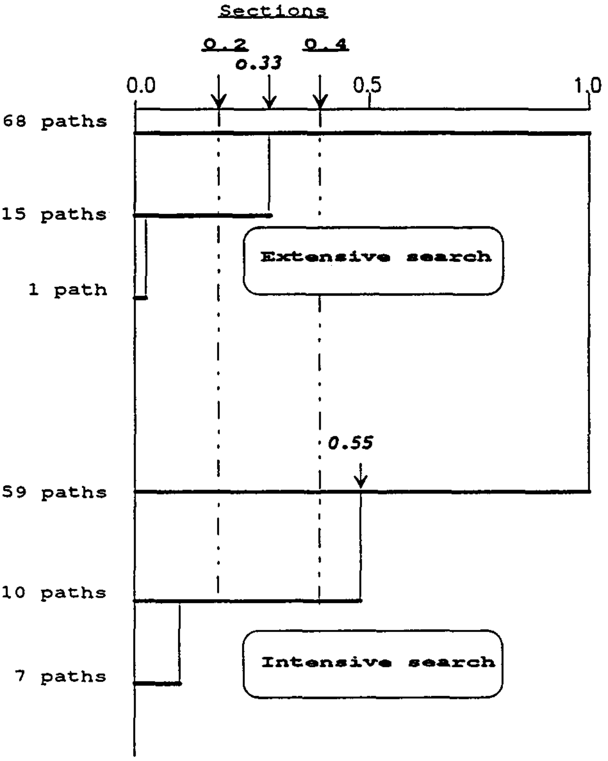


Fig. 1. Last instar larvae of *H. axyridis*. Hierarchical classification of individual paths to define movement patterns.

TABLE 3

Grouping of H. axyridis *larval paths using the hierarchical classification. The groups are characterized by the mean for each parameter (division of fig. 1 at the level 0.4)*

Path Groups	Number of stops	Linear speed (mm/s)	Angular speed (degrees/s)	Straightness rate	Number of larvae
G. 1	0.32 ± 0.07[a]	12.8 ± 0.4	15.8 ± 0.7	0.31 ± 0.02	84
G. 2	2.2 ± 0.3	9.0 ± 0.3	30.7 ± 2.0	0.19 ± 0.04	59
G. 3	5.1 ± 0.4	6.9 ± 0.3	72.4 ± 6.8	0.08 ± 0.04	17

[a] : confidence interval at $p < 0.05$.

and E populations. The two other groups were the same as those obtained in the previous section (0.4). Considering the low number (20) of larvae in each combination, path analysis was done only in the first division (0.4).

Before feeding, 95 % of A larvae and 80 % of E larvae used extensive search (G1), whereas 5 % to 20 % used the intermediate intensive search (G2), and none used intensive search (G3), (fig. 2). The difference in G2 between the two larval populations can be explained by the two sub-groups in figure 1.

After feeding, AA larvae adopted intensive searching (G1 : 0 %, G2 : 45 %, G3 : 55 %). Prey changing (AE larvae) induced important modifications in larvae searching; 25 % retained the extensive search (G1), 75 % the intermediate intensive search (G2) and none the intensive search (G3).

The EE larvae, behaved like the AA larvae and adopted the two forms of intensive search (G1 : 0 %, G2 : 75 %, G3 : 25 %). However, the percentage movement in classes G2 and G3 were note similar to those for the AA larvae. Prey changing (EA larvae) resulted in the same modifications as in AE larvae. These were the maintenance of extensive search (G1 : 45 %) and adoption of intermediate search (G2 : 50 %). These was also some intensive search (G3 : 5 %), probably due to the return to the natural prey (aphids).

The above results were consistent with those obtained by path parameter analysis. The only difference between the analytical methods occured between the AE and EA larvae. Their path parameters were not significantly different, though the frequencies of movement patterns were. Nevertheless, the extensive search (G1) and the intermediate intensive search (G2) predominated in both populations of larvae.

DISCUSSION

In last instar larvae of *Harmonia axyridis* (Pallas), the search paths varied according to the food used during larval rearing and the prey species the larvae encountered subsequently. Comparison of path parameters showed that different prey resulted in different movement patterns during intensive search. The classification of paths made it possible to distinguish three movement patterns (the extensive search, the intensive search, and an intermediate intensive search) and to compute the frequency of such patterns in the different groups of larvae.

Before feeding, most larvae used extensive search. Previous diet (aphids or eggs) had little effect on movement prior to feeding. However, larvae reared on moth eggs seemed more frequently to adopt the intermediate intensive search. During movement prior to feeding, some larvae of the coccinellid *Semiadalia undecimnotata* (Schn.) carried out intensive search movements (Ferran *et al.*, in press).

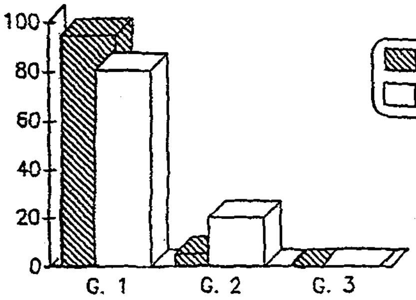
After feeding on the prey item on which they were reared, larvae gave up the extensive search and carried out the two forms of intensive search. The classical effect of prey feeding on larvae movements is upheld. Although prey-changed larvae retained the extensive search, most of them adopted the intermediate intensive search and none of them the intensive search. In these larvae, the classical effect that feeding has on subsequent movement was not shown. Thus, in searching behaviour, the continuous rearing of *H. axyridis* on a given prey conditions the larvae to that prey.

The effect of the prey given to larvae to test the change in movement pattern was not obvious. It would seem that natural prey stimulated the larvae to adopt the intensive searching behaviour.

In a biological control scheme, the larvae of this coccinellid would be reared on the substitute prey and released into orchards against different aphid species. However, conditioning to such prey may decrease the efficiency of the released predators. Larvae which retain the extensive search, may soon leave the trees, while larvae using intermediate intensive search may not be as effective in exploiting aphid colonies. Consequently, it is

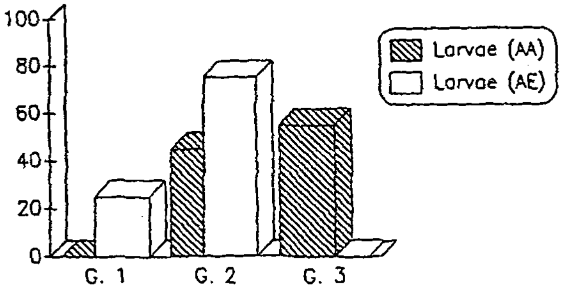
1. PATTERNS OF MOVEMENT BEFORE FEEDING

Larvae reared on the aphid Acyrthosiphon pisum (A) or on eggs of the floor moth (E).



2. PATTERNS OF MOVEMENT AFTER FEEDING

Larvae reared on the aphid (A) and fed subsequently on the same prey (AA) or on eggs (AE).



Larvae reared on eggs (E) and feed subsequently on the same prey (EE) or on aphids (EA).

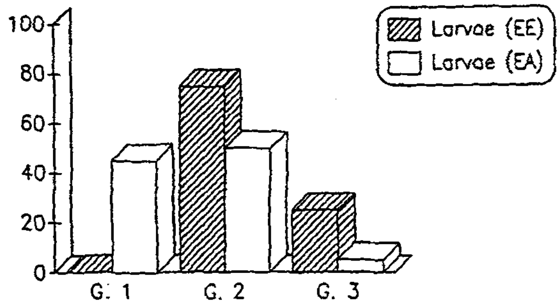


Fig. 2. Last instar larve of *H. axyridis*. Distribution (in %) of larval paths in the different locomotory patterns defined by the hierarchical classification according to before (1) and after (2) feeding. Movement paths: G1, extensive search; G2, intermediate intensive search; and G3, intensive search.

important to suppress this conditioning even if the absence of the reinforcing factor, the substitute prey, induces its progressive disappearance (Kaas *et al.*, 1990). Starving the larvae prior to release, which favours the adoption of intensive search, is perhaps the best means (pers. com.).

This polyphagous predator is perhaps more apt to conditioning than monophagous species (Vinson, 1976). Thus this above feeding adaptation may also occur with natural preys.

The capacity to condition depends on insect age (Wardle & Borden, 1985). Larvae and adults of *Semiadalia undecimnotata* (Schn.) retain the extensive pattern if they ingest an aphid during the three hours following moulting or following emergence (Ettifouri & Ferran, in press) hours following moulting or following emergence (Ettifouri & Ferran, in press). Induction or conservation of conditioning may occur during this period, and this would appear to be the "critical period" of Eibl-Eibesfeldt (1970).

RÉSUMÉ

Influence des conditions trophiques de l'élevage sur l'apparition de la recherche intensive chez les larves d'*Harmonia axyridis*

Chez les larves de dernier stade de la coccinelle *Harmonia axyridis* Pallas (Col., *Coccinellidae*) l'ingestion d'une proie entraîne une modification du comportement locomoteur : la recherche extensive est remplacée par la recherche intensive. L'élevage permanent de cette espèce sur une proie particulière, les œufs d'*Ephestia kühniella* Zell. (Lep., *Pyralidae*) ou, le puceron *Acyrthosiphon pisum* Harris (Homop., *Aphidae*) aboutit, au travers de l'apprentissage, à un conditionnement trophique. Seules les larves qui reçoivent une proie identique à celle de l'élevage, abandonnent la recherche extensive et adoptent la recherche intensive. L'utilisation en lutte biologique contre les pucerons de larves produites sur la proie de substitution peut se traduire par une diminution de leur efficacité prédatrice.

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