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MECHANISMS OF PREY FINDING IN THE APHIDOPHAGOUS LADYBIRD BEETLE, *HARMONIA AXYRIDIS* [*COLEOPTERA : COCCINELLIDAE*]

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The mechanism of prey finding in adult *Harmonia axyridis* was investigated by presenting beetles with small gauze or polyethylene bags containing either aphid-infested leaves or healthy leaves alone together with empty control bags. Beetles were attracted to bags by olfactory and visual cues suggesting the presence of aphids, including the odor of aphid-infested leaves, the odor of aphids, the odor of healthy leaves, and the sight of leaves. Particularly effective in prolonging beetles's stay on bags visited was the odor of aphid-infested leaves. It is suggested that adults of *H. axyridis* use olfactory and visual cues to detect prey before actual contact occurs. Use of such cues seems more advantageous than random searching, as has been reported in many previous studies of coccinellid foraging.

KEY-WORDS : lady beetle, *Harmonia axyridis*, prey-finding.

The searching behavior of predatory coccinellids has been thought to be random, with detection of prey occuring only at actual contact, without aid of previous visual or olfactory orientation to prey (e.g., **Hodek,** 1973). Many studies have dealt consequently with factors enhancing the efficiency of such random searching by coccinellid beetles (particularly larvae). Factors identified include : (1) Searching larvae, like their prey, are negatively geotactic and positively phototactic (**Fleschner,** 1950 ; **Banks,** 1957 ; **Dixon,** 1959). (2) Following prey capture, several predatory coccinellids tend to search the local area intensively, which is advantageous if their prey live in clusters (**Banks,** 1957 ; **Dixon,** 1959 ; **Nakamuta,** 1982).

The ability of randomly searching larvae to find their prey is enhanced by ovipositing females since egg batches are usually laid either on or close to leaves infested with aphids (e.g., **Dixon,** 1959). But newly emerged adults of *Harmonia axyridis* must seek out new concentrations of prey because they have generally consumed most prey nearby during development (unpublished field observations). What serves as the cue in their search for prey ? Previous studies of adult coccinellids have not addressed this question, except for the study by **Colburn & Asquith** (1970) who found that *Stethorus punctum* adults were attracted to their prey by smell.

In this paper I describe results of laboratory experiments suggesting that adults of *H. axyridis* may locate sites where aphids are likely to occur by means of olfactory and visual cues.

MATERIALS AND METHODS

Pupae and prepupae of *H. axyridis*, attached to leaves of Japanese pittosporum, *Pittosporum tobira*, were collected in Kyoto from late May to early June. When the adults emerged in the laboratory, they were given honey but not aphids until the experiments started 7-10 days later.

The aphids used for experiments, *Aphis citricola*, were collected together with their host plant, annual fleabane, *Erigeron annuus*. Experiments were carried out in the daytime (11:00-17:00) at room temperature (20-25 °C).

EXPERIMENT I

In this experiment, I used model "trees" each made of 3 plastic straws (diam. 4 mm × length 20 cm), to the top of which 1 of 6 variants of small bags (diam. ca. 5 cm) was attached (fig. 1). The 6 variants included 3 gauze bags for testing olfactory factor apart from visual cues, and the 3 polyethylene bags for testing visual apart from olfactory cues. The 3 gauze bags contained aphid-infested leaves (AO), healthy leaves (LO) or nothing for control (CO), respectively. Each bag for AO contained about 100 aphids and 3 pieces of leaves (about 6 cm^2 in total), and for LO the same amount of leaves as AO. The 3 polyethylene bags had similar contents (AV, LV and CV).

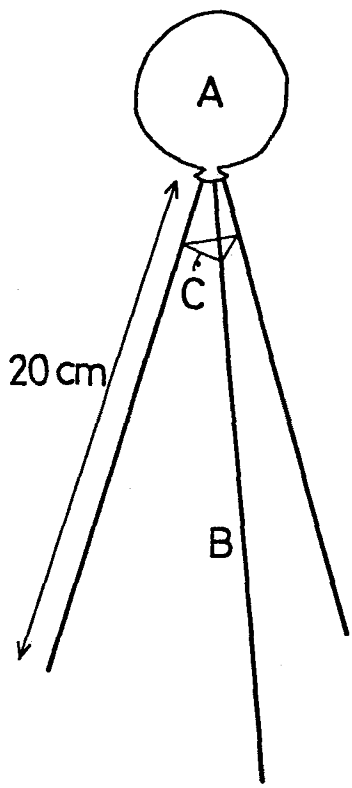

Fig. 1. Model tree used for *Experiment I*. A : gauze or polyethylene bag - B : plastic straw - C : pin.

These 6 "trees" were placed together in a large plastic cage (20.0 × 36.0 × 26.5 cm) and the 25-30 beetles of the same sex were then introduced into the cage. Observations were made for 1 h and the total number of visits by beetles to bag of each tree, whether by climbing the straw or by flying directly, were counted. This experiment was repeated 8 times, i.e. 4 times for each sex and the arrangement of 6 trees was changed every time according to the random table.

EXPERIMENT II

Two small bags (diam. ca. 3 cm), an experimental bag and its control, were attached to a plastic straw (diam. 4 mm × length 18 cm) and the straw was placed upright in cylinder of transparent vinyl chloride (0.3 mm in thickness) (fig. 2). Five combinations of bags were tested : *a*) a gauze bag containing aphid-infested leaves (AO) vs. an empty gauze bag (CO), *b*) a gauze bag containing healthy leaves (LO) vs. CO, *c*) a polyethylene bag containing healthy leaves (LV) vs. an empty polyethylene bag (CV), *d*) LV applied with ethyl ether extract of aphids on the polyethylene-bag surface (LV + AE) vs. CV applied with ether on the polyethylene-bag surface (CV + E) and *e*) CV applied with ether extract of aphids on polyethylene-bag surface (CV + AE) vs. CV + E. Ether extract of aphids was prepared by rinsing them with a volume of about 1 cm^3 in 5 ml of ethyl ether. For each combination, a pair of cylinders with bags placed at complementary positions on straw was observed at the same time.

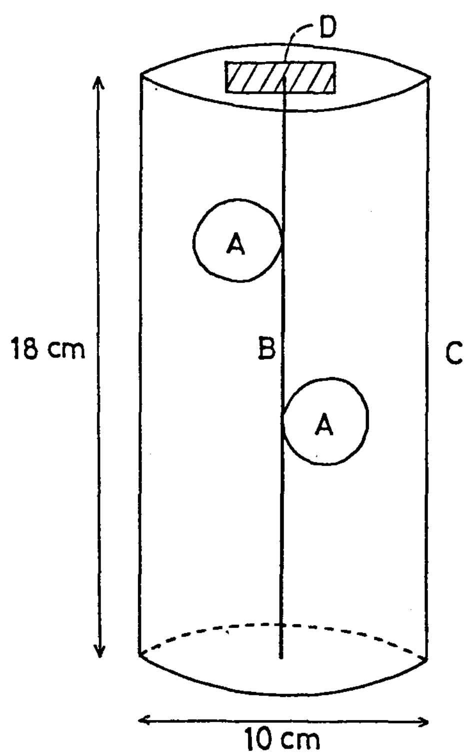

Fig. 2. Observation apparatus for *Experiment II*. A : experimental or control bag - B : plastic straw - C : transparent vinyl chloride wall - D : nylon net for ventilation on top of the cylinder.

Five to 7 beetles of the same sex were introduced in each cylinder and observed for 1 h. When individuals walked along the straw, their wandering paths were traced and the numbers of passes by (P) and visit to (V) each bag were counted. A "pass" indicates that a beetle passed by the point on the straw where the bag was attached but did not visit the bag, whereas a "visit" indicates that the beetle changed its course and left the straw to visit and walk on it. The duration of each stay on a bag was also recorded. The 1-h observations were repeated, bags and experimental insects being renewed every time, 5 times for combination *a*, 6 times for combination *b*, 4 times for combination *c*, 6 times for combination *d* and 4 times for combination *e*.

RESULTS

EXPERIMENT I

Table 1a shows the total number of times beetles visited each of 6 bags during 1-h experimental period and the statistical analysis of the date by analysis of variance is summarized in table 1b. Although males were used in trials No. 1-4 and females in No. 5-8, no major differences between the 2 sexes' responses to bags were detected ($P > 0.25$). Beetles did not visit the 6 kinds of bags randomly ($P < 0.001$). Further comparisons by analysis of variance were then made between 2 types of bags of reasonable combination (table 1c). Among the 3 types of gauze bag (AO, LO and CO), bags containing aphid-infested leaves (AO) attracted significantly more beetles than did the others ($P < 0.005$ for both AO vs. LO and AO vs. CO). The attractiveness of bags containing healthy leaves (LO) is similar to empty control bag (CO) ($P > 0.25$). Among the 3 types of polyethylene bags (AV, LV and CV), bags containing aphid-infested leaves (AV) and healthy leaves (LV) were more attractive for beetles than empty control bag (CV) ($P < 0.05$ for AV vs. CV, $P < 0.005$ for LV vs. CV). No difference was detected in attractiveness among AO, AV and LV ($P > 0.25$ for both AO vs. AV and AV vs. LV, $P > 0.10$ for AO vs. LV). Since there was no significant difference between CO and CV ($P > 0.25$), beetles seemed to have no particular choice between gauze and polyethylene bags.

TABLE 1a

Frequency of beetles' visits to the bag on each tree in Experiment I

Ser. No.	Sex of beetles	Gauze bags containing			Polyethylene bags containing			total
		Aphid-infested leaves (AO)	Healthy leaves (LO)	Nothing (CO)	Aphid-infested leaves (AV)	Healthy leaves (LV)	Nothing (CV)	
1	male	28	15	14	33	21	17	128
2	male	17	3	9	8	19	2	58
3	male	12	8	8	25	20	11	84
4	male	50	11	7	30	29	10	137
5	female	30	5	9	18	21	7	90
6	female	20	14	14	50	20	7	125
7	female	30	13	4	13	15	10	85
8	female	44	17	4	26	20	19	130
male total		107	37	38	96	89	40	407
female total		124	49	31	107	76	43	430
Total		231	86	69	203	165	83	837

TABLE 1b

Analysis of variance on frequency of beetles' visits to the bag on each tree in Experiment I

Source	Sum of squares	Degree freedom	Mean square	F	Probability
Sex of beetles	11.021	1	11.021	0.138	$P > 0.25$
Type of bag	3009.94	5	601.99	7.535	$P < 0.001$
Interaction	86.604	5	17.32	0.234	$P > 0.25$
Deviation	2876.25	36	79.896	–	–
Total	5983.81	47			

TABLE 1c

Analysis of variance on frequency of beetles' visit to the bag in Experiment I *; comparisons between 2 types of bags of reasonable combinations*

Comparison	Degree of freedom	F	Probability
AO vs. LO	(1, 12)	12.109**	$P < 0.005$
AO vs. CO	(1, 12)	15.816**	$P < 0.005$
LO vs. CO	(1, 12)	0.856	$P > 0.25$
AV vs. LV	(1, 12)	0.856	$P > 0.25$
AV vs. CV	(1, 12)	7.761*	$P < 0.05$
LV vs. CV	(1, 12)	17.066**	$P < 0.005$
CO vs. CV	(1, 12)	0.477	$P > 0.25$
AO vs. AV	(1, 12)	0.253	$P > 0.25$
AO vs. LV	(1, 12)	2.653	$P > 0.10$

** 0.5 % significant * 5 % significant

EXPERIMENT II

Table 2 shows the numbers of passes (P) and visits (V) for the experimental bag and control bags, together with the ratio of V to P (V/P). The numbers of passes and visits in table 2 are the sum total of all replicates for each combination. I did not observe long-range orientation to bags, and I use V/P as an index of the short-range orientation to bags. In all combinations, V/P for the experimental bag was significantly higher than that for the control ($P < 0.001$, by X^2-test).

Table 3 shows the distribution of the staying duration on experimental and control bags for each combination. The time range is divided into every 5 s, and durations longer than 120 s are combined. Lengths of visits to experimental bags vs. control bags were compared for each combination by Kolmogolov-Smirnov 2-sample test using this table. In all combinations beetles stayed significantly longer on the experimental bags than on its control ($P < 0.001$ for combination *a, b, d* and *e*, $P < 0.01$ for combination *c*). Furthermore, the lengths of visits to experimental bags were compared between combination *a* and *b*, and between combination *d* and *e*, because only these 2 pairs had the same type of control bags and were

TABLE 2

Frequency of pass (P) and of visit (P) to experimental (E) and control (C) bags in Experiment II

Combination	Experimental bag (E) vs Control bag (C)	No of replicates	Total no of pass (P)	Total no of visit (V)	Ratio of V to P (V/P) (%)
a	AO (E) CO (C)	5	217 183	149 80	68.7 43.7
b	LO (E) CO (C)	6	249 213	144 81	57.8 38.0
c	LV (E) CV (C)	4	291 274	180 106	61.9 38.7
d	LV + AE (E) CV + E (C)	6	248 195	180 55	72.6 28.2
e	CV + AE (E) CV + E (C)	4	152 136	98 43	64.5 31.6

reasonable for comparison (table 3). For the experimental bags of combination *a* and *b*, AO effectively prolonged beetles' staying time compared with LO ($P < 0.001$) and for combination *d* and *e*, LV + AE was more effective for prolongation of beetles' stay on the bag than CV + AE ($P < 0.001$).

DISCUSSION

In EXPERIMENT I, beetles were especially attracted to 3 types of bags : 1) AO, the gauze bag containing aphid-infested leaves, through which the beetles could not see the leaves but could detect the odor of aphids, their host plant and honeydew, 2) AV, the transparent polyethylene bag containing aphid-infested leaves, 3) LV, the polyethylene bag containing healthy leaves. The polyethylene bags of AV and LV allowed beetles only to see and not smell leaves and aphids. Thus adults of *H. axyridis* were attracted by the odor of aphid-infested leaves and by a leaves (regardless of whether or not these were aphid-infested). The odor of healthy leaves seemed less effective because gauze bag with leaves alone (LO) attracted few beetles.

In each of the 5 combinations in EXPERIMENT II, the experimental bag provided some information reflecting the presence of aphids while the control bag provided none. Although LO and LV did not have any information related directly to aphids, they contained leaves on which aphids might occur.

The ratio of V/P is an index of the extent to which beetles are apt to visit each bag when they pass by it. V/P comparisons of experimental vs. control bags confirm that adults of *H. axyridis* particularly orient to sites likely to harbor aphids. In making choices in EXPERIMENT II, beetles seemed to utilize various cues, including the odor of aphid-infested leaves (AO), the odor of healthy leaves (LO), the odor of aphids (LV + AE, CV + AE) and the sight of leaves (LV, LV + AE). Beetles were particularly likely to visit experimental bags in trials with combination *d*, where this polyethylene bag was contained leaves and coated with aphid extract. This may suggest that adults of *H. axyridis* orient particularly to sites where green leaves and the odor aphids co-exist. Indeed, such sites are quite likely to be aphid-infested.

TABLE 3

Distribution of the staying duration for every 5 seconds on experimental and control bags for each combination in Experiment II. *Figures represent percentage to the number of visits for each bag*

Combination	Experimental bags (E) vs. Control (C)									
	a		*b*		*c*		*d*		*e*	
time range (second)	AO (E)	CO (C)	LO (E)	CO (C)	LV (E)	CV (C)	LV + AE (E)	CV + E (C)	CV + AE (E)	CV + E (C)
0-5	0	2.50	5.56	9.88	1.11	0.94	0.56	5.45	0	0
5-10	2.01	12.50	9.03	35.80	5.00	19.81	1.11	14.55	3.06	6.98
10-15	6.71	22.50	11.11	18.52	16.67	21.70	3.89	20.00	5.10	4.65
15-20	8.05	17.50	15.97	6.17	13.33	16.04	5.00	14.55	7.14	13.95
20-25	6.71	20.00	10.42	9.88	11.67	9.43	7.22	5.45	10.20	16.28
25-30	8.72	2.50	6.25	2.47	7.78	8.49	6.11	5.45	10.20	11.63
30-35	8.05	5.00	4.86	2.47	5.00	1.89	4.44	10.91	5.10	11.63
35-40	5.37	5.00	6.94	0	7.22	6.60	8.88	0	7.14	13.95
40-45	2.01	5.00	3.47	0	3.89	4.72	6.11	1.82	4.08	6.98
45-50	3.36	1.25	2.78	1.23	2.22	5.66	4.44	7.27	8.16	0
50-55	2.01	1.25	0.69	0	2.22	0	1.67	3.64	6.12	2.33
55-60	2.01	0	4.17	0	2.78	1.89	3.33	0	4.08	0
60-65	2.68	2.50	2.78	0	3.33	0	7.22	1.82	2.04	6.98
65-70	2.01	1.25	2.08	1.23	2.22	0.94	2.22	0	8.16	0
70-75	0.67	0	0	1.23	2.22	0	2.78	0	6.12	0
75-80	4.03	0	0	2.47	1.67	0	2.78	1.82	1.02	0
80-85	4.03	0	0.69	0	2.22	0.94	1.11	0	0	0
85-90	2.01	0	0.69	0	1.67	0	2.78	1.82	2.04	0
90-95	2.68	0	0	1.23	0.56	0	1.11	1.82	0	0
95-100	2.68	0	0.69	0	1.11	0	2.22	1.82	0	0
100-105	2.68	0	1.39	0	0.56	0.94	1.67	0	2.04	2.33
105-110	1.34	0	1.39	0	0.56	0	1.67	0	1.02	0
110-115	2.01	0	1.39	0	0.56	0	1.11	0	1.02	0
115-120	1.34	0	0	0	0.56	0	3.89	0	2.04	0
120 <	16.78	1.25	7.64	7.41	3.89	0	16.67	1.82	4.08	2.33
Number of visits	149	80	144	81	180	106	180	55	98	43

Kolmogolov-Smirnov two sample test

i) Experimental vs. Control bag

combination *a* : AO vs. CO	$X^2 = 55.24$, $P < 0.001$
combination *b* : LO vs. CO	$X^2 = 30.74$, $P < 0.001$
combination *c* : LV vs. CV	$X^2 = 13.37$, $P < 0.01$
combination *d* : LV + AE vs. CV + E	$X^2 = 38.87$, $P < 0.001$
combination *e* : CV + AE vs. CV + E	$X^2 = 13.82$, $P < 0.001$

ii) Experimental vs. Experimental bag

AO (combination *a*) vs. LO (combination *b*)	$X^2 = 23.95$, $P < 0.001$
LV + AE (combination *d*) vs. CV + AE (combination *e*)	$X^2 = 11.99$, $P < 0.001$

As for staying duration, beetles tended to stay on the experimental bag for a longer time than on the control one in all combinations. Various cues reflecting the presence of aphid, mentioned above, seemed to have the effect for prolongation of beetles' stay as well as the effect of V/P increase. Comparison between AO and LO suggests that either aphid odor and/or aphid plus leaf odor is more important factor influencing the length of stay than odor of healthy leaves. Ether extract of aphid (AE) seemed to prolong beetles' stay, but view of leaves plus aphid extract (LV + AE) induced beetles to linger longer than aphid extract alone (CV + AE) did. This suggests a synergistic effect of these factors on beetles' behavior.

Based on these experiments, the mechanisms of prey finding by adults of *H. axyridis* can be explained as follows. Beetles searching for aphids do not stop at sites randomly ; rather they tend to visit sites where aphids are likely to exist. Olfactory cues including the odor of the aphid-infested plant help beetles select sites to visit as **Colburn & Asquith** (1970) suggested in *Stethorus punctum*. Responding both to the odor of healthy leaves and to the odor of aphids can make beetles' search for prey more effective. The beetles also appear capable of utilizing visual cues such as the view of green leaves in deciding to visit a site.

When visiting a site, beetles stay longer when the site provides information indicating aphids may be present. The most important cue seems to be the complex odor of aphids and host plant.

The behaviors reported here are more advantageous than the random searching reported in many studies of coccinellid larvae, e.g. *Stethorus picipes, Coccinella septempunctata* and *Pharoscymnus numidicus* (**Fleschner,** 1950 ; **Banks,** 1957 ; **Kehat,** 1968). The time for searching can be considerably reduced when beetles use olfactory and visual cues to assess whether or not aphids are likely to exist at particular sites. The results of this study, however, can be applicable for relatively short-distance orientation of this coccinellid because of restriction of experimental style in laboratory. In long-distance orientation of this beetle, a mode of response to aphid seems of the same kind as that of this study, but a detailed study in natural conditions will be necessary.

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RÉSUMÉ

Mécanismes de découverte de la proie chez la Coccinelle aphidiphage
Harmonia axyridis [*Coleoptera : Coccinellidae*]

Le mécanisme de découverte de la proie chez l'adulte d'*Harmonia axyridis* est étudié en donnant aux Coccinelles de petits sacs en gaze ou en polyéthylène contenant soit des feuilles infestées de pucerons, soit des feuilles saines seules, avec des sacs vides comme témoins. Les coccinelles sont attirées vers les sacs par des indicateurs olfactifs ou visuels suggérant la présence de pucerons, incluant l'odeur des feuilles infestées de pucerons, l'odeur des pucerons, l'odeur des feuilles saines et la vue des feuilles. L'odeur des feuilles infestées de pucerons est particulièrement efficace en prolongeant le séjour des Coccinelles sur le sac visité. Il est suggéré que les adultes d'*H. axyridis* utilisent des indicateurs olfactifs et visuels pour détecter leur proie avant que s'établisse le véritable contact. L'emploi de tels indicateurs semble plus avantageux que la recherche au hasard, comme cela a été indiqué dans de nombreuses études antérieures sur la quête alimentaire des Coccinelles.

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