

Laboratory studies of plant searching behaviour by *Coccinella septempunctata* L. larvae

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Abstract

Movements of individual fourth instar *Coccinella septempunctata* L. larvae on pot-grown broad bean plants in England were recorded continually to assess plant recognition and the effects of prey capture. Prey capture was also studied in a 25×25 cm artificial arena. Approximately 80% of the total distance moved by larvae on plants was along leaf edges and mid-veins. Typical search speeds were 0.38–0.51 cm s⁻¹. An average of only 3.2% of larval search time on plants was spent on leaf laminae on which movement was considerably slower, about 0.15 cm s⁻¹. Discovery of *Acyrtosiphon pisum* (Harris) nymphs on plants induced intensive re-searching of the general area of discovery for periods of up to 12 min whereas in the arena the intensive search following prey capture lasted only up to 72 s and was much more localised. Examination of larval search times on plants revealed that 64% and 36% of maximum search times were recorded for the first and for the second or third searches, respectively, in a sequence of searches of the same plant. Only after prey capture was a fourth or subsequent search the longest. Plants previously searched unsuccessfully were recognised by detection of a chemical marker, specific for each individual, secreted via the anal disc during searching. Since *C. septempunctata* larvae are unable to detect aphids by sight or smell, recognition of areas previously searched unsuccessfully minimises wasteful energy expenditure.

Introduction

Although the Coccinellidae are an important family of aphidophagous insects, only a few studies of predator behaviour on plants have been published (Fleschner, 1950; Banks, 1957; Dixon, 1959). During a study of predation by *Coccinella septempunctata* L. on the aphid species *Aphis fabae* Scop. and *Acyrtosiphon pisum* (Harris) on small bean plants, *Vicia faba* (Marks, 1970; Murdoch & Marks, 1973), it was noticed that sequential introductions of a larva to the same plant frequently resulted in reduced search times if the larva was unsuccessful in obtaining food. This paper presents results of experiments done in England with *C. septempunctata* larvae in which behaviour on plants and in an artificial arena were compared and the possibility examined that larvae could recognise areas of plants which they had previously searched.

Methods

Three-day-old fourth instar *C. septempunctata* larvae were used in all experiments. Larvae were maintained in a greenhouse on individual potted bean plants infested with

A. pisum until about 16 h before use. They were then transferred individually to glass tubes and held in the laboratory without food until use.

Large uninfested bean plants in pots (leaf area 1600–1900 cm²) were used to study searching behaviour and the effect of *A. pisum* capture. Smaller uninfested bean seedlings in pots (leaf area about 102 cm²) were used for experiments on plant recognition. Sequential search times for six or more re-introductions of a larva to the same plant were recorded. Following the final search, larvae were transferred within 5–15 s to a second plant and the process of sequential introductions was repeated. Occasionally the procedure was extended to third and fourth plants. Predator movement was recorded by a shorthand notation which was later transformed into distances moved, as measured on pressed plants using a planimeter. Leaf areas were measured using a graduated opisometer. All experiments were done under laboratory conditions of daylight, temperature (21–23°C) and humidity, except for a simple arena experiment which used white blotting paper as the substrate and diffuse overhead lighting.

In the arena experiment predators were released singly from the centre quadrat of a 25×25 cm arena divided into 400 12.5×12.5 mm quadrats. In each of 17 randomly-chosen quadrats two *A. pisum* nymphs were secured by glue, initially 2 mm apart. Times to first effective contacts and discovery of the second prey after completion of the first meal were recorded for pairs of aphids placed various distances apart. Failure to capture the second aphid in a pair was scored when the predator moved outside a 2.5 cm boundary line and resumed its normal search pattern. Ten replications for each separation were examined.

C. septempunctata larvae have no visual or olfactory perception of prey and must touch an aphid before they can detect its presence (Marks, 1970). Since objects touched by the prothoracic legs were invariably seized by the mouthparts and examined, width of the prothoracic legs and not the head (Banks, 1957) was taken as a measure of the width of the perceptive field of larvae in the calculation of search areas.

Results

Behaviour of hungry larvae on plants

Searching larvae moved almost continuously, searching leaves and stipules both when ascending and descending the plant. On small plants up to 84% of leaves were visited once, whereas on larger plants fewer leaves were visited once and a larger proportion left unvisited (Table I). However, similar proportions of leaves on both large and small

TABLE I. *Frequency of leaf visits on broad bean plants, Vicia faba, by fourth-instar larvae of C. septempunctata*

Leaf area (cm ²)	Mean search time (s)	% leaves searched the indicated number of times*				Search time/unit area (s/cm ²)
		0	1	2–3	4	
Small plants						
46	126	10 <i>0–33</i>	47 <i>17–84</i>	33 <i>0–50</i>	10 <i>0–50</i>	2.7
102	620	10 <i>0–40</i>	33 <i>0–70</i>	28 <i>0–66</i>	29 <i>0–90</i>	6.1
296	1755	31 <i>18–68</i>	19 <i>14–23</i>	25 <i>0–45</i>	25 <i>14–45</i>	5.9
Larger plants						
1320	3405	38 <i>28–51</i>	29 <i>20–44</i>	23 <i>18–28</i>	10 <i>0–28</i>	2.6
1908	6960	23	14	39	16	3.7

*Ranges of values in each frequency class in italics. Data based on 10 replicates except for 1320 and 1908 cm² plants for which there were 5 and 1 replicates, respectively.

plants were visited 2–4 times. Consideration of the search time per unit area of plant (Table I) revealed that larvae did not become trapped on larger, more complex plants, nor search them more thoroughly than smaller plants. On large plants the area searched by larvae (distance moved \times perceptive field) was as high as 93% of total plant area (Table II). The proportion of the area searched which was traversed more than once

TABLE II. *An analysis of searching behaviour of five C. septempunctata larvae on uninfested broad bean plants* †

% Time on leaf lamina (s)	3.2 \pm 0.72
% Total distance moved	
Leaf edges*	71.0 \pm 6.36 (84.2–91.8)
Mid-veins*	9.2 \pm 1.17 (7.1–18.8)
Stems	17.8 \pm 4.43
Total distance moved (cm)	1667 \pm 403.3
Mean search speed (cm/s)	0.41 \pm 0.036
Mean search time (s/cm ²)	2.85 \pm 0.604
% Total plant area searched*	66.5 \pm 12.38 (45.2–92.9)
*Approximate leaf area 1600 cm ²	
*Values in parentheses indicate range of percentages based on total distances moved on leaves only.	

was not estimated. An average of 71.0 and 9.2% of the total distance moved by larvae on plants was along leaf edges and mid-veins, respectively (Table II), whereas only 3.2% of total search time was spent on the flat surface of leaf laminae. Search speed of larvae on the leaf lamina, however, was only about 0.15 cm s⁻¹ compared to typical search speeds on leaf edges and veins of between 0.38–0.51 cm s⁻¹.

Effect of prey discovery on search behaviour

Arena experiment.—A wide range of search times existed between replicates before discovery of the second aphid in a pair (Fig. 1), depending largely upon which direction

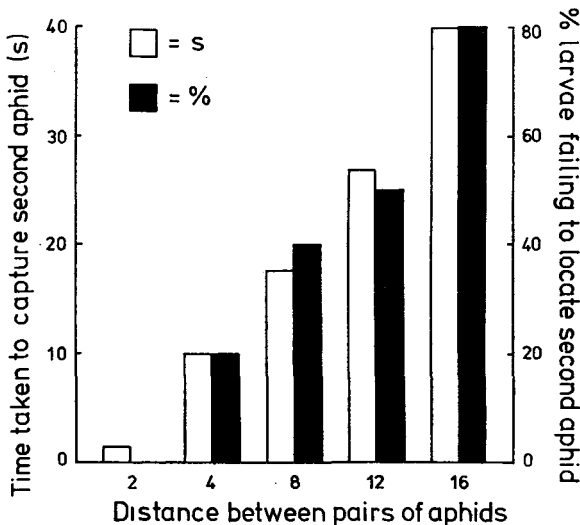


Fig. 1.—The effect of aphid capture on the ability of *C. septempunctata* larvae to locate adjacent aphids in a uniform arena (distances between pairs given in mm). Unsuccessful captures were not considered in the computation of means (based on 15–20 observations).

the predator turned on completion of its first meal. The longest intensive search was for 72 s. As the aphids were moved further apart in the pairs, the intensive search induced by the first meal became less effective. The distance apart at which the second aphid in a pair was not found in 50% of cases, was 12 mm.

The intensive search induced by a successful attack in the arena consisted of two components, on the completion of which random searching with reduced turning was resumed. An intense localised search in the immediate vicinity of prey discovery, followed by random searching with reduced turning, has been described for other coccinellid larvae (Fleschner, 1950; Banks, 1957) and for 24-h-old larvae of *Episyrphus balteatus* (Degeer) (Chandler, 1969). In the case of *Adalia bipunctata* (L.) this lasts for about 15 s (Banks, 1957). With *C. septempunctata*, the first component of the intensive search was extremely localised, the abdomen remaining fixed to the surface by the anal-disc whilst the head and the prothoracic legs were repeatedly moved from side to side through an arc of less than 180°. This, the shortest component of the two, lasted for 5–10 s. The second component of the intensive search (50–60 s) was more extensive, the larvae frequently turning through 360° with head and thorax moving to alternate sides of a line drawn down the mid-line of the abdomen. During this phase the entire abdomen of the predator was extremely sensitive to prey contact.

Prey discovery on plants.—The behaviour of larvae before and after finding an aphid secured by glue at two different locations on a bean plant was recorded in detail. In the first situation the aphid was secured on a leaflet, and in the second on a stipule at the junction of a leaf and the stem. The effect of prey discovery at these two locations was assessed either by comparing: (i), the distance moved on the leaf on which the capture was made with the mean distance moved per leaf prior to prey discovery, or (ii), the distance moved on the stem after prey discovery with the mean distance moved on the stem between leaf visits prior to the aphid discovery.

TABLE III. *The effect of prey capture on searching behaviour of five C. septempunctata larvae on plants* *

Mean distance moved per leaf before aphid capture (cm)	Distance moved on single leaf after aphid capture (cm)	Mean distance moved on stem between leaf visits before aphid capture (cm)	Distance moved on stem after aphid capture (cm)
42.1 (17–102)	388	16.75 (4–33)	86
53.1 (20–129)	275	11.25 (3–17)	73
50.5 (20–97)	302		

*In this table a leaf refers to a group of four leaflets. Figures in parentheses indicate the range of observed values in each category. Each set of data refers to one larva.

In both situations (Table III), the effect of prey discovery was to make the larva search the 'general area of discovery' more thoroughly, in the one case a leaf consisting of four leaflets and in the second a particular region of stem. Observations with very small plants (unpublished data) revealed that the entire plant is frequently re-searched, the actual leaf on which the capture was made often being vacated almost immediately. In one observation, the period of intensive search on the leaf of a large bean plant lasted for 12 min. The large distance moved by predators after prey discovery on a leaf was accounted for by repeated visits to the four leaflets comprising the leaf so that the intensive search was not confined to the immediate area of discovery, *viz.* the leaflet. Similarly, prey discovered on the stem induced an intensive searching of the stem both above and below the point of discovery. The extremely localised searching behaviour after prey capture described for the arena experiment was observed on plants only when captures were made on the surface of the leaf lamina.

Host plant recognition

Search times recorded for larvae on single bean plants (Fig. 2) showed that 64% of maximum search times recorded in a sequence of searches of the same plant were recorded on the first introduction of a larva to the plant. On only 36% of 178 recorded searches did the second or third introduction generate a maximum search time in a sequence and on no occasion, except after prey capture, was a fourth or subsequent

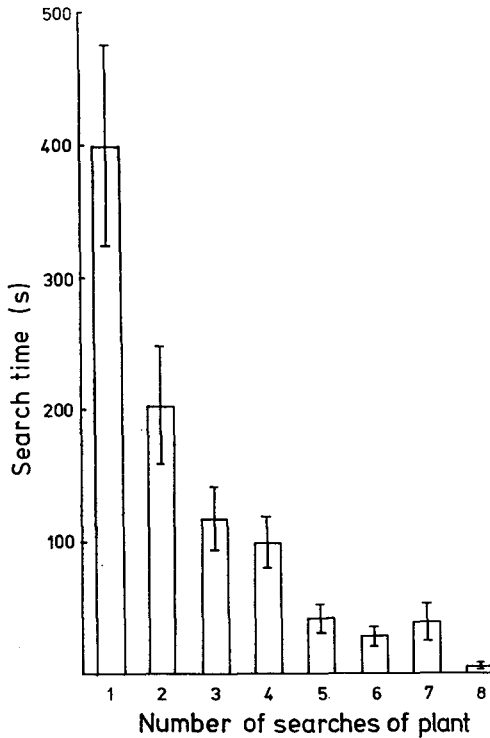


Fig. 2.—Search times (\pm s.e.) resulting from sequential introductions of *C. septempunctata* larvae to an uninfested bean plant. Data relate to nine larvae subjected to a total of 178 plant searches.

search the longest. There are two possible explanations for this, both involving a consideration of leaf visits, and neither mutually exclusive: (i), the first leaves encountered by a larva had not previously been searched and were not therefore recognised, (ii), since approximately 57% of leaves are visited 2–4 times in the course of searching a plant (Table I), recognition of a previous visit may not be firmly established until a given leaf has been visited several times. In no case did larvae reject plants previously visited by other larvae.

A further experiment was done to determine if plant recognition was due to a chemical marker left by individual predators during their searching activity on plants. In this experiment, search times were recorded for sequential introductions of a single larva to the same plant but between each series of sequential introductions plants were

wiped with acetone-soaked pads and dried quickly in a stream of fresh air. The larva was then immediately reintroduced to the base of the plant and sequential search times noted as before. On each occasion (Table IV) the predator re-searched the same plant

TABLE IV. *Sequential search times (s) by a single larva of C. septempunctata on an uninfested bean plant washed with acetone on five occasions*

Treatment with acetone	Sequence of introductions to plant after initial treatment with acetone*						
	1	2	3	4	5	6	7
1	<i>215</i>	5	15	19	40	5	15
2	<i>183</i>	15	5	10	5	0	0
3	<i>85</i>	40	5	4	5	0	12
4	<i>70</i>	5	35	22	12	5	0
5	<i>173</i>	15	98	15	5	13	4

*Maximum search times in italics.

thoroughly on the first introduction. Simultaneous reintroductions of larvae to a control plant that had been treated with acetone only before the first introductions produced a series of low search times or complete rejections.

Discussion

The aphidophagous larvae of *C. septempunctata* have no visual or olfactory perception of prey. However, these experiments suggest that larvae may mark plants chemically while searching, a procedure which enables subsequent short-term recognition of areas which have previously been searched unsuccessfully and which can therefore be avoided. Wasteful expenditure of energy (Banks, 1957; Dixon, 1959) is thus minimised. Dixon (1959) concluded from observations on wood and glass cylinders that the motivating influence on coccinellid behaviour is that larvae exhibit a negative geotaxis, as a result of which they move upwards on plants. The gradual decline of this geotactic response, influenced by hunger, eventually makes larvae move off plants. However, if this were so, then treatment of plants with acetone should not have affected a geotactic response.

Suggestions may be made as to the source of the marker and its method of detection. Coccinellid larvae frequently dab the surface on which they are moving with the anal-disc, an evagination of the rectum on the last abdominal segment, used for support when moving on plants (Gage, 1920). If larvae are held in petri dishes containing filter paper, the paper is soon marked by many yellow spots produced by anal-disc secretions. It is suggested that the anal-disc secretion may be used by larvae to mark plants with a discontinuous odour trail. When larvae are moving on plants, the maxillary palps, bearing fine setae distally, are always held close to or touching the substrate being searched, behaviour consistent with trail perception. Unfortunately, attempts to excise maxillae of larvae during these experiments were unsuccessful. Detection of marked plants by larvae was clearly specific since visits of a larva to a plant did not affect the searching activity of a second larva introduced to the same plant, a situation analogous to the detection by individual mammals of their own scent and implying slight chemical differences between the markers produced by individual larvae.

The entirely random movement ascribed to aphidophagous Coccinellidae (Banks, 1957; Bänsch, 1965; Murdie, 1969; Dixon, 1970) has been accepted largely as a result of work on artificial surfaces. However, the ability of larvae to sense previously visited areas on plants confers some system to their searching behaviour and to extrapolate the random movement observed on plain surfaces to the whole plant would not appear to be valid. The observations on plants have also shown that the effect of prey discovery is to produce an increased turning, often for considerable periods, in the 'general area

of discovery' rather than short-term behaviour in the 'immediate area of discovery', as observed in artificial arenas.

The behaviour of coccinellid larvae on plants has been described as inefficient (Banks, 1957; Dixon, 1970) since larvae occasionally recross their tracks. Murdie (1969) developed a model based on the assumption that the predator was a 'blundering idiot'. There is, however, adaptive value in this aspect of behaviour. Nymphs of *Acyrtosiphon pisum* or *Aphis fabae*, for example, may be sufficiently visually or physically disturbed by a predator to withdraw their stylets and move from the area in which they were feeding. Frequently these individuals are successfully captured by larvae traversing their original search path a second or a third time.

Murdie (1969) has suggested that an attacker specifically tied to one prey or host would be expected to have evolved, or be in the process of evolving, its own efficient search for its food source. Chandler (1969) found evidence of gustatory stimulation of the syrphid larva *S. balteatus* prior to contact with an aphid and a modification of its subsequent movement through an area. In this slow-moving predator there would be adaptive value in this. Similarly, with coccinellid larvae any mechanism which makes the location of prey more efficient in the absence of visual or olfactory clues, particularly when prey are present at low densities, must be of considerable adaptive value.

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