Res. Popul. Ecol. (1976) 18, 123–134.

ANALYSIS OF THE AGGREGATION BEHAVIOR IN THE LARVAE OF *HARMONIA AXYRIDIS* PALLAS (COLEOPTERA: COCCINELLIDAE) TO PREY COLONY

Akira KAWAI[1]

Entomological Laboratory, College of Agriculture, Kyoto University, Kyoto 606, Japan

INTRODUCTION

In natural predator-prey systems, prey are seldom distributed uniformly and often congregate in restricted space, forming a colony to which predators aggregate and feed on them. The pattern of spatial distribution of predator individuals is greatly influenced by that of prey individuals. The response of predators to the change of prey density is recognized as the functional and numerical responses by SOLOMON (1949, 1964). Change in the number of prey eaten by an individual predator is interpreted as the functional response, and change in the number of predators through the change in survival, reproduction and immigration of predators is interpreted as the numerical response. HASSELL (1966) stated the change of percentage mortality in prey in one predator generation at the different prey densities is caused through two kinds of behavioral responses: an individual response and an aggregative response. HOLLING (1959, 1961) showed that the functional response curve is influenced by the searching and handling behavior of predator. Change in the number of predators to a prey colony is also reinforced not only by the attraction through stimuli emanated from prey colony but also by the searching and handling behaviors of predators.

Alate aphids settled on a plant reproduce parthenogenetically and form a colony with their progeny. Therefore, aphids are distributed in colonies of various sizes on their host plants in the field. Most coccinellids are well-known predators of aphids and feed on them voraciously when they are plenty. BANKS (1956) stated that old larvae of *Adalia bipunctata* and other coccinellids tended to concentrate on stems infested more intensively by *Aphis fabae*. However, from the observation on searching behavior of coccinellids, most of their larvae do not perceive until they have physical contact with them (FLESCHNER 1950; BANKS 1954, 1957; PUTMAN 1955; DIXON 1959; KADDOU 1960). Concentration of coccinellid larvae to aphid colony is not necessarily due to the attraction by prey colony.

In the present paper the author examined experimentally the searching behavior of *Harmonia axyridis* larvae to the colony of *Rhopalosiphum padi* and analyzed the processes of their aggregation to prey colony. It is concluded from the result that the

[1] Present address: Entomological Laboratory, Kyushu National Agricultural Experiment Station, Chikugo, Fukuoka 833, Japan.

larval movement in searching prey is at random, but that the expense of time to eat prey (in the 2nd and 3rd instars) and the change of searching movement for prey (in the 3rd and 4th instars) contribute the larval concentration to prey colony as a trapping effect for predators to prey colony.

Materials and Methods

Materials used in this study were *Harmonia axyridis* as predator, *Rhopalosiphum padi* as prey and yellow dent corn, *Zea mays* as host plant of the prey. A number of *Harmonia axyridis* eggs were collected from cherry trees in Kyoto City in May 1973 and reared under laboratory conditions at 25°C and 16L with a sufficient number of *R. padi*. The predators used in each experiment were those within 24 hours after larval moulting, and they were deprived of food for about 24 hours prior to the experiments.

A population of *Rhopalosiphum padi* was collected in corn field at Tanba Cho, Kyoto Pref. in May 1973. The 3rd instar larvae were mainly used in the experiments, but occasionally the 2nd and 4th instar larvae were mixed.

Seedlings of yellow dent corn with 6 leaves (about 40cm in height) grown in pots (15cm in diameter) were prepared for the experiments.

Aphids were inoculated on a leaf as a colony with a leaf cage (Fig. 1), which

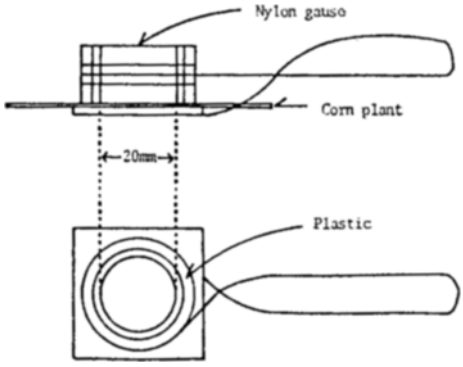

Fig. 1. A leaf cage used for inoculating aphids.

was removed several hours later. There was only one colony on an "infested" plant and the colony size represents the density per plant in this study. Each experiment was conducted at 25°C and 50-60% R. H. under continuous illumination of fluorescent light.

Experiment 1: Time spent in handling a prey and number of prey consumed

One hundred aphids were inoculated in a colony on a leaf of an individual potted corn plant. A white paper was placed on the bottom of the plant and tangle foot was put on the edge of the paper. After 24 hours (16L8D), the number of aphids on the

plant and paper was counted. When the predator captured a prey for the first time, the time consumed for eating was also measured.

Experiment 2 : Aggregation to prey colony

Six corn plants were arranged in a box as shown in Fig. 2-a. The number of plants on which aphids were released was different in three experiments:

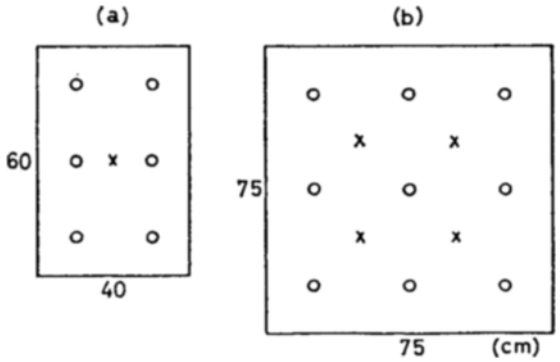

Fig. 2. The box used for the experiments.

(a) The box used for Expt. 2.
Four to six predator individuals were released at the center.
(b) Thebox used for Expt. 3.
Two predator individuals were released at the four points.
o : Corn plant
X : Point of releasing predators

a) no aphids were inoculated on the 6 plants
b) 100 aphids were inoculated on each of the 6 plants
c) 100 aphids were inoculated on 3 of the 6 plants

From 4 to 6 predators of the same instar were released one after another in the middle of the box. The predators were observed every 30 minutes for 6 hours to record their number on each plant. They were prevented to escape from the box by tangle-foot placed on the edge around the box.

Experiment 3 : Response to the size of prey colony

Nine corn plants were planted in a box as shown in Fig. 2-b, and 1, 10 and 100 aphids were inoculated on 3 of the 9 plants respectively in a Latin square method. Tangle-foot was used in the same manner as Experiment 2. Eight individuals of each instar of the predator were released from the points described in Fig. 2-b.

Results

Experiment 1 : Time spent in handling a prey and number of prey consumed

Table 1 shows the time spent handling a prey by a hungry *Harmonia axyridis* larvae when they first captured a prey. The time spent handling a prey was defined

Table 1. The time spent by a hungry *Harmonia axyridis* larva in handling a single prey.

Instar	No. of larvae observed	Time (min.)		
		Range	Mean (t)	S. D.
1st	6	137-321	237. 2	76. 55
2nd	9	59-132	88. 2	22. 67
3rd	12	11- 71	34. 3	16. 19
4th	12	5- 15	8. 9	2. 91

Table 2. The number of prey consumed by a hungry *Harmonia axyridis* larva during 24 hours.

Instar	No. of larvae observed	No. of prey consumed		
		Ramge	Mean	S. D.
1st	8	0- 3	1. 87	0. 89
2nd	8	7-13	10. 12	2. 29
3rd	8	12-32	21. 50	7. 34
4th	8	23-52	42. 00	9. 53

as the length of time from touching a prey to the release of it or to the entire consumption of it. The younger the larvae, the longer time was required by them. Table 2 shows the number of prey consumed daily by each instar larva of the predator. The older the larvae, the more aphids they consumed.

Experiment 2 : Aggregation to prey colony

The distribution of predators on the plants with various prey densities was shown in Fig. 3. Changes in proportions of the predator observed on the plants with prey, on those without prey and on the ground to the total predators were shown with 30 minutes' intervals.

When no aphid was inoculated (Expt. 2-a, Fig. 3-a), the proportion of the 1st instar larvae of predator found on plants rapidly increased with time. Once the larvae climbed up the plants after searching on the ground, they usually remained on them, and all the larvae had been staying on the plants after 2. 5 hours. However, the 2nd to 4th instar larvae sometimes left the plant once again. Movements of older larvae between plants and ground were observed more frequently than that of younger ones, but about 50% of the larvae of each instar were found on plants throughout the observation.

When aphids were inoculated on all of the 6 plants (Expt. 2-b, Fig. 3-b), the 1st instar larvae remained on the plants on which they once located and all the larvae had been staying on plants in 2 hours as in the previous experiment. With the 2nd and 3rd instars, the proportion of the larvae found on the plants increased rapidly at

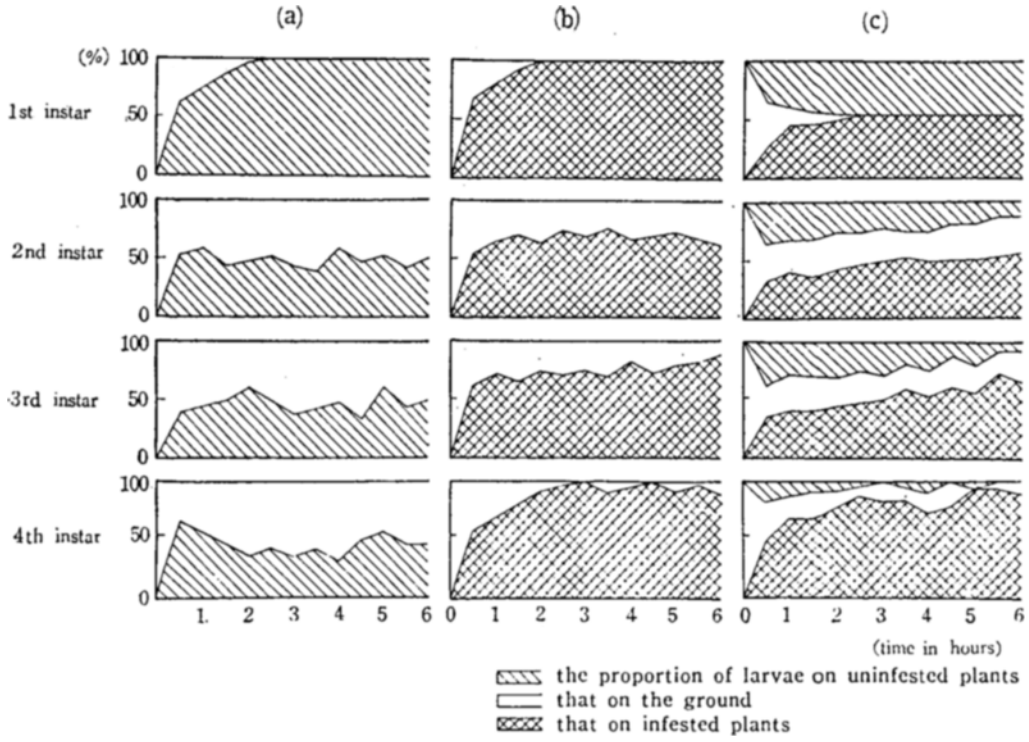

Fig. 3. Aggregation of predaters to prey colony.
(a) No aphids were inoculated on the 6 plants. (Expt. 2-a)
(b) 100 aphids were inoculated on each of the 6 plants. (Expt. 2-b)
(c) 100 aphids were inoculated on 3 of the 6 plants. (Expt. 2-c)

first, and was then kept around 70%, though movements between plants and ground were frequently observed. The proportion of the 4th instar larvae on plants increased rapidly at first, and then all the larvae moved on plants after 3 hours. However, unlike the lst instar, movements between plants and ground were frequent and, consequently, less than 10% of the larvae were found on the ground.

When aphids were inoculated on only 3 plants (Expt. 2-c, Fig. 3-c), the results of the lst instar larvae were similar to those in the previous two experiments. The proportion of the larvae on infested plants was nearly equal to that on uninfested plants. With the 2nd and 3rd instars, the proportion of the larvae on infested plants was almost equal to that on uninfested plants during the first 30 minutes, but the former gradually increased while the latter decreased. However, the proportion of the larvae on the ground remained constant at about 30%, although there were frequent movements between plants and ground. With the 4th instar, the proportion of the larvae on infested plants increased rapidly than that with the 3rd instar.

The rates of emigration of predators from infested plants (a), and those from uninfested plants (n) per unit time (30 min.) were calculated with the results of

Experiment 2-c. It is regarded that the decrease in the number of predators on each plant during the 30 minutes period equals the number of emigrants. When the number increased or showed no change, it was assumed that no emigration occurred. When the emigration and immigration occurred simultaniously, the rate of emigration should have been estimated to be sumewhot smaller than actually occurred. The number of larvae on each plant and that of emigrants observed in every 30 minutes were totaled, and the ratio of the number of emigrants to the total number was defined as the rate of emigration (a, n in Table 3). The lst instar larvae rarely emigrated from plants which they once located as stated before. With the 2nd to 4th

Table 3. The time spent for predation and the rate of emigration during the unit time (30 min.) in Expt. 2-c. (see text)

Instar	No. of prey consumed per 30 min. (N)	Total time spent for predation (min.) (T)	Actual searching time on infested plants (min.) ($30-T$)	Rate of emigration (%)		
				from uninfested plants (n)	from infested plants (a)	estimated from the searching time (a')
1st	0.05	11.9	18.1	0.34	0.87	0.21
2nd	0.27	22.3	7.7	10.88	3.54	2.20
3rd	0.58	19.9	10.1	20.25	2.71	7.95
4th	1.13	10.1	19.9	28.63	2.26	19.05

instars, the rates of their emigration from infested plants were kept at almost constant level as low as 2-3%. However, the rate of emigration from uninfested plants was greater than those with infested ones.

In order to eximine if the difference between (a) and (n) is due to the difference in time spent handling prey, the mean number of prey consumed by individual predator per unit time (30 min.) was estimated from the number of prey consumed by individual predator during 24 hours. BANKS (1957) found that the activity of the larvae of *Propylea quatuordecempunctata* in the dark was about one third of that in the light. A correction was applied to the present analysis: the estimated number of prey consumed per 30 minutes in the light (N) was determined as follows:

$N=$(the number consumed per 24 hours)$\times 1/(16+8\times 1/3)\times 30/60$ (See Table 3)

A total time spent for predation (T) was estimated from the time spent consuming consuming a single prey (t) which is shown in Table 1:

$$T=N\times t$$

The total time spent for predation was estimated as over 60% of total time in the 2nd and 3rd instars. However, that was only obout one-third in the lst and 4th instars, because the number of prey consumed by the lst instar was very small and the time spent consuming a prey by the 4th instar was very short.

When the actual searching time on uninfested plants is supposed to be 30 minutes, that on infested plants is reduced to $(30-T)$ minutes by the expense of time for

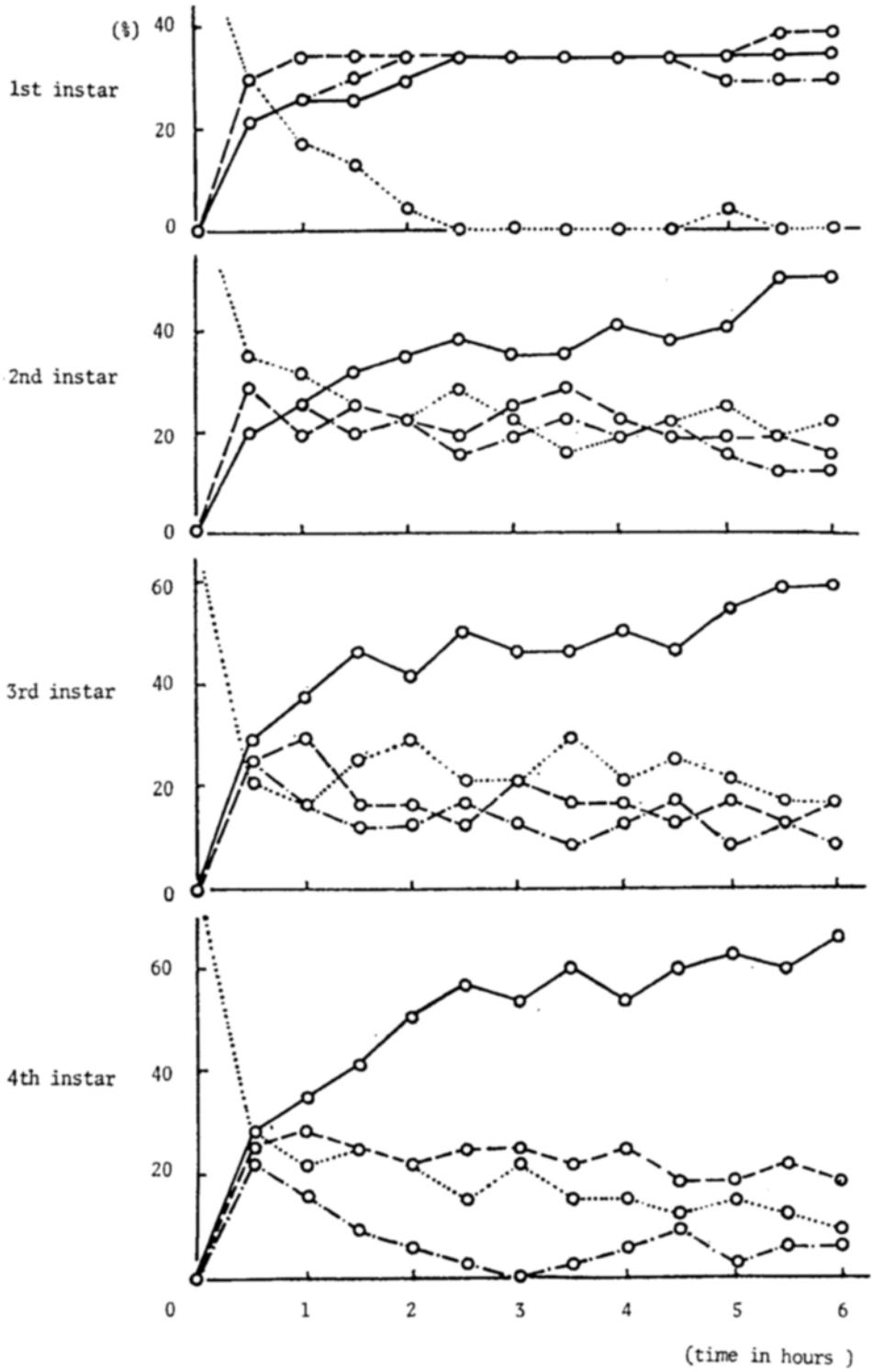

Fig. 4. Aggregation of predators to prey colonies of different sizes. ○——○ : the proportion of larvae on plants with 100 prey, ○---○ : that on plants with 10 prey, ○-·-·-○: that on plants with 1 prey, ○····○: that on the ground.

predation. If the rate of emigration is proportional to the searching time, the rate of emigration on infested plants is estimated as follows:

$$a' = n \times (30 - T)/30$$

As shown in Table 3, the difference between the rate of emigration from uninfested plants and that from infested ones in the 2nd instar is explained by the reduction of the actual searching time due to the increase of the handling time. However, in the 3rd and 4th instars, the reduction of the rate of emigration from infested plants is not explained by this mechanism.

Experiment 3 : Response to the size of prey colony

Fig. 4 shows the changes in the proportion of predator larvae observed on the plants with each of the three different densities (1, 10 and 100 individuals per plant) of prey and on the ground. Because the lst instar larvae tended to stay on the plants on which they once located, all of them were on the plants after 2.5 hours. They were distributed almost evenly on the plants with each of the three different densities. With the 2nd and 3rd instars, the proportion of the larvae on the plants with 100 prey gradually increased. On the other hand, that on the plants with 10 or 1 prey gradually decreased, but there was no clear difference between these two proportions. With the 4th instar, the proportion of the larvae on the plants with 100 prey rapidly increased, but that on the plants with 10 or 1 prey decreased. However, in this case the proportion on the plants with 1 prey decreased more rapidly. The proportion of the larvae on the ground gradually decreased.

The rate of emigration from plants with the different number of prey during the unit time (30 min.) was calculated in the same manner as Experimnt 2 (Table 4). The rate of emigration by the lst instar was almost nil. In the 2nd and 3rd instars the rate of emigration decreased as prey densities increased. However, in the 4th instar the difference between the rates of emigration from plants with 1 prey and with 10 prey was greater than that between the rates with 10 prey and with 100 prey.

Table 4. The relationship between the prey density per plant and the rate of emigration (%) (Expt. 3).

Instar	Prey densisy per plant		
	100	10	1
1st	0.63	1.21	0.92
2nd	3.62	7.02	9.26
3rd	2.96	12.63	20.16
4th	3.36	5.73	21.63

Discussion

In this study, the processes of larval concentration in *Harmonia axyridis* to the colony of their prey, *Rhopalosiphum padi*, was experimentally analyzed. The result

showed that larvae of all the instars except the lst tend to concentrate on prey colony. This coincided with the field observation by BANKS (1956) that older larvae of *Adalia bipunctata* and other coccinellids tended to occur more often on stems where aphids were numerous. This process has been considered to be the numerical response of predators through their immigration (SOLOMON 1964).

The author considered four possible mechanisms of the aggregation of predators to the area where prey density is high:

A) similarity in tactic response between predators and their prey

B) attraction for predators to their prey

C) mutual attraction among individual predators

D) trapping effect for predators to prey colony

In (A), predator and prey have similar requirement for environmental conditions and are, thereby, distributed in the same area. DIXON (1959) found that *Adalia decempunctata* and its prey, *Microlophium evansi*, concentrate on the apex of a plant because of their positive phototaxis and negative geotaxis. The searching by *Propylea quatuordecempunctata* and the distribution of its prey, *Aphis fabae*, (BANKS 1957) and the searching by *A. decempunctata* and the distribution of its prey, *M. evansi* (DIXON 1959) were concentrated heavily along veins of leaves.

Harmonia axyridis has a distinct positive phototaxis and a negative geotaxis and these act effectively on the process in climbing up a plant. Besides, the predator tends to search along the vein and edge of leaves, but *Rhopalosiphum padi* mainly reproduces in lower parts of a plant and is not distributed along the vein and edge of leaves. Therefore, the concentration by the mechanism (A) is not applicable to this case.

(B) is a mechanism in which predator gains information on its prey from a distance by the sense organs and is attracted to the prey. The larvae of most predatory coccinellids do not seem to perceive the prey until they make physical contact with prey (FLESCHNER 1950; BANKS 1954, 1957; PUTMAN 1955; DIXON 1959; KADDOU 1960). The present study shows that the larvae of *Harmonia axyridis* search for the prey at random and the selection of the plant they climbed up is simply by chanse. The 4th instar larvae apparently seemed to prefer the infested plants from the beginning as shown in Fig. 3-c. But in this case the individual predators had already moved from uninfested plants to infested ones during the first 30 minutes. The selection of plants by the larvae in the first time was at random, and thus there was no preference to prey colony.

(C) is the case in which predators attract each other by mutual communication. The concentration in this case may occur, for example, through sounds, pheromones, biological conditioning and so on. In the present observation, however, the larvae of *Harmonia axyridis* did not perceive the other individuals of the same species until making a physical contact with them. In addition, when the individuals of this species

happen to meet with each other, they repel each other. Therefore, it is unlikely that the concentration was because of the attraction between predators themselves.

The author considers (D) as the mechanism which is responsible for the concentration of *Harmonia axyridis* larvae. This means that in spite of random searching predators remain in areas where prey density is high, and thus they concentrate there.

Though the 2nd to 4th instar larvae of *Harmonia axyridis* searched for the prey at random, the rate of emigration from infested plants was smaller than those from uninfested ones (Table 3). In the 2nd instar the difference between these two rates is explained by the reduction of the actual searching time due to the increase of the handling time, but in the 3rd and 4th instars, it is not explained by this mechanism alone. The 4th instar larvae of *Propylea quatuordecempunctata* (BANKS 1957) and *Hippodamia quinquesignata* (KADDOU 1960) change their searching behavior before and after feeding a prey. They searched precisely where they once ate a prey with short turnings of movement after feeding. This increases predator's chance to encounter aphids in a colony. Observation showed that the 4th instar larvae of *Harmonia axyridis* also exhibited this behavior. In the 4th instar this behavioral change seems to be more important in decreasing the rate of emigration rather than searching time, because the time consuming a single prey was very short and the speed of moving is very rapid. The 3rd instar larvae also exhibited this behavior a little but it was not clear in the lst and 2nd instars.

In the 2nd and 3rd instars, the rate of emigration from plants with the different number of prey decreased as prey densities increased (Table 4). This decrease is also explained mainly by the reduction of actual searching time due to the handling time. However, in the 4th instar the difference between the rates of emigration from plants with 1 prey and with 10 prey was greater than that between the rates with 10 prey and with 100 prey (Table 4). This was because predators easily found another prey on plants with 10 or 100 prey by short turnings but on plants with 1 prey they could not find another prey and they resume long turnings.

The 2nd and 3rd instar larvae tended to concentrate on prey colony for some time but this tendency was gradually decreased. This is because aphids in colony dispersed by dropping and walking to escape from the attack by the predators.

In conclusion, the larvae of *Harmonia axyridis* except the lst instar search at random, and after encountering a prey colony, they remain there because of trapping effect. However, because the trapping effect is not perfect, the predators often emigrate, leaving some individuals of prey in a colony uneaten. This behavior of the predator is very effective in increasing the possibility of its own survival without eliminating the prey colony when prey density is low, but such a predator does not utilize the prey effectively when prey density is high.

Summary

The searching and handling behaviors of *Harmonia axyridis* larvae to the colony of *Rhopalosiphum padi* were experimentally examined and the processes of their aggregation to the prey colony was analyzed.

All the instar larvae searched for the prey at random and they have no preference to the prey colony, but except the lst instar they tend to aggregate to the plants with prey colonies. The lst instar larvae tend to stay on the plants they once located. The 2nd to 4th instar larvae often emigrate from the plants without prey colony but seldom emigrate from the plants with prey colonies, and consequently, they aggregate to the plants with prey colonies. The expense of time to eat prey (in the 2nd and 3rd instars) and the change of searching behavior for the prey after feeding (in the 3rd and 4th instars) are responsible for the larval concentration to prey colony as a trapping effect for predators to prey colony.

Acknowledgement: The author wishes to thank Dr. F. Takahashi for his invaluable advices and critical reading of the manuscript. Thanks are also due to Dr. A. Takafuji for his critical reading of the manuscript. The author also wishes to thank Prof. S. Utida for his invaluable advices in the course of this work.

References

Banks, C. J. (1954) The searching behaviour of coccinellid larvae. *Brit. J. Anim. Behav.* 2: 37-38.

Banks, C. J. (1956) The distribution of coccinellid egg batches and larvae in relation to numbers of *Aphis fabae Scop.* on *Vicia faba*. *Bull. Ent. Res.* 47: 47-56.

Banks, C. J. (1957) The behaviour of individual coccinellid larvae on plants. *Brit. J. Anim. Behav.* 5: 12-24.

Dixon, A. F. G. (1959) An experimental study of the searching behaviour of the predatory coccinellid beetle *Adalia decempunctata* (L.) *J. Anim. Ecol.* 28: 259-281.

Fleschner, C. A. (1950) Studies on searching capacity of three predators of the citrus red mite. *Hirgardia* 20: 233-265.

Hassell, M. P. (1966) Evaluation of parasite or predator responses. *J. Anim. Ecol.* 35: 65-75.

Holling, C. S. (1959) Some characteristics of simple types of predation and parasitism. *Can. Ent.* 91: 385-398.

Holling, C. S. (1961) Principles of insect predation. *Ann. Rev. Ent.* 6: 163-182.

Kaddou, I. K. (1960) The feeding behaviour of *Hippodamia quinquesignatata* (Kirby) larvae. *Univ. Calif. Publ. Entomol.* 16: 181-232.

Putman, W. L. (1955) Bionomics of *Stethorus punctillum* Weise (Coleoptera : Coccinellidae) in Ontario. *Can. Ent.* 87: 9-33.

Solomon, M. E. (1949) The natural control of animal populations. *J. Anim. Ecol.* 18: 1-35.

Solomon, M. E. (1964) Analysis of processes involved in the natural control of insects. *Adv. Ecol. Res.* 2: 1-58.

ナミテントウ幼虫の餌コロニーへの集中過程の解析

河合　章

ナミテントウ幼虫の餌であるムギクビレアブラムシのコロニーへの集中，捕食行動を実験的に観察し，餌コロニーへの集中過程を解析した。

ナミテントウ幼虫の餌探索は各令ともランダムであり，餌コロニーへの選好性はないにもかかわらず，1令幼虫を除き，餌コロニーのある株への集中が見られ，令が進むほど集中は顕著であった。この集中は，餌の捕食に要する時間により実際の探索に費される時間が短縮されること（主に2・3令）や，捕食後の探索行動の変化によって摂食した付近を細かく探索すること（主に3・4令）により，餌コロニー付近に留まることが多くなる結果である。

このような，各個体の探索行動がランダムであっても餌の処理に伴なう時間の消費やその後の行動の変化の結果として捕食者が餌密度の高い場所に集中するという機構は，コロニーを形成する餌を捕食する本種のような捕食者にとって適応的であると考えられる。