

on each *D. racemosum* tree (mean number of galls per leaf=1.2; KIBAYASHI and ITÔ, unpublished), *N. yanonis* galls provide enough foods for the first generation larvae of *Nola*.

The fact that *Nola innocua* can complete its annual life cycle depending on galls of *N. yanonis* in spring, *N. distyliicola* in summer and *M. globuli* and *Metanipponaphis* sp. A in autumn indicates that this species has evolved a unique life history strategy as a kleptoparasite of gall aphids [at least two other gall aphids, *Sinonipponaphis monzeni* (TAKAHASHI) and *Metanipponaphis* sp. B, causing galls on *D. racemosum* trees, are also attacked by *Nola*; HATTORI and

ITÔ, unpublished]. Analysis of the evolution of the kleptoparasitic life history of this species is an interesting subject for future studies.

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Aphid Body Fluid Stimulates Feeding  
 of a Predatory Ladybeetle,  
*Coccinella septempunctata* L.  
 (Coleoptera: Coccinellidae)<sup>1</sup>

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The sequence of predatory behavior of a ladybeetle, *Coccinella septempunctata* L., consists of searching, capture, consumption and grooming (NAKAMUTA, 1983), and visual perception in the close proximity of the prey plays an important role in eliciting the prey capture (NAKAMUTA, 1984).

Unfed first instar larvae of *Prophylea quatuordecempunctata* examined aphid exuviae but did not eat them (BANKS, 1957). This phenomenon suggests the presence of another cue for prey recognition of the ladybeetle.

**Methods:** The ladybeetle, *C. septempunctata*, and the green peach aphid, *Myzus persicae* SULZER, were reared in the laboratory with the method described by NAKAMUTA (1983). The ladybeetles were deprived of food for 24 hr before the experiments. All experiments were conducted at  $25 \pm 2^\circ\text{C}$  from 10:00 to 17:00 during which time the ladybeetle was active.

Apterous adults of the green peach aphid were

desiccated with a vacuum pump for 48 hr ("desiccated aphid"). The desiccated aphids were soaked in distilled water for 30 min ("humidified aphid"). A live, desiccated or humidified *M. persicae* was fixed at the center of a filter paper (11 cm in diameter) with adhesive (G-17, KONISHI Co. Ltd., Osaka, Japan). A *C. septempunctata* adult was released onto the filter paper and the paper was covered with a petri dish (9 cm in diameter, 1.5 cm in height). Subsequent behavior was recorded and classified as contact, capture and consumption. Capture means that the ladybeetle captured but promptly left the aphid.

A droplet of body fluid was pressed out of an adult *M. persicae* with forceps. At the center of the filter paper, this droplet or a drop of distilled water was spotted. A ladybeetle was released onto the paper and the duration of the attempt to feed on the area of paper with the droplet by the adult coccinellid was recorded.

A droplet of either aphid body fluid or distilled water was dropped on the upper side of an agar block (ca.  $2 \times 2 \times 2$  mm, agar powder: water=1:40 W/W) and the block was set at the center of the filter paper. An agar block without fluid was used as a control. A ladybeetle was released onto the filter paper and the paper was covered with a petri dish (9 cm in diameter, 1.5 cm in height). When the ladybeetle reached the objective, the behavior on such "aphid-agar" dummy was recorded. If the ladybeetle did not reach the point within 5 min, observation was terminated.

**Results:** On touching the prey, 100% (72/72)

<sup>1</sup> *Appl. Ent. Zool.* **19** (1): 123-125 (1984)

Table 1. Feeding response of *Coccinella septempunctata* to an "aphid-agar" dummy

	Frequency				No. of ladybeetles
	Pass	Capture	Drink	Consumption	
Agar + body fluid of <i>M. persicae</i>	6 (10%)	16 (27%)		38 (63%)	60
Agar + distilled water	15 (37%)	6 (15%)	20 (48%)		41
Agar	61 (100%)				61

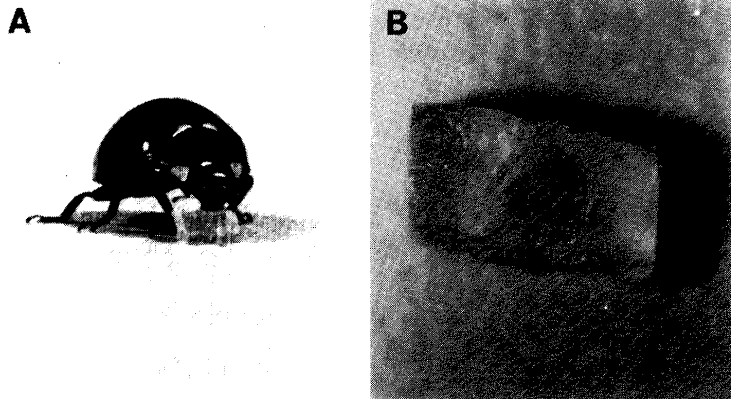


Fig. 1. A: the adult of *Coccinella septempunctata* which is eating an "aphid-agar" dummy. B: vestiges of *Coccinella septempunctata* biting the surface of a dummy.

of the ladybeetles captured a live aphid and 94% (68/72) consumed it. Although 78% (61/78) of the ladybeetles captured the desiccated aphid, only one consumed it. Ninety percent (60/67) of the ladybeetles captured the humidified aphid and 58% (39/67) consumed it.

When the ladybeetle came to the location of the aphid body fluid, it remained there, contacted the spot with its mouthparts and tried to bite it; duration of these biting attempts lasted from 1 to 258 sec ( $\bar{X} \pm S.D. = 25 \pm 61$  sec,  $n=20$ ). However, the ladybeetle passed over the spot of the distilled water or control paper without response ( $n=20$ ).

Sixty-three percent of the ladybeetles used in the experiment consumed the aphid-agar dummy, but none consumed the control agar dummy (Table 1.). The ladybeetle held the aphid-agar dummy with its forelegs and bit it with its mandibles (Fig. 1A), leaving vestiges of the biting on the surface of the agar block (Fig. 1B). About half of the ladybeetles drank the distilled water spotted on the upper side of the agar block, but no vestiges of biting could be found. These results suggest that the body fluid is the final cue for the ladybeetle to

recognize the aphid as a potential prey.

Chemical component of the host plays an important role in host recognition by many insects (VINSON, 1976; DETHIER, 1982). It was shown that, although involvement of chemical component of the prey in prey recognition has not been proved in predatory insects, at least some predatory coccinellids use prey body fluid as a cue for recognition. Which chemical component of the body fluid stimulates the feeding is the subject of further study but, as *C. septempunctata* feeds on a large number of aphid species (HODEK, 1973), a substance common to many aphid species might play this role.

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### Early Development of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoi- didae) Population in the Inoculated Branches of Pine Seedlings<sup>1</sup>

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Pine wilt is caused by the pine wood nematode, *Bursaphelenchus xylophilus*, and the dispersal forth-stage larvae of the nematode are transmitted to pine trees by the Japanese pine sawyer, *Monochamus alternatus*. When emerged adult beetles feed on the branches for maturation, the dispersal larvae leave the bodies of the beetles and invade trees through their scars. It has been assumed that the dispersal forth-stage larvae molt after the branch invasion.

In the present study, observations were conducted in detail on molting and early distribution of the pine wood nematode in the inoculated branches of pine trees.

Five-year-old seedlings of *Pinus thunbergii* growing in a nursery were used. The dispersal forth-stage larvae of the pine wood nematode were collected from the emerged beetles.

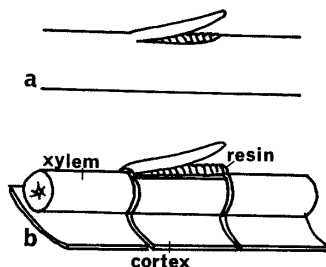


Fig. 1. Inoculation site (a) and division of inoculated branch for nematode examination (b).

<sup>1</sup> *Appl. Ent. Zool.* **19** (1): 125–129 (1984)

Inoculation was done to two- or three-year-old branches through a wound made by peeling the bark and cortex 1 cm and indenting the xylem with a small saw counterfeiting the feeding scar of the beetle (Fig. 1-a). In the first experiment, 0.1 ml of a nematode suspension containing 600 larvae was inoculated onto the wound on June 17, 1983 and in the second experiment, 1,000 larvae on June 28. The inoculation site was covered with parafilm. At each examination, an inoculated branch was cut off and divided into 2 cm long pieces.

Each piece was divided into cortex and xylem (Fig. 1-b). The peeled bark and thin resinous surface layer of the wound, the cortex and the xylem of the inoculation site were examined separately.

In the second experiment, the cultured nematodes were inoculated onto similar branches in the same way for comparison with the dispersal forth-stage larvae.

Nematode extraction was done by dipping chopped pieces in water in Syracuse dishes for five hours.

In the first experiment, the dispersal forth-stage larvae didn't molt one day after inoculation (Table 1). Most of the larvae were trapped in the oleoresin exudated on the wound, and their distribution was restricted in the inoculation site. Three days after inoculation, most of the larvae developed into adults. From the forth day, the adults began to pass through the cortex and dispersed among 4 cm of the branch. One of the females had an egg in the uterus and a few second-stage larvae were observed. Most of the adults had stayed at the surface of wound for two weeks and some of them oviposited. Two weeks after inoculation, nematodes were found in a current shoot, but not in the rest of the examined branch.

In the second experiment, a lot of the dispersal forth-stage larvae were molting in the inoculation site and their distribution was almost restricted there two days after inoculation (Table 2).

Some of the larvae developed into adults and