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Feeding Preferences of Adults and Larvae of *Epilachna admirabilis* (Coleoptera: Coccinellidae) to Their Host Plant at Different Leaf Ages

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Abstract. This study investigated the feeding preferences of adults and larvae of *Epilachna admirabilis* to young and old leaves of *Trichosanthes kirilowii* var. *japonica*. Both adults and larvae preferred the young leaves to the old. Penetrometer showed that old leaves were about twice as hard as young leaves. Old leaves were scarcely fed on by both adults and larvae within short test periods but were well accepted by them during 24hr. In rearing tests that provided young and old leaves for larvae as food, there was no obvious difference in growth performance. The no-choice assay with methanol extracts of young and old leaves showed that both adults and larvae were stimulated to feed by young and old leaf extracts. The choice assay revealed that larvae much preferred the extract of old leaves to that of young leaves compared with adults. These results indicate that the chemical feeding stimulant and physical toughness in old leaves are related to the difference in feeding preferences between adults and larvae.

Key words: *Epilachna admirabilis*, feeding stimulant, leaf toughness.

Introduction

Phytophagous insects are known to use various plant features such as chemical or physical property when selecting host plants. Among those features, chemical features are considered to play an important role for herbivorous insects in deciding the host plant range (Fraenkel, 1959; Beck, 1965; Hedin *et al.*, 1977). Physical property in plants, such as hair on plant surface and hardness of plants, is also related to host preference of phytophagous insects. For example, a larva, especially in an early instar stage, usually feeds on young and soft leaves because the size and strength of their mandibles are less sufficient to eat old and tough leaves (Patanakamjorn & Pathak, 1967; Bernays & Chapman, 1970a, b; Cuthbert & Davis, 1972; Coley, 1983; Raupp, 1985; Godfrey *et al.*, 1989).

Epilachna admirabilis Crotch is a phytophagous lady beetle species which feeds mainly on *Tricosanthes kirilowii* var. *japonica* leaves. We observed the feeding behavior of *E. admirabilis* in fields, and found that the feeding behavior was different between adults and larvae. Adults feed almost solely on young and soft leaves, while larvae eat not only young leaves but also old and tough leaves. Although food plant preference changes during larval development or among the reproductive status of adults in several herbivorous insects (Browne, 1995), it has never been reported that larvae feed more on old and tough leaves than adults do. Confirming this feeding behavior will contribute to an understanding of insect-host plant relationships. Thus, this study aims to detect causative factors responsible for the difference in feeding preferences between adults and larvae of *E. admirabilis*.

Materials and Methods

Insects and plants

E. admirabilis was collected at an experimental field of the Faculty of Agriculture, Tohoku University. The beetles were reared at 24±1℃, 16L–8D photoregime and provided *T. kirilowii* var. *japonica* leaves as food. Plants were cultivated at the experimental field of Faculty of Agriculture, Tohoku University. Adults of *E. admirabilis*, 7–14 days old, and last instar larvae of *E. admirabilis*, 2 days old after 3rd eclosion, were used for feeding tests (mixed geuder). Adults were previously starved for 24 hr before feeding tests.

A young leaf was defined as a soft and yellow green colored leaf within about the 10th leaf from the shoot

apex. An old leaf was defined as a tough and dark green leaf lower than about the 10th leaf from the shoot apex. A vague leaf that could not be distinguished as young or old was not used for assays.

Measurement of leaf toughness

To clarify the definition between young and old leaves, the toughness of leaves was measured using a penetrometer as described by Williams (1954) and Tanton (1962). The hardness was shown by the weight by which a pin just penetrated the leaves.

Rearing test

To investigate whether 1st instar larvae can feed on old leaves and grow to adults, rearing test was conducted. A filter paper (Toyo, 1, 7 cm d.) was placed in the bottom of a petri dish (6 cm d.) and moistened with distilled water to provide humidity. Young or old leaves were then placed into the petri dish, and ten first instar larvae that had emerged within 24 hr were released in it. Leaves were renewed and suitable water to provide humidity was added every day. Each test was replicated three times.

Feeding preference to fresh leaves

To clarify difference of feeding preference between young and old leaves, feeding test was conducted as follows: A filter paper (Toyo, 1, 7 cm d.) was previously placed in the petri dish (6 cm d.) and distilled water was added (0.3 m*l*) to provide humidity. Young or old leaves of *T. kirilowii* var. *japonica* were punched out in circle of 2 cm by a cork borer. Two disks were weighed and put on the bottom of the petri dish. Three adults or three last instar larvae were released in the petri dish and allowed to feed for 2 hr under a light condition. After the test was finished, the disks were removed and reweighed.

Furthermore, to estimate the feeding acceptance of old leaves, a feeding test using old leaves was con ducted at different test duration by the same manner mentioned above. The number of test leaf disks was three if the test duration was 12 hr and four if the test duration was 24 hr. A test for 24 hr was conducted under 16L–8D condition.

Feeding preference to plant extracts

To clarify whether leaf constituents affect food preference between adults and larvae, feeding test with methanol extracts of young and old leaves were conducted. Young and old leaves were extracted with methanol (fresh leaves 1 g/20 m*l*). The methanol extract was evaporated *in vacuo* to dryness less than 40℃. A methanolic test solution (75 μl, 1 g fresh leaf equivalent/m*l*) was applied to a square piece of filter paper (Toyo roshi, 50, 20×20 mm) and the solvent was removed by air-drying. Two treated and two control (applied solvent only) papers were placed equidistantly (same treatment was opposite) on a doughnut-like plastic disk (20 mm i.d. and 70 mm o.d.) and put on a plastic petri dish (90 mm d.) in which were placed three filter papers (70 mm d.) moistened with 3 m*l* distilled water on the bottom. Distilled water (75 μl) was added to the test filter paper immediately before the test. Five adults or five last instar larvae were released into each dish. The petri dishes were placed in the condition of 24±1℃, 16L–8D photoregime, for 24 hr. After the test was finished, each test filter paper was divided into 100 sections (each 2×2 mm) and the number of sections with bites in them was counted. Each test was replicated five times. The preference for control versus treated or young versus old leaves extract was evaluated using Mann–Whitney test.

Results

Toughness of young and old leaves

The critical weight for which young leaves were penetrated was 6.8±0.1 g (mean±SE), whereas the critical weight for old leaves was 13.7±0.2 g. This result showed that old leaves were about twice as hard as the young leaves.

Table 1. Development of *E. admirabilis* provided young or old leaves of *T. kirilowii* var. *japonica* as food.

Leaves	Developmental time from 1st instar[a] (N)[b]					Adult weight (mean±SE, mg)	
	2nd	3rd	4th	Pupa	Adult	Male (N)[b]	Female (N)[b]
Young leaves	8.7±0.1 a[c] (30)	16.9±0.3 a (30)	26.4±0.4 a (30)	50.2±1.0 a (30)	59.6±1.0 a (29)	43.0±1.0 (13)	46.5±1.2 (16)
Old leaves	9.4±0.1 a[b] (30)	16.1±0.2 b (30)	25.2±0.3 b (30)	48.4±1.0 a (26)	58.5±1.0 a (25)	42.5±0.9 (18)	49.1±1.4 (7)

[a] Mean of number of days±SE.
[b] Number of individuals that survived.
[c] Means followed by the same letter in the same column are not significantly different at $P<0.05$ (Mann–Whitney test).

Rearing test

Although the survival rate of larvae provided young leaves was slightly higher than of those served old leaves, there was no obvious difference in survival, duration of growth or weight of adults (Table 1).

Feeding preference to fresh leaves

Both adults and larvae much preferred young leaves to old leaves (Fig. 1). In the feeding test using old leaves at different test duration, the feeding amount by larvae was positively correlated with the length of test period, whereas the consumption by adults hardly increased until the test duration of 12 hr and suddenly increased when the test duration was 24 hr (Fig. 2).

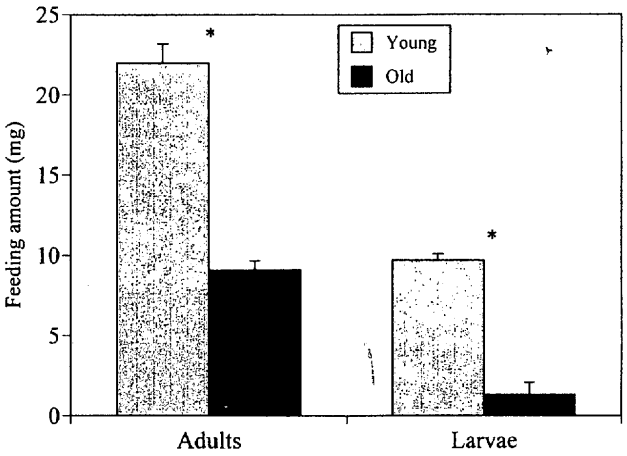

Fig. 1. Feeding amount (mean±SE) on young and old leaves of *T. kirilowii* var. *japonica* by adults and larvae of *E. admirabilis*. * significantly different at $P<0.05$ by Mann–Whitney test.

Feeding preference to methanol extracts

In the no-choice test, there was no difference for both adults and larvae in feeding stimulant activity between the young and old leaves extracts (Fig. 3). However, in the choice test, there was no difference for adults in feeding stimulant activity between young and old leaves extracts, and larvae were more stimulated to feed by the old leaves extract than the young leaves extract (Fig. 4).

Discussion

In many herbivorous insects, it is difficult for larvae to feed on old and hard leaves because their mandibles are not developed enough compared with those of adults. In lepidopterous larvae, some species have a pattern of diet change during development because nutritional needs vary with age (Claridge & Wilson, 1978; Bernays & Janzen, 1988). The plant availability for some lepidopterous larvae varies before and after diapause (Gaston *et al.*, 1991).

Wilson (1986) reported that larvae of *Epilachna cucurbitae*, a cucurbitaceous feeding lady beetle spe-

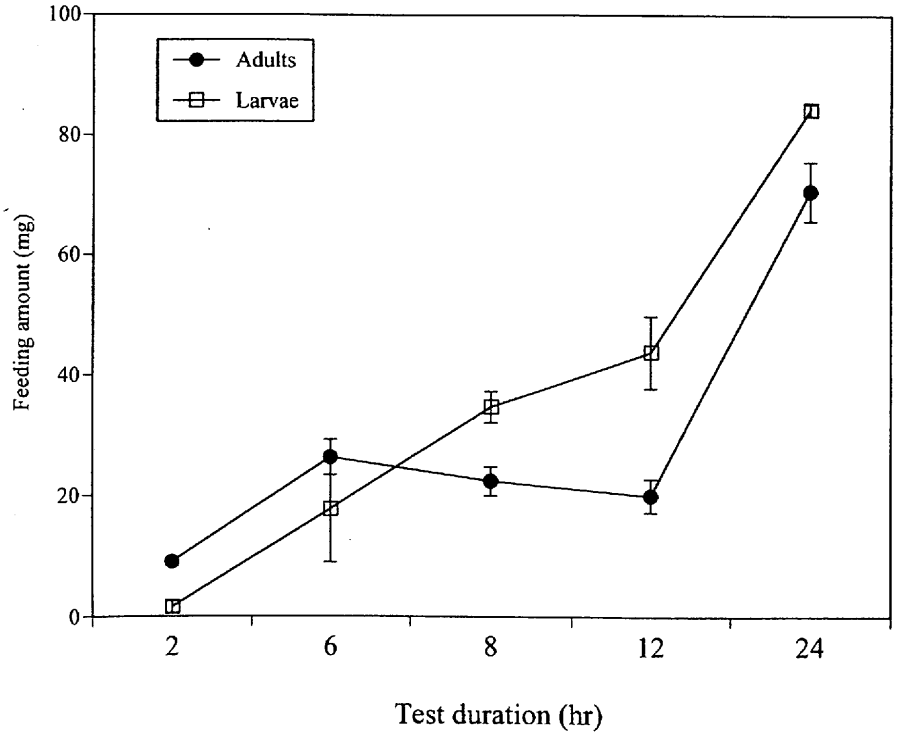

Fig. 2. Feeding amount (mean±SE) on old leaves of *T. kirilowii* var. *japonica* by adults and larvae of *E. admirabilis* at different test duration.

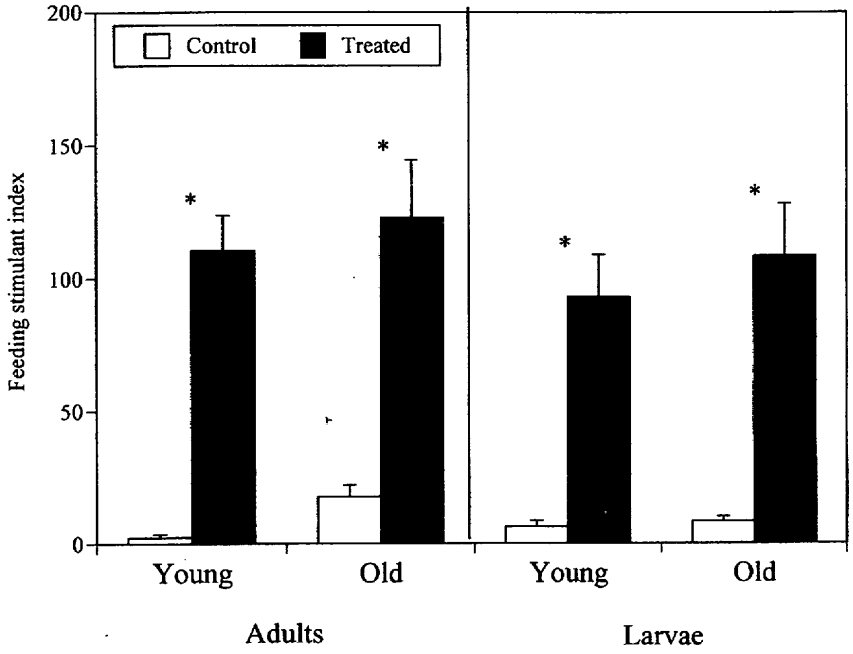

Fig. 3. Feeding responses of adults and larvae of *E. admirabilis* to methanol extracts (no-choice test). Data are indicated as feeding stimulant index (mean±SE). * significantly different at $P<0.05$ by Mann–Whitney test.

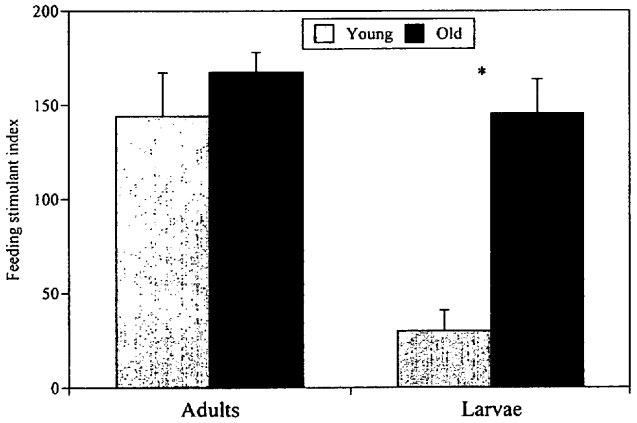

Fig. 4. Feeding responses of adults and larvae of *E. admirabilis* to methanol extract (choice test). Data are indicated as feeding stimulant index (mean± SE). * significantly different at $P<0.05$ by Mann–Whitney test.

cies, fed on lower old leaves rather than upper young leaves because they avoid hairs on young leaves. Since the surface of both young and old *T. kirilowii* var. *japonica* leaves is smooth and has few hairs, it is unlikely that the feeding behavior of *E. admirabilis* is affected by the hairs on leaves.

Some lady beetle species feeding on cucurbitaceous plant perform "trenching behavior" when they feed on host plant leaves, and this behavior is regarded as counteradaptation against protective substances in cucurbitaceous plants (Charroll & Hoffman, 1980; Tallamy, 1985; McCloud & Tallamy, 1995). *E. admirabilis* also exhibits trenching behavior (Katakura, 1988). Adults and larvae of *E. admirabilis* perform trenching on leaves of *T. kirilowii* var. *japonica* in nature, especially larvae do trenching on both young and old leaves. Therefore, it seems that trenching behavior is not related to the difference in feeding palatability between young and old leaves.

Feeding test using both young and old leaves suggested that young leaves contained larger amount of some feeding stimulants than old leaves and that old leaves possessed feeding resistant factors against both adults and larvae. Because old leaves was about twice as hard as young leaves, it seemed that toughness functioned as an important feeding resistant factor and mainly prevented the beetle from feeding on old leaves. Feeding test using old leaves at different test duration indicated that larvae are more likely to conquer the toughness than adults and that adults also can overcome the toughness by chewing for longer times.

Rearing tests revealed that even first instar larvae, which seem to have difficulty feeding on tough leaves, could feed on them and develop into adults. These results indicate that there is no difference in nutrition necessary for larval growth between young and old leaves. Thus, it is suggested that larvae adapt to the toughness of old leaves more than adults do.

Feeding tests with methanol extracts revealed that old leaves contain a stronger feeding stimulant for larvae than young leaves. This may be the reason why larvae can sooner accept and eat more old leaves than

adults can by the presence of some feeding stimulant (s). Because there was no difference in feeding stimulant activity for adults between young and old leaves extracts, adults were probably reduced feeding on old leaves by the toughness at a short period.

For these reasons, it is concluded that adults differ from larvae in the feeding preference between young and old leaves of *T. kirilowii* var. *japonica*. But further studies are needed to clarify the details of difference in feeding preference between adults and larvae.

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