Feeding responses of four phytophagous lady beetle species (Coleoptera: Coccinellidae) to cucurbitacins and alkaloids

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Abstract

Feeding responses of adults and larvae of *Epilachna admirabilis*, *E. boisduvali*, *E. vigintioctomaculata*, and *E. vigintioctopunctata* to four cucurbitacins (B, E, I and E-glucoside) were investigated. Both adults and larvae of *E. admirabilis*, which mainly feeds on the genus *Trichosanthes* (Cucurbitaceae), were strongly stimulated to feed by these cucurbitacins, especially by cucurbitacin E-glucoside. *E. boisduvali* feeds on *Diplocyclos palmatus* (Cucurbitaceae). Larvae of this species were stimulated to feed by all four cucurbitacins, especially by cucurbitacin I, and adults were stimulated to feed by cucurbitacin B only. *E. vigintioctomaculata* and *E. vigintioctopunctata*, which usually feed on solanaceous plants, were also stimulated to feed by cucurbitacins. They were not stimulated to feed by solanine and tomatine, which are usually contained in solanaceous host plants.

INTRODUCTION

Cucurbitaceous plants usually contain cucurbitacins which serve as feeding or oviposition deterrents for herbivorous insects (Nielsen et al., 1977; Nielsen, 1978; Sachdev-Gupta et al., 1993; Tallamy et al., 1997). However, cucurbitacins act as feeding stimulants or attractants for cucurbitaceous feeding insects such as *Acalymma*, *Aulacophora* and *Diabrotica* leaf beetle species (Chambliss and Jones, 1966; Shinha and Krishna, 1969, 1970; Metcalf et al., 1980). Cucurbitaceous feeding lady beetle species have only been studied on *Epilachna borealis* and *E. tredecimnotata* (Carroll and Hoffman, 1980; Tallamy, 1985; Tallamy and McCloud, 1991; McCloud et al., 1995), and the relationship between cucurbitaceous feeding lady beetle species and cucurbitacins is not yet clear.

E. admirabilis (Crotch) and *E. boisduvali* (Mulsant) are phytophagous lady beetle species which feed on cucurbitaceous plants. *E. vigintioctomaculata* (Motschulsky) and *E. vigintioctopunctata* (Fabricius) are known as solanaceous feeding lady beetle species, but these adults also attack cucurbitaceous plants such as cucumber, melon, and pumpkin. Their solanaceous host plants, *Solanum tuberosum* and *S. nigrum* commonly contain an alkaloid, solanine, and *Lycopersicon esculentum* also contains an alkaloid, tomatine. Because these alkaloids are characteristic in these solanaceous plants, it is thought that they play a role in host selection of these solanaceous feeding lady beetles. The alkaloids have never been reported to act as feeding stimulants. The feeding responses of *E. vigintioctomaculata* and *E. vigintioctopunctata* to alkaloids have never been investigated.

In this study, we conducted feeding tests on the feeding responses of the four lady beetle species to cucurbitacins B and E, which commonly occur in Cucurbitaceae, and to their derivatives, cucurbitacins I and E-glucoside (Rehm et al., 1957), and evaluated whether the feeding responses are elicited by cucurbitacins. Solanine and tomatine, which are commonly contained in potatoes and tomatoes, respectively, were further tested on *E. vigintioctomaculata* and *E. vigintioctopunctata* to determine whether these alkaloids were stimulate feeding.

MATERIALS AND METHODS

Insects and plants. *E. admirabilis* adults were collected at the experimental field of the Faculty of Agriculture, Tohoku University, Sendai. *E. boisduvali* adults were collected at Ishigaki Island, Okinawa Prefecture. *E. vigintioctomaculata* adults were collected at Natori City, Miyagi Prefecture. *E. vigintioctopunctata* adults were collected at Naha City, Okinawa Prefecture. All species were reared continuously under 16L-8D, 24±1°C conditions. *E. admirabilis* were provided with *Cucurbita pepo, Sicyos angulatus* and *Trichosanthes kirilowii* leaves, *E. boisduvali* with *Cucurbita pepo, Diplocyclos palmatus* and *Sicyos angulatus* leaves, and *E. vigintioctomaculata* and *E. vigintioctopunctata* with *Solanum tuberosum* and *S. nigrum* leaves for food. Adults were used for bioassay within 2 weeks after eclosion and larvae of each stadium were used within 2 days of ecdysis.

Host plants were cultivated at the experimental field of the Faculty of Agriculture, Tohoku University.

Bioassay for adults and 4th stadium larvae. Feeding tests of adults and 4th stadium larvae were conducted with a filter paper assay (Fig. 1A). Cucurbitacins, solanine and tomatine were dissolved in methanol at each concentration (1.0, 0.1, and 0.01 mg/ml), and a square piece of filter paper (Toyo roshi, 50, 2×2 cm) was treated with 75 μl of the test solution and allowed to dry. Three filter papers (7 cm each) were placed on the bottom of a plastic petri dish (9 cm i.d.) and moistened with 3 ml distilled water. A doughnut-like plastic disc (2 cm i.d. and 7 cm o.d.) was set on three filter papers. Two treated and two control (treated with solvent only) filter papers were placed equidistantly on the plastic disc. Distilled water (75 μl) was added to the filter paper immediately before the test. Five adults (mixed gender, starved for 24 h) or five 4th stadium larvae were introduced into the petri dish. The petri dish was placed under conditions of 24±1°C and a 16L-8D photoperiod, for 24 h. Each test was replicated three times. After the test was finished, each filter paper square was divided into 100 sections (each 2×2 mm) and the number of sections with bites

in them was counted (max: 100/filter paper square, and 200/petri dish).

Bioassay for 3rd stadium larvae. Feeding tests for 3rd stadium larvae were conducted with a filter paper assay (Fig. 1B). A filter paper (Toyo roshi, 50, 2×2 cm) was treated with 75 μl of the test solution and allowed to dry. One treated and one control (treated with solvent only) paper were placed oppositely on the bottom of a plastic petri dish (6 cm i.d.). Distilled water (75 μl) was added to each filter paper square immediately before the test. Three 3rd stadium larvae were released into the petri dish. Each test was replicated five times. The petri dish was placed under the same conditions described above for 24 h. Each test was replicated five times. After the test was finished, each filter paper square was divided into 100 sections (each 2×2 mm) and the number of sections with bites in them was counted (max: 100/petri dish).

Bioassay for 1st and 2nd stadium larvae. Feeding tests for 2nd and 1st stadium larvae were conducted with a filter paper assay (Fig. 1B). A filter paper square (Toyo roshi, 50, 1×1 cm) was treated with 18.75 μl of the test solution and allowed to dry. One treated and one control (treated with solvent only) paper were placed oppositely on the bottom of a glass petri dish (3 cm i.d.). Distilled water (18.75 μl) was added to each filter paper square immediately before the test. Five 1st or 2nd stadium larvae were released into the petri dish. Each test was replicated five times. The petri dish was placed under the same conditions described above for 24 h. Each test was replicated five times. After the test was finished, each filter paper was divided into 100 sections (each 1×1 mm) and the number of sections with bites in them was counted (max: 100/petri dish).

Statistical analysis. The results from each feeding test are represented as a numerical value by the number of sections with bites and shown as feeding stimulant response in each table. The preference for control versus treated was evaluated using Mann-Whitney test.

Chemicals. Cucurbitacins B, E, and E-glucoside were isolated from *Ecballium elaterium* (Abe et al., 2000). Cucurbitacin I and solanine were purchased from Funacoshi Co. Ltd., and tomatine was obtained from Tokyo Kasei Co.
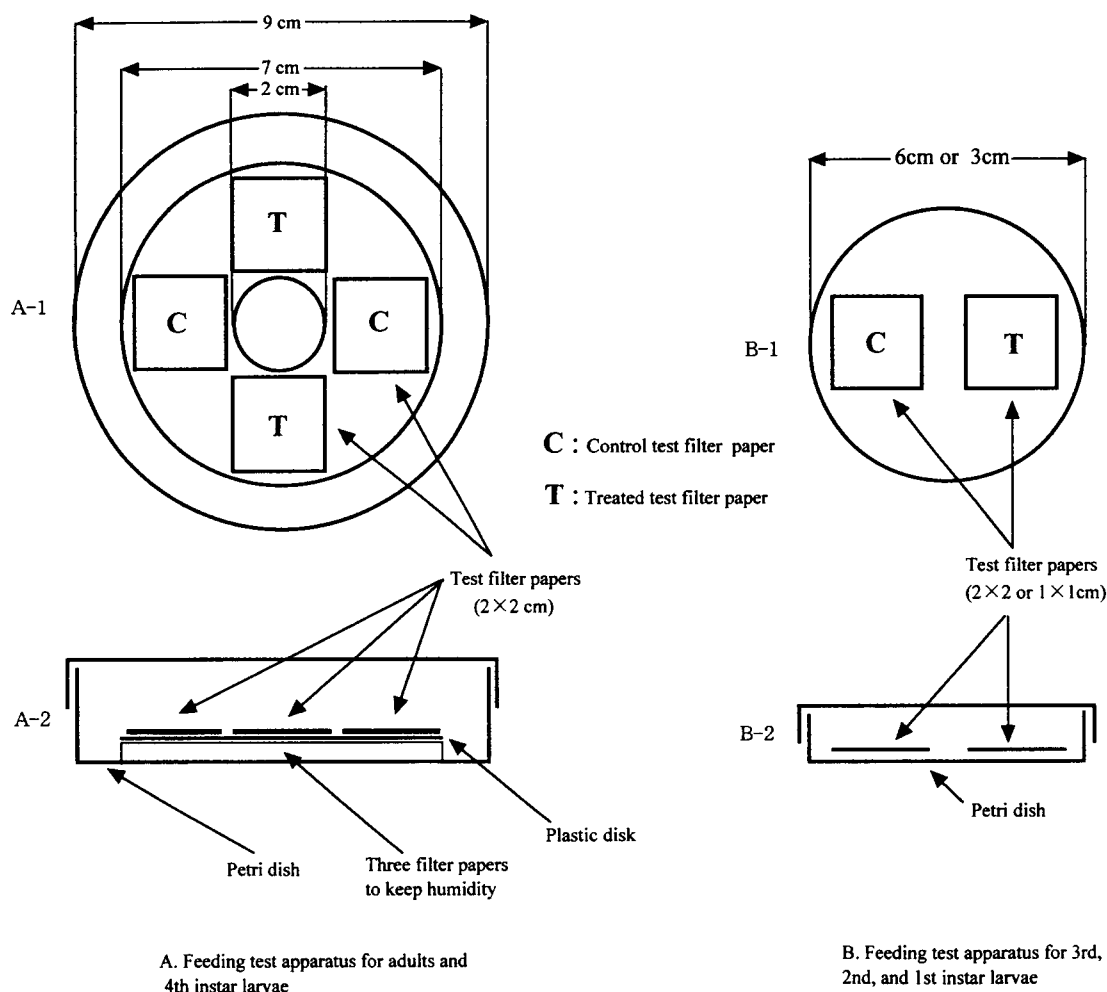
Fig. 1. Feeding test apparatuses for lady beetles. A-1 and B-1 indicate overlook view, and A-2 and B-2 indicate side view. Six centimeters i.d. petri dish and 2 × 2 cm test filter papers were used for 3rd stadium larvae. Three centimeters i.d. petri dish and 1 × 1 cm test filter papers were used for 2nd and 1st stadium larvae.

Ltd.

RESULTS

Feeding responses of beetles

The results from feeding tests of *E. admirabilis* to cucurbitacins are shown in Table 1. The adults were strongly stimulated to feed by all the cucurbitacins tested. Among cucurbitacins, E-glucoside at a concentration of 1 mg/ml elicited the strongest feeding stimulant activity. The larvae were also stimulated to feed by all the cucurbitacins, but early (1st and 2nd) stadia larvae were less stimulated to feed by cucurbitacins than old (3rd and 4th) stadia larvae. In particular, 1st stadium larvae were only slightly stimulated to feed by all the cucurbitacins.

The results from *E. boisduvali* are shown in Table 2. The adults were weakly stimulated to feed by only cucurbitacin B at 1 mg/ml and did not respond to the other cucurbitacins. In contrast, 4th stadium larvae were strongly stimulated to feed by all the cucurbitacins, especially by cucurbitacin I at a concentration of 1 mg/ml. Third and 2nd stadium larvae were also stimulated to feed by all the cucurbitacins but responded less to lower concentrations of cucurbitacins than 4th stadium larvae. First stadium larvae were not stimulated to feed by any of the cucurbitacins.

The results from *E. vigintioctomaculata* and *E. vigintioctopunctata* are shown in Table 3. *E. vigintioctomaculata* adults and larvae were strongly stimulated to feed by all the cucurbita-

Table 1. Feeding responses of *E. admirabilis* to cucurbitacins (feeding stimulant response: mean ± SE)

Cucurbitacins (mg/ml)		Adults		4th stadium larvae		3rd stadium larvae		2nd stadium larvae		1st stadium larvae	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
E	1.0	1.3±0.9	51.7±9.8*[a]	1.7±1.2	60.7±13.2*	0.8±0.5	44.4±6.9*	0.4±0.4	26.4±14.0*	0.8±0.6	0.8±0.5 ns[b]
	0.1	0	49.3±3.2*	1.0±1.0	72.7±12.4*	0.4±0.4	47.2±7.5*	0	5.8±1.4*	0	1.6±1.0 ns
	0.01	12.7±8.6	64.0±24.1*	0	1.0±1.0 ns	0.2±0.2	5.2±4.0 ns	0	1.0±0.4 ns	0	0 ns
B	1.0	1.0±1.0	76.0±6.4*	3.3±3.3	68.7±17.7*	0	52.4±8.4*	1.0±0.8	54.2±12.4*	0	5.2±1.4*
	0.1	4.0±3.5	67.0±3.5*	0	50.7±26.1*	0	22.4±6.9*	0.4±0.4	14.4±3.3*	0.4±0.4	1.2±0.8 ns
	0.01	8.0±5.3	88.3±29.3*	0	1.0±0.6 ns	0	0 ns	0	0 ns	0	0.8±0.6 ns
I	1.0	0	92.3±21.4*	0	43.3±22.0*	0.4±0.4	57.2±8.6*	2.6±2.6	3.4±1.7 ns	0.6±0.6	2.8±1.2 ns
	0.1	0	81.3±7.1*	0	15.7±13.7*	0	19.0±5.3*	0	1.4±1.0 ns	0	1.4±0.9 ns
	0.01	3.3±1.3	17.7±6.7*	1.3±1.3	1.3±1.3 ns	0	0 ns	0	0.2±0.2 ns	0	0 ns
E-glu.[c]	1.0	0	135.7±11.3*	0	144.7±9.1*	0.4±0.4	47.8±11.7*	0.8±0.8	44.8±9.9*	0	6.8±4.0 ns
	0.1	2.3±1.5	104.3±29.9*	3.0±2.1	69.3±21.2*	0	42.0±10.4*	0	5.8±1.8*	0	9.6±5.7 ns
	0.01	0.7±0.7	50.0±16.9*	1.3±1.3	20.3±19.3 ns	0.2±0.2	4.6±4.4 ns	0	6.4±2.9*	0	0 ns

[a] Significant difference between control and treated at 5% by Mann-Whitney test.
[b] Not significant.
[c] E-glu.: cucurbitacin E-glucoside.

Table 2. Feeding responses of *E. boisduvali* to cucurbitacins (feeding stimulant response: mean ± SE)

Cucurbitacins (mg/ml)	Adults		4th stadium larvae		3rd stadium larvae		2nd stadium larvae		1st stadium larvae	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
E 1.0	1.3±1.3	0 ns	0	58.7±11.2 *[a]	0	22.8±3.5 *	2.6±1.9	22.4±5.4 *	0	0 ns[b]
0.1	0	0.3±0.3 ns	0	32.3±6.7 *	0	0.6±0.6 ns	0	5.8±1.4 *	0	0 ns
0.01	—[c]	—	0	6.0±2.1 ns	0	0.4±0.2 ns	0	0.4±0.4 ns	0	0 ns
B 1.0	0	40.3±8.4 *	0	64.7±18.6 *	0	21.6±3.0 *	0.4±0.4	27.8±4.9 *	0	3.6±1.9 ns
0.1	0	8.0±8.0 ns	0	55.0±8.1 *	0	0 ns	0.4±0.4	14.4±3.3 *	0	0.4±0.4 ns
0.01	—	—	0	0 ns	0	0 ns	0	0.6±0.4 ns	0	0.6±0.6 ns
I 1.0	0	0 ns	0.7±0.7	113.0±19.7 *	0	8.4±2.3 *	0.4±0.4	13.0±6.0 *	0	0 ns
0.1	0	0 ns	0	9.3±4.7 *	0	0 ns	0	1.4±1.0 ns	0	0 ns
0.01	—	—	0.7±0.7	14.0±1.7 *	0.8±0.8	0 ns	0	0 ns	0	0 ns
E-glu.[d] 1.0	0	14.7±12.3 ns	0	52.7±4.3 *	0	25.6±2.5 *	0	13.6±2.9 *	1.0±0.8	9.0±3.8 ns
0.1	0	1.0±1.0 ns	0	51.0±9.1 *	0	0 ns	0	5.8±1.8 *	0	0 ns
0.01	—	—	0	10.3±2.3 *	0	0.4±0.2 ns	0	0 ns	0	0 ns

[a] Significant difference between control and treated at 5% by Mann-Whitney test.
[b] Not significant.
[c] Not tested.
[d] E-glu.: cucurbitacin E-glucoside.

Table 3. Feeding responses of *E. vigintioctomaculata* and *E. vigintioctopunctata* to cucurbitacins and alkaloids (feeding stimulant response: mean±SE)

Chemicals[a] (mg/ml)	E. vigintioctomaculata				E. vigintioctopunctata			
	Adults		4th stadium larvae		Adults		4th stadium larvae	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
E 1.0	10.3±5.3	156.7±13.4*[b]	1.7±1.7	41.7±17.7*	3.3±2.8	83.0±17.4*	0	8.6±3.8 ns[c]
0.1	1.3±1.3	161.7±2.4*	2.0±1.0	48.0±30.1*	0	39.0±5.7*	—	—
0.01	6.0±5.0	135.7±19.2*	—[d]	—	0	16.7±10.2 ns	—	—
B 1.0	14.3±6.7	160.3±26.2*	1.0±0.6	57.3±20.7*	1.0±0.6	86.3±28.1*	0	42.0±29.4*
0.1	16.3±10.8	156.3±33.4*	0.3±0.3	25.3±14.3*	3.0±1.7	109.0±22.4*	—	—
0.01	1.3±0.7	64.3±17.3*	—	—	3.0±2.1	91.0±18.9*	—	—
I 1.0	3.0±2.1	116.0±40.0*	1.3±0.9	58.7±17.1*	2.7±1.5	90.7±14.7*	4.3±1.2	53.0±23.1*
0.1	0.3±0.3	22.3±3.8*	0.7±0.7	20.7±9.6*	6.0±5.0	98.0±30.0*	—	—
0.01	4.0±0.6	17.3±4.4*	—	—	4.3±4.3	67.7±32.5 ns	—	—
E-glu. 1.0	7.7±6.2	179.3±8.2*	0	111.3±11.5*	3.3±1.9	81.7±16.2*	7.0±7.0	25.0±8.6 ns
0.1	11.0±2.9	149.7±12.7*	0	35.3±17.3*	20.7±10.7	85.0±25.6 ns	—	—
0.01	0.7±0.7	128.3±35.3*	—	—	9.3±7.0	103.7±23.4*	—	—
Solanine 1.0	6.3±6.3	9.0±7.0 ns	0	2.7±2.7 ns	0	1.7±1.7 ns	3.0±2.1	1.0±1.0 ns
0.1	0	0 ns	0.3±0.3	1.0±0.6 ns	9.7±9.7	5.7±4.7 ns	—	—
Tomatine 1.0	1.0±0.6	5.3±3.1 ns	0	0 ns	0.3±0.3	1.7±1.7 ns	7.6±1.9	5.0±1.7 ns
0.1	1.0±1.0	4.3±2.2 ns	0	0 ns	0	0 ns	—	—

[a]E: cucurbitacin E, B: cucurbitacin B, I: cucurbitacin I, E-glu.: cucurbitacin E-glucoside.
[b]Significant difference between control and treated at 5% by Mann-Whitney test.
[c]Not significant.
[d]Not tested.

cins, especially by E-glucoside, at a concentration of 1 mg/ml. Adults were more strongly stimulated to feed by cucurbitacins than adults of the other three species. Adults of *E. vigintioctopunctata* were also strongly stimulated to feed by cucurbitacins, whereas the larvae were less stimulated to feed by cucurbitacins than the adults, and they were not stimulated to feed by cucurbitacins E and E-glucoside. Neither species was stimulated to feed by solanine or tomatine.

DISCUSSION

The role cucurbitacins might play in the host selection of cucurbitaceous feeding lady beetles has been discussed (Carroll and Hoffman, 1980; Tallamy, 1985; Tallamy and McCloud, 1991; McCloud et al., 1995). In cucurbitaceous feeding lady beetle species, genus *Epilachna*, there are species which chew circular trenches prior to feeding on their host plant leaves. Carroll and Hoffman (1980), and Tallamy (1985) described how the trenching behavior by *E. borealis* and *E. tredecimnotata* prevents cucurbitacins from

increasing. On the contrary, McCloud et al. (1995) reported that cucurbitacins B and I acted as feeding stimulants rather than feeding deterrents against *E. borealis*, and did not affect trenching behavior. *E. admirabilis* and *E. boisduvali* also exhibit trenching behavior when they feed on host plant leaves, while *E. vigintioctomaculata* and *E. vigintioctopunctata* do not exhibit trenching behavior.

Our results revealed that cucurbitacins act as feeding stimulants to four lady beetle species and the activity was different among species or between adults and larvae within species. These four beetle species were stimulated to feed by the high concentration of cucurbitacins, and there were no trenching marks on any of the test filter papers. Although cucurbitacin contents in many cucurbitaceous plant species, especially in fruits and roots, have been reported (Rehm et al., 1957; Lavie and Glotter, 1971; Kitajima and Tanaka, 1989), their host plant leaves, especially genus *Trichosanthes* or *Diplocyclos* leaves, were not analyzed. Thus, it is unknown whether cucurbitacins are involved with trenching be-

havior. In cucurbitaceous feeding lady beetle species, the feeding responses to cucurbitacins were quite different between *E. admirabilis* and *E. boisduvali*. Compared with the host plant range of both species, *E. admirabilis* feeds on genera *Trichosanthes, Melothria* and *Gynostemma*, while *E. boisduvali* feeds only on *Diplocyclos palmatus*. The differences in responses to cucurbitacins are probably related to the host plant range of the two species. Although adults or 4th stadium larvae of both beetle species were strongly stimulated to feed by relatively higher concentrations of cucurbitacins, 1st stadium larvae were scarcely stimulated. It is known that food preference is changed by chemicals present in the food (Städler and Hanson, 1978; Blaney and Simmonds, 1987; Szentesi and Jermy, 1989). Thus, 1st stadium larvae may gradually adapt to cucurbitacins by the continuing ingestion of host plant leaves and use cucurbitacins as a feeding stimulant. *E. boisduvali* larvae, especially 4th stadium larvae, were more stimulated by cucurbitacins than adults. The adults may be more stimulated to feed by substances in their host plant other than cucurbitacins.

Among the solanaceous feeding lady beetle species, both species were stimulated to feed by cucurbitacins at lower concentrations than cucurbitaceous feeding lady beetle species. In particular, *E. vigintioctomaculata* adults responded strongly to cucurbitacins. Adults of both species attack many cucurbitaceous plants when they cannot obtain solanaceous plants as hosts, and *E. vigintioctomaculata* feeds on many more cucurbitaceous plant species than *E. admirabilis, E. boisduvali* and *E. vigintioctopunctata*. The adaptation of *E. vigintioctomaculata* and *E. vigintioctopunctata* to cucurbitacins may be related to their range of food plants among Cucurbitaceae. *E. vigintioctopunctata* larvae were slightly stimulated to feed by cucurbitacins. It is possible that larvae of *E. vigintioctopunctata* responded to cucurbitacins less than adults because they do not feed on cucurbitaceous plants. The larvae of *E. vigintioctomaculata* seem to adapt to cucurbitacins more easily than the larvae of *E. vigintioctopunctata*. This is supported by the fact that *E. vigintioctomaculata* feeds on a cucur-

bitaceous plant, *Schizopepon bryoniaefolius* as a host in Hokkaido (Katakura, 1975). Because neither species was stimulated to feed by solanine or tomatine, they are possibly stimulated to feed by some other substances contained in solanaceous host plants such as the potato and tomato.

ACKNOWLEDGEMENTS

REFERENCES

Abe, M., K. Matsuda and Y. Tamaki (2000) Differences in feeding response among three cucurbitaceous feeding leaf beetles to cucurbitacins. *Appl. Entomol. Zool.* 35: 137–142.

Blaney, W. M. and M. S. J. Simmonds (1987) Experience, a modifier of neural and behavioural sensitivity. In *Insects-Plants* (V. Labeyrie, G. Fabres and D. Lachaise eds.). Junk, Dordrecht, pp. 237–241.

Carroll, D. R. and C. A. Hoffman (1980) Chemical feeding deterrent mobilized in response to insect herbivory and counter adaptation by *Epilachna tredecimnotata. Science* 209: 414–416.

Chambliss, O. L. and C. M. Jones (1966) Cucurbitacins: specific insect attractants in Cucurbitaceae. *Science* 153: 1329–1393.

Katakura, H. (1975) *Schizopepon bryoniaefolius* (Cucurbitaceae) as a native host plant for *Henosepilachna vigintioctomaculata* (Coleoptera: Coccinellidae) in Hokkaido. *Appl. Entomol. Zool.* 10: 103–107.

Kitajima, J. and Y. Tanaka (1989) Studies on the constituents of *Trichosanthes* root. I. Constituents of roots of *Trichosanthes kirilowii* Maxim. var. *japonicum* Kitum. *Yakugaku Zasshi* 109: 250–255 (in Japanese with English summary).

Lavie, D. and E. Glotter (1971) The cucurbitacins, a group of tetracyclic triterpenes. *Forts. Chem. Organ. Naturstoffe.* 29: 306–362.

McCloud, E. S., D. W. Tallamy and F. T. Halaweish (1995) Squash beetle trenching behaviour: avoidance of cucurbitacin induction or mucilaginous plant sap? *Ecol. Entomol.* 20: 51–59.

Metcalf, R. L., R. A. Metcalf and A. M. Rhodes (1980) Cucurbitacins as kairomones for diabroticite beetles. *Proc. Natl. Acad. Sci. U.S.A.* 77: 3769–3772.

Nielsen, J. K. (1978) Host plant discrimination within Cruciferae: feeding responses of four leaf beetles (Coleoptera: Chrisomelidae) to glucosinolates, cucurbitacins and cardenolides. *Entomol. Exp. Appl.* 24: 41–54.

Nielsen, J. K., M. Larsen and H. J. Sorenson (1977) Cucurbitacins E and I in *Iberis amara* feeding inhibitors for *Phyllotreta nemorum. Phytochemistry* 16: 1519–1522.

Rehm, S., P. A. Enslin, A. D. J. Meeuse and J. H. Wessels (1957) Bitter principles of the Cucurbitaceae. VII. The distribution of bitter principles in this plant family. *J. Sci. Food Agric.* 8: 679–686.

Sachdev-Gupta, K., C. D. Radke and J. A. Renwick (1993) Antifeedant activity of cucurbitacins from *Iberis amara* against larvae of *Pieris rapae. Phytochemistry* 6: 1385–1388.

Shinha, A. K. and S. S. Krishna (1969) Feeding of *Aulacophora foveicollis* on cucurbitacin. *J. Econ. Entomol.* 62: 512–513.

Shinha, A. K. and S. S. Krishna (1970) Further studies on the

feeding behavior of *Aulacophora foveicollis* on cucurbitacin. *J. Econ. Entomol.* 63: 333-334.

Städler, E. and F. E. Hanson (1978) Food discrimination and induction of preference for artificial diets in the tobacco hornworm, *Manduca sexta. Physiol. Entomol.* 3: 121-133.

Szentesi, A. and T. Jermy (1989) The role of experience in host plant choice by phytophagous insects. In *Insect-Plant Interactions, Vol. 2* (E. A. Bernays ed.). CRC Press, Boca Raton, pp. 39-74.

Tallamy, D. W. (1985) Squash beetle feeding behavior: an adaptation against induced cucurbit defenses. *Ecology* 66: 1574-1579.

Tallamy, D. W. and E. S. McCloud (1991) Squash beetles, cucumber beetles, and inducible cucurbit responses. In *Phytochemical Induction by Herbivores* (D. W. Tallamy and M. J. Raupp eds.). John Wiley & Sons, New York, pp. 155-181.

Tallamy, D. W., J. Stull, N. P. Ehresman, P. M. Gorski and C. E. Mason (1997) Cucurbitacins as feeding and oviposition deterrents to insects. *Environ. Entomol.* 26: 678-683.