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Host use in relation to food availability and larval development in the specialist herbivore *Epilachna admirabilis* (Coleoptera: Coccinelidae)

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Abstract

We compared the effects of intrinsic quality and seasonal abundance of two host plants, *Gynostemma pentaphyllum* and *Trichosanthes cucumeroides* (Cucurbitaceae), on host use by a phytophagous lady beetle, *Epilachna admirabilis*. Although, only *G. pentaphyllum* exists as an available host in April, *T. cucumeroides* sprouts from late May and its leaf abundance surpasses that of *G. pentaphyllum* in August, the egg-laying period for *E. admirabilis*. In the laboratory, *T. cucumeroides* supported higher growth than did *G. pentaphyllum* and larvae grew faster as the dietary proportion of *T. cucumeroides* increased. We expected females to switch to *T. cucumeroides* for feeding and egg deposition. However, most adults remained and laid eggs on *G. pentaphyllum*. There is a poor correlation between larval performance and adult egg-laying site selection. On the other hand, first and second instar larvae showed a greater preference for *T. cucumeroides* in a host preference test. Additionally, 70–80% of first instar larvae were found on *G. pentaphyllum* in the field, and the number of larvae on *T. cucumeroides* increased with developmental stage. We suggest that larvae often switch food sources from *G. pentaphyllum* to *T. cucumeroides* depending on the intrinsic quality and food availability of these two host plants.

Key words: Leaf abundance; host use; host switching; larval performance; mixed diet

INTRODUCTION

The specialist phytophagous lady beetle *Epilachna admirabilis* depends on wild liana cucurbit plants, such as *Gynostemma pentaphyllum*, *Trichosanthes cucumeroides*, *T. kirilowii* var. *japonica* and *Schizopepon bryoniaefolius*, and produces one generation per year. We previously reported the seasonal occurrence of a population using *G. pentaphyllum* and *T. cucumeroides* at Hadano in Kanagawa Prefecture (Takeuchi, 1994; Takeuchi and Tamura, 1994). Newly emerged females pass through the pre-egg deposition period for approximately three months and lay eggs from late July to September on *G. pentaphyllum* and *T. cucumeroides* at a ratio of 4 : 1 when both host plants occur in the same place. Therefore, this population shows an oviposition preference for *G. pentaphyllum*, and most hatching larvae feed and grow on this host plant. However, our laboratory rearing tests showed that the developmental duration of larvae on *G. pentaphyllum* was 1.4 times that of *T. cucumeroides* (Takeuchi and Tamura, 1994).

Previous reports suggest that growth retardation can result in greater enemy-influenced mortality (e.g. Leather, 1985; Benrey and Denno, 1997) and in a relative shortage of suitable host plants. In this situation, *G. pentaphyllum* is not a good food plant for larvae when compared to *T. cucumeroides*. If large differences in growth and survival occur between host plants, consumers should spend time feeding and egg-laying on the more suitable host plant.

Cunningham et al. (2001) proposed an additional benefit based on the idea that host suitability varies with host species abundance. If consumers are required to forage on more than one host, their fitness will be higher when they commence development on the most abundant host.

In this study, we investigated the host use patterns of *E. admirabilis*, both adults and larvae, and assessed the intrinsic quality and seasonal availability of two co-occurring host plants.

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MATERIALS AND METHODS

Study area. Field observations were conducted in Hadano City, Kanagawa Prefecture. The study area (17.5 ha) is at an altitude of approximately 140–200 m and is covered with temperate semideciduous lowland forests and crop fields composed of satsuma mandarin orchard, chestnut orchard, plowed fields and abandoned satsuma mandarin orchards. Two host plant species for *E. admirabilis*, *G. pentaphyllum* and *T. cucumeroides*, occur in discrete units of different sizes over the study area. A unit with an aggregation of host plants is referred to as a 'site' in this article. We selected seven study sites (A–G; Fig. 1). Sites A and B had been used for cultivation of satsuma mandarin until approximately 20 y ago. Sites E and G were positioned on the edge of the orchard where satsuma mandarins are still grown. Sites C, D and F were on the edge of the secondary forest. *G. pentaphyllum* and *T. cucumeroides* often grew together in all study sites except site F, where we found *T. cucumeroides* only. Site A had a high abundance of both host plants and was divided into 100 patches of standing satsuma mandarin trees in 1991. Both host plants often occurred in the same patch and were intertwined with each other. Three quarters of the area in site A was cleared and plowed in 1998, and the host plant resources decreased greatly. Sites B and C had a greater abundance of *G. pentaphyllum* and D, E, F and G had a greater abundance of *T. cucumeroides*. This study was carried out in 1991, 1993, 1997, 1998, 1999, and 2000.

Spatial abundance of host plants and larval density over the study area. To clarify the spatial distribution and abundance of host foods and *Epilachna* larvae, we divided the whole study area into 70 units (50 m×50 m). In October 1999, we counted all mature leaves and fourth (final) instar larvae feeding on each host plant in every unit. To estimate the leaf abundance and larval density, we collected 300 mature leaves of both host plants from several populations adjoining the study area and measured the leaf area with a 'leaf area measuring instrument' (Hayashi Denkoh Co., Ltd. AAM-8). The total leaf area of the two hosts in each unit was estimated from 'the number of leaves in the unit' × 'mean leaf area.'

Seasonal trends in host abundance and adults. We investigated whether the host usage by the beetle was synchronized with the abundance of the host plant. In 1991 and 1993 from April to September, 14 and 12 patches covered with abandoned satsuma mandarin trees were selected at site A. Leaf abundance was estimated 1 to 4 times a month by counting the number of mature leaves in each patch. Simultaneously, we estimated the visible percentage injury of host plants (no. of leaves damaged/no. of leaves in total). Leaves were partially eaten by herbivorous insects, including *E. admirabilis* and two specialist leaf beetles, *Paridea angulicollis* on *G. pentaphyllum* and *Aulacophora nigripennis* on *T. cucumeroides*. In 2000, we similarly investigated leaf abundance and injury at the seven study sites from April to October.

We estimated the seasonal occurrences of adults on *G. pentaphyllum* and *T. cucumeroides*. Eight and 12 patches at site A were selected in 1991 and 1993. The numbers of *E. admirabilis* adults on each host plant were counted 2 to 4 times a month from May to late August. In 2000, we similarly counted the number of adults at six sites (A–F).

Larval stage-specific host use. Developmental changes in larval host usage on the two host plants were determined for 4 y (1993, 1997, 1998, and 1999). The numbers of larvae and their developmental stages were assessed 1 to 3 times a month from August to December at site A in five patches where both plants grew in sufficient quantities.

Growth and host choice experiments under laboratory conditions. In 1997, we assessed the impact of experimental food treatments on larval survival, developmental duration and weight. We conducted a series of rearing experiments on *G. pentaphyllum* and *T. cucumeroides*. Several egg batches were collected from the field in early August. To avoid genetic polarization, newly hatched larvae from each egg batch were divided equally into the five groups referred to below. Larvae were introduced singly for rearing in Petri dishes (9 cm diameter) and conditioned at 24°C with a photoperiod of 12L:12D. Leaves of *G. pentaphyllum* or *T. cucumeroides* cut from the petiole and covered with water-soaked absorbent paper were supplied as food. The larval developmental stage and survival were recorded every other day. The molting day was decided from larval body color (a newly molting individual is bright yellow).

To evaluate the intrinsic quality of the two host plants, we compared larval growth on mixed food with that on a single species: (1) *T. cucumeroides* only (TC), (2) *G. pentaphyllum* only (GP), (3) two host plants alternately given every other day (EX), (4) transfer from *G. pentaphyllum* to *T. cucumeroides* at the molt to the third instar stadium (G→T), and (5) transfer from *T. cucumeroides* to *G. pentaphyllum* at the molt to the third instar stadium (T→G). Twenty-five to 51 larvae represented each possible diet sequence. When all larvae were full grown and had stopped feeding for more than 4 d, body weight was measured and the experiments ended.

In 1998, we supplied two mixed food treatments with different food plant-distances; either both hosts in contact with each other in a 9 cm Petri dish (MIXC) or placed separately in a 15 cm Petri dish (MIXS). Larvae were introduced singly onto *G. pentaphyllum* and were reared as above. Under these conditions, the larvae were free to feed on either food source. The plants on which they remained and fed were noted every other day until they were full-grown. Larvae were returned to fresh leaves of the same host plant they were found on. We observed 49 larvae in MIXC and 50 larvae in MIXS.

RESULTS

Spatial abundance of host plants and larval density over the study area

The number of mature host leaves and 4 th instar larvae were counted in the 70 study units. Figure 1 illustrates the leaf and larval distributions. *T. cucumeroides* had a wider distributional area than *G. pentaphyllum*. Among the 70 units, 14 had *T. cucumeroides* only, 2 had *G. pentaphyllum* only and 13 had both (29 units in total). The mean total leaf area (±SE) in the 29 units was $0.48 \pm 0.28\,\mathrm{m}^2$ for *G. pentaphyllum* and $4.48 \pm 0.91\,\mathrm{m}^2$ for *T. cucumeroides* (Mann-Whitney *U*-test, $U=107$, $p<0.001$).

Larvae were found on *G. pentaphyllum* in 10 units and on *T. cucumeroides* in 17 units. Larval density (No. individuals/m^2 host leaf) was 12.99 ± 3.41 (±SE; $n=15$) on *G. pentaphyllum* and 4.12 ± 1.41 ($n=27$) on *T. cucumeroides* (Mann-Whitney *U*-test, $U=251.5$, $p<0.025$). However, the density of larvae in all units (No. individuals/(50 m×50 m)) was 12.92 ± 6.50 on *G. pentaphyllum* ($n=15$; 168 individuals in total) and 20.63 ± 8.36 on *T. cucumeroides* ($n=27$; 557 individuals in total) (Mann-Whitney *U*-test, $U=181.0$, $p=0.871$).

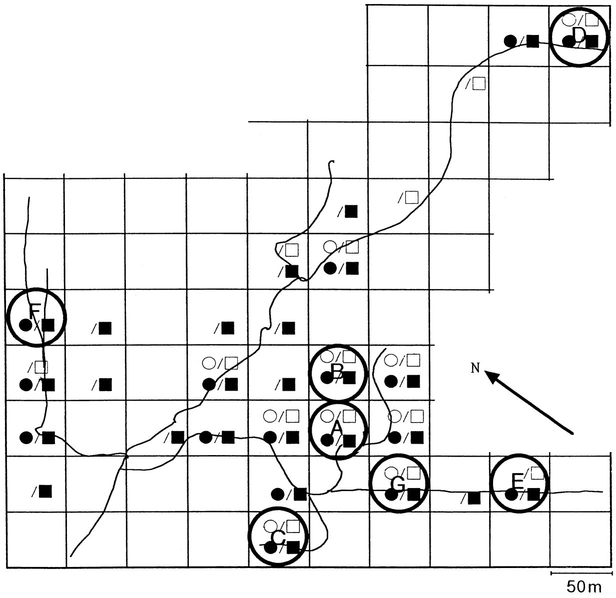

Fig. 1. Spatial distribution of host plants and fourth instar larvae in the study area. The entire study area was divided into 70 units (50 m×50 m). The freehand line indicates a sidewalk. Symbols indicates the presence of host plants and fourth instar larvae: □, *G. pentaphyllum*; ■, *T. cucumeroides*; ○, larvae on *G. pentaphyllum*; ●, larvae on *T. cucumeroides*.

Seasonal trends in host abundance and adults

Figure 2 shows the seasonal changes in total leaf area and percentage injury in both host species. *G. pentaphyllum* sprouts in April and grows evenly throughout the season. *T. cucumeroides* leaves appear and expanded from late May and leaf abundance greatly surpasses that of *G. pentaphyllum* in July. *T. cucumeroides* was still overwhelmingly predominant from August to November, the egg-laying and the larval developmental period. Additionally, leaf injury of *G. pentaphyllum* was consistently higher than that of *T. cucumeroides* throughout the season, because damage by the leaf beetle *P. angulicollis* was conspicuous.

The seasonal occurrence of *E. admirabilis* adults is outlined in Fig. 3. The numbers of both sexes were pooled. The largest number of newly emerged adults was found from late May to early June, after a temporary decrease in mid June. Since only *G. pentaphyllum* is available as a host in spring, newly emerged adults fed on it. Although some adults moved to *T. cucumeroides* in June, most remained on *G. pentaphyllum* except during late

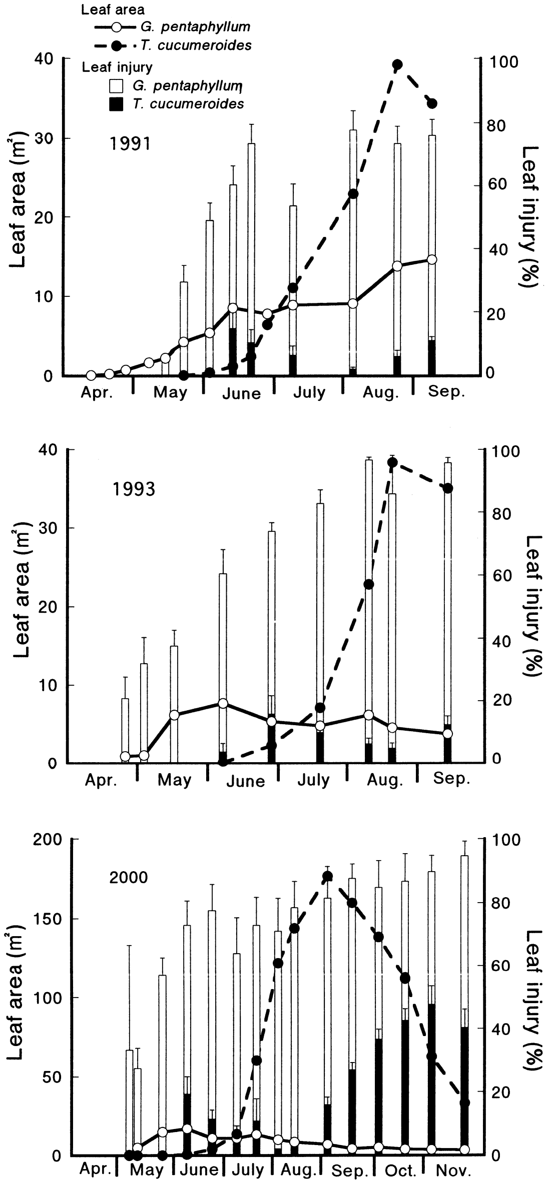

Fig. 2. Seasonal changes in leaf abundance and percentage of leaf injury in the two host plants. Leaf abundance represents the total leaf area. Leaf injury is the mean±SE over the investigated patches in 1991 and 1993 and at the seven study sites in 2000.

August, the latter part of the egg-laying period. Consequently, the number of adults on each host plant after July showed a poor association with the abundance of host plants.

Larval stage-specific host use

To clarify the host use of larvae, we surveyed larval stage-specific changes in feeding preference at site A, where there were sufficient leaves on both

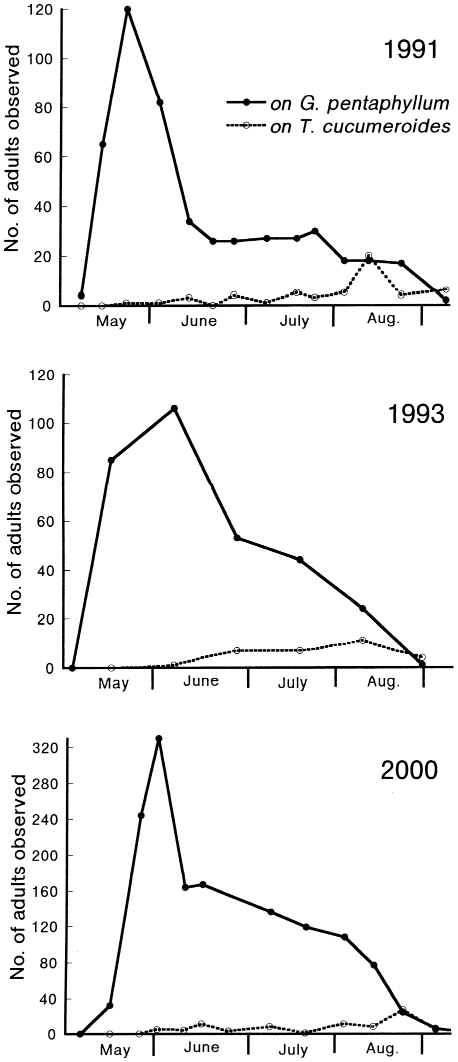

Fig. 3. Seasonal changes in the number of adults on the two host plants.

species in 1993, 1997, 1998 and 1999 (Fig. 4). Larval host use patterns between the two plants differed among developmental stages. Seventy to 80% of first instar stadium were found on *G. pentaphyllum*. After the second instar stadium, the number of larvae on *T. cucumeroides* gradually increased with developmental stage, then 60% to 90% of 4th instar larvae were found on *T. cucumeroides*.

Growth and host choice experiments

Table 1 shows the survival rate and developmental duration of immature stages and the weight of full-grown larvae raised on different food treatments. Differences in developmental duration between food treatments were detected by Tukey's

HSD multiple comparison test. The larval period (first to fourth instar) was significantly shorter on *T. cucumeroides* as a single food (average of 66 d) than on *G. pentaphyllum* (average of 98 d). However, the difference in growth performance became less clear at the fourth instar stadium. The three food switching treatments showed intermediate development. Larvae initially reared on *G. pentaphyllum* had a high developmental performance after transfer to *T. cucumeroides*. Conversely, development of larvae initially reared on *T. cucumeroides* suddenly slowed when transferred to *G. pentaphyllum*. Consequently, larvae grew faster as the dietary proportion of *T. cucumeroides* increased.

The survival rate during treatments also varied. Larvae reared on *G. pentaphyllum* showed a higher mortality than larvae reared on other food sources. However, body weight showed no clear difference between the two host plants.

When the larvae were free to feed on both foods, they switched food sources. The host selectivity of larvae was similar between the two food-distance treatments (MIXC and MIXS). The average proportion of feeding on *T. cucumeroides* per individual (frequency of feeding on *T. cucumeroides*/frequency of feeding on either host) over the developmental period (first to fourth instar) was 0.64±0.03 (mean±SE; $n=31$) on MIXC and 0.62±0.03 ($n=29$) on MIXS. These values indicate that larvae slightly preferred *T. cucumeroides* ($F=37.12$; $p<0.001$ in MIXC; $F=35.59$; $p<0.001$ in MIXS). However, regardless of the distance between the two foods, first and second instar larvae showed a greater preference for *T. cucumeroides* (Fig. 5).

On the other, larvae switched more often when the two foods were placed in contact with each other. The frequency of food switching over the larval period was 6.58±0.47 (mean±SE; $n=31$) in MIXC and 4.34±0.38 ($n=29$) in MIXS (Mann-Whitney *U*-test; $U\text{cal}=673.0$, $p<0.01$).

DISCUSSION

We investigated the interaction between seasonal abundance of the two host plants and *E. admirabilis* occurrence, and the effects of the intrinsic quality of each host plant on larval feeding.

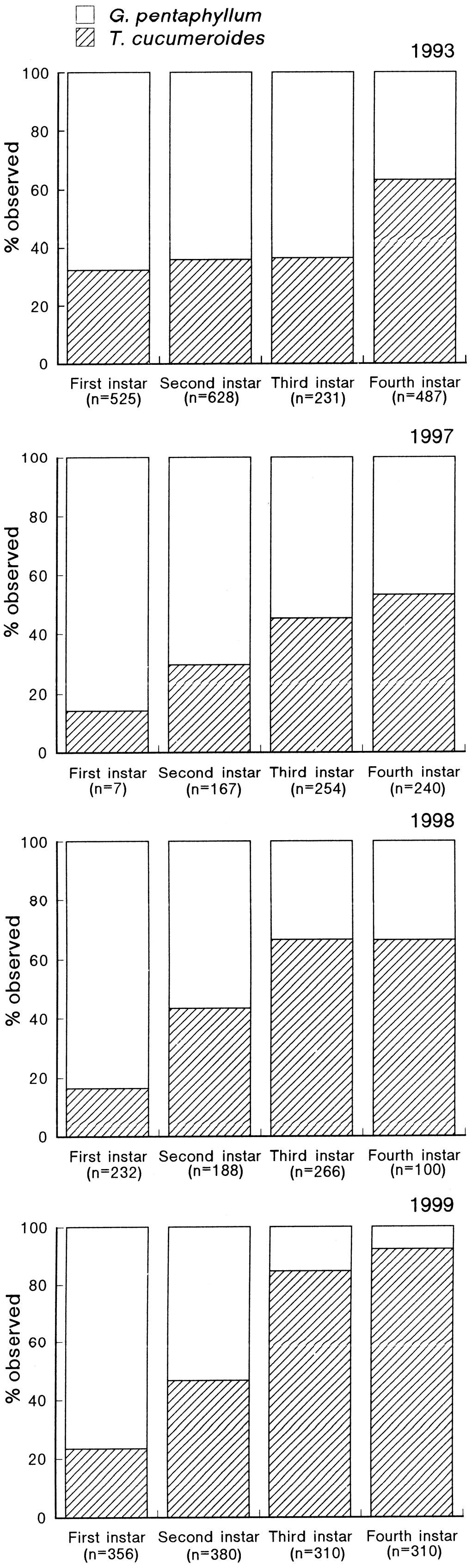

Fig. 4. Larval stage-specific host use in the field.

Table 1. Stage-specific development and survival rate of larvae in a laboratory experiment

	Food plant treatments				
	TC (50)	GP (51)	EX (25)	T→G (25)	G→T (25)
Developmental period (d)					
First instar	12.13±0.22 a	20.90±0.92 c	15.22±0.31 b	12.48±0.25 a	20.07±1.19 c
Second instar	12.98±0.23 a	25.18±1.72 c	15.90±0.39 b	12.92±0.34 a	22.92±1.41 c
Third instar	17.42±0.39 a	26.00±1.74 b	19.26±0.72 a	39.43±1.54 c	16.29±0.81 a
Fourth instar	23.91±1.11 a	26.48±1.81 ab	33.24±1.28 c	30.38±2.17 bc	26.33±1.90 abc
First instar to feeding quiescence	66.40±1.30 a	98.23±2.69 c	83.68±1.49 b	95.14±2.10 c	88.58±2.49 bc
Survival rate (%)	90.0	43.1	88.0	84.0	56.0
Weight of full-grown larvae (g)	0.052±0.01	0.051±0.01	0.049±0.01	0.038±0.01	0.045±0.01

Mean±SE. TC: *T. cucumeroides*, GP: *G. pentaphyllum*, EX: two host plants alternately given every other day, T→G: transfer of food plants from *T. cucumeroides* to *G. pentaphyllum* at the molt to the third instar stadium, G→T: transfer of food plants from *G. pentaphyllum* to *T. cucumeroides* at the molt to the third instar stadium. Numbers in parentheses indicate the number of individuals introduced initially. Means of developmental period followed by different letters in the same row are significantly different ($p<0.05$ by Tukey's HSD multiple comparison). The data of developmental period were transformed to logarithmic scale.

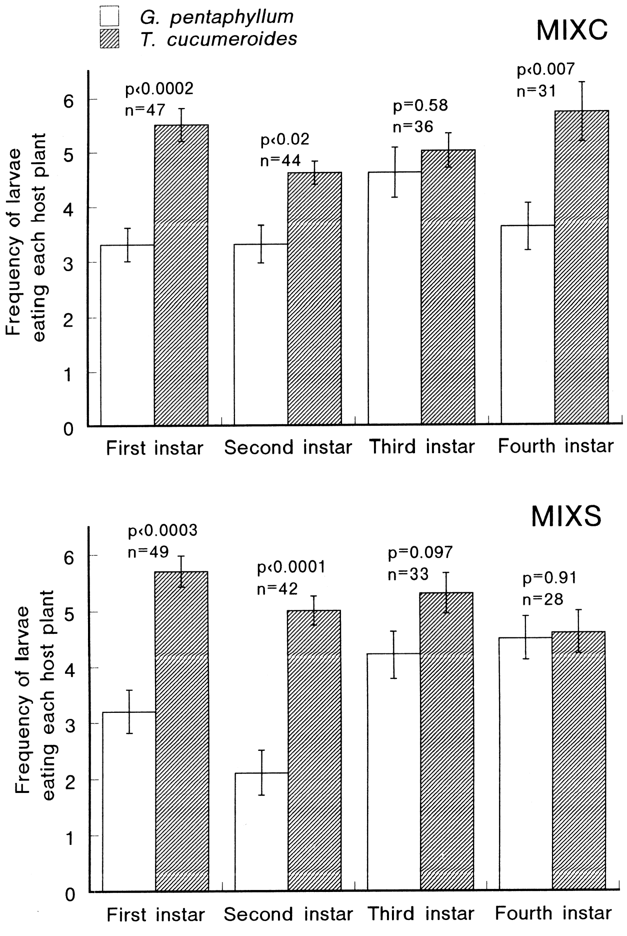

Fig. 5. Host plant selectivity of larvae in two mixed plant treatments. MIXC: two hosts were placed in contact with each other; MIXS: two hosts were placed separate from each other. Mean±SE. Statistical analyses were performed by paired *t*-test.

Adult host use

First, the effects of phenological constraints of the two host plants in relation to adult feeding and egg-laying site selection are discussed. Newly emerged adults of *E. admirabilis* feed on only *G. pentaphyllum* because it is the only available host in April. Adults pass through the pre-egg deposition period for approximately three months and the feeding activity temporary decreases in May and June (Takeuchi, unpublished data). After this feeding interruption, most adults remained and laid eggs on *G. pentaphyllum*, even if there is enough *T. cucumeroides* available. Recently, researchers have proposed that more abundant (Cunningham et al., 2001) and long-lasting (Hellmann, 2002) host species are more essential to herbivore fitness. In *E. admirabilis*, the number of adults on each host plant showed a poor correlation to the abundance of host plants. It is unclear why many adults remained on *G. pentaphyllum*, even though there was low leaf abundance and high injury. Concerning the fitness of the adults, if sufficient nutrition is obtained from a primary host food, females usually remain on that food irrespective of its abundance. Females were collected from the field in July and reared singly on different food treatments controlling abundance and injury level of *G. pentaphyllum*. Preliminary observations showed no difference in number of eggs and longevity, irrespective food condition (Takeuchi, unpublished data). In addition, phenological variation of intrinsic quality such as deterrent chemicals and nutritional content of host plants also seemed another alternative factor in host usage. If some deterrent chemicals were abundant in the leaves of *T. cucumeroides* in July and August, adults did not seem to utilize this host. We feel it is necessary to assess the qualitative effects of both host plants on preference and reproduction of the adult stages.

Feeding experience can significantly change the host selection of adults. Continual conditioning to *G. pentaphyllum* from emergence may contribute to the abundance of adults on this host plant and lead to an increased preference for it. When adults fed on one of the two host plants in the field were given a choice between both foods under laboratory conditions, the adults fed on the *G. pentaphyllum* showed a preference for the same plant. In contrast, adults fed on *T. cucumeroides* showed no preference (Takeuchi, unpublished data).

Next, the effects of the two host plants on egg-laying site selection is discussed. Our laboratory experiments showed that the two host plants had clearly different developmental effects on larval performance. *T. cucumeroides* supported higher larval survivorship and developmental rates than did *G. pentaphyllum* under both single- and mixed-food conditions. Growth was clearly accelerated in the larval stages by eating *T. cucumeroides*. In addition, when we compared the adult body lengths between the six study sites, there was a significant difference in both sexes (Takeuchi, unpublished data). Body length was smaller in sites where *G. pentaphyllum* was dominant. This was probably due to food shortage for larval stages on this host plant. Therefore, females of *E. admirabilis*, which were fed on *G. pentaphyllum* from emergence, should switch host plants and deposit eggs on *T. cucumeroides*. However, there was a remarkably large proportion of adults that remained on *G. pentaphyllum* and the number of eggs deposited was similar in both hosts (Takeuchi, 1994). The factor that determines this poor correlation between larval performance and adult egg-laying site selection in unclear. However, plant traits often have the potential to alter the success of the natural enemy herbivores. In this population, predator assassin bugs, *Sphedanolestes impressicollis* (Homoptera: Reduviidae), and parasitoid wasps, *Nothiserphus afissae* (Hymenoptera: Proctotrupidae) were observed as natural enemies of the larval stages. If predation and parasitic risk is associated with a particular plant, the plant traits may play a crucial role in host selection.

An another viewpoint concerning the egg-laying site selection is that the females often lay eggs on linear objects, such as the tendrils of the crane host plants. More crane tendrils were found on *G. pentaphyllum* than *T. cucumeroides*, so this may have increased the motivation of females to lay eggs on *G. pentaphyllum*.

Scheirs and De Bruyn (2002) proposed that optimal foraging and optimal oviposition by the adults needs to be integrated in order to understand the complex interactions between plants and insects. It is possible that alternative factors also have a large effect on host selection by *Epilachna*, and it is necessary to investigate multiple aspects of the plants and natural enemies to better understand host plant availability.

Larval host use

Larvae may feed more on plants that are the best food sources of total nutrients. This ‘complementary diet’ is known to be used by several generalist insects, such as grasshoppers (Raubenheimer and Simpson, 1993; Bernays et al., 1994; Behmer et al., 2002) and moths (Stockhoff, 1993; Singer et al., 2002).

On the other hand, the specialist herbivores do not appear to compensate for dietary defects by eating multiple host plants, but have adapted to only one or few host plants to obtain the required nutrition. In our rearing experiments, dietary switching between plants was not superior to a single food source of *T. cucumeroides*. Larvae grew faster as the proportion of *T. cucumeroides* increased. Specialist herbivores are clearly different from generalist herbivores that often show compensatory feeding. However, as an exception, Moreau et al. (2003) proposed that a similar compensatory mechanism exists for the specialist Diprionid sawfly that feeds on highly variable food resources, such as different age classes of foliage on coniferous trees.

In *E. admirabilis*, two host liana plants co-occur in the same habitat and intertwine with each other, larvae on one host plant can switch to the other. Since, females showed a preference to oviposit on *G. pentaphyllum*, hatching stages feed and grow on *G. pentaphyllum*, 70% to 80% of the first instar larvae were found on this plant. Then larvae switched food sources, the number of larvae on *T. cucumeroides* increased with developmental stage reaching 60% to 90% in 4th instar larvae. In our laboratory tests that allowed free choice between the two food plant hosts, the larvae switched food sources and showed a preference for *T. cucumeroides*. Additionally, the larvae switched food sources more in treatments when the two hosts were placed in contact with each other (MIXC). We suggest that larvae switched food sources often from *G. pentaphyllum* to *T. cucumeroides*, dependent on intrinsic quality and food availability (abundance and distribution in the range of which the transfer was possible) of these two host plants.

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REFERENCES

Behmer, S. T., S. J. Simpson and D. Raubenheimer (2002) Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology* 83: 2489–2501.

Benrey, B. and R. F. Denno (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. *Ecology* 78: 987–999.

Bernays, E. A., K. L. Bright, N. Gonzalez and J. Angel (1994) Dietary mixing in a generalist herbivore: tests of two hypotheses. *Ecology* 75: 1997–2006.

Cunningham, J. P., S. A. West and M. P. Zalucki (2001) Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos* 95: 537–543.

Hellmann, J. J. (2002) The effect of an environmental change on mobile butterfly larvae and the nutritional quality of their hosts. *J. Anim. Ecol.* 71: 925–936.

Leather, S. R. (1985) Oviposition preferences in relation to larval growth rates and survival in the pine beauty moth, *Pannolis flammea*. *Ecol. Entomol.* 10: 213–217.

Moreau, G., D. T. Quiring, E. S. Eveleigh and É. Bauce (2003) Advantages of a mixed diet: feeding on several foliar age classes increases the performance of a specialist insect herbivore. *Oecologia* 135: 391–399.

Raubenheimer, D. and S. J. Simpson (1993) The geometry of compensatory feeding in the locust. *Anim. Behav.* 45: 953–964.

Scheirs, J. and L. De Bruyn (2002) Integrating optimal foraging and optimal oviposition theory in plant-insect research. *Oikos* 96: 187–191.

Singer, M. S., E. A. Bernays and Y. Carriére (2002) The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. *Anim. Behav.* 64: 629–643.

Stockhoff, B. A. (1993) Diet heterogeneity: implications for growth of a generalist herbivore, the gypsy moth. *Ecology* 74: 1939–1949.

Takeuchi, M. (1994) Host utilization of phytophagous ladybird-beetle: host selection of phytophagous insects. *Biol. Sci.* (Tokyo) 46: 69–74 (in Japanese).

Takeuchi, M. and M. Tamura (1994) Life cycle of two populations of *Epilachna admirabilis* feeding on different host plants. *Jpn. J. Appl. Entomol. Zool.* 38: 79–84 (in Japanese with English summary).