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The Effect of Leaf Surface Wax on Feeding of the Strawberry Leaf Beetle, *Galerucella Vittaticollis*, with Reference to Host Plant Preference

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Summary

The relationship between host plant preference and feeding reduction by the removal of leaf surface wax from plant was investigated in the strawberry leaf beetle, *Galerucella vittaticollis*. In the host plants such as *Rumex acetosa*, *Persicaria thunbergii*, *Fragaria ananassa*, and *Rumex obtusifolius*, which the beetle preferred to feed in the leaf choice test, feeding amount was significantly decreased when the wax was removed, and the rate of the feeding reduction was 53-60%. While in *Reynoutria japonica* and *Persicaria lapathifolia*, which were less preferred, and in the non-host, *Salix gracilistyla*, there was no significant feeding reduction even when the wax removed. These results suggest the possibility that the feeding stimulant(s) contained in the leaf surface wax of host plant affects the host preference of the beetle.

Introduction

Due to the progress in the study on plants surface characteristics, it has been recognized that there are specific differences in chemical and morphological characters of plant surface wax (Jeffree, 1986). Several reports have demonstrated that the plant surface wax plays imporant roles in insect-plant interactions (Städler, 1986; Eigenbrode and Espelie, 1995).

We previously reported that the strawberry leaf beetle, *Galerucella vittaticollis* Baly is stimulated to feed by the leaf surface wax of the strawberry, *Fragaria ananassa* which is a host plant of the beetle (Adati and Matsuda, 1993). But the effects of the leaf suface wax of the host plants other than strawberry have not been studied anthough the beetle feeds on several polygonaceous plants in the field (Suzuki, 1987).

In the present paper, to clarify the role of leaf surface wax in host recognition of *G. vittaticollis*, relations between the beetle's host preference and feeding reduction by removal of the wax from host and non-host plants are examined.

Materials and Methods

Insects

Adults beetles were collected from the spontaneous communities of *Rumex obtusifolius* L. in our faculty campus. Succesive laboratory culture was maintained on fresh leaves of *Ru. obtusifolius* under 16L : 8D photoperiod at 24± 1°C.

Plants

The plants tested in this study are listed in Table 1. Matured leaves of each species were collected in our campus except for *Salix gracilistyla*, which was obtained from the Botanical Garden, Faculty of Science, Tohoku University, Sendai. The distinction of host and non-host was judged from the result of preliminary rearing experiment, in which the survival rates of larvae on the strawberry and all polygonaceous plants were 71-96%. Whereas on *S. gracilistyla*, the larvae fed a little on the leaves and never pupated.

Test of feeding preference

The plants leaves were cut into disks (2 cm dia.) with a cork borer. A leaf disk of each plant species was weighed and radially placed on the moist filter paper in a petri dish (12 cm dia.) with 3 cm of distances between the disks. Twenty adult beetles starved for 30 h were released into the test chambers, and control chambers without release of the beetle were left as they were. Twenty-four hours after release, all those leaf disks were weighd again. Feeding amount on each plant was caluculated as follows :

TABLE 1. *List of plants*

Species	Family	Host/Non-host[1]
Fragaria ananassa Duchesne	Rosaceae	Host
Persicaria lapathifolia (L.) S.F. Gray	Polygonaceae	Host
Persicaria thunbergii (Sieb. et Zucc.) H. Gross	Polygonaceae	Host
Reynoutria japonica Houtt.	Polygonaceae	Host
Rumex acetosa L.	Polygonaceae	Host
Rumex obtusifolius L.	Polygonaceae	Host
Salix gracilistyla Miq.	Salicaceae	Non-host

[1] See text.

$$F = T - T' \ (\Sigma C / \Sigma C')$$

Where F is feeding amount, T and T' are weight of the test disk before and after the release, respectively, and ΣC and $\Sigma C'$ are total weight of control disks before and after the test, respectively. Six replications of the test were run with three control chambers.

Comparison of feeding on intact and wax-removed leaves

The plant leaves were dipped in chloroform for 1 min. After the solvent on leaves dried at room temperature, the leaves were cut into disks (2 cm dia.). For each plant, six disks from an intact or wax-removed leaf were equidistantly placed, and the twenty adults starved for 24h were released in a petri dish (12 cm dia.). After 24h, the leaf disks were collected from the dish, and the feeding area was traced on section paper ruled into 1-mm square. For the mesurement of feeding area, complete and incomplete squares within the outline on section paper were measured as 1 and 0.5 mm^2, respectively, and total area was calculated by counting those squares. The test were run with four replications.

Results

Feeding preference

The result of choice test for feeding preference is shown in Table 2. The leaf disks from *Rumex acetosa*, *Persicaria thunbergii*, and *Ru. obtusifolius* ware chosen most preferably. The beetles only fed on little amount of *Reynoutria japonica*. The beetles did not feed on *S. gracilistyla* actually but data indicated a negative value of feeding amount because of the compensation for weight loss by drying. The feeding preference for *F. ananassa* and *Persicaria lapathifolia* was intermediate.

TABLE 2. Host preference of *G. vittaticollis* among seven plants species

Plant	Feeding amount (mg)[1]
Ru. acetosa	25.7 ± 15.8^{a}
P. thunbergii	20.7 ± 6.1^{ab}
Ru. obtusifolius	20.2 ± 5.2^{abc}
F. ananassa	14.9 ± 4.0^{abc}
P. lapathifolia	11.6 ± 1.5^{bcd}
Re. japonica	6.8 ± 7.7^{cd}
S. gracilistyla	-1.8 ± 1.0^{d}

[1] Mean $\pm$ SD. Means followed by the same letter are not significantly different at $P < 0.05$ by the Tukey-Kramer's test.

Feeding on intact and wax-removed leaves

The result from feeding and its difference between intact and wax-removed leaves are shown in Table 3. Significant difference in feeding amounts between intact and wax-removed leaves was observed on *Ru. acetosa*, *P. thunbergii*, *Ru. obtusifolius* and *F. ananassa*. The rate of feeding reduction in those plants was 53-60%. However, there is no significant feeding reduction in *Re. japonica*, *P. lapathifolia* and *S. gracilistyla*, and the reduction was relatively low.

Discussion

Two experiments in this study showed that the more a plant was preferred by the beetle, the more feeding reduction occured when leaf surface wax was removed from the plant. For example, *Ru. acetosa*, *P. thunbergii* and *F. ananassa* in which the highest reduction rates were indicated, were the most preferable (Table 2 and 3). While in the less preferred hosts such as *Re. japonica*, and in *P. lapathifolia* and the non-host *S. gracilistyla*, the reduction rates were low or very low. Woodhead (1983) reported a similar phenomenon. The unpalatable sorghum seedings became acceptable by nymphs of *Locusta migratoria* when the surface wax was removed. It seems likely that the wax of the plant leaves mentioned above does not contain feeding stimulants, or contains feeding deterrents to the beetle.

Our results indicated that the change of feeding amount by the wax removal differed between plants. The previous study showed that the beetle was stimulated to feed by the *n*-alkane and *n*-carboxylic acid containing fractions which dominantly exsited in the wax of strawberry leaf (Adati and Matsuda, 1993). It

TABLE 3. *Feeding of adult beetles on intact and wax-removed leaf disks of seven plant species*

Plant	Area eaten on disk (cm^2)[a]		Feeding reduction[b]
	Intact (I)	Wax-removed (R)	
Ru. acetosa	4.3±1.1	1.7±0.3	0.60**
P. thunbergii	11.5±2.5	4.7±1.6	0.59**
F. ananassa	6.6±1.4	2.8±1.4	0.58**
Ru. obtusifolius	3.8±1.5	1.8±0.2	0.53*
Re. japonica	5.9±2.5	4.7±1.6	0.20
P. lapathifolia	4.7±0.9	4.6±0.4	0.02
S. gracilistyla	0.2±0.1	0.9±0.9	−3.50

[a] Mean±SD.

[b] Feeding reduction $=(I-R)/I$, where I and R are the mean areas eaten on intact and wax-removed leaves, respectively. Means are significantly different between I and R by the unpaired-sample t test at $P<0.05$ (*) and $P<0.01$ (**).

may be possible that the feeding stimulant(s) contained in the leaf surface wax of plants contribute to the host preference of the beetle in terms of quality and/or quantity, although the other feeding stimulat(s) contained in the plant affects the feeding of the beetle. Further investigation of the beetle to the wax in the broad range of host and non-host plant is necessary.

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