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A Leaf-rolling Caterpillar Improves Leaf Quality

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Abstract. Larvae of *Rhopobota naevana* (Lepidoptera: Tortricidae) make leaf shelters by rolling leaves of the Japanese lilac, *Syringa reticulata* (Oleaceae). Compared with fully expanded leaves, rolled leaves showed a 21% reduction in toughness, a 55% reduction in total phenolic content and a 20% increase in nitrogen content. A bioassay using free-living larvae of *Samia ricini* (Lepidoptera: Saturniidae) was performed. *Samia ricini* larvae that were reared on rolled leaves grew up faster than those reared on expanded leaves. These results clearly show that rolled leaves are superior food for lepidopteran larvae and suggest that leaf rolling made by lepidopteran species would induce beneficial changes in other lepidopteran species.

Key words: anti-herbivore defenses, leaf roller, *Rhopobota naevana*, phytophagy, plant-insect interactions, *Syringa reticulata*.

Introduction

The anti-herbivore defenses of plants have been reported to have important effects on herbivore fitness (Fritz & Simms, 1992). There is considerable evidence that secondary compounds (chemical defense) and plant physical characteristics (physical defense) reduce herbivore survival, reproductive output, or plant preferences (Fritz & Simms, 1992; Karban & Baldwin, 1997). On the other hand, there is also evidence for counter-adaptation by phytophagous insects to plant defenses, e.g., detoxifying anti-herbivore chemicals or inducing favorable changes in the host (Karban & Baldwin, 1997). One example is the vein-cutting behavior of milkweed feeders on a latex-producing plant, *Asclepias syriaca* (Dussourd & Eisner, 1987; Malcom & Zalucki 1996). Vein cutting blocks latex flow to the feeding sites and induces beneficial changes in the host plant for the milkweed feeders.

Making leaf shelters, such as leaf-folds, -galls, -rolls, -sandwiches, and -ties, is widespread in herbivorous arthropods. It has been reported that leaf shelters reduce the anti-herbivore defenses of host plants; concentrations of anti-herbivore defensive compounds were reduced in leaf shelters compared to normal leaves (Berenbaum, 1978; Sandberg & Berenbaum, 1989). The presence of hypericin in St. John's -wort, *Hypericum perforatum* (Guttiferae), reduced the survivorship of tortricid larvae (*Heliothis zea* and *Platynota flavedana*) that fed on leaves in the light and prolonged their development. When the light wavelength that photoactivates the toxin hypericin was excluded by filters, larval survival was enhanced (Sandberg & Berenbaum, 1989). In the wild, *P. flavedana* successfully survived on the plant by tying leaves together and feeding on the ties inside.

Sagers (1992) reported that artificial binding of leaves reduced the physical and chemical defenses of the neotropical shrub, *Psychotria horizontalis* (Rubiaceae), without altering its nutritional status. Artificially binding the leaves of *P. horizontalis* decreased toughness by 31% and leaf tannin concentration by 15% compared with expanded leaves, while having no effect on nitrogen concentration and water content. Caterpillars, *Desmia* sp. (Lepidoptera: Pyralidae) preferred to feed on artificially bound leaves.

This study examines whether leaf rolling induces changes in leaf quality of the Japanese lilac, *Syringa reticulata* Hara (Oleaceae), and how the changes affect the performance of lepidopteran larvae.

Materials and Methods

Observations and experiments were conducted in the Tomakomai Experimental Forest, Hokkaido University (42°43′N, 141°36′E), Hokkaido, northern Japan, from May to June 2000. Larvae of *Rhopobota naevana* (Huebner) (Lepidoptera: Tortricidae) are one of the dominant phytophagous species on the Japanese lilac tree, *S. reticulata*, a common understory shrub in the study site. *R. naevana* larvae attack the lilac buds and roll pairs of leaves together into corn-cob shapes. They feed, develop and pupate within the leaf-shelters. After the larvae leave the leaf-rolls, vacant leaf-rolls remain on the tree throughout the growing season.

To equalize environmental conditions between samples of expanded and rolled leaves, one expanded leaf and one rolled leaf were taken from the top of each shoot on the same trees from 9 June to 17 June. The examined characteristics were leaf toughness, total phenolic content, nitrogen and carbon concentration and water content, all of which are often negatively or positively correlated with insect performance (White, 1993). Leaf toughness and total phenolics concentration were measured to assess the physical and chemical defenses of leaves (Karban & Baldwin 1997). Leaf toughness was measured with a penetrometer that records the force necessary to punch a hole (3 mm in diameter) through a leaf blade (rolled leaves: $N=15$, fully expanded leaves: $N=15$). The total concentration of phenolics was measured as shown in Julkunen-Titto (1985), Orians (1994), and Orians & Fritz (1995). Leaves of the lilac trees did not contain tannin. The concentrations of nitrogen and carbon and water content were measured to assess the nutritional status. Nitrogen concentration is defined here as nitrogen weight to dry leaf weight. Carbon concentration is defined similarly. Nitrogen and carbon concentrations were measured with a C/N analyzer (Sumitomo NC-900, Osaka, Japan). Water content is defined here as the proportion of water weight to fresh leaf weight. Data were analyzed using the paired *t*-test between expanded and rolled leaves. The proportion of water content was transformed to arcsine square-root in order to satisfy the requirement of normality.

To determine leaf quality as a food, we carried out a rearing experiment with the larvae of *Samia ricini* Donovan (Lepidoptera: Saturniidae), which had been reared at Laboratory of Insect-Plant Interactions in National Institute of Agrobiological Sciences. The larvae of this species are suitable for bioassays because they feed on other plants of their natural hosts (Konno, unpublished data). Furthermore, they have a favorable habit for bioassay in that they usually show a particular symptom corresponding to each plant species on which they feed, e.g., larval body weight (Konno, personal communication). A total of 500 eggs from two clutches were split evenly into two groups; in one group larvae were reared on fully expanded lilac leaves (control: 250 larvae) and in the other group larvae were reared on rolled leaves of *R. naevana* (leaf-roll group: 250 larvae). Expanded leaves and rolled leaves were taken from the top of shoots on the same trees. The larvae of *S. ricini* hatched on May 23 and we started the rearing experiment immediately. They were kept at 25C° and under 12L 12D during this experiment. Fifty larvae were weighed five times throughout the experimental period (on the 9th, 12th, 15th, 18th, and 21st days after hatching). The larvae weighed were removed from the experiment after measuring. All of the larvae survived through this experimental period. The experiment terminated on June 12. The data were analyzed using Two-way ANOVA, with leaf type and days treated as independent variables.

Results

The leaf toughness and total phenolic content of rolled leaves decreased significantly compared to those of the expanded leaves (Table 1). The rolled leaves contained significantly more nitrogen than did expanded leaves. The carbon and water contents did not differ significantly between the two types of leaves (Table 1). Larvae of the leaf-roll group were significantly heavier than those of control group (Fig. 1 and Table 2), and the growth rate differed significantly between the two groups (for leaf type×days, $P<0.0001$).

Discussion

This study clearly demonstrated that the leaves rolled by *R. naevana* larvae decreased leaf toughness and total phenolic content but increased nitrogen concentration (Table 1), resulting in a significant gain in weight for *S. ricini* larvae that were reared on rolled leaves as compared with larvae that were reared on expanded leaves (Fig. 1). It is most likely that the reduction in the physical and chemical defense traits and the increase in nutritional quality of the leaves provided positive effects on the *S. ricini* larval growth. Thus, we suggest that leaf rolling induces beneficial changes in the host plant for lepidopteran larvae.

Table 1. Comparison of characteristics between expanded leaves and rolled leaves. Each value is the mean ±1 SE. DW = dry weight. FW = fresh weight.

Characteristics	Expanded leaves (n)	Rolled leaves (n)	Paired-*t*	*P*
Leaf toughness (mg mm^{-2})	167.55±5.91 (15)	132.37±6.05 (15)	4.16	<0.001
Total phenolics (mg g^{-1} DW)	35.53±2.65 (6)	24.20±1.42 (6)	3.54	0.006
Nitrogen content (mg g^{-1} DW)	18.57±1.13 (6)	22.19±0.86 (6)	6.59	0.029
Carbon content (mg g^{-1} DW)	460.01±2.59 (6)	466.48±3.28 (6)	2.40	0.15
Water content (% FW)	65.89±0.57 (9)	67.14±1.49 (9)	0.93	0.39

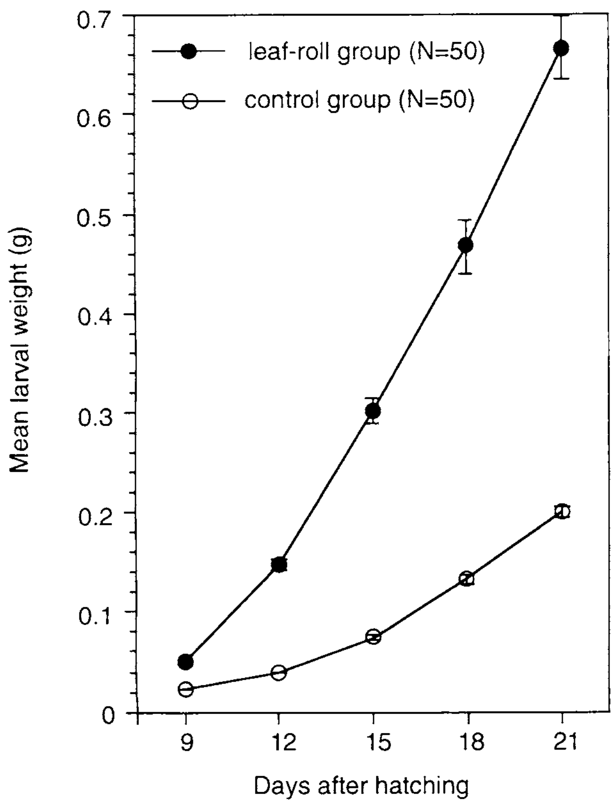

Fig. 1. Mean weight of *S. ricini* larvae of rolled group (N=50) and control group (N=50). Vertical bars indicate SE.

Table 2. Two-way ANOVA of the effect of leaf type as a food for *S. ricini* larvae on body weight over 9th, 12th, 15th, 18th, 21st day after hatching.

Source	df	Mean square	*F*-ratio	*P*
leaf type	1	6.32	661.62	<0.001
days	4	2.43	254.54	<0.001
leaf type x days	4	0.80	83.62	<0.001
Error	490	0.01		

Our results also suggest that leaf rolling behavior might be a counter-adaptation against host-plant defenses. Sagers (1992) found artificially binding leaves of *P. horizontalis* decreased anti-herivore defence, without altering nutritional status. The present study supports the results of Sagers (1992), except that for nitrogen concentration (Table 1). The reason why rolled leaves of the Japanese lilac showed a rise in nitrogen concentration while those of *P. horizontalis* did not, may be that leaf rolling does not induce identical changes in each plant species. There may be another possibility. Sagers (1992) used artificially bound leaves for the experiment, while we used leaves rolled by larvae. Therefore, it is possible that larvae can influence the nitrogen concentration of rolled leaves.

Occupants of leaf shelters benefit from a favorable microhabitat, protection from natural enemies, and reduction in anti-herbivore defenses (Damman, 1993; Fukui 2001). Previous studies reported that architecturally modified leaves, such as those rolled by lepidopteran larvae, frequently contain organisms that did not build the shelters (Mani, 1964; Damman, 1993; Fukui, 2001), which are called secondary users. Free-living species of secondary users consist of a wide range of taxa including Coleoptera, Dermaptera, Diptera, Hemiptera, Homoptera, Hymenoptera Lepidoptera, Orthoptera, spiders, and terrestrial isopods (Fukui, 2001). Some studies showed that not only shelter makers but also secondary users could take advantage of leaf shelters. The neonate larvae of the leaf beetle *Galerucella lineola* (Fabricius) inhabit and grow up in artificially rolled shelters or leaf-galls made by the cecidomyiid *Dasineura marginemtorquens* (Bremi) to escape from desiccation (Larsson *et al.*, 1997). Leaf-rolls made by caterpillars reduce the leaf toughness of the sunflower *Helianthus annuus* and provide more easily consumable food for the grasshopper *Melanoplus differentialis* (Thomas) (Lewis, 1977). Kudo (1994) reported that nymphs and newly-molted adults of the acanthosomatid bug *Elasmucha putoni*

(Scott) are more often found in leaf shelters than on leaves, suggesting that this behavior might protect molting bugs from predators and adverse weather conditions.

It has been pointed out that one mechanism of the positive interactions among herbivore species is the mutual use of shelters (Cappuccino, 1993; Damman, 1993). Even though *S. ricini* is not a leaf roller and cannot make leaf rolls by itself, the larvae could take advantage of the leaves rolled by *R. naevana*. Other phytophagous insects and snails were frequently observed as secondary users in rolled leaves made by larvae of *R. naevana* (Fukui, unpublished). The increase in larval weight of *S. ricini* suggests that the nutritional status of rolled leaves improves the performance of the secondary leaf-roll users on lilac trees in general.

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