

Leaf Miner as a Physical Ecosystem Engineer: Secondary Use of Vacant Leaf Mines by Other Arthropods

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ABSTRACT We have documented the secondary use of vacant leaf mines of a lepidopteran leaf miner, *Phyllonorycter pastorella* (Zellar), by other arthropods. At least six species were identified as secondary users of the vacant mines. Among secondary users, aphids and springtails were most abundant. Distribution patterns of these two insects showed that aphids used vacant mines by chance and that springtails used them selectively as feeding and reproductive sites. Hole diameter of mines did not affect the use of vacant leaf mines by aphids and springtails. We believe this is the first report on leaf miners as physical ecosystem engineers that provide modified habitats to other organisms.

KEY WORDS aphids, habitat modification, indirect interaction, springtails

PHYSICAL ECOSYSTEM ENGINEERS ARE defined as organisms that directly or indirectly control the availability of resources to other organisms by causing physical changes in biotic or abiotic materials (Jones et al. 1997). There is increasing evidence that physical ecosystem engineering plays an important role in structuring of both plant and animal communities (Martinsen et al. 2000, Reichman and Seabloom 2002, Wright et al. 2002). Leafrollers that change plant leaves physically (i.e., leaf rolls, folds, ties, and sandwiches) are good examples of physical ecosystem engineers in terrestrial arthropod communities (Martinsen et al. 2000, Fournier et al. 2003, Lill and Marquis 2003, Nakamura and Ohgushi 2003). The principal function of leafrollers is to provide leaf rolls as habitats for themselves. Other organisms that secondarily use leaf rolls may receive some benefits, including favorite microclimates (Larsson et al. 1997), high-quality food (Sagers 1992, Fukui et al. 2002), and escape from natural enemies (Cappuccino 1993, Jones et al. 2002), without the costs of making leaf rolls. Therefore, many organisms of several taxa use leaf rolls made by primary leafrollers to receive these benefits (Fukui 2001). Leaf rolls have been shown to increase species richness and abundance of arthropods, including both herbivores and their predators on plants (Martinsen et al. 2000, Lill and Marquis 2003).

Another type of architectural modification of plant leaves is leafmining by various insects, including Lepidoptera, Diptera, Coleoptera, and Hymenoptera. When leafminers complete their development, they leave the leaf mine, making an emergence hole at the leaf epidermis (Auerbach et al. 1995). Subsequently, a vacant mine with an entrance hole remains inside the leaf. This vacant leaf mine may have a similar function to leaf rolls in that the vacant leaf mines may be used

by other arthropods. However, no studies have yet been reported on the secondary use of vacant leaf mines by other organisms that do not make leaf mines. In addition, if the availability of vacant leaf mines is dependent on entrance hole size, hole size may be critical for different secondary users that inhabit vacant mines. Entrance holes of leaf mines vary in size because they are created not only by the emergence of leafminers but also by parasitic wasps, and enlarged preexisting holes due to physical damage.

Here, we have documented arthropod secondary users of vacant leaf mines that were made by the lepidopteran leafminer *Phyllonorycter pastorella* (Zellar) (Gracillariidae) on willow (*Salix* spp.) trees. We then determined 1) whether arthropods use the vacant leaf mines selectively or by chance, and 2) whether secondary use of vacant mines is dependent on hole size of mine entrances.

Materials and Methods

Study System. This study was conducted in 2003 on the flood plain along the Yasu River in Shiga Prefecture, central Japan. Willows are the predominant woody plants in the area, and at least seven species of willows occur sympatrically: *Salix chaenomeloides* Kimura, *Salix eriocarpa* Franchet et Savatier, *Salix gilgiana* Seemen, *Salix gracilistyla* Miquel, *Salix integra* Thunberg, *Salix serissaefolia* Kimura, and *Salix subfragilis* Andersson. Of these willows, *S. eriocarpa*, *S. integra*, and *S. serissaefolia* occur most frequently in this area (Inui et al. 2003).

The leafminer *P. pastorella* uses exclusively several willow and poplar species as host plants (Kuroko 1982). In the study area, the leafminer used *S. eriocarpa*, *S. gilgiana*, and *S. serissaefolia*, and in 2003 was

observed most frequently on *S. eriocarpa*. A larva of *P. pastorella* mines the underside of a leaf, forming a blotch-type mine. Whereas young larvae are sap feeders and expand the area of mines, old larvae are tissue feeders that excavate the mines without increasing the area of mines, reaching the upper side of leaf epidermis. In Hokkaido, northern Japan, this leafminer consumed $\approx 1.0 \text{ cm}^2$ of leaf area of *Salix miyabeana* Seemen before pupating (Kagata and Ohgushi 2001). Pupation occurs within the mine, and the adult moth ecloses through the underside of the leaf epidermis, making an emergence hole. The pupal exuvium protrudes from the leaf epidermis for several days after eclosion.

Survey of Secondary Users. We randomly collected mined leaves of *P. pastorella* from 18 trees of *S. eriocarpa* in late September to early October, which corresponds to the period after the eclosion peak of the third generation of the leafminer. In addition, we collected the nearest unmined leaves from the same shoot as controls. In total, 328 leaves (164 mined leaves and 164 unmined leaves) were collected from the study trees. Leaf size did not differ significantly between mined and unmined leaves (analysis of variance [ANOVA], $df = 1$, $F = 1.44$, $P > 0.05$; mean leaf length \pm SE, 7.32 ± 0.14 and 7.55 ± 0.13 cm for mined and unmined leaves, respectively). Leaves were collected carefully without disturbing arthropods on the leaves and were individually packed into small plastic bags. All sampled leaves were immediately brought to the laboratory. Species and abundance of arthropods on leaf surfaces were recorded. The mined leaves were subsequently dissected in 70% ethanol under a binocular microscope. Species and abundance of arthropods in the mines were recorded, and hole diameters of mines were measured. When a pupal exuvium of the leafminer was still attached to the leaf epidermis, the hole was regarded as the emergence hole of the leafminer. Arthropods on leaves or within mines were fixed in 70% ethanol and identified later. For abundant species of aphid and springtails, body width of 100 randomly selected individuals was measured. In addition, the major axis length and area of 15 leaf mines were measured using the public domain software NIH Image (<http://www.tsb.info.nih.gov/nih-image>).

Data Analyses and Statistics. Differences in the proportion of leaves with arthropods and the number of arthropods per habitat were tested by χ^2 test and Mann-Whitney *U* test with sequential Bonferroni correction ($P = 0.05$), respectively, for multiple comparisons among the three habitats. To determine whether the arthropods used vacant leaf mines selectively or by chance, we compared the frequency of occupation and the abundance of aphids and springtails among three types of habitats (within mines, leaf surface of mined and unmined leaves) by χ^2 test and ANOVA, respectively. Scheffé test ($P = 0.05$) was performed as a post hoc test after ANOVA. Abundance data were log-transformed before analysis when necessary to meet the assumptions of ANOVA. To determine whether the secondary use of a vacant mine was de-

Table 1. Arthropod fauna and number of *S. eriocarpa* leaves with arthropods within mines and on the surface of mined and unmined leaves

Arthropod species	Mined leaves		Unmined leaves
	Surface	In mine	Surface
Aphid	41 (322)	41 (91)	32 (281)
<i>C. saliniger</i>			
Springtails	31 (92)	64 (950)	0
<i>Entomobrya</i> sp. 1			
<i>Entomobrya</i> sp. 2			
Lace bug	1 (1)	0	4 (16)
<i>M. populi</i>			
Thrips	1 (1)	6 (6)	1 (1)
Caterpillar	0	5 (5)	0
Spider	2 (2)	1 (1)	0
<i>Lysiteles</i> sp.			
<i>Tetragnatha</i> sp.			
<i>Clubiona</i> sp.			
Ant	0	0	3 (3)
<i>L. sakagamii</i>			

Values in parentheses show number of arthropods. Sample size was 164 leaves each. Arthropods without species name were not identified to species level.

pendent on its hole size, hole size distribution was compared between mines occupied by secondary users and all mines by a Kolmogorov-Smirnov test. In addition, Mann-Whitney *U* test was performed to determine whether the hole size of mines used by aphids differs from that used by springtails, and whether the body width of aphids differs from that of springtails. Springtail species were pooled in the analyses.

Results

Secondary Users. We found 1772 individual arthropods on and within leaves (Table 1). They included at least 10 species from six orders: aphid *Chaitophorus saliniger* Shinji (Aphididae: Hemiptera); springtails, *Entomobrya* sp. 1 and 2 (Collembola: Entomobryidae); lace bug *Metasalis populi* Takeya (Hemiptera: Tingidae); thrips, unknown species (Thysanoptera); caterpillars, unknown species (Lepidoptera); spiders *Lysiteles* sp. (Araneae: Thomisidae), *Tetragnatha* sp. (Araneae: Tetragnathidae), and *Clubiona* sp. (Araneae: Clubionidae); and ant *Lasius sakagamii* Yamachi & Hayashida (Hymenoptera: Formicidae). Although the caterpillars could not be identified to species, they were not leafminers and were identified based on morphology and size to belong to Yponomeutidae, Oecophoridae, Gelechiidae, Tortricidae, Pyralidae, and Crambidae (Y. Sakamaki, personal communication). Thrips and spiders were not identified to species because they were immatures. All ants collected were attending to aphid colonies on leaf surfaces.

Arthropods were more frequent (χ^2 tests with Bonferroni correction, $P < 0.05$ for each comparison; Fig. 1a) and abundant (Mann-Whitney *U* tests with Bonferroni correction, $P < 0.05$ for each comparison; Fig. 1b) within mines than on the surface of mined and unmined leaves. Of the 164 leaf mines, 90 mines (54.9%) were secondarily occupied by other arthro-

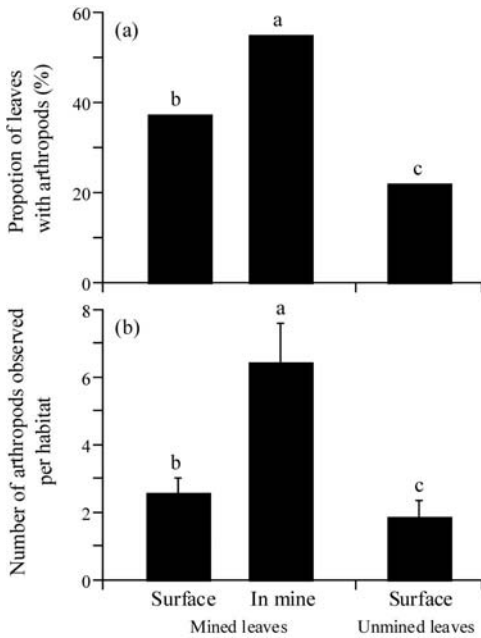


Fig. 1. Proportion of leaves with arthropods (a) and mean number of arthropods on the surface and inside mines of mined leaves, and on the surface of unmined leaves (b). Sample size was 164 leaves each. Vertical bars show SE. Different letters show significant difference ($P < 0.05$).

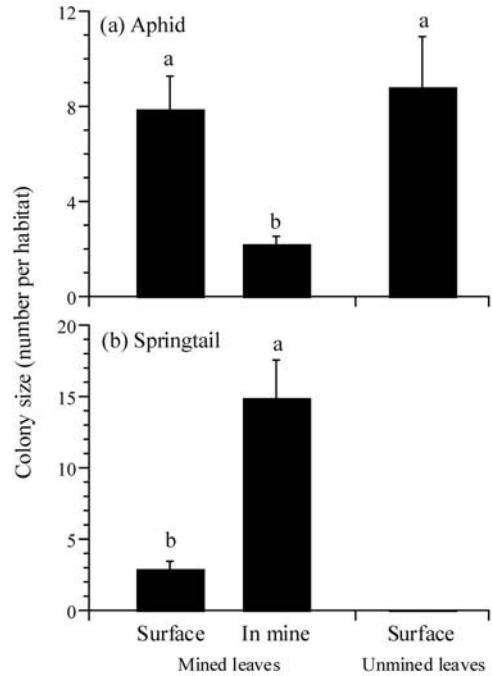


Fig. 2. Colony size of aphids (a) and springtails (b) on the surface and in the mines of mined leaves, and on the surface of unmined leaves. Vertical bars show SE. Different letters show significant difference ($P < 0.05$).

Pods, and 1,053 individuals of at least six species were found within mines, i.e., an aphid, springtails, thrips, caterpillars, and a spider (Table 1). The aphid *C. saliniger* and springtails *Entomobrya* sp. 1 and 2 were the most abundant species in the mines, occupying 25.0% (91 individuals in 41 mines) and 39.0% (950 individuals in 64 mines) of the vacant leaf mines, respectively. In the mines, other arthropods were observed at a low frequency, and only one predator, the spider *Clubiona* sp., was found. In addition, several mites were observed in the mines, but we failed to capture them.

Specific Use of Leaf Mines. Aphids were observed in all three habitats, i.e., within mines and on the surface of both mined and unmined leaves, and the frequency of occupation did not differ among habitats ($\chi^2 = 1.85$, $df = 2$, $P > 0.05$; Table 1). However, the springtails were observed within mines and on the surface of mined leaves, but not on the surface of unmined leaves (Table 1). They were found more frequently within mines than on the surface of mined leaves ($\chi^2 = 15.08$, $df = 1$, $P = 0.0001$). The habitat choice of other species was not examined because of small sample size.

Aphid colony size, i.e., number of individuals in each habitat, differed among habitats, and it was significantly smaller in mines compared with on the surface of mined or unmined leaves (ANOVA with Scheffé test: $df = 2$, $F = 7.72$, $P = 0.0007$; Fig. 2a). In contrast, colony size of the springtails was significantly larger within mines than on the leaf surface

(ANOVA with Scheffé test: $df = 1$, $F = 32.79$, $P < 0.0001$; Fig. 2b). The springtail colonies included many nymphs and adults. The largest springtail colony in a single mine contained 141 nymphs and adults. Springtail eggs also were found within the mines.

Hole Size and Secondary Users. The mean mined area \pm SE of the leafminer was 1.15 ± 0.04 cm², and mine length ranged from 13.8 to 23.5 mm (mean \pm SE, 18.0 ± 0.6 mm). Hole diameter of mines ranged from 0.1 to 16.8 mm, with three peaks in frequency at ≈ 0.2 –0.4, 1.2–1.5, and 10.0–11.0 mm. Emergence holes of the leaf miner measured 1.2 ± 0.1 mm in diameter on average, corresponding to the second peak of the hole size distribution. There was no significant difference in hole size distribution between all vacant mines and mines with secondary users (Kolmogorov–Smirnov test: $P > 0.05$). The body width of the aphids (including nymphs and adults) was significantly larger than that of the springtails (Man–Whitney *U* test: $U = 647$, $P < 0.0001$; mean body size \pm SE = 0.51 ± 0.02 and 0.26 ± 0.01 mm for aphids and springtails, respectively). There was no significant difference in hole size of mines used by the aphids and the springtails. (Mann–Whitney *U* test: $U = 1163$, $P > 0.05$, mean hole size = 3.75 ± 0.63 and 2.68 ± 0.41 mm for aphids and springtails, respectively).

Discussion

Secondary Users of Leaf Mines. Our results clearly documented that at least six species of arthropods

secondarily used vacant leaf mines formed by *P. pastorella*. We believe this is the first report of the secondary use of leaf mines. Leaf miners gain some benefits from inhabiting leaf mines, which is a major reason for the evolution of the leaf-mining habitat (Connor and Taverner 1997). They also pointed out these benefits include protection from unfavorable environmental conditions (such as desiccation, UV radiation, rain, and wind), avoidance of plant defenses of leaf epidermis layers (such as tough leaf epidermis or defensive chemicals on the leaf surface), and protection from attack by natural enemies and pathogens. However, leafminers are known to often suffer from heavy attack by parasitic wasps, compared with externally feeding herbivores (Hawkins 1994). Secondary users of leafmines also would gain some of these benefits by inhabiting vacant leafmines.

Of the arthropods observed in the leafmines, the aphid *C. saliniger* and two species of springtails, *Entomobrya* spp., were the most dominant. However, distribution patterns of the two insects were different. Whereas aphids occurred within mines and on surface of both mined and unmined leaves at similar proportions, colony size was smaller within mines than in the other habitats. Hence, the aphid is not likely to have a strong preference for vacant leaf mines. *C. saliniger* colonies were frequently observed in leaf rolls of a willow, *S. miyabeana* (Nakamura and Ohgushi 2003). Vacant leaf mines may be less favorable to the aphid than leaf rolls, because the leaf mines could be too small to permit the aphid to develop colonies through reproduction; the average area of *P. pastorella* mines was 1.15 cm². However, the springtails showed a strong preference for vacant leaf mines, and most individuals on willows were found within leaf mines. Because we found few springtails on leaf surfaces when sampling in the field, individuals observed on the leaf surface in the laboratory probably escaped from leaf mines during transportation. They feed on fungi on leafminer frass in the mines (H. Takeda, personal communication). In addition, the springtails must use vacant leaf mines as not only feeding sites but also reproductive sites, because many eggs and nymphs were observed together with adults in the mines. These findings indicate that the vacant leaf mines provided a more favorable habitat than leaf surface for springtails.

Hole Size and Secondary Users. Hole diameter of leaf mines varied widely from 0.1 to 16.8 mm. Of these, holes of diameter 0.8–1.5 mm were emergence holes of the leafminer *P. pastorella*. Small holes, <0.8 mm, were due to parasitic wasps, and large holes, >1.5 mm, probably resulted from expansion by abiotic factors or other organisms after insect emergence. We had expected that the availability of vacant leafmines would be different for secondary users, depending on hole size, because small holes may prevent large secondary users from entering leafmines. In addition, mines with a large hole may be unfavorable microclimates and unsuitable as shelters against natural enemies. However, the hole sizes of leafmines used by the two insects did not differ, although the two insects differed

appreciably in body size. Furthermore, aphids and springtails used leafmines with holes far larger than their body width. Thus, hole size did not affect the use of leafmines, at least by the aphid and springtails that were dominant secondary users of leafmines that we examined. However, relatively large insects, such as ants and lace bugs that were not found in the mines, may not use vacant mines due to hole size limitation.

Leafminers as Ecosystem Engineers. The ecological impacts of leaf miners have often been studied from the viewpoint of direct interactions (such as inter- and intraspecific competition) (Stiling et al. 1984, Sato 1991), parasitism (Connor and Cargain 1994, Kato 1994, Rott and Godfray 2000), and the initiation of premature leaf abscission by host plants (Auerbach and Simberloff 1989, Preszler and Price 1993). However, a few studies have demonstrated that leafminers indirectly affect the distribution and performance of other herbivores, mediated by changes in nutritional quality of host plants (Masters and Brown 1992, Johnson et al. 2002). For example, Johnson et al. (2002) reported that damage to the midrib by lepidopteran leafminers resulted in poor survival of an aphid that occurred on the mined leaf. This could be due to a physical disruption of phloem hydraulics by leafminers, which lowered the quality of phloem sap for the aphid. In contrast, our results indicate that the leafminer modified habitat resource availability for other arthropods via architectural changes in leaf structure. This meets the definition of physical ecosystem engineers (Jones et al. 1997) and has often been reported as a function of leafrollers (Fukui 2001). Resource modification by leafrollers has been shown to positively or negatively affect many species of arthropods, and the impacts spread from individuals living in and around leaf rolls to affect arthropod community structure on a tree-wide scale, through interaction chains between species (Fournier et al. 2003, Lill and Marquis 2003, Nakamura and Ohgushi 2003). Compared with the leafrollers, the impact of individual leafminers as ecosystem engineers may be weak, because the new habitat created by leafminers (i.e., vacant leaf mines) is relatively small. However, these small habitats may be more important for small arthropods, such as springtails, than leaf rolls, because they may be able to monopolize new habitats without relatively large competitors. To better understand the role of ecosystem engineers in structuring arthropod communities, it is important to focus on how organisms function as ecosystem engineers on different spatial scales.

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