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Positive and negative effects of leaf shelters on herbivorous insects: linking multiple herbivore species on a willow

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Abstract We experimentally examined the effects on other herbivorous insects of leaf shelters constructed by lepidopteran larvae on a willow, *Salix miyabeana*. Several insect species occupied the vacant leaf shelters. Our experiment using artificial leaf shelters showed that the number of aphids increased with the number of artificial leaf shelters on a shoot, as did the numbers of three ant species (*Camponotus japonicus*, *Lasius hayashi*, and *Myrmica jessensis*) that entered leaf shelters to collect aphid honeydew. To determine the ant-mediated effect of leaf shelters on herbivorous insects that do not use leaf shelters, we transferred newly hatched larvae of a common leaf beetle, *Plagiodera versicolora*, to the leaves of shoots with and without artificial leaf shelters. One day after the transfer, larval survival rate was significantly lower on shoots with shelters than on those without shelters, and shoots with shelters had significantly more ants than did shoots without shelters. Our field experiments demonstrated clearly that shelter-making lepidopteran larvae increased the abundance of both aphids and ants and decreased the survival rate of leaf beetle larvae, probably because the larvae were removed by ants that were attracted to the leaf shelters by the aphid colonies.

Keywords Ant-aphid mutualism · Habitat resources · Shelter-makers · Secondary users · Shelter-mediated interactions

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Introduction

The prevalence of interspecific interactions among herbivorous insects has long been discounted in insect community ecology (Hairston et al. 1960; Lawton and Strong 1981). Recently, however, there has been increasing evidence that herbivorous insects sharing a host plant often do affect one other considerably by changing the quality or quantity of the host plant (Faeth 1986, 1987; Karban and Myers 1989; Hunter and West 1990; Damman 1993; Denno et al. 1995; Ohgushi 1997). Herbivory by one species often induces morphological, phenological, and chemical changes in the host plant that, in turn, alter the availability of resources for other species that feed at different times or on different parts of the plant. Such indirect plant-mediated interactions may commonly occur between species that are separated by time or space (Faeth 1986, 1987; Harrison and Karban 1986; Gange and Brown 1989; Moran and Whitham 1990; Masters and Brown 1992; Masters et al. 1993; Ohgushi 1997).

In the study of plant-mediated interactions, much attention has been paid to changes in the quality or quantity of host plants following herbivory, because plants have mainly been considered simply as food for herbivorous insects (Faeth 1986, 1987; Karban and Myers 1989). However, plants provide not only food but also microhabitats that protect many insect species against natural enemies or environmental stress (Strong et al. 1984; Hunter and West 1990).

Several studies have reported that leaf shelters, such as leaf rolls and leaf galls made by herbivorous insects, are later used by other arthropods as microhabitats (Carroll and Kearby 1978; Carroll et al. 1979; Akimoto 1981; Hajek and Dahlsten 1986; Cappuccino 1993; Cappuccino and Martin 1994; Kudo 1994; Larsson et al. 1997; Alper 1998; Martinsen et al. 2000). For example, Akimoto (1981) reported that the aphid *Eriosoma yangi* inhabited leaf-roll galls on elm trees made by other *Eriosoma* species. Since herbivorous insects commonly make shelters (Martinsen et al. 2000), shelter-mediated interactions between arthropods that make shelters and those that do not may occur frequently on terrestrial plants.

In Hokkaido, northern Japan, numerous species of lepidopteran larvae construct leaf shelters such as leaf folds, rolls, sandwiches, and ties on a species of willow tree, *Salix miyabeana* (Seemen). Several species of arthropods later occupy the vacant leaf shelters after the lepidopteran larvae have emerged or made new shelters. In particular, we have frequently observed an aphid, *Chaitophorous saliniger* (Shinji), in leaf shelters. After these aphids have colonized the leaf shelters, ants visit the shelters, probably to obtain aphid honeydew. Therefore, we predict that leaf shelters on *S. miyabeana* decrease the larval survivorship of a common leaf beetle, *Plagiodera versicolora* (Laicharting) because attending ants remove the larvae.

The aims of this study were to determine whether leaf shelters affect the abundance of the aphid and its attending ants and whether the presence of leaf shelters subsequently affects the larval survivorship of *P. versicolora*, which does not use leaf shelters. We determined the number and species of leaf-shelter occupants, tested the effect of artificial leaf shelters on the abundance of aphids and attending ants, and examined the subsequent effects on the survivorship of leaf beetle larvae.

Materials and methods

Study site and host plant

This study was performed in 1999 on the banks of the Ishikari River (43°N, 141°E) in Hokkaido, the northern island of Japan. Six willow species (*Salix miyabeana* Seemen, *S. sachalinensis* Fr. Schmidt, *S. integra* Thunberg, *S. hultenii* Floderus, *S. subfragilis* Anders, and *S. pet-susu* Kimura) were sympatric at the study site. Field observations and experiments were conducted on *S. miyabeana*, the most abundant willow species in the study area (Ishihara et al. 1999), which supported a high density of leaf shelters built by lepidopteran larvae.

Shelter-makers and secondary users

A number of lepidopteran larvae, mainly in the Tortricidae and Pyralidae families, make leaf shelters on *S. miyabeana* by folding or rolling a single leaf or by tying two or three leaves together with silk. The larvae either develop in the leaf shelters until pupation or move on and make new shelters. Vacant shelters are often occupied by other arthropods (secondary users).

Leaf beetle

The leaf beetle *Plagiodera versicolora* Laicharting is a dominant specialist herbivorous insect that feeds on *Salix* and *Populus* leaves (Kimoto and Takizawa 1994). Overwintering adults begin to emerge in late May; females lay egg clutches on the undersurface of leaves from early June to late August; and larvae pass through three instars and then pupate (Ishihara et al. 1999).

Survey of secondary users

To determine the species and numbers of secondary users of leaf shelters, we randomly selected 10–15 trees on which we sampled leaf shelters from mid-May until late September. In mid-May and late September, we sampled only 50 leaf shelters, because relatively few were found, but otherwise we sampled 100 leaf shelters three times a month. While sampling, we lifted leaf shelters carefully to avoid displacing secondary users and immediately packed the shelters in small vinyl bags. The bags were kept in a laboratory freezer at –30°C. We classified the leaf shelters into the following categories: Containing lepidopteran larvae, *Chaitophorous saliniger*, *C. saliniger* with ants, other arthropods, and without secondary users.

Effects of leaf shelters on abundance of aphids and ants

To determine whether leaf shelters affect the abundance of *C. saliniger* and its attending ants, we conducted a field experiment involving artificial leaf shelters. Each artificial leaf shelter was constructed by rolling a single leaf and maintaining the rolled shape by binding the ends with two plastic tubes (6 mm diameter, 10 mm length). On 12 July 1999, we randomly selected 23 trees that were 2–3 m in height. On each tree, we selected four current-year shoots of similar size. On each shoot, we set 0, 1, 3, or 6 artificial leaf shelters, with each shoot receiving a different number. Two weeks later, the shoots were individually packed in vinyl bags and frozen at –30°C. We then counted the number of *C. saliniger* aphids and their attendant ants on each shoot. To determine the relationships between leaf shelters and aphids and between leaf shelters and ants, we used a linear regression of log (n+1)-transformed numbers of aphids and ants. To determine the relationship between aphids and ants, we used a linear regression of the raw data.

Effects of leaf shelters on survival of leaf beetle larvae

We conducted a larval transfer experiment to determine how leaf shelters affect the survival rate of *P. versicolora* larvae, which may be removed by ants attending aphid colonies in the leaf shelters. On 15 July 1999, we randomly selected 11 trees that were 2–3 m in height. On each tree, we selected two current-year shoots of similar size. On each shoot, we set four artificial leaf shelters on one shoot (the shelter shoot) and none on the other (the non-shelter shoot). Two weeks later, newly hatched beetle larvae [11.3±2.2 larvae (mean±1 SD)] were transferred to a leaf on each shoot. During the first 6 h after the transfer, we counted the number of ants on each shoot every hour. One day later, we counted the number of remaining beetle larvae on each shoot to estimate the larval survival rate. We used the Mann-Whitney U test to examine differences in larval survival rates and in the cumulative number of ants on shelter shoots and non-shelter shoots.

Results

Secondary users

Of the 7,927 arthropods in the sampled leaf shelters, 94.4% were aphids (*Chaitophorous saliniger*), 5.0% were ants (*Camponotus japonicus*, *Lasius hayashi*, and *Myrmica jessensis*) that were attending aphid colonies in the shelters, and 0.6% were other arthropods.

Species composition in leaf shelters changed with season (Fig. 1). The proportion of leaf shelters housing lepidopteran larvae decreased consistently from mid-May to mid-July because of adult emergence. In late July, second-generation lepidopteran larvae began to construct leaf shelters. The number of lepidopteran larvae was highest in mid-August and declined thereafter. In contrast, *C. saliniger* began to colonize vacant leaf shelters in mid-June; in mid-July and mid-September, the aphid had colonized approximately 75% of all leaf shelters.

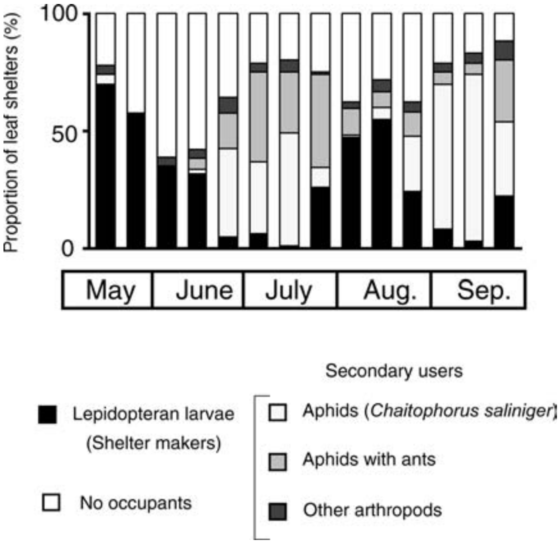

Fig. 1 Proportion of leaf shelters made on *Salix miyabeana* containing different types of occupants. Shelters were classified into five categories: (1) lepidopteran larvae, (2) aphids (*Chaitophorus saliniger*), (3) aphids with ants, (4) other arthropods, and (5) no occupants

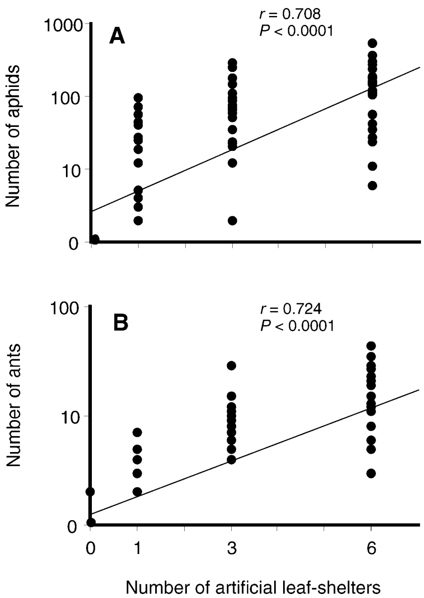

Fig. 2A, B Effect of artificial leaf shelters on the abundance of aphids (*C. saliniger*) and its attending ants. Relationship between number of artificial leaf shelters and **A** aphids and **B** ants

Effects of leaf shelters on aphid and ant abundance

The number of aphids and associated ants significantly increased with increasing number of artificial leaf shelters on a shoot (aphid: n=92, r=0.708, P<0.0001; ants; n=92, r=0.724, P <0.0001) (Fig. 2). The number of ants increased significantly with the number of aphids (n=92, r=0.790, P<0.0001) (Fig. 3).

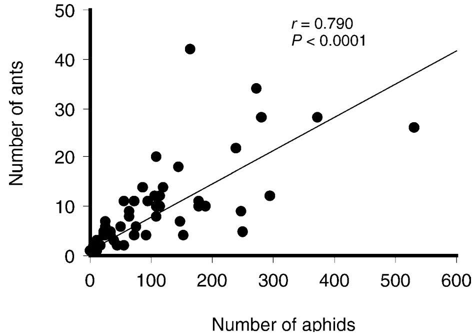

Fig. 3 Relationship between number of aphids (*C. saliniger*) and attending ants

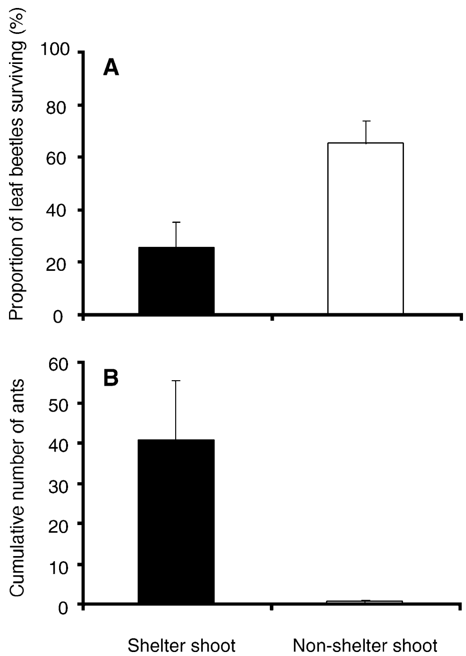

Fig. 4A, B Ant-mediated effect of artificial leaf shelters on the survival rate of transferred larvae of the leaf beetle, *Plagiodera versicolora*, near leaf shelters. **A** proportion of leaf beetles surviving one day after transfer to shelter shoots and non-shelter shoots, and **B** cumulative number of ants on shelter shoots and non-shelter shoots over the first six hours after larval transfer. Means ±1 SE are presented

Effects of leaf shelters on survival of leaf beetle larvae

The presence of leaf shelters negatively affected the survival rate of beetle larvae near leaf shelters. One day after larval transfer, the larval survival rate was significantly lower on shelter shoots than on non-shelter shoots (n=11, U=19.5, P=0.0065) (Fig. 4A). In contrast to the few ants observed on non-shelter shoots, ants visited artificial leaf shelters continuously. Thus, the cumulative number of ants during the first 6 h was over 74 times higher than the number on non-shelter shoots (n=11, U=8.5, P=0.0003) (Fig. 4B).

Discussion

Effects of leaf shelters on other arthropods

This study showed that the leaf shelters constructed by lepidopteran larvae were used as microhabitats by several insect species, the most abundant of which was the aphid, *Chaitophorous saliniger*. Similarly, recent studies have shown that shelter-makers interact positively with secondary users by providing leaf shelters, which increase microhabitat availability (Cappuccino 1993; Cappuccino and Martin 1994; Larsson et al. 1997; Martinsen et al. 2000; Fukui 2001).

We also found that leaf shelters positively affected the abundance of an aphid and attending ants that collected honeydew. This increase in ant numbers subsequently negatively affected the larval survival rate of a leaf beetle, *Plagiodera versicolora*, near leaf shelters.

Positive effects of leaf shelters on aphid and ant abundance

Several authors have suggested that most interactions among herbivorous insects are indirect and that such interactions can be positive as well as negative (Damman 1993; Denno et al. 1995). Our experiments clearly illustrated that the lepidopteran larvae positively affected aphid abundance by providing leaf shelters. This might be due to increased survival of the aphids and a subsequent increase in reproduction. Living in a leaf shelter has several benefits: refuge from natural enemies (Fowler and MacGarvin 1985; Damman 1987; Atlegrim 1989; Cappuccino 1993; Eubanks et al. 1997), protection against adverse microclimatic conditions (Henson 1958; Hunter and Willmer 1989; Larsson et. al. 1997), and acquisition of high-quality food (Lewis 1979; Dussourd and Eisner 1987; Sandberg and Berenbaum 1989; Sagers 1992; Fukui et al. 2002).

Ants are often strongly associated with aphid colonies, because honeydew is a stationary and renewable food resource (Way 1963; Carroll and Janzen 1973; Buckley 1987). In this study, three ant species were found with *C. saliniger*, the aphid that colonized leaf shelters, and the abundance of these ants correlated highly with the number of the aphid. We conclude, therefore, that the ants were probably gathering aphid honeydew.

Some authors have argued that bottom-up regulation by host-plant characteristics is the primary factor controlling herbivorous insect population dynamics (Hunter and Price 1992; Ohgushi 1992; Harrison and Cappuccino 1995). This argument focuses on the quantity and quality of host plants as food for herbivores (Ohgushi and Sawada 1985; Schultz 1988). We showed, however, that an increase in leaf shelters increased aphid abundance. Therefore, the aphid population may be limited by the abundance of leaf shelters. This conclusion implies that, in addition to food availability, microhabitat availability on host plants is also an important factor in determining the population dynamics of secondary users.

Negative effects of leaf shelters on survival of leaf beetle larvae

Leaf shelters may negatively affect herbivorous insects near leaf shelters if predators are attracted to the shelters (Martinsen et al. 2000; Fukui 2001). In our larval transfer experiment, attending ants were often observed patrolling outside leaf shelters. These ants frequently encountered leaf beetle larvae and removed them from the trees. One day after larval transfer, survival was much lower on shelter shoots, where the attending ants probably removed the transferred beetle larvae. Many authors have reported negative effects of the ant-aphid association on other herbivorous insects (Messina 1981; Fritz 1983; Fowler and MacGarvin 1985; Ito and Higashi 1991; Floate and Whitham 1994; Wimp and Whitham 2001), because attending ants remove herbivorous competitors of the aphid food resources (Way 1963; Buckley 1987). In the field, we commonly found one or two leaf shelters on a shoot during the experimental period (mid-July), which is somewhat lower than the density used in the larval transfer experiment.

In conclusion, our field experiments suggest that shelter-makers provide critical microhabitats to other herbivorous insects. These positive shelter-mediated interactions among herbivorous insects on terrestrial plants may be much more common and widespread than previously thought. This is because insects including Lepidoptera, weevils, sawflies, and aphids construct leaf shelters on a wide variety of plants, from trees and shrubs to herbs and even ferns (Martinsen et al. 2000). On the other hand, this study revealed that by attracting predators leaf shelters can also negatively affect herbivorous insects that do not use the leaf shelters. Future studies need to focus on both direct and indirect effects of leaf shelters on herbivorous insects, to establish the prevalence of positive and negative effects.

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