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Aphid-tending Ants Affect Secondary Users in Leaf Shelters and Rates of Herbivory on Salix hookeriana in a Coastal Dune Habitat

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ABSTRACT.—The interactions between keystone species and ecosystem engineers may have important community-level consequences. We studied the effects of a keystone species, the aphid-tending ant (*Formica obscuripes*), on the abundance of engineered leaf shelters, the levels of herbivory on leaf tissue and the structure of arthropod communities within leaf shelters on Hooker's willow (*Salix hookeriana*) in a coastal dune ecosystem in northern California. Leaf shelters on branches with aphid-tending ants had 54% more individuals than shelters on branches without ants, possibly because shelters are used as a refuge from predation. Levels of herbivory were 2× greater on branches without aphid-tending ants than branches with ants. Our study suggests that aphid-tending ants may actually increase the abundance of arthropods at small spatial scales within leaf rolls while simultaneously reducing rates of herbivory at the branch level.

INTRODUCTION

Through their interactions with other members of a community, particular species can have profound effects on community structure (Kareiva and Levin, 2003). These species are sometimes called keystone species and can also be called ecosystem engineers if they modify, maintain or create habitats that are used by other species (Paine, 1966; Jones *et al.*, 1994). One situation in which keystone species may interact with ecosystem engineers is in the context of aphid-tending ants and shelter-building insects. These are two common keystone species and ecosystem engineers within insect communities (Lill and Marquis, 2003; Wimp and Whitham, 2001) and can occur together on the same plant (Nakamura and Ohgushi, 2003).

By excreting resource-rich honeydew, some aphid species attract ants that protect them from their predators and parasitoids (Hölldobler and Wilson, 1990). By defending aphids, aphid-tending ants can dramatically alter the structure of arthropod communities associated with their hostplant (Schultz and McGlynn, 2000). For example, Wimp and Whitham (2001) found that arthropod species richness increased by 57%, and abundance by 80%, on trees with aphid-ant mutualists removed. Aphid-tending ants also remove competing herbivores from the aphids' hostplant (Messina, 1981; Fowler and MacGarvin, 1985; Floate and Whitham, 1994), which can lead to reduced herbivory on hostplants (Ito and Higashi, 1991).

By modifying leaves into leaf rolls, ties, folds and tents, shelter-building insects create new habitats on plants that are often used concurrently and subsequently by other arthropods. An organism that occupies a shelter made by another species is called a secondary user (Cappuccino, 1993; Fukui, 2001). Thus, shelter-builders can have positive effects on community structure by providing shelter from enemies or the environment (Martinsen

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et al., 2000; Lill and Marquis, 2003). Shelter-builders may also negatively influence some species by attracting predators (Martinsen *et al.*, 2000) or by increasing the abundance of aphid-tending ants, which would remove herbivores (Nakamura and Ohgushi, 2003).

The interactions between keystone species and ecosystem engineers may have important community-level consequences. In this study, we examine the effects of ant-aphid interactions on the structure of arthropod communities within leaf rolls and levels of herbivory on leaf tissue on Hooker's willow. Specifically, we ask three related questions: (1) What are the relationships between aphid and ant abundance and proximity to the nearest nest of the aphid-tending ant, *Formica obscuripes*? (2) Is the presence of aphid-tending ants associated with the abundance of leaf rolls or the level of herbivory on Hooker's willow? (3) Does the structure of the secondary user community within leaf rolls differ on branches and entire trees with and without aphid-tending ants?

STUDY SYSTEM

We conducted this work at Lanphere-Christensen Dunes Preserve in Humboldt County, northern California in July–Aug. 2003. This 260-ha restored reserve is one of the largest remaining pristine coastal dunes in the Pacific Northwest. At the site, we focused our work on interactions between the thatch ant *Formica obscuripes*, the aphid (*Aphis* sp.), they regularly tend and the other arthropods associated with Hooker's Willow (*Salix hookeriana*). Several species of lepidopteran larvae are suspected of creating leaf shelters on Hooker's willow at this site starting in the late spring. These shelters are often secondarily colonized by a variety of arthropods including spiders, other caterpillars, various homopterans, beetles and mites. Aphids in this system rarely occupy leaf shelters, and we only rarely detected ant workers in the shelters. All of the trees we studied were between 1 and 3 m in height and occurred mainly in dune hollows.

Formica obscuripes is the dominant ant species at this site. *Formica obscuripes*, like many ants in the *Formica rufa* group, aggressively tends aphids on a variety of hostplants (Way, 1963; McIver and Yandell, 1998). At our site, *F. obscuipes* is diurnally active year round, though tending of aphids does not begin until the early to mid-summer. *Formica obscuripes* foragers were very conspicuous and were common on approximately half of the Hooker's Willow trees during the study.

METHODS

Relationships between aphid and ant abundance and proximity to the nearest nest.—To examine the relationship between ant and aphid abundance and proximity to the nearest *F. obscuripes* nest, we measured the distance to the nearest *F. obscuripes* nest and calculated the average number of *F. obscuripes* workers and aphids present for 5 min on 7 trees over 4 d. Censuses were taken in the afternoon when foragers were most active. Because the data were distributed non-randomly, we used Spearman's rank correlation to examine the relationships between distance to the nearest nest and the average number of ants and aphids present.

The association of aphid-tending ants with the abundance of leaf rolls or levels of herbivory.—To examine whether the abundance of aphid-tending ants is related to the abundance of leaf rolls on *Salix hookeriana*, we haphazardly chose six trees with ants and aphids and five trees without. On each tree with ants and aphids, we haphazardly selected three to five branches with ants and aphids and 5 branches without. We then counted 25 consecutive leaves on each branch, starting with the first thumb-sized leaf from the distal end of the branch. For all branches, we recorded the number of rolled leaves present out of the 25 leaves examined. On each of the trees without ants and aphids, we haphazardly selected

10 branches. We repeated the same methods and recorded the number of rolled leaves out of 25 leaves. We used paired *t*-tests to compare the arcsine-transformed proportion of leaf shelters on branches with and without aphid-tending ants and *t*-tests to compare the arcsine-transformed proportion of shelters on trees with aphid-tending ants to those without aphid-tending ants.

We examined whether the presence of aphid-tending ants was associated with levels of herbivory on 10 trees with ants and aphids present and 10 trees without ants and aphids. We compared braches with and without ants by haphazardly choosing two similarly sized branches on each tree, one with ants and aphids present and one without. We then examined every 3rd leaf from the distal end of each branch for a total of 10 leaves/branch. We scored a leaf as attacked if it had recently been damaged by a tissue-removing herbivore. We did not measure rates of herbivory to the leaf rolls themselves. We used paired *t*-tests to compare the arcsine-transformed proportion of damaged leaves on branches with and without aphid-tending ants and *t*-tests to compare damaged leaves on trees with to trees without aphid-tending ants.

The effects of aphid-tending ants on the structure of the secondary user community within leaf rolls on branches and entire trees.—To examine if the structure of secondary user communities in leaf rolls on branches with aphid-tending ants differed from those on branches without aphid-tending ants, we collected 10 haphazardly selected leaf rolls (five from branches with ants and five from branches without ants) from each of six trees. We placed each leaf roll in a separate plastic bag with a label and brought them directly to the lab. We opened each roll under a dissecting scope and recorded arthropod species richness and abundance. All occupants within the rolls were considered secondary users as it was unknown who originally established the leaf roll or if any of the species present were capable of maintaining the leaf roll. We compared the total richness and abundance (by combining the occupants from all of the leaf rolls on a particular branch) for each branch type using paired *t*-tests. To examine whether communities in leaf rolls on entire trees differed, we also collected 10 haphazardly selected leaf rolls from another 5 haphazardly selected trees without aphid-tending ants present on them. We compared the total richness and abundance of arthropods in leaf rolls on 5 trees without ants and aphids to the 6 trees with ants and aphids (sum of the branch-level) using a *t*-test.

RESULTS AND DISCUSSION

Relationships between aphid and ant abundance and proximity to the nearest nest.—Like other studies (*e.g.,* Floate and Whitham, 1994; Nakamura and Ohgushi, 2003) we found a strong positive relationship between the number of aphids on a hostplant and the number of ants (Fig. 1, $r_s = 0.94$ $P = 0.005$). However, in contrast to at least one previous study (Wimp and Whitham, 2001), there was no signification relationship between the number of aphids and distance to the nearest *Formica obscuripes* nest ($r_s = 0.49$, $P = 0.33$) or distance to the nest and the number of ants ($r_s = 0.20$, $P = 0.70$). Our findings suggest other factors may shape the dynamics and spatial distribution of ant-aphid interactions. For example, microclimate, plant stress and seasonality in host plant quality have been shown to influence aphid distributions (Dixon and McKay, 1970; Dixon, 1975). Thus, it seems likely that the distribution of aphids (and therefore ants) could be influenced by characteristics of Hooker's willow that we did not examine in this study. Future research should address the mechanisms driving the distribution of ant-aphid mutualisms, and the consequences of such variation at the community and landscape scale.

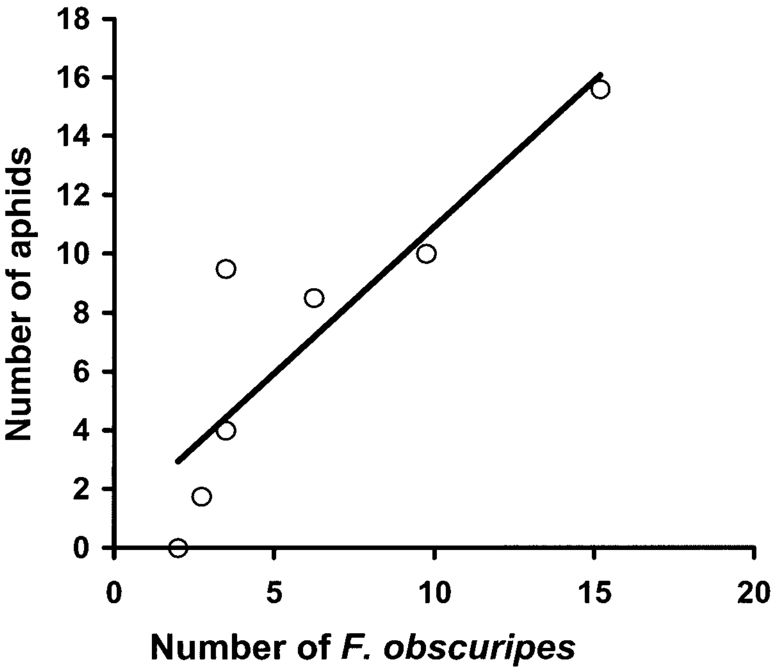
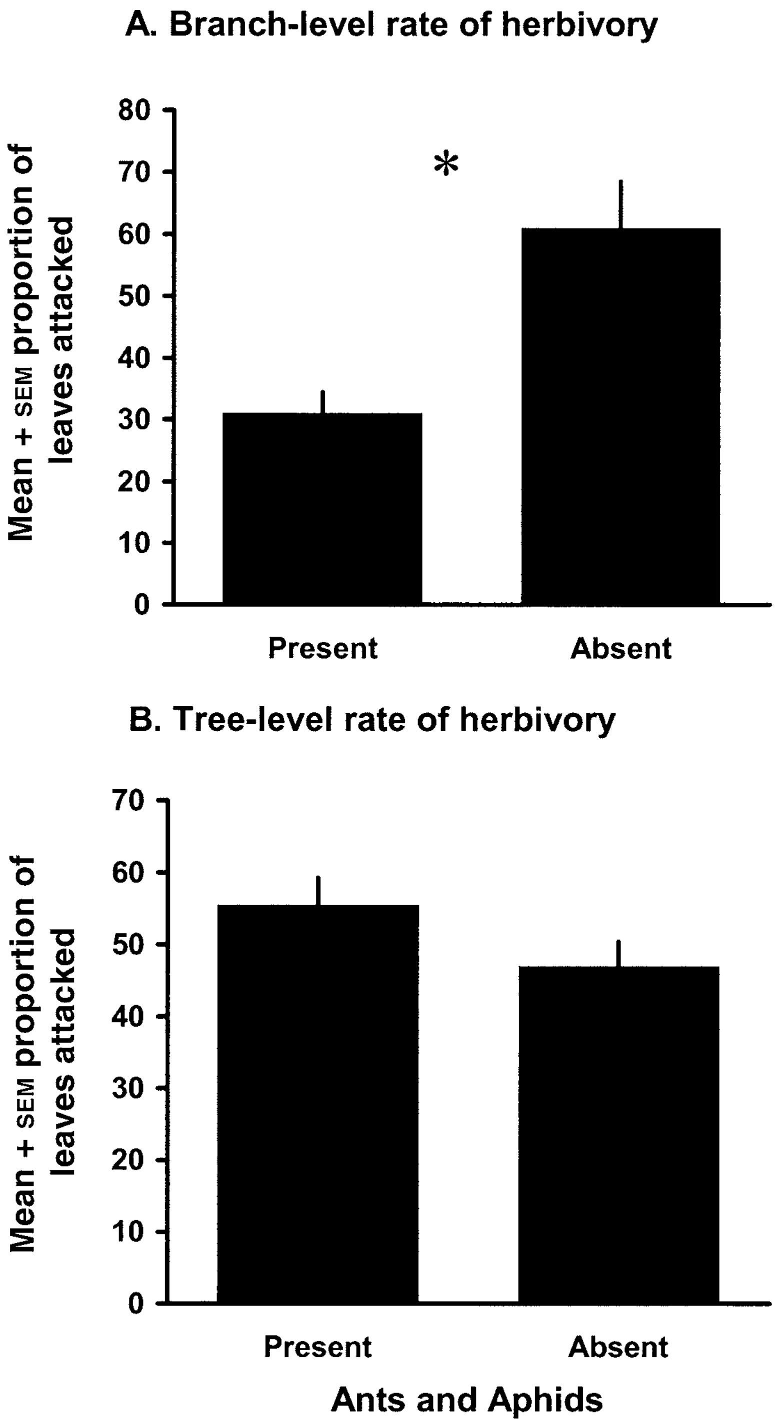


FIG. 1.—The relationship between the number of *Formica obscuripes* workers and the number of aphids on Hooker's willow. Circles represent total number of individual aphids per tree

The association of aphid-tending ants with the abundance of leaf rolls or levels of herbivory.—There was no effect of the presence of aphid-tending ants on the number of leaf rolls per branch on trees with ants (paired $t = -0.77$, df = 12, P = 0.45). There was also no effect of the presence of aphid-tending ants on the number of leaf rolls per tree ($t = 0.37$, df = 10, P = 0.71). Though other studies have indicated that aphid-tending ants reduce the abundance of shelter building insects (Fowler and Macgarvin, 1985), this was not the case at either the level of branches or whole trees in our study. Hostplant architecture and leaf age has been shown to influence the abundance of shelter building insects in other systems (Damman, 1987; Marquis *et al.*, 2002). Hostplant characteristics might be more important factors than the presence of aggressive ants in determining leaf roll abundances. Additionally, leaf shelters may have been initiated prior to the establishment of the aphid-ant mutualisms. Leaf shelters may have been constructed in early spring at our site before either ants or aphids were active.

Levels of herbivory were 2× greater on branches without ants than branches with ants (Fig. 2a, paired $t = 3.80$, df = 9, P = 0.004). Rates of herbivory on entire trees did not depend on the presence of aphid-tending ants (Fig. 2b, $t = 1.58$, df = 9, P = 0.15). These results suggest that ant aggression by *Formica obscuripies* reduces the abundance of herbivores locally. Similarly, Floate and Whitham (1994) found that ants attracted to experimentally established aphids reduced herbivory from leaf-feeding beetles on cottonwood trees by 50%. Our results are also consistent with other studies that experimentally excluded *Formica* ants


A. Branch-level rate of herbivory
Mean + SEM proportion of leaves attacked
80
70
60
50
40
30
20
10
0
*
Present
Absent
B. Tree-level rate of herbivory
Mean + SEM proportion of leaves attacked
70
60
50
40
30
20
10
0
Present
Absent
Ants and Aphids

(Ito and Higashi, 1991) and provide further support for positive interactions between ants and the plants they tend insects on, but only at a branch level in our system.

The effects of aphid-tending ants on the structure of the secondary user community within leaf rolls on branches and entire trees.—Total richness within leaf rolls did not differ between branches with aphid-tending ants and without aphid-tending ants (Fig. 3a, paired $t = 1.77$, $df = 5$, $P = 0.14$). However, leaf rolls on branches with aphid-tending ants had 54% more individuals than leaf rolls on branches without aphid-tending ants (Fig. 3b, paired $t = 2.43$, $df = 5$, $P = 0.05$). Neither richness ($t = 1.76$, $df = 9$, $P = 0.11$) nor abundance ($t = 0.96$, $df = 8$, $P = 0.37$) of secondary leaf roll users differed between trees with and without aphid-tending ants. The higher abundance when ants are present is surprising because tending by ants typically causes a decline in both arthropod species richness and abundances on plants (Wimp and Whitham, 2001; Fowler and Macgarvin, 1985; Messina, 1981). One possibility is that more arthropods use pre-established leaf rolls when ants are present on a branch as a kind of refuge. Damman (1987) found that caterpillars in leaf shelters suffered less predation and had higher survival rates than those caterpillars not in shelters. The secondary user community on Hooker's willow in our system may be using leaf rolls as enemy-free space to escape aggression from ants. Since we found no difference between the leaf roll communities at the tree level, the impacts of ants on the secondary user communities is very localized within individual trees that maintain aphid-ant mutualisms. Nakamura and Ohgushi (2003) found that leaf shelters on a different willow species positively affected the abundance of an aphid and tending ants, which had negative impacts on the survival rate of a leaf beetle. However, we observed very few aphids within leaf rolls and there was no indication that shelters were influencing ant abundance.

In conclusion, our results indicate that tending of aphids by ants influences a variety of aspects of the species interactions and communities found on willows, including rates of herbivory on Hooker's willow and the abundance of arthropods within leaf rolls. Neither the abundance of leaf rolls nor the number of species within them was related to the presence of aphid-tending ants. In some ways, however, it is not surprising that ants differentially affect members of the community with which they interact, and that these effects, whatever they may be, vary temporally and spatially. In other systems, keystone species and ecosystem engineers can drive patterns of diversity within a community and across spatial scales (Wimp and Whitham, 2001; Wright *et al.*, 2003). In this study, we showed that a keystone species, aphid-tending ants, can mediate the effects of an ecosystem engineer, shelter-building insects on the density of individuals within leaf shelter. Ant-aphid mutualisms may actually increase the abundance of arthropods at small spatial scales within leaf rolls while simultaneously reducing the abundance of herbivores attacking outside of shelters. It is possible, however, that the aphid-tending ants have only limited effects on either the herbivores outside of the leaf shelters or the ecosystem engineers that construct the leaf shelters. An experiment that manipulated the timing of leaf shelter construction or excluded aphid-tending ants for an entire season might test this hypothesis. Future studies aimed at elucidating how such interactions depend on scale, and interact with the influence of ecosystem engineers, will be necessary to understand the myriad of direct and indirect factors that shape community structure.

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FIG. 2.—Rates of herbivory on branches and entire trees with and without aphid-tending ants. Bars indicate the mean (± SE) proportion of leaves attacked. Note that we performed analyses on the arcsine-transformed proportions, but for clarity we present untransformed values

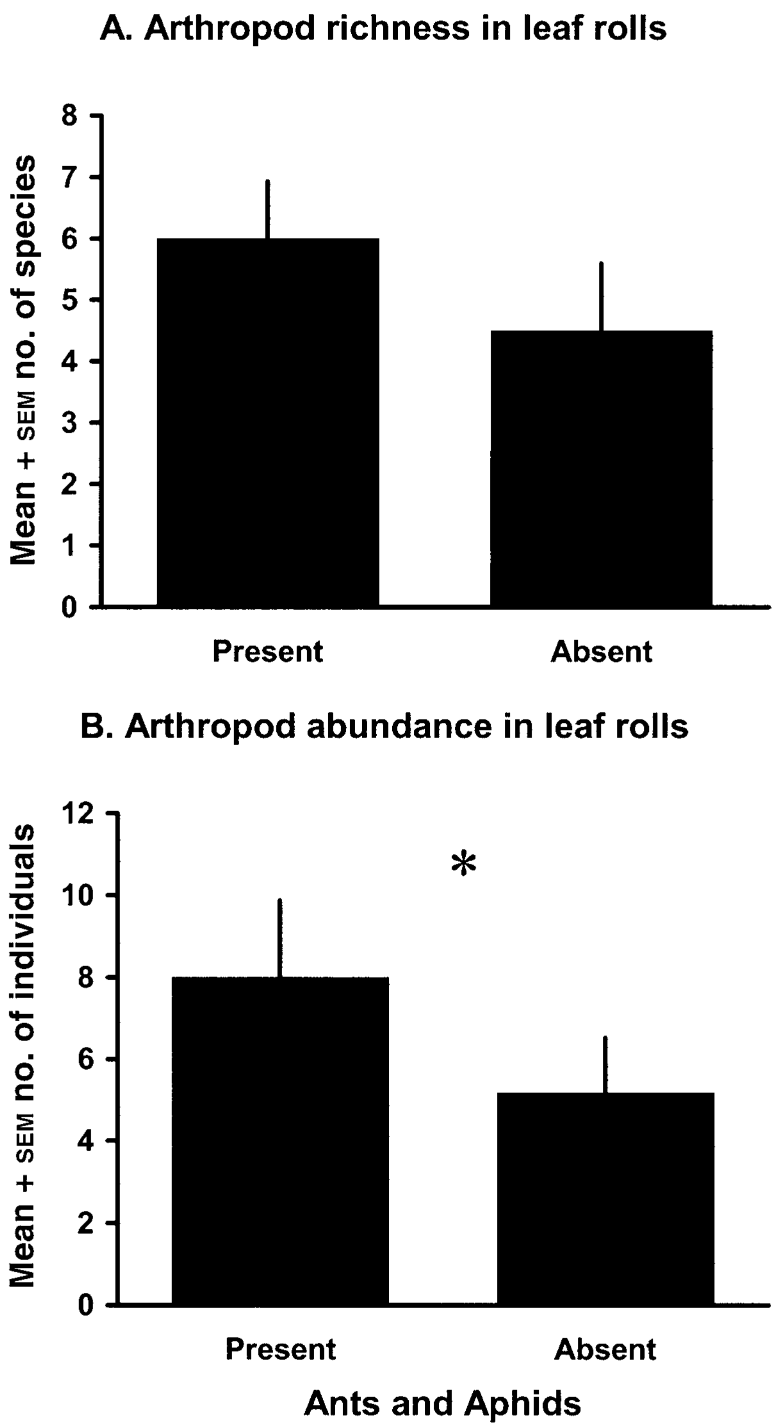
A. Arthropod richness in leaf rolls
Mean + SEM no. of species
0
1
2
3
4
5
6
7
8
Present
Absent
B. Arthropod abundance in leaf rolls
Mean + SEM no. of individuals
0
2
4
6
8
10
12
*
Present
Absent
Ants and Aphids

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FIG. 3.—The richness and abundance of arthropods within leaf rolls on branches with and without aphid-tending ants. Bars indicate the mean (± SE) number of total species or individuals for 5 leaf rolls/branch. The asterisk indicates that the means are statistically different from one another

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