CONTRASTING EFFECTS OF ANTS ON THE HERBIVORY AND GROWTH OF TWO WILLOW SPECIES

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Abstract. This study examines the effects of two predatory ants, *Myrmica rubra* L. and *Formica aquilonia* Yarr. on the herbivory and growth of two phytochemically different willow species: low-salicylate *Salix phylicifolia* L. and high-salicylate *Salix myrsinifolia* Salisb. The net influence of ants on willow growth will depend on a balance between the benefits ants provide to the plant through predation on herbivores and the costs to the plant through the protection of aphids. Using both field observations and experimental manipulations of the densities of ants and tended aphids, I tested the hypothesis that this balance differs between the two willow species. On both observational and experimental willows, I followed the insect densities throughout one growing season and measured leaf damage and willow growth. The results from both data sets suggest that the effects of ants on the herbivory and consequent growth of these willows differ. Especially *F. aquilonia* had a positive effect on the growth of *S. phylicifolia*, whereas the effect on *S. myrsinifolia* was negligible or even slightly negative. These differences can be derived from three factors: (1) the ant-tended aphid *Pterocomma salicis* L. was very abundant on both willow species, leading to strong negative impact of ants on the willows, (2) *S. myrsinifolia* harbored lower densities of leaf-chewing insects, which made the positive effect of ants through reduced leaf damage less likely, and (3) the dominant leaf-chewing herbivore on *S. myrsinifolia*, a leaf beetle *Phratora vitellinae* L., is chemically defended against predatory ants, being thus less preyed on than the predominating generalist leaf-chewing herbivores on *S. phylicifolia*. Furthermore, when ants were not attending aphids, they even facilitated *P. vitellinae*, apparently by removing other predators. This study provides one explanation for the debate of whether ants benefit or harm plants: differences in herbivore resistance of plants may lead to diverging or even opposite results in tritrophic interactions between plants, their herbivores, and predatory ants.

Key words: Ants; Formica aquilonia; *herbivory;* Myrmica rubra; Phratora vitellinae; *predation;* Salix myrsinifolia; Salix phylicifolia; *tritrophic interactions; willows.*

INTRODUCTION

Predatory ants are often considered to be keystone species, greatly affecting the numbers and the community composition of herbivorous insects in temperate and boreal woodlands. Extensive literature exists on the subject (reviewed by Hölldobler and Wilson 1990, Whittaker 1991), debating whether ants are beneficial or harmful insects for trees and whether they can be used as agents for biological control of forest pests (Way and Khoo 1992). Numerous successful attempts have been made to introduce ant colonies to new forest stands or even new continents to control populations of herbivorous insects (Gösswald 1951, Hölldobler and Wilson 1990). These introductions provide some evidence that ants may have a positive contribution to tree growth even in cases where trees do not provide any additional resources for ants. Some studies have also demonstrated the crucial role of ants in the survival of trees during insect outbreaks (Laine and Niemelä 1980, Karhu 1998). On the other hand, several studies have

found no effect of ants on plant performance (e.g., Mahdi and Whittaker 1993) and there are cases where the effect has shown to be negative (Adlung 1966, Otto 1967).

Beneficial effects of ants arise when they reduce the number of herbivorous insects and subsequent damage in plant tissue. However, if herbivorous insects are scarce or plants are tolerant to the prevailing level of insect damage (see Strauss and Agrawal 1999), the effect of ants may be neutral or obscured by incidental variation. Herbivorous insects may also survive ant predation if ants find better food sources elsewhere (e.g., Skinner 1980), or if these insects live in shelters or are otherwise defended against ants (Faeth 1980, Lawton and Heads 1984, Fowler and MacGarvin 1985, Heads and Lawton 1985, Pasteels et al. 1988, Ito and Higashi 1991). Furthermore, ants may have a substantial positive effect on the performance of some herbivores. Namely, many ant species commonly tend aphids and other phloem-feeding Homoptera in order to gain honeydew rich in carbohydrates and some other nutrients (Beattie 1985, Cushman and Addicot 1991). The tended Homoptera gain from this interaction especially by getting protection against natural enemies

(Beattie 1985; see discussion of other benefits in Hölldobler and Wilson 1990), and thus increase in numbers. While foraging or defending Homoptera colonies, ants also remove or disturb other predators and parasitoids (Bartlett 1961) or competitors of herbivores (Fritz 1983), which may also benefit leaf-chewing insects and lead to increased leaf damage. As a conclusion, ants are likely to have negative effects on plants when the positive effects on aphids override the negative effects on leaf-chewing insects (Messina 1981), or when the exclusion of natural enemies or competitors compensate for the negative effects of ants on nontended herbivores. Apparently, the final effect of ants on the growth of plants is an outcome of the balance between these factors (Messina 1981, Whittaker 1991).

Plants vary in respect to the traits affecting the components of the plant–herbivore–ant interaction. First, plants may differ in their susceptibility to ant-tended Homoptera (e.g., Whitham 1983), which is likely to affect the ant visitation rates and the potential of negative influence on plants (Floate and Whitham 1994). Second, plants may differ in their susceptibility to nontended herbivores (e.g., Sipura 1999), which clearly alters the potential of ants to help plants through reduction of leaf damage. Third, plants may differ in their potential to provide safe habitats for herbivorous insects (e.g., Price et al. 1980, Boethel and Eikenbary 1986). Some plants provide physical barriers for the enemy avoidance of herbivores (e.g., Damman 1987), and some herbivorous insects use plant-derived precursors for their own defense (e.g., Pasteels et al. 1988). Finally, physical and chemical properties of plants can affect the densities of the ants for example by providing shelters (Janzen 1966) or food (Janzen 1966, Bentley 1976, Tilman 1978) for them.

Recently, Sipura (1999) found that the effect of insectivorous birds on the herbivory and growth of willows depends on willow species; birds had a stronger positive effect on willows with high herbivore densities, apparently due to density-dependent foraging. When this kind of variation is taken into account, the effect of generalist predators, such as ants, can lead to widely divergent outcomes in different systems. In this paper, I propose and test a hypothesis stating that the differences in the level of herbivore resistance and the consequent differences in the composition of the herbivore communities of two chemically different willow species lead to different impact of predatory ants on the herbivore densities, leaf damage, and growth of these willow species.

Materials and Methods

Study organisms

The two willow species, the tea-leaved willow *Salix phylicifolia* L. and the dark-leaved willow *Salix myrsinifolia* Salisb., used in this study are among the most common willow species in northern Europe, and can be found growing abundantly on shores and human habitats including cultivated fields and roadsides (Hämet-Ahti et al. 1998, Skvortsov 1999). These closely related (Nyman 2000) willow species are remarkably similar in their ecology and morphological appearance, but show dramatic differences in the defensive chemistry of their leaves. Bitter-tasting leaves of *S. myrsinifolia* contain approximately fifty-fold concentrations of phenolic glucosides (including salicylates salicin and salicortin) compared to the leaves of *S. phylicifolia* (Tahvanainen et al. 1985, Julkunen-Tiitto 1986, Rank et al. 1998, Nyman and Julkunen-Tiitto 2000). It has been shown that generalist herbivores are usually repelled by these compounds (e.g., Tahvanainen et al. 1985, Kolehmainen et al. 1995), whereas some specialists may use them as feeding cues (Soatens and Pasteels 1994, Kolehmainen et al. 1995, Roininen et al. 1999). Consequently, fewer generalist herbivores are found on *S. myrsinifolia* (Sipura 1999).

A specialist leaf beetle, *Phratora vitellinae* L. prefers salicylate-rich willows (Soatens and Pasteels 1994, Rank et al. 1998) and usually predominates in the leaf-chewer communities of *S. myrsinifolia*. It made up 20–98% (mean 76%) of the individuals of leaf-chewing insects observed on 37 *S. myrsinifolia* populations censused throughout the growing seasons in 1998 and 1999, but occurred only rarely on *S. phylicifolia* (M. Sipura, *unpublished data*). Larvae of this leaf beetle use salicylates as precursors when producing salicylaldehyde, which has been shown to be a strong repellent against generalist predators, especially ants (Pasteels et al. 1988). Another common herbivore, the ant-tended aphid *Pterocomma salicis* L., formed 0–97% (mean 36%) of the herbivorous insects on *S. myrsinifolia* populations mentioned above and 0–94% (mean 25%) of the insects on *S. phylicifolia* at the same sites. This aphid forms dense colonies on willow stems preferring 2-yr-old branches of both weakly and strongly defended willows (Heie 1986), where it sucks phloem fluids and excretes honeydew, which is readily used by ants.

Large and aggressive *Formica aquilonia* Yarr. ants live in large colonies and are usually extremely abundant when present (see Karhu 1998 for a review of the life history traits and ecological importance of this species). *Formica* ants are known to be effective foragers: one average-sized colony has been estimated to collect over 20 000 moth caterpillars and sawfly larvae during a day (Hölldobler and Wilson 1990). Smaller *Myrmica rubra* L. ants live in much smaller colonies than *F. aquilonia* (Collingwood 1979) and occur in much lower densities on willows, even when the nest is right beneath the willow (M. Sipura, *personal observation*). However, both ant species were seen to kill herbivorous insects in the study areas.

Study areas

The study was conducted in two separate areas. The first area was located along the shore of a shallow lake

on an artificial embankment constructed to prevent flooding of the fields nearby (see detailed description in Sipura 1999) and along a forest edge adjacent to the embankment in Parikkala, southeastern Finland (61°33′ N, 29°33′ E). Young willows (age 3–8 yr) from both species were growing sympatrically throughout the area. Nests of the ant *M. rubra* were patchily distributed across the 2-ha study area providing natural gradients in ant densities. The other study area (~2 ha) was an abandoned field near the city of Joensuu, eastern Finland (62°37′ N, 29°40′ E). Several thousand 4–7-yr-old willows from both species were growing evenly in the field forming dense early successional vegetation. There were no ant colonies in the field, but individuals of *F. aquilonia* from at least 16 nests located in an adjacent forest visited the field in large numbers. The field was surrounded by a ditch, and the ants were forced to use only a few bridges of fallen trees to enter the field. This caused many steep natural gradients in ant densities in the study field.

Observations and experimental procedure

I collected observational data from 66–80 randomly selected willow clones, representing both species, in both study areas. For the manipulative experiments I selected 78–180 willows from both species and both study areas, concentrating in areas with high ant densities. Just before the budbreak in 1998 I protected approximately half of the experimental willows from ants by placing 10 cm wide plastic tapes lubricated with liquid teflon (Fluon, Whitford Plastics, Chesire, UK) around the base of all stems. The other half of the bushes was designated for controls. The control willows were not taped, as the tape itself seemed to repel foraging ants. Unlike problematic grease bands used in many similar experiments (e.g., Fowler and MacGarvin 1985, Mahdi and Whittaker 1993), Fluon provides a conservative method. It forms a one-way barrier allowing insects to crawl down from the willow, but preventing them from going upwards. I changed the tape at least three times during the growing season to prevent possible harmful effects on willows. In the Joensuu study area, I also removed all tended aphids from randomly selected half of treated and control willows using a soft brush, while the other half served as controls. During the growing season, I checked all willows at least 16 times and repeated the aphid removal whenever new stem mothers of aphids appeared.

I censused the insect densities on both observational and experimental willows five times during the 1998 growing season (see census dates in Fig. 1) by counting all individuals or their constructions (galls or leaf ties). To obtain a comparable measurement of insect densities, I calculated the number of insects for 1 m² of leaf area. I counted the number of shoots and measured the length of 10–20 randomly selected shoots from every willow at each census time. At each occasion, I also collected a sample of ~40 shoots from adjacent wil-

lows in both sites. After measuring the lengths of the sampled shoots, I dried the leaves under moderate pressure, took a photocopy of each leaf, and measured the total leaf area of each shoot using a planimeter. The shoot length explained 76–97% of the variation in the total leaf area in linear regression models. Therefore, on the basis of the number of shoots and the mean shoot length, I was able to obtain a reliable estimate of leaf area of each observational and experimental willow at each census time.

For the estimation of herbivore damage I used two variables. Because willows tend to abscise their leaves prematurely when badly damaged or stressed by aphids (M. Sipura, *personal observation*), I determined the proportion of premature abscission at the fourth census by counting the leaves and leaf scars from 20 randomly selected shoots per willow. After the last census, I collected all remaining leaves from 50 randomly selected shoots per willow and determined the total leaf area and damaged or missing leaf area from 50 randomly selected leaves per willow using transparent scale paper as in Sipura (1999). In the analysis, I used the mean proportion of premature abscission of the 20 counted shoots and the mean proportion of damaged leaf area of the 50 leaves as variables.

At the end of the growing season, I measured the lengths of 50 randomly selected shoots from each willow and used the mean final shoot length of each willow as the first growth variable. Also, I cut down all the willows and took a disk from the basal stem at the height of 2 cm to determine the annual radial growth. I measured the thickness of the previous and current year growth rings under a stereomicroscope and used the relative radial growth (current year growth divided by previous year growth) as a second growth variable.

Data analysis

Herbivorous insects other than ant-tended aphids, leaf-chewing beetles, moths, and sawflies made up only 1.4% of the whole herbivore guild in Parikkala and 0.8% in Joensuu, and, for clarity, I did not include them in the analysis. I analyzed the observational data for the effect of ants on herbivore densities (mean of the five censuses), leaf damage, and willow growth using linear regression analysis with logarithmic mean ant density as the independent variable. I followed Zar (1999:360–364) when testing the differences of the regression slopes between the willow species. I analyzed the experimental data of insect densities using GLM repeated measures MANOVA with willow species, ant exclusion and aphid removal (in Joensuu only) as fixed factors and census occasion as the repeated measurement. When comparing the survival of *P. vitellinae* larvae on experimental *S. myrsinifolia*, I used the maximum number of third instar larvae observed as the test variable, as the actual survival rates could not be estimated. Other experiments conducted in Joensuu site suggest that late mortality of *P. vitellinae* larvae is
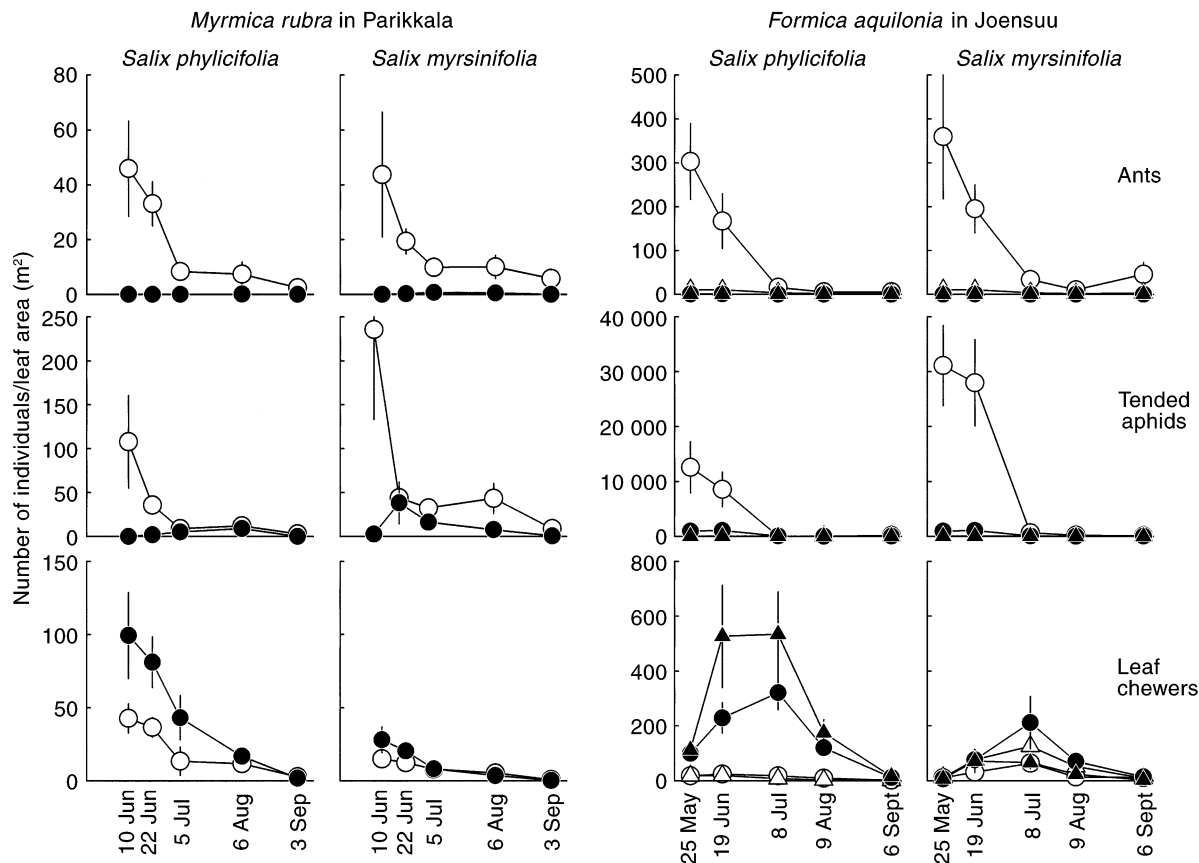
FIG. 1.   Densities of ants, tended aphids, and leaf chewing on experimental willows (mean ± 1 SE). Open circles, control; open triangles, aphids removed; filled circles, ants excluded; and filled triangles, both treatments. See the number of replicates in Fig. 3. Note the different scales of axis used for *Myrmica* and *Formica* sites.

relatively rare, and this variable correlates closely to the number of larvae entering to pupal stage (M. Sipura, *unpublished data*). When testing the differences in invertebrate predator densities, I used mean densities of all censuses as a variable to obtain normally distributed data that allows testing with parametric ANOVA. For the leaf damage and willow growth variables, I used a two- or three-way GLM MANOVA with type III sum of squares and eta squared (η^2) values as estimates of effect sizes. Eta squared gives the ratio of between-groups sum of squares and the total sum of squares and is thus comparable to R^2-values (Keppel 1982). Before the analyses, I tested all variables for the heteroscedasticity or normality of distributions and made appropriate transformations to meet the criteria of the tests. All values given are mean ± 1 SE.

RESULTS

Observational data

The densities of the aphid *Pterocomma salicis* (individuals/m²) were slightly but not significantly higher on *S. myrsinifolia* than on *S. phylicifolia* both in Parikkala (112.2 ± 20.0, $N = 72$ vs. 56.8 ± 9.7, $N = 66$;

$t_{136} = 1.47$, $P = 0.15$) and Joensuu (4489.0 ± 1244.6, $N = 74$ vs. 1380.8 ± 466.4, $N = 80$ respectively; $t_{152} = 0.20$, $P = 0.84$). The densities of the aphids were strongly related to the ant densities in both sites (Table 1). On the other hand, leaf-chewing insects were less abundant on *S. myrsinifolia* in both sites. The mean densities were 48.7 ± 8.8 vs. 9.3 ± 1.3 individuals/m² ($t_{136} = 4.63$, $P < 0.001$) in Parikkala and 224.9 ± 59.0 vs. 45.9 ± 15.6 individuals/m² ($t_{152} = 2.93$, $P = 0.004$) in Joensuu on *S. phylicifolia* and *S. myrsinifolia*, respectively.

In Parikkala, the logarithmic ant density significantly explained logarithmic leaf-chewer densities on *S. phylicifolia* but not on *S. myrsinifolia* (Table 1). Consequently, ants also had a significantly stronger effect on the leaf damage levels on *S. phylicifolia*. Ant density negatively affected the premature leaf abscission on *S. phylicifolia*, but positively on *S. myrsinifolia*. The early abscission of leaves is apparently induced both by leaf-chewing insects and aphids. In a regression model where the early abscission is explained by mean aphid densities and mean leaf-chewer densities, both insect groups are needed in the model ($P < 0.05$) to explain

TABLE 1. Observations on the effects of $\log(x + 1)$-transformed ant densities on $\log(x + 1)$-transformed mean insect densities, leaf damage, and growth of the two willow species in two study areas.

| Variable | Salix phylicifolia | | | | |
	df	Equation	R^2	F	P
Myrmica rubra in Parikkala	1, 65				
Log(tended aphids)		$0.11 + 1.26x$	0.66	121.87	<0.001
Log(leaf chewers)		$1.88 - 0.73x$	0.53	72.84	<0.001
Log(early abscission)		$0.10 - 0.031x$	0.15	11.05	0.001
Log(% of leaf area damaged)		$1.10 - 0.37x$	0.43	48.12	<0.001
Log(final shoot length)		$0.38 + 0.055x$	0.05	3.28	0.075
Log(relative radial growth)		$1.19 + 0.12x$	0.13	9.72	0.003
Formica aquilonia in Joensuu	1, 79				
Log(tended aphids)		$0.53 + 1.24x$	0.48	70.84	<0.001
Log(leaf chewers)		$2.22 - 0.83x$	0.53	89.15	<0.001
Log(early abscission)		$0.13 - 0.015x$	0.04	2.85	0.095
Log(% of leaf area damaged)		$1.40 - 0.65x$	0.66	148.61	<0.001
Log(final shoot length)		$0.26 + 0.09x$	0.17	16.37	<0.001
Log(relative radial growth)		$1.096 + 0.10x$	0.14	12.84	0.001

Note: Regression slopes were tested for both deviance from zero and from each other following Zar (1999).

the level of early abscission in both willow species and sites. In both sites, ants had a positive effect on the growth of *S. phylicifolia* whereas the effects on the growth of *S. myrsinifolia* were negative.

Insect densities on experimental willows

On experimental willows, the aphid *P. salicis* was slightly more abundant on *S. myrsinifolia*, but the difference was significant only in Joensuu ($F_{1,82} = 2.47$, $P = 0.12$ in Parikkala and $F_{1, 168} = 4.11$, $P = 0.044$ in Joensuu; Fig. 1). There were no significant differences in ant densities between the willow species ($F_{1,82} = 0.05$, $P = 0.83$ in Parikkala and $F_{1, 168} = 0.03$, $P = $

FIG. 2. Maximum number of third instar larvae of *Phratora vitellinae* observed per square meter of leaf area on experimental *Salix myrsinifolia* (mean $\pm$ 1 SE). The numbers within or above the bars indicate sample sizes.

0.86 in Joensuu). Ant exclusion caused a dramatic decrease in aphid densities at both sites, and nearly as dramatic decrease occurred in ant densities in Joensuu when aphids were removed (Fig. 1).

In Parikkala, ants had a clear negative effect on leaf-chewer densities on *S. phylicifolia*, but subtle effect on *S. myrsinifolia* (main effect: $F_{1,82} = 9.60$, $P = 0.003$, $\eta^2 = 0.11$; Fig. 1). However, the willow-species $\times$ ant-exclusion interaction was not significant ($F_{1,82} = 2.53$, $P = 0.12$). The maximum number of third instar larvae of *P. vitellinae* was not affected at all by ant removal in Parikkala ($t_{44} = 0.73$, $P = 0.47$; Fig. 2).

In Joensuu, the exclusion of *F. aquilonia* caused a tremendous increase in the densities of leaf-chewing insects on *S. phylicifolia* (Fig. 1). The increase was significantly smaller on *S. myrsinifolia* (willow-species $\times$ ant-exclusion interaction $F_{1, 168} = 54.96$, $P < 0.001$, $\eta^2 = 0.25$; Fig. 1). Ants had no overall effect on the mean larval densities of *P. vitellinae* on *S. myrsinifolia* ($F_{1,89} = 0.68$, $P = 0.41$; Fig 2). In addition, ants did not significantly affect the densities of the rest of the leaf-chewing guild on *S. myrsinifolia* in either of the study areas ($F_{1,44} = 3.26$, $P = 0.078$ in Parikkala and $F_{1,89} = 0.004$, $P = 0.95$ in Joensuu). When the entire data set was analyzed using repeated-measures ANOVA, there was a significant willow-species $\times$ ant-exclusion interaction in the rest of the densities of leaf-chewer guild in Joensuu ($F_{1, 168} = 38.03$, $P = 0.001$, $\eta^2 = 0.13$). In Parikkala, the effect is marginally significant ($F_{1,82} = 3.66$, $P = 0.059$).

Although the ant densities significantly decreased after aphid removal, the aphid treatment had no significant effects on leaf-chewer densities in Joensuu ($F_{1, 168} = 0.29$, $P = 0.59$), and there were no significant interactions between aphid removal and willow species or ant exclusion ($P > 0.25$). However, the ant exclusion increased the density of the third instar larvae of *P.*
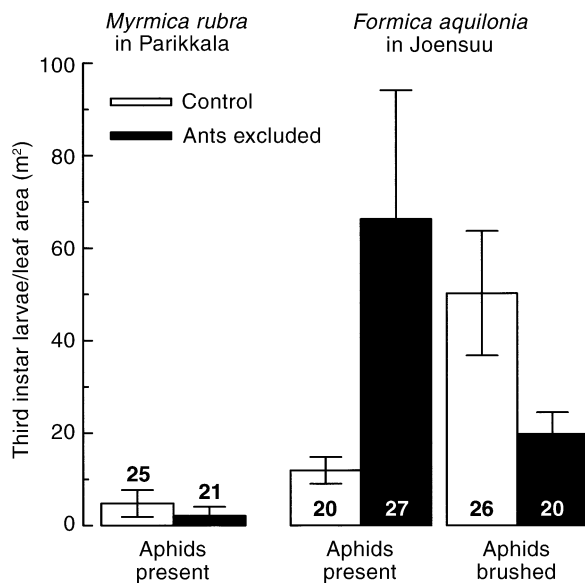
TABLE 1. Extended.

		Salix myrsinifolia			Difference between slopes	
df	Equation	R^2	F	P	t	P
1, 71						
	$0.22 + 1.29x$	0.69	154.52	<0.001	0.29	0.39
	$0.79 + 0.051x$	0.006	0.45	0.50	10.09	<0.001
	$0.056 + 0.015x$	0.06	4.54	0.037	6.82	<0.001
	$0.30 + 0.014x$	0.001	0.08	0.78	8.91	<0.001
	$0.44 - 0.022x$	0.02	1.60	0.21	3.84	<0.001
	$1.33 - 0.078x$	0.10	7.81	0.007	7.17	<0.001
1, 73						
	$0.47 + 1.48x$	0.57	94.91	0.001	1.49	0.069
	$1.17 - 0.094x$	0.014	1.00	0.32	7.32	<0.001
	$0.042 + 0.06x$	0.54	85.78	<0.001	8.56	<0.001
	$0.71 - 0.15x$	0.07	5.44	0.022	7.64	<0.001
	$0.38 - 0.038x$	0.06	4.64	0.034	6.09	<0.001
	$1.27 - 0.098x$	0.23	21.05	<0.001	7.56	<0.001

vitellinae only when aphids were present, but decreased when aphids were removed (Fig. 2; the interaction between aphid removal and ant exclusion $F_{1,89} = 8.55$, $P = 0.004$, $\eta^2 = 0.09$).

The ant exclusion increased the density of other invertebrate predators (individuals/m^2) both in Parikkala (0.13 $\pm$ 0.02, $N = 48$ vs. 0.50 $\pm$ 0.09, $N = 38$ in control and exclusion treatments respectively; $F_{1,82} = 21.51$, $P < 0.001$, $\eta^2 = 0.21$) and in Joensuu (0.16 $\pm$ 0.04; $N = 96$ vs. 0.70 $\pm$ 0.12, $N = 80$; $F_{1,168} = 38.98$, $P < 0.001$, $\eta^2 = 0.19$). There were no willow-species $\times$ ant-exclusion interactions in predator densities ($F_{1,168} = 0.32$, $P = 0.57$ in Joensuu and $F_{1,82} = 0.001$, $P = 0.97$ in Parikkala).

Leaf damage on experimental willows

In Parikkala, the exclusion of the ant *M. rubra* led to a significant increase in the percentage of leaf area damaged but had no effect on the rate of premature leaf abscission (Fig. 3, Table 2). Although the effect of ants on leaf damage was somewhat more pronounced on *S. phylicifolia*, the willow-species $\times$ ant-exclusion interaction was not significant. In Joensuu, the exclusion of *F. aquilonia* significantly increased leaf damage and the effect was significantly larger on *S. phylicifolia*. The exclusion of aphids significantly decreased the level of premature leaf abscission in both species when ants were present. The effect was larger on *S. myrsinifolia* but the willow-species $\times$ aphid-exclusion interaction was not quite significant. In early abscission there also was a significant interaction between the ant and aphid exclusions, reflecting the more positive effect of the ant exclusion for willows when the aphids have been removed.

Growth of the experimental willows

In Parikkala, the exclusion of *M. rubra* had no effects on the growth of the willows (Table 3, Fig. 4). In Joen-
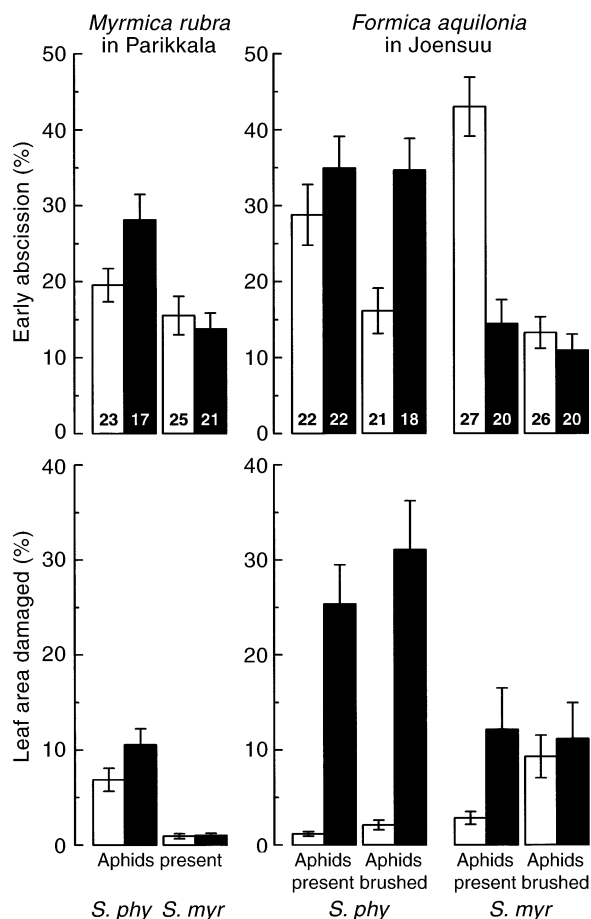

FIG. 3. Percentage of premature leaf abscission and the percentage of leaf area damaged on the experimental willows (mean $\pm$ 1 SE). White bars, control; black bars, ants excluded. The numbers within bars indicate sample sizes and are the same for both panels. *S. phy* = *Salix phylicifolia*; *S. myr* = *S. myrsinifolia*.

TABLE 2. Analysis of variance on the effects of willow species (W), aphid exclusion (''Brushing,'' B), and ant exclusion (A) on the percentage of premature leaf abscission and the percentage of leaf area damaged of the experimental willows in two study areas.

	df	Premature abscission (%)†				Leaf area damaged (%)†			
		MS	F	P	η^2	MS	F	P	η^2
Myrmica rubra in Parikkala									
Model	3	0.143	5.63	0.001	0.17‡	0.282	33.40	<0.001	0.55‡
Willow species	1	0.354	13.94	<0.001	0.145	0.818	96.79	<0.001	0.541
Ant exclusion	1	0.047	1.86	0.176	0.022	0.041	4.81	0.031	0.055
W × A	1	0.077	3.04	0.086	0.036	0.027	3.14	0.08	0.037
Error	82	0.025				0.008			
Formica aquilonia in Joensuu									
Model	7	0.561	13.76	<0.001	0.36‡	0.622	17.25	<0.001	0.42‡
Willow species	1	0.568	13.93	<0.001	0.077	0.300	8.31	0.004	0.047
Aphid exclusion (Brushing)	1	0.881	21.62	<0.001	0.114	0.157	4.35	0.039	0.025
Ant exclusion	1	0.046	1.13	0.289	0.007	2.647	73.37	<0.001	0.30
W × B	1	0.148	3.62	0.059	0.021	0.0043	0.12	0.73	0.001
W × A	1	1.362	33.43	<0.001	0.166	1.34	37.23	<0.001	0.18
B × A	1	0.630	15.47	<0.001	0.084	0.023	0.64	0.42	0.004
W × B × A	1	0.078	1.91	0.168	0.011	0.074	2.05	0.15	0.012
Error	168	0.041				0.036			

† Data were arcsine square-root transformed before the analyses.

‡ R^2 values.

suu, the exclusion of *F. aquilonia* had a clear effect both on the shoot length and the relative radial growth (Table 3, Fig. 4). There was a significant willow-species × ant-exclusion interaction in both variables indicating more profound negative effect of the ant exclusion in *S. phylicifolia* than in *S. myrsinifolia*. The aphid exclusion increased the shoot lengths, but in the relative radial growth the effect was not significant. There also was a nearly significant willow-species × aphid-exclusion interaction in shoot length, indicating that aphid removal increased the shoot growth more in *S. myrsinifolia*. When aphids were present on *S. myrsinifolia*, the presence of ants decreased rather than increased willow growth.

DISCUSSION

The results show that the effect of predatory ants on the herbivory and growth of willows under study is strongly dependent on the willow species. Both ant species decreased leaf damages on *S. phylicifolia* more effectively than on *S. myrsinifolia*, and in the *F. aquilonia*-inhabited site this led to increased growth of experimental *S. phylicifolia*. Both observational and experimental data from both study sites suggest that ants have a negligible or even negative effect on the growth of *S. myrsinifolia*. There are three major factors to explain the results. First, tended aphids were equally abundant on both willow species, which is likely to promote the negative effect of ants through phloem

TABLE 3. Analysis of variance on the effects of willow species (W), aphid exclusion (''Brushing,'' B), and ant exclusion (A) on shoot length and relative radial growth of the experimental willows in two study areas.

	df	Shoot length†				Relative radial growth†			
		MS	F	P	η^2	MS	F	P	η^2
Myrmica rubra in Parikkala									
Model	3	0.044	1.09	0.357	0.04‡	0.028	1.58	0.201	0.055‡
Willow species	1	0.006	0.14	0.709	0.002	0.012	0.68	0.412	0.008
Ant exclusion	1	0.010	0.26	0.615	0.003	0.050	2.79	0.099	0.033
W × A	1	0.113	2.83	0.096	0.033	0.034	1.90	0.172	0.023
Error	82	0.040				0.018			
Formica aquilonia in Joensuu									
Model	7	0.280	9.65	<0.001	0.29‡	0.089	4.57	<0.001	0.16‡
Willow species	1	0.140	4.82	0.029	0.028	0.025	1.29	0.258	0.008
Aphid exclusion (Brushing)	1	0.659	22.71	<0.001	0.119	0.068	3.53	0.062	0.021
Ant exclusion	1	0.182	6.28	0.013	0.036	0.239	12.32	0.001	0.068
W × B	1	0.111	3.83	0.052	0.022	0.002	0.08	0.772	0.001
W × A	1	0.627	21.61	<0.001	0.114	0.259	13.35	<0.001	0.074
B × A	1	0.071	2.46	0.118	0.014	0.014	0.71	0.400	0.004
W × B × A	1	0.059	2.02	0.157	0.012	0.016	0.799	0.373	0.005
Error	168	0.029				0.019			

† Data were $\log(x + 1)$ transformed before the analyses.

‡ R^2 values.

herbivory on both willow species. Second, there were clearly fewer leaf-chewing insects on *S. myrsinifolia*, making the potential positive effect of ant predation less likely. Third, the dominant leaf-chewing insect on *S. myrsinifolia*, the leaf beetle *P. vitellinae*, is better defended against generalist predators than the dominant generalist herbivores on *S. phylicifolia* (Sipura 1999). I argue that the balance between the first and the other two factors explains the differences in the observed effects of ants on the herbivory and growth of the studied willow species.

Pterocomma salicis, which was the only ant-tended insect species in both study areas, seems to be almost completely dependent on the attendance provided by ants. In this system, the most obvious benefit obtained by the aphids was the protection against natural enemies. The number of generalist predators increased when ants were excluded and, for example, predatory larvae of syrphid flies were never found in the aphid colonies that ants were tending. Thus, the presence of ants apparently increased the density of tended aphids, and because aphids seemed to retard willow growth, ants had a negative effect on willows susceptible to these aphids.

The densities of the aphid *P. salicis* did not differ between the willow species in the observational data, and there were slightly more aphids on the experimental *S. myrsinifolia* suggesting that the aphids colonizing stems are not harmed by phenolic glucosides found both in the leaves and bark of *S. myrsinifolia* (Julkunen-Tiitto 1986). It is possible that phloem sap of *S. myrsinifolia* does not contain phenolic glucosides or is otherwise more nutritious, but there are no studies available comparing the composition of the phloem fluids in these willows. However, it is also possible that the observed variation in aphid densities between willow individuals was originally launched by ants. *Salix phylicifolia* produces flowers early in the spring before budbreak, a few days earlier than *S. myrsinifolia*. The nectar of these flowers attracts ants. In the spring of 1998, the hatching of aphids occurred in synchrony with the flowering of *S. myrsinifolia*, when many flowers of *S. phylicifolia* had already withered. Therefore, there were more tending ants on *S. myrsinifolia* during the hatching of small, wingless, and vulnerable stem-mothers of aphids.

Salix phylicifolia harbored approximately five times higher densities of leaf-chewing insects than *S. myrsinifolia* when ants were excluded and the leaves were far more damaged by herbivores. The difference was largest in June during the most extensive shoot growth with an apparent consequence of reduced shoot growth of *S. phylicifolia*. The observed difference in leaf-chewer densities between the willow species is apparently a consequence of the striking differences in leaf chemistry (Tahvanainen et al.1985). Some of the chrysomelid beetle species observed in the study sites (in-
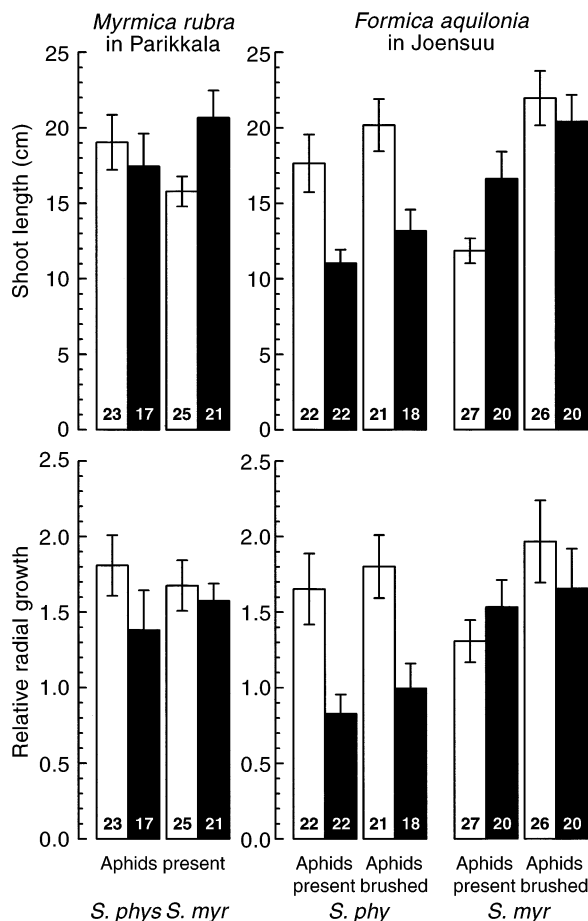

FIG. 4. The final shoot length and the relative radial growth of the experimental willows (mean ± 1 SE). White bars, control; black bars, ants excluded. The numbers within bars indicate sample sizes. *S. phys = Salix phylicifolia*; *S. myr = S. mysinifolia*.

cluding leaf beetles *Galerucella lineola* F. and *Lochmaea capreae* L.), have been shown to avoid feeding on salicylate-rich willows like *S. myrsinifolia* (Tahvanainen et al. 1985, Kolehmainen et al. 1995) and, in some studies, generalist herbivores were shown to perform worse on willows rich in phenolic glucosides (Roininen and Tahvanainen 1989, Denno et al. 1990, Matsuki and MacLean 1994).

Phratora vitellinae, which leaves distinctive feeding marks on the leaves, was responsible for ~84% of the leaf damage on *S. myrsinifolia*. I observed that the ovipositing females of *P. vitellinae* were less disturbed by foraging ants than the females of the abundant leaf beetles, *Galerucella lineola* and *Lochmaea caprea*, on *S. phylicifolia*. Therefore, large amounts of eggs were present on *S. myrsinifolia* also with high ant densities. The larvae of *P. vitellinae* were conspicuously less affected by ants than leaf-chewing herbivores on *S. phylicifolia*. When ants encounter larvae of *P. vitellinae*, they usually retreat immediately cleaning themselves

vigorously, but especially in the vicinity of aphid colonies they usually attack several times (M. Sipura, *personal observation*). I suggest that ants do not kill the larvae of *P. vitellinae* primarily for food, but kill or molest them in great numbers to protect aphid colonies. Floate and Whitham (1994) have shown earlier that another salicylaldehyde-producing leaf beetle *Chrysomela confluens* can suffer high mortality through ant predation, but the effect is strongly mediated by the presence of tended aphids. In this study, the exclusion of aphids enhanced the survival of the leaf beetle larvae when ants were present. This is probably caused by decreased aggressiveness of ants against *P. vitellinae* larvae while the natural enemies of the larvae are still killed. It has been shown earlier that generalist predators like bugs with piercing mouthparts, are not affected by the larval secretion (Rank et al. 1998) and some specialist predators, like a syrphid fly *Parasyrphus nigritarsis* Zett. are strongly attracted by salicylaldehyde (Köpf et al. 1997). It is also known that the sole presence of ants can repel parasitoids (e.g., Bartlett 1961) and even insectivorous birds (Haemig 1996). If the predators excluded by ants are a more serious threat for the larvae of *P. vitellinae* than the ants themselves, the positive effect of ants on larval survival seems logical.

In addition to *P. vitellinae*, ants did not significantly affect the densities of other leaf-chewers on *S. myrsinifolia*. The functional group of ''leaf-chewers'' consisted almost completely of generalist adults or larvae of leaf beetles, lepidopteran caterpillars, and sawfly larvae, which are not known to have any active plant-derived defense mechanisms against predators. It has been suggested, however, that also the generalist herbivores are partially safe from predators when feeding on chemically strongly defended plants, due to plant material in their gut or plant chemicals in their haemolymph (Brower 1984, Bowers 1993). However, the lower predation rates on *S. myrsinifolia* can be caused by a response of the ants to the densities of their prey or possible enemies of aphids (see also Jones 1987). It is known that ant individuals are specialized to different functions like aphid tending or predating (Rosengren and Sundström 1987, Gösswald 1989, Hölldobler and Wilson 1990). Consequently, it is possible that the ants on *S. myrsinifolia* were mainly aphid-milkers, since this willow species provided very few acceptable prey items or potential threats to aphids, but offered relatively more aphids than *S. phylicifolia*.

The results obtained in this study are similar the findings of Sipura (1999) on the impacts of insectivorous birds on the same two willow species. Both ants and birds have a negligible or even negative effect on the performance of the more strongly defended *S. myrsinifolia* with very low densities of herbivorous insects even when predators are excluded. It is clear that predators cannot have a strong positive effect on plant per-formance, if the number of herbivores is as low as on *S. myrsinifolia*, or if the herbivores are effectively defended against predators, as in the case of *P. vitellinae*. It seems that *S. phylicifolia* and *S. myrsinifolia* represent the extremes of a continuum created by the antagonistic relationship between direct plant defense and indirect defense provided by predators. *Salix phylicifolia*, which is susceptible to herbivores, clearly gain from the presence of predators, whereas *S. myrsinifolia*, which uses an armory of secondary chemicals against generalist herbivores, obtains no benefits from predators. Therefore, the third trophic level deserves to be considered as a component of plants defense when seeking to explain and predict the patterns of multitrophic systems such as the effect of ants on plants.

LITERATURE CITED

Adlung, K. G. 1966. A critical evaluation of the European research on the use of red wood ants (*Formica rufa* group) for the protection of forests against harmful insects. Journal of Applied Entomology **57**:167–189.

Bartlett, B. R. 1961. The influence of ants upon parasites, predators, and scale insects. Annals of the Entomological Society of America **54**:543–551.

Beattie, A. J. 1985. The evolutionary ecology of ant–plant mutualism. Cambridge University Press, Cambridge, UK.

Bentley, B. 1976. Plants bearing extrafloral nectaries and the associated ant community: interhabitat differences in the reduction of herbivore damage. Ecology **54**:815–820.

Boethel, D. J., and R. D. Eikenbary. 1986. Interactions of plant resistance and parasitoids and predators of insects. Ellis Horwood Entomology Series. John Wiley and Sons, New York, New York, USA.

Bowers, M. D. 1993. Aposematic caterpillars: life-styles of the warningly colored and unpalatable. Pages 331–371 *in* N. E. Stamp and T. M. Casey, editors. Caterpillars: ecological and evolutionary constraints on foraging. Chapman and Hall, New York, New York, USA.

Brower, L. B. 1984. Chemical defence in butterflies. Pages 109–132 *in* R. I. Vane-Wright and P. R. Ackery, editors. The biology of butterflies. Academic, London, UK.

Collingwood, C. A. 1979. The Formicidae (Hymenoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica 8. Scandinavian Science, Klampenborg, Denmark.

Cushman, J. H., and J. F. Addicott. 1991. Conditional interactions in ant–plant–herbivore mutualism. Pages 92–103 *in* C. R. Huxley and D. F. Cutler, editors. Ant–plant interactions, Oxford University Press, Oxford, UK.

Damman, H. 1987. Leaf quality and enemy avoidance by larvae of a pyralid moth. Ecology **68**:87–97.

Denno, R. F., S. Larsson, and K. L. Olmstead. 1990. Role of enemy-free space and plant quality in host–plant selection by willow beetles. Ecology **71**:124–137.

Faeth, S. H. 1980. Invertebrate predation of leaf miners at low densities. Ecological Entomology **5**:111–114.

Floate, K. D., and T. G. Whitham. 1994. Aphid–ant interaction reduces chrysomelid herbivory in a cottonwood hybrid zone. Oecologia **97**:215–221.

Fowler, S. V., and M. MacGarwin. 1985. The impact of hairy wood ants, *Formica lugubris*, on the guild structure of herbivorous insects on birch, *Betula pubescens*. Journal of Animal Ecology **54**:847–855.

Fritz, R. S. 1983. Ant protection of a host plant's defoliator: consequences of ant–membracid mutualism. Ecology **64**:789–797.

Gosswald, K. 1951. Die rote Waldameise im Dienste der Waldhygiene. Forstwirtschaftliche Bedeutung, Nutzung, Lebensweise, Zucht, vermehrung und Schultz. Metta Kinau, Lüneburg, Germany.

Gosswald, K. 1989. Die Waldameise. Biologie, Ökologie, und forstliche Nutzen. 1. Biologische Grundlagen, Ökologie und Verhalten. Aula, Wiesbaden, Germany.

Haemig, P. D. 1996. Interference from ants alters foraging ecology of great tits. Behavioural Ecology and Sociobiology **38**:25–29.

Hämet-Ahti, L., J. Suominen, T. Ulvinen, and P. Uotila. 1998. Retkeilykasvio. Luonnontieteellinen keskusmuseo, Kasvimuseo, Helsinki, Finland.

Heads, P. A., and J. H. Lawton. 1985. Bracken, ants and extrafloral nectaries. III. How insect herbivores avoid ant predation. Ecological Entomology **10**:29–42.

Heie, O. E. 1986. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. III. Family Aphididae: subfamily Pterocommatinae and tribe of subfamily Aphidinae. Fauna Entomologica Scandinavica 17. E. J. Brill/Scandinavian Science, Copenhagen, Denmark.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Springer-Verlag, Berlin, Germany.

Ito, F., and S. Higashi. 1991. Variance of ant effects on the different life forms of moth caterpillars. Journal of Animal Ecology **60**:327–334.

Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution **20**:249–275.

Jones, R. E. 1987. Ants, parasitoids, and the cabbage butterfly *Pieris rapae*. Journal of Animal Ecology **56**:739–749.

Julkunen-Tiitto, R. 1986. A chemotaxonomic survey of phenolics in leaves of northern Salicaceae species. Phytochemistry **25**:663–667.

Karhu, K. J. 1998. Green islands: top-down and bottom-up effects of wood ants in forests under folivore attack. Dissertation. University of Turku, Turku, Finland.

Keppel, G. 1982. Design and analysis: a researchers handbook. Second edition. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Kolehmainen, J., R. Julkunen-Tiitto, H. Roininen, and J. Tahvanainen. 1995. Phenolic glucosides as feeding cues for willow-feeding leaf beetles. Entomologia Experimentalis et Applicata **74**:235–243.

Köpf, A., N. A. Rank, H. Roininen, and J. Tahvanainen. 1997. Defensive larval secretions of leaf beetles attract a specialist predator *Parasyrphus nigritarsis*. Ecological Entomology **22**:176–183.

Laine, K. J., and P. Niemelä. 1980. The influence of ants on the survival of mountain birches during an *Oporinia autumnata* (Lep., Geometridae) outbreak. Oecologia **47**:39–42.

Lawton, J. H., and P. A. Heads. 1984. Bracken, ants and extrafloral nectaries. I. The components of the system. Journal of Animal Ecology **52**:995–1014.

Mahdi, T., and J. B. Whittaker. 1993. Do birch trees (*Betula pendula*) grow better if foraged by wood ants? Journal of Animal Ecology **62**:101–116.

Matsuki, M., and S. F. MacLean, Jr. 1994. Effects of different leaf traits on growth rates of insect herbivores on willows. Oecologia **100**:141–152.

Messina, F. J. 1981. Plant protection as a consequence of an ant–membracid mutualism: interactions on goldenrod (*Solidago* sp.). Ecology **62**:1433–1440.

Nyman, T. 2000. Phylogeny and ecological evolution of gall-inducing sawflies (Hymenoptera: Tenthredinidae). Dissertation. University of Joensuu, Joensuu, Finland.

Nyman, T., and R. Julkunen-Tiitto. 2000. Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. Proceedings of the National Academy of Sciences (USA) **97**:13184–13187.

Otto, D. 1967. Die Bedeutung der Formica-Vöker für die Dezimierung der Wichtigsten Schadinsekten—ein Literaturbericht. Waldhygiene **7**:65–90.

Pasteels, J. M., M. Rowell-Rahier, and M. J. Raupp. 1988. Plant-derived defense in chrysomelid beetles. Pages 235–271 *in* P. Barbosa and D. K. Letourneau, editors. Novel aspects of insect–plant interactions. Wiley, New York, New York, USA.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics **11**:41–65.

Rank, N. E., A. Köpf, R. Julkunen-Tiitto, and J. Tahvanainen. 1998. Host preference and larval performance of the salicylate-using leaf beetle *Phratora vitellinae*. Ecology **79**:618–631.

Roininen, H., P. W. Price, R. Julkunen-Tiitto, J. Tahvanainen, and A. Ikonen. 1999. Oviposition stimulant for a gall-inducing sawfly, *Euura lasiolepis*, on willow is a phenolic glucoside. Journal of Chemical Ecology **25**:943–953.

Roininen, H., and J. Tahvanainen. 1989. Host selection and larval performance of two willow-feeding sawflies. Ecology **70**:129–136.

Rosengren, R., and L. Sundström. 1987. The foraging system of a red wood ant colony (*Formica* s. str.): collecting and defending through an extended phenotype. Experientia Supplemental **54**:117–137.

Sipura, M. 1999. Tritrophic interactions: willows, herbivorous insects and insectivorous birds. Oecologia **121**:537–545.

Skinner, G. J. 1980. The feeding habits of the wood-ant *Formica rufa* in limestone woodland in Northwest England. Journal of Animal Ecology **49**:381–394.

Skvortsov, A. K. 1999. Willows of Russia and adjacent countries. Taxonomical and geographical revision. University of Joensuu, Faculty of Mathematics and Natural Sciences report series number 39, Joensuu, Finland.

Soatens, P., and J. M. Pasteels. 1994. Synergistic effect of secondary compounds and nutrients in the host plant choice of a salicaceous-feeding leaf beetle: *Phratora vitellinae* (Coleoptera: Chrysomelidae). Mededelingen van de Faculteit Landbouwwetensschappen Rihksuniversiteit Gent **59/2b**:685–689.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution **14**:179–185.

Tahvanainen, J. R., R. Julkunen-Tiitto, J. Kettunen. 1985. Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. Oecologia **67**:52–56.

Tilman, D. 1978. Cherries, ants and tent caterpillars: timing of nectar production in relation to susceptibility of caterpillars to ant predation. Ecology **59**:686–692.

Way, M. J., and K. C. Khoo. 1992. Role of ants in pest management. Annual Review of Entomology **37**:479–503.

Whitham, T. G. 1983. Host manipulation of parasites: within-plant variation as a defense against rapidly evolving pest. Pages 15–41 *in* R. F. Denno and M. S. McClure, editors.

Variable plants and herbivores in natural and managed systems. Academic, New York, New York, USA.

Whittaker, J. B. 1991. Effect of ants on temperate woodland trees. Pages 67–79 *in* C. R. Huxley and D. F. Cutler, editors.

Ant–plant interactions, Oxford University Press, Oxford, UK.

Zar, J. H. 1999. Biostatistical analysis. Fourth edition. Prentice Hall, New Jersey, New Jersey, USA.