Herbivory on willows: abiotic constraints and trophic interactions

by

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One of the major goals of the discipline of ecology is to find out why our terrestrial world remains green amongst a myriad of herbivorous animals. Both bottom-up effects through host plant quality and top-down effects through natural enemies of herbivores have been proposed as global answers to the surprisingly low herbivore densities, and relatively low levels of damage suffered by plants. However, there is currently a consensus among ecologist agreeing that a combination of these and many other factors need to be considered simultaneously, and that the relative importance of these factors is likely exhibit both spatial and temporal variation between and within the systems studied.

In this thesis I have studied the effects of abiotic conditions, bottom-up impacts and top-down impacts on the distribution, abundance and performance of willow-feeding herbivorous insects, and their effects on two chemically different host species; a weakly defended Salix phylicifolia L. and strongly defended Salix myrsinifolia Salisb. It appeared that no one of the three factors exhaustibly could explain the distribution and abundance of willow-feeding herbivores. Although willows growing in shaded habitats provided high-quality food for the two leaf beetle species studied, both species were more abundant on willows in open habitats. This is probably due to the higher and more variable temperature in open habitats, which enhanced larval performance. Similarly, one of these species, Galerucella lineola F., occurred at high densities on S. phylicifolia growing in wetlands which, however, provided poor quality food for both adults and larvae of this beetle. The primary reason for this seems to be the better abiotic conditions in wetlands, affecting especially the performance of neonate larvae. Furthermore, it appeared that the factors affecting larval abundance and performance can interact, and their relative importance show spatial and temporal variation.

Both birds and ants enhanced the growth of S. phylicifolia by reducing densities of leaf-chewing insects, but their impact on S. myrsinifolia were either negligible or, in the case of ants, negative. The reason for this was the higher density of leaf-chewers on S. phylicifolia, and the consequent aggregative response by predators. Similar results were obtained when the predation on clones of S. phylicifolia were examined. When equal densities of G. lineola were used, predators reduced leaf damage more on strongly defended clones, but under natural densities, the strongest impact of predators was found on weakly defended clones. Therefore, predation should be considered a substitutive component of willow defence.

This thesis reveals the multiplicity of factors affecting herbivory on willows. Firstly, some of the willows providing high-quality food for herbivores grow under abiotic habitat not accessible for the herbivores. Secondly, when occurring abundantly on otherwise adequate willows, herbivores face high predation rates or intense intraspecific competition. Moreover, due to high variation, it may be impossible for ovipositing females to distribute their eggs optimally between the host plants. Therefore, I conclude that abiotic factor, host quality, top-down impacts, interactions between them and variation in all of these all deserve to be considered in attempting to explain the ecology of herbivorous insects and their interactions with plants. There is no optimal host plant for the willow-feeding insects and, if there is one, the herbivores most probably don’t know it! Therefore, the world of willows remains green.

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This thesis is based on the following articles and previously unpublished results. The articles are referred to in the text by the Roman numerals I-V


IV Sipura, M.: Contrasting effect of ants on the herbivory and growth of two willow species. Accepted after revision (Ecology).

1. Introduction

“Why don’t all the herbivores eat up all green world? Because most of the green is inedible to any given species of herbivore. Why don’t the herbivores that can readily consume a species of plant eat their host into oblivion? Because the carnivory and climate regimes stop them.” (Janzen 1988).

Above, Janzen (1988) provides a straightforward answer to the classical question of why our terrestrial world remains green amongst a myriad of herbivorous animals. Being aware of the high diversity and enormous reproductive potential of herbivorous insects, it is certainly surprising that only about 5-20% of green plants are consumed by herbivorous insects whereas the rest ends up directly with decomposers (Crawley 1983; Strong et al. 1984b; Crawley 1997; Hartley and Jones 1997). However, spatially and temporally restricted outbreaks of populations of herbivorous insects do occur, destroying whole areas of vegetation, completely defoliating some plant species or otherwise reducing plant performance (Tenow 1972; Berryman 1987; Crawley 1997; Tikkanen and Roininen 2000). These, often unexpected, eruptions are warning examples of the capriciousness of the pattern of low herbivory; the greenness of our world is not a self-evident fact. Therefore, in order to understand and try to predict the changes in herbivore populations, we need to distinguish between the factors that usually keep the level of herbivory so low.

Traditionally, there have been three lines of thought to explain the control of herbivore populations and consequent low level of herbivory. The top-down view proclaimed by Hairston et al. (1960) states that as herbivores eat only a fraction of the plant material available, they are not limited by the availability of food, and it must be predators acting in a density-dependent way that suppresses herbivore populations to the observed low level. The other view advocated by Southwood (1973), Strong et al. (1984a), White (1993) and many others appreciate bottom-up factors. Their view is based on the indisputable fact that plant tissues are generally very poor food for animals (Crawley 1983; Strong et al. 1984a; Hartley and Jones 1997). Furthermore, plants defend themselves effectively against many herbivores and together exhibit a great diversity in defence mechanisms, including morphological structures such as thorns and hairs, and an enormous number of secondary chemicals (Harborne 1997). Consequently, omnipotent herbivores mastering all plant defences have not evolved, and those that are able to circumvent the defences of some plants are limited by the availability of those plants. Moreover, herbivores may cause the deterioration their own food when they occur at high densities through selective mortality of the host plants (Berryman 1987; Marquis 1992) or through induced defences (Haukioja and Niemelä 1977; Karban and Baldwin 1997). Therefore, density-dependent bottom-up impacts (i.e. competition) among herbivores may be more common than previously judged (Hairston et al. 1960; Strong et al. 1984a versus Denno et al. 1997; Masters and Brown 1997).

Figure 1. Adult Galerucella lineola copulating on a leaf of Salix phylicifolia (Photo by MS).
Although both bottom-up and top-down views have gained support (e.g. Strong et al. 1984a; Hunter and Price 1992; Power 1992; Hunter et al. 1997; Stiling and Rossi 1997), ever since the seminal papers by Lawton and McNeill (1979), and Price et al. (1980), it has become progressively clearer that both bottom-up forces arising from plants and top-down forces cascading from upper trophic levels must be taken into account when considering the evolution and ecology of plant-insect interactions (e.g. Hartley and Jones 1997; Karban 1997; Sabelis et al. 1999; Forkner and Hunter 2000). As Lawton and McNeill (1979) put it, herbivorous insects are squeezed between the devil and deep blue sea … as they have to … live in a world dominated on the one hand by their natural enemies and on the other by a sea of food that, at best, is often nutritionally inadequate and at worst is simply poisonous.

Furthermore, there is increasing awareness that plant quality and the impact of natural enemies on herbivores are likely to interact (Price et al. 1980; Sabelis et al. 1999; Forkner and Hunter 2000). For example, direct plant defences may act antagonistically with top-down impacts if foraging predators are harmed by them (Price et al. 1980; Boethel and Eikenbary 1986; Damman 1987; Pasteels et al. 1988; Hare 1992) or if predators aggregate to plants with low defence and, consequently, high herbivore density (Schultz 1983; Haukioja 1993; III). On the other hand, top-down and bottom-up impacts on herbivory may act synergistically when weak and easily detectable herbivores on well-defended plants are more vulnerable to predation (Bergelson and Lawton 1988; Häggström and Larsson 1995; Bernays 1997; III). The tight link between bottom-up and top-down impacts has become so apparent that the impact of predators and parasitoids are currently interpreted as an essential components of plant defence (van der Meijden 1996; Sabelis et al. 1999; Speight et al. 1999; Agrawal et al. 2000; III).

The third environmental factor suggested as controlling populations of herbivorous insects, announced by Andrewartha and Birch (1954; 1960), represents a criticism of the advocates of density-dependent biotic factors (sensu Nicholson 1933). Andrewartha and Birch (1954; 1960) emphasized the role of density-independent abiotic factors (especially temperature), stating that population growth is irregularly interrupted by harsh environmental conditions, suppressing the average populations sizes to the usually observed low level. They concluded that no biotic factors acting in a density-dependent way are needed to control herbivore populations, i.e. to keep the world green. However, there is currently a consensus among population ecologists agreeing that at least some form of density-dependence is needed to control populations (Hanski 1990; Cappuccino 1995; Turchin 1995). This may be the principal reason why abiotic factors have recently been largely ignored in the study of processes in populations and communities of terrestrial organisms, especially in the case of herbivorous insects (Kingsolver 1989; Dunson and Travis 1991; Hunter and Price 1992). However, abiotic factors can be important components in both spatial and temporal density-dependent ecological processes, for example by suppressing herbivore populations to levels that can be regulated by predators (Kidd and Jervis 1997; Dunson and Travis 1991) and by restricting the spatial distribution of herbivores to regions where bottom-up or top-down impacts may predominate (Dunson and Travis 1991; Hunter and Price 1992; Virtanen and Neuvonen 1999; Solbreck 1995; II; III).

In addition to the multiplicity of interacting factors affecting herbivores described above, these factors exhibit both spatial and tempo-
ral variation within and between systems (Hunter and Price 1992; Hartley and Jones 1997). For example, changes in abiotic conditions may shift the balance between bottom-up and top-down forces; the strength of top-down forces may increase with increasing primary productivity driven by plant resources and climate (Fretwell 1977; Price et al. 1980; Hunter and Price 1992; Hunter et al. 1997; Forkner and Hunter 2000). As emphasized by Hunter and Price (1992), incorporating such environmental heterogeneity in our synthesis may be the key issue in understanding the relative roles of bottom-up and top-down forces in natural communities. We should not ask which factors predominate in the system under study, but under what conditions these factors predominate and how these factors interact.

In this thesis I shall explore factors that might be responsible for the greenness of the world of willows (Salix spp.; Salicaceae). My main focus will be on proximate and ultimate factors affecting spatial distribution and variation in the performance of willow-feeding herbivorous insects, and their impact on their host plants. In order to answer the fundamental question, I have implicitly assumed that the spatial processes mirror the temporal patterns of herbivore populations and communities (see Steward-Oaten and Murdoch 1990; Cappuccino et al. 1995). The ecological factors explored here include bottom-up impacts through variable plant quality, constraining abiotic factors conceivably affecting willows; as well as herbivores and predators, and top-down impacts through the third trophic level possibly interacting with willow quality. Article I focuses on the effect of shading on habitat selection of leaf beetles, both directly and indirectly through host plant quality. Article II investigates why one species of these leaf beetles prefers damp wetland habitats, and discusses how the observed pattern of habitat selection may affect the ecology of the beetle. In chapter 8 of this thesis, I will further discuss several ecological and evolutionary implications of the results obtained in articles I and II on the life of the leaf beetles. Article III turns to the interaction between willows and the third trophic level, asking whether the plants need the third trophic level to support their direct defense against herbivores, or to substitute for it. Articles IV and V examine the effects of predatory ants (IV) and insectivorous birds (V) on the herbivory and growth of two phytochemically different willow species. In order to fill the gaps left by these articles and to provide further information about the relationships between willows, their herbivores and predators, I have also included several previously unpublished results in this thesis. Finally, I will try to provide a simplified answer to the question; why the world of willows is green.

2. The three trophic levels studied

2.1. Willows

The tea-leaved willow Salix phylicifolia L. is a common and widespread shrub in Scandinavia, growing abundantly in wet meadows, wastelands, along lakesides and drainage ditches (Jalas and Suominen 1974; Hämet-Ahti et al. 1998; Skvortsov 1999). S. phylicifolia is a typical early successional shrub, which readily colonizes bare ground and uncultivated arable land. It grows fast, and often forms dense but relatively low vegetation together with other willows. The leaves of S. phylicifolia contain only a small amount in phenolic glucosides (Tahvanainen et al. 1985b; Julkunen-Tiitto 1989; Rank et al. 1998), the proposed major components of herbivore resistance of willows (e.g. Tahvanainen et al. 1985b; Denno et al. 1990; Matsuki and MacLean 1994; Kolehmainen et al. 1995; Kendall et al. 1996). Instead, its leaves contain considerable amounts of other phenolics, including ampelopsin, a flavonoid characteristic to the leaves S. phylicifolia, and condensed tannins (Rank et al. 1998: II). Apparently, due to its relatively low concentrations of detrimental secondary chemicals, S. phylicifolia harbors a rich community of herbivorous insects (Seppänen 1970; Lii-ikanen 1997).

Dark-leaved willow Salix myrsinifolia Salisb. regularly forms mixed vegetation with S. phylicifolia, but is usually somewhat less abundant, and does not occupy the dampest wetland habitats. Although S. myrsinifo-olia appears to be morphologically and ecologically similar to S. phylicifolia, there
is a striking difference in their leaf chemistry. Generally, the phenolic glucoside concentrations in the bitter-tasting leaves of *S. myrsinifolia* are about fifty-times higher than those in the mild-tasting leaves of *S. phylicifolia* (Tahvanainen et al. 1985b; Julkunen-Tiitto 1989; Rank et al. 1998). Salicylates, salicin and salicortin are the main secondary compounds found in the leaves (Julkunen-Tiitto 1986; 1989; Julkunen-Tiitto and Meier 1992), and apparently give them their bitter taste protecting *S. myrsinifolia* against insect herbivores. As observed in this thesis (I; IV; V), only a small number of generalist herbivorous insects feed upon its leaves. However, as phenolic glucosides may also act as feeding cues for specialist herbivores (Tahvanainen et al. 1985b; Pasteels et al. 1988; Rowell-Rahier and Pasteels 1990; Roininen et al. 1999), *S. myrsinifolia* may occasionally suffer severe leaf damage caused by specialist herbivores, especially by the leaf beetle *Phratora vitellinae*.

2.2. Herbivores and their enemies

The brown willow leaf beetle *Galerucella lineola* F. (Coleoptera: Chrysomelidae: Galerucinae) is one of the 10 species of the genus *Galerucella* usually inhabiting moist habitats and feeding on aquatic plants (Koch 1992). It is a univoltine species which overwinters in litter or bark crevices as an adult. Adults colonize their host plants in early spring at the budbreak, after which they mate (Fig. 1) and feed for several weeks before oviposition. The eggs, laid in batches of 10-20, hatch after a few weeks, and the larvae pass through three instars, feeding mainly on the lower surface of leaves. After more than 11 days, the fully-grown larvae crawl down the stem to enter a preupal stage in the soil. A new generation of adults emerges after a short pupal stage in late autumn. Some populations have been found to feed on *Alnus* spp. (Maisner 1974; Ikonen and Sipura, unpublished data), but in Finland most populations prefer *S. phylicifolia* growing in wetlands as host plants (II). *G. lineola* usually makes up more than 95% of the community of willow-feeding insects in wetlands (Sipura, unpublished data). However, adults and larvae of *G. lineola* also made up 20-70% (mean 44%) of the guild of leaf-feeding insects on 37 haphazardly chosen *S. phylicifolia* populations growing on well-drained soils, censused in Eastern Finland during the years 1998 and 1999 (Sipura, unpublished data). Several invertebrate predators (Kanervo 1946; Häggström and Larsson 1995) and birds (III; V) have been observed to prey upon *G. lineola* larvae with ants (*Formica aquilonia* Yarr. and *Myrmica rubra* L.), hemipteran bugs (especially *Anthocoris nemorum* L.), spiders, lacewigs and insectivorous birds forming the most numerous group of potential predators in my study areas. The larvae studied in this thesis were also attacked by a parasitoid wasp (assumed to be *Asecodes mentho*; Eulophidae; Entedontinae). However, it appeared to be scarce in my study sites (parasitism rate 0-2.2%). Nothing is known about pupal predation of *G. lineola*, which has been observed to play an important role in the population dynamics of some other insects (Hanski 1992; Tanhuapää et al. 1999).

Another common leaf beetle, *Phratora vitellinae* L. (Coleoptera: Chrysomelidae) is specialized in willows and poplars containing large amounts of phenolic glucosides (Tahvanainen et al. 1985a; Pasteels et al. 1988; Denno et al. 1990; Rank et al. 1998). The life cycle of *P. vitellinae* resembles that of *G. lineola*, but *P. vitellinae* occurs somewhat later in the summer. In my study areas most adults colonize *S. myrsinifolia* (Fig. 2).
but adults, eggs and larvae are frequently found on other high-salicylate willows, such as *S. fragilis* (Fig. 3). *P. vitellinae* is seldom found on *S. phylicifolia*, but it made up 20-98% (mean 76%) of the guild of leaf chewing insects on 37 haphazardly chosen *S. myrsinifolia*-populations censused in Eastern Finland during the years 1998-1999. The larvae of *P. vitellinae* convert salicylates derived from their host plants into salicylaldehyde, which has been shown to repel many natural enemies of the larvae (Pasteels et al. 1988; Rowell-Rahier and Pasteels 1990; Rank et al. 1996). Thus, it has been suggested that *P. vitellinae* benefits from feeding on high-salicylate willows, as these plants provide an enemy-free space for the larvae (Pasteels et al. 1988; Denno et al. 1990). However, Rank et al. (1998) found that there were no differences in mortality rates through predation when larvae were grown on different willow species, although they grew better on *S. myrsinifolia* compared to *S. phylicifolia*. Larval secretion has been found to attract specialist predators including the larva of the syrphid fly (*Parasyrphus nigritarsis* Zett.) (Köpf et al. 1997; Rank et al. 1996). Consequently, these specialist predators and generalist predators with the ability to avoid contact with the secretion while attacking (see Rank et al. 1996) may cause considerable mortality in *P. vitellinae* populations (Rank et al. 1998).

The orange willow aphid *Pterocomma salicis* L. (Fig. 4) occurs patchily, but when present, abundantly on willows (Heie 1986). As a sap-sucking insect it might not be harmed by phenolic glucosides, especially found on the leaves of *S. myrsinifolia* and in the bark of both *S. myrsinifolia* and *S. phylicifolia* (Tahvanainen et al. 1985b). *P. salicis* formed 0.97% (mean 36%) of the total number of herbivorous insects in the *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 (Fig. 4) preferring two-year old branches of both mild and strongly defended willows (Heie 1986; Sipura, personal observation), where it sucks phloem fluids and excretes honeydew readily used by ants. Apparently, ants provide efficient protection against the predators of aphids (IV). *P. salicis* overwinters as eggs attached to willow stems and the stem-mothers hatch before budbreak early in spring. Under favorable conditions, the colony grows to 100 000 individuals within a few weeks, and may cover entire bark surfaces on the most preferred willow clones. *P. salicis* is most abundant in early spring, but occurs on willow stems throughout the summer (IV).

### 3. General methodology

Throughout this thesis I have applied both observational and experimental methods, as well as combinations of the two (III). The observational protocols were planned on the one hand to realistically describe patterns in nature (I; II; IV), and on the other, to reveal factors worthy of more rigorous examination by experiments (I; II; III; IV). The quality of willows as food for herbivores was defined under controlled conditions in the laboratory (I; III; III), or in the field by attempting subjectively to minimize variation in abiotic...
conditions between the experimental units (III). The role of abiotic conditions was examined by simultaneously establishing analogous experiments under the controlled conditions of the laboratory and under natural microclimatic conditions in the field (I; II). Although, the microclimate surrounding the herbivores may affect the quality of the leaves of willows as well as the herbivores themselves (Stamp 1993), I have assumed that the differences between laboratory and field experiments essentially reflect the direct impacts of microclimate on the performance of herbivores. The role of predators was defined by using cages or sleeve bags excluding either the whole predator community (I; II; III), only the crawling predators such as ants (IV) or only the largest predators, insectivorous birds (V). Depending on the objectives of the experiments, the exclosures covered either entire plants (I; III; IV; V) or a part of a ramet (II). I have paid special attention to the impacts of exclosures themselves (cage effect) to the performance of plants and herbivores (see Hairston 1989; Harrison and Cappuccino 1995). Especially, I have made effort to minimize biases imposed by differential migration of herbivores between exclosures and control plants.

The spatial scale adopted for a study significantly affects the results obtained (Heads and Lawton 1983; Whitham 1983; Wiens 1989; Kouki 1991; Harrison and Cappuccino 1995; but see Connor et al. 1999), and should therefore always be considered in ecological studies. In this thesis I have focused on the variation between individual plants and clones, and at the landscape level the variation between habitats (I; II; III). However, I have made no attempt to examine within-plant variation, through some fundamental ecological processes can take place within plants, within shoots, or even within individual leaves (Whitham 1983; Hassell et al. 1987; Steward-Oaten and Murdoch 1990; Kouki 1991). This does not imply that I consider the small-scale variation insignificant. However, I have assumed that, as most herbivores under study in this thesis are not sedentary, but have a restricted ability to disperse (by walking), they can more easily cope with intra-plant variation than with variation between separate bushes.

Below, I describe the methods of previously unpublished results carefully enough to allow rigorous evaluation of the arguments that have been made. However, in the cases of the studies presented in the original articles, the article referred to should be consulted. The results presented in this thesis are mean ± standard error of mean (SE). Eta squared values ($\eta^2$) are used for estimates of effect magnitudes in ANOVA-models (Keppele 1982).

4. Bottom-up impacts: willows as food for their herbivores

4.1. Genetic variation

It has been incontrovertibly shown that willow species differ in their quality as food for both generalist and specialist herbivorous insects (e.g. Tahvanainen et al. 1985b; Rowell-Rahier and Pasteels 1990; Kelly and Curry 1991; Kirsten and Topp 1991; Matsuki and MacLean 1994; Kendall et al. 1996; Rank et al. 1996; IV; V), and that phenolic glucosides are the most obvious candidates for causing these differences (e.g. Tahvanainen et al. 1985b; Rowell-Rahier and Pasteels 1990; Matsuki and MacLean 1994; Kendall et al. 1996; IV; V), and that phenolic glucosides are the most obvious candidates for causing these differences (e.g. Tahvanainen et al. 1985b; Rowell-Rahier and Pasteels 1990; Matsuki and MacLean 1994; Kendall et al. 1996; Roininen et al. 1999; Ruuhola and Tikkanen, in preparation). However, despite the established fact that $S$. phylicifolia and $S$. myrsinifolia lie at the ends of a continuum of concentrations of phenolic glucosides (Tahvanainen et al. 1985b; Julkunen-Tiitto 1989), no studies exist that compare the differences in herbivory between these willow species.

This thesis shows that, when growing sympatrically, the total leaf area damaged by herbivores in the absence of certain predators was from 2.8 (V; birds excluded) to 11.0 (IV; ants excluded) times higher on $S$. phylicifolia. The leaf-chewer community on $S$. myrsinifolia consisted primarily of the specialist leaf beetle P. vitellinae whereas the community of $S$. phylicifolia was dominated by G. lineola (I; IV; V). However, on $S$. phylicifolia (V) there were also significantly more generalist herbivores, especially lepidopteran larvae, in which the salicylates may severely reduce larval performance (Lindoeh et al. 1988; Lindroth and Peterson
In order to examine whether the low density of generalist caterpillars on *S. myrsinifolia* could be due to host plant selection behavior or higher mortality of the larvae, I performed laboratory experiments with two generalist lepidopteran larvae, *Orthosia gothica* L. (Lepidoptera, Noctuidae) and *Lacanobia thalassina* Hufnagel (Lepidoptera, Noctuidae), which were both present on the willows studied in articles IV and V, and appeared to be more abundant on *S. phylicifolia*. These species are known to feed on plants from several families of trees and herbs (Seppänen 1970; Sipura, personal observations) and are therefore true generalists. The females of these moths lay their eggs indiscriminately in batches of dozens of eggs, and the neonate larvae search for appropriate food plants.

The experiments with *O. gothica* were performed in June 1997 and the experiments with *L. thalassina* in July 1998. In the first experiment, I collected a sample from the largest fully expanded leaves from both willow species growing near the University of Joensuu, and performed a two-choice experiment using the methods described in article I. Five neonate larvae were placed on pairs of leaves under plexiglass plates with two holes exposing an area of 201 mm² of both willow species. The leaf area eaten was determined after 24 hours as in article I. As shown in Fig. 5, young larvae of both moth species preferred the leaves of *S. phylicifolia*. It is therefore probable that *S. myrsinifolia* bushes originally harbor smaller number of these larvae due to their discriminatory behavior.

In the second experiment, studying larval performance, I placed 10-20 neonate larvae on Petri dishes with one fully expanded leaf from one of the willow species placed on a moistened filter paper. The larvae were allowed to feed for five days in a growth chamber at +18°C (+22°C in *L. thalassina*), >90% RH and 8:16 L:D light rhythm, after which the percentage of larvae surviving was recorded. As shown in Fig. 5, the first instar larvae of *O. gothica* survived significantly better on *S. phylicifolia*. In *L. thalassina*, the overall larval survival was very high and the difference between the willow species was not statistically significant. Thus, the results implicate that at least some generalist caterpillars may perform better on *S. phylicifolia* than on *S. myrsinifolia*. However, when larval stages from third instar to pupae were reared individually on 0.33-L plastic cups at normal room temperature on these willow species (the experiment lasting for 14–32 days), only few larvae died, and the moth

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**Table 1.** Survival of two moth species from third instar larvae to pupae on two willow species when reared individually in the laboratory. The P-values refer to Fisher’s exact tests.

<table>
<thead>
<tr>
<th>Species</th>
<th>Died</th>
<th>Survived</th>
<th>S.phyl</th>
<th>S.myrs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Orthosia gothica</em></td>
<td>4</td>
<td>70</td>
<td>5</td>
<td>72</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Lacanobia thalassina</em></td>
<td>2</td>
<td>154</td>
<td>7</td>
<td>162</td>
<td>0.18</td>
</tr>
</tbody>
</table>

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**Figure 5.** Host plant selection (upper panels) and survival (lower panels) of neonate larvae of two generalist moths, *Orthosia gothica* and *Lacanobia thalassina* on *Salix phylicifolia* (open bars) and *Salix myrsinifolia* (black bars). The P-values refer to exact significances of Wilcoxon tests. The figures in bars give the number of replications.
species survived equally well on these willow species (Table 1)

As found in the case of *G. lineola* (II; III), the defences of willows may act primarily through the behavior or mortality of young insects. This is evolutionarily sound, as the benefits of the defences affecting early developmental stages may be especially high in relation to costs (Agrawal 2000). Overall, the results above, and those in articles II and III point out the importance of understanding processes taking place during early developmental stages for assessing the ecology of herbivorous insects and their interactions with plants (Reavey 1992; 1993).

Several studies have also shown that willow species show a genetical intra-species variation measured as leaf secondary chemistry (e.g. Julkunen-Tiitto and Meier 1992; Nichols-Orians et al. 1993) and insect densities (e.g. Liikanen 1997; Kendall et al. 1996; Roche and Fritz 1997). Although the unusually high genetic correlation between potted *S. phylicifolia* of the same clonal origin in their susceptibility to attack by *G. lineola*, found in article III may include a phenotypic component, the result is nonetheless consistent with findings by Liikanen (1997), who found out that *S. phylicifolia* clones differ considerably in their ability to defend themselves against *G. lineola*. As a conclusion, the willow species studied in this thesis show both between-species and within-species genetic variation in their susceptibility to herbivorous insects.

4.2. Phenotypic variation

4.2.1. Shading enhances the quality of willow leaves to leaf beetles

Environmental factors such as light, temperature, nutrients and flooding can create phenotypic variation in a plant’s direct defences against herbivores (see Hartley and Jones 1997 for a review). In this thesis I focused on three abiotic factors, all of which might affect the balance between mineral nutrients and carbon available for plants. The theory of resource allocation by plants in relation to balance between carbon and mineral nutrients (CNB -hypothesis; Bryant et al. 1983; 1987; Tuomi et al. 1984), as well as some related hypotheses (Price 1991; Herms and Matson 1992; Tuomi 1992) predict that leaves of plants growing (slowly) under sub-optimal levels of photosynthetically active radiation should contain relatively more mineral nutrients (especially nitrogen) and relatively fewer carbon-based secondary compounds compared to plants growing in direct light. Herbivore performance has usually been found to correlate positively with plant nitrogen content (e.g. McNeill and Southwood 1978; Matson 1980; Slansky 1993) and negatively with the content of carbon-based compounds (Scriber and Slansky 1981; Bryant et al. 1983; Larsson 1986; Ayres et al. 1997), predicting that shaded plants should provide better food for herbivorous insects. Shading has therefore served as a tool for testing the CNB-hypothesis in terms of herbivore preference and performance (e.g. Larsson et al. 1986; Ruohomäki et al. 1996; Crone and Jones 1999; McDonald et al. 1999). For example, Larsson et al. (1986) compared phenolic chemistry and feeding preference of the leaf beetle *G. lineola* F. on *Salix dasyclados* Wimm. grown under low or high light under the controlled laboratory conditions, and found that *S. dasyclados* grown under low-light conditions contained fewer phenolic compounds and more nitrogen, and were preferred by the adults of *G. lineola*.

In article I, *G. lineola* showed a preference for leaves of *S. phylicifolia* obtained from shaded habitats over the leaves from open habitats, and its larvae also performed better on those leaves. In *P. vitellinae* there also was a similar tendency, though the preference for shaded *S. myrsinifolia* by adults was only marginally statistically significant. These results are consistent with the CNB-hypothesis, but because the chemical characteristics of the leaves were not measured, proximate factors affecting preference and performance cannot be ascertained. However, since *G. lineola* is found to be affected more by water and nitrogen than secondary compounds found on leaves of *S. phylicifolia* (II), and *P. vitellinae* is attracted by salicylates (Tahvanainen 1985b; Pasteels et al. 1988; Rank et al. 1998), which are assumed to decrease as a result of shading, I suggest that the increased concentration of nutrients
and decreased relative amount of supporting tissues and protecting wax layer on the leaves play more important roles than secondary chemicals (I).

4.2.2. Flood stress causes deterioration of the quality Salix phylicifolia leaves for herbivores

The second abiotic factor examined here is the effect of flooding on the leaf chemistry and herbivore damage in *S. phylicifolia*. Since *S. phylicifolia* has no apparent morphological or physiological adaptation for growing under anoxic conditions in waterlogged soils, such as root structure allowing oxygen transportation, surface-sprouting adventitious roots or pneumatophores (Kozlowski 1981; Good et al. 1990), its metabolism and nutrient acquisition is expected to be debilitated by submersion or waterlogging (Kozlowski 1981). This may both enhance and weaken the quality of its leaves for herbivores. The plant stress hypothesis (White 1984; 1993) states that leaves of stressed plants may contain relatively more of the soluble amino acids that may be essential especially for early developmental stages of herbivorous insects (White 1993). In the contrary, the willows growing under anoxic condition in waterlogged soil may suffer from nutrient and oxygen deficit, and their leaves may contain relatively less nitrogen essential for herbivorous insects as predicted by the carbon nutrient balance hypothesis. Good et al. (1990) have previously found that that waterlogging reduces the growth of willows (*Salix cinerea* L. and *Salix caprea* L.), but that the effect differs between species and clones.

In article II we tested whether the difference in the densities of *G. lineola* between three habitats, wetlands (Fig. 9), flood zones and dry zones, were due to variation in leaf quality of *S. phylicifolia*. The results showed that the leaves of *S. phylicifolia* grown in waterlogged soils in wetlands contain relatively small amount of water and nitrogen, and relatively high amounts of condensed tannins, their precursor (+)-catechin and amnelopsin, and are poor quality food for both adults and larvae of *G. lineola*. Multiple regression analysis revealed that water content was the most probable factor affecting poor larval performance on wetland willows, but adults seemed to choose their host plants according to the nitrogen content of the leaves. However, since we made no attempt to experimentally alter the level of flood stress, no strong inferences can be made of the role of flood itself. The impact of differences in the genetic structure of *S. phylicifolia* between habitats caused by flooding, or the impact of *G. lineola* on both phenotypes and genetic structure of the populations of their host plants cannot be completely excluded (see chapter 8.3).

4.2.3. Complex effect of fertilization on the herbivory of willows

The CNB-hypothesis predicts that fertilization should decrease the carbon:nutrient ratio, making plants better food for herbivorous insects. This is often found to be true, but results to the contrary have also been obtained (Waring and Cobb 1992; Hartley and Jones 1997; V). In willows, Hakulinen et al. (1995) found only slight changes in phenolic chemistry after fertilization of *S. myrсинифолия* clones, and concluded later (Hakulinen 1998), that the resistance of *S. myrсинифолия* against *Melampsora* -rusts cannot be explained in terms of phenolic chemistry altered by fertilization. Fritz (1990) has demonstrated that different herbivore species showed varying response to fertilization on arroyo willow *Salix lasiolepis* Bentham, but up to the present, no studies exist that explicitly evaluate the role of fertilization in overall herbivore damage in willows.

Although the CNB-hypothesis is intuitively sound and widely accepted as a part of the theory of insect-plant interactions, and even though the relative availability of resources obtained by plants obviously play an important role in plant-insect interactions (Hartley and Jones 1997; Tuomi 1992), I shall here stress the more complex effects of soil fertility on herbivore density and consequent damage caused to the host plants. Firstly, inherent responses by plants to fertilization may be complicated (e.g. Haukioja et al. 1998) and genotypes may show different responses (e.g. Hakulinen et al. 1995), making the outcomes unpredictable in
practice (Hartley and Jones 1997). For instance, as suggested by Haukioja et al. (1998), some unexpected results challenging the CNB-hypothesis may arise from the competing biochemical pathways of primary and secondary metabolism. Other inherent factors, such as the differential responses of leaves depending on their ontogeny (Wait et al. 1998), have also been suggested as explanations for the variable outcomes in fertilization experiments. Secondly, as will be proposed in the following chapters of this thesis, the responses of herbivorous insects to the availability of mineral nutrients for their host plants may be non-linear. Therefore, when only a few levels of fertilization are used in experiments, misleading conclusions can easily be drawn. Thirdly, as will be shown below, soil fertility and enhanced plant growth also change traits that are linked to plant size, such as microclimate and density-dependent responses by predators (Hartvigsen et al. 1995; III; V; see below). Herbivores preferring sunny but moist habitats near the ground, such as \textit{G. lineola}, may therefore occur in low densities on vigorously growing fertilized bushes which could, nevertheless, provide high-quality food for the beetles. Finally, different insect species may respond differently to changes in plant quality. Consequently, the effects of soil fertility on the amount of damage suffered by plants depend on the structure of the herbivore community of a particular site (see also Fritz 1990; Ritchie 2000).

As a part of this thesis, I fertilized willows with complete NPK-fertilizer and found that the addition of 100 g m$^{-2}$ before budbreak in early spring decreased insect densities on \textit{S. phylicifolia} and, consequently, the level of leaf area damaged, especially when the impact of insectivorous birds was excluded (V). However, no clear effects were found on \textit{S. myrsinifolia}. At the same time, I also conducted an analogous fertilization experiment in the Parikkala study area (see IV; V) using six fertilization levels (see Fig. 6 for the fertilization levels), and counted insect densities and determined leaf damage as in article V. The insect densities in \textit{S. phylicifolia} were highest when only 25 g m$^{-2}$ of fertilizer were added and tended to decrease with higher fertilization levels (One-way ANOVA; $F_{5,68} = 3.34, P = 0.009, \eta^2 = 0.20$; Fig. 6). In \textit{S. myrsinifolia} no such peak was found (Fig. 6) but, on the contrary to results obtained in article V, the willow species x fertilization interaction of the two-way ANOVA was not significant ($F_{5,134} = 0.85, P = 0.52$), indicating that the responses of herbivores on the willow species were in practice similar. The leaf-chewer densities tended to decrease with an increasing level of fertilization in \textit{S. myrinifolia} (One-way ANOVA; $F_{5,68} = 3.48, P = 0.007, \eta^2 = 0.20$; Fig. 6). Consequently, little support was found in the field for the linear predictions of the CNB-hypothesis.

However, as illustrated by the contrasting impact of fertilization between the predation treatments in article V, and the discussion in articles I, II and III, several factors other
than plant quality considerably affect the observed patterns of herbivore distributions in the field. Such results were obtained when host plant selection by *G. lineola* between differently fertilized *S. phylicifolia* was studied both in the field and in the laboratory. When the fertilization treatments 0, 50, 100 and 200 g m$^{-2}$ were compared using another random set of *S. phylicifolia* at the same site in Parikkala, *G. lineola* eggs reached the highest densities on the control willows (Fig. 7; One-way ANOVA; $F_{3,76} = 7.56, P < 0.001, \eta^2 = 0.23$). However, when the food choice by adult *G. lineola* was tested using a four-choice experiment under controlled conditions in the laboratory following the methods described in article II, the adults preferred the fertilization level of 100 g m$^{-2}$ (Fig. 7; Friedman’s test, $\chi^2 = 14.87, P = 0.002$). These results suggest that 1) fertilization does not linearly change the quality of *S. phylicifolia* leaves to *G. lineola* and 2) factors other than the plant quality must be involved in the host plant choice behavior of *G. lineola* choosing between differently fertilized *S. phylicifolia* in the field.

In order to investigate whether plant size itself affects the density of herbivores on the fertilized willows, I applied the ANOVA-model to the data with six fertilization levels. In the analysis I used willow species and the level of fertilization as fixed factors, and willow age (see IV, V for age determination) and size (total leaf area) as covariates. The size of each bush was estimated by counting the number of shoots, measuring the lengths of 20 randomly selected shoots, and calculating the mean leaf area of the shoots at the third census (see Fig. 1 in V) using the standard equations described in article V. Table 2 shows that willow size was the most important factor explaining herbivore densities on both willow species; smaller bushes had higher insect densities. Consequently, it seems that the changes in herbivore densities after fertilization may be caused by the increased growth itself, and not entirely by changes in the inherent plant quality.

But why does the largest and most heavily fertilized bush harbor fewer herbivores than the smaller, unfertilized ones? There are at least four possible explanations for this: 1) Large, fertilized bushes grow faster under the load of insects with poor ability to disperse, and consequently bear relatively sparse insect populations, regardless of their potentially better quality as food and oviposition sites. 2) The smaller, unfertilized bushes have larger exterior surface area compared to their biomass or leaf area. Con-

### Table 2

Analysis of variance on the effect of willow species (*S. phylicifolia* and *S. myr-"sinißfolia*; fixed factor), fertilization (six levels; fixed factor), willow age (covariate) and the total estimated leaf area of the bush (covariate) on the mean density of leaf chewing insects.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>13</td>
<td>2.48</td>
<td>11.51</td>
<td>&lt;0.001</td>
<td>0.53</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>10.84</td>
<td>50.27</td>
<td>&lt;0.001</td>
<td>0.28</td>
</tr>
<tr>
<td>Fertilization</td>
<td>5</td>
<td>0.89</td>
<td>4.12</td>
<td>0.002</td>
<td>0.14</td>
</tr>
<tr>
<td>S x F</td>
<td>5</td>
<td>0.11</td>
<td>0.53</td>
<td>0.75</td>
<td>0.02</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>0.13</td>
<td>0.61</td>
<td>0.44</td>
<td>0.005</td>
</tr>
<tr>
<td>Leaf area</td>
<td>1</td>
<td>9.72</td>
<td>45.07</td>
<td>&lt;0.001</td>
<td>0.26</td>
</tr>
<tr>
<td>Error</td>
<td>132</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Large, fertilized bushes grow faster under the load of insects with poor ability to disperse, and consequently bear relatively sparse insect populations, regardless of their potentially better quality as food and oviposition sites. 2) The smaller, unfertilized bushes have larger exterior surface area compared to their biomass or leaf area. Con-

![Figure 7: Food choice in the laboratory, and maximum density of eggs of Galerucella lineola observed on Salix phylicifolia in the field under four levels of fertilization. The number of replicates is 15 in each bar. See text for statistical analyses.](image-url)
sequently, they passively gather more herbivores with restricted ability to locate their host plants (Bell 1991; Schoonhoven et al. 1996), than the larger and vigorously growing fertilized bushes. 3) On average, the leaves of smaller trees are nearer the ground and therefore provide better abiotic conditions for insects than leaves of larger bushes (I; II). 4) Large fertilized trees shade themselves, providing a cooler microclimate for herbivores (I; II). Although some circumstantial evidence exists for all of the four hypotheses (Sipura, personal observations), further examinations are needed to separate these factors and to evaluate their relative importance. However, these results may provide some explanation for the observed behavior pattern of many herbivorous insects showing a positive response to fertilization at the individual level (often tested in the laboratory conditions), although there is little evidence at the population level (usually studied in the field; Kytö et al. 1996; Hartley and Jones 1997; but see Hartvigsen et al. 1995).

In order to test whether different herbivore species show diverse responses to fertilization, we (MS and Arsi Ikonen) performed a similar fertilization experiment to the one described above in 1999. In this experiment we used *S. phylicifolia* growing in an abandoned field in Joensuu (62°37’N, 29°40’E), fertilized with 0, 100 and 200 g m⁻² of the complete NPK-fertilizer. The 7-12 years old *S. phylicifolia*, selected randomly from among several hundred clones in the field, received the fertilization treatment before budbreak both in 1998 and 1999. The mean shoot lengths measured from a sample of 25 clones from each treatment at the end of the growing season 1998 were (mean ± SE) 26.23 ± 0.98 cm in control willows, 41.00 ± 1.77 cm in the medium fertilization treatment and 44.12 ± 3.26 cm in the highest fertilization treatment respectively. In this experiment we focused on the differences in responses to the fertilization of *S. phylicifolia* by four willow-feeding leaf beetles. For this we collected adults of *Agelastica alni* (L.), *Plagiodera versicolora* (Laich.), *Lochmaea capreae* (L.) and *Galerucella lineola* from Joensuu and Parikkala. *A. alni* is specialized in *Alnus* spp. (Baur and Rank 1996), but is frequently found on *Salix* spp. (Tahvanainen et al. 1985b). Leaves number 5 and 9 from the

![Figure 8](image-url)

*Figure 8* Host selection by four willow-feeding leaf beetle species in relation to fertilization of *Salix phylicifolia*. For clarity the age classes used in the analyses are pooled leading to a total number of 32 replicates for each bar. The P-values refer to the main effects of block-design ANOVAs applied for each species separately.
apex of the shoots were used in the three-choice experiment performed in July 1999, following the methods described in article I. The experimental arenas were arranged as hierarchical blocks with beetle species, leaf age and fertilization treatment as within-subject factors (Split-plot design; Zar 1999).

Overall, fertilization had a significant positive effect on food selection (\(F_{6,30} = 3.54, P = 0.042\)), but a significant beetle species x fertilization interaction (\(F_{2,90} = 2.33, P = 0.035\); Fig. 8) is also found. These results demonstrate that leaf beetle species differ in their reaction to the fertilization of *S. phyllicifolia*; *A. alni* clearly preferred leaves from the highest fertilization treatment, whereas non-linear (though not significantly so) preference was observed in *G. lineola*. The result is not intuitively surprising but, to my knowledge, has not been explicitly tested until now.

In conclusion, the studies about bottom-up impacts presented above have two central findings. Firstly, the fertilization experiments show the shortcomings of hypotheses with linear and oversimplified predictions such as “the susceptibility of plants to attack by herbivores increases with increasing availability of nutrients for the host plants”. However, complex nonlinear responses can frequently occur, which are intuitively sound and also suggested by the results of the total herbivore abundance and the host choice by *G. lineola*. Such non-linear responses severely complicate interpretations of experiments with few levels of treatment; and contradictory results can be obtained from experiments with different resolution (compare V and Fig. 6 of this thesis), or experiments with plants having different nutritional backgrounds. Secondly, the results show that environmental factors other than those directly related to plant quality may easily override the direct effects of plant quality. Little can be inferred about plant quality on the basis of the distribution of an herbivore in the field and *vice versa*. The impacts of these factors will be the subject matter of the following chapters.

Overall, this chapter provides support for the first part of Janzen’s (1988) explanation: the world of willows is green because herbivores are capable of efficiently utilizing only some willow species, genotypes within species and phenotypes grown under different environmental conditions. Most of the willow leaves available are likely to be unpalatable to any given species of willow-feeding insects.

5. Role of abiotic habitat

The results and the discussion above leave a great deal of the variation in the host plant choice and performance of herbivorous insects to be explained by factors other than host plant quality. Although abiotic factors, especially temperature, have generally been recognized as one of the most important ecological factors affecting the distribution and abundance of organisms (e.g. Begon et al. 1990; Krebs 1994), small-scale variation in these factors has largely been ignored in terrestrial systems (Willmer 1986; Dunson and Travis 1990), especially in the case of herbivorous insects (see the tables of contents in Bernays and Chapman 1995, and Schoonhoven et al. 1996 for examples). However, there is no reason to believe that abiotic factors play a less important role among terrestrial herbivorous insects than in marine or freshwater arthropods, among which the impact of the abiotic environment has been studied more intensively (Dunson and Travis 1990; Brönmark and Hansson 1998).

In article I we found that although willows growing under the shade of other trees provided better quality food for both *G. lineola* on *S. phyllicifolia* and *P. vitellinae* on *S. myrsinifolia*, more beetle individuals were harbored by willows growing in open habitats. When larvae were simultaneously reared in the laboratory and in the field, they performed relatively poorly on shaded willows despite the high quality of food. The apparent reason for this is the higher and more variable temperature, which enhanced larval performance in open habitats.

Despite the facts that *S. phyllicifolia* growing in wetlands provide very poor-quality food for both adults and larvae of *G. lineola* (II), and that high-quality *S. phyllicifolia* are usually abundantly available on drier meadows near a shoreline, *G. lineola* is about 16 times more abundant on *S. phyllici-
Since we found no differences in predation rates between the habitats (II), the abiotic habitat remains the only adaptive explanation for this. Larsson et al. (1997) have previously found that the neonate larvae of *G. lineola* (Fig. 10) are susceptible to desiccation, and actively seek moist microhabitats within their host plants. In a rearing experiment performed in both laboratory and field during a rainless period in 1998, we found that the first instar larvae of *G. lineola* performed relatively poorly when growing in drier habitats, despite the high food quality (II). When ramets of *S. phylicifolia* were transferred between the habitats and neonate larvae were reared on these ramets, larvae performed best on ramets from dry habitats; placed in a sunny places in wetlands. When supplementary moisture was supplied for neonate larvae, their performance improved most in drier habitats. These results evidently show that *G. lineola* attack wetland willows because these habitats provide better abiotic conditions for the neonate larva.

It also seems that *G. lineola* adults avoid highly exposed wetlands and prefer damp patches that are sheltered, but not shaded by larger trees (Sipura, personal observation). These habitats are likely to be moist and warm, but also less windy and better protected from environmental hazards such as thunder- and hailstorms. On 16th June 1998 in Värsilä (see Fig. 1. in II), a short (lasting for about 20 min) but violent hailstorm occurred immediately after the third field census, beating down some ramets of willows, and seriously damaging their leaves. I counted the number of *G. lineola* larvae both before and after the storm in order to measure larval mortality inflicted by such a unique environmental hazard. As seen in Fig. 11, the larval destruction inflicted by wind and rain was very high, and appeared to be higher on *S. phylicifolia* growing in dry habitats (Table 3). Contrary to intuition, larval disappearance increased with increasing leaf size (Fig. 11, Table 3). This is probably because the *S. phylicifolia* growing at higher altitudes (in dry habitats), and those bearing larger leaves, were almost completely beaten down, exposing larvae feeding on the lower side of the leaves to the rain, hail and wind, whereas smaller *S. phylicifolia* with smaller leaves growing in wetlands were practically unaffected.

Consequently, I argue that both *G. lineola* and *P. vitellinae* first choose the abiotic

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**Figure 9.** Representative wetland habitats in southeastern Finland. Totally skeletonized *S. phylicifolia* in Raikanniemi, Parikkala, where the experiments with naturally growing willows under very high *Galerucella lineola* herbivory were performed in article III and chapter 8.3 of this thesis (upper panel). Lush aquatic vegetation with low *Salix phylicifolia* bushes in Tiviänluhta, Parikkala, with low density of *Galerucella lineola* (III; chapter 8.3 of this thesis) (middle panel). An artificial pond in Penttilä, Joensuu, where most of the experiments in article II, and the experiment in chapter 6 of this thesis were performed (lower panel) (Photos by MS).
habitat, and only then the best possible host plant within the preferred habitat. As the adults preferred open habitats, but changed their habitat preference towards shaded sites at very high ambient temperatures (I), both beetle species seem to prefer warm but not too exposed, sun-drenched, habitats. For *G. lineola*, wetlands seem to provide the best compromise. Consequently, abiotic factors can partially explain the greenness of willows, providing support for the last part of the Janzen’s (1988) explanation. The world of willows is, at least partly, green because climate regimes stop the herbivores from efficiently circumventing the direct defences of some willow phenotypes.

6. Performance of *G. lineola* larvae under variable environmental conditions

In this thesis I have shown that bottom-up factors (I; II; III; IV; V), climatic factors (I; II) and top-down factors (I; II; III; IV; V) all play a central part in the life of *G. lineola*. But what is the relative importance of these factors, and does their relationships always remain the same? In order to incorporate the between-plant and between-habitat heterogeneity to the analysis (see Hunter and Price 1992), I performed an extensive set of experiments on the 60 willow clones used in the analyses of leaf chemistry in article II. The main objectives were to find out which factors are the most important contributors to differences between the performance of the larvae of *G. lineola* in different *S. phylicifolia* bushes, and how the relative roles of these factors vary between habitats.

To obtain realistic data on the larval performance in the field, I counted the numbers of eggs and larvae of *G. lineola* on ten occasions from 200-400 randomly selected shoots on the southern side of each clone. To get a reliable measurement of larval survival and growth, I collected pupae from 5 ramets randomly selected from among those bearing larvae of *G. lineola* on each willow, by attaching a funnel lubricated with liquid Teflon around the stems (see III for the method). I then used the number of pupae collected,

| Table 3. Analysis of variance on the effects of habitat (fixed factor), shoot length (covariate) and mean leaf size (covariate) on the loss of *G. lineola* larvae during a thunderstorm in Värsilä 16th June 1998. |
|------------------|-----|------|-----|---|
|                  | df  | MS   | F   | p  |
| Habitat          | 2   | 0.049| 6.70| 0.003 |
| Shoot length     | 1   | 0.002| 0.37| 0.55  |
| Leaf size        | 1   | 0.080| 10.88| 0.002 |
| Error            | 40  | 0.007|     |     |

Figure 10. A neonate larva and eggs of Galerucella lineola (Photo by Mika Lännenpää).

Figure 11. The relationships between mean leaf area of Salix phylicifolia and the survival of the larvae of Galerucella lineola in three habitats during a violent hailstorm in Värsilä 16th June 1998. White dots = wetland, gray dots = flood zone and black dots = dry zone.
Using a set of manipulative experiments, I then independently separated four components of larval performance: 1) food quality, 2) abiotic factors, 3) density-independent factors affecting larval performance. The circles indicate the residual variances \((1 - \sqrt{R^2})\). See text for the full description of the factors.

**Figure 12.** Path diagrams for the factors affecting survival and growth of *G. lineola* larvae in three habitats. The figures associated with arrows provide path coefficients (see III for further information). The figures on lines with no arrowhead are Spearman correlations. The gray boxes refer to host selection determined in the laboratory by a multiple-choice experiment, and in the field by counting the maximum egg density. PCA1 is the first principal component axis of leaf chemistry (Table 4). Host = host plant quality, predation = the impact of predation, abiotic = the impact of abiotic factors and density = density-dependent factors affecting larval performance. The circles indicate the residual variances \((1 - \sqrt{R^2})\). See text for the full description of the factors.
predation and 4) factors depending on larval density (density-dependent component of predation and competition). 1) In order to measure the quality of the host plant, I established a laboratory rearing experiment covering the entire larval period in a growth chamber at +18°C, >90% relative humidity and 12:12 photoperiod, following the methods described in article II. 2) I simultaneously performed a similar experiment in the field by attaching four egg patches to a ramet covered with a sleeve bag containing a small piece of Sphagnum moss, to provide a pupating matrix for the larvae. 3) The predation experiment was performed by removing all eggs from two adjacent ramets, attaching three egg batches to each, and using a sleeve bag to exclude predators from experimental ramets (see II for a full description of the method). 4) The density experiment was performed by removing all but two egg batches from a randomly selected ramet, and employing one of the ramets used for field observations as a control. The pupae of the laboratory rearing experiment, the growth experiment in the field, the predation experiment and the density experiment were collected using the funnels described in articles II and III, after which they were dried, counted and weighed.

As an estimate of host plant quality (see Fig. 12), I used the pupal dry weight and larval survival (number of pupae / number of eggs) measured in the laboratory. The other three factors were obtained as relative values using residuals from least square regression performed for each habitat separately. The relative effect of abiotic habitat was determined using performance indices measured in the laboratory as independent variables, and indices measured in sleeve bags in the field as dependent variables in regression analyses. To obtain an estimate of the relative clone-specific predation, I used the indices measured inside the sleeve bags for predation treatment as independent variables and variables measured in the control ramets with fixed larval densities as dependent variables. Finally, I determined the effect of larval density using indices under fixed density as dependent variables and indices under natural larval densities as dependent variables. It is worth noting that the residuals are measured as survival units (not mortality). For instance, high predation values in larval survival denote that the larvae could effectively avoid the impact of predation. After the regression analyses, I visually checked the linearity of the regressions and the distribution of the obtained residuals to evaluate the reliability of the method.

I reduced the data on leaf chemistry by applying principal component analysis (Table 4) for the five main chemical components measured from the leaves (II). As a consequence, a great deal of the variation in leaf chemistry was reduced to the first principal component axis (PCA1; Table 4). To assemble all relationships to the same figure, I constructed path diagrams for each habitat (Fig. 12; see article III for further information about path analysis), supposing that leaf chemistry may affect larval performance through host plant quality, and the host plant quality may further affect the other, possibly interacting, components of larval performance.

The path diagrams (Fig. 12) show that the importance of the ecological factors affecting larval performance varied between habitats, and had a different effect on the components of larval performance, survival and growth. The low number of indirect effects implies that the factors varied relatively independently. Host plant quality was the most important factor in larval growth but affected larval survival only in wetland habitats. The abiotic factors became more important to-

<table>
<thead>
<tr>
<th>Component</th>
<th>PCA 1</th>
<th>PCA 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (%)</td>
<td>-0.89</td>
<td>0.11</td>
</tr>
<tr>
<td>Total nitrogen (mg g⁻¹)</td>
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</tr>
<tr>
<td>Condensed tannins (mg g⁻¹)</td>
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<td>0.61</td>
</tr>
<tr>
<td>(+)-catechin (mg g⁻¹)</td>
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<td>0.36</td>
</tr>
<tr>
<td>Ampelopsin (mg g⁻¹)</td>
<td>0.56</td>
<td>-0.75</td>
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</tbody>
</table>

% variance explained   60.83 23.06
Eigenvalue            3.04 1.15
wards drier habitats but density-independent predation played an important role only in the flood zone. It is also worth noting that the leaf chemistry (PCA1) correlated with larval performance only in the wetland habitat, providing more support for the hypothesis of non-linear host-plant relationships of *G. lineola*, discussed in chapter 2.3.3.

These results illustrate the vast variation in ecological factors contributing between-plant differences in the performance of *G. lineola* larvae. In general, abiotic factors seem to predominate in drier habitats whereas variation in food quality and density-dependence are the key factors in wetlands. Adopting the terminology of Karban (1997), in order to understand the patterns of the performance of *G. lineola* larvae ultimately affecting adult habitat preference (I; II; see below), the processes should be considered as pluralistic and conditional (see also Hunter and Price 1992). By pluralistic, I mean several important factors acting simultaneously, and by conditional, the relative forces of these factors showing spatial and temporal variation.

7. Tri-trophic interactions

7.1. Substitutive and complementary predation

One of the major goals of this thesis was to study the impact of predators on the herbivory of willows and, especially, to examine how predators interact with the direct defences of willows. It is accepted that predators such as ants (Beattie 1985; Hölldobler and Wilson 1990; Whittaker 1991) and insectivorous birds (Holmes et al. 1979; Adlegrim 1991; Marquis and Whelan 1994) may enhance the growth and increase the fitness of plants thanks to reduced damage by herbivores. However, the impact of predators may not be constant across the range of direct plant defences (Price et al. 1980; Hare et al. 1992; van der Meijden 1996; Sabelis et al. 1999). Consequently, predation has to be seen as a component of plant defence.

Physical or chemical traits of plants may directly or indirectly decrease predation rates, leading to an antagonistic relationship between the direct plant defences and the indirect defence provided by the third trophic level. Since predation can be seen here as a defence component substituting direct defences of susceptible plants, I will hereafter refer to this type of predation as a substitutive component of plant defence. On the other hand, herbivores living on plants that have strong direct defences may be more susceptible to predators, which may lead to a synergistic impact of direct and indirect defences. In this case predators complement the ineffective direct defences of plants, i.e. predation acts as a complementary component of plant defence. Although predation as a component of plant defence has rarely been examined from the perspective of the plants, both substitutive and complementary impacts have gained support in studies examining herbivore performance in relation to the level of direct plant defence (Hare 1992; van der Meijden 1996; Sabelis et al. 1999). In a review, Hare (1992) found that, out of 16 cases, there were six negative (substitutive), eight neutral and two positive (complementary) interactions between plant resistance and the efficiency of biological control using natural enemies.

In order to examine these relationships in willows, I compared the impact of predation on the two chemically different willow species (IV; V) and between individuals or genotypes of *S. phylicifolia* (III). Specifically, I asked the following questions: 1) are predators abundant enough to affect herbivory and, consequently, willow growth? and 2) do predators act as a complementary or supplementary component of willow defences, or do they contribute to defence at all?

7.2. Ants on willows

Earlier results on the role of predatory ants in the herbivory of plants have been controversial, and despite the number of studies carried out, it is still unclear whether ants positively affect plant performance, and whether they can universally be used as agents for biological control (Gösswald 1951; Adlung 1966; Otto 1967; Whittaker 1991; Way and Khoo 1992; Karhu 1998). Especially in tight mutualistic relationships, where plants provide additional food or
shelter for patrolling ants, the effect of ant predation has usually, though not always, shown to be positive (Janzen 1966; Beattie 1985; Hölldobler and Wilson 1990). However, in cases where no such investment is made by plants, there are many occasions where ants have had a negligible (Mahdi and Whittaker 1993) or even negative impact on plant performance (Adlung 1966; Otto 1967). This is apparently due to increased densities of ant-tended aphids (Messina 1981; Whittaker 1991), or to the reduced number of other predators or parasitoids that are killed or harassed by ants (Whittaker 1991). In article IV, I experimentally tested the effect of two predatory ant species, *Myrmica rubra* and *Formica aquilonia* on insect numbers, leaf damages and growth of *S. phylicifolia* and *S. myrsinifolia* attacked both by honeydew exuding aphids *P. salicis*, and leaf-chewing insects.

Both observational data and ant exclusion experiments (IV) showed that especially wood ants *F. aquilonia* considerably reduced herbivore numbers, which in turn led to a decreased level of leaf damage and increased growth in *S. phylicifolia*, whereas the effect on the growth of *S. myrsinifolia* was negligible or even negative (IV). This difference can be attributed to three factors. First, the tended aphid *P. salicis* was equally abundant or, if anything, more abundant on *S. myrsinifolia* resulting in an equal or slightly stronger negative impact of ants on the growth of *S. myrsinifolia*. Second, *S. myrsinifolia* harbored lower densities of leaf-chewing insects (due to their stronger direct defences), which reduced the potential positive effect of ants trough decreased leaf damage. Last, the community of leaf-chewing insects on *S. myrsinifolia* was dominated by chemically defended larvae of *P. vitellinae*, which were apparently subject to less predation than herbivores on *S. phylicifolia*. Moreover, the relationships between *P. vitellinae* larvae and ants were strongly mediated by the presence of aphids; in fact when *P. salicis* were removed, ants even enhanced the survival of *P. vitellinae*, probably by removing other natural enemies of the larvae. Consequently, as revealed by a significant willow species x ant exclusion interaction (IV), ant predation acts as a substitutive component of the defence in the two willow species; when measured in terms of willow growth.

### 7.3. Insectivorous birds on willows

In article V, I established a bird exclusion experiment in order to examine the interactions between bottom-up forces and bird predation on two willow species, *S. phylicifolia* and *S. myrsinifolia*. In this experiment both fertilized and control clones of both willow species were protected from insectivorous birds using cages made of gill nets stretched on wooden side poles. The effects of insectivorous birds on insect numbers, leaf damage and growth of the willows closely resembled those observed in the ant exclusion experiment (IV), although the mechanisms leading to the results were apparently different. No impact was found in sap-sucking or concealed feeding insects (but see Tscharntke 1992; Abrahamsson and Weis 1997; Connor et al. 1999; Murakami 1999). However, birds reduced the number of leaf-chewing herbivores and subsequent leaf damage more on unfertilized *S. phylicifolia* than on fertilized ones or on *S. myrsinifolia*. It is not clear whether the birds preferred unfertilized *S. phylicifolia* because of the larger number of prey available or because of the lower quality of herbivores grown on bitter-tasting leaves of *S. myrsinifolia* (Brower 1984; Topp and Bell 1990; Bowers 1993). Perrins (1976; 1979) have found that condensed tannins isolated from oak (*Querqus robur* L.) leaves and inoculated into the food of *Parus caeruleus* L. nestlings reduced chick growth, suggesting that changes in leaf chemistry inducible by fertilization (Hunter and Schultz 1995; Forkner and Hunter 2000) may affect the quality of insects as food for birds. However, the changes in leaf chemistry due to fertilization are probably rather low, especially in *S. phylicifolia*, leading me to suggest that herbivore density is the key factor affecting the foraging behavior of birds (V). It is known that birds forage using both direct prey observations and visual cues provided by leaf damage, and that they may prefer plants with more damaged leaves (Heinrich and Collins 1983; Bell 1991; Heinrich 1993; Murakami
In article III, I also observed that birds preferred foraging on *S. phylicifolia* bushes, which provided high-quality food for the larvae of *G. lineola* and therefore harbored higher larval density. However, birds were also seen to forage more often on high-quality *S. phylicifolia* bushes with fixed densities of larvae, but damaged by the colonizing adults earlier in the season, than on low-quality bushes with few signs of herbivores (III). Therefore, I argue that insectivorous birds show positively density-dependent predation on willow bushes by foraging more on bushes with higher insect densities and more damaged leaves (but see Tanhuanpää 2000). Consequently, bird predation seems to act as a substitutive component of willow defence.

**7.4. Predation in relation to host quality in *S. phylicifolia***

In article III, I examined the relationship between bottom-up and top-down impacts in individuals and clones of *S. phylicifolia*. When the densities of *G. lineola* were fixed, the predation appeared to contribute more to larval mortality on low-quality (measured independently as pupal weight attained) willows. This may be due to both 1) altered behavior of the larvae on poor-quality hosts enhancing their detectability by predators (Bergelson and Lawton 1988; Bernays 1997), or 2) weakness and poor ability of the larvae to defend themselves when fed on inadequate food (Isenhour et al. 1989; Montllor and Bernays 1993; Häggström and Larsson 1995). However, when testing the first hypothesis, Bergelson and Lawton (1988) found no unequivocal difference in the predation rate of moth larvae under altered food quality. Instead, the larvae of *G. lineola*, which defend themselves by moving their abdomen vigorously when attacked by a predator (Häggström and Larsson 1995; Sipura, personal observation; Fig. 13), seemed more phlegmatic on low-quality hosts, providing a potential explanation for the observed pattern. Therefore, I tested the second hypothesis experimentally.

During the common garden experiment described in article III, I performed a similar experiment using a set of 50 potted clones of *S. phylicifolia*. Larval performance (covering the entire development period) on each clone was measured in the laboratory, following the methods described in article I. During this experiment, I actively observed predation events on the *S. phylicifolia* clones and recorded the outcomes of the battles between predators and larvae of *G. lineola*. If predators retreated, and did not return within 5 minutes, I interpreted the larva as a survivor. As a great diversity (ants, hemipteran bugs, spiders, lacewings etc.), but a relatively low number of predators was observed, I pooled all predation events in the same analysis. I included the clones with at least five predation observations in the analysis using the larval growth rate (pupal weight / developmental time), indicating the quality of the host plant, as an independent variable, and the clone-specific proportion of surviving larvae as a dependent variable in the logistic regression analysis. As seen in Fig. 13, the probability of larvae surviving a predation event increased with increasing host plant quality. This suggests that the higher observed survival from predation on high-quality clones under fixed larval densities is at least partly due to the weakness of the larvae on low-quality clones when attacked by a predator. Consequently, when the density

![Figure 13. A nymph of a hemipteran bug attacking a second instar larva of *G. lineola*](Photo by MS)
of \textit{G. lineola} is constant, predation acts as a complementary component in the defence of \textit{S. phylicifolia}.

However, under equal abiotic conditions the females of \textit{G. lineola} preferred to oviposit on the \textit{S. phylicifolia} clones where the larval performance was highest (III). Predators, at least birds and hemipteran bugs (III), seemed to aggregate on those high-quality clones with high larval densities, reducing the leaf damage more than on low-quality willows with low larval densities. Although predators could not totally compensate for the shortage of direct defences, the total predation can be seen as a partially substitutive component of defence in \textit{S. phylicifolia}. Therefore, the results obtained in articles III, IV and V are consistent with the second part of Janzen’s (1988) explanation; predators seem to reduce leaf damage more on willows with a low level of direct defence i.e. on willows, which could otherwise be “consumed into oblivion”.

8. Ecological and evolutionary consequences of habitat selection by \textit{G. lineola}

8.1. Environmental variability and preference-performance linkage

Considering that multiple and varying factors affect the suitability of willows as food for the leaf beetles, one may ask, whether the female beetles can always find the best compromise among this variability? Do mothers always know what is good for their children? The relationship between oviposition preference and the performance of offspring has been one of the central issues in the evolution and ecology of insect-plant interactions (Thompson 1988; Courtney and Kibota 1990; Thompson and Pellmyr 1991; Ohgushi 1995; Abrahamsson and Weis 1997). The evolutionary logic predicts that in herbivore species where larval mobility is restricted, and the ovipositing females select the host plant for the larvae, the selection should favor female behavior that maximizes offspring fitness. Positive correlations have often been found (e.g. Whitham 1980; Roinininen and Tahanainen 1989; Denno et al. 1990; Minkenberg and Ottenheim 1990; Kouki 1993; Ohgushi 1995), but a large number of studies exists where the correlation has been found to be poor or totally lacking (see references in Thompson 1988). These apparent anomalies have been explained by the novelty of host plants (Chew 1977), the relative shortage of suitable host plants or plant parts (Thompson 1988), asynchronous evolution between preference and performance (Roininen and Tahanainen 1989), constraining life-history traits such as short life span or restricted flight capacity (Larsson and Ekbom 1995), the impact of natural enemies (Courtney 1988; Valladares and Lawton 1991), or density-dependent processes obscuring the preference-performance correlation (Valladares and Lawton 1991).

A number of studies investigating the relationship between oviposition preference and offspring performance have been conducted under controlled laboratory conditions where variation in ecological factors such as abiotic conditions, competition and predation are absent (Thompson 1988). However, as these factors may be important in the host selection process (Janzen 1988; Valladares and Lawton 1991; Yamaga and Ohgushi 1999; I; II; III; IV; V), and do not necessarily correlate positively with host plant quality, different results can be obtained in the laboratory and in the field (I; II). These factors may also be poorly predictable for ovipositing females interfering with the female’s ability to track suitable host plants for larval performance.

In order to evaluate the preference-performance linkage both under controlled laboratory conditions and under variable conditions in the field, I used the data for larval performance described in chapter 6. I used the maximum density of eggs counted from each of the 60 bushes as an estimate of female choice in the field, and the results of the 60-choice experiment described in article II as a measure of female choice in the laboratory (Fig. 12). When the three moisture habitats were tested separately, the correlation between female preference and larval survival was significant only in the flood zone, whereas the only significant correlation in the laboratory was obtained on leaves from the water zone (Fig. 12). In general, the correlations tended to be stronger when pu-
pal weight was considered instead of survival. However, when the habitats were pooled in the same analysis, there was no correlation between female preference in the field and larval survival ($r = 0.07$, $P = 0.58$; Spearman correlation) or larval growth in the field ($r = -0.04$, $P = 0.79$). The same correlations in the laboratory are $r = 0.41$, $P = 0.001$ for the survival and $r = 0.80$, $P < 0.001$ for the larval growth, respectively.

Since most of the important ecological factors are highly variable and may be impossible for ovipositing females of *G. lineola* to predict (see Kouki 1991; Valladares and Lawton 1991; Ohgushi 1995), a wide range of variation in the preference-performance linkage is expected under field conditions. The total lack of this relationship in *G. lineola* in the pooled data apparently reflects the rule of thumb followed by females, always to select moist habitats which, on average, will provide the best habitat for the larvae (II). In 1998, the wetland habitats did not provide sufficiently good abiotic conditions to override the impact of poor food quality, which apparently led to the relatively poor larval performance on the willows preferred by ovipositing females.

The low correlation between adult preference and larval performance may have profound consequences for the population level, as relatively poor-quality host plants are easily overcrowded, leading to high mortality (II; III). On the other hand, many high-quality host plants harbor relatively few individuals. Suboptimal host selection due to the unpredictability of habitat quality may therefore suppress population growth despite the fact that good-quality host plants growing in a mild microclimate with a low number of predators are abundantly available at the time of larval development. It sees that mother’s of *G. lineola* do not always know what is good for their children.

### 8.2. Competition among *G. lineola*

The results in articles II and III suggest that when occupying wetland habitats, the larvae of *G. lineola* easily face intense intraspecific competition, leading to increased mortality and lower pupal weight. It is therefore probable that most individuals of *G. lineola* often face a shortage in food supply, even though good-quality food is available in excess in drier habitats. Therefore, the habitat selection pattern driven by an apparent ancestral constraint may lead to density-dependent processes, not intuitively obvious when wrong spatial scales are concerned (Steward-Oaten and Murdoch 1990; Taylor 1993). Consequently, it may play a substantial role in the population dynamics of this leaf beetle.

### 8.3. Selective mortality of *S. phylicifolia*

Above, I have shown that *G. lineola* strongly prefer moist habitats, where they can completely defoliate their host plants *S. phylicifolia* (II). During this study the highest densities of *G. lineola* were found in the northern part of Lake Siikalahti, in Parikkala (Fig. 9), where the beetles defoliated on average of 82% of the leaf area of *S. phylicifolia* (Fig. 14). I visited the site for the first time in 1994, and found only slight leaf damage. Furthermore, according to leaf samples collected from the bottom of the lake in 1998, the level of leaf damage in 1997 was on average 41% in 1997, suggesting that the outbreaks of *G. lineola* at this site had occurred recently. Since a very high mortality rate in *S. phylicifolia* was also observed in the southern part of Lake Siikalahti with an outbreak of *G. lineola* in the early 1990’s, I was curious to find out whether the high defoliation by this leaf beetle always leads to selective mortality of *S. phylicifolia*, and whether the quality of *S. phylicifolia* as food for the beetles changes as a consequence of defoliation. I focused on the following four questions: 1) Does *G. lineola* affect the fitness of its host plants?, 2) is this effect selective i.e. is the negative effect of the beetles greater on more susceptible phenotypes?, 3) does the population structure of *S. phylicifolia* change in relation to their susceptibility to *G. lineola*? and 4) does *G. lineola* change the quality of its host plants phenotypically?

In 1998, I randomly selected about 40 willows both from northern Siikalahti (High beetle density in Fig. 14) and from southern Siikalahti (Low beetle density in Fig. 14), and performed a short-term RGR-experiment with second instar larvae fol
lowing methods described in article II. After the pupation of *G. lineola* in the field, I measured the leaf area damaged from a sample of 30 leaves from each willow clone. About half of the willows in northern Siikalahti died before the growing season of 1999, when I checked the conditions of the willows previously used in RGR -experiments. As seen in Fig. 14, the probability of mortality clearly increased with increased defoliation. Also, *S. phylicifolia* providing high-quality food for the larvae (and attracting more ovipositing females) seemed to suffer the highest mortality (Fig. 14). Therefore, it seems that *G. lineola* can induce selective mortality among a population of *S. phylicifolia* by killing the most susceptible phenotypes.

In order to explicitly test this hypothesis, I collected another sample of willows from both sites in 1999 and performed an RGR -experiment as in 1998. I also included results obtained similarly from adjacent drier habitats with low densities of *G. lineola* in the analysis (Fig 15). If the quality of willows as food for *G. lineola* truly decreased between the years 1998 and 1999 as a consequence of defoliation, a significant three-way year x site x habitat interaction was expected. As seen in table 5, this interaction was not statistically significant, although Fig. 15 suggests some evidence for the hypothesis. However, the differences in quality of *S. phylicifolia* between habitats was far greater in the high-density site, probably due to earlier mortality inflicted by defoliating *G. lineola* in this high-density site (Fig. 15; Table 5).

The RGR -experiments performed on the same willows in both years give a ubiquitous picture of whether defoliation increased or reduced the quality of individual phenotypes of *S. phylicifolia* as food for the leaf beetles (Fig. 16). In 1998, all RGR experiments were performed on the largest fully expanded leaves from shoots of relatively old ramets. However, some of the *S. phylicifolia* surviving defoliation initiated their subsequent growth from root-collar shoots and allowed the older ramets to die, whereas others continued their growth from buds on the older ramets. Therefore, I used the form of re-growth as a factor, and leaf damage in 1998 as a covariate when analyzing changes in quality of leaves.

![Figure 14](image1.png)

**Figure 14.** The effect of defoliation by *G. lineola* on the mortality of *S. phylicifolia* in Tiviänluhta (*b*; Low-density site) and Raikanniemi (*d*; High-density site), and the relative growth rate (RGR) of the second instar larvae of *G. lineola* on willows that died, and on those that survived (small plates a and c). The iterated logistic regression in Figure d is $y = 59933/1+(x/250)^{12.07}$. $F_{2,71} = 22.00, \ P < 0.001, R^2 = 0.38$. The vertical lines show the mean level of leaf damage for both sites in 1998. NS $P > 0.01$, **$P < 0.01$.

![Figure 15](image2.png)

**Figure 15.** Relative growth rate of second instar larvae of *G. lineola* in laboratory experiment performed on leaves from two habitats in Tiviänluhta (Low-density site) and Raikanniemi (High-density site) in 1998 and 1999. The numbers inside symbols give the number of replications. See table 5 for statistical analysis.
in larval growth between years. As seen in Fig. 16, the change in leaf quality between years showed an overall decrease with increasing defoliation rate in 1998 (leaf damage in 1998; \( F_{1,16} = 9.53, P = 0.007, \eta^2 = 0.37 \)). However, the type of re-growth affected the change as well (\( F_{1,16} = 20.62, P < 0.001, \eta^2 = 0.56 \); Fig. 16). In willows with re-growth from root-collar shoots, the quality of leaves available were as good as or slightly better than food for \( G. lineola \) as their leaves in 1998, whereas leaves from willows with re-growth from older shoots were clearly poorer than before the defoliation in 1998 (see also Neuvonen et al. 1987).

Regardless of the ubiquitous results obtained, I suggest that the lower food quality in the high-density site (Fig. 15; Table 5) can be a result of selective mortality or phenotypic changes in \( S. phylicifolia \) inflicted by defoliating \( G. lineola \). This means that \( G. lineola \) occurring in the patchy mosaic of adequate abiotic habitats (II) may cause the deterioration of their own habitat patches, which may have profound consequences for the population dynamics of this leaf beetle (see also chapter 8.2). The dynamically changing population densities of \( G. lineola \) in different parts of Siikalahti wetland area during the 1990’s (Sipura, unpublished data), give an indication that such density-dependent mechanisms may exist in the population dynamics of \( G. lineola \), calling for further examination.

8.4. Variation in willow defence: do severe microclimate and predators help the helpless?

8.4.1. The hypothesis

Recognizing the enormous between- and within-species variation in the susceptibility of willows to attack by herbivores one may ask: 1) why all individuals are not strongly defended, and 2) what factors maintain the observed variance (see van der Meijden 1996). One of the easiest ways to explain why some plants are weakly defended against herbivory is that defence incurs costs (Coley et al. 1985; Herms and Mattson 1992; Simms 1992). It has been suggested that the metabolic costs or toxicity associated with the defensive agents, or the trade-off between herbivore resistance and tolerance, can balance the fitness of differently defended plants (e.g. Coley 1985; Simms 1992; Agrawal and Strauss 1999). However, many empirical studies have found that the immediate costs of direct defences tend to be sur-

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prisingly low (Zangerl and Bazzaz 1992; Ågren and Schemske 1992), and furthermore the universality of resistance-tolerance trade-offs has recently been questioned (Mauricio et al. 1997). In *S. phylicifolia*, the resistance against *G. lineola* seems to be determined by water and nitrogen content or some physical properties of the leaves rather than purposely produced chemical or physical defences. These characters are likely to correlate negatively with plant growth, and resistant individuals may lose to the competition in early successional habitats. Therefore, the cost of being unpalatable to *G. lineola* can be surprisingly high. However, direct defences may have indirect costs as well (e.g. Vrieling et al. 1991; van der Meijden 1996; Sabelis et al. 1999).

As shown earlier, *G. lineola* may change the population structure of *S. phylicifolia* in wetlands by killing the most susceptible host individuals. As a consequence, the willows growing in wetlands can be genetically highly resistant to *G. lineola*. On the other hand, *S. phylicifolia* growing in drier habitats suffer only little defoliation by *G. lineola*, and are not selected to be unpalatable. Similarly, susceptible willows suffer a relatively low amount of leaf damage when predators are present, but still far more than strongly defended willows when predators are excluded (III; IV; V). This raises the question whether variation in abiotic conditions (I; II) or predator densities (Fig. 8 in article II) can act as a factor maintaining the variation in herbivore resistance of *S. phylicifolia* observed by Liikanen (1997) and article III of this thesis.

In this chapter I shall present a graphical model explaining how variation in abiotic conditions and predator densities can maintain variance in plant defences against herbivores, and test the model’s qualitative predictions using *G. lineola* feeding on *S. phylicifolia* as a model system. According to the hypothetic model (Fig. 17), even low physiological cost of direct defence can increase variance in defence when the density of herbivores varies. The model predicts that without any internal costs, resistant (strongly directly defended) and susceptible clones will perform equally well in the absence of herbivores, but otherwise the resistant genotype will be superior. However, when resistance incurs some costs, the susceptible clone will perform better when herbivores are scarce. I suggest that variation in predator densities or abiotic conditions is a sufficient source of this variation.

8.4.2. Climate

Earlier in this thesis I demonstrated that abiotic conditions are central in the habitat selection and larval performance of *G. lineola* and, consequently, greatly affect the level of leaf damage suffered by *S. phylicifolia* (I; II).

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**Figure 17.** A graphical model modified from Abrahamsson and Weis (1997) and van der Meijden (1996) for the reaction norms of differently defended plants in the gradient of herbivore density. The density of herbivores is assumed to be determined by suitability of abiotic habitat and density of predators. The light gray curve shows the response curve of an imaginary highly resistant clone with no direct cost of resistance, and the dark gray curve the response of similarly defended clone with some cost of resistance. The black curve presents the response of a susceptible clone. The model predicts that resistant clones should perform better than susceptible clones in habitats that are preferred by herbivores or habitats where predators are scarce, whereas the reverse is true in habitats avoided by herbivores or in habitats where predators are abundant.
Therefore, I suggest that abiotic variation can partly maintain the variation in herbivore defence in *S. phylicifolia*. To test the hypothesis, I established an experiment where the same clones were reared both along a waterline and in a drier habitat. Among the 60 clones collected as cuttings from a wide variety of habitats in Parikkala in 1998, and cultivated in the botanical garden of the university of Joensuu, I selected 10 resistant and 10 susceptible clones of *S. phylicifolia*. The susceptibility to *G. lineola* was tested in 1998 using a 60-choice laboratory experiments replicated six times (see I for the method). As the difference in susceptibility to *G. lineola* was very great, it is possible that some of the resistant clones were hybrids of *S. phylicifolia* and *S. myrsinifolia*. However, they did not differ morphologically from the susceptible ones, and I believe that this possible shortcoming does not radically violate the assumptions of the hypothesis. All shoots were cut before the growing season in order to level their size. Before the peak of the oviposition period of *G. lineola*, I transferred one pot from each clone to about 1 meter distance from the shoreline of Lake Pyhäselkä (62°37’N, 29°39’E). The other pot from each clone was placed in a drier, open site 50-80 meters from the shoreline. The willows were fertilized with complete NPK-fertilizer (see V) and watered regularly during the experiment. Thus, the two pots from each clone had similar resources, but grew under different abiotic environments. I counted the number of *G. lineola* regularly, and used the number of hatched larvae and the survival estimate (maximum number of third instar larvae observed / number of hatched larvae; see II) as variables. When the larvae had pupated, I measured the dry weight of the above-ground biomass and took a sample of fifty leaves from each clone, in order to determine the leaf area damaged as in articles I-V. The data were analyzed using two-way ANOVA with susceptibility as a between-subject factor and habitat as a within-subject factor.

Susceptible clones bore markedly more hatched larvae of *G. lineola* (Fig. 18; $F_{1,18} = 31.78, P < 0.001$), and pots placed near the shoreline harbored markedly more *G. lineola* individuals ($F_{1,18} = 45.02, P < 0.001$). Furthermore, the larval survival was higher in the moist habitat (susceptibility x habitat interaction: $F_{1,16} = 8.54, P = 0.01$), which resulted in vast differences between the levels of leaf damage in the different habitats. The susceptible clones were almost completely defoliated in the moist habitat but suffered a relatively low level of damage in the drier habitat, compared to resistant clones (susceptibility x habitat interaction: $F_{1,18} = 52.17, P < 0.001$). There was a significant susceptibility x habitat interaction ($F_{1,18} = 19.65, P < 0.001$) in the biomass produced. As suggested by the model, the susceptible clones tended to grow better in the drier habitat, whereas resistant clones tended to grow markedly better near the shoreline.

![Figure 18](image-url)
growth is correlated with the fitness of *S. phylicifolia*, the variation in abiotic habitats can maintain the variance in resistance to *G. lineola*, as suggested by the model.

As a conclusion, these results clarify the patchy nature of the relationship between *S. phylicifolia* and its major leaf herbivore *G. lineola*. The system driven by the poor ability of the neonate larvae to tolerate desiccation leads to high beetle densities in moist habitats, and consequently severe leaf damage in the willows. At their highest densities the beetles defoliate the most susceptible clones totally, leading to increased probability of death of these willows. As a consequence of its patchy distribution, *G. lineola* can clearly promote patchy selection pressures on the populations of its host plant *S. phylicifolia*.

8.4.3. Predators

The model presented in Fig. 17 suggests that, besides variation in abiotic conditions, variation in predation intensity may also act as a factor maintaining genetic variation in herbivore resistance. Observations in article II (Fig. 8) show that predator densities show great spatial variation. Furthermore, inherent variation in willow defences is shown to induce variation in predation pressure (III). In some places and on susceptible clones, predators may therefore contribute greatly to plant growth (III; IV; V), but they will have a negligible effect in some habitats (I) and on resistant clones. As predation acts as a substitutive component of the defence against herbivores in *G. lineola*, lack of the predation component may lead to selection towards stronger defence and *vice versa*, as suggested by the model in the Fig. 17 (see van der Meijden 1996). Some evidence for this was provided by articles IV and V: *S. phylicifolia* grew better when predators were present, whereas *S. myrsinifolia* tended to grow better when predators were excluded.

In order to test this hypothesis using clonal variation within *S. phylicifolia*, I established an experiment using the same clones as when testing the effect of abiotic variation (chapter 8.4.2.). Before the peak of egg-laying period of *G. lineola* I transferred both pots of each clone to about 1 meter from shoreline, and allowed *G. lineola* to oviposit for a week. Then I transported the pots to the common garden described in article III, and protected one of the two pots from predation with a sleeve bag as in article III. I then followed the methods described in chapter 8.4.2. The data were analyzed using two-way ANOVA with susceptibility as a between-subject factor and predation treatment as a within-subject factor.

As seen in Fig. 19, the susceptible clones had markedly more hatched larvae of *G. lineola* (*F* < 0.001). The larval mortality was higher in non-protected plants

![Figure 19](image-url)
There was no susceptibility x predator treatment interaction ($F_{1,18} = 1.09, P = 0.31$). The presence of predators reduced leaf damage ($F_{1,18} = 37.50, P < 0.001$), and the effect was significantly stronger on susceptible clones (susceptibility x predation treatment interaction: $F_{1,18} = 25.56, P < 0.001$). There was a significant susceptibility x predation treatment interaction ($F_{1,18} = 6.77, P = 0.018$) in the biomass produced, indicating that predators did not affect biomass produced by resistant genotypes, but greatly increased the growth of susceptible genotypes (Fig. 19).

As suggested by the model (Fig. 17), the susceptible clones tended to grow better in the presence of predators, whereas the resistant clones tended to grow better when predators were eliminated. Consequently, the variation in predator densities, as well as the variation in the abiotic conditions, can maintain the variance in resistance to $G. lineola$. Moreover, as the predation mortality of herbivorous insects on $S. phylicifolia$ appeared to be density-dependent (III), willows apparently face an evolutionary trade-off between direct and indirect defenses. Should they defend themselves using phenolic glucosides, or should they prefer the cheap, but probably uncertain and ineffective protection provided by predators?

9. Conclusion: therefore is the world of willows green

Despite the fact that herbivores can inflict severe spatially restricted defoliations on the willows studied in this thesis (II; III), overall the world of willows remains predominantly green. Line transects conducted by me over a wide range of habitats in August 1998 and 1999 in Eastern Finland revealed that although $S. phylicifolia$ is the most severely damaged willow species in Eastern Finland, the average level of leaf damages did not exceed 15% in either of these years (Sipura, unpublished data). As a conclusion of this thesis, I shall suggest a generalized explanation for the greenness of the studied willows.

Basically, this thesis has revealed the variety of interacting, and both spatially and temporally varying, ecological factors determining the distribution and abundance of willow-feeding insects. I propose that none of these single factors (sensu Hairston et al. 1960; White 1993), or even combinations of several factors (sensu Lawton and McNeill 1979) alone can explain the greenness of the world of willows. Instead, I argue that the world of willows is green because of the inability of herbivores to optimally track the variation among these factors and their interactions on different spatial and temporal scales. Firstly, due to phylogenetical, physiological or behavioral constraints, herbivores are adapted to optimally use only a small fraction of the available host plants. For instance, the adults of $G. lineola$ are adapted to select willows with small concentrations of phenolic glucosides, where their larvae also perform best, whereas $P. vitellinae$ occurs exclusively on willows rich in phenolic glucosides. There is also genetic and phenotypic variation within the preferred host plants, possibly partly induced by the herbivores themselves, which make some individuals or genotypes inappropriate host plants. Secondly, other factors than food quality also affect larval performance. Some host plants may be unavailable if they grow in habitats with an unsuitable microclimate, or if they are loaded with natural enemies of herbivores or their competitors. Thirdly, predators tend to respond positively to density of herbivores, and to cause higher mortality on good-quality host plants. Fourthly, if reaching high densities on otherwise appropriate host plants, the herbivores will face exploitative intra-specific competition that can cause very high mortality, though the accompanying host plants may suffer negligible leaf damage and the landscape remains green. Finally, due to the spatially and temporally heterogeneous environment, it may be difficult or impossible for herbivore females to optimally distribute their eggs even when the host plants 1) provide high-quality food, 2) bear sparse predator community, 3) grow under satisfactory abiotic conditions and 4) are abundantly available at the time of oviposition. To exaggerate, there exists no ideal host plant for herbivorous insects, and if there is one, the herbivores most probably do not know it! I therefore agree with the explanation provided by Janzen (1988) in his article “On the broadening of insect-plant
research” for the greenness of the world of willows. However, I would broaden the explanation further by adding one word: variation!

Acknowledgments

First of all, I should like to thank my wife Jaana for all her love, patience and encouragement during this trip to places I have never visited before and will never return again, and for sharing all the ups and downs with me during these years. Jaana helped greatly with the fieldwork especially by constructing birdcages and sewing sleeve-bags. She also read all my texts with great enthusiasm, eagerly improving my less than perfect English. This thesis would never have seen daylight without my supervisors Prof. Jorma Tahvanainen and Doc. Heikki Roininen, who repeatedly begged me not to take on too much! They provided me with an endless amount of advice, refereed my texts and, first and foremost, obtained the funds necessary for finishing my work. Arsi Ikonen initially pointed out to me the mystery of wetland willows and gave his professional advice throughout this work. I also thank Prof. Heikki Hokkanen and Prof. Jari Kouki for commenting the earlier version of this thesis. Rosemary Mackenzie kindly checked my English. I also thank Satu Kuntsi for the nice cover illustration. Without naming anybody specifically, I sincerely want to thank the personnel of our department and the members of our herbyory project for always being accessible when I needed them. I also wish to express sincere thanks to all my relatives, colleagues and friends for their endless support and friendly smiles. The experiments in the natural reserve of Siikalalhti were performed with a kind permission of Forest and Park Service of Finland. The Academy of Finland (Finnish Centre of Excellence Program 2000-2005, project no. 51997), the Faculty of Science of the University of Joensuu, VAPO Oy, Kemina Agro Oy, Kivilangas Oy and Enso Oy supported my work financially. These acknowledgements are heart-felt, as I know that my name alone on the cover of this thesis is inadequate — no one can do it alone. As Heikki once said: “I have never seen anyone for whom producing a PhD thesis was easy”. And it certainly was not!

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Shading enhances the quality of willow leaves to leaf beetles – but does it matter?

Mika Sipura and Jorma Tahvanainen

In press (Oikos)
Shading enhances the quality of willow leaves to leaf beetles – but does it matter?

Mika Sipura and Jorma Tahvanainen

Plant shading is commonly recognised as a factor, which increases susceptibility of plants to attack by herbivorous insects. In this study we experimentally investigated the effect of host plant shading on two willow-feeding leaf beetles, *Galerucella lineola* F. feeding upon *Salix phylicifolia* L. and *Phratora vitellinae* L. feeding upon *Salix myrsinifolia* Salisb. Both beetle species were more abundant on potted willows growing in open habitats than on the same clones placed under the shade of trees. However, in the laboratory the food preference by adults and larval performance showed that the shaded willows are actually better food for both beetle species. On the contrary, when larvae were reared in the field under natural abiotic conditions, we found no difference in larval performance, or if any, even better performance in open habitats. Apparently, higher and more variable daily temperatures in open habitats accelerated the growth of the larvae. When adults were let to emigrate from or immigrate to potted willows, which had been grown in the same conditions but placed either in the open or shady habitats, adults preferred exposed willows. Invertebrate predators were more abundant in open habitats, but we found no differences in leaf beetle mortality by natural enemies between the habitats. Although the larval performance appeared to be approximately equal in the two habitats during the unusually warm study period, we suggest that under suboptimal temperatures the better abiotic conditions of open sites can easily override the better food provided by shaded habitats. The selection of abiotic habitat thus plays a significant role in the adaptive habitat and host plant selection of these beetles within the gradient of shadiness.

**Keywords:** Habitat selection, leaf beetles, willows, shading, abiotic factors, herbivory

Introduction

When an herbivorous insect colonises a potential host plant, it encounters not only the food that is often inadequate or even poisonous, but also climatic conditions, predators, competitors and mutualists associated with the plant (Janzen 1988). Ecologists studying plant-insect interactions have conventionally been concentrating on bottom-up impacts mediated by host plants when explaining spatial distribution and host plant utilization of herbivorous insects (Price et al. 1980, Bernays and Graham 1988, Janzen 1988, Bernays and Chapman 1994). Recently, the importance of higher trophic levels (e.g. Price et al. 1980, Bernays and Graham 1988, Hare 1992, Sabelis et al. 1999), competitors (Denno et al. 1995) and mutualists (e.g. Baylis and Pierce 1993, Müller and Godfray 1999) has also been frequently recognised. The impact of spatial variation in abiotic conditions has also been found to affect abundance and performance of herbivores, both directly (e.g. Lewis 1965, Shreeve 1986, Martinat 1987) and indirectly through host plants (e.g. Larsson et al. 1986, Hartley and Jones 1997, Crone and Jones 1999). However, studies that have explicitly assessed the relative roles of indirect and direct influences of abiotic factors on host plant use by herbivorous insects are still scarce (but see Moore et al. 1988, Louda and Rodman 1996).

The theory of resource allocation of plants in relation to balance between carbon and mineral nutrients (Bryant et al. 1983, 1987, Herms and Mattson 1992) predicts that the leaves of plants growing under suboptimal levels of photosynthetically active radiation should contain relatively more mineral nutrients (especially nitrogen) and relatively less carbon-based secondary compounds compared to plants growing in direct light. Insect performance has usually been found to correlate positively with nitrogen content (e.g.
Mattson 1980, Scriber and Slansky 1981, Slansky 1993) and negatively with the contents of carbon-based compounds (Scriber and Slansky 1981), predicting that shaded plants should be better food for herbivorous insects. Shading has therefore served as a tool for testing the carbon/nutrient balance hypothesis in terms of herbivore preference and performance (e.g. Larsson et al. 1986, Ruohomäki et al. 1996, Crone and Jones 1999). In a laboratory experiment, Larsson et al. (1986) compared phenolic chemistry and feeding preference of the leaf beetle *Galerucella lineola* F. on *Salix dasyclados* L. grown under low or high light, and found that willows growing under low-light conditions contained smaller amount of phenolics and were preferred by the adults of *G. lineola*. In the field, however, shading is likely to affect insect food preference and performance also indirectly through abundance of natural enemies (Harrison 1987) or competitors, or directly through altered microclimatic conditions which affect the orientation behaviour and growth of herbivorous insects (Singer 1971, Hicks and Tahvanainen 1974, Lincoln and Mooney 1984, Willmer 1986, Moore et al. 1988, Weis et al. 1988, Louda and Rodman 1996).

The objective of this study is to experimentally test the balance between direct and indirect effects of shading on host plant selection, abundance and larval performance of two leaf beetle species feeding upon two willow species. Our preliminary observations suggested that willows growing under the shade of other trees harbour clearly fewer herbivores and their leaves are less damaged than in those of the willows growing in open habitats. To explain this we constructed four hypotheses. 1.) On the contrary to expectations, especially to carbon/nutrient balance - hypothesis, shaded willows are worse food for these leaf beetles. This can be caused by unexpected response by plants to shading or specialised feeding habits of the beetles. It has been shown earlier that willow-feeding leaf beetles can use phenolic glycosides, a group of carbon based secondary compounds including the salicylates salicin and salicortin, as feeding cues (e.g. Tahvanainen et al. 1985). Adult beetles specialised on willows may therefore prefer leaves with higher concentrations of these compounds. 2.) Shaded willows under dense vegetation bear more predators than isolated willows in open habitats and are therefore more risky habitats for leaf beetles. 3.) Shaded habitats provide worse abiotic environment for the beetles. 4.) Surrounding vegetation in shaded habitats obscures host seeking behaviour of the beetles. The first three hypotheses are tested here experimentally, but only circumstantial evidence is available for the last one.

**Study species**

*Galerucella lineola* F. (Coleoptera: Chrysomelidae) feeds on alder (*Alnus* spp.) and several low-salicylate willow species (Maisner 1974) but is in our study area most abundant on *S. phylicifolia* L. *S. phylicifolia* is an abundant and widespread willow in northern Europe usually growing in moist early successional habitats (Hämälä-Ahti et al. 1998, Skvortsov 1999). Leaves of *S. phylicifolia* contain very low levels of phenolic glucosides (especially salicylates), which have been shown to affect host selection and larval performance of many chrysomelid beetles (Tahvanainen et al. 1985, Denno et al. 1990). Instead, leaf water and nitrogen contents, or some physical traits not measured from the leaves have been found to play more important role in host selection and larval performance of *G. lineola* on *S. phylicifolia* (M. Sipura et al., unpublished data). The adults of *G. lineola* overwinter in litter or bark crevices and colonise willows by the bud break in early spring. Adults feed on their host plant few weeks before females oviposit their eggs in batches on the lower surface of the leaves. Females usually lay their eggs while feeding, and therefore their food preference correlates strongly with oviposition preference (M. Sipura, personal observation). Larvae hatch after a few weeks, and pass through three instars before pupating in the soil. *G. lineola* is only occasionally encountered on high-salicylate willow species (including *S. myrsinifolia*), but made up 20-70% (mean 44%) of the guild of leaf-chewing insects on 37 haphazardly chosen *S. phylicifolia* -populations censused in Eastern Finland during the years 1998-1999 (M. Sipura, unpublished data).
Phratora vitellinae L. (Coleoptera: Chrysomelidae) is specialized on willows and poplars containing large amounts of phenolic glucosides (Tahvanainen et al. 1985, Pasteels et al. 1988). The life cycle of P. vitellinae resembles that of G. lineola, but P. vitellinae occurs somewhat later in summer. In our study area most adults colonize S. myrsinifolia Salisb., which generally has about fifty times higher concentrations of phenolic glucosides (including salicin and salicortin) in its leaves compared to S. phylicifolia (Tahvanainen et al. 1985, Rank et al. 1998). P. vitellinae and other specialist herbivores may use phenolic glucosides as stimulants for feeding or oviposition (Tahvanainen et al. 1985, Rank 1992, Soetens and Pasteels 1994, Roininen et al. 1999). The larvae of P. vitellinae convert salicylates derived from their host plants into salicylaldehyde that has been shown to repel many natural enemies of larvae (Pasteels et al. 1988). Therefore, it has been suggested that P. vitellinae benefits from feeding on high-salicylate willows, since these plants provide protection for the larvae (Pasteels et al. 1988, Denno et al. 1990). However, Rank et al. (1998) found that there were no differences in predation when grown on different willows, but larvae grew better on S. myrsinifolia compared to S. phylicifolia. P. vitellinae is rarely found on S. phylicifolia, but made up 20-98% (mean 76%) of the guild of leaf-chewing insects on 37 haphazardly chosen populations censused in Eastern Finland during the years 1998-1999. The observations and experiments in this study are conducted correspondingly for G. lineola on S. phylicifolia and for P. vitellinae on S. myrsinifolia.

Methods

Field observations

For observational data we used potted willows of four clones of both species, propagated from cuttings and cultivated in the botanical garden of the University of Joensuu in 1998. We selected 13 pairs of study sites in the vicinity of the city of Joensuu (62°37’N, 29°40’E) and transported the potted willows to the field just before bud break in 1999. At each site one pot of each clone was placed in open sunny habitat with no shadowing vegetation (e.g. in meadows, wastelands and industrial districts) and one pot of each clone under the shade of trees. The shading trees consisted mainly of Alnus incana L. (72% of the coverage above the experimental willows), Sorbus aucuparia L. (19%) and other willows (6%). The distance between the open and shady habitats varied between 5 and 30 metres in each site and the distance between the sites was at least 500 metres. The light intensity was measured with a portable Li 250 Light meter (LI-COR Inc.) by taking ten measurements from each site at haphazardly chosen order during two sunny days. The light intensity was 2008.85 ± 17.74 µmol m⁻²s⁻¹ in open sites and 122.00 ± 22.73 µmol m⁻²s⁻¹ in shaded sites.

During the summer 1999 we censused the number of adults, eggs and larvae of both beetle species 16 times, and used the maximum number of individuals observed as the measurement of abundance. We used the maximum number of third instar larvae observed as a variable because it provides a reliable estimate for both larval survival and the amount of leaf area damaged by the larvae (M. Sipura, unpublished data). After pupation of the beetle larvae, we collected the shoots and a random sample of 50 leaves from each pot and measured shoot length, mean leaf size and the mean proportion of leaf area damaged using methods described in Sipura (1999). The preliminary examination of the data suggested that the effect of shading was similar in all clones, so we used mean values of all four clones of both species in the analyses.

Host plant selection and larval performance

For the experimental data we used pairs of potted plantlets from 20 clones of both willow species (40 pots/willow species) originated, cultivated, and placed in the field in a similar way as the observational willows. The experimental willows were somewhat larger containing at least ten ramets sprouting from at least eight cuttings. This allowed us to use separate willow individuals in different experiments, thereby reducing possible damage-induced responses by the wil-
The clones were placed in pairs in 20 pairs of habitats. Temperature and relative humidity were measured at a height of 20 cm using a Wilh. Lambrecht thermohygrograph, placed in each pair of habitats for one randomly selected day during the experiment. The light intensity above the experimental willows during the two open days was 2005.80 ± 17.52 µmol m⁻² s⁻¹ in open sites and 118.65 ± 16.19 µmol m⁻² s⁻¹ in shaded sites.

About five weeks after bud break we tested the food preference of adult leaf beetles in the laboratory using two-choice tests. The youngest fully expanded leaves from a clone growing either in open or shady habitats were placed in pairs under Plexiglass plates with two holes exposing an area of 201 mm² of the upper side of the leaf. One starved individual of G. lineola (in S. phylicifolia) or P. vitellinae (in S. myrsinifolia) was placed in the middle of the arena, which was then covered with the lid of a Petri dish. Adults were allowed to feed for 30 hours, after which the total leaf area eaten was measured using a transparent scale-paper.

We measured the performance of larvae both in the laboratory and in the field sites. Two leaves from each clone were collected and placed on a well-moistened filter paper into a Petri dish of 15 cm in diameter. About 35 eggs (2-4 batches), laid by 2-4 females originating from laboratory stocks obtained from several populations, were placed on the leaves. The dishes were then placed in a growth chamber with +28°C temperature, >90% relative humidity and 12 hours light period. Fresh food, obtained from three separate willow individuals from each pot, was offered to the larvae every third day. In the field, about 40 eggs (3-5 batches/females) were attached to leaves of one ramet so that fresh food was readily available for neonate larvae. The ramet with eggs was covered with a sleeve bag with 1 mm mesh size and a piece of Sphagnum moss was placed in the bag to offer a matrix for pupation. Each dish and sleeve bag was checked regularly to obtain median dates of hatching and pupating for each rearing. When all larvae had pupated, we collected, dried and weighed the pupae. We used larval survival (percentage of pupae/hatched larvae), developmental period and pupal dry weight as variables when assessing larval performance. We performed statistical analyses for laboratory and field experiments separately applying paired t-tests, and using these growing conditions as a factor to examine the significance of interactions. A randomised block design ANOVA with type III sum of squares was applied in the latter analysis.

**Predator densities and predation experiment**

We counted the numbers of invertebrate predators on the control ramets of experimental willows ten times during the larval rearings and used the mean number of predators observed on each willow as an estimate of predator density. The predation experiment was conducted on the same potted willows as the larval rearing. About 30 eggs were attached on two ramets of each willow. One of the ramets was covered with a sleeve bag whereas the other one served as a control. A funnel made of sticky flypaper was affixed to the stem of the bagged ramet to catch escaping or accidentally fallen individuals. When larvae had moulted to third instars we removed the sticky funnel and

![Figure 1. The host plant selection of Gale-rucella lineola and Phratora vitellinae adults in the laboratory on plants grown in open and shady habitats. Numbers in bars show sample sizes. Wilcoxon tests are Z = 2.82, exact P = 0.001 for G. lineola and Z = 1.91, exact P = 0.056 for P. vitellinae.]
counted the number of larvae stuck in the glue, which allowed us to eliminate the effect of emigration from the experiment. We then placed a plastic funnel lubricated with liquid Teflon (Fluon®) and filled with Sphagnum moss to the stem of each ramet to collect pupating larvae. This procedure may have led to overestimation of the larval survival, since the slithery funnel may have impeded crawling natural enemies. However, we believe that the effect was similar for both habitats. Furthermore, earlier results suggest that at least in Phratora vitellinae, the larvae in late developmental stages are relatively safe from predators (Rank et al. 1998, M. Sipura, unpublished data). There was no block effect in arcsine square root transformed survival, so we omitted blocks from the analysis and applied a two-way ANOVA with type III sum of squares.

### Habitat selection in field

We conducted both emigration and immigration experiments to assess the colonization activity of adult beetles in open and shady habitats. The emigration experiment was conducted near the observational sites described above. Twelve sites were selected for the experiment so that there were no willows growing within 40 metres of the site. Potted willows of the same clonal origin were placed in open and shaded habitats and covered with a sleeve bag. Fifty adult beetles were released into the bag and were let to settle down till the next morning when the bags were removed. The number of individuals remaining was counted after 6 hours. 

The immigration experiment was conducted in an open wasteland area near the city of Joensuu with no willows within 40 m

<table>
<thead>
<tr>
<th>Table 1. The size of the willows, maximum number of adults, eggs and third instar larvae of the beetles, and the percentage of leaf area damaged on the potted willows placed in two habitats. The within-plant CV% of shoot length is the coefficient of variation (standard deviation/mean) × 100] of the shoot lengths calculated for each replicate. Mean values of the four clones were used as variables.</th>
<th>Habitat</th>
<th>Wilcoxon test</th>
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<tbody>
<tr>
<td>Sunny (n = 13)</td>
<td>Shady (n = 13)</td>
<td>Z</td>
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<td><strong>Galerucella lineola on Salix phylicifolia</strong></td>
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<td>The plants</td>
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<td>Mean shoot length (cm)</td>
<td>14.14 ± 0.78</td>
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<td>Within-plant CV % of shoot length</td>
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<td>71.58 ± 7.98</td>
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<tr>
<td>Mean leaf size (cm²)</td>
<td>10.57 ± 1.55</td>
<td>8.06 ± 0.97</td>
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<td>The beetles</td>
<td></td>
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<td>5.61 ± 1.20</td>
<td>1.00 ± 0.49</td>
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<tr>
<td>Number of eggs</td>
<td>65.08 ± 16.29</td>
<td>10.31 ± 3.85</td>
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<tr>
<td>Number of III instar larvae</td>
<td>19.69 ± 7.15</td>
<td>2.31 ± 1.17</td>
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<td>% of leaf area damaged</td>
<td>11.66 ± 1.67</td>
<td>5.61 ± 1.95</td>
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<td><strong>Phratora vitellinae on Salix myrsinifolia</strong></td>
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<td>The plants</td>
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<td>10.61 ± 1.5</td>
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<td>Mean leaf size (cm²)</td>
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<td>9.41 ± 0.41</td>
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<tr>
<td>% of leaf area damaged</td>
<td>1.65 ± 0.46</td>
<td>1.22 ± 0.74</td>
</tr>
</tbody>
</table>
of the experimental site, during one week without rain in June 1999. Two potted willows of the same clonal origin were placed in pairs 3 meters apart. One of the willows was shaded with a plywood board of 80 cm in diameter and placed about 20 cm above the willow, which was observed to lower the temperature approximately 4°. Fifty individuals of beetles were released from a Petri dish placed between the willows and individuals present on willows were counted after 2 hours. The experiment was replicated 12 times in both species using different willows and beetles in each replicate. Air temperatures during the experiment were measured both in open and shaded sites.

The results presented here are mean ± standard error of mean (SE). All tested variables were examined for the distribution of residuals and homoscedasticity, and standard transformations were used if necessary. Nonparametric tests were applied when appropriate.

**Results**

On average, *S. phylicifolia* produced slightly shorter shoots but not significantly smaller leaf area as a result of shading (Table 1). In *S. myrsinifolia* the differences were not significant. There was greater within-plant variation in shoot lengths in both species in the shade. Adults, eggs and larvae of both beetles were more abundant on willows placed in open sites. In *S. phylicifolia* the leaves were also more damaged in open sites, but in *S. myrsinifolia* the difference was not significant.

In the laboratory, adult *G. lineola* preferred leaves from willows grown in shaded habitats (Fig. 1). There was a similar tendency in *P. vitellinae* but the difference was only marginal (Fig. 1). When reared in the laboratory both beetles performed better on leaves from shaded willows, but in the field the difference disappeared or became reversed (Fig. 2). In *P. vitellinae* the habitat (sun or shade) × growing conditions (laboratory or field) interactions were significant both for survival (*F*₁,₁₀ = 10.65, *P* = 0.004), developmental time (*F*₁,₁₆ = 78.21, *P* < 0.001) and pupal weight (*F*₁,₁₆ = 9.45, *P* = 0.007). In *G. lineola* the interaction was significant only for developmental time (*F*₁,₁₉ = 15.10, *P* = 0.001). In the field both beetles developed longer in shaded habitats (Fig. 2).

Daily variation in temperature and relative humidity are presented in Fig. 3.

In *S. phylicifolia* there were 1.34 ± 0.22 versus 0.50 ± 0.11 (*t*₁₉ = 3.62, *P* = 0.002, paired *t*-test) individuals of invertebrate predators and on *S. myrsinifolia* 3.22 ± 0.22 versus 0.56 ± 0.18 (*t*₁₉ = 2.54, *P* = 0.02, paired *t*-test) individuals in open and shaded habitats respectively. On *S. myrsinifolia* all individuals (*n* = 27 on observational and 5 on experimental willows) of a specialist predator of *P. vitellinae*, *Parasyrphus nigritarsis* (Diptera: Syrphidae), were observed in
open habitats. The experimental predator exclusion increased survival in *G. lineola* ($F_{1,76} = 10.75, P = 0.002$), but not in *P. vitellinae* ($F_{1,76} = 0.41, P = 0.53$)(Fig. 4). There were no significant habitat × predator exclusion interactions (*G. lineola*, $F_{1,76} = 1.93, P = 0.17$ and *P. vitellinae*, $F_{1,76} = 1.26, P = 0.27$).

In the emigration experiment there were only $0.25 ± 0.18$ *G. lineola* left on shaded willows but $12.50 ± 2.75$ on willows in open sites ($Z = 2.94$, exact $P = 0.001$, Wilcoxon test). In *P. vitellinae* there were $9.50 ± 3.76$ individuals in shady and $18.42 ± 4.12$ in open sites ($Z = 2.71$, exact $P = 0.004$, Wilcoxon test) respectively. In the immigration experiment adults preferred willows placed in open sites, though in *P. vitellinae* the difference was not significant ($Z = 2.08$, exact $P = 0.037$ for *G. lineola* and $Z = 1.69$, exact $P = 0.092$ for *P. vitellinae*, Wilcoxon test, Fig. 5). However, at the highest ambient temperatures adults showed preference for shaded willows (Fig. 5).

**Discussion**

**Shading enhances leaf quality**

As expected on the basis of the carbon/nutrient balance hypothesis (Bryant et al. 1983, 1987) the quality of willows for both leaf beetles was enhanced when the willows were grown under the shade of other plants. The adult beetles preferred the shaded leaves (Fig. 1), and in the controlled laboratory conditions the larvae showed better survival, grew faster and attained higher pupal weight on leaves of shaded plants (Fig. 2). It may be that reduced solar radiation led to lowered concentrations of harmful phenolic secondary metabolites, e.g. salicylates or condensed tannins, or to higher contents of water and nitrogen (see Larsson et al. 1986). Earlier laboratory results (Sipura et al., unpublished data) suggest that the differences in host plant preference and performance of *G. lineola* on *S. phylicifolia* clones is primarily determined by water or nitrogen content. Similarly, *P. vitellinae*, which actually prefers willow species containing high concentrations of salicylates (Tahvanainen et al. 1985, Rank et al. 1998) also had a tendency to prefer shaded *S. myrsinifolia* in this study. It may therefore be that the apparent relative increase in water or nitrogen concentrations in shaded willows is more important for leaf beetle performance here than the possible impacts of reduced concentra-
tions of secondary phenolics. We also observed a distinguishable reduction in the waxiness of the leaf surfaces when willows were growing in shaded sites. Especially the leaves of *S. phylicifolia* growing in open sites are covered by waxes, which are usually composed of long-chained carbon-based molecules that apparently protect leaves from desiccation or ultraviolet radiation (Taiz and Zeiger 1991, Eigenbrode and Espelie 1995), but can also be harmful for insects (Woodhead 1983, Eigenbrode et al. 1991, Eigenbrode and Espelie 1995). The larvae of the beetles often fed through the leaves of shaded willows but skeletonized the leaves of willows in open sites by feeding only a layer from the lower sides of the leaves (Fig. 6). As a conclusion, the results obtained in laboratory are consistent with the predictions of the carbon/nutrient balance hypothesis but do not explain the distribution of the leaf beetles in the field.

But climatic factors are more important

Despite the enhanced food quality in the shaded willows, both beetle species were more abundant on willows in open habitats and selected preferentially open habitats in the colonisation experiments. In the field experiment, the larvae also performed relatively better in open habitats suggesting that some factors associated with shading itself (e.g. temperature, irradiation, humidity, olfactory or visual environment, etc.) play a crucial role in the habitat and host plant selection of these leaf beetles. Although we made no attempt to separate these factors we suggest that the differences were primarily caused by the interaction of temperature and humidity. Earlier studies have shown that especially the neonate larvae of *G. lineola* are vulnerable to desiccation and seek for humid habitats (Larsson et al. 1997, M. Sipura et al. unpublished data). The adults also avoid sun-drenched habitats, and prefer equally warm but damp wetlands where their larvae perform best (M. Sipura et al., unpublished data). In our study there were no clear differences between the habitats in relative humidity but a moderate difference in temperature (Fig. 3.), which can be the primary reason for the observed behaviour and performance of the beetles.

The difference in daily mean temperature between the habitats was only two degrees, and therefore the considerable effect of habitat on larval performance in the field seems exaggerated. However, there was also a clear difference in the amplitude of daily fluctuation in temperature (Fig. 3.), which

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**Figure 5.** Microhabitat preference by the adult leaf beetles in relation to ambient temperature. Spearman correlations: *G. lineola* $r_s = -0.66, P = 0.019$ and *P. vitellinae* $r_s = -0.71, P = 0.01$.

**Figure 6.** Second instar larvae of *P. vitellinae* skeletonizing a leaf of *S. myrsinifolia* (upper panel). Third instar larvae that have fed through a leaf of *S. myrsinifolia* (lower panel) (Photos by MS and Jaana Sipura).
can alter the foraging behaviour of larvae (Casey 1993) or affect the larval development through non-linear growth responses (Worner 1992, Ruel and Ayres 1999). Ruel and Ayres (1999) have suggested that if the growth response curve of larvae to temperature is accelerating, insects growing in sub-optimal temperature gain from fluctuating temperatures. There is also some empirical evidence that fluctuating temperatures accelerate the growth of several insects (e.g. Tingle and Copland 1988), but to our knowledge there is no evidence for chrysomelids.

Both beetle species developed slower in shaded habitats but, perhaps due to lower abundance of predators, this did not convert to increased mortality. There was no significant difference in larval mortality between the habitats when the effect of predators was removed. However, it is worth noting that the period in which this study was made was unusually warm and dry (Meteorological Institute of Finland 1999). It is probable that under lower ambient temperatures, the superiority of open habitats can be even more pronounced.

The importance of environmental heterogeneity in plant-insect interactions

This study questions the common, but often implicit conception, that the intrapopulation variation in the abundance and spatial distribution of herbivorous insects is primarily influenced by variation in nutritional quality of their host plants (but see examples from mountainous habitats, e.g. Smiley and Rank 1986, Suzuki 1998, Virtanen and Neuvonen 1999). Using a relatively small spatial scale, this study shows that the chrysomelid beetles studied select at first climatically appropriate habitat and then the host plant within the suitable habitat. Previously, Alliende (1989) found that shading increased leaf damage within Salix cinerea L. shrubs, but that compass orientation had no effect. Quite different results have been obtained by Moore et al. (1988) who found that western tent caterpillars (Malacosoma californicum pluviale Dyar) prefer their host plants, red alder (Alnus rubra Bong.), growing in isolated and exposed sites and branches facing to the south despite small differences in leaf quality. This kind of patchiness is likely to lead to decreased plant growth and reproduction in habitats preferred by herbivorous insects and thereby to affect the distribution of plants as suggested by Harper (1969). Louda and Rodman (1996) have tested this hypothesis on a crucifer (Cardamine cordifolia A. Grey) and found that the level of herbivore damage was clearly higher in open habitats and that the removal of shade entailed a dramatic increase in herbivore numbers. They concluded that the distribution of the crucifer, which usually grows only in the shade of willows or at forest edges, is largely determined by destructive feeding by herbivores in open habitats. Thereby, their results suggest that spatially explicit variation in insect herbivory induced by abiotic variation may exert considerable effects on the community structure of plants.

As pointed out by Hunter and Price (1992), we should expect the relative roles of different ecological forces to vary among systems and even within the same system when environmental heterogeneity is taken into account. In spite of many classical examples of population and community ecology that show the importance of variable abiotic factors in constraining and interacting with biotic interactions (e.g. Birch 1953, Connell 1961), there are surprisingly few studies investigating the role of abiotic habitat in species interactions (Dunson and Travis 1991). This is especially pronounced in the case of herbivorous insects (e.g. Willmer 1986, Bernays and Chapman 1994). This study demonstrates that herbivorous insects are not able to use plants growing in unsuitable habitats and plants may obtain a substantial gain from growing in a habitat which is evaded by herbivorous insects. Therefore, the role of abiotic factors need to be considered along with biotic factors when explaining the patterns in plant-insect interactions observed in nature.

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References


Why does the leaf beetle *Galerucella lineola* F. attack wetland willows?

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Why does the leaf beetle *Galerucella lineola* F. attack wetland willows?

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The willow-feeding leaf beetle *Galerucella lineola* F. is regularly observed to attack heavily its host plant tea-leaved willow *Salix phylicifolia* L. growing in damp habitats. Although *G. lineola* is common on *S. phylicifolia* also in drier habitats, it has not been observed to inflict as serious leaf damage on willows growing in well-drained soils. In order to confirm the existence of this pattern and to explore the ultimate factors affecting the habitat and host plant use by this beetle, we collected data from twelve study sites in Finland during two consecutive years. All sites included three habitats: 1) water, where the willows were growing in permanently waterlogged soils, 2) flood zone, where the willows underwent periods of flooding and 3) dry zone, where the willows grew in well-drained soils.

*G. lineola* was on average 16 times more abundant on willows growing directly from water as compared to willows in well-drained soils nearby. In order to explain this we proposed three hypotheses. 1) The food quality hypothesis states that the apparently stressed willows growing in waterlogged soils provide superior food for the beetles. 2) The predation hypothesis states that open water surface around the willows in wetlands impedes dispersal of predators providing enemy-free space for the beetles. 3) The climate hypothesis states that wetlands provide better abiotic conditions for the adults, eggs or larvae of this leaf beetle. These hypotheses were tested using observational data and performing experiments both under the controlled laboratory conditions and in the field.

In the contrary to the food quality hypothesis adult beetles preferred *S. phylicifolia* grown in dry habitats both for food and oviposition medium in the laboratory, and produced fewer eggs when fed on willow leaves from wetlands. Consistently, larvae performed poorly on leaves from wetland willows in the laboratory, most probably due to low water content of the leaves, also rich in secondary phenolics and poor in nitrogen. The densities of crawling predators did not differ between the habitats, and the predation experiments revealed no difference in predation pressure between the habitats. However, during a rainless period, the first instar larvae performed relatively poorly in drier habitats despite of the high quality of food. When ramets of *S. phylicifolia* were transported between the habitats the first instar larvae performed best in sunny wetland habitats on willows originally from dry sites. Spraying with water improved the performance of neonate larvae more on willows growing in dry habitat than on wetland willows.

The results show that *G. lineola* does not attack *S. phylicifolia* growing in wetlands because of the superior food quality or lower predation pressure, but because of the strong affinity by adult beetles to the moist habitat where also the first instar larva, vulnerable to desiccation, usually perform best. An apparently ancestral physiological constraint hinders the beetles to use the best food resources restricting their occurrence in moist habitat patches with poor quality food and high risk of intraspecific competition.

Keywords: *Salix phylicifolia*, willows, wetlands, waterlogging, plant stress, *Galerucella lineola*, leaf beetles, habitat selection, herbivory, food quality, secondary chemistry, predation, enemy-free space, abiotic habitat, humidity, desiccation

Introduction

The abundance of herbivorous insects shows vast spatial variation within the range of occurrence even when apparently suitable host plant species are available (e.g. Denno and McClure 1983, White 1993). One of the central themes in the history of the discipline of ecology has been the discussion whether the bottom-up impacts trough host plant quality (McNeill and Southwood 1978, Janzen 1988, White 1993), top-down impacts trough mortality inflicted by enemies (Hastings et al. 1960, Price 1987), competition (Denno et al. 1995) or abiotic environment (Andrewartha and Birch 1954, Kingsolver 1989) control populations of herbivorous insects. Apparently, spatial variation in these “regulating” factors generates spatial variation in herbivore abundance giving us an indication of what might be central factors in the ecology of insects. There is an extensive
literature on the impact of genetically and environmentally determined plant traits on the abundance, habitat and host plant selection, and performance of herbivorous insects (e.g. McNeill and Southwood 1978, Denno and McClure 1983, Bernays and Graham 1988, Bernays and Chapman 1992, White 1993). However, as stated by Janzen (1988), as an herbivore colonizes a host plant, it encounters not only the internal quality of the plant but also the carnivory and variable climate regimes engaged to the plant. Since the seminal paper by Price et al. (1980) it has been frequently shown that natural enemies can play significant role in shaping the patterns of host plant selection and abundance of herbivorous insects (Smiley et al. 1985, Denno et al. 1990; Roininen et al. 1993; Kendall et al. 1996), but the impact of predators, competition and abiotic habitat has not yet been incorporated in the analyses. This study was designed to reveal the spatial pattern of the abundance of G. lineola and to explore the multiplicity of the ultimate factors governing the habitat and host plant use by this leaf beetle. Firstly, we documented the existence and predictability of the phenomenon using field observations from twelve sites in Finland. Secondly, we tried to discover the ultimate reasons for the observed patterns using extensive experimental settings both in the field and under the controlled conditions in the laboratory. To explain the habitat preference of G. lineola we constructed three hypotheses.

1. The food quality hypothesis states that willows growing in moist habitats are superior food for the beetles. S. phylicifolia with no apparent adaptations to anaerobic conditions (see Keeley 1979, Kozlowski 1984, Good et al. 1992) may suffer from nutrient and oxygen deficiency and is apparently stressed when growing submerged or in waterlogged soil (Good et al. 1992, see Kozlowski 1980 for the definition of the concept of plant stress). It has been suggested that plants under abiotic stress can provide better food for herbivorous insects due to increased amount of soluble amino acids (White 1984, 1993; Rhoades 1983), which are especially critical to young developmental stages of herbivorous insects (White 1984, 1993). However, the willows growing in wetlands form low and scattered vegetation and their photosynthesis is apparently not limited by the photosynthetically active solar irradiation. Consequently, the nutrient deficit may shift carbon-nutrient balance (see

2) The enemy-free space hypothesis states that wetlands provide safer habitats for adults, eggs or larvae of the insects, because open water areas impede dispersal of predators, especially crawling ones. Predation has been observed to considerably contribute larval mortality in *G. lineola* (Häggström and Larsson 1995, Larsson et al. 1997, Sipura 1999) and it is suggested to hinder the colonisation of con-generic leaf beetles *Galerucella* spp. used for biocontrol (Nechols et al. 1996).

Finally, 3) the climate hypothesis states that open and moist wetlands are abiotically better habitats for adults, eggs or larvae of this beetle. Previously, Larsson et al. (1997) have demonstrated that especially neonate larvae of *G. lineola* are susceptible to desiccation and actively seek for moist micro-habitats within their host plants. Similar observations have also been made in con-generic *Galerucella* species e.g. *G. nymphaeae* L. and *G. sagittariae* L. (Christina Nokkala, pers. comm., M. Sipura, pers. obs.). Moreover, Sipura and Tahvanainen (2000) have shown that abiotic factors can greatly affect both adult host plant preference and larval performance of *G. lineola* and they can easily overdrive the impact of plant quality.

We believe that careful testing of these hypotheses provide an exhaustive set of possible explanations for understanding of the habitat selection and host utilization pattern of *G. lineola*. Finally, we briefly discuss the consequences of the habitat selection for both ecological and evolutionary interactions between *S. phylicifolia* and *G. lineola*.

**The study system**

*Salix phylicifolia* L. is a common and widespread shrub in Scandinavia growing abundantly in wet meadows, lakesides and drainage ditches, but also in much drier artificial habitats such as forest edges, abandoned fields and wastelands (Jalas and Suominen 1974, Hämet-Ahti et al. 1999, Skvortsov 1999). Despite of the abundance in habitats exposed to flooding, *S. phylicifolia* shows no apparent morphological adaptation, such as root morphology allowing oxygen transportation or surface-sprouting adventitious roots (Keeley 1979, Kozlowski 1984), for waterlogging. However, it can grow on wet meadows and along lakeshores waterlogged most of the year, though there seems to be considerable genetic variation in the tolerance against submersion (M. Sipura, unpublished data, see also Good et al. 1992).

Only small amounts of phenolic glucosides, the major components in herbivore resistance of willows (e.g. Tahvanainen et al. 1985, Kelly and Curry 1991, Matsuki and MacLean 1994, Orians et al. 1997), have been found in the leaves of *S. phylicifolia* (Julkunen-Tiitto 1989, Rank et al. 1998). Instead, leaves contain considerable amounts of some other phenolics, including a flavonoid, amelopsin, characteristic to leaves of *S. phylicifolia* (Rank et al. 1998) and phenolic polymers, condensed tannins (Julkunen-Tiitto 1989; Rank et al. 1998).

The brown willow beetle *Galerucella lineola* F. (Coleoptera: Chrysomelidae: Galerucinae) is a univoltine species, which overwinters as an adult in litter or bark crevices. Adults colonise their host plants by the bud-break in early spring, and feed for few weeks before the oviposition. Some populations are found to feed also on *Alnus* spp. (Maisner 1974, Ikonen and Sipura, unpublished data), but in Finland most populations seem to occur on *S. phylicifolia* growing in wetlands. *G. lineola* generally prefers willows with low concentrations of phenolics glucosides (Tahvanainen et al. 1985, Denno et al. 1990, Kendall et al. 1996) and probably avoid some other phenolics as well (Larsson et al. 1986). The eggs laid in batches of 10-20 hatch after a few weeks, and the larvae pass through three instars feeding mainly on the lower surface of the leaves. After more than 11 days the fully-grown larvae crawl down the stem into the soil or litter debris to enter a prepupal stage. The larvae are poor dis-
persers (M. Sipura, pers. obs.) and therefore complete their development on a plant chosen by the egg-laying females. The new generation of adults emerges after a short pupal stage in August. Several invertebrate predators (Kanervo 1946) and birds (Sipura 1999) have been observed to prey upon G. lineola larvae. Ants, hemipteran bugs (especially Anthocoris nemorum), spiders, lacewings, sedge warblers (Aceroccephalus schoenobaenus) and willow warblers (Phylloscopos trochilus) are the most abundant potential predators in our study areas.

Methods

Distribution of G. lineola across habitats

We selected twelve study sites (Fig. 1) providing a representative sample of the habitats of S. phylicifolia in Finland. The sites number 1, 5, 6 and 8 in Parikkala, site 2 in Lempäälä, site 7 in Värsilä and sites 9, 11 and 12 in Joensuu are all natural damp meadows on the river or lake shore bordered by abandoned or uncultivated fields, whereas the sites 3, 4 and 10 in Joensuu consist of industrial districts and wastelands. At each site we selected at least 60 small clones of S. phylicifolia and divided them individually into one of the three following habitats. 1) In the moistest habitat, called hereafter as water or wetland, the willows were growing with their roots continuously submerged or in a permanently waterlogged soil. 2) In the flood zone root necks of the willows were 0-10 cm above the water surface during the first census in early June indicating that the willows undergo more or less regular periods of waterlogging annually. 3) The willows in dry zone grew over 30 cm above the water surface in a well-drained soil, and have therefore never experienced waterlogging. We did not include intermediate habitats in the analysis. The sample sizes in each habitat varied between 20 and 52 leading to the total number of 847 clones of S. phylicifolia included in the analyses of field surveys.

We counted the number of adults, eggs and larvae of G. lineola at seven sites in 1998 (sites number 1-7) and at five sites in 1999 (sites number 8-12). All individuals were counted from 20 to 150 shoots of each willow three times during the reproductive cycle of G. lineola. The adults and eggs were abundantly present during the first census occasions (between 3rd and 17th of June). Neonate larvae predominated during the second census occasions (between 13th June and 2nd July), whilst the older instars were chiefly observed during the third census occasions (between 26th June and 17th July). In order to calculate beetle densities we measured the lengths of 20 shoots at each census occasion and calculated the total leaf area censused using standard equations for each site and habitat separately as in Sipura (1999). We used the maximum density of each developmental stage observed per square meter of leaf surface at the time of second census occasions by calculating the number of individuals per shoot for each census, determining the mean growth rate of the shoots between the census occasions and estimating the leaf area of these shoots during the second census according to standard equations mentioned above. At the end of the study we collected a sample of 30 leaves from each willow and estimated the percentage of leaf area damaged using methods described in Sipura (1999). In both years we also used data from five sites to estimate larval survival by counting the maximum number of third instar larvae observed and calculating the percentage of third instar larvae of the maximum density of eggs counted. Although the number of censuses was quite low, this index has been previously found to correlate well with actual larval survival (M. Sipura, unpublished data).

Testing the three hypotheses

Testing food quality hypothesis

Host plant traits. At the end of the field censuses we measured the shoot length from twenty randomly selected shoots and estimated the mean size of leaf lamina from 30-50 leaves of each 847 observational clone. More intensive measurements of S. phylicifolia traits were made at Penttilä in Joensuu 1998 (Site 3; 62°37’N, 29°40’E). It was selected as the main study area because it provides a diverse mosaic of artificial and natural ponds, ditches and natural wet meadows
along a lake and a river. The dry habitats in Penttilä consist of an old industrial district, wastelands, small meadows and fields. At Penttilä we selected 20 large (>20 ramets) clones of *S. phylicifolia* in each three habitat. The number of shoots, maximum height of the shrub, shoot lengths (a sample of 100 shoots), leaf size (50 leaves), the radial stem growth (measured from discs of five randomly selected stems) and age of these clones were measured after the growing season using the methods described in Sipura (1999).

On the 20th of July 1998, we collected >200 leaves (leaves number 4-9 from the apex of shoots) from each of the 60 clones in Penttilä and dried the sub-samples of about 100 leaves in 60°C for four days to obtain leaf water content by weighing them before and after drying. The other sub-samples for the chemical analyses were dried in well-ventilated room at normal room temperature (+20-22°C). Dry samples were then milled to fine powder. Then leaf phenolics were extracted for 15 minutes at +4°C in 20 ml of 100% methanol from weighed amount (about 150 mg) of each sample. After the extraction period, sample under processing were homogenized twice for 3 minutes with Ultra-Turrax homogenizator. Solvent phases from both homogenisation events were combined, and the solvent was evaporated by rotate-evaporator. Low-molecular secondary phenolics of these extracts were analysed with the Hewlett-Packard HPLC-instrument described by Keinänen and Julkunen-Tiitto (1998). Identification of individual compounds was based on their UV-vis spectra and on retention times. Condensed tannins were measured using butanol-HCl-assay described in Julkunen-Tiitto (1989). The total nitrogen content of the leaves was measured using automatic CHN-analyser.

**Food choice of adults in the laboratory.**
About three weeks after the budbreak in both years we tested the food preference of adult leaf beetles in the laboratory using leaves collected from three habitats of the moisture gradient (see Fig. 4 for details). In 1998 we conducted tests with leaf material from four study sites and in 1999 from two sites. The beetles were collected at the same sites as the leaves, and originated either from only one of the habitats, or from all of them. In 1999 we also performed an experiment where same leaves (from site 1) were tested separately using beetles from all three habitats. Entire shoots were collected in the field and transported to the laboratory in a cooler. The experiment was initiated within four hours. The youngest fully expanded leaves from a *S. phylicifolia* growing in one of the moisture zones were placed in triplets under the Plexiglass plates with three holes each exposing an area of 201 mm². One individual of *G. lineola* starved over one night was placed in the middle of the arena, which was then covered with the lid of a Petri dish. The experiments were conducted under the normal room temperature (+20-24°C) in continuous light. Adults were allowed to feed for 30-40 hours, after which the total leaf area eaten was measured using a transparent scale-paper. The results were then calculated as the total leaf area eaten in 24 hours.

**Oviposition choice of females in the laboratory.** The oviposition choice experiments were performed simultaneously with food choice experiments under the same conditions, and using the same beetles and leaves from the same sites (Fig. 4b). One twig from a *S. phylicifolia* from each moisture zone was placed under 3-L plastic chambers placed upside down on a plastic foam sheet. The bases of willow twigs were placed in 50-mL plastic vials containing water. Two copulating pairs of the beetles were released into the chamber, and females were let to oviposit for 60 hours. The number of eggs on each twig was counted, and the results are expressed as the number of eggs laid by two females per 24h. Leaf areas of the twigs from different habitats measured after the experiment did not differ (Friedman’s test, *P* > 0.15 in every experiment).

**Adult fecundity in the laboratory.** To examine the effect of food quality on adult fecundity we collected newly emerged adults from each moisture zone at Penttilä in early spring 1999. The beetles were kept in three plastic chambers for three weeks with leaves from the habitat where they were collected from and allowing them to choose their mates freely. When adults started to copulate, we transferred each copulating pair in to a Petri dish which contained two leaves of
the type food they had been feeding on. Food was changed every third day, but the leaves containing eggs were not removed. After 18 days the experiment was ended, the number of eggs was counted and the hatching success was estimated. To get an estimate of larval ability to survive during the first days we collected two egg groups from each Petri dish before the hatching. We took the egg groups apart from the original leaves and moved them to new Petri dishes with fresh leaves of *Salix caprea*. This willow species was earlier found to be adequate food for adult beetles (Tahvanainen et al. 1985), but because of hairiness of its leaves we supposed it to be suboptimal food for the neonate larvae. To reduce variation, all leaves were collected from a single tree. The number of surviving larvae was counted after five days.

Larval performance in the laboratory. In 1998 we performed a laboratory experiment to explore the effect of food plant quality on larval performance using leaf material from Penttilä. Two leaves from a *S. phylicifolia* collected from one of the three habitats were placed on a well-moistened filter paper into a Petri dish of 15 cm in diameter. About 35 eggs (2-4 batches) were placed on the leaves, and the dishes were placed in a growth chamber with +22°C temperature, >90% relative humidity and 12:12 light period. Fresh food was offered for larvae every third day. Each dish was checked regularly to obtain median dates of hatching and pupating for each rearing. When all larvae had pupated, we collected, dried and weighed the pupae. We use larval survival (percentage of pupae/hatched larvae), developmental period and pupal dry weight as variables when assessing larval performance. To estimate within-season changes in food plant quality, we also performed four short term RGR-experiments during the summer 1999 (see Fig. 7 for the dates) using leaf material from willows where the samples for leaf chemistry were obtained. In the RGR-experiments one newly moulted second instar larva was weighed, placed in a Petri dish with fresh leaf material from one of the habitats and placed in the growth chamber with +20°C temperature, >90% relative humidity and 12:12 light period. The larvae were re-

weighed after 48 hours. The relative growth rate was calculated for 24 hours as: \((ln \text{ final weight} - ln \text{ initial weight}) / 2\).

The effect of leaf chemistry on adult choice and larval performance. We also performed multiple-choice experiment with adults using the leaf material from willows of which the samples for leaf chemistry were collected. We collected one leaf (5th from the apex) from each willow trying to minimize variation in leaf sizes and placed all 60 leaves in a cardboard box (size 20 x 30 x 6 cm) lined with a well-moistened filter paper and covered with a plexiglass. The leaves were fastened to the box using insect pins with a numbered tag. Sixty adults of *G. lineola* starved over night were released to a box and let to feed for 30 hours. The total size of leaf lamina and the leaf area damaged was then measured using a transparent scale paper. The experiment was replicated for 10 times and the mean value of area eaten was used as a variable in the regression analysis described below. The total area eaten did not correlate with leaf size (\(P > 0.08\) for each replicate, Pearson correlation). We also performed a similar larval performance experiment covering whole larval period as described earlier using the same leaf material as in the adult choice experiment above. The leaves were collected from different ramets in each food replacement, and only the fifth and sixth leaves from the apex of the shoots in ramets in southern side of the willow were used. Using each willow clone as an individual observation, we performed a multiple regression for both adult choice, arcsin square-root transformed larval survival, logarithm transformed larval developmental period and pupal weight to investigate, how leaf clonespecific chemical properties of *S. phylicifolia* leaves affected the adult choice and larval performance of *G. lineola*.

Testing predation hypothesis

Observations. The number of crawling invertebrate predators was counted in the field censuses and the densities were calculated for a square meter of leaf surface (see above). During the censuses we also recorded the number of occasions when we saw predation towards the adults. To study
egg predation, we tagged 60-80 newly laid egg groups in each habitat in 1998 at Penttilä and 30 egg groups in each habitat at Marjala 1999. A small plastic tag was attached to a twig about 30 cm beneath the egg groups trying to avoid any disturbance towards predators. The fate of the egg groups was followed regularly, and the number of disappeared or damaged eggs was recorded.

Predator exclusion experiments. To test experimentally the effect of natural enemies in the three habitats we performed exclusion experiments in Penttilä (site 3) in 1998 and in Marjala (site 10) in 1999. We selected 60 S. phylicifolia in each habitat at both sites. We then cleared one ramet of each willow from adults, eggs and larvae of G. lineola and attached 4 egg groups (about fifty eggs) on the ramet so that neonate larvae had free access to leaves. One half of the ramets were then covered with a sleeve bag whereas the other half served as control. The sleeve bag apparently eliminated emigration, and to separate emigrating or accidentally fallen individuals from survivors, we attached a funnel made of sticky flypaper at the base of each stem of the experimental ramets. In Penttilä we checked the willows regularly and used the maximum number of third instar larvae observed divided by the number of hatched larvae as an estimate of survival. In Marjala we replaced the sticky funnel by a funnel lubricated with liquid Teflon (Fluon®) and endowed the funnels with Sphagnum moss to offer pupating matrix for the larvae. In Marjala we used the number of pupae divided by the number of hatched larvæ as a survival variable. Because different survival estimates were used, we analysed the experiments separately using two-way ANOVAs with habitat and predation treatments as fixed factors. If predation pressure differs between the habitats, a significant interaction between the factors is expected.

Testing climate hypothesis

Climate measurements. In 1999 we measured the temperature and relative humidity in all sites excluding site 2 using HOBO® data-loggers. The measurements were taken at each 11 sites by attaching the loggers on wooden poles at the height of 1 metre and 1 metre southwards of a haphazardly selected willow shrub in each habitat. The data-loggers took a measurement at five minute intervals for four days at each site, after which the mean values for all 288 times of the day were calculated (see Fig. 10). The measurements were apparently conservative because most G. lineola in wetlands inhabit willows shorter than one metre and are therefore closer to water surface (M. Sipura, pers. obs.). Furthermore, the moisture is likely to be higher inside the bushes (Willmer 1986) especially in wetlands where we observed condensed water under the leaves during warm and sunny days.

Adult fecundity in the field. We measured the fecundity of adults in the field in the three habitats at the time of the laboratory experiment using the same willows. Two copulating pairs were collected from adjacent willows and placed on the ramet, which was then covered with a sleeve bag. The pairs were let to stay in the bags for 18 days after which the eggs were counted and the hatching success was measured. The survival of neonate larvae was tested on S. caprea in laboratory using methods described above. To evaluate the effect of abiotic environment we entered the experimental condition (laboratory or field) as a factor to the ANOVA-model. If the abiotic environment affects adult fecundity, a significant habitat x experimental conditions –interaction is expected.

Larval growth in the field. The larval performance experiment was performed in both years in Penttilä. For these experiments we selected 30 S. phylicifolia from each habitat. Two egg groups (about 30 eggs) were taped to the leaves of one ramet on each willow so that hatching larvae could readily obtain fresh food. The ramets were then covered with sleeve bags and a piece of Sphagnum moss was placed as matrix for pupation. We checked all bags regularly to obtain median hatching and pupating day. When all larvae were pupated, we collected, counted, dried and weighed the pupae. The variables (arcsin transformed percentage of larvae survived, logarithm transformed developmental time and pupal dry weight) were analysed using two-way ANOVA with the habitat as a fixed factor and the year as a random factor.
The growth of young larvae in the field during dry weather conditions. The summer 1998 was unusually rainy (Meteorological Institute of Finland 1998) and only one about six days long period of clear weather occurred during the larval period of *G. lineola*. During this period we conducted an experiment where we reared larvae of all three instars simultaneously on the same clones both in the laboratory and in the field. Twenty *S. phylicifolia* was selected from each habitat in Penttilä. Three egg groups ready to hatch, thirty newly moulted and weighed second instar larvae, and about twenty weighed third instar larvae were attached to the leaves of one ramet which were then covered with a sleeve bag. Leaves from adjacent ramets of the same willows were then collected and transported to the laboratory. Similar sets of larvae were reared on these leaves in Petri dishes in the laboratory (±20°C, >90% RH, 12:12 photoperiod). The experiment lasted for five days after which we counted the number of survivors and re-weighed the second and third instar larvae. The first instar larvae are too small to be reliably weighed, and therefore we use the proportion of larvae moulting to second instars at the end of the experiments as the measure of larval growth rate (see Larsson et al. 1997). For the older larvae we calculated the relative growth rate using methods described above.

The effects of habitat, host plant and shading. In order to evaluate the relative importance of host plant quality and the impact of abiotic factors and interactions between them, we conducted a willow ramet transportation experiment during the clear weather period described above. We selected about 70 willows from each three habitats, collected one about 0.5 m high ramet from each clone and placed the ramets in glass jars with water. We then placed half of the willows in wetland habitat and the other half in dry habitat. Furthermore, half of the willows in each habitat were placed either in open habitat with no shading vegetation or under the shade of other willows. This led to three-way factorial design with willow origin, habitat and shading as fixed factors in the ANOVA model. Three patches of newly hatched (<12 hours ago) larvae were attached on all ramets. No sleeve bags were used to protect the larvae from predators, but

*FIG. 1. Densities of adults (A), eggs (E) and larvae (L) of *G. lineola* in twelve study sites censused during the years 1998 (upper panels) and 1999 (lower panels). The values are mean ± 1SE. Open symbols indicate water, hatched symbols flood zone and black symbols dry zone.*
we lubricated the glass jars with Fluon® hindering crawling predators to climb to experimental shoots. The experiment was ended after four days when first willow leaves showed signs of wilting and about half of the larvae were moulted to second instar. We used the percentage of larvae survived and the percentage of second instar larvae (i.e. growth) as variables.

The water spraying experiment. To test the effect of moisture on the performance of first instar larvae we performed an experimental moisturizing of larvae during the clear weather period in 1998. We selected 15 willows from each habitat in Penttilä for the experiment. About twenty newly hatched larvae were placed to two ramets on each willow. Both ramets were covered with a sleeve bag and a piece of a moistened Sphagnum moss was placed in one of the sleeve bags. The bags with moss were then sprayed with water 4-6 times a day to increase the moisture of the microhabitat. The experiment was stopped after four days and the number of survived larvae and the number of larvae moulted to second instar was counted.

Statistical analyses

In ANOVA models we use eta squared ($\eta^2$) 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). All tested variables were examined for the distribution of the data, the distribution of residuals and homoscedasticity. Standard transformations were used if necessary and nonparametric tests were applied when appropriate. The results presented in the text, tables and figures are mean ± standard error of mean (SE).

Results

Field observations

Adults, eggs and larvae of G. lineola were about 11, 22 and 16 times more abundant in water habitats than in dry ones, respectively (Fig. 1; MANOVA; Wilks’ $\lambda = 0.63$, $F_{2,753} = 218.05$, $P < 0.001$, $\eta^2 = 0.20$). The densities in flood zones were typically intermediate (Fig. 1), but varied (MANOVA; habitat x site –interaction, Wilks’ $\lambda = 0.63$, $F_{2,753} = 218.05$, $P < 0.001$, $\eta^2 = 0.09$) probably depending on local topography. Consistently, willows growing in wetlands were far more damaged than willows in dry habitats (Fig. 2; ANOVA $F_{2,22.16} = 22.16$, $P < 0.001$, $\eta^2 = 0.67$). There was no significant difference in overall larval survival between habitats (Fig. 3., mixed-model ANOVA with sites nested within years, $F_{2,560} = 1.34$, $P = 0.26$), but there was a significant year x habitat interaction (mixed model ANOVA, $F_{2,560} = 11.29$, $P < 0.001$, $\eta^2 = 0.05$) i.e. the larvae performed relatively better in moist habitats in 1999. The non-linear relationship between egg density and larval survival in Fig. 3 suggests that there may have been some density-dependent mortality lowering the larval survival in moist habitats.

The impact of food quality

S. phylicifolia growing in moist habitats produced clearly shorter shoots; mean shoot lengths across habitats when the sites were pooled were $5.4 \pm 0.2$ cm ($N=307$) in water, $11.3 \pm 0.3$ cm ($N=269$) in flood zone and $15.3 \pm 0.4$ cm ($N=271$) in dry zone respectively ($F_{2,844} = 228.18$, $P < 0.0001$, $\eta^2 = 0.35$). Correspondingly, the leaf blades in
wetland willows were smaller; 4.14 ± 0.1 cm² in water, 7.61 ± 0.2 cm² in flood zone and 11.96 ± 0.3 cm² in dry zone respectively 

\( F_{2,844} = 298.53, P < 0.0001, \eta^2 = 0.42 \), suggesting that individuals growing in water-logged soils could not grow efficiently. In Penttilä S. phylicifolia growing in water were smaller, they grew slower, and their leaves contained less water and nitrogen, and more condensed tannins, (+)-catechin and ampelopsin than the approximately same aged willows in dry habitats (Table 1). The amounts of three structurally unidentified secondary substances in leaves did not differ between habitats.

Contrary to the food quality hypothesis, adults preferred to feed on leaves from dry habitats in the laboratory experiments excluding the experiment performed with leaves from site 7 (Fig. 4a). The results from oviposition choice experiments gave the same result (Fig. 4b). There were no signs that beetles preferred the type of willow leaves they had been eating in the field in site 1 (Fig. 4); beetle origin × leaf origin – interaction for food choice is \( F_{4,116} = 0.50, P = 0.74 \) and for oviposition choice \( F_{4,116} = 1.00, P = 0.41 \) (between subject interactions in the block-design ANOVA). The multiple

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**FIG. 3.** Estimated larval survival in five study sites in 1998 and 1999. Open symbols indicate water, hatched symbols flood zone and black symbols dry zone. The different shapes of symbols refer to different sites. The best-fitting log-normal regressions are:

For 1998: \( y = 35.07e^{0.75 \ln[(x/1.18)/0.76]^2} \),

\( F_{2,12} = 1.69, P = 0.23, R^2 = 0.22 \)

For 1999: \( y = 45.42e^{0.88 \ln[(x/2.55)/0.30]^2} \),

\( F_{2,12} = 4.45, P = 0.035, R^2 = 0.43 \)

---

**FIG. 4.** Food (a) and oviposition (b) choices by adult G. lineola tested in three-choice experiments using leaves from six study sites. Open bars refer to water, hatched bars to flood zone and black bars to dry zone. The number on each panel gives the number of the study site/year (see map in FIG. 1.). The text beneath each panel gives the habitat from which the beetles were collected (mixed = mixed stock of beetles from every habitat). The sample size is 30 in every bar. The asterisks indicate statistical significance: NS \( P \geq 0.1 \), (*) \( P \geq 0.05 \), * \( P \leq 0.05 \), ** \( P \leq 0.01 \) and *** \( P \leq 0.001 \).
regression analysis performed with a leaf material from Penttilä suggested that the nitrogen content of the leaves was the major chemical component affecting adult choice (Table 2).

Feeding on *S. phylicifolia* originating from drier habitats tended to increase fecundity of *G. lineola* in the laboratory though the differences with all three variables included in the model were only marginally significant (MANOVA; Wilk’s $\lambda = 0.81$, $F_{6,110} = 1.98$, $P = 0.074$, $\eta^2 = 0.10$, Fig. 5). Females feeding on willows from dry and flood zones laid significantly more eggs than those fed on wetland willows (ANOVA $F_{2,57} = 51.89$, $P < 0.001$). There was a significant difference both in survival (ANOVA $F_{2,57} = 13.84$, $P < 0.001$, $\eta^2 = 0.33$), developmental time (ANOVA $F_{2,57} = 13.31$, $P < 0.001$, $\eta^2 = 0.32$) and pupal weight ($F_{2,57} = 4.27$, $P = 0.02$, $\eta^2 = 0.13$), but there were no significant differences in hatching success ($F_{2,57} = 1.64$, $P = 0.17$) or in early larval survival between the treatments ($F_{2,57} = 1.81$, $P = 0.64$). In the field the differences disappeared ($P > 0.59$), but there was no significant experimental conditions (laboratory or field) x habitat interaction in the MANOVA model (Wilk’s $\lambda = 0.94$, $F_{6234} = 1.14$, $P = 0.34$).

In the laboratory larvae performed clearly better on leaves from willows grown in dry habitats (Fig. 6; MANOVA; Wilk’s $\lambda = 0.59$, $F_{6,112} = 9.81$, $P < 0.001$, $\eta^2 = 0.35$). There was a significant difference both in growth (ANOVA $F_{2,57} = 13.84$, $P < 0.001$, $\eta^2 = 0.33$) and development time (ANOVA $F_{2,57} = 13.31$, $P < 0.001$, $\eta^2 = 0.32$) and pupal weight ($F_{2,57} = 29.91$, $P < 0.001$, $\eta^2 = 0.51$) between the habitats. The results from short term RGR-experiments show that the quality of the leaves as food for *G. lineola* decreased during the course of growing season in 1998 (rmANOVA; $F_{3,171} = 31.44$, $P < 0.001$, $\eta^2 = 0.36$), that leaf quality decreased from dry zone to water ($F_{2,57} = 13.90$, $P < 0.001$, $\eta^2 = 0.32$) and that the difference between the habitats remained relatively constant during the course of growing season (Fig. 7; date x habitat interaction $F_{6,171} = 1.37$, $P = 0.23$). The multiple regression analysis shows that the water content and the content of (+)-catechin were the most important measured chemical factors affecting larval performance (Table 2).

### Table 1. Size, growth and leaf chemistry of *S. phylicifolia* growing in three habitats in the Penttilä study area. Different letters in superscripts indicate statistical significance between the habitats ($P < 0.05$; Scheffe’s test).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Water (N = 20)</th>
<th>Flood zone (N = 20)</th>
<th>Dry zone (N = 20)</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.0 ± 0.6$^a$</td>
<td>16.8 ± 1.0$^a$</td>
<td>14.7 ± 0.8$^a$</td>
<td>1.92</td>
<td>0.16</td>
</tr>
<tr>
<td>Number of ramets</td>
<td>25.2 ± 0.8$^a$</td>
<td>26.1 ± 1.0$^a$</td>
<td>25.4 ± 0.8$^a$</td>
<td>0.34</td>
<td>0.71</td>
</tr>
<tr>
<td>Maximum height (m)</td>
<td>2.09 ± 0.08$^a$</td>
<td>2.78 ± 0.11$^b$</td>
<td>2.93 ± 0.14$^b$</td>
<td>15.77</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>5.12 ± 0.31$^a$</td>
<td>11.09 ± 0.91$^b$</td>
<td>22.06 ± 1.83$^c$</td>
<td>51.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Leaf size (cm$^2$)</td>
<td>4.37 ± 0.35$^a$</td>
<td>8.49 ± 0.97$^b$</td>
<td>18.06 ± 1.13$^c$</td>
<td>63.20</td>
<td>0.001</td>
</tr>
<tr>
<td>Radial growth (mm)</td>
<td>0.69 ± 0.13$^a$</td>
<td>1.74 ± 0.19$^b$</td>
<td>3.54 ± 0.37$^c$</td>
<td>32.95</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Leaf chemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water content (% of fw)</td>
<td>53.07 ± 0.83$^a$</td>
<td>55.84 ± 0.84$^a$</td>
<td>61.29 ± 0.70$^c$</td>
<td>27.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Total nitrogen (% dw)</td>
<td>1.26 ± 0.04$^a$</td>
<td>1.60 ± 0.10$^b$</td>
<td>2.18 ± 0.07$^b$</td>
<td>39.44</td>
<td>0.001</td>
</tr>
<tr>
<td>Condensed tannins (mg g$^{-1}$)</td>
<td>89.29 ± 4.91$^a$</td>
<td>81.96 ± 5.79$^a$</td>
<td>60.95 ± 4.63$^b$</td>
<td>8.21</td>
<td>0.001</td>
</tr>
<tr>
<td>(+)-catechin (mg g$^{-1}$)</td>
<td>8.43 ± 0.85$^a$</td>
<td>7.99 ± 0.59$^a$</td>
<td>4.39 ± 0.45$^b$</td>
<td>11.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Ampelopsin (mg g$^{-1}$)</td>
<td>173.05 ± 5.96$^a$</td>
<td>143.82 ± 11.78$^b$</td>
<td>130.09 ± 9.20$^b$</td>
<td>5.58</td>
<td>0.006</td>
</tr>
<tr>
<td>Ampelopsin derivative (mg g$^{-1}$)</td>
<td>5.17 ± 0.58$^a$</td>
<td>3.98 ± 0.44$^a$</td>
<td>4.02 ± 0.51$^a$</td>
<td>1.73</td>
<td>0.18</td>
</tr>
<tr>
<td>Myricetin derivative I (mg g$^{-1}$)</td>
<td>0.73 ± 0.06$^a$</td>
<td>0.64 ± 0.09$^a$</td>
<td>0.59 ± 0.07$^a$</td>
<td>0.90</td>
<td>0.41</td>
</tr>
</tbody>
</table>
The impact of natural enemies

There was no overall difference in the density of crawling predators between the habitats (Fig. 8; mixed model ANOVA $F_{2,22.25} = 1.56$, $P = 0.23$). However, there was a significant site x habitat interaction ($F_{2,81} = 5.62$, $P < 0.001$) suggesting that in some habitats (especially sites 8, 9, 10 and 11 in Fig. 8) the preconditions for the enemy-free habitat hypothesis may hold. The water habitats were generally dominated by spiders (73% of the observed individuals) whereas ants (41%) dominated in dry habitats. In site 10, where there was 50 times higher density of predators in dry habitat, the predator community in dry zone was clearly dominated by ants (84% of the observed individuals), whereas no ants were observed in wetland dominated by spiders (98%). During the censuses only 6 occasions of predation toward adults were observed in wetlands, 4 in flood zones and 5 in dry zones respectively. In Penttilä 1998 the egg predation was very low; only 3.1% of egg-groups were predated in water, 9.5% in flood zone and 6.0% in dry zone respectively ($G^2 = 2.37$, $df = 2$, $P = 0.31$). In site 12 (Marjala) in 1999 no egg predation was observed. Predator exclusion clearly increased larval survival in both experimental sites (Fig. 9, Table 3). In Marjala the habitat x predation treatment interaction was only marginally significant despite the large difference in observed density of

![FIG. 5](image.png)

**FIG. 5.** Fecundity of G. lineola in each three habitat tested both in the laboratory and in the field. a) Number of eggs laid by two females during 18 days, b) hatching success of the eggs and c) early larval survival measured on Salix caprea in the laboratory. See text for statistical tests. The numbers inside the bars indicate sample sizes.

![FIG. 6](image.png)

**FIG. 6.** Larval survival, developmental time and pupal dry weight of G. lineola larvae reared both in the laboratory (1998) and in the field (1998 and 1999). Open bars refer to water, hatched bars to flood zone and black bars to dry zone, respectively. The numbers inside the bars indicate sample sizes.
crawling predators (Fig. 8), and no interaction was found in Penttilä (Fig. 9, Table 3). Thus, little support for the enemy-free habitat hypothesis was found.

There was no marked difference in temperature between the three habitats although wetlands tended to be about 0.6°C warmer during the night (Fig. 10). In daytime the relative humidity was about 10% higher in the wetland than in the two other habitats. In sunny days there was also a clear difference in daily minimum of relative humidity.

When the three variables of adult fecundity were tested in the field, there was no difference between the habitats in the number of eggs laid (MANOVA; Wilks’ $\lambda = 0.95$, $F_{6,110} = 0.47$, $P = 0.83$). When the experimental conditions (laboratory or field) were entered as a factor to MANOVA-model, there was no significant habitat x experimental conditions interaction (Wilks’ $\lambda = 0.94$, $F_{6, 224} = 1.14$, $P = 0.34$). The hatching success of eggs in the field did not differ between the habitats in the eggs used in the fecundity experiment (Fig. 5, $F_{2,57} = 0.53$, $P = 0.59$). The mean hatching success in the eggs tagged for the predation experiment in Penttilä was 92.8 ± 1.3 ($N = 65$) in water, 93.3 ± 1.4 ($N = 63$) in flood zone and 89.3 ± 2.0 ($N = 65$) in dry zone respectively (Kruskal-Wallis test, $H = 1.12$, $df = 2$, $P = 0.57$). Hatching success in

### TABLE 2. The results of multiple regression analysis on the effect of clone-specific leaf chemistry on the adult host preference and on the performance larvae of G. lineola on 60 S. phyllicifolia clones under the controlled conditions in the laboratory. *** $P \leq 0.001$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adult preference†</th>
<th>Larval survival‡</th>
<th>Developmental time</th>
<th>Pupal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Std. coeff. t p</td>
<td>Std. coeff. t p</td>
<td>Std. coeff. t p</td>
<td>Std. coeff. t p</td>
</tr>
<tr>
<td>Water</td>
<td>0.09 0.70 0.49</td>
<td>0.43 2.41 0.02</td>
<td>-0.28 1.47 0.15</td>
<td>0.33 1.99 0.05</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.57 4.31 &lt;0.001</td>
<td>-0.37 2.12 0.04</td>
<td>0.09 0.47 0.64</td>
<td>0.18 1.07 0.29</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>-0.12 0.84 0.41</td>
<td>-0.09 0.47 0.64</td>
<td>0.28 1.38 0.17</td>
<td>-0.03 0.16 0.87</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>-0.21 1.61 0.11</td>
<td>-0.44 2.55 0.01</td>
<td>0.14 0.74 0.46</td>
<td>-0.23 1.42 0.16</td>
</tr>
<tr>
<td>Ampelopsin</td>
<td>0.08 0.72 0.48</td>
<td>-0.07 0.47 0.64</td>
<td>0.05 0.29 0.77</td>
<td>-0.05 0.33 0.74</td>
</tr>
<tr>
<td>Model</td>
<td>$F_{5,54} = 21.62^{***}$</td>
<td>$R^2 = 0.67$</td>
<td>$F_{5,54} = 7.91^{***}$</td>
<td>$R^2 = 0.42$</td>
</tr>
</tbody>
</table>

† The data was log (x+1) transformed before the analysis
‡ The data was arcsin square root transformed before the analysis

### FIG. 7. Relative growth rates of second instar larvae of G. lineola on the leaves at the same willows at four times during the year 1998. See text for statistical analyses. The sample size is 20 for each dot. The arrow indicates the time when the leaf samples for the chemical analysis were collected.
Marjala 1999 was 95.1 ± 3.8 in water, 93.3 ± 2.0 in flood zone and 97.9 ± 3.5 in dry zone respectively (Kruskal-Wallis test, $H = 0.51, df = 2, P = 0.78$).

When the larvae were reared from eggs to pupae simultaneously in the laboratory and the field in 1998 there was a significant habitat x experimental condition –interaction in larval performance (Fig. 6; MANOVA; Wilks’ $\lambda = 0.89, F_{6,282} = 2.84, P = 0.011, \eta^2 = 0.057$) suggesting that the wetland habitat was somewhat better environment for the larvae. When the field experiments performed in two consecutive years were entered into a same MANOVA-model, there was no significant year x habitat –interaction (Wilks’ $\lambda = 0.98, F_{2,171} = 2.09, P = 0.13$). However, there was a significant year x habitat –interaction in larval survival ($F_{2,171} = 3.09, P = 0.048, \eta^2 = 0.04$) suggesting that the wetland habitat was relatively better for larval survival in 1999 (see also Fig. 3). The total precipitation and average daily temperatures during the experimental periods in June and July were 247 mm and 15.4°C in 1998, and 129 mm and 17.8°C in 1999 respectively, measured at the airport of Joensuu, about 10 km from the study site (Meteorological Institute of Finland 1998, 1999).

In the short term rearing experiment performed both in the laboratory and field during dry weather conditions in 1998, there was a significant habitat x experimental conditions (laboratory or field) –interaction in the survival and growth of first instar larvae (Fig 11; MANOVA; Wilks’ $\lambda = 0.79, F_{4,218} = 6.83, P < 0.001, \eta^2 = 0.11$); the neonate larvae performed better on willows grown in drier habitats when tested in the laboratory but clearly better in wetland when tested in situ in the field. No significant interactions were observed in the second (Fig. 11; Wilks’ $\lambda = 0.93, F_{4,226} = 2.00, P = 0.10$) or in the third instar larvae (Fig. 11; Wilks’ $\lambda = 0.95, F_{4,226} = 1.46, P = 0.21$).

In the transfer experiment where host plant quality, habitat and shading were tested simultaneously larvae performed best when growing on willows transported from drier habitats to wetlands (Fig 12, Table 4). Shading affected negatively the larval growth but not the survival (Fig 11, Table 4). In larval growth, there was also a significant habitat x shading –interaction (Table 4) showing that shading decreased larval growth in wetland but not in the dry habitat (Fig 12).

Spraying with water and the presence of wet Sphagnum moss increased larval survival and growth (Fig 13; Wilks’ $\lambda = 0.38, F_{2,83} = 67.14, P < 0.001, \eta^2 = 0.62$).
The effect was stronger in dry habitat (Fig 13; the habitat x spraying interaction, Wilks’ $\lambda = 0.79$, $F_{4,166} = 5.39, P < 0.001$, $\eta^2 = 0.11$).

### Discussion

The field observations showed that *G. lineola* is dramatically more abundant on *S. phylicifolia* growing in water than on those growing in well-drained soils. At the time of budbreak of willows in early spring adult beetles have to actively orientate towards damp habitats since they cannot overwinter in flooded litter debris of wetlands. Following the evolutionary logic we assumed that this strong affinity to moist habitats has to be driven by some benefit to the beetles associated with such habitats. The experimental results evidently show that *G. lineola* does not attack *S. phylicifolia* growing in wetlands because of higher nutritional quality of those willows. In sharp contrast to the food quality hypothesis adults preferred to feed on and oviposit on leaves from willows growing in well-drained soils, and produced slightly more eggs when fed on those willows under controlled laboratory conditions. Consistently, larvae performed better on leaves from willows grown in drier habitats as well. Neither do wetland habitats provide better option for surviving from predation. In contrast to the predation hypothesis there was no consistent pattern in predator densities, and the effect of predation did not differ between the habitats. Instead, it seems that especially the first instar larvae of *G. lineola* are especially vulnerable to desiccation (Larsson et al. 1997) constraining their ability to live in drier habitats where more nutritious food is available. However, as illustrated by the significant interactions between habitat and year in larval survival, wetlands appear to be rather inferior habitats in rainy (and cold) years possibly leading to negative relationship between adult preference and larval performance.

### TABLE 3. The effects of habitat (H) and predation treatment (P) on the survival *Galerucella lineola* larvae in two predator exclusion experiments. The data was arcsin square root – transformed before the analyses.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Model</th>
<th>Habitat</th>
<th>Predation treatment</th>
<th>H x P</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
<td>P</td>
<td>$\eta^2$</td>
<td>MS</td>
</tr>
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<tr>
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<td>2.929</td>
</tr>
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<td>Predation treatment</td>
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<td>0.127</td>
<td>3.696</td>
</tr>
<tr>
<td>H x P</td>
<td>2</td>
<td>0.098</td>
<td>0.44</td>
<td>0.65</td>
<td>0.006</td>
<td>0.523</td>
</tr>
<tr>
<td>Error</td>
<td>144</td>
<td>0.225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIG 10.** Daily variation in mean temperature and mean relatively humidity in each three habitats measured during the egg and larval stages of *G. lineola* in 1999. The small panel give the daily minimums of relative humidity (mean ± 1SE, *N* = 11 for each habitat) when only the rainless days were included.
Growing in waterlogged soil apparently stresses *S. phylicifolia* which does not have any apparent adaptation for growing in waterlogged soil or with their roots submerged (Fig. 14). The willows growing in wetlands produced shorter shoots and smaller leaves, which were yellowish and contained less water and nitrogen, and more carbon-based secondary compounds like condensed tannins, (+)-catechin and ampelopsin. We do not know whether the differences of willow quality are entirely phenotypic induced by submersion, or an outcome of selective mortality (M. Sipura, pers. obs.), suggesting that the waterlogging may act as the primary stressing factor in wetlands. It is not known whether the slow growth leading to death and to low nitrogen content of the leaves is due to low nitrogen availability in waterlogged soil or malfunction in primary metabolism possibly due to populations between the habitats. Our preliminary experiments suggest that at least in sites with high beetle density all three factors are likely to contribute to the variation in willow quality as food for *G. lineola*. However, reclamation of the wetland area in Parikkala (site 6) in 1980’s increased radial growth of willows, while a considerable mortality among wetland willows occurred in sites 5 and 6 soon after the artificial lifting of water surface in early 1990’s (M. Sipura, pers. obs.), suggesting that the waterlogging may act as the primary stressing factor in wetlands. It is not known whether the slow growth leading to death and to low nitrogen content of the leaves is due to low nitrogen availability in waterlogged soil or malfunction in primary metabolism possibly due to

**FIG 11.** Larval performance of *G. lineola* in a short term rearing experiments performed simultaneously in the laboratory (a) and in the field (b) during a 5-day rainless period. The rate of development of the first instar larvae was estimated by the percentage of larvae which reached the second instar during the five day experiment. The numbers inside the bars indicate sample sizes. See text for statistical analyses.

Growing in waterlogged soil apparently stresses *S. phylicifolia* which does not have any apparent adaptation for growing in waterlogged soil or with their roots submerged (Fig. 14). The willows growing in wetlands produced shorter shoots and smaller leaves, which were yellowish and contained less water and nitrogen, and more carbon-based secondary compounds like condensed tannins, (+)-catechin and ampelopsin. We do not know whether the differences of willow quality are entirely phenotypic induced by submersion, or an outcome of selective mortality (M. Sipura, unpublished data) or delayed induced response [not observed in *S. phylicifolia*; Neuvonen et al. (1987), but see Raupp and Sadof (1989)] by outbreaking populations of *G. lineola*, or whether there exist genetical differences in *S. phylicifolia* populations between the habitats. Our preliminary experiments suggest that at least in sites with high beetle density all three factors are likely to contribute to the variation in willow quality and food for *G. lineola*. However, reclamation of the wetland area in Parikkala (site 6) in 1980’s increased radial growth of willows, while considerable mortality among wetland willows occurred in sites 5 and 6 soon after the artificial lifting of water surface in early 1990’s (M. Sipura, pers. obs.), suggesting that the waterlogging may act as the primary stressing factor in wetlands. It is not known whether the slow growth leading to death and to low nitrogen content of the leaves is due to low nitrogen availability in waterlogged soil or malfunction in primary metabolism possibly due to

**FIG 12.** Performance of the first instar larvae of *G. lineola* on willows originated either from water or dry zone and transferred both to wetland and to dry zone, and in an open habitat or under the shade of other bushes. The numbers inside the bars give the number of replicates. The rate of development of the larvae was estimated by the percentage of larvae which reached the second instar during the four-day experiment. See Table 4 for statistical analyses.
depletion of oxygen after flooding (Kozlowski 1984). We did not measure contents of soluble nitrogen of the leaves so we do not know the ratio of soluble versus non-soluble nitrogen of the leaves suggested to be important for young herbivorous insects (White 1984; 1993). However, our results evidently show that the apparently stressed willows in wetlands were poor food for *G. lineola* probably due to low water content of the leaves (see also Connor 1988, Horton 1989, McQuate and Connor 1990) or due to leaf toughness (not measured here) that usually correlates negatively with water content (Slansky 1993, Rank et al. 1998). Even though the hypothesis stating that stressed plants are more susceptible to be attacked by herbivorous insects because of higher nutritional quality (White 1984; 1993) seems apparently sound when observing the distribution of *G. lineola* in the field, our results give no mechanistic support for the hypothesis. Instead, the results support the opposing plant-vigour hypothesis by Price (1991) suggesting that vigorously growing plants provide better food especially for insects whose larval development is associated with plant growth. Consistently, *G. lineola* is shown to prefer young leaves abundant in vigorously growing shoots as food (A. Ikonen et al. unpublished manuscript).

The negligible impact of the predation on the differential abundance of *G. lineola* in different habitats is rather surprising considering that many predator groups including ants cannot disperse to willows growing directly from water. Moreover, probably due to poor overall food quality, *G. lineola* predominates herbivore guild in wetland habitat almost completely (Sipura, unpublished data), whereas the willows in drier habitats harbour a rich and abundant herbivore fauna that could be expected to attract generalist predators. However, the predator guild of wetland habitat seems to be dominated by spiders which can disperse through air as nymphs or using silk threads as adults and even move on water surface. Furthermore, even if the willows were growing directly from water, there was dense floating vegetation on water surface providing connections between the willows. Also birds that can cause considerable mortality in *G. lineola* populations (Sipura 1999) were seen to forage on beetle larvae in wetlands.

Although vulnerability to desiccation is generally believed to be a major environmental jeopardy for soft-bodied insects like

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**Table 4.** The effects of habitat (H), willow origin (O) and shading (S) on the survival and growth (percentage of larvae moulting to second instars at the end of the experiment) of neotenous larvae of *G. lineola.* The data was arcsin square-root transformed before the analyses.

<table>
<thead>
<tr>
<th></th>
<th>Survival</th>
<th></th>
<th></th>
<th></th>
<th>Growth</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>F</td>
<td>P</td>
<td>η²</td>
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<tr>
<td>Model</td>
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<td>Habitat</td>
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<td>32.74</td>
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<td>0.225</td>
<td>1.555</td>
<td>17.13</td>
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<td>Origin</td>
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<td>9.55</td>
<td>0.003</td>
<td>0.078</td>
<td>1.372</td>
<td>15.12</td>
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<td>0.48</td>
<td>0.004</td>
<td>0.825</td>
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</tr>
<tr>
<td>H x O</td>
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<td>0.002</td>
<td>0.04</td>
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<td>0.0001</td>
<td>0.003</td>
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<tr>
<td>H x S</td>
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<td>0.072</td>
<td>1.66</td>
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<td>0.922</td>
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<tr>
<td>O x S</td>
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<td>0.005</td>
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<td>0.108</td>
<td>1.19</td>
<td>0.28</td>
</tr>
<tr>
<td>H x O x S</td>
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<td>0.88</td>
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<td>0.005</td>
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<tr>
<td>Error</td>
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<td></td>
<td></td>
<td>0.091</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
young larvae of beetles (Wilmer 1986, Pelletier 1995), there exist few reports to consider it in ecological (Larsson et al. 1997) or evolutionary (Price et al. 1987) context. Even though the results of this study unambiguously demonstrate the importance of aerial moisture affecting the distribution of \( G. \text{lineola} \), earlier results (Sipura and Tahvanainen 2000) and the transfer experiment in this study (Fig. 12) also show that adult \( G. \text{lineola} \) seek for warm habitats and even a small difference in temperature greatly affects larval performance. Therefore, it seems that wetlands provide the best compromise between temperature and relative humidity. Moreover, we also observed that due to low altitude, wetlands can be less windy habitats and provide refuges for larvae during accidental environmental hazards such as hailstorms (see Raupp and Denno 1983).

![FIG 13. Performance of the first instar larvae of \( G. \text{lineola} \) in each three habitat when additional moisture was applied (white bars) and on control shoots (black bars). The rate of development of the larvae was estimated by the percentage of larvae which reached the second instar during the four-day experiment. The numbers inside the bars give the number of replicates. See text for statistical analyses.](image-url)

Apparently, spatially variable abiotic conditions can constrain the ecology and evolution host plant use by \( G. \text{lineola} \) in dry microhabitats. This is illustrated by some populations of \( G. \text{lineola} \), which have shifted to feed preferentially on alder \( \text{Alnus} \) spp. in damp habitats with no willows, although the larval performance on alder is very poor as compared to that on \( S. \text{phylicifolia} \) growing in dry habitats nearby (Ikonen and Sipura, unpublished data, Sipura, pers. obs.). But why don’t the larvae evolve to tolerate drier habitats? Obviously, genotypes with an ability to tolerate drier environments would be superior in \( G. \text{lineola} \) populations since such a trait would readily offer access to better quality host plants. There are several possibilities why this has not yet been realized. First, poor tolerance against desiccation can be primitive trait in the genus \( \text{Galerucella} \), as 8 out of 10 species of this genus in Eurasia colonise wetland plants (Koch 1992). It may be that the physiological ability to tolerate dry conditions is phylogenetically constrained and there may be no additive genetic variation left to produce tolerant genotypes. Second, there may be a physiological trade-off between tolerance against desiccation and some other fitness-related trait. Third, the evolution of tolerance against desiccation may just begin to evolve and the beetles are extending their distribution in novel habitats. It is reasonable to assume that before human influence most \( S. \text{phylicifolia} \) grew along shorelines and in wet meadows, and \( S. \text{phylicifolia} \) growing in drier habitats may be a new phenomenon in the evolutionary scale. When inhabiting patches of moist habitats \( G. \text{lineola} \) face an intense intraspecific competition. As suggested by Fig. 3 and confirmed by unpublished results by the authors the larval mortality in the most dense populations can lead to very low number of recruits. In the wetland site with the densest \( G. \text{lineola} \) population (site 1 in Parikkala) only 0.1% of hatched larvae were observed to survive to pupation and only a portion of these attained pupal weight >1.5 mg (compare to Fig. 6). When the density of eggs was experimentally reduced to match to the density in dry field nearby, 36% of larvae survived to pupal stage and the mean pupal dry weight was 2.2 mg.
As a conclusion, waterlogged habitats colonized by adult beetles in the spring provide favourable microhabitat to the first instar larvae which do not tolerate desiccation. At the same time, however, the larvae are forced to feed on low quality food and are susceptible to intense intraspecific competition while better quality food is often abundantly available perhaps closer than 10 meters. Therefore, the habitat selection behaviour by adults of *G. lineola* seems to play surprisingly important role in the local distribution and population dynamics of this abundant leaf beetle (see also Sipura and Tahvanainen 2000).

This study provides an example of the multitude of factors that ought to be considered in studies on the distribution and abundance of herbivorous insects. As stated by Hunter and Price (1992) the relative roles of different ecological factors are expected to vary among and within biological systems when environmental heterogeneity is taken into account. We do not argue that the variation in abiotic conditions provides a universal explanation to the distributional pattern of *G. lineola*. When there is larger variation in plant quality (for example when comparing willow species instead of clones within species) or in predator density (for example when ants are abundant; Laine and Niemelä 1981, Sipura, unpublished manuscript) they may easily overdrive the impact of abiotic conditions. However, we argue that small-scale spatial variation in abiotic factors like relative humidity, precipitation, temperature and windiness affects the ecology of herbivorous insects more than usually thought (see also Raupp and Denno 1983). The abiotic factors should not be dealt as a background against which species interactions are played out (Dunson and Travis 1991). Instead, they must be incorporated as important and interacting variables in the analyses of trophic interactions controlling the populations of herbivorous insects.

**Acknowledgements**

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III

Predation as a component of defence against the major defoliator *Galerucella lineola* F. in *Salix phylicifolia* L.

Mika Sipura

Submitted manuscript (*Oecologia*)
Predation as a component of defence against the major defoliator *Galerucella lineola* F. in *Salix phylicifolia* L.

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Plant defences against herbivores can be divided into two components: direct defences affecting directly the behaviour and performance of herbivores through chemical or physical plants properties, and indirect defences affecting through natural enemies of herbivores or other environmental factors. These components can act both synergistically and antagonistically. For example, mortality of herbivores through predators may increase with decreasing quality of the host plants. However, predators may also be more effective or prefer foraging on poorly defended plants leading to antagonistic relationship between direct and indirect defences. In this study I examine the relative roles and interactions between direct defences and the impact of natural enemies in the interaction between tea-leafed willow *Salix phylicifolia* L. and its major defoliator, a leaf beetle *Galerucella lineola* F. In laboratory rearing experiments performed to evaluate the role of direct defences I found that damage inflicted by beetle larvae increased with host quality (measured as pupal weight attained or relative growth rate of larvae) though the relationship was not linear across the continuum of host quality. The laboratory experiments also suggested that the direct defences act primarily by increasing mortality of neonate larvae or by impairing larval ability to compensate poor food quality by increasing food consumption. In a field experiment performed in a wetland and in a common garden, predators affected larval mortality more on poor-quality willows when larvae were reared under a fixed density. However, ovipositing females of *G. lineola* preferred high-quality willows where the larvae grew best leading to high variation in larval density between willow individuals and clones. Predators showed an aggregative response to the beetle density causing directly density-dependent larval mortality. Consequently, predators decreased leaf damage inflicted by *G. lineola* more on high-quality willows but could not totally compensate for the shortage of direct defences. Predation can therefore be considered being a substitutive but uncompensatory component of the defence against the major herbivore in *S. phylicifolia*.

**Keywords:** *Galerucella lineola*, herbivory, host selection, *Salix phylicifolia*, willows, direct defence, indirect defence, sub-lethal defence, quantitative defence, predation, density-dependence

**Introduction**

Plants defend themselves against herbivorous insects using a wide array of chemicals and physical structures deterring feeding, reducing digestibility of the plant tissue or being directly toxic (Denno and McClure 1983; Fritz and Simms 1992; Schoonhoven et al. 1997; Sleight et al. 1997; Sipura et al., unpublished data). Substantial amount of research on these so-called direct plant defences has revealed that plants may really obtain fitness benefits through these defences (Fritz and Simms 1992; Marquis 1992; Schoonhoven et al. 1996). Consequently, these plant traits appear to be under the selection pressures inflicted by herbivores. However, the concept of defence can be understood in a broader sense including any plant character, which has a potential to be under these selection pressures (see Karban and Baldwin 1997; Abrahamsson and Weis 1997; Sleight et al. 1999). For example, plants may escape from their herbivorous enemies by growing in a microhabitat inadequate for the herbivores (e.g. Stiling 1994; Sipura et al., unpublished data) or in the vicinity of strongly defended plants (Sabelis and De Jong 1988; Hjältén and Price 1997; Sabelis et al. 1999), or they may tolerate the damage when attacked (Strauss and Agrawal 1999). Furthermore, plants may actively attract natural enemies of the herbivores by providing food or shelter (Beattie 1985; Sabelis et al. 1999; Agrawal et al. 2000) or just offering a good habitat for them (Boethel and Eikenbary 1986). It has recently become increasingly obvious that plants benefit from indirect defence provided
by natural enemies of their herbivores (Sabelis et al. 1999), and that the direct defences can interact with the efficiency of natural enemies (Price et al. 1980; Boethel and Eikenbary 1986; Barbosa and Letourneau 1988; Hare 1992). For example, direct plant defence and indirect defence through natural enemies may act synergistically i.e. predators may contribute more to herbivore mortality on poor-quality host plants where herbivores can be weaker (Loader and Damman 1991; Håggström and Larsson 1995) and more exposed to predators due to altered foraging behaviour (Bergelsson and Lawton 1988; Bernays 1997). On the other hand, the direct defence may harm foraging predators (Price et al. 1980; Boethel and Eikenbary 1986; Damman 1987; Pasteels et al. 1988; Hare 1992) or the predators may prefer foraging on high quality plants providing more prey (e.g. Schultz 1983; Haukioja 1993). Obviously, this represents frequently observed (see Hare 1992; van der Meijden and Klinkhamer 2000) antagonistic relationship between direct and indirect defences. As emphasised by Price et al. (1980) and Sabelis et al. (1999) to fully understand the ecological and evolutionary patterns of plant defence, we need to consider the interactions between all trophic levels involved in the system.

One classic but still unsolved question, the paradox of sub-lethal plant defence (e.g. Clancy and Price 1987; Leather and Walsh 1993), draws our attention to synergistic impacts between the components of defence. The paradox arises when the defences of the host plant do not kill herbivores but reduce food manageability or digestibility leading to compensatory feeding by herbivores (Feeny 1976; Moran and Hamilton 1980; Slansky 1993). Investing in such non-lethal (or quantitative) defence by plants may therefore increase herbivore damage to plants, but inflict no disadvantageous effects on the fitness of the herbivore. In order to solve this problem, it has been suggested that direct defences and natural enemies act in concert. Retarded growth, prolonged developmental time and possibly increased activity on sub-optimal host plants may increase the exposure of herbivores to their natural enemies (e.g. Feeny 1976; Moran and Hamilton 1980; Price et al. 1980; Schultz 1983; Grossmuller and Lederhouse 1985). The paradox has later been reformulated as an insect-centred slow growth/high mortality hypothesis (Clancy and Price 1987). Many studies have since documented the negative relationship between insect growth and susceptibility to enemies (e.g. Loader and Damman 1991; Håggström and Larsson 1995; Benney and Denno 1997) emphasising the importance of the synergistic impact of direct defences and predation on plants with non-lethal defence.

Although both direct and indirect defences can have important consequences both to the theory of plant-insect interactions and to practical applications in agriculture and forestry (e.g. Hare 1992; Walde 1995), we are still lacking critical studies concerning their importance and relationships, especially those focusing on natural systems (van der Meijden and Klinkhamer 2000). Firstly, although the concept defence refers to plant fitness, the roles of direct and indirect plant defences have been rarely addressed from plants perspective (van der Meijden and Klinkhamer 2000; Agrawal et al. 2000). Second, the tests have usually been conducted by placing a fixed number of herbivores on each plant, though under natural conditions better hosts are likely to harbour more individuals. Consequently, predators can respond directly to herbivore density causing higher mortality on high quality plants with high herbivore densities. Finally, the demarcation of the concepts lethal and non-lethal or quantitative and qualitative defence have been recently impugned (e.g. Hartley and Jones 1997), calling for both theoretical and empirical reassessment of their usefulness and questioning the importance of synergistic predation. Therefore, it is still not known whether the plants need the third trophic level to support their direct defence or to substitute it (Hare 1992; Sabelis et al. 1999).

The primary objectives of the study are to examine the relative roles and interactions between direct and indirect defence in the relationships between tea-leaved willow, Salix phylicifolia L. and its major defoliator, the brown leaf beetle Galerucella lineola F, and to reconsider the existence of the paradox of sub-lethal defence. Previously,
Häggström and Larsson (1995) have conducted an experiment with *G. lineola* where they tested the slow growth/high mortality hypothesis using larvae growing in fixed densities on potted plantlets of two willow species. They found that predators contributed to mortality more on *Salix dasyclados* Wimmer where the performance of larvae was worse and concluded that the paradox of sub-lethal defence was solved through this synergistic impact of direct and indirect defence. Here I present a series of experiments which: 1.) consider the consequences of the interaction both from insect’s and plant’s perspective, 2.) were conducted both in laboratory, in seminatural conditions in common garden and on naturally growing individuals of the preferred host plant growing under conditions where these organisms usually interact i.e. in wetlands and 3.) were conducted using both fixed and natural larval densities allowing the assessment of the roles of density-dependent factors.

The study species

*Salix phylicifolia* L. is a common and widespread shrub in Scandinavia growing abundantly in wet meadows, wastelands, along lakesides and drainage ditches (Jalas and Suominen 1974; Hämet-Ahti et al. 1998; Skvortsov 1999). Only small amounts of phenolic glucosides, the apparent major components of enemy resistance of willows (e.g. Tahvanainen et al. 1985; Denno et al. 1990; Kelly and Curry 1991; Matsuki and MacLean 1994; Kolehmainen et al. 1995), have been found on the leaves of *S. phylicifolia* (Rank et al. 1998). Instead, leaves contain considerable amounts of some other phenolics, including a flavonoid, ampelopsin, and condensed tannins (Julkunen-Tiitto et al. 1989; Rank et al. 1998). However, earlier results (Sipura et al., unpublished data) have suggested that the performance (measured in terms of mortality, developmental time and pupal weight) of *G. lineola* larvae in laboratory is affected primarily by the water content, or physical traits such as leaf toughness (Raupp 1985) or pubescence (Soetens et al. 1991) not measured from the leaves. However, nitrogen content was observed to be the major factor affecting adult choice amongst sixty individuals of *S. phylicifolia* (Sipura et al., unpublished data). Probably due to relatively low amounts of detrimental secondary chemicals a rich community of herbivorous insects harbour on *S. phylicifolia* (e.g. Seppänen 1970). *S. phylicifolia* has no apparent adaptation for attracting predators or parasitoids.

The brown leaf beetle *Galerucella lineola* F. (Coleoptera: Chrysomelidae: Galerucinae) is a univoltine species that overwinters as an adult in litter or bark crevices. Adults colonise their host plants by the budbreak in early spring, and feed for few weeks before the oviposition. Some populations are found to feed on *Alnus* spp. (Maisner 1974; Ikonen and Sipura, unpublished data), but in Finland most populations prefer *S. phylicifolia* growing in wetlands. The densities of *G. lineola* are about 16 times higher in wetlands compared to adjacent drier habitats, apparently due to high susceptibility to desiccation of the neonate larvae (Sipura et al., unpublished data). The apparently stressed *S. phylicifolia* growing in shallow water with their roots continually submerged provide nutritionally poor food for herbivorous insects (Sipura et al., unpublished data). Therefore, *G. lineola* that seeks moist habitats usually makes up more than 95% of the community of herbivorous insects in wetlands (Sipura, unpublished data). However, adults and larvae of *G. lineola* made up 20-70% (mean 44%) of the guild of leaf-chewing insects also on 37 haphazardly chosen *S. phylicifolia* –populations growing in well-drained soils censused in Eastern Finland during the years 1998 and 1999 (Sipura, unpublished data). Consequently, most variation in the amount of leaf damage on *S. phylicifolia* can be explained in terms of abundance of *G. lineola*. The eggs, laid in patches of 10-20, hatch after a few weeks, and the larvae pass through three instars feeding mainly on the lower surface of the leaves. After more than 11 days the fully-grown larvae crawl down the stem to enter a prepupal stage into the soil. The new generation of adults emerges after a short pupal stage in August. Several invertebrate predators (Kanervo 1946) and birds (Sipura 1999) have been observed to prey upon *G. lineola* larvae, hemipteran bugs, spiders and sedge...
warblers (*Acrocephalus schoenobaenus*) being the most abundant in the wetland study area. The predator community in the common garden was dominated by wood ants (*Formica aquilonia*), spiders, and a hemipteran bug (*Anthocoris nemorum*). Common warblers (*Sylvia communis*), whinchats (*Saxicola rubetra*) and blue tits (*Parus caeruleus*) were also observed to forage regularly on willows in the study area. The larvae were also attacked by a parasitoids wasp (supposed to be *Asecodes mentho*; Eulophidae; Entedontinae) which was, however, very scarce in my study sites (parasitism rate 0-0.4%).

**Methods**

**Laboratory experiments**

The objectives of laboratory experiments were to answer the following questions: 1.) is the direct defence of *S. phylicifolia* against *G. lineola* primarily lethal or non-lethal, 2.) do larvae show compensatory feeding on poor quality *S. phylicifolia*, and 3.) what is the relationship between host plant quality and leaf damage inflicted by herbivores under fixed densities and in the absence of natural enemies i.e. what is the impact of direct defences on leaf damage? For this I performed a rearing experiment covering whole larval period in 1998 using leaf material from an abandoned industrial district in the city of Joensuu (62°37'N, 29°40'E). The leaf material originated from 20 *S. phylicifolia* individuals in each of the following three habitats: 1.) the willows growing in a permanently waterlogged soil (*water*), 2.) the willows growing near the shoreline and were therefore prone to flooding (*flood zone*) and 3.) the willows growing in a well-drained soil (*dry zone*). Leaves number 5-9 from the apex of the shoot were collected from each willow, transported to the laboratory in a cooler and placed in Petri dishes (Ø 15 cm) on a well-moistened paper towel. From 3 to 5 egg groups was then placed on the leaves. When 30 larvae hatched, the unhatched eggs were removed. The larvae were then reared in a growth chamber with +22°C temperature, >90% relative humidity and 12:12 light period. The food was changed every third days. The removed leaves skeletonized by larvae were dried under moderate pressure in a plant press and stored in a freezer. The total leaf area eaten was measured later using transparent scalepaper. When larvae had pupated, larval survival, developmental time and pupal dry weight were assigned. I use the pupal dry weight as an indicator of plant quality since it varies in relation to leaf chemistry even when no mortality occurs under optimal laboratory conditions (Sipura, unpublished data).

During the experiment described above, I also performed an experiment with leaf material from the same willows to test whether the plant quality affects differently the mortality of larvae at different developmental stages. In this experiment I used the leaves number 10-13 from the shoot apex. I placed 20 larvae of each instar collected from the field in Petri dishes and let them to feed for five days in a growth chamber (+18°C, >90% RH, 12:12 light period) after which I counted the number of survivors.

To assess the relationship between larval growth and relative food consumption rate more accurately I also performed two short-term laboratory experiments using second instar larvae. The first experiment was performed in 1998 using the same leaf material as above. I collected leaves (fifth leaf from the apex) from every 20 *S. phylicifolia* in each habitat, and placed them in Petri dishes. One newly moulted second instar larvae, reared on *Salix caprea* L. before the experiment, was then weighed and placed on the leaf. The larvae were let to feed in a growth chamber (+22°C, >90% RH, 12:12 light period) for three days after which the larvae were re-weighed and the damaged leaf area was measured using transparent scalepaper. I then calculated the relative growth rate of larvae (RGR) as follows: \[
\ln(\text{weight at the end}) - \ln(\text{weight at the beginning}) / \text{length of the experiment (days)},
\]
and the relative consumption rate (RCR) as: leaf area consumed / mean larval weight during the experiment. The second experiment was performed in 1999 using leaf material from willows studied in the common garden experiment (see below). The methods were same as described above, but ten larvae were placed in each Petri dish instead of one, and mean values of
the relative larval growth and relative leaf area consumed were used as variables.

**Natural willows**

The experiment using natural willows was designed to reveal the relationship between direct and indirect defenses through predation under natural conditions. I conducted the experiment in a shallow eutrophicated lake in Parikkala, southeastern Finland (61°33'N, 29°33'E) in 1998. *G. lineola* occurred at low densities in the southern part of the study area, but at very high densities in the northern part of the area. After the peak of the oviposition period, I selected 128 approximately 60 cm high *S. phylicifolia* individuals from both areas for the experiment trying to minimise abiotic variation between the shrubs. Before the experiment I removed all detritus around the willows so that they were growing directly from water. This eliminated migration of the larvae and allowed collection of the pupae. The larvae showed no signs of active migration but to separate foolishly emigrating or accidentally fallen individuals from the survivors I attached a funnel lined with a sticky paper at the base of each willow. Before the experiment, I estimated the proportion of leaf area damaged by adults *in situ* from ten randomly chosen leaves from each willow using a transparent scalepaper. Half of the willows were protected from predators using a large sleeve bag (1 mm mesh size) supported by two wooden side poles. The willows were further divided into two density treatments: from one half of the willows I removed all *G. lineola* eggs and attached three new egg batches to each. The other half of the willows was not manipulated, but the egg batches that appeared during the experiment were removed. This produced a 2 x 2 x 2 factorial experiment with site, density treatment and predator treatment as fixed factors. To obtain an independent estimate of the plant quality because it correlates strongly both with larval developmental time i.e. predisposition to predators (this study) and larval ability to defend themselves when attacked by a predator (Häggström and Larsson 1995; Sipura, unpublished results). It is possible that leaf damage outside the sleeve bag may have affected the quality of leaves inside the bag for example through induced defense (e.g. Haukioja 1991). However, such inductions have rarely been observed on willows (Neuvonen 1987; Julkunen-Tiitto et al. 1995; but see Raupp and Sadof 1989) and the defoliation of some ramets was not observed to cause immediate changes in the quality of adjacent ramets as food for *G. lineola* (Sipura, unpublished data). My preliminary experiments also suggest that the effect of bag itself on leaf quality is negligible and becomes detectable after more than a month after the start of experiment.

After 15 days, I lined the sticky funnels with *Sphagnum* moss to offer pupating matrix for the larvae. I collected the pupae and counted the number of hatched larvae by examining egg remnants about one week after the pupation. Immediately after the experiment, I collected all leaves from the experimental willows and determined the proportion of leaf area damaged from randomly selected sample of 100 leaves/willow as in Sipura (1999). I counted the number of the dominating invertebrate predators (spiders and bugs) on control plants at 7 occasions and used their mean density as a variable. During the insect censuses, I actively monitored the foraging of insectivorous birds in the study area and noted the number of occasions when a bird foraged on an experimental willow. The total observation time was approximately 30 hours.

In order to analyse the relationship between plant quality and the effect of natural enemies on larval mortality, I applied linear regressions for both low- and high-density populations and for both density treatments. I used logarithm-transformed density of hatched larvae and pupal weight as independent variables and the arcsin square root -transformed larval mortality and leaf damage as dependent variables. If the impact of predators on larval mortality or leaf damage
depends on willow quality or larval density, the regression slopes of predator exclusion treatment and control plants should differ. The difference of the slopes was tested following Zar (1999; pp. 360-364).

**Common garden experiment**

In 1999 I conducted a study in a common garden in order to examine the relationship between direct defense and the impact of predators focusing particularly on the role aggregative response of predators and density-dependent larval mortality. In 1998 I collected cuttings from 30 *S. phylicifolia* clones growing both in wetlands, lush meadows and drier forest clearings in eastern Finland. The cuttings were then rooted in a greenhouse and cultivated in 5 litres plastic pots at the botanical garden of the University of Joensuu (62°37'N, 29°40'E) on a small grassy field trying to minimise spatial variation in growing conditions. About one week after the budbreak in 1999 I transported all pots to a damp meadow near the city of Joensuu where *G. lineola* had colonised the natural *S. phylicifolia* in high densities. The pots were positioned randomly within a small area (about 5x20 m) trying to minimise spatial variation in abiotic conditions and in the abundance of beetles. *G. lineola* females were let to oviposit on potted willows for one week, after which the pots were transformed to an adjoining meadow with relatively low density of *G. lineola*. The females caused some leaf damage (0.03-11% of leaf area damaged) while laying their eggs. I estimated this by measuring the level of damage from 15 randomly selected leaves from every pot in situ. The numbers of eggs were counted from each pot after which all eggs were pierced or removed from three pots of every clone. *S. phylicifolia* in one of these three pots was used for larval growth experiment. It received about 50 *G. lineola* eggs attached on the leaves using Scotch Magic Tape®, and was then covered with a sleeve bag. The other two received about 30 eggs of *G. lineola*. Half of the natural density willows and half of the willows with fixed density of 30 eggs were then covered with a sleeve bag described earlier to exclude predators. All pots were placed randomly in the meadow within an area of 20 x 30 metres. The pots for larval growth were checked frequently and on the 15th day the larvae were collected, reared to pupae in the laboratory, and finally the dried pupae were weighed. At this time a sample of 50 leaves were collected from each willow, and the percentage of leaf area damaged was determined as in Sipura (1999). The estimated amount of leaf damage inflicted by adults was reduced from the final leaf damage estimate. When all larvae had pupated on experimental pots, I covered every pot with a smaller sleeve bag. Few weeks later, I counted the number of emerging adults, and used the number of adults/number of hatched larvae as an estimate of survival.

The number of eggs laid by *G. lineola* on potted willows showed very strong genetic correlations between the five 5 pots containing the same *S. phylicifolia* clone (Kendall’s coefficient of concordance; \( W = 0.94, P = 0.86 \)). This allowed the use of clones as observational units in the analyses. Although most of the variation observed is apparently genetic, the phenotypic impact through cuttings cannot be completely excluded. The number of *G. lineola* eggs and leaf damage inflicted by adults were unusually low in some clones and it is possible that these clones are hybrids between *S. phylicifolia* and high-salicylate *S. myrsinifolia* avoided by *G. lineola*. However, I suppose that these possible shortcomings have a negligible effect on the interpretation of the results. I used the difference (percentage units) in mortality or leaf damage between control and predator exclusion treatments as a variable for the effect of predation. I applied path analysis to examine the relative roles and indirect effects linking 1) the plant quality (estimated as pupal weight), 2) the contribution of predators and 3) density (number of eggs) to clone specific mortality and leaf damage measured on control plants. First, I assumed following causal effects: 1.) all three factors can affect directly the dependent variables, 2.) plant quality can have indirect effects through predation and density of *G. lineola* and 3.) density of *G. lineola* can affect predation rates. Then I calculated the path coefficients (standardised regression coefficients) and residual variation for the
full model using Statistica 6.0 software. Finally, I removed weaker relationships and tested the models using goodness-of-fit statistics, until there was a significant or otherwise unambiguous change in χ²-values. It appeared that the best models were always those including all significant paths.

Results

Laboratory experiments

In the rearing experiment covering whole larval period the larvae had higher mortality ($F_{2,57}=4.18$, $P=0.02$, $\eta^2=0.13$; see also Fig. 1) and attained higher pupal weight ($F_{2,57}=8.07$, $P=0.001$, $\eta^2=0.22$; Fig. 2) when grown on leaves of S. phylicifolia growing in drier habitats. The more accurate results of these rearings will be published elsewhere. Pupal weight correlated significantly with larval mortality on wetland willows ($r_s=-0.61$, $P=0.005$, $N=20$) but not on willows from flood zone ($r_s=-0.37$, $P=0.11$, $N=20$) or dry zone ($r_s=0.31$, $P=0.19$, $N=20$). The mean pupal weight correlated significantly with developmental time both on wetland willows ($r_s=-0.73$, $P<0.001$, $N=20$) and on willows from flood zone ($r_s=-0.55$, $P=0.01$, $N=20$) but not on willows from dry zone ($r_s=-0.11$, $P=0.63$, $N=20$).

The mortality rate of the first instar larvae growing on relatively old leaves were far
higher than that of later instars, and they also suffered most from the low food quality in wetland willows (Fig 1). There was a clear correlation between pupal weight and mortality of the first instar larvae measured on leaves of the same willow individuals (Spearman correlation; \( r_s =-0.63, P <0.001 \)) but no correlations were found in the second \( r_s =-0.08, P =0.68 \) or third \( r_s =0.10, P =0.61 \) instar larvae respectively. These results suggest that direct defences affecting larval mortality may occur, but not linearly across the continuum of host plant quality, and that the defence is most effective against the first instar larvae.

The larvae consumed larger leaf area when grown on high-quality willows from drier habitats as compared with willows from other habitats \( (F_{2,57}=18.93, P <0.001, \eta^2=0.40; \text{Fig.2a}) \). The overall leaf area consumed increased with plant quality only on willows grown in water (Fig. 2a). When larval mortality was statistically controlled using the residual variation as variable (Fig. 2b) there was a clear negative relationship between residual leaf area consumed and plant quality on willows from dry zone suggesting that larvae compensated low food quality on these willows by increased feeding. Similar non-linear response was found also in short term experiments; second instar larvae increased their relative consumption rate with decreasing plant quality on wetland willows but decreased it on willows from dry zone (Fig.2a) and on potted willows (Fig. 3b). Therefore, without any additional sources of mortality, the paradox of sub-lethal defence can occur but only on willows growing in the optimal habitats in well-drained soils.

**Natural willows**

On the willows with the fixed number of *G. lineola* larvae, the number of hatched larvae per plant varied between 18 and 50 (mean=31.2, Coefficient of Variation=0.21) in the low-density area and between 19 and 51 (\( \kappa=31.2, CV=0.20 \)) in the high-density area. On willows with natural densities the same ranges were 5-422 (\( \kappa=65.1, CV=1.44 \)) and 32-1980 (\( \kappa=770.5, CV=0.71 \)) respectively. In the data pooled over the sites and the predation treatments there was no significant correlation between the number of hatched larvae and pupal weight in fixed density (Pearson correlation; \( r =-0.12, P =0.20, N=119 \)) but a strong correlation in natural densities \( (r =0.65, P <0.001, N=104) \). Both predation exclusion and fixing of the

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**Table 1.** Analysis of variance on the effects of study site (S), density treatment (D) and predation treatment (P) on the larval mortality and leaf damage inflicted by the larvae of Galeucilla lineola. The eta squared (\( \eta^2 \)) gives the ratio of between-groups sum of squares and total sum of squares and can be interpreted as R²-values (Keppel 1982). The data is arcsin square root transformed before the analysis.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>( \eta^2 )</th>
<th></th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>( \eta^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>7</td>
<td>1.63</td>
<td>22.47</td>
<td>&lt;0.001</td>
<td>0.42</td>
<td>Mortality</td>
<td>5</td>
<td>0.07</td>
<td>0.02</td>
<td></td>
<td>0.027</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>0.34</td>
<td>4.64</td>
<td>0.032</td>
<td>0.02</td>
<td>Leaf area damaged</td>
<td>16.30</td>
<td>609.72</td>
<td>&lt;0.001</td>
<td>0.74</td>
<td></td>
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<tr>
<td>Density treatment</td>
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<td>3.51</td>
<td>48.34</td>
<td>&lt;0.001</td>
<td>0.18</td>
<td></td>
<td>12.99</td>
<td>485.77</td>
<td>&lt;0.001</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Predation treatment</td>
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<td>3.35</td>
<td>46.17</td>
<td>&lt;0.001</td>
<td>0.18</td>
<td></td>
<td>0.75</td>
<td>28.11</td>
<td>&lt;0.001</td>
<td>0.12</td>
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</tr>
<tr>
<td>S x D</td>
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<td>3.55</td>
<td>48.90</td>
<td>&lt;0.001</td>
<td>0.19</td>
<td></td>
<td>8.22</td>
<td>307.44</td>
<td>&lt;0.001</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>S x P</td>
<td>1</td>
<td>0.09</td>
<td>1.18</td>
<td>0.28</td>
<td>0.005</td>
<td></td>
<td>0.22</td>
<td>8.21</td>
<td>0.005</td>
<td>0.037</td>
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</tr>
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<td>0.23</td>
<td>3.13</td>
<td>0.078</td>
<td>0.014</td>
<td></td>
<td>0.13</td>
<td>4.84</td>
<td>0.029</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>S x D x P</td>
<td>1</td>
<td>0.56</td>
<td>7.72</td>
<td>0.006</td>
<td>0.035</td>
<td></td>
<td>0.06</td>
<td>2.48</td>
<td>0.12</td>
<td>0.011</td>
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<td>Error</td>
<td>215</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.027</td>
</tr>
</tbody>
</table>
larval density decreased larval mortality (Table 1, Fig. 4). Many interactions between the factors in the ANOVA-table (Table 1) apparently implicate the variability in the responses to the treatments under different initial conditions. For example, the fixed density represents an unnaturally low density in high-density site probably relaxing larval competition but the same fixed density changed only slightly larval densities on the willows in the low-density site.

When the arcsin square root transformed larval mortality was regressed against the logarithm-transformed number of hatched larvae, the regression slopes between predator exclusion and control treatments did not differ either in the fixed densities or in the natural densities at high-density site ($P>0.2$). In natural densities at the low-density site the slopes ($-0.09$ vs. $-0.21$ in the control and predator exclusion treatments respectively) differed significantly ($t_{52}=18.18$, $P<0.001$), suggesting that mortality due to predation acted in a positively density-dependent way.

In the fixed larval densities the predators caused greater mortality and reduced leaf damage more on poor-quality willows (Figs 1a-b and 2a-b). When tested in natural larval densities, there were opposite (Figs 1c-d and 2c) or insignificant (Fig 2d) relationships between plant quality and the effect of predators. There was a significant relationship between the pupal weight and larval mortality or leaf damage in most cases (but see Fig 1c). The relationship between pupal weight and leaf damage remained significant ($P<0.01$) also when the proportion of leaf area damaged before the experiment was included as an independent variable in the regression analysis. At the highest densities larval mortality was positively related to the pupal weight (Fig 1d). Unfortunately, there is no statistical method known to me to rigorously evaluate whether the larval density or plant quality was the key factor affecting predation mortality.

Birds were seen to forage more often on willows where G. lineola larvae grew best even when there were no differences in larval densities (Fig. 6c). The number of hemipteran bugs showed a positive response to the larval growth under the natural larval

Figure 3. The relationship between relative growth rate and relative consumption rate in second instar larvae of G. lineola during short experiment in laboratory using leaf material from a) naturally growing and b) potted S. phylicifolia. The regressions for figure a) are: leaves from water zone (white dots) $y=1.00x+0.04$, $R^2=0.72$, $F_{1,18}=45.75$, $P<0.001$, leaves from flood zone (grey dots) $y=0.52x+0.18$, $R^2=0.20$, $F_{1,18}=4.52$, $P=0.05$ and leaves from dry zone (black dots) $y=-0.43x+0.49$, $R^2=0.20$, $F_{1,18}=4.50$, $P=0.05$. The regression for figure b) is $y=-0.29x+0.42$, $R^2=0.27$, $F_{1,28}=10.51$, $P=0.003$.

Table 2. Matrix of Pearson correlation analysis for the clone-specific oviposition preference (number of eggs laid), larval mortality, larval developmental time and pupal weight measured in the common garden experiment. The number of replicates is 30 clones for each case.

<table>
<thead>
<tr>
<th></th>
<th>Preference</th>
<th>Mortality</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>$-0.61 ***$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>$-0.69 ***$</td>
<td>$0.82 ***$</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>$0.75 ***$</td>
<td>$-0.80 ***$</td>
<td>$-0.90 ***$</td>
</tr>
</tbody>
</table>
densities but not in the fixed density (Fig. 6b). Spiders showed a slight negative relationship to the larval growth in the fixed density (Fig. 6a).

Common garden experiment

On potted willows the mean number of hatched larvae varied between 28 and 31.5 (x=29.87, CV=0.03) on clones with fixed larval densities and between 17.5 and 589.5 (x=213.42, CV=0.87) under natural densities. Females of *G. lineola* clearly preferred plants where the larvae performed best as the mean egg density on a pot used for larval growth experiment correlates strongly both with larval mortality, developmental time and pupal weight measured under fixed densities on the same pots (Table 2). The density was not related to the effect of predation in larval mortality or leaf damage in fixed density, but showed a strong (direct or indirect) influence under natural densities both on mortality and leaf damage (Fig. 8). Plant quality affected negatively the effect of predation in fixed densities but positively in natural densities (Fig. 7). Consequently, predators decreased the level of leaf damage more on better quality clones, whereas no relationship was found between plant quality and the effect of predators in fixed density (Fig. 7). However, plant quality (i.e. pupal weight) affected positively the level of leaf area damaged on control plants of both treatments (fixed density; $\beta=1.70\pm1.93$, $R^2=0.66$, $F_{1,28}=54.37$, $P<0.001$ and natural

**Figure 4** The relationships between independently measured pupal weight (an indicator of plant quality) and arcsin square root -transformed larval mortality of *G. lineola* when predators were excluded (white dots) and controls (black dots) in two study areas with low and high-density populations. All regression slopes except the one indicated by NS differ significantly ($P<0.05$) from zero. The tests for differences in regression slopes are: a) $t_{56}=2.69$, $P=0.005$; b) $t_{55}=10.75$, $P<0.001$; c) $t_{54}=4.22$, $P<0.001$ and d) $t_{44}=4.42$, $P<0.001$. Note the different scales used in upper and lower panels.
densities; \( y = 11.22 \times 10.79, \quad R^2 = 0.53, \quad F_{1, 26} = 30.89, \quad P < 0.001 \) showing that predators can not totally substitute for the low level of direct defence. Consistently, under natural densities the number of eggs laid by *G. lineola* correlates clearly positively both with the number of surviving larvae (Pearson correlation; \( r = 0.70, \quad P < 0.001, \quad N = 30 \)) and the level of leaf area damaged (\( r = 0.73, \quad P < 0.001, \quad N = 30 \)).

The path analysis (Fig. 8) demonstrates that when the density-loop is missed (under the fixed densities), the plant quality and predation act synergistically leading to clearly larger mortality on low-quality willows. However, when the loop of natural variation in larval density is included, the plant quality affects predation mortality both directly (negatively) and indirectly (positively) trough density-dependent predation. This means that plant quality and predation interact antagonistically; the mortality by predators is higher on high quality clones with high larval densities.

**Discussion**

This study provides several insights to the relationship between *S. phylicifolia* and its major defoliator *G. lineola*. First, the results demonstrate that at least under controlled conditions in laboratory *S. phylicifolia* is directly defended against *G. lineola* causing mortality during the early development of the larvae and consequently decreasing the level of leaf damage. However, above a certain level of leaf quality, no correlation between larval growth and mortality was observed. Instead, it appeared that on high-quality leaves the larvae try to compensate
poorer food quality by consuming more plant material. Secondly, under equal abiotic conditions in the field *S. phylicifolia* providing high-quality food for the larvae also harbour more adults and eggs, and even excluding the possible compensatory feeding behaviour, experience more leaf damage than poor-quality willows. Thus, *S. phylicifolia* are directly defended against *G. lineola* also in field because, all other things being equal, individuals resisting adults and providing worse food for the larvae suffer least from leaf damage. Third, it was also found that *G. lineola* individuals growing under fixed densities on poor-quality leaves were more susceptible to predation than those on high-quality leaves. Therefore, under fixed density the direct defence deteriorating larval growth act in concert with predation leading to lower level of leaf damage in poor-quality willows despite the possible compensatory feeding by the larvae. Finally, predators tend to aggregate on willow individuals with high prey densities i.e. individuals attracting adult beetles and providing high-quality food for the larvae. This leads to directly density-dependent but uncompensatory (see Oedekeven and Joern 2000) mortality of larvae and larger impact of predators on leaf damage on high-quality plants. In the evolution of host choice, ovipositing leaf beetle females apparently face a trade-off between direct and indirect effects of the host quality.

**Figure 6.** The relationships between pupal weight (an indicator of willow quality) of *G. lineola* and mean number (or dichotomous observed/not observed for birds) of dominating predators on control plants with fixed (white dots) or natural (black dots) densities during seven census occasions. The lines give the best fitting logistic regressions. The thinner lines are not statistically significant at the risk level of 0.05.

**Figure 7.** The impact of plant quality on the mortality (upper panel) and on the reduction of leaf damage (lower panel) inflicted by predators when fixed (black dots) and natural (white dots) larval densities were used. The regression for the mortality are \( y = -16.6x + 60.4, R^2 = 0.38, F_{1,28} = 17.10, P < 0.001 \) for the fixed density and \( y = -4.82x + 16.7, R^2 = 0.33, F_{1,28} = 13.72, P = 0.001 \) for the natural densities. The regression for the leaf damage for fixed density is \( y = 0.36x - 2.1, R^2 = 0.003, F_{1,28} = 0.78, P = 0.39 \). The iterated (SigmaPlot 5.1 software) logistic regression for natural densities is \( y = 313/[1+(x/528)^{4.15}], R^2 = 0.71, F_{2,27} = 33.54, P < 0.001 \).
illustrated by the lack of correlation between female choice (number of eggs laid) and larval mortality \((r=0.27, P=0.15, N=30\) Pearson correlation\) in the common garden experiment. However, the surviving larvae attained higher pupal weight that may increase their fitness. This has not been ascertained in *G. lineola* but is found to be the rule in other species including some feeding as adults (e.g. Honěk 1993; Tammaru et al. 1996; but see Leather 1988). Accordingly, the high pupal weight obtained may ultimately favour the evolution of oviposition preference observed in this study. As a conclusion, the adult preference and aggregative response by predators leads to antagonistic relationship between the direct and indirect defences on *S. phylicifolia*. The poorly defended individuals of *S. phylicifolia* with no ability to resist adult beetles or kill early instar larvae obtain partial aid from predators. Predation can therefore be seen as a substitutive component of the herbivore defence in this willow species.

Earlier studies have shown that the larval performance of *G. lineola* is primarily affected by water content of the leaves, but chemicals measured from the leaves explain less than half of the variation of larval performance (Sipura et al. *unpublished data*). I suggest that the performance of *G. lineola* larvae is more likely to be affected by some physical properties (e.g. toughness or fibre content) of leaf tissue not measured in earlier studies. When observing the behaviour of neonate larvae, I found that the larvae attempted to start feeding within few hours after the hatching on every host plant, but the gut was filled sooner when growing on leaves from drier habitats. Therefore, some factor affecting the larval ability to ingest food affects early larval mortality and may also prohibit the compensatory feeding of older instars on poor-quality hosts. Since the mortality of *G. lineola* larvae due to low food quality occurred during the first days of their development, the impact of direct defences is easily missed if focusing only on older and more manageable larval stages. Therefore, this study emphasises the necessity to follow whole developmental period when assessing the performance in the larvae of herbivorous insects (Reavey 1993).

![Figure 8](image_url)

**Figure 8.** Path models showing the causal influences between clone specific plant quality, impact of predation and density of hatched larvae on the larval mortality and leaf damage tested both in fixed (a and c) and natural densities (b and d). The path coefficients associated with the causal paths parallel to standardised regression coefficients and give the predicted change in the dependent variable, in standard deviations, buy one standard deviation change in the independent variable. The thicknesses of the lines indicate the strength of the dependence. The circles denote variation not explained by the path model. The tests for the models are: a) \(\chi^2=0.20, df=3, P=0.98\), b) \(\chi^2=0.04, df=1, P=0.85\) and c) \(\chi^2=1.15, df=4, P=0.89\). The high P-values indicate that the relationships predicted by the path model do not differ from those observed in field. In path d) the degrees of freedom equals to zero, and the path cannot be tested using goodness-of-fit criteria. In the coefficients (*) \(P \leq 0.10\), *\(P \leq 0.05\), **\(P \leq 0.01\) and ***\(P \leq 0.001\).

Although a possible role of microbial infections cannot be excluded, I suggest that the problem of quantitative plant defence leading to the paradox of non-lethal plant defence is not relevant in the relationship between *S. phylicifolia* and its major insect herbivore *G. lineola*. Although, the larvae showed some compensatory feeding in relation to their own body weight when growing on relatively good-quality leaves, leaf dam-
age increased with increasing plant quality in wetland as well as on potted willows in the common garden even when predators were excluded. Because the leaves of *S. phylicifolia* are known to contain only so-called quantitative defensive compounds (especially condensed tannins and flavonoids; Rank et al. 1998), but nevertheless appeared to inflict considerable mortality in neonate larvae of *G. lineola* this study also questions the practicability of the concepts of quantitative or non-lethal plant defence.

Both in naturally growing wetland willows and in potted willows in common garden the larvae growing on sub-optimal host plants under fixed densities suffered more from predation, as found earlier by Häggström and Larsson (1995). This led to noticeable decrease in the leaf damage on these willows. The higher mortality may be due to both prolonged larval period and to less effective enemy avoidance or resistance of the slow growing larvae as suggested by Häggström and Larsson (1995). However, when larvae grew under the natural densities, predators (at least birds and bugs) seemed to aggregate on willows with high beetle densities and had stronger effect on mortality and leaf damage on the high quality willows. However, other directly density-dependent processes, especially intraspecific competition, may have enhanced the susceptibility of larvae to predators as well. Especially on natural willows, some clones in the high-density site were overcrowded which apparently led to high mortality due to exhausted food resources. When all leaves had been skeletonized most larvae remained conspicuously at the tip of the shoots and were apparently extremely susceptible to the predators.

Spatially density-dependent predation by insectivorous birds on differently defended willows has previously been found by Sipura (1999). In that study birds reduced the leaf damage more on willows where densities of leaf chewing insect were higher i.e. on willows that were apparently better food for the insects. In general, the density-dependent responses of predators seem to be highly variable both between (e.g. Sipura 1999; Connor et al. 1999) and within (e.g. Hunter and Elkinton 1999) different systems studied, and the results obtained here probably cannot be generalized to other systems. However, it is noticeable that the same proportional larval mortality on low and high quality plants leads to stronger reduction in the amount of leaf damage in high quality plants. Predators reduce leaf damage where there is something to reduce, even without any density dependence in the predation mortality.

This study provides strong evidence for the antagonistic relationship between direct and indirect defence in *S. phylicifolia*. Similar results have been presented earlier (e.g. Price et al. 1980; Barbosa and Letourneau 1988; Hare 1992; Agrawal et al. 2000), but usually the mechanisms have been different. In many cases predators are harmed directly by the defensive characters of plants (Price et al. 1980; Boethel and Eikenbary 1986; Hare 1992; Malcolm 1992; Walde 1995) or indirectly through active plant-derived defence by the herbivore (Brower 1984; Bowers 1993; Pasteels et al. 1988). However, there are no reports known to me to explicitly examine the roles of density-dependent responses. As shown previously, both insectivorous birds (Sipura 1999) and predatory ants (Sipura, unpublished manuscript) affect herbivory more on willow species with high herbivore densities, and consequently increase the growth only of willows susceptible to herbivores. This study shows the same pattern between phenotypes and genotypes within a willow species: the predators are likely to benefit *S. phylicifolia* susceptible to herbivores, whereas resistant clones obtain no benefits from predators in terms of decreased leaf damage. Because nothing is known about the relationship between herbivore resistance and tolerance (components of direct defence; see Abrahamsson and Weis 1997; Strauß and Agrawal 1999) in *S. phylicifolia*, the effect of predation cannot be evaluated in terms of plant fitness. However, I suggest that variation in predator densities or their efficiency can act as an evolutionary agent maintaining variation in direct plant defences observed in willows (Julkunen-Tiitto and Meier 1992; Orians and Fritz 1996; Roche and Fritz 1997) as suggested previously in other systems (e.g. Vrieling et al. 1991; van der Meijden 1996; van der Meijden and Klinkhamer 2000). Therefore, the third trophic level have to be considered as a compo-
ment of plant defence that can help us to understand the variation in direct defences in plant genotypes.

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IV

Contrasting effects of ants on the herbivory and growth of two willow species

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Contrasting effects of ants on the herbivory and growth of two willow species

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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. Using both field observations and experimental manipulations of the densities of ants and ant-tended aphids, I tested the hypothesis stating that the differences in defense against herbivores between these plants are reflected to the mode of willow-herbivore-ant interaction. 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, thus being less preyed on than the dominating 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 plant traits, especially herbivore resistance of plants may lead to diverging or even opposite results in tritrophic interactions between plants, their herbivores and predatory ants.

Keywords: Ants, Formica aquilonia, Myrmica rubra, willows, Salix phylicifolia, Salix myrsinifolia, herbivory, Phratora vitellinae, predation, tritrophic interactions

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 been 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, Heads and Lawton 1985, Fowler and MacGarvin 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 Hölldöpler 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 (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 non-tended 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).

It is clear that 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 non-tended 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 enemy-free space 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.

Material 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 road-sides (Hämet-Ahti et al. 1998, Skvortsov 1999). These 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). 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 feed 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, an ant-attended 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 two-year-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 (Fig. 5).

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, south-eastern Finland (61°33’N, 29°33’E). Young willows (age 3-8 years) 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 (about 2 ha) was an abandoned field near the city of Joensuu, eastern Finland (62°37’N, 29°40’E). Several thousand 4-7 years 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*®) around the base of all stems. The other half of the bushes were designated for controls. 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 an one-way barrier that allows insects to crawl down from the willow, but preventing them to go 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 growing season 1998 (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 a square meter of leaf area. I counted the number of shoots and measured the length of 10-20 haphazardly selected shoots from every willow at each census time. At each occasion I also collected a sample of about 40 shoots from adjacent willows 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 haphazardly selected shoots per willow. After the last census, I collected all remaining leaves from 50 haphazardly 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/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; pp. 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. Other experiments conducted in Joensuu site suggest that late mortality of *P. vitellinae* larvae is 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 two- or three-way GLM MANOVA with type III sum of squares and eta squared (\(\eta^2\)) -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 ± standard error of mean (SE).

Results

Observational data

The densities of the aphid *Pterocomma salicis* per square meter 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 average densities were 48.7 ± 8.8 vs. 9.3 ± 1.3 individuals m⁻² (*t*₁₃₆ = 4.63, *P* < 0.001) in Parikkala and 224.9 ± 59.0 vs. 45.9 ± 15.6 individuals m⁻² (*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, and the effect was significantly stronger on *S. phylicifolia* (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 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 $F_{1,168} = 0.03$, $P = 0.86$ 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 x 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 x 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, but see below). 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 mANOVA, there is a significant willow species x ant exclusion interaction in the rest of the densities of leaf chewer guild in Joensuu ($F_{1,168} =

### 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. Regression slopes are tested for both deviance from zero and from each other following Zar (1999).

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<td></td>
<td>Equation</td>
<td>$R^2$</td>
<td>$F$</td>
<td>$P$</td>
<td>Equation</td>
</tr>
<tr>
<td><strong>Myrmica rubra</strong></td>
<td>N = 66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tended aphids</td>
<td>0.11+1.26x 0.66 121.87 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.22+1.29x 0.69 154.52 &lt;0.001</td>
</tr>
<tr>
<td>Leaf chewers</td>
<td>1.88-0.73x 0.53 72.84 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.79+0.051x 0.01 0.45 0.50</td>
</tr>
<tr>
<td>Early abscission</td>
<td>0.10-0.031x 0.15 11.05 0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.056+0.015x 0.06 4.54 0.037</td>
</tr>
<tr>
<td>Leaf damage</td>
<td>1.10-0.37x 0.43 48.12 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.30+0.014x 0.00 0.08 0.78</td>
</tr>
<tr>
<td>Shoot length</td>
<td>0.38+0.055x 0.05 3.28 0.075</td>
<td></td>
<td></td>
<td></td>
<td>0.44-0.022x 0.02 1.60 0.21</td>
</tr>
<tr>
<td>Radial growth</td>
<td>1.19+0.12x 0.13 9.72 0.003</td>
<td></td>
<td></td>
<td></td>
<td>1.33-0.078x 0.10 7.81 0.007</td>
</tr>
<tr>
<td><strong>Formica aquilonia</strong></td>
<td>N = 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tended aphids</td>
<td>0.53+1.24x 0.48 70.84 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.47+1.48x 0.57 94.91 0.001</td>
</tr>
<tr>
<td>Leaf chewers</td>
<td>2.22-0.83x 0.53 89.15 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>1.17-0.094x 0.01 1.00 0.32</td>
</tr>
<tr>
<td>Early abscission</td>
<td>0.13-0.015x 0.04 2.85 0.095</td>
<td></td>
<td></td>
<td></td>
<td>0.042+0.06x 0.54 85.78 &lt;0.001</td>
</tr>
<tr>
<td>Leaf damage</td>
<td>1.40-0.65x 0.66 148.61 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.71-0.15x 0.07 5.44 0.022</td>
</tr>
<tr>
<td>Shoot length</td>
<td>0.26+0.09x 0.17 16.37 &lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td>0.38-0.038x 0.06 4.64 0.034</td>
</tr>
<tr>
<td>Radial growth</td>
<td>1.096+0.10x 0.14 12.84 0.001</td>
<td></td>
<td></td>
<td></td>
<td>1.27-0.098x 0.23 21.05 &lt;0.001</td>
</tr>
</tbody>
</table>
In Parikkala the effect is marginally significant ($F_{1,82} = 3.66, P = 0.001, \eta^2 = 0.13)$. 

Although the ant densities significantly decreased after aphid removal, the aphid treatment had no significant effects on leaf chews in Joensuu ($F_{1,168} = 0.33, P = 0.035, \eta^2 = 0.001$), 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 \(P.\ vitellinae\) 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 number of other invertebrate predators per square meter in 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 x ant exclusion interactions.

### 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.

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

† Data was arcsin square root -transformed before the analyses
‡ R²-values

**FIG. 2.** Maximum number of third instar larvae of *Phratora vitellinae* observed per square meter of leaf area on experimental *Salix myrsinifolia* (mean ± SE). The figures within or above the bars indicate sample sizes.
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 x 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. The effect was larger on *S. myrsinifolia* but the willow species x aphid exclusion interaction was not quite significant. In early abscission there also was a significant interaction between the ant and aphid exclusions, apparently 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 Joensuu, 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 x ant exclusion interaction in both variables indicating more profound negative effect of the ant exclusion in *S. phylicifolia*. 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 x aphid exclusion interaction in shoot length, indicating that aphid removal increased the shoot growth more on *S. myrsinifolia*. When aphids were present on *S. myrsinifolia*, the presence of ants rather decreased 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 *F. aquilonia* -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*. The results provide three major factors to explain the results. First, tended aphids were equally abundant on both willow species or somewhat more abundant on *S. myrsinifolia*, which is likely to promote the negative effect of ants through phloem herbivory on both willow species. Second, there were clearly less leaf chewing insects on *S. myrsinifolia* making the potential positive effect of ant predation less likely. Third, the dominant leaf chewing insect on *S. myr-
*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. 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 significantly between 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 caused 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 may be a consequence of the striking differences in leaf chemistry. Some of the chrysomelid beetle species observed in the study sites (including 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).

![Graph](image-url)

**FIG. 4.** The final shoot length and the relative radial growth of the experimental willows (mean ± SE). White bars = control, black bars = ants excluded. The figures within bars indicate sample sizes.

*Salix phylicifolia*, the leaf beetle *P. vitellinae*, is better defended against generalist predators than the dominant generalist herbivores on *S. phylicifolia*. 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. 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.
Phratora vitellinae, which leaves distinctive feeding marks on the leaves, was responsible for about 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, such as bugs with piercing mouthparts, are not affected by the larval secretion (Rank et al. 1998). On the other hand, some specialist predators, such the syrphid fly Parasyrphus nigrararis Zett. is strongly attracted by salicylaldehyde (Köpf et al. 1997). It is also known that the 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

| 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. |
|-------------------------------------------------|-------------------------------------------------|
| Shoot length† | Relative radial growth† |
| df | MS | F | P | η² | df | MS | F | P | η² |
|---------------------------------|---------------------------------|
| **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 x 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 x B                          | 1                              | 0.111 | 3.83 | 0.052 | 0.022 | 0.002 | 0.08 | 0.772 | 0.001 |
| W x A                          | 1                              | 0.627 | 21.61 | <0.001 | 0.114 | 0.259 | 13.35 | <0.001 | 0.074 |
| B x A                          | 1                              | 0.071 | 2.46 | 0.118 | 0.014 | 0.014 | 0.71 | 0.400 | 0.004 |
| W x B x 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 was arcsin squareroot -transformed before the analyses
‡ R²-values
of \textit{P. vitellinae} than the ants themselves, the positive effect of ants on larval survival seems logical.

In addition to \textit{P. vitellinae}, ants did not significantly affect the densities of other leaf chewers on \textit{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 \textit{S. myrsinifolia} can be caused by a response of the ants to the densities of their prey or potential 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 \textit{S. myrsinifolia} were mainly aphid-milkers, since this willow species provided very few acceptable prey items or potential threat to aphids, but offered relatively more aphids than \textit{S. phyllicifolia}.

The results obtained in this study are similar to 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 \textit{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 performance, if the number of herbivores is as low as on \textit{S. myrsinifolia}, or if the herbivores are effectively defended against predators, as in the case of \textit{P. vitellinae}. It seems that \textit{S. phyllicifolia} and \textit{S. myrsinifolia} represent the extremes of a continuum created by the antagonistic relationship between direct plant defense and indirect defense provided by predators. \textit{Salix phyllicifolia}, which is susceptible to herbivores clearly gain from the presence of predators, whereas \textit{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.

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References


Tritrophic interactions: willows, herbivorous insects and insectivorous birds

Mika Sipura

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Tritrophic interactions: willows, herbivorous insects and insectivorous birds

Mika Sipura

Insectivorous birds can increase plant growth by consuming herbivorous insects and reducing insect damage. However, plant traits such as the level of chemical defense may affect the quantity and quality of insects, and alter the foraging behavior of birds. Therefore, I predicted that plant traits can also modify the effect of birds on leaf damage and plant growth. In this study I compared the effect of insectivorous birds on the herbivory and growth of two chemically different willow species, weakly defended Salix phylicifolia and strongly defended Salix myrsinifolia under two fertilization levels. Half of the willows were protected from birds using a translucent gill-net, which did not limit access of insects. The effect of birds on the densities of leaf chewing insects and leaf damages was considerable on unfertilized S. phylicifolia but less obvious on fertilized ones. The effect of bird predation was negligible on S. myrsinifolia, which had very low insect densities in all treatments. Birds increased the growth of the experimental willows, but the effect clear only in unfertilized S. phylicifolia. I suggest that birds avoided foraging on willows with low populations of insects and little visible damage. The study shows that bird predation can alter the patterns of insect densities we see on willows, thus emphasizing the importance of considering multitrophic effects when studying plant-insect interactions.

Keywords: Willows, herbivory, insectivorous birds, predation, multitrophic interact.

Introduction

Several studies have demonstrated that insectivorous birds can remarkably reduce the number of their insect prey (e.g. Holmes et al. 1979; Kroll and Fleet 1979; Loyn et al. 1983) and leaf damage of plants through consumption of leaf chewing insects (Atlegrim 1989; Marquis and Whelan 1994). Although using birds in biocontrol has been attempted much earlier than using invertebrate predators or parasitoids (Otvos 1979), the study by Marquis and Whelan (1994) was the first to show that birds can increase plant growth by consuming herbivorous insects. In their experiment, exclusion of birds from white oaks (Quercus alba L.) increased the number of leaf chewing insects and leaf damage. Since insect damage retarded the growth of the white oaks, bird exclusion led to considerable decrease in leaf size and biomass production of these trees.

Overall, the existing evidence suggests that birds have a potential to benefit plants (see Marquis and Whelan 1994 for references). However, this may not always occur even if both birds and herbivorous insects are abundant (see Mattson 1974 for example). Firstly, plants may be tolerant to moderate levels of insect damage (Owen and Wiegert 1976; Paige and Whitham 1987; Strauss and Agrawal 1999), which is likely to obscure the indirect effect of birds on plant growth. Secondly, birds attack both phytophagous and entomophagous prey, and may prefer parasitised or otherwise predation-prone insects (Betts 1955; Tscharntke 1997). Birds that consume relatively large amounts of predatory invertebrates may therefore even increase the numbers of herbivorous insects (Kristin 1991; Tscharntke 1997). Thirdly, birds may prefer some plants as foraging sites because of the expected quantity of insect prey and discard less profitable or unpredictable feeding sites. Since plants differ in their acceptability to herbivorous insects (e.g. Fritz and Simms 1992), different amounts of prey can obviously be obtained on different plants. Fourthly, plants may affect the detectability of insect prey. For example increased movements (Bergellson and Lawton 1988) or active feeding (Bernays 1997) by insects may affect their vulnerability to their natural enemies. Plants may also
differ in architecture and foliar structure, which may either facilitate or constrain the way birds detect or capture their prey (Holmes 1990). Finally, herbivorous insects may use the defensive compounds of plants for their own defense (Smiley et al. 1985; Pasteels et al. 1988; Pasteels and Rowell-Rahier 1991) being lower quality food for predators if growing on strongly defended plants. As a result, genetically and environmentally determined traits of plants may modify the degree in which birds can benefit them.

In this study I examined the impact of avian predators on insect herbivore densities, leaf damage and growth of two willow species under two fertilization levels. The two willow species used in this study, the tea-leaved willow (Salix phylicifolia L.) and the dark-leaved willow (Salix myrsinifolia Salisb.) are among the most widespread willow species in Northern Europe and can be found abundantly along streams, lakesides and other open habitats including cultivated fields (Hämet-Ahti et al. 1998). The willow species are morphologically similar but show remarkable differences in the secondary chemistry of their leaves. Generally about fifty-fold difference in concentrations of phenolic glycosides (including salicin and salicortin) have been found between bitter tasting S. myrsinifolia and mild S. phylicifolia (Tahvanainen et al. 1985; Julkunen-Tiitto 1986; Rank et al. 1998). Phenolic glycosides have been shown to repel generalist herbivores (e.g. Tahvanainen et al. 1985; Dodge et al. 1990), whereas some specialists may use them as stimulants for feeding or oviposition (Tahvanainen et. al 1985; Soatens and Pasteels 1994; Kolehmainen et al. 1995). On the other hand, considerable amounts of a flavonoid, ampelopsin, has been found in the leaves of S. phylicifolia but not in S. myrsinifolia (Rank et al. 1998). However, this compound has been observed to be rather inert to some generalist insects (M. Sipura and A. Ikonen, unpublished data). Therefore I predict that when generalist herbivores predominate S. phylicifolia should suffer more severely from insect damage than S. myrsinifolia.

In addition to species-specific differences, there is environmental factors, such as soil fertility, that may affect both insect herbivores and their enemies (e.g. Strauss 1987; Haartvigsen et al. 1995). In S. myrsinifolia nitrogen fertilization has been observed to increase the nitrogen content in the leaves, and to cause slight decrease in the concentration of phenolic glycosides (Hakulinen et al. 1995). Basically, fertilization could affect insect densities and change the foliar architecture of willows as well. These changes may affect foraging of insectivorous birds.

Here I will concentrate on four questions relevant to tritrophic interactions among willows, willow feeding insects and insectivorous birds. First, do the two willow species have different herbivore loads and do they suffer from different amounts of herbivore damage? Second, how does fertilization alter insect densities on these willows? Third, do insectivorous birds reduce insect densities and leaf damage on the two willow species and do bird predation on herbivorous insects indirectly affect willow growth? And fourth, how does the effect of birds on the herbivory and growth differ between willow species and how do birds respond to fertilization of willows?

Methods

Study site and experimental design

The study was conducted in Parikkala, SE Finland (61°33’N, 29°33’E). The experimental willows were growing on the shore of a highly eutrophicated lake on a narrow embankment constructed to prevent flooding of the fields surrounding the lake. There was a belt of larger trees growing on both sides of the embankment, and younger (< 10 years) S. phylicifolia and S. myrsinifolia on the top of the embankment.

The bird community in the area was censused using a standard mapping method (Enemar 1959). The study area was long but very narrow (about 1600 x 4 meters), and it was not possible to determine the actual bird densities. However, the most common bird species in the area were sedge warbler (Acrocephalus schoenobaenus L.) with 7 pairs, pied flycatcher (Ficedula hypoleuca Pallas) with 6 pairs, willow warbler (Phylloscopus trochilus L.) with 4 pairs and reed bunting
(Emberiza schoeniclus L.) with 4 pairs. Altogether, individuals from sixteen species of insectivorous birds were observed to forage regularly in the study area.

Just before budbreak in spring 1998, I selected 12 blocks with four S. phylicifolia and four S. myrsinifolia individuals in each and randomly divided the willows into fertilization and bird exclusion treatments. The ages of the willows (determined from annual rings) varied from four to nine years. Willows in fertilization treatment received 100 g of complete fertilizer (N:P:K 8:4:14; Puutarhan yleisnäos, Kemira Agro Oy, Finland) per square meter, and unfertilized willows served as controls. The cages for excluding birds were made of four wooden side poles (diameter 3-5 cm) erected on the ground. The side of the cages varied between 0.8 to 2.5 m, depending on the size of the willow. A completely translucent monofilament gill-net (Pietarinverkko, Kivikangas Oy, Finland) with 22 mm diameter holes and 0.2 mm thread was stretched upon the side poles. Although the hole size is rather small for the largest insects, some butterflies with a wingspan of 40 mm were seen to fly through the net. Also, caterpillars of the largest insects on willows, Smerinthus ocellatus L. and Cerura vinula L., were observed both on caged and control willows. Because the erection of the side poles for the net may cause root damage for experimental willows, I also pierced four holes on the ground next to the control willows. At the end of the growing season some shoots (less than 3% of the estimated shoot biomass) of three fertilized willows of both species grew out of the cages. However, I observed that birds avoided foraging near the cages, and assume this error to be negligible.

**Insect censuses**

Insect populations were censused by counting all herbivorous insects on the experimental willows five times during the growing season 1998 (see Fig 1. for census dates). Because it was not possible to open the cages, some of the smallest insects, such as thrips and smallest nymphs of aphids, had to be omitted from the censuses. I avoided disturbing insects and counted the constructions of concealed insects instead of individuals.

I determined insect densities per leaf area by counting the number of shoots and measuring the length of 20 randomly selected shoots from each willow at each census time. At each time I also collected a sample of 40 shoots from both species from adjacent willows. After measuring the lengths of these sampled shoots I dried the leaves under a moderate pressure, took a photocopy of each leaf and measured the leaf surface area of each shoot with a planimeter. The shoot length significantly explained 76-94% of the variation in the total leaf area in regression models. Therefore, on the basis of the number of shoots and the mean shoot length I was able to obtain a good estimate of leaf area of each experimental willow at each census time. I calculated all insect densities for a square meter of leaf surface area.

**Measuring insect damage and willow growth**

I measured the leaf damage caused by leaf chewing insects in two ways. Because willows tend to abscise badly damaged leaves early in the growing season, I determined the proportion of early-abscised leaves at the fourth census by counting the leaves and leaf scars from 20 randomly selected shoots per willow. At the last census I collected all remaining leaves from 50 randomly selected shoots per willow, and stored them into a freezer. I determined the total leaf area and damaged or missing leaf area from 50 randomly selected leaves per willow using transparent scale paper. I used a sample size of 50 leaves, since primary observations showed that fluctuations of means diminished sharply after 40 leaves. In the analyses I used the mean proportion of early abscission and the mean proportion of damaged leaf area as variables.

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

**Data analysis**

The experiment was originally planned as a completely randomized block design with three factors. I tested the block effects prior to final analyses. Because no significant effects were found, I omitted blocks from the analysis to save the degrees of freedom. I analyzed all insect densities using a three-factor repeated measures GLM ANOVA, with censuses as the within-subject measures and willow species, fertilization treatment and predation treatment as fixed factors. I use eta squared \( \eta^2 \) as estimates of effect sizes. Eta squared is the squared ratio of the between-groups sum of squares and the total sum of squares (Keppel 1982), and can be interpreted as \( R^2 \)-values. Most variables were log \((x + 1)\) or arcsin-square-root transformed to meet the criteria of the analysis of variance.

**Results**

**Insect densities**

There was no significant difference in total herbivore density between *S. phylicifolia* and *S. myrsinifolia* \((F_{1,88} = 0.48, P = 0.49)\). Concealed feeding and leaf chewing insects were, however, more abundant on *S. phylicifolia* whereas sap sucking insects were more abundant on *S. myrsinifolia* (Fig. 1, Table 1). An aphid, *Pterocomma salicis* L., that consisted 89.9% of the sap sucking guild, was clearly more abundant on *S. myrsinifolia* \((F_{1,88} = 16.9, P < 0.001, \eta^2 = 0.16)\), whereas there was no difference in densities

**Table 1.** Between subject effects of the repeated measures analysis of variance on the effects of willow species (W), fertilisation treatment (F) and bird predation treatment (P) on the insect densities of experimental willows.

<table>
<thead>
<tr>
<th>Source</th>
<th>Concealed feeders</th>
<th>Sap suckers</th>
<th>Leaf chewers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Willow species</td>
<td>3.49</td>
<td>32.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilisation</td>
<td>0.51</td>
<td>4.78</td>
<td>0.031</td>
</tr>
<tr>
<td>Predation</td>
<td>0.35</td>
<td>3.30</td>
<td>0.073</td>
</tr>
<tr>
<td>W x F</td>
<td>0.046</td>
<td>0.43</td>
<td>0.51</td>
</tr>
<tr>
<td>F x P</td>
<td>0.028</td>
<td>0.27</td>
<td>0.61</td>
</tr>
<tr>
<td>W x F x P</td>
<td>0.242</td>
<td>2.27</td>
<td>0.14</td>
</tr>
<tr>
<td>Error</td>
<td>88.016</td>
<td>1.095</td>
<td>0.167</td>
</tr>
</tbody>
</table>

\(^{1}\) Data was log \((x + 1)\) -transformed before the analyses.
Fertilization caused a significant reduction in overall insect density per leaf area ($F_{1,88} = 7.45, P = 0.008$) on the studied willows, but explained only 7.8% of the variance. The difference disappeared when mean leaf area was used as a covariate ($F_{1,88} = 0.91, P = 0.76$), suggesting that the difference in overall insect densities resulted mainly from the increased leaf area in fertilized willows. The reduction in densities due to fertilization was significant in concealed feeders and leaf chewers but not in sap suckers (Fig. 1, Table 1). There was also a significant willow species x fertilization interaction in leaf chewer densities (Fig. 1, Table 1), showing that fertilization caused stronger reduction in the densities of leaf chewing insects on *Salix phylicifolia* than on *S. myrsinifolia*.

Exclusion of birds caused a significant increase in overall herbivore insect density ($F_{1,88} = 7.85, P = 0.006$), but the treatment explained only 8.2% of the variance. Concealed feeders were slightly but not significantly (Fig. 1, Table 1) more abundant on control bushes, whereas bird exclusion treatment increased significantly the densities of free-living leaf chewers (Table 1). In leaf chewers there is a significant willow species x predation treatment interaction (Table 1). Caged *S. phylicifolia* had far higher insect densities than caged *S. myrsinifolia* (Fig. 1). It can also be seen that bird exclusion had a strong effect on unfertilized *S. phylicifolia*, but only slight effect on fertilized ones. A slight effect of predation treatment occurred in fertilized *S. myrsinifolia* whereas no effects could be seen in unfertilized ones (Fig. 1). Therefore, there is also a significant three-way interaction between willow species, fertilization treatment and predation treatment (Table 1).

To clarify the effect of birds on herbivore densities, I picked up three abundant subgroups of leaf chewers for closer examination. Generalist lepidopteran caterpillars consisted 14% of leaf chewing individuals on *S. phylicifolia* and 21% on *S. myrsinifolia*, and because of their large size, they may cause considerable leaf damage on willows. As can be seen in Fig. 2a the caterpillars were significantly more abundant on *S. phylicifolia* ($F_{1,88} = 56.60, P < 0.001, \eta^2 = 0.39$). Although the main effect of birds was clear ($F_{1,88} = 36.75, P < 0.001, \eta^2 = 0.30$), it was significantly more pronounced on *S. phylicifolia* (willow species x predation treatment interaction: $F_{1,88} = 10.22, P = 0.002, \eta^2 = 0.10$). There is also a significant willow species x fertilization interaction ($F_{1,88} = 11.57, P = 0.002, \eta^2 = 0.10$) and a significant three-way interaction between all three factors ($F_{1,88} = 7.72, P = 0.007, \eta^2 = 0.08$).

Larvae of the leaf beetle *Galerucella lineola* F. consisted 34% of leaf chewers on *S. phylicifolia*, but this beetle never occurred on *S. myrsinifolia*. Another common leaf beetle, *Phratora vitellinae* L. consisted 22%...
of leaf chewers on *S. myrsinifolia* but only few individuals were observed on *S. phylicifolia*. Fertilization affected negatively on the densities of *G. lineola* (*F*$_{1,44}$ = 9.18, *P* = 0.004, $\eta^2$ = 0.17). Predator exclusion treatment caused a dramatic increase in the densities of *G. lineola* on unfertilized *S. phylicifolia*, whereas the effect was negligible on fertilized bushes (main effect: *F*$_{1,44}$ = 13.83, *P* = 0.001, $\eta^2$ = 0.24; Fig 2b). However, the fertilization x bird predation interaction was not significant (*F*$_{1,44}$ = 2.84, *P* = 0.099). On the other hand, fertilization and predation treatments did not have any effects on the densities of *P. vitellinae* on *S. myrsinifolia* (*P* > 0.43 in every case; Fig. 2c).

Ants (*Myrmica rubra* L.) and spiders formed 97% of the individuals of invertebrate predators observed on experimental willows. Although cages may have provided additional substrates for web-building spiders, the bird predation treatment did not affect spider densities (*F*$_{1,88}$ = 0.42, *P* = 0.52), nor was there any interaction between willow species and predation treatment (*F*$_{1,88}$ = 0.10, *P* = 0.75). Likewise, birds did not affect densities of ants (*F*$_{1,88}$ = 0.48, *P* = 0.49) and plant species did not interact with predation treatment (*F*$_{1,88}$ = 1.71, *P* = 0.20). Ants were, however, significantly more abundant on *S. myrsinifolia* than on *S. phylicifolia* (*F*$_{1,88}$ = 7.67, *P* = 0.007, $\eta^2$ = 0.08).

In a data pooled over experimental treatments there were on average 32.32 ± SE 6.03 ants on *S. myrsinifolia* and 13.78 ± SE 3.11 on *S. phylicifolia*.

### Leaf damage

Leaves of caged willows abscised prematurely significantly more than leaves of control willows (Table 2, Fig. 3.). In *S. phylicifolia* leaves from unfertilized bushes abscised prematurely more than leaves from fertilized bushes, whereas the reverse was true in *S. myrsinifolia* (Table 2, Fig. 3.). This may have been caused by *Melampsora* rust that mostly attacked fertilized *S. myrsinifolia*. In a data pooled over willow species and experimental treatments the percentage of early abscission was significantly correlated with the mean density of leaf chewers during first three censuses (*r*$_s$ = 0.53, *P* < 0.001, Spearman correlation), but even more closely with the mean density of an abundant leaf-tier moth *Brachylomia viminalis* F. (Lepidoptera, Noctuidae) (*r*$_s$ = 0.62, *P* < 0.001).

The results of the percentage of early abscission and the percentage leaf area damaged were strongly correlated with the results of the densities of leaf chewing insects (Fig. 3, Table 2). There was significantly more damage on *S. phylicifolia* than on *S. myrsinifolia*. Fertilization decreased the level of damage in *S. phylicifolia* but not in *S. myrsinifolia*. Predator exclusion significantly increased the amount of leaf damage, and the effect was more pronounced in *S. phylicifo-
The significant three-way interaction of all factors (Table 2) can be interpreted so that the more there were insect damage, the larger was the effect of bird predation (Fig. 3).

**Willow growth**

Predation treatment had a significant effect on willow growth (MANOVA for shoot length and relative radial growth, Wilks' $\lambda = 0.89$, $F_{2,87} = 5.61$, $P = 0.005$, $\eta^2 = 0.12$). There was no significant willow species x predation treatment interaction ($Wilks' \lambda = 0.95$, $F_{2,87} = 2.17$, $P = 0.12$). Predation treatment did not have a significant effect on the length of shoots, but there was a significant willow species x bird predation treatment interaction (Table 3). When the relative radial growth is considered (Table 3), bird predation seems to have had a significant overall effect on willow growth, but the willow species x predation treatment interaction was not significant. As seen in Fig. 4, the effect of birds on willow growth is most evident in unfertilized *S. phylicifolia* that also had the largest herbivore load.

**Discussion**

The results show that birds decrease densities of leaf chewing insects but have a negligible effect on concealed feeding and sap sucking insects. There is many studies to show that insectivorous birds can greatly attack insect galls that may lead to remarkable

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**Table 2.** Analysis of variance on the effects of willow species (W), fertilisation treatment (F) and predation treatment (P) on early leaf abscission and leaf damages on experimental willows.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>% Early abscission</th>
<th>% Leaf area damaged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS  F  P $\eta^2$</td>
<td>MS  F  P $\eta^2$</td>
</tr>
<tr>
<td>Willow species</td>
<td>1  0.156  7.70 0.007 0.08</td>
<td>0.524 185.34 &lt;0.001 0.68</td>
</tr>
<tr>
<td>Fertilization treatm</td>
<td>1  0.131  6.49 0.013 0.07</td>
<td>0.013  4.55 0.035 0.05</td>
</tr>
<tr>
<td>Predation treatment</td>
<td>1  0.145  7.14 0.009 0.08</td>
<td>0.048  17.15 &lt;0.001 0.16</td>
</tr>
<tr>
<td>W x F</td>
<td>1  0.406 20.04 &lt;0.001 0.19</td>
<td>0.040  14.03 &lt;0.001 0.14</td>
</tr>
<tr>
<td>W x P</td>
<td>1  0.021  1.05 0.31 0.01</td>
<td>0.027  9.60 0.003 0.10</td>
</tr>
<tr>
<td>F x P</td>
<td>1  0.030  1.46 0.23 0.02</td>
<td>&lt;0.001  0.001 0.98 &lt;0.001</td>
</tr>
<tr>
<td>W x F x P</td>
<td>1  0.076  3.74 0.056 0.04</td>
<td>0.011  3.96 0.05 0.04</td>
</tr>
<tr>
<td>Error</td>
<td>88  0.020</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*a* Data was arcsin square-root transformed before the analyses.
consequences on the ecology and evolution of some galler systems (Tscharntke 1992; 1997; Abrahamson and Weis 1997). In these systems, however, the galls and gallmakers are usually larger than those of studied willows, and the action of birds takes place during fall or winter when alternative food is probably scarce. It may therefore be that insects in galls and other shelters may escape birds when alternative and more profitable prey sources are available. On the other hand, the dominant sap-sucking insects on willows are either quick-moving (leaf hoppers) or protected vigorously by ants (aphids; see below).

The increase of leaf chewers due to bird exclusion was more pronounced on *S. phylicifolia* compared to *S. myrsinifolia*, which clearly had lower insect densities even when birds were excluded. The difference in insect densities can be reduced to leaf chemistry of the willows. Some of the chrysomelid beetle species [including *G. lineola* and *Lochmaea caprea* (L.)] observed on the experimental willows, 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 glycosides (Roininen and Tahvanainen 1989; Denno et al. 1990; Matsuki and MacLean 1994). The specialist leaf beetle *Phratora vitellinae* that usually predominates in the leaf-chewing guild on *S. myrsinifolia* was rather scarce in this study area. In sites where *P. vitellinae* is abundant, *S. myrsinifolia* may have even more leaf damage than *S. phylicifolia* (M. Sipura, unpublished data).

There are at least three possibilities why birds prefer foraging on weakly defended *S. phylicifolia* over strongly defended *S. myrsinifolia*. First, aphid-tending ants that were more abundant on *S. myrsinifolia* may interfere foraging birds. Haemig (1996) have shown that great tits (*Parus major* L.) avoided foraging on artificial trees with *Formica*-ants, which may lead to increased survival of insects from bird predation. In my study, however, using mean ant density as a covariate in the analyses of leaf chesher densities or leaf damage did not cause any notable changes to the results of ANOVA, proposing that the effect of ants on bird predation was insignificant in this case. Second, insects on *S. myrsinifolia* may be less palatable for birds because they use plant secondary chemicals for their own defense. It is well-known that larvae of *S. myrsinifolia* feeding *P. vitellinae* convert salicylates from their host into salicylaldehyde, that has been shown to repel many natural enemies including one bird species, blackcap (*Sylvia atricapilla*) (Topp and Bell 1992). It has also been suggested that generalist herbivores

**Table 3.** Analysis of variance on the effects of willow species (W), fertilization treatment (F) and predation treatment (P) on shoot length and relative radial growth of the experimental willows.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Shoot length <em>a</em></th>
<th>Relative radial growth <em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>Willow species</td>
<td>W</td>
<td>0.058</td>
</tr>
<tr>
<td>Fertilization treatm</td>
<td>1</td>
<td>1.70</td>
</tr>
<tr>
<td>Predation treatment</td>
<td>1</td>
<td>0.031</td>
</tr>
<tr>
<td>W x F</td>
<td>1</td>
<td>0.0012</td>
</tr>
<tr>
<td>W x P</td>
<td>1</td>
<td>0.068</td>
</tr>
<tr>
<td>F x P</td>
<td>1</td>
<td>0.0172</td>
</tr>
<tr>
<td>W x F x P</td>
<td>1</td>
<td>0.0195</td>
</tr>
<tr>
<td>Error</td>
<td>88</td>
<td>0.0158</td>
</tr>
</tbody>
</table>

* Data were log (x+1) transformed before the analyses.
may be either actively or passively defended just by having plant chemicals in their haemolymph or gut (Brower 1984; Bowers 1993). There are, however, no studies showing the role of plant derived chemicals on defense of generalist herbivores, and even the role of larval secretions of specialist herbivores is still controversial (Rank et al. 1996; Rank et al. 1998). The third possibility is that birds respond to herbivore densities in a density-dependent way (Dickson and Whitham 1996) by staying longer times in profitable bushes or flying more often to bushes with higher expected prey densities. Birds may use leaf damage as a cue for profitable foraging sites and therefore prefer foraging on willows with high damage levels. Heinrich and Collins (1983) have shown that black-capped chickadees (Parus atricapillus L.), learn to use trees with damaged leaves as foraging sites and may even recognize the tree species where they have previously found food. To what extent birds use leaf damage as a cue in the field is not known (Heinrich 1993; but see Greenberg and Gradwohl 1980), but the hypothesis provides a promising explanation for the phenomenon observed in this experiment.

The results of this study contradict the hypothesis suggesting that predation on herbivores is higher on suboptimal host plants (Feeny 1976; Clancy and Price 1987; Häggström and Larsson 1995). As shown earlier, the presence of phenolic glycosides have been found to have a negative effect on the growth of generalist herbivores. Prolonged development time, increased time devoted to foraging and increased movements on poor quality host plants may lead to increased exposure to natural enemies (Feeny 1976; Price et al. 1980; Bergelsson and Lawton 1988; Häggström and Larsson 1994; Bernays 1997). However, here generalist lepidopteran caterpillars escaped bird predation better on S. myrsinifolia where their densities were lower.

The negative effect of fertilization on insect densities on S. phylicifolia is rather surprising. In their review Waring and Cobb (1992) report that 67% of the studies have found herbivores to perform better on fertilized plants. These results can be explained by increased nitrogen contents and decreased concentrations of carbon based secondary compounds in plants after fertilization (Hartley and Jones 1997). So far, no studies are available to show the effect of fertilization on the food choice or insect performance on S. phylicifolia. So, it is not clear whether the observed decrease in the densities of leaf chewers after fertilization is due to lowered food quality, altered composition of feeding or oviposition stimulants, or some external factors. Because fertilization affected only leaf chewing insects, it may be that insects responded to the physical changes instead of nutritional alteration of host plants after fertilization. It is possible that fertilization changed the structure of leaves, which made them less edible for herbivores. Because of the vigorous shoot growth it is also possible that fertilization changed abiotic conditions thus making the willows less suitable substrates for exposed leaf chewing insects.

The differences between willow species and the effect of fertilization on the bird pre-
...dation leads me to suggest that the low insect densities and low level of leaf damage make *S. myrsinifolia* and fertilized *S. phylicifolia* less attractive to foraging birds. It may therefore be that density-dependent predation provides partial refuges for herbivorous insects on strongly defended plants. The study of Hartvigsen et al. (1995) demonstrated that as predatory mites responded to prey increase on *Populus deltoides* Marsh., they masked the positive effect of fertilization to herbivorous mites. The similar indirect effect of fertilization was found by Strauss (1987). In her study the number of predatory ants increased in fertilized plots of *Artemisia ludoviciana* Nutt. leading to increased mortality of chrysomelid beetles and decreased leaf damage in fertilized plots. In my study birds appeared to have the most profound effect on unfertilized *S. phylicifolia*, thus masking the negative effect of fertilization on insect densities. The effects of fertilization on bird predation may be affected by architectural changes of the bushes or altered levels of foraging cues for birds. Because fertilized *S. phylicifolia* produced more and larger leaves, and had less leaf-chewing insects per leaf area, the level of visible insect damage was clearly lower on fertilized willows. In *S. myrsinifolia* the leaves were somewhat more damaged on fertilized bushes, which may explain the difference in the response of birds to fertilization of these willow species.

This study emphasizes the necessity to consider multitrophic interactions when trying to understand relationships between plants and insects. The species-specific and fertilization-induced differences in willow resistance to herbivores would have looked totally different if the effect of insectivorous birds had not been included. It is generally agreed that both the evolution of insect-plant interactions (Bernays and Graham 1988; Strong 1988; Schultz 1992) and control of herbivore populations (Lawton and McNeill 1979; Hunter and Price 1992; Karban 1997) result from a combination of bottom-up and top-down forces (Hartley and Jones 1997). This study evidently shows the dual control of the herbivore densities on willows: the effect of predatory birds was unimportant on chemically strong *S. myrsinifolia* but substantial on milder *S. phylicifolia*, which is favored by generalist herbivores.

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