Habitat difference in abundance of willow leaf beetle *Phratora vulgatissima* (Coleoptera: Chrysomelidae): plant quality or natural enemies?

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Abstract

Herbivorous insects are influenced by both 'bottom-up' forces mediated through host plants and 'top-down' forces from natural enemies. Few studies have tried to evaluate the relative importance of the two forces in determining the abundance of insects. The leaf beetle *Phratora vulgatissima* Linnaeus sometimes occurs at high densities and severely damages the willow *Salix cinerea* in forest habitats. For willows growing in open agricultural landscapes (farmland *S. cinerea*), the leaf beetle generally occurs at low densities and plants receive little damage. The purpose of the present study was to evaluate the relative importance of host plant quality and natural enemies behind the observed difference in *P. vulgatissima* abundance. Female egg-laying and larval performance (growth and survival) were studied on caged willow branches in the field to investigate if plant quality differs between *S. cinerea* trees growing in forest and farmland habitats. The survival of eggs exposed to natural enemies was examined to see if predation could explain the low abundance of leaf beetles on farmland willows. The results indicated no difference in plant quality; female egg laying and larval performance did not differ between the forest and the farmland. However, heteropteran predators (true bugs) were more abundant, and the survival of eggs was lower, on plants in the farmland habitat than in the forest habitat. The data suggest that the low abundance of *P. vulgatissima* on farmland willows could not be explained by a poor quality of plants, but more likely by high predation from heteropterans.

Keywords: bottom-up, Heteroptera, *Phratora vulgatissima*, *Salix cinerea*, top-down

Introduction

Herbivorous insects often show great variation in abundance, both in time and space (Berryman, 1988). The causes behind these patterns have puzzled ecologists for decades and a central question has been whether herbivorous insects are influenced by 'bottom-up' forces mediated through host plants (Rhoades, 1983) or by 'top-down' forces from natural enemies (Hairston *et al.*, 1960). Today, the general conception is that bottom-up and top-down forces are acting in concert in influencing the distribution and abundance of herbivorous insects (Hawkins, 2001). Few studies have, however, tried to evaluate the relative importance of the two forces in natural populations (Walker & Jones, 2001). Also, little is known about how host plant quality and predation can vary spatially for insects among habitats (Preszler & Boecklen, 1996).

An extensive number of studies show that variation in plant traits, such as morphology, nutrients, and secondary metabolites, influence host plant selection and performance

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of herbivorous insects (Bernays & Chapman, 1994; Schoon-hoven *et al.*, 1998). Variation in plant traits that affect individual insects may then also translate into effects at the population level (Larsson *et al.*, 2000). Because important plant traits can be determined not only genetically but also environmentally (Koricheva *et al.*, 1998), it is not unlikely that plants can vary in quality for insects among habitats (Louda *et al.*, 1987). For example, the availability of soil nitrogen may influence concentrations of nutrients (Gratton & Denno, 2003) and secondary metabolites (Björkman *et al.*, 1998) in plants. In addition, several studies have shown that herbivorous insects often increase in abundance in the absence of natural enemies (Kidd & Jervis, 1997; Symondson *et al.*, 2002). Localized high population densities may, for example, be due to spatial escapes from natural enemies (Björkman *et al.*, 2000; Maron *et al.*, 2001). As a consequence, observed variations in insect abundance might be attributed to the combination of both host plant quality and natural enemy factors.

The purpose of the present study was to evaluate the relative importance of host plant quality and natural enemies on the abundance of the leaf beetle *Phratora vulgatissima* Linnaeus (Coleoptera: Chrysomelidae) in natural stands of *Salix cinerea* L. (Salicaceae). In south central Sweden, beetles sometimes increase to damaging levels in natural stands of *S. cinerea* growing in forest landscapes (i.e. stands surrounded by conifer dominated forests), whereas stands growing in open agricultural landscapes (from now on referred to as 'farmland willows') rarely receive any significant damage and population densities of leaf beetles are generally low (Dalin, 2004). Previous studies have highlighted the role of plant secondary metabolites, especially phenolic glycosides, in influencing host plant selection and performance of willow leaf beetles (Koleh-mainen *et al.*, 1995; Rank *et al.*, 1998; Peacock *et al.*, 2004). Thus, female fecundity and larval performance are para-meters commonly used for investigating the role of host plant quality for willow leaf beetles. In addition, studies suggest that predation by heteropteran predators can cause variation in beetle abundance among individual plants within stands (Björkman *et al.*, 2000) and influence popula-tion growth of leaf beetles in willow plantations (Björkman *et al.*, 2004). These heteropterans mainly attack eggs and young larvae of *P. vulgatissima* (Björkman *et al.*, 2003). Variation in predation pressure could, therefore, partially explain the difference in leaf beetle abundance between the two habitats.

Two hypotheses were formulated to evaluate the rela-tive effects of bottom-up and top-down forces. The plant quality hypothesis states that *S. cinerea* growing in forest habitats is of superior quality for *P. vulgatissima* compared with plants in the farmland habitat. To test this hypothesis, female egg-laying and larval performance (survival and growth) were studied in the field. The prediction was that female beetles would lay more eggs, and/or that larvae would perform better on plants in the forest than in the farmland habitat. The natural enemy hypothesis states that *P. vulgatissima* becomes more abundant in the forest habitat because the density of heteropteran predators is lower than in the farmland habitat. To test this hypoth-esis, the survival of eggs exposed to natural enemies was studied in the field and the prediction was that egg survival should be higher in the forest than in the farmland habitat.

Materials and methods

Insects and study sites

The leaf beetle *Phratora vulgatissima* is a major pest in willow plantations grown for biomass production in north-ern Europe (Kendall *et al.*, 1996b; Björkman *et al.*, 2004). It is univoltine in Sweden and adults appear on plants in the beginning of May after emergence from winter hibernation. The egg-laying period extends from late May to the middle of June and the eggs are laid on the underside of leaves. Females lay the eggs in clusters consisting of 10–50 eggs per cluster and the eggs hatch after approximately 15–20 days (Kendall *et al.*, 1996b). Larvae feed on leaves in aggregations during first and second instars, whereas feeding becomes solitary during the third instar. After passing through three instars, larvae pupate in the soil and the adults emerge in August. After a short period of feeding, adult beetles leave the plants in late season to find hibernation sites under the bark of trees, cracks of wood materials and in the ground vegetation (Kendall & Wiltshire, 1998; Björkman & Eklund, 2006). The three heteropterans: *Orthotylus marginalis* Reuter (Miridae), *Closterotomus fulvomaculatus* De Geer (Miridae), and *Anthocoris nemorum* Linnaeus (Anthocoridae) are common predators of *P. vulgatissima* (Björkman *et al.*, 2003).

The willow *Salix cinerea* grows into bushes 1–4 m high and is characterized by densely hair-covered current-year shoots and inversely egg-shaped (obovate) leaf blades (Jonsell, 2000). In the experiment, 20 stands of *S. cinerea* located in an area close to Uppsala, south central Sweden, were used. The distance between individual stands was at least 1 km and the size of the stands ranged between 10 and 50 m^2. Ten stands were located in mixed forests dominated by conifers (forest habitats), and ten stands were located in open agricultural landscapes (farmland habitats). All stands were growing on relatively wet soils along ditches, wet meadows or damp forest areas. The stands consisted of plants that were 1–3 m high, except in one of the farmland stands, which had been cut down by the landowners during the previous summer and, therefore, mainly consisted of young shoots.

Densities of leaf beetles and heteropteran predators

Densities of leaf beetles and heteropteran predators were measured in each of the 20 willow stands in late May/early June 2003 and 2004. The purpose was to investigate if the previously observed difference in leaf beetle abundance was consistent over several years. The purpose was also to investigate if predator abundance differs between the two habitats. To measure densities, knock-down samples were taken from 35-cm parts of plants containing current-year shoots by knocking off all insects by hand inside a plastic cylinder (length 35 cm, diameter 25 cm). Shoots for sampling were evenly distributed among bushes within a stand in order to reduce possible sampling error due to spatial variability of insect abundance. At least 30 samples per stand were taken (mean = 62, range = 32–81). It has been shown that 22–24 samples are sufficient for obtaining a stable estimate of leaf beetle density in willow stands (Björkman *et al.*, 2004). For each sample, the number of leaf beetles (*P. vulgatissima*) and heteropteran predators (*O. marginalis*, *C. fulvomaculatus* and *A. nemorum*) that had fallen off the shoots inside the cylinder were counted and released again at the base of the plants. Density was calculated as the

average number of individuals per 35-cm parts of plants containing leaves. Because the data did not fulfil the requirements for parametric analyses (not even after data transformation), non-parametric Mann-Whitney U-tests (adjusted for ties) were used to test for differences in densities between the two habitats. Due to flooding, one of the farmland stands could not be sampled properly in 2004 and therefore lacks data from the last year.

Female egg-laying and larval performance

Adults of *P. vulgatissima* were allowed to feed and lay eggs on *S. cinerea* branches inside cages in the field for seven days in late May 2003. In each of the 20 willow stands, four cages containing two males and two females were placed on randomly selected willow branches. The cages used in the experiment were plastic bags (width 30 cm, length 50 cm), which contained many small ventilation holes. The micro-climate in such cages is not significantly different from the outside (Öberg, 2002). Beetles used in the experiment were collected from an outbreak population in a willow plantation (*Salix viminalis*), situated 30 km south of Uppsala. The beetles had recently emerged from winter hibernation and started to mate. All leaves inside the cages were removed after the experiment and dried in an oven (80°C, 48 h) to measure leaf biomass. This was done to investigate if female egg-laying was affected by the amount of leaf resources inside the cages. To test for differences between habitats, nested ANOVAs using type III sum of squares were carried out with the factor 'habitat' as fixed, and the factor 'stand' as a random nested factor (nested within 'habitat'). The mean square for the nested factor was used as error term when calculating *F*-values for the fixed factor (Zar, 1999). Pearson correlation was used to investigate the relationship between female egg-laying and leaf biomass inside cages. Because some beetles escaped or died during the experiment, six of the observations were excluded. Consequently, 74 observations were used in the analyses of female egg-laying.

Larval performance was studied by following larvae enclosed in cages from newly moulted second instars to third instars in the field. Larval groups were collected from a willow plantation (*S. viminalis*), 10 km west of Uppsala. Leaves containing, on average, nine larvae per leaf (range = 6–15 larvae per leaf, *n* = 80 larval groups) were pinned onto leaves of four randomly selected willow branches in each of the 20 willow stands in the middle of June. All naturally occurring eggs and larvae were removed from branches prior to the experiment. Initially, eggs were used instead of larvae and the aim was to follow larvae from hatching to third instars. However, a high proportion of eggs died by unlucky coincidence due to predation inside the cages, although an attempt were made to remove predators from the branches prior to the experiment. Small nymphs of the heteropteran predator species *A. nemorum* and *O. marginalis*, which probably had hatched inside the cages, were found feeding on the eggs. However, in the middle of June, when second instars larvae were put out in cages in the field, natural enemies were large enough to be more easily removed from experimental branches. The larvae were allowed to feed on the plants for 16 days. The percentage of remaining leaf area was at least 50% in all cages at the end of the experiment, indicating that larvae had a surplus of food throughout the experimental period. Larval weight and

proportion of surviving larvae at the end of the experiment were used as measurements of larval performance. Data on larval survival were arc-sine square root transformed before analysis to meet the assumptions of normality and homoscedacity in ANOVA. Larval weight was measured to the nearest 0.1 mg (fresh weight) for every individual larva and the average from each larval group was used as an independent observation in a nested ANOVA (see above). Because some cages were found damaged by the end of the experiment (probably because of heavy wind), data from four cages were excluded from the analyses. Temperature was measured six times daily from 1 May until the 31 July 2003 using data loggers (TINYTALK datalogger, Gemini data loggers Ltd, UK). The purpose was to investigate if the climate differed between the forest and farmland habitats. The data loggers were wrapped in aluminium foil and placed in the central part of the stands at approximately 1 m height under the leaf canopy. Mean day temperatures were compared between habitats using t-tests.

Survival of eggs exposed to natural enemies

To study predation, willow leaves containing fresh eggs (maximum two days old) were pinned onto plants in the field. The eggs used in the experiment were laid by female beetles on willow leaves in the laboratory. In each willow stand, six egg clusters with, on average, 13 eggs per cluster (range = 8–20 eggs per egg cluster; *n* = 120 egg clusters) were pinned onto leaves of six randomly chosen branches in late May 2003. The branches onto which experimental eggs were placed were cleaned from other leaf beetle eggs and the experimental eggs were left exposed to natural enemies for 14 days. The experiment was terminated after 14 days to reduce the risk of eggs hatching. No caged control eggs were used to compare survival with exposed eggs since it seemed impossible to avoid egg predation inside cages (see above). However, as noted in a previous study (Björkman *et al.*, 2003), predated eggs are easy to recognize and distinguish from non-predated eggs. Furthermore, survival of eggs not exposed to natural enemies is normally very high, around 90% (C. Björkman *et al.*, unpublished data).

The predation data did not meet the assumption of normality for ANOVA. Survival of eggs was either high (e.g. 100% for egg clusters that escaped predation), or low (0% in many egg clusters found by predators), resulting in a distribution that looked more dichotomous (binomial) than normal. To allow modelling of data with a distribution other than normal, the proportion of surviving eggs was analysed with a generalized linear model using the logit link function for binomial data (Olsson, 2002). The effects of the two categorical variables 'habitat' and 'site' were analysed with type 1 analysis of logit regression using the factor 'site' as a nested factor. The data were descaled to meet the criteria for assessing the goodness of fit (scaled deviance < 2). To investigate whether the model was appropriate for the data collected, the observed survival values were compared with the values predicted by the model. The maximum difference between observed and predicted survival in willow stands was 0.053, indicating a reasonably good model fit.

Mean egg survival was calculated for each stand to be correlated with predator density using Spearman rank correlations.
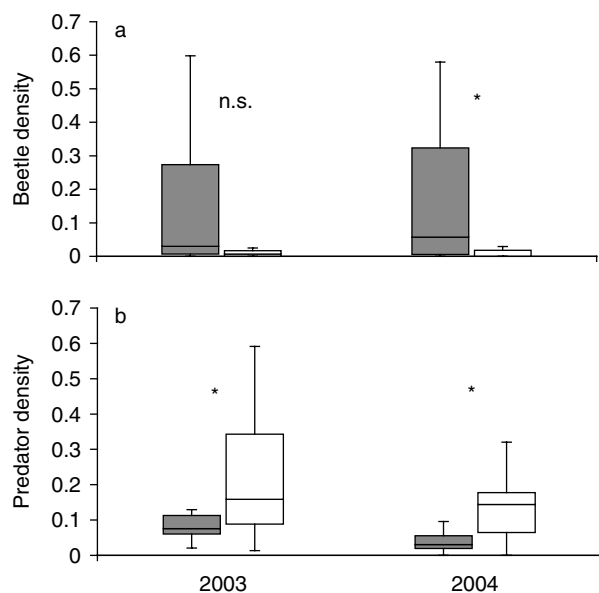
Fig. 1. Box-plots showing densities of the leaf beetle *Phratora vulgatissima* (a) and its most common predator, the mirid *Orthotylus marginalis* (b), over two years in natural stands of *Salix cinerea* growing in forest (■, $n = 10$ stands) and farmland (□, $n = 10$ stands) habitats. Densities are presented on a logarithmic scale (Log_{10} (density + 1)) and illustrate the number of individuals per 35-cm sections of willow shoots containing leaves. Boxes show the interquartile ranges (i.e. 75% data point interval) of densities in the two habitats. Horizontal lines within boxes show the medians. Extreme values are not shown; error bars represent the whiskers (i.e. 95% data point interval). Asterisk indicates statistical significance at the 0.05 level as revealed by Mann-Whitney U-tests.

Results

Densities of leaf beetles and heteropteran predators

Beetle density varied considerably, especially among forest willow stands (fig. 1a). The leaf beetle *Phratora vulgatissima* was more abundant on forest willows than on farmland willows in 2004 (fig. 1a; $U_{9, 10} = 73$, $P = 0.025$). The same trend, although not significant, was found in 2003 (fig. 1a; $U_{10, 10} = 76$, $P = 0.052$). The density of the most common predator, *Orthotylus marginalis*, was higher on farmland willows than on forest willows in both 2003 (fig. 1b; $U_{10, 10} = 82$, $P = 0.017$) and 2004 (fig. 1b; $U_{9, 10} = 74$, $P = 0.022$). However, the lowest density of *O. marginalis* was found in the farmland stand that had been cut down in the previous year. Densities of the other two main predators, *Closterotomus fulvomaculatus* and *Anthocoris nemorum*, did not differ between the forest and the farmland habitats (*C. fulvomaculatus* 2003: $U_{10, 10} = 58$, $P = 0.443$; *A. nemorum* 2003: $U_{10, 10} = 60$, $P = 0.585$; *C. fulvomaculatus* 2004: $U_{9, 10} = 53$, $P = 0.568$; *A. nemorum* 2004: $U_{9, 10} = 66$, $P = 0.094$).

Female egg-laying and larval performance

There was no significant difference between forest and farmland habitats in the number of eggs laid by female leaf beetles (table 1). Mean number of eggs laid was 44 ± 5 (mean ± SE) in the forest, and 46 ± 6 (mean ± SE) in the

Table 1. ANOVAs on results estimating the effects of host plant quality on the leaf beetle *Phratora vulgatissima* studied in willow stands (*Salix cinerea*) growing in two types of habitats: forest and farmland.

Effect	df	MS	F	P
Female egg-laying				
Habitat	1	252.284	0.25	0.626
Stand (habitat)	18	1025.131	1.02	0.450
Error	54	1001.332		
Larval survival				
Habitat	1	0.857	3.00	0.101
Stand (habitat)	18	0.286	2.77	0.002
Error	56	0.103		
Larval weight				
Habitat	1	$1.962 \cdot 10^{-5}$	4.06	0.059
Stand (habitat)	18	$0.484 \cdot 10^{-5}$	3.94	< 0.001
Error	48	$0.123 \cdot 10^{-5}$		

Larval survival (proportion surviving larvae) arc-sine square-root transformed before analysis. The factor 'stand' nested within 'habitat'.

farmland. In addition, there was no significant stand effect in the analysis of female egg-laying (table 1). Leaf biomass inside cages did not differ between habitats ($F_{1, 18} = 0.46$, $P = 0.506$), but there was a significant stand effect ($F_{18, 54} = 3.86$, $P < 0.001$). However, the amount of leaf resources did not seem to influence female egg-laying since no correlation was found between leaf biomass and the number of eggs laid ($R_P = -0.012$, $n = 74$, $P = 0.921$).

No difference in larval survival was detected for caged larvae between habitats (table 1). Mean survival was 0.79 ± 0.05 (mean ± SE) in the forest, and 0.67 ± 0.04 (mean ± SE) in farmland. A significant stand effect was detected in the analysis of larval survival (table 1). Every individual larva that survived to the end of the experiment had moulted to the third instar. There was no significant difference in larval weight between forest and farmland habitats (table 1). Mean weight of larvae was 8.5 ± 0.3 mg (mean ± SE) in the forest, and 9.3 ± 0.2 mg (mean ± SE) in the farmland. However, similar to larval survival, a significant stand effect was detected in the analysis of larval weight (table 1). There was no significant relationship between performance parameters: survival and weight ($Rp = -0.135$, $n = 68$, $P = 0.271$). No significant relationship was found between the number of larvae released inside cages and larval survival ($R_S = 0.144$, $n = 76$, $P = 0.215$), or between number of larvae and their weight at the end of experiment ($R_S = -0.060$, $n = 68$, $P = 0.626$).

Mean day temperatures were lower in the forest habitat ($9.94 \pm 0.09°C$ (mean ± SE)) than in the farmland habitat ($10.47 \pm 0.10°C$ (mean ± SE)) in May 2003 ($t = 4.13$, $P = 0.001$). The maximum day temperatures measured were on average 1.31°C higher in the farmland habitat in May (average daytime maximum for May: $15.04 \pm 0.77°C$ and $16.34 \pm 0.79°C$ in forest and farmland, respectively). The lowest temperatures measured during night were, however, similar in the two habitats (average for May: $4.90 \pm 0.57°C$ in the forest, and $5.06 \pm 0.57°C$ in the farmland). In June and July, no significant differences in mean day temperatures were found between habitats (June: $t = 1.53$, $P = 0.147$; July: $t = 1.25$, $P = 0.233$). In June, mean day temperatures were $13.85 \pm 0.08°C$ (mean ± SE) in the forest, and $14.04 \pm 0.10°C$ (mean ± SE) in
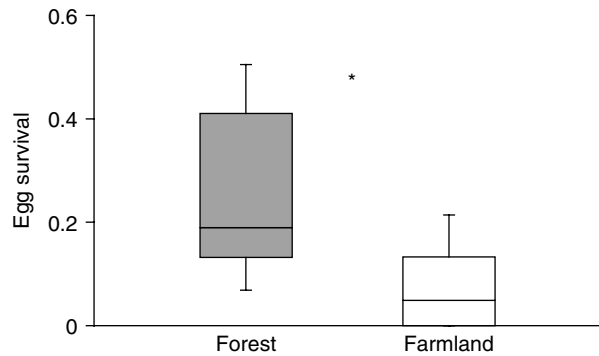
Fig. 2. Proportion of leaf beetle *Phratora vulgatissima* eggs that survived 14 days in the field when exposed to natural enemies in natural stands of *Salix cinerea* growing in forest ($n = 10$ stands) and farmland ($n = 10$ stands) habitats. Box-plots show the interquartile ranges of egg survival (i.e. 75% data point interval) in the two habitats with the horizontal line in each box representing the medians. Error bars below and above the boxes are the whiskers (i.e. 95% data point interval). Asterisk indicates statistical difference at the 0.05 level as revealed by Type 1 logit linear regression model.

the farmland. The maximum day temperatures during June were similar in the in the two habitats (average for June: $18.33 \pm 0.70°C$ in the forest, and $18.50 \pm 0.69°C$ in the farmland). Also, the lowest temperatures measured were almost identical in June ($9.22 \pm 0.50°C$ and $9.20 \pm 0.52°C$ for forest and farmland, respectively). In July, mean day temperatures were $18.63 \pm 0.09°C$ (mean $\pm$ SE) in the forest, and $18.43 \pm 0.13°C$ (mean $\pm$ SE) in the farmland. Both maximum day temperatures ($23.63 \pm 0.67°C$ and $23.36 \pm 0.63°C$ for forest and farmland, respectively) and lowest night temperatures ($13.61 \pm 0.42°C$ for forest, and $13.20 \pm 0.43°C$ for farmland) were similar in the two habitats for July 2003.

Survival of eggs exposed to natural enemies

The survival of eggs exposed to natural enemies was higher in the forest than in the farmland habitat (fig. 2, $F_{1,1} = 4.41$, $P = 0.033$). In addition, there was a significant stand effect in the analysis of egg survival ($F_{18,18} = 2.12$, $P = 0.004$). The highest survival was observed in the farmland stand that had been cut down the previous year and contained the lowest density of predators. When calculating mean survival for each stand to be correlated with predator density, the survival of eggs was negatively correlated with the density of heteropteran predators (fig. 3; $R_S = -0.681$, $n = 20$, $P = 0.001$). Survival of eggs was mainly correlated with the most common predator, the mirid *O. marginalis* ($R_S = -0.680$, $n = 20$, $P = 0.001$). All three predators, *O. marginalis*, *C. fulvomaculatus* and *A. nemorum* were observed to feed on experimental eggs in the field, but no other enemies were observed attacking the eggs.

Discussion

Consistent with observations in previous years (Dalin, 2004), high population densities of the leaf beetle *Phratora vulgatissima* were only found on *Salix cinerea* growing in forest habitats. Plant quality did not seem to explain the generally low abundance of leaf beetles on farmland
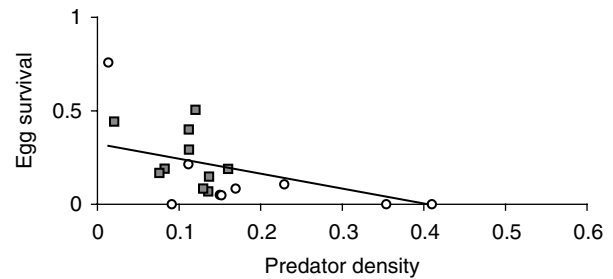

Fig. 3. Correlation between predator density (densities of the three heteropteran predators *Orthotylus marginalis*, *Closterotomus fulvomaculatus* and *Anthocoris nemorum* added together) and the survival of leaf beetle *Phratora vulgatissima* eggs studied on *Salix cinerea* growing in forest (■) and farmland (○) habitats. Predator density presented on a logarithmic scale (Log_{10} (density $+ 1$)).

willows. Female beetles laid equal numbers of eggs and larvae performed equally well on *S. cinerea* in the two habitats. The most common natural enemy, the mirid predator *Orthotylus marginalis*, was more abundant on farmland willows than on forest willows. Also, the predation rate on eggs was higher in the farmland habitat. The results suggest that the low abundance of leaf beetles on farmland willows is most likely explained by high predation, and not by poor plant quality.

The experimental results gave no support for the hypothesis that plants should be of superior quality in the forest habitat. The design of the experiment contains, however, a number of potentially confounding factors that need to be discussed before rejecting this hypothesis. Firstly, female egg-laying was only tested in cages, implying that female beetles had no choice to lay eggs on plants differing in quality within stands. Secondly, the beetles were reared on another willow species (*S. viminalis*) prior to experimentation, suggesting that there could be carryover effects from natal host plants. Lastly, larval performance was tested on newly moulted second instar larvae, whereas greater effects might have been detected if larvae had been studied from hatching (even though first instar larvae moult to second instar only a few days after hatching). Although these potentially confounding factors might have obscured differences between habitats, there is nothing in the data suggesting that farmland willows, by definition, should be of poor quality for the leaf beetle *P. vulgatissima*. There was rather a trend for a greater larval weight in the farmland habitat (table 1; $P = 0.059$). In addition, larval performance was studied over a relatively long time period (16 days), which should reduce potential carry-over effects from natal host plants. There were, however, significant stand effects in the analyses of both larval weight and survival. It is known that willow plants can vary in quality for *P. vulgatissima* when growing under different environmental conditions (Glynn *et al.*, 2004). Waterlogging may, for example, influence the levels of water, nitrogen and secondary metabolites in willow plants (Sipura *et al.*, 2002). It is also well known that *P. vulgatissima* avoids feeding on willow genotypes containing high concentrations of phenolic glycosides (Kolehmainen *et al.*, 1995). Thus, plant quality probably influences *P. vulgatissima* in the present system but does not seem to explain the low abundance of leaf beetles on farmland willows.

The mirid *O. marginalis* was the most common predator and the survival of *P. vulgatissima* eggs was negatively

correlated with its abundance. The predator was more abundant on farmland willows and is probably the main reason for the difference in egg survival between the two habitats. Although there is little knowledge about why the mirid *O. marginalis* was more abundant on farmland willows than on forest willows, the species is a broad diet omnivorous predator that probably can feed and survive on other prey and plant items when leaf beetle abundance is low on farmland willows. The lowest density of *O. marginalis* was found on the farmland stand that had been cut down the previous summer. It is known that harvesting of willow plants often results in a reduced density of heteropteran predators (Björkman *et al.*, 2004). However, due to compensatory factors, a reduced enemy attack rate does not necessarily translate into increased herbivore density (Chase, 1996; Stiling & Moon, 2005). In the present study, there were several willow stands with relatively low densities of both heteropteran predators and leaf beetles. Other factors than heteropteran predators and host plant quality that might influence the abundance of *P. vulgatissima* include the availability of over-wintering sites (e.g. larger trees) and how isolated the willow stands are in the landscapes. Forest willows are surrounded by many trees and, thereby, many possible over-wintering sites. The willow *S. cinerea* may also grow more continuously in forests compared with the rather patchy distribution of plants in the farmland habitats. However, although not studied here, the dispersal capacity of *P. vulgatissima* seems to be sufficiently good for beetles to also colonize relatively isolated willow stands. For example, *P. vulgatissima* sometimes occur at high densities in willow plantations, which can be more or less isolated in agricultural landscapes. In addition, the leaf beetle can be found overwintering in many types of substrates, such as cracks of fence posts, dried reed stems and in the ground vegetation in the vicinity of willow plantations (Kendall & Wiltshire, 1998; Björkman & Eklund, 2006). Thus, the indications of a reasonably good dispersal capacity, and the ability to use many types of hibernation substrates, imply that these factors are not likely to explain the low abundance of leaf beetles on farmland natural willows. However, it is possible that populations take longer to build up in density in isolated willow stands compared with stands growing in landscapes with beetles in the close vicinity.

Other types of natural enemies than heteropteran predators might also have influenced the patterns of leaf beetle abundance. The three heteropteran species *O. marginalis*, *C. fulvomaculatus* and *A. nemorum* have been shown to be among the most important enemies of *P. vulgatissima* in willow plantations (Björkman *et al.*, 2003). Parasitism rates are, for example, extremely low in the system (Maisner, 1974). Generalist predators, such as spiders, ants and ladybirds, occurred on the willows but were never observed attacking the eggs. One study suggests that ants might be important predators of larvae of the willow leaf beetle *Galerucella lineola* Fabricius (Sipura, 2002). Ants can be common on forest willows (P. Dalin, unpublished data) and it is possible that larval predation by ants could explain some of the variation in beetle abundance found among the forest willow stands. Further studies are, therefore, needed that also include predation on other life stages than eggs of *P. vulgatissima*.

Climatic factors are also likely to influence the distribution and abundance of herbivorous insects (Azerefegne *et al.*, 2001). For example, the willow leaf beetle *G. lineola*, which is a common herbivore on *S. cinerea* in the study area, was shown to occur at higher densities on willows (*S. phylicifolia*) growing in damp habitats than on plants growing in dry habitats (Sipura *et al.*, 2002). This habitat difference in leaf beetle abundance was not explained by differences in predation, nor in plant quality, but rather because larvae suffered from desiccation in dry habitats (Sipura *et al.*, 2002). Although the farmland habitat was slightly warmer than the forest habitat early in the season, there were no indications that beetles suffered from desiccation in the present study. All willows were growing on relatively wet soils, indicating similarities in humidity. This highlights that the relative importance of various factors may differ for insects with similar biology, feeding on the same type of host plants, when studied under different circumstances.

The purpose of the present study was to evaluate the relative importance of host plant quality and natural enemies on the abundance of *P. vulgatissima* in natural stands of *S. cinerea*. Although a good correspondence was found between high predation rates by heteropterans and low abundance of leaf beetles, further studies are needed to test whether heteropteran predators actually can control beetle abundance. In addition, plant quality may become more important when studied under other circumstances; for example, when comparing beetle abundance among different willow species (Kelly & Curry, 1991; Kendall *et al.*, 1996a), or when plants are growing under more extreme conditions (Lower *et al.*, 2003). Also, variations at smaller spatial scales, e.g. within willow stands, might be influenced by the aggregative behaviour of *P. vulgatissima* (Peacock *et al.*, 2001). It is also likely that *P. vulgatissima* is limited in certain habitats because of climatic factors; e.g. suffer from desiccation in dry areas (Sipura *et al.*, 2002). To conclude, the results of this study suggest that heteropteran predators influence the abundance of leaf beetles on natural willows.

References

Azerfegne, F., Solbreck, C. & Ives, A.R. (2001) Environmental forcing and high amplitude fluctuations in the population dynamics of the tropical butterfly *Acraea acerata* (Lepidoptera: Nymphalidae). *Journal of Animal Ecology* **70**, 1032–1045.

Bernays, E.A. & Chapman, R.F. (1994) *Host-plant selection by phytophagous insects*. 312 pp. New York, Chapman and Hall.

Berryman, A.A. (*Ed.*) (1988) *Dynamics of forest insect populations: patterns, causes, implications*. 624 pp. New York, Plenum Press.

Björkman, C. & Eklund, K. (2006) Factors affecting willow leaf beetles (*Phratora vulgatissima*) when selecting overwintering sites. *Agricultural and Forest Entomology* **8**, 97–101.

Björkman, C., Kytö, M., Larsson, S. & Niemelä, P. (1998) Different responses of two carbon-based defences in Scots pine needles to nitrogen fertilization. *Ecoscience* **5**, 502–507.

Björkman, C., Bengtsson, B. & Häggström, H. (2000) Localized outbreak of a willow leaf beetle: plant vigor or natural enemies? *Population Ecology* **42**, 91–96.

Björkman, C., Dalin, P. & Eklund, K. (2003) Generalist natural enemies of a willow leaf beetle (*Phratora vulgatissima*): abundance and feeding habits. *Journal of Insect Behaviour* **16**, 747–764.

Björkman, C., Bommarco, R., Eklund, K. & Höglund, S. (2004) Harvesting disrupts biological control of herbivores in a short-rotation coppice system. *Ecological Applications* **14**, 1624–1633.

Chase, J.M. (1996) Abiotic controls of trophic cascades in a simple grassland food chain. *Oikos* **77**, 495–506.

Dalin, P. (2004) *Food-web interactions and population variability of leaf beetles in managed and natural willow stands*. PhD thesis, Silvestria 303. Uppsala, Swedish University of Agricultural Sciences.

Glynn, C., Rönnberg-Wästljung, A.-C., Julkunen-Tiitto, R. & Weih, M. (2004) Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomologia Experimentalis et Applicata* **113**, 1–14.

Gratton, C. & Denno, R.F. (2003) Seasonal shift from bottom-up to top-down impact in phytophagous insect populations. *Oecologia* **134**, 487–495.

Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960) Community structure, population control, and competition. *American Naturalist* **94**, 421–425.

Hawkins, B.A. (2001) Top-down and bottom-up forces in the population and community ecology of insects. *Basic and Applied Ecology* **2**, 293–294.

Jonsell, B. (*Ed*.) (2000) *Flora Nordica*. 368 pp. Stockholm, Bergius Foundation.

Kelly, M.T. & Curry, J.P. (1991) The influence of phenolic compounds on the suitability of three *Salix* species as hosts for the willow beetle *Phratora vulgatissima*. *Entomologia Experimentalis et Applicata* **61**, 25–32.

Kendall, D.A. & Wiltshire, C.W. (1998) Life-cycles and ecology of willow leaf beetles on *Salix viminalis* in England. *European Journal of Forest Pathology* **28**, 281–288.

Kendall, D.A., Hunter, T., Arnold, G.M., Liggitt, J., Morris, T. & Wiltshire, C.W. (1996a) Susceptibility of willow clones (*Salix* spp.) to herbivory by *Phyllodecta vulgatissima* (L.) and *Galerucella lineola* (Fab.) (Coleoptera, Chrysomelidae). *Annals of Applied Biology* **129**, 379–390.

Kendall, D.A., Wiltshire, C.W. & Butcher, M. (1996b) *Phenology and population dynamics of willow leaf beetles (Coleoptera; Chrysomelidae) in short-rotation coppiced willows at Long-Ashton*. ETSU (DTI) Biofuels Study B/M4/00487/14/REP. Bristol, IACR Long Ashton Research Station.

Kidd, N.A.C. & Jervis, M.A. (1997) The impact of parasitoids and predators on forest insect populations. pp. 49–68 *in* Watt, A.S., Stork, N.E. & Hunter, M.D. (*Eds*) *Forests and insects*. London, Chapman and Hall.

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

Koricheva, J., Larsson, S., Haukioja, E. & Keinänen, M. (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos* **83**, 212–226.

Larsson, S., Ekbom, B. & Björkman, C. (2000) Influence of plant quality on pine sawfly population dynamics. *Oikos* **89**, 440–450.

Louda, S.M., Farris, M.A. & Blua, M.J. (1987) Variation in methylglucosinolate and insect damage to *Cleome serrulata* (Capparaceae) along a natural soil moisture gradient. *Journal of Chemical Ecology* **13**, 569–581.

Lower, S.L., Kirshenbaum, S. & Orians, C.M. (2003) Preference and performance of a willow-feeding leaf beetle: soil nutrients and flooding effects on host quality. *Oecologia* **136**, 402–411.

Maisner, N. (1974) Chrysomelidae, Blattkäfer. pp. 202–236 *in* Schwenke, W. (*Ed*.) *Die Forstschädlinge Europas*, vol. 2. Hamburg, Parey.

Maron, J.L., Harrison, S. & Greaves, M. (2001) Origin of an insect outbreak: escape in space or time from natural enemies? *Oecologia* **126**, 595–602.

Öberg, S. (2002) *Interactions between two ladybird species attacking a host alternating aphid*. Master thesis, 52, Department of Ecology and Crop Production Science, Uppsala, Swedish University of Agricultural Sciences.

Olsson, U. (2002) *Generalized linear models – an applied approach*. 232 pp. Lund, Studentlitteratur.

Peacock, L., Lewis, M. & Herrick, S. (2001) Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima*. *Entomologia Experimentalis et Applicata* **98**, 195–210.

Peacock, L., Harris, J. & Powers, S. (2004) Effects of host variety on blue willow beetle *Phratora vulgatissima* performance. *Annals of Applied Biology* **144**, 45–52.

Preszler, R.W. & Boecklen, W.J. (1996) The influence of elevation on tri-trophic interactions: opposing gradients of top-down and bottom-up effects on a leaf-mining moth. *Ecoscience* **3**, 75–80.

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

Rhoades, D.F. (1983) Herbivore population dynamics and plant chemistry. pp. 155–220 *in* Denno, R.F. & McClure, M.S. (*Eds*) *Variable plants and herbivores in natural and managed systems*. New York, Academic Press.

Schoonhoven, L.M., Jermy, T. & van Loon, J.J.A. (1998) *Insect-plant biology*. 409 pp. London, Chapman and Hall.

Sipura, M. (2002) Contrasting effects of ants on the herbivory and growth of two willow species. *Ecology* **83**, 2680–2690.

Sipura, M., Ikonen, A., Tahvanainen, J. & Roininen, H. (2002) Why does the leaf beetle *Galerucella lineola* F. attack wetland willows? *Ecology* **83**, 3393–3407.

Stiling, P. & Moon, D. (2005) Are trophodynamic models worth their salt? Top-down and bottom-up effects along a salinity gradient. *Ecology* **86**, 1730–1736.

Symondson, W.O.C., Sunderland, K.D. & Greenstone, M.H. (2002) Can generalist predators be effective biocontrol agents? *Annual Review of Entomology* **47**, 561–594.

Walker, M. & Jones, T.H. (2001) Relative roles of top-down and bottom-up forces in terrestrial tritrophic plant–insect herbivore–natural enemy systems. *Oikos* **93**, 177–187.

Zar, J.H. (1999) *Biostatistical analysis*. 4th edn. 663 pp. New Jersey, Prentice Hall.