Differential performance of two geometrids on previously defoliated Scots pine

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We studied the effect of previous defoliations of *Pinus sylvestris* on the performance of two geometrid moths, *Bupalus piniarius* and *Macaria liturata*, using a field experiment. Prior to the experiment, we found defoliation to increase the concentrations of certain secondary chemicals and reduce the N (nitrogen) concentration of needles. Hence, it was predicted that defoliation-induced changes should decrease the performance of geometrids feeding on *P. sylvestris*. As *B. piniarius* is a monophagous species on *P. sylvestris*, and *M. liturata* is oligophagous feeding also on *Larix* and *Juniperus* species, we predicted that *M. liturata* should suffer more than *B. piniarius* from defoliation-induced changes in needle chemistry. As predicted, defoliation had a negative effect on the performance, the oligophagous *M. liturata* being more vulnerable. Defoliation resulted in decreased sex-specific survival of *M. liturata*, but did not affect the survival of *B. piniarius*. In *M. liturata*, no males survived to the pupal stage in previously defoliated trees. Defoliation also resulted in lower pupal weights in both species. We conclude that defoliation-induced changes on the performance of insects with different host ranges merit more attention.

Introduction

Defoliation often causes changes in the chemistry of plant foliage. In conifers, however, the evidence of the negative impact of induced response on the performance of herbivores is relatively scarce and contradictory (Niemelä *et al.* 1984, 1991, Leather *et al.* 1987, Niemelä & Tuomi 1993, Lyytikäinen 1994, Raffa *et al.* 1998, Šmits & Larsson 1999, Hódar *et al.* 2004, Nykänen & Koricheva 2004). These divergent results may indicate variation in the inducible resistance of conifers, but also reflect differential sensitivity of herbivore species to changes in the needle chemistry (*see* e.g. Niemelä *et al.* 1991).

Induced resistance is a phenomenon in which changes in plants following damage or stress reduce herbivore survival, reproductive output

or preference for a plant (Karban & Baldwin 1997). The tolerance of a herbivore to induced resistance is known to depend on its degree of specialisation (Berenbaum & Zangerl l999). In general, if a species is monophagous, it has to develop a higher tolerance for the host-specific defensive chemicals than oligophagous and polyphagous species. The choice of a host plant is made mainly by a female during oviposition and, according to Nylin and Janz (1999), oligophagous species may express a clear-cut preference towards different plant species. Although this preference rank within species is regarded fairly conservative, it does sometimes change during evolution (Singer *et al.* 1992). A female may use chemical cues for host plant selection (*see* e.g. Renwick & Chew 1994) and select host plants that are either higher in nutrients or lower in defensive chemicals. If the selection of host plants covers several species, there is naturally less evolutionary pressure for tolerance against some defensive chemicals characteristic to a single host species.

In order to study the impact of defoliation-induced changes in Scots pine *Pinus sylvestris* L. foliage on the performance of two geometrid moths, *Bupalus piniarius* L. and *Macaria liturata* Clerck, we reared larvae on pines that were artificially defoliated in two consecutive summers prior to the experiment. In the autumn of 1999 defoliation resulted in significantly increased concentrations of certain phenolics and 20% lower nitrogen (N) concentration in the needles (Roitto *et al.* 2003). Corresponding analyses conducted in August 2000 indicated that, of the nine flavonoids detected in the previous-year needles, quercetin-3-rhamnoside (quercitrin), quercetin-3-galactoside (hyperin), myricetin-3-galactoside and monocoumaroyl-isoquercitrin were still significantly elevated by 94%, 89%, 70% and 71%, respectively, as compared with controls. No further difference between the defoliated and control trees in respect to foliar N was detected, as N concentration showed only slight non-significant reduction by 7% (M. Roitto *et al.* unpubl. data). We predicted that if these changes reflect induced resistance in the studied pines the performance of larvae should be worse on defoliated trees than on control trees. Moreover, as *B. piniarius* is a specialist on *P. sylvestris*, defoliation should cause a larger negative effect on the performance of oligophagous *M. liturata* than monophagous *B. piniarus* (*see* e.g. Nykänen & Koricheva 2004).

Materials and methods

The study was carried out in northern Finland on the island of Hailuoto (65°03´N, 24°36´E) where thirty 8–25-year-old and 2-m tall pines were selected for the experiment. From these 30 trees, 15 randomly selected ones were defoliated using scissors after annual shoot had elongated fully, between 22 and 26 June 1998 (Kuikka *et al.* 2003). All needles except the current developing ones were clipped totally off simulating herbivory by pine sawfly (Diprionidae) larvae which are the most common natural defoliators of Scots pine in northern Europe (Viitasaari & Varama 1987). Previous studies have demonstrated similar responses to natural *vs.* artificial defoliation among evergreen coniferous trees (Lyytikäinen 1992, Raffa *et al.* 1998), although some deciduous species are sensitive to the agent of defoliation (Karban & Baldwin 1997). Defoliation of the same study trees was repeated between 21 and 26 June 1999. Fifteen undefoliated trees served as controls.

Study species *Bupalus piniarius* and *M. liturata* are univoltine moth species, the adults of which fly in the study area mainly in June. Larvae forage from June to September prior to pupating in soil. *B. piniarius* is a monophage in our study area feeding only on *P. sylvestris* whereas *M. liturata* is an oligophage that feeds mainly on *P. sylvestris* but also on *Juniperus communis* and *Larix* spp.

We collected adult moths from different locations of Hailuoto and brought them to the laboratory where one male and one female from the same location were placed in a plastic box containing a pine branch as an oviposition site. Neonate larvae were transported to the field on 28 and 29 June 2000. The same number of larvae was placed on control and defoliated trees and 90 larvae of *B. piniarius* from 10 females and 74 of *M. liturata* from 4 females were used in the experiment. One larva was placed on a branch that was enclosed in a mesh bag. Enclosed

branches had all the needles formed during the previous growing season and new developing needles. Pupated specimens and some still at the larval stage were taken to the laboratory at the end of September. If the larvae were not yet pupated (few larvae of both species) the shoot was cut off and placed in the bottle filled with water and a larva on the shoot enclosed in a mesh bag was reared in the laboratory at room temperature to the pupal stage. Survival and the pupal weight were measured immediately after transportation to the laboratory, or if pupation occurred in the laboratory, five days after pupation.

Survival of the moth larvae was analysed as a generalized linear mixed model (GLMM) using a penalized quasi-likelihood, quasi-binomial link-function and F-statistic for the analysis of deviance change in R statistical package (Ihaka & Gentleman 1996, Venables & Ripley 2002). The counts of the living and dead larvae per tree formed a binomial response variable. Model fitting was carried out sequentially starting from a model containing the random variable (female nested in species), which forms the intercept and ending with a model:

$$Y(\text{alive, dead}) \approx \text{female (species)} + \text{species} + \text{treatment} + \text{treatment} \times \text{species}$$

Further, contrasts in the GLMM analysis were constructed to find out if the survival of larvae differs in control *vs.* defoliated trees. As the sex of the dead larvae was not determined we could not test its potential impact on larval survival.

In order to find out whether the defoliation had a statistically significant effect on pupal weight, we tested the data using a mixed effects nested ANOVA model where treatment (control *vs.* defoliation), species, and sex were grouping factors. Furthermore, because the larvae were reared from every single female on both control and defoliated trees we considered 'female' in the ANOVA model as a blocking (random) factor (nested within the species). Of the collected 14 females, 71 larvae of 13 females (nine *B. piniarus* and four *M. liturata*) survived (Fig. 2). ANOVA was performed using the tree-specific means for each female (usually several larvae

per female within a tree survived to the pupal state). The number of trees in which survived larvae were found varied from 1 to 4 per female. Due to the complete absence of males of *M. liturata* on defoliated trees (only females survived to pupae) we performed ANOVA by using SS type IV (Tabachnick & Fidell 1996). Furthermore, we did not include species × sex interaction and treatment × sex interaction terms in the ANOVA model:

$$Y = \mu + \text{female (species)} + \text{species} + \text{treatment} + \text{sex} + \text{treatment} \times \text{species} + \varepsilon$$

where 'female' (nested within the species) is used as the error term for the species term, while the other terms are tested against ε (residual variation).

Because males of *M. liturata* did not survive to the pupal stage and because all studied main effects (treatment, species and sex) were significant (Table 2), the significance of difference in weight of pupae grown in defoliated *vs.* control trees was tested separately for sexes within both studied species with the one-tail *t*-test (H_0: no reduction in pupal size of specimens reared on defoliated trees, H_1: pupae from larvae grown in defoliated trees are lighter than those grown in control trees). The results of these comparisons are reported in Fig. 2. Statistical significance ($\alpha < 0.05$) from these comparisons was corrected for three comparisons, according to the sequential Bonferroni method (aka Dunn-Sidák; Sokal & Rohlf 1995).

Results

Defoliation had a major impact on the survival of moth larvae. Treatment itself had no statistically significant main effect as there was a strong interaction between moth species and treatment on survival of larvae (Table 1) due to lower survival of *M. liturata* than *B. piniarius* on defoliated trees (Fig. 1). On the basis of contrasts in the GLMM analysis the survival of *M. liturata* on defoliated trees was significantly lower ($t_{19} = -3.19$, $p = 0.0048$) than predicted on the basis of the main effects of species and defoliation.
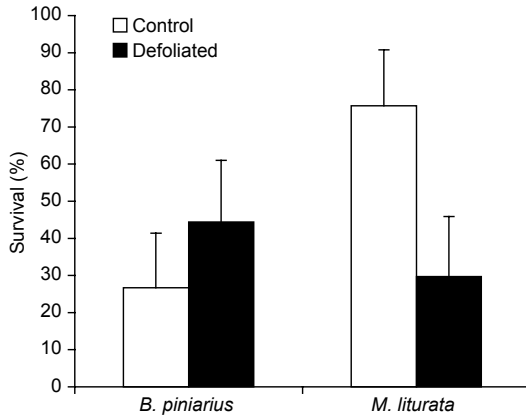
Defoliation had also a significant effect on

Fig. 1. Mean ± 1 S.E. survival (%) of *B. piniarius* and *M. liturata* larvae on defoliated and control trees.

pupal weights (Table 2 and Fig. 2). Females of *M. liturata* grew bigger on control trees than on defoliated trees. Comparisons of pupal weights of *M. liturata* males were not possible because males living on defoliated trees did not survive to the pupal stage. Pupal weights of *B. piniarius* males were significantly lower on defoliated trees than on control ones. There was a similar trend for females but the difference was not statistically significant (Fig. 2).

Discussion

As a result of defoliation larvae performed less well on defoliated trees than on undefoliated trees. This is either due to change in the needle chemistry of the defoliated pines or alternatively larval performance responded to changes in the relative availability of different needle age-classes (*see* Šmits & Larsson 1999). Our results are in contrast with those of Šmits and Larsson (1999), who studied the performance of *B. pin-*

iarius on naturally defoliated Scots pines. In their study, N concentration in the needles was 38% higher on previously defoliated trees as compared with that in the needles of trees that had escaped from *B. piniarius* damage. Accordingly, in the study by Niemelä *et al.* (1991), artificial defoliation tended to increase N concentration and decrease dry matter content of Scots pine needles. In contrast, in our study defoliation resulted in reduced foliar N (Roitto *et al.* 2003; M. Roitto *et al.* unpubl.). The outcome of defoliation may depend on a number of external factors such as plant type (deciduous *vs.* evergreen), growth rate, the type and timing of damage and the ability to recover lost nutrients (e.g. Niemelä & Tuomi 1993, Tuomi *et al.* 1990, Nykänen & Koricheva 2004). The nutrient concentration in the sandy soil of our study area is very low (N concentration below the accurate detection level of 0.01% dry mineral soil) (Kuikka *et al.* 2003). Defoliation in two successive years may have depleted a major part of nutrient reserves in the older needles leading to growth reduction by nutrient limitation and increased allocation of carbon to secondary chemicals. The suggestion of Tuomi *et al.* (1984, 1988, 1990) in the case of mountain birch that, in nutrient-poor conditions, intense defoliation may deplete nitrogen reserves more than carbon reserves may in fact be applicable to our pines too. The recovery of carbon reserves in pines growing in an open sandy habitat in latitudes with long daylight hours during the growing season may be profoundly faster than, for example, in conditions where plants are competing for light (i.e. carbon). Although, as predicted, oligophagous *M. liturata* suffered more from defoliation than monophagous *B. piniarius*, one has to be careful in doing generalisations between responses of monophagous and oligophagous species as our results are based only on one species pair.

Table 1. The GLMM model for larval survival on defoliated *vs.* control trees. The variables are fitted sequentially in the order given.

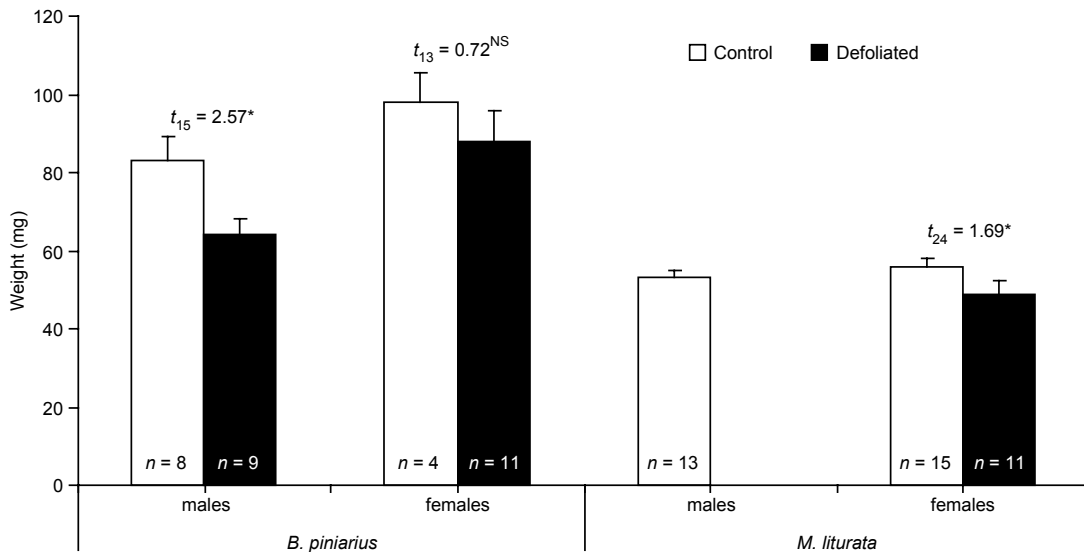|	df (numerator)	df (denominator)	*F*	*p*
Intercept [inc.Female(Species)]	1	19	1.46	0.24
Species	1	11	1.73	0.22
Treatment	1	19	0.58	0.46
Treatment × Species	1	19	10.2	0.0048

Fig. 2. Mean (± S.E.) of pupal weights (in mg) of *B. piniarius* and *M. liturata* on defoliated and control trees. * stands for $\alpha < 0.05$ according to Dunn-Sidák method, NS = not significant. Numbers within bars indicate the number of survived larvae.

Only females of oligophagous *M. liturata* were able to survive to the pupal stage on defoliated trees whereas the sex ratio of the species was even on control trees, which, on the basis of the survival data, was due to increased mortality of males. In Lepidoptera, females are generally bigger than males and if this difference occurs already at the egg stage, it may have a remarkable impact on sex-dependent survival on tough pine needles as in general bigger eggs contain bigger larvae with wider head capsules and mandibles (e.g. Braby 1994). It is possible that when some repellent or deterrent is present in foliage in large quantities, larvae are at first reluctant to feed on needles and their development is hindered. When needles mature and become tougher, small larvae (mainly males) are no longer able to feed on them due to their small mandibles. This idea is supported by Leather and Burnand (1987) who found that *Panolis flammea* females laid many small eggs on "good" hosts whereas "poor" hosts received fewer but larger eggs. The results of Braby's (1994) study on *Mycalesis terminus* and *M. sirius* showed that heavier eggs with bigger neonate larvae had a much better chance of reaching adulthood on tougher hosts than lighter eggs with smaller larvae. Accordingly, Niemelä *et al.* (1991) found that most of the larval mortality of

diprionid sawflies on previously defoliated Scots pine took place within a few days at the beginning of experiments when small larvae easily stick to the resinous surface of the needles.

Phenolic secondary chemicals may have variable effects on insect herbivores. First, several flavonoid glycosides, such as quercetin derivatives, modify insect feeding by either deterring or stimulating it, but the effect may be dependent on the specific compound, its concentration and the species and age of the insect (Simmonds 2001). For example, rutin (quercetin-3-*O*-rhamnosyl-1,6-glucoside) in high concentrations was reported to deter *Heliothis zea* and *Heliocoverpa armigera* larvae from feeding, whereas in low

Table 2. ANOVA for pupal weights of geometrid moths on defoliated and control trees.

Source	df	F	p
Species +	1	20.28	0.001
Female (species)	11	2.4	0.037
Treatment	1	7.26	0.013
Sex	1	8.95	0.007
Treatment × species	1	0.11	0.916
Error	23		

+ For species, the female (nested within species) is used as the error term.

concentrations it stimulated feeding (Simmonds 2001). Second, flavonoids may have direct toxic effects on larval survival and growth. Thus, the growth of gypsy moth (*Lymantria dispar*) was reduced by quercetin and its derivatives rutin (quercetin-3-*O*-rhamnosyl-1,6-glucoside) and quercetin-*O*-glucoside (Beninger & Abou-Said 1997). Unfortunately, no published data are available on the effects of the specific flavonoid compounds on the performance of geometrid larvae used in this experiment.

Although many studies have reported induced resistance in conifers, in relatively few cases damage to needles have caused induced resistance (Niemelä & Tuomi 1993, Karban & Baldwin 1997). Moreover, several studies which have been carried out with different species of needle-feeding sawflies have not found significant deleterious effects of defoliation on insect performance (Niemelä *et al.* 1984, 1991, Lyytikäinen 1994, Raffa *et al.* 1998). According to Géri *et al.* (1993) Diprionidae seem to be more tolerant of phenolics and defoliations than other insect families. For example, Watt (1987) reported that survival of *Panolis flammea* on Scots pine decreased during the shoot and needle growth phase, whereas sawflies generally exhibit a preference for older foliage (Géri *et al.* 1993). Results parallel to this study have been reported by Harris (1960) and Leather *et al.* (1987) in studies on the noctuid moth *Panolis flammea* and tortricid moth *Rhyacionia buoliana*. Their results indicated that larval growth and survival were lower on defoliated *Pinus contorta* and *Pinus sylvestris* than on undefoliated trees. Survival of *B. piniarius* larvae was lower on defoliated *P. sylvestris* also in the study carried out by Šmits and Larsson (1999), but they concluded that it resulted from removal of preferred type of needles.

Whatever is the mechanism underlying the responses in the present study, defoliation had a great impact on both studied geometrids, of which the oligophagous species (*M. liturata*) was more vulnerable. The fact that defoliation had a great impact on the sex ratios suggests that it may also have a marked effect on the demographic structure of populations of the studied species.

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