Ann. Zool. Fennici 31:307–318
Helsinki 7 October 1994
ISSN 0003-455X

Effects of natural and artificial defoliations on sawfly performance and foliar chemistry of Scots pine saplings

P. Lyytikäinen

Lyytikäinen, P., Finnish Forest Research Institute, Department of Forest Ecology, P.O. Box 18, FIN-01301 Vantaa, Finland

Received 20 April 1994, accepted 1 June 1994

Induction of short- (present year) and long-term (subsequent year) resistance reactions in *Pinus sylvestris* (L.) saplings was assessed by rearing two species of diprionid sawflies, (*Neodiprion sertifer* Geoffr. and *Gilpinia pallida* Klug), on previously artificially or naturally defoliated trees. Both treatments consisted of three defoliation levels based on the amount of removed needle biomass per whole canopy (25%, 50% and 75%), carried out during the early and late periods of the growing season. Defoliation treatments had no significant effect on most of the growth variables of *N. sertifer* or *G. pallida*. In most cases there was no consistent pattern to either both sawfly species or in all treatment groups. Artificial defoliation tended to induce stronger effects than insect-made defoliation. By the end of the second year artificial defoliation increased needle water contents, but the values were reversed in respect to natural defoliation. In both years, during the early season, manual removal of foliage induced the highest nutrient concentrations in needles on adjacent branches, but by late season this was the case in the insect-made defoliation treatments. Elevated contents of soluble tannins and soluble sugars were induced by natural and artificial damage, respectively. The concentrations of N, P, Cu, Fe and glucose and fructose, representing the soluble sugars, appeared to be the best indicators of sawfly performance.

1. Introduction

The various damage-induced defense reactions that adversely effect herbivores have been mainly identified in deciduous trees (Haukioja & Niemelä 1979, Schultz & Baldwin 1982, Raupp & Denno 1984, Neuvonen et al. 1987), although qualitative changes in evergreen foliage, in response to herbivory, have also been reported in *Pinus sylvestris* (Thielges 1968), *P. ponderosa* (Wagner & Evans 1985), *P. contorta* (Leather et al. 1987) and *P. radiata* (Karban 1990). The types of re-

sponse, induced in trees can be very diverse. In addition to changes relating to current growth, phenology and morphological traits, other physico-chemical responses (e.g. fiber, secondary chemicals and nutrients) have been well documented (Fowler & Lawton 1985, Haukioja & Neuvonen 1987, Myers & Bazely 1991). Resistance mechanisms that are rapidly activated (short-term) (within one insect generation) are designed to stabilize insect populations whereas the delayed mechanisms (long-term), operate by destabilizing insect populations (Haukioja & Neuvonen 1987).

Defoliation following insect herbivory usually induces stronger responses than comparable artificial damage (e.g. tearing) (Haukioja & Neuvonen 1985, Neuvonen et al. 1987, Hartley & Lawton 1987, 1991), supporting the hypothesis that the chemical changes expressed are specifically targetted against herbivorous insects. Similar (Raupp & Denno 1984, West 1985) or opposing (Baldwin 1988) reactions have also been identified after manual damage. Differences in real and simulated herbivory relating to timing, amount and spatial distribution (Baldwin 1988, 1990) could induce the expression of defensive chemical compounds (e.g. phenols) of different quantities or molecular weights (Hartley & Firn 1989, Hartley & Lawton 1991).

The differences in qualitative changes and/or insect response may be related to the extent of defoliation. It is clear that the level of damage needed to trigger induction in deciduous trees is lower than in evergreen trees (Haukioja & Neuvonen 1987, Wagner 1988). Wagner & Evans (1985) had observed, that the synthesis of phenols, proteins and procyanidins were most affected following moderate artificial defoliation of ponderosa pine seedlings. Previous larval feeding resulting in a 50% defoliation of lodgepole pine saplings lowered the success of *Panolis* larvae (Leather et al. 1987). This defoliation level was also found to be effective in a preliminary study that examined sawfly success following artificial defoliation of pole-size Scots pine trees (Lyytikäinen 1992b).

Water and mineral nutrient contents (e.g. concentrations of N, P, and K), the relative ratios of nutrients, and other nutritional or secondary compounds (water, fiber, allelochemicals) may be the most profound factors influencing folivorous insects (Mattson & Scriber 1987, Clancy et al. 1988, Mattson et al. 1991, Clancy 1991). Tannins have been found to play a major role in plant-herbivore interactions (e.g. Feeny 1976). Through their antidigestive, antifeedant and toxic properties, tannins can significantly reduce insect growth (Berenbaum 1983, Bernays et al. 1989, Karowe 1989). Conversely, soluble sugars act as phagostimulants and promote growth of herbivorous insect (Albert & Parisella 1985), although they may not be essential nutritional components (Van der Meijden et al. 1989). Glucose, fructose and sucrose have been found to be particularly good nutritional sources for *Choristoneura fumiferana* (Clemens) (Harvey 1974), but the recent studies of Clancy (1992) seem to contradict this view.

In this study the experiments were designed to test for the presence of rapid and delayed induced responses in Scots pine saplings and to examine the connection between the performance of diprionid sawflies and qualitative foliar changes. Saplings were investigated because earlier studies had failed to uncover any significant induced reactions in pole-size or mature trees (Niemelä et al. 1984, 1991). Particular attention was paid to 1) type of damage, 2) defoliation level, 3) timing of the treatments, 4) needle chemistry and 5) response of different sawfly species.

2. Materials and methods

2.1. Experimental saplings and defoliation treatments

The study was conducted at the Punkaharju Research Station (61°75′N, 29°08′E) of the Finnish Forest Research Institute in 1989 and 1990. The Scots pine (*Pinus sylvestris*) saplings used in the experiments, formed part of a naturally reforested even aged stand growing on a *Vaccinium* type forest site. Scots pine was the dominant tree species in the stand mixed with a low proportion of white birch (*Betula pubescens* Ehrh.). In 1990 the saplings were 2.58 m tall (SD = 0.46) with a mean age of 14.7 years (SD = 2.5).

The experimental saplings (N = 120) were subjected to either manual (N = 60) or larval (N = 60)

defoliation. In 1989, manual defoliation treatments to randomly selected saplings were made from 24th May to 15th June (N = 30) and from 10th July to 3rd August (N = 30). During the former period mature needles were cut-away with a pair of scissors in order to simulate sawfly feeding. During the latter period mature and current needles were similarly cut. Simultaneously, larval-made defoliations were carried out with *Neodiprion sertifer* (early summer) (N = 30) or *Diprion pini* L. (late summer) (N = 30) with groups of approximately 100 larvae consuming needles contined within each mesh-bag (30 × 70 cm). In the summer of 1989 there were, on average, 60000 larvae contributing to the defoliation of experimental saplings. Defoliation levels in the two different defoliation groups were visually estimated and placed into three categories (25%, 50% and 75%) on the basis of the amount of needle biomass removed from the whole canopy. Ten saplings, five saplings in 1989 and a further five in 1990, with artificial or natural defoliation levels corresponding to each of the three defoliation categories were bioassayed. Each randomly selected experimental sapling was located in the close vicinity of a control tree of the same age.

2.2. Sawflies and larval bioassays

The bioassays were carried out using two sawfly species (Hymenoptera, Diprionidae), which are gregarious and univoltine in Finland (Schwenke 1982). *Neodiprion sertifer* larvae hatch in May–June after overwintering as the egg-stage and pupate in July. *Gilpinia pallida* overwinters at the cocoon-stage, the larvae hatch in July and pupate in late August. The sawfly cultures originated from southern Finland (*N. sertifer*, Nauvo 60°14′N, 21°58′E; *G. pallida*, Köyliö 61°05′N, 22°29′E; *D. pini* (as a damage agent), Pyhtää 60°40′N, 26°66′E). The field-collected larvae were reared to adulthood and freshly emerged adult pairs were introduced onto pine branches which were then enclosed within mesh-bags (50 × 28 cm). The resulting first instar larval progenies were apportioned by individually placing larvae to similar experimental groups for the bioassays.

Branches adjacent to the defoliated branches were cut from the experimental trees and brought to the laboratory for the larval bioassays after approximately a two week (rapid reactions) or a one year (delayed reactions) induction period. Similar branches were also taken from control trees for the bioassays. Groups of both sawfly species were reared in 5-litre plastic containers, covered with nylon netting and containing a 0.2-litre glass bottle filled with water. Each defoliated sapling, branches of which were subjected to a single bioassay, were exposed to two groups (replicates) of 20 larvae, and each control tree to one larval group. Larvae were fed once a week by replacing branches, cut a week earlier, with pieces of unattacked, fresh branches supporting current and one-year-old needles (cf. Niemelä et al. 1984, 1991). These branches were mounted to stand vertically in glass bottles in 5-litre containers. *N. sertifer* preferred the mature needles, but by late summer *G. pallida* was capable of consuming both age-classes. Old twigs, faeces and dead larvae were regularly removed and the water simultaneously changed. The larvae were weighed in groups at the beginning of the bioassays, singly after two weeks, as approximately third-instar larvae, and as cocoons after being maintained in plastic vials filled with damp moss. All containers with larvae groups were maintained at field temperature.

2.3. Needle sampling and foliar analysis

All needle samples for the analysis comprised one-year-old needles from branches adjacent to the defoliated branches, from the south-west oriented middle third of the crown, of the experimental saplings. Corresponding samples were taken from control trees. Samples for needle water content measurements were collected weekly during the sawfly bioassays. Samples for needle nutrient determinations were taken on two occasions in 1989 (13th June and 24th July) and 1990 (11th June and 23th July). The soluble tannin and sugar content of needles was only measured from samples collected in 1990. The needles were dried for 24 h at 85°C and 48 h at 40°C for nutrient and tannin analysis, respectively, and stored in paper bags. Fresh one-year-old needles for sugar analysis were immediately deep-frozen and stored at –20°C until required.

The total concentrations of C, N, P, K, Ca, Mg, Mn, Fe, Al, Cu and Na were determined on an inductively coupled plasma atomic emission spectrophotometer and a CHN elemental analyzer after dry ashing according to the methods described by Halonen et al. (1983). Soluble tannin content was analysed using the Folin-Dennis method as described by Allen et al. (1974). Soluble sugars (saccharose, glucose, galactose and fructose) were determined by liquid chromatography (Voipio & Laakso 1992).

2.4. Statistical analyses

The larval relative growth rate (RGR) was calculated as:

$$(\ln W_t - \ln W_0)/T$$

where W_t is the larval fresh weight on final weighing, W_0 the larval fresh weight at the beginning and T the time between the two measurements (Radford 1967). The initial larval group weights were divided by n (n = group size) to obtain the mean individual larval weight. The larval RGR was calculated for the first 14 days of the bioassay when there was no cocoon formation yet. The larval survival data were arcsine square-root transformed for the statistical analyses. The values for the experimental trees were means of the two larval replicate groups. Needle water content per cent was a mean of the values of the needle samples collected weekly during bioassays. All statistical tests were based on tree specific means (Hurlbert 1984, Neuvonen & Haukioja 1985).

The statistical tests were applied using the BMDP statistical package (BMDP 1988), according to Sokal and Rohlf (1981). The treatment effect on sawfly growth variables (Table 1, Figs. 1–2) and water, nutrient, tannin and sugar concentrations of the trees (Tables 2–3, Fig. 3) were tested by factorial ANOVA. The treatment (Tre)

Table 1. ANOVA analysis (*F*-values) for sawfly growth characteristics in the bioassays on experimental and control trees during the summers of 1989 and 1990. Experimental trees were defoliated during the early or late season in 1989 either artificially or naturally and divided to three levels (25%, 50% and 75%). Tre = treatment, lv = defoliation level. (···P < 0.001, ··P < 0.01, ·P < 0.05, NS = not significant)

Source	df	Larval weight	Cocoon weight (males)	Cocoon weight (females)	RGR	Larval period	Survival
1989							
Neodiprion sertifer							
Tre	2,51	3.51·	2.73 NS	0.16 NS	7.80···	2.27 NS	0.31 NS
Lv	2,51	2.42 NS	1.74 NS	1.12 NS	4.51·	2.43 NS	0.36 NS
Tre*Lv	4,51	4.14··	0.65 NS	1.70 NS	3.21·	0.39 NS	1.66 NS
Gilpinia pallida							
Tre	2,51	1.96 NS	2.56 NS	4.50·	4.72·	2.31 NS	0.67 NS
Lv	2,51	2.19 NS	0.61 NS	0.21 NS	0.11 NS	1.94 NS	0.81 NS
Tre*Lv	4,51	1.70 NS	1.82 NS	0.94 NS	1.30 NS	0.73 NS	0.15 NS
1990							
Neodiprion sertifer							
Tre	2,51	6.31··	0.30 NS	0.67 NS	9.79···	1.61 NS	0.01 NS
Lv	2,51	1.72 NS	0.08 NS	3.95·	7.49···	1.12 NS	0.42 NS
Tre*Lv	4,51	1.11 NS	0.38 NS	2.06 NS	8.49···	1.11 NS	1.24 NS
Gilpinia pallida							
Tre	2,51	0.50 NS	0.62 NS	1.60 NS	0.53 NS	1.12 NS	0.15 NS
Lv	2,51	2.93 NS	0.54 NS	1.00 NS	0.76 NS	1.59 NS	1.79 NS
Tre*Lv	4,51	0.35 NS	0.47 NS	0.76 NS	0.05 NS	0.42 NS	1.11 NS

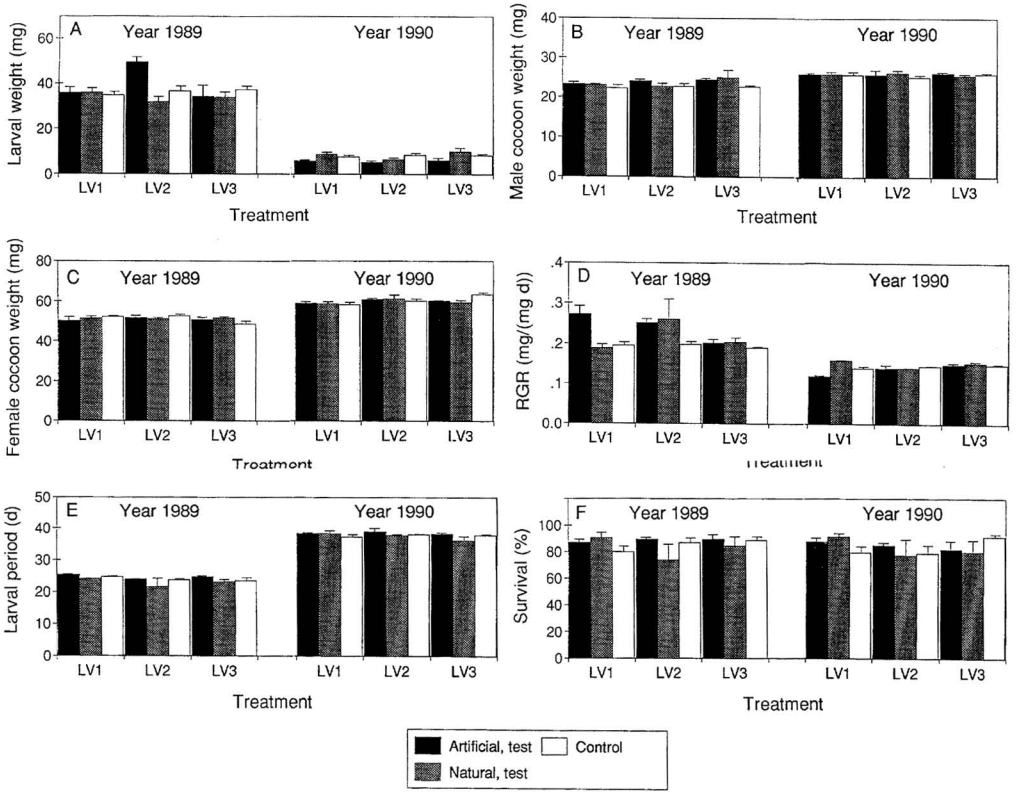

Fig. 1. Performance of *Neodiprion sertifer* (mean ± *SE*) on artificially or naturally defoliated and control trees. Early season damage, dividing into three levels (Lv1 = 25%, Lv2 = 50% and Lv3 = 75%), was performed in 1989. A) larval weight, B) male cocoon weight, C) female cocoon weight, D) RGR, E) larval period and F) survival. N = 5 in each defoliation treatment and N = 10 in control treatment (controls of both damage type combined).

tests variation in relation to artificial damage, natural damage and control, the level (Lv) tests variation in relation to the three levels of defoliation, and the interaction (Tre*Lv) tests if artificially and naturally defoliated trees responded differently to the different levels of defoliation. To assess the factors influencing sawfly performance the contents of water and biochemical elements were used as predictors in multivariate regression analysis (Table 4).

3. Results

3.1. Insect response

Defoliation treatments had no significant effect on most growth characteristics of *N. sertifer* or *G. pallida* (Table 1). Morover, in most cases where significant effects were found, there was no consistent pattern in both sawfly species or in all treatment groups (Figs. 1–2). Removal of the cases with a significant interaction effect (Tre*Lv; $P < 0.05$), revealed three significant treatment effects (Table 1). Larval weight of *N. sertifer* was reduced one year after the defoliation (Fig. 1A). The female cocoon weight (Fig. 2C) and RGR (Fig. 2D) of *G. pallida* were reduced during the first summer after the treatments. In contrast, heavier *N. sertifer* female cocoon weights were recorded at the higher levels of previous defoliation (Fig. 1C). A consistent pattern that emerged was that, compared to natural defoliation, artificial defoliation tended to induce stronger insect responses.

In three cases, a significant interaction effect on *N. sertifer* was detected (Table 1). In 1989, defoliation affected both larval weight and RGR,

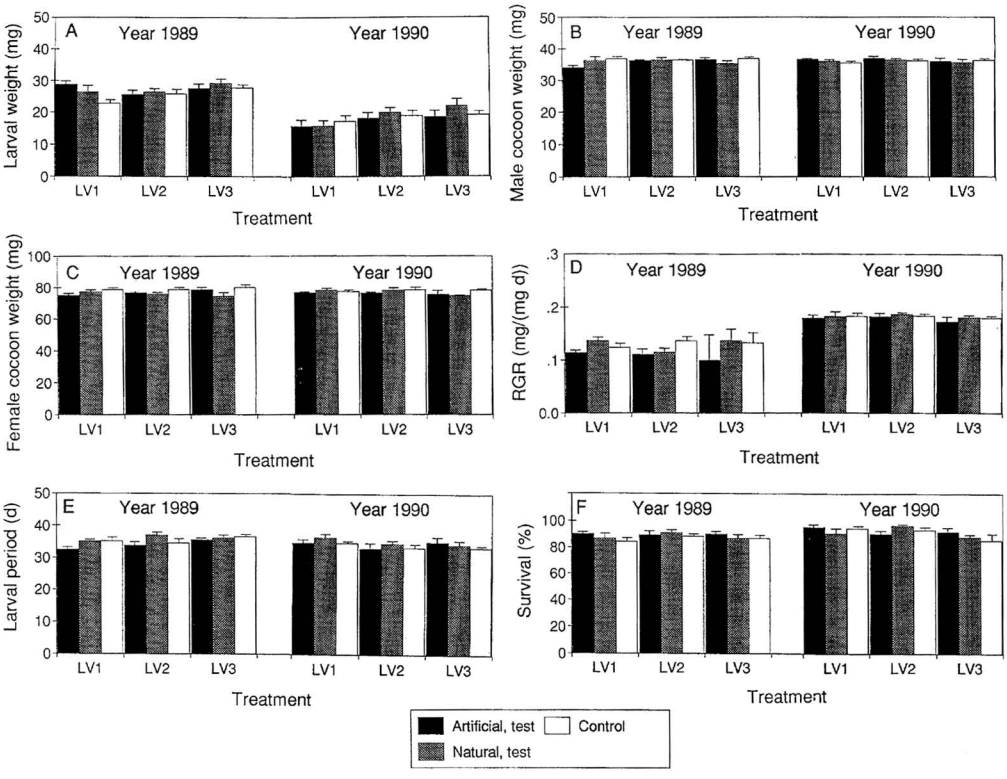

Fig. 2. Performance of *Gilpinia pallida* (mean ± *SE*) on artificially or naturally defoliated and control trees. Late season damage, dividing into three levels (Lv1 = 25%, Lv2 = 50% and Lv3 = 75%), was performed in 1989. A) larval weight, B) male cocoon weight, C) female cocoon weight, D) RGR, E) larval period and F) survival. N = 5 in each defoliation treatment and N = 10 in control treatment (controls of both damage type combined).

Table 2. ANOVA analysis (*F*-values) of water and mineral nutrient concentrations in the foliage of the trees used in the sawfly bioassays in 1989 and 1990. For categories, see Table 1. Tre = treatment, Lv = level, df = 2,51 in the treatments and df = 4,51 in the interactions. ($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, NS = not significant)

Source	Water content	C	N	P	K	Ca	Mg	Mn	Fe	Al	Cu	Na
1989 (E)												
Tre	2.35 NS	106.25***	4.50*	7.17**	18.75***	16.55***	0.29 NS	0.77 NS	25.08***	3.40*	13.41***	9.84*
Lv	2.68 NS	7.12**	7.32**	2.33 NS	0.24 NS	0.89 NS	5.39**	1.96 NS	0.27 NS	0.17 NS	1.94 NS	1.95 NS
Tre*Lv	0.62 NS	1.91 NS	5.18**	1.23 NS	2.84*	2.21 NS	2.79*	2.27 NS	6.01***	1.31 NS	3.89**	2.81*
1989 (L)												
Tre	2.34 NS	4.96*	9.40***	26.43***	4.25*	3.44*	0.73 NS	0.25 NS	9.64***	1.46 NS	45.95***	9.30***
Lv	4.11*	2.59 NS	0.31 NS	2.88 NS	3.53*	4.96*	0.90 NS	0.92 NS	0.28 NS	1.67 NS	2.23 NS	2.19 NS
Tre*Lv	0.18 NS	7.13***	1.74 NS	2.03 NS	3.97**	6.98***	3.05*	2.37 NS	1.47 NS	0.77 NS	2.64*	2.33 NS
1990 (E)												
Tre	2.93 NS	2.97 NS	6.28**	5.25**	1.69 NS	0.36 NS	0.73 NS	3.20*	2.93 NS	1.47 NS	1.52 NS	4.54*
Lv	0.40 NS	2.11 NS	4.66*	1.01 NS	1.03 NS	0.04 NS	1.02 NS	9.83***	0.40 NS	1.09 NS	2.17 NS	1.72 NS
Tre*Lv	1.71 NS	1.51 NS	0.29 NS	0.76 NS	1.62 NS	0.78 NS	0.89 NS	1.98 NS	1.30 NS	1.35 NS	2.10 NS	3.41*
1990 (L)												
Tre	10.89***	1.23 NS	2.90 NS	8.24***	1.35 NS	2.31 NS	0.97 NS	1.89 NS	6.30**	4.18*	2.33 NS	27.99***
Lv	2.52 NS	9.17***	0.50 NS	0.24 NS	0.42 NS	3.03 NS	2.15 NS	4.30*	3.42*	1.01 NS	0.79 NS	8.00***
Tre*Lv	3.63*	0.47 NS	0.51 NS	0.25 NS	0.42 NS	2.00 NS	2.57*	1.79 NS	0.96 NS	0.80 NS	1.28 NS	6.92***

(E) - early season, (L) - late season

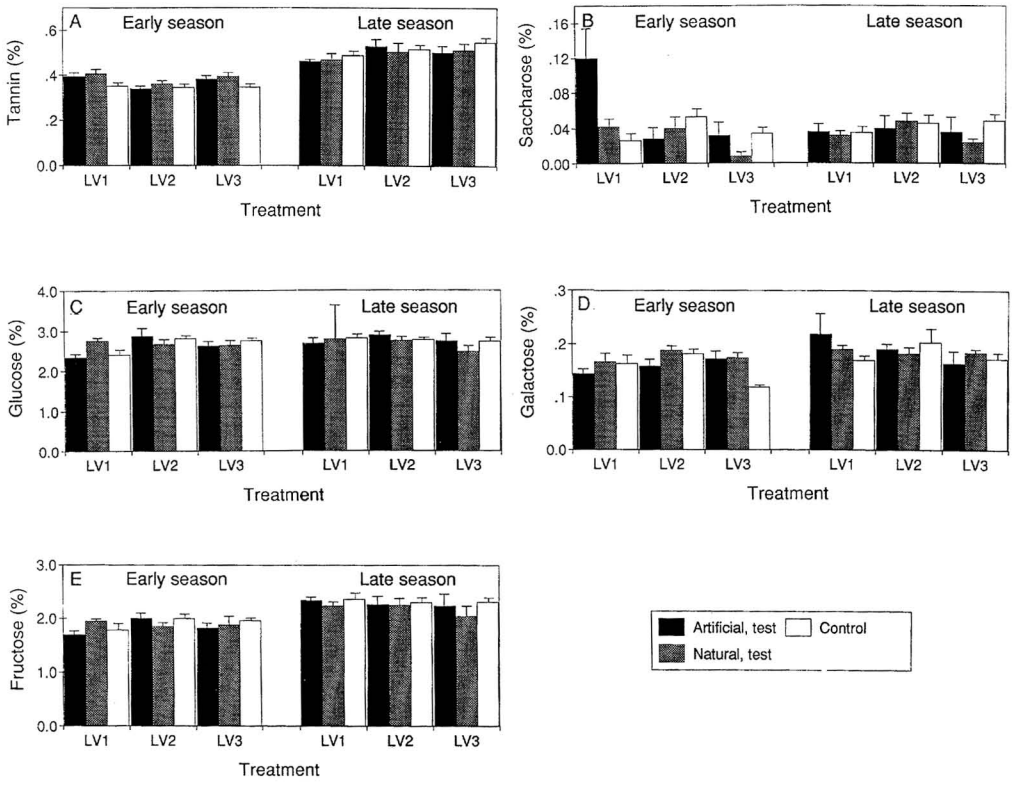

Fig. 3. Soluble tannin and sugar contents (mean ± *SE*) of the one-year-old foliage of the artificially or naturally defoliated and control trees in 1990. Previous early and late season damage divided into three levels (Lv1 = 25%, Lv2 = 50% and Lv3 = 75%). A) tannin content, B) saccharose content, C) glucose content, D) galactose content and E) fructose content. N = 5 in each defoliation treatment and N = 10 in control treatment (controls of both damage type combined).

although these changes were partially contradictory. A reduction in larval weight was detected on defoliated trees, except in the case of the group with moderate (50%) artificial damage (Fig. 1A). On the other hand, defoliation raised the RGR following low (25%) level artificial damage and moderate (50%) levels of both types of damage (Fig. 1D). Defoliation also affected the RGR of *N. sertifer* in 1990 (Fig. 1D), but there was no consistency in the pattern. The major effect seemed to be that artificial and natural defoliation had opposite effects at the low damage level. Artificial damage reduced and natural damage improved RGR. The different mean temperatures in both years could have been a contributory factor affecting growth of the larvae.

3.2. Foliar chemistry

Differences between the levels or the treatments on the water content of the foliage were detected during the late season of both years (Table 2). In 1989, the water content was highest at the low (25%) damage level and defoliated saplings contained the lowest amounts of water towards the end of the following summer. The water content in artificially defoliated saplings was increased with increased damage levels, but the values were reversed in the natural defoliation groups.

In most of the cases in 1989, mineral nutrient contents (C, N, K, Ca, Mg, Fe, Cu and Na) suffered from a significant interaction effect (Table 2). After early season defoliation the con-

centrations of C, N, P, Cu and Na increased in the defoliated saplings and contrasted with lowered K, Ca, Fe and Al concentrations. Concentrations of C were reduced, as the defoliation level increased. The moderate (50%) defoliation level induced the highest N and Mn concentrations. During the late season elevated concentrations of C, N, P, K, Ca, Fe and Na were recorded. Concentration of K was highest at moderate (50%) natural defoliation, and Ca was decreased with increased defoliation levels.

A year after treatments (Table 2) three cases had a significant interaction effect (Mg and Na). Concentrations of N, P, Mn and Na were increased in defoliated trees during the early part of the season. Increased concentration of N was correlated with increased damage level, and Mn level was highest at the moderate (50%) defoliation level. After previous late season damage, concentrations of P, Fe, Al and Na were elevated in defoliated trees. Moderate (50%) and high (75%) defoliation levels induced the highest concentrations of C, Mn, Fe and Na. A consistent pattern in both years seemed to be that, during the early season, artificial defoliation induced the higher elemental concentrations than natural defoliation, but this was reversed during the late season.

The concentrations of soluble tannin and sugars were altered after previous early season defoliation (Table 3, Fig. 3). Defoliation, particularly in the natural treatments, induced elevated tannin concentrations at low (25%) and high (75%) defoliation levels (Fig. 3A). The contents of saccharose, suffering from a significant interaction effect, were elevated, particularly at the low (25%) artificial defoliation level, but were reversed at the 50% defoliation level. The concentrations of glucose increased following moderate (50%) defoliation.

Concentrations of C, water, Ca, Cu, N and P (early season) and Fe, Na, Cu, Ca, N and Mn (late season) attained the highest values of squared multiple correlations (SMC) with the other predictive variables in 1989 (Table 4). RGR of the dependent variables had a significant SMC compared with the independent variables during the early summer, but later in the summer any of the sawfly variables were significant. In 1990, concentrations of P, Cu, N, K, glucose and fructose (early season) and K, fructose, Mn, Na, glucose and P (late season) proved to be the most significant independent variables. Larval weight and period (early season) and female cocoon weight (late season) showed significant SMCs in relation to the predictive variables.

4. Discussion

The results of this investigation differ slightly from earlier studies (Niemelä et al. 1984, 1991, Lyytikäinen 1992a, b) in showing that under certain contitions Scots pine may exhibit weak damage-induced reactions, that can affect sawfly performance. The damage type is a key factor affecting performance. Artificial defoliation, in particular, increased the success of sawflies after a short induction period (cf. Niemelä et al. 1984). The present results did not provide evidence that

Table 3. ANOVA analysis (*F*-values) for soluble tannin and sugar contents in the foliage of the experimental and control trees used in the sawfly bioassays in 1990. For categories, see Table 1. Tre = treatment, lv = level. (••$P < 0.01$, •$P < 0.05$, NS = not significant)

Source	*df*	Tannin	Saccharose	Glucose	Galactose	Fructose
Early season						
Tre	2,51	5.13••	3.64•	0.25 NS	1.83 NS	0.48 NS
Lv	2,51	3.84•	6.32••	4.01•	1.72 NS	1.71 NS
Tre*Lv	4,51	0.85 NS	6.58•••	1.92 NS	2.62•	1.03 NS
Late season						
Tre	2,51	0.78 NS	0.74 NS	0.70 NS	0.18 NS	1.09 NS
Lv	2,51	2.98 NS	0.93 NS	1.31 NS	0.84 NS	0.61 NS
Tre*Lv	4,51	0.37 NS	0.66 NS	0.79 NS	0.99 NS	0.20 NS

sawfly-specific cues are a prerequisite for triggering response. More often artificial damage seemed to have a greater adverse effect on sawfly performance than natural defoliation by the larvae. An apparent trend was that during the early season the artificial defoliation treatment induced the highest nutrient concentrations, but later during the season the larval defoliation induced the greatest concentrations. Contents of soluble tannin and sugars in defoliated trees were significantly different to those of the controls, and highest in the insect defoliated saplings.

Table 4. Multivariate regression analysis of the sawfly bioassays in 1989 and 1990. The variation in sawfly growth characteristics were explained by biochemical element and water contents in the foliage of the experimental and control trees. All significant variables ($P < 0.05$) have been included in every bioassays. RGR = relative growth rate, LARVA = larval weights two weeks after beginning, LARPE = larval period and FEMA = female cocoon weight. N = 30 in each bioassay. ($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$)

Species	Indep. var.	Dep. var.	Multiple R^2 (%)	corr.	F
1989					
Neodiprion	C		82.78	0.910	6.41***
sertifer	Water		79.53	0.892	5.18**
	Ca		71.97	0.848	3.42*
	Cu		67.30	0.820	2.74*
	N		66.89	0.818	2.69*
	P		66.43	0.815	2.64*
		RGR	75.39	0.868	3.53*
Gilpinia	Fe		89.31	0.945	10.44***
pallida	Na		84.92	0.922	7.04***
	Cu		84.72	0.920	6.93***
	Ca		78.07	0.884	4.45**
	N		77.79	0.882	4.38**
	Mn		74.81	0.865	3.71**
	P		73.26	0.856	3.42*
		—			
1990					
Neodiprion	P		95.08	0.975	10.74***
sertifer	Cu		91.32	0.956	5.85**
	N		87.61	0.936	3.93*
	K		86.63	0.931	3.60*
	Glucose		85.22	0.923	3.20*
	Fructose		84.47	0.919	3.02*
	Fe		84.09	0.917	2.94*
	Mg		83.64	0.915	2.84*
		LARVA	94.72	0.973	8.50**
		LARPE	94.03	0.970	7.46**
Gilpinia	K		97.05	0.985	20.09***
pallida	Fructose		95.75	0.979	13.75***
	Mn		94.71	0.973	10.94***
	Na		93.17	0.965	8.34***
	Glucose		92.68	0.963	7.74***
	P		92.66	0.963	7.72***
	Cu		91.27	0.955	6.39**
	Mg		89.46	0.946	5.19**
	N		88.21	0.939	4.57**
	Fe		85.29	0.924	3.54*
	Tannin		85.04	0.922	3.47*
		FEMA	84.69	0.920	2.91*

The artificial simulations may not exactly mimic insect feeding. Even if the amount, age and spatial pattern of the removed foliage is similar to that in real herbivory, timing can be a serious problem. Insect larvae eat continuously bite by bite, while in the artificial treatments the needles were cut once a day over a three week period. Moreover, in eating, larval mouthparts shear cells in a manner different to that resulting from scissor cuts (Baldwin 1988) and this may affect the quantity and quality of the cues eliciting the resistance reactions. Insects may also support fungal spores, hyphae or bacterial products in the vicinity of their mouthparts which, in addition to the direct effect of insect saliva, may trigger a larger or altered plant response (Baldwin 1988, 1990, Hartley & Firn 1989, Hartley & Lawton 1991).

The preliminary experiments indicated that artificial defoliation mainly affected sawfly performance following a 50% removal of needles, but similar lower removal (under 25%) did not cause any response (Lyytikäinen 1992b). The results of the present study differ from those on *Pinus ponderosa* (Wagner & Evans 1985, Wagner 1988) and *P. contorta* (Leather et al. 1987). In 1989, the better success of *N. sertifer* was found to be linked to low and medium defoliation levels, but a year later there was no consistent pattern. After a short-term induction period, nutrient and water contents tended to decrease in the higher defoliation levels, but a year later the situation was reversed, showing similarity to that observed by Piene & Percy (1984) from *Abies balsamea* (L). In many cases, the medium levels of defoliation induced the highest nutrient concentrations. However, tannin contents were lowest in the medium damage level, and saccharose and glucose at the high and low damage levels. The results showed that the direction and intensity of responses in foliage quality and insect perform-

ance were totally dependent on the type of defoliation procedure used and the damage level attained.

The timing of defoliation may also be of fundamental importance. Induced reactions in birch to folivorous insects were most apparent after leaf damage in spring, and similar late season defoliation invoked weaker responses (Haukioja & Niemelä 1979). Observations on *Pinus pinaster* provide evidence that the contents of sugars, lipids, starch and terpenes vary according to season and age of the foliage, but tannins remain permanently in vacuoles (Bernard-Dagan 1988), which was partly evident in the present results. Defensive responses could be more profound in current-year foliage, but they are avoided by diprionids (Niemelä et al. 1984). Ericsson et al. (1980) found that the deleterious effects to Scots pine carbohydrate dynamics were most pronounced in trees that had been defoliated during the late season.

Levels of N, in particularly, but also P, K, sugar and water contents are known to significantly affect the success of folivorous insects (Harvey 1974, Leather et al. 1987, Mattson & Scriber 1987, Clancy et al. 1988). Mattson et al. (1991) assumed that tannins can reduce the utilization rates of Fe and Zn in *Choristoneura fumiferana*, and Cu may interfere with the uptake of several foliar elements. In particular, ratios of N to P, K, Mg, Zn and sugars predisposed Douglas firs to spruce budworm defoliations (Clancy 1991, 1992). In the present trials the foliar levels of N, P, Cu and Fe were most often the best predictive variables of sawfly growth characteristics, but K, Mg, Mn, Ca, Na, glucose and fructose were also significant in this respect. The results were partly in accordance with the abovementioned earlier findings.

The foliar C content showed significant variation only after a short-term, early summer defoliation period, which could have led to improved *N. sertifer* success on defoliated saplings as a consequence of the resulting elevated sugar contents (Bernard-Dagan 1988). Also the relatively fertile forest site may also have led to sufficient nutrient uptake and low carbon/nutrient-ratio and contents of carbon-based secondary compounds (Tuomi et al. 1984). The mesh-bags used on naturally defoliated saplings, but not on the controls, may have affected plant biochemistry through shading of the foliage (Bryant et al. 1983, Larsson et al. 1986).

The differences in species sensitivity to variation in foliage quality may also explain the observations (Hanski 1987, Haukioja 1990). Compared to uncommon species, outbreak species may be less affected by needle quality but the results did not totally confirm this hypothesis. Besides, differences in larval feeding periods may also influence the response pattern. Larvae of *N. sertifer* only consumed mature needles during the early season, whereas larvae of *G. pallida* were also able to feed on current needles later in the season. Needle age classes could react to defoliation in different ways (Niemelä & Tuomi 1993).

Pre-reproductive pine seedlings of *Pinus contorta*, *P. ponderosa* and *P. radiata*, resembling deciduous trees in their fast growth, have been found to show delayed induced resistance (Wagner & Evans 1985, Leather et al. 1987, Karban 1990). However, the pole-sized pines did not show this type of response pattern (Wagner 1988, Niemelä et al. 1991, Watt et al. 1991) but possibly expressed different defensive tactics. Tall saplings may respond in a similar manner as pole-size trees. Constitutive defenses are expected to be high in evergreens, which have considerable resources locked into the foliage (Bryant et al. 1983, 1988, Neuvonen & Niemelä 1991). Consequently, based on present analysis, Scots pine evidently has no effective, or at best only weak, short- or long-term induced resistance against diprionid sawflies.

Acknowledgements. I wish to thank Petri Martikainen, Rauno Roine and Heli Vainio for their hard-working assistance with the field work, and the staff of the Punkaharju Research Station of The Finnish Forest Research Institute. Marja Aittamaa, Tuula Jusko and Raija Yksjärvi prepared and skillfully analysed the needle samples with the supervision of Seija Sirén. The data was carefully prepared by Sauli Härkönen and Leila Korpela. Erkki Annila, Pekka Niemelä and Juha Tuomi critically reviewed previous versions of the manuscript and Robin Sen kindly revised the English text. The study was financially supported by The Niemi Foundation, The Foundation for Research of Natural Resources in Finland, The Leo and Regina Wainstein Foundation and The Foundation of Foresters.

References

Albert, P. J. & Parisella, S. 1985: Feeding preferences of eastern spruce budworm larvae in two-choice tests with combinations of host-plant extracts. — Ent. Exp. Appl. 38: 221–225.

Allen, S. J., Grimshaw, M., Parkinson, J. R. & Quarmby, C. 1974: Chemical analysis of ecological methods. — Blackwell, Oxford.

Baldwin, I. T. 1988: The alkaloidal response of wild tobacco to real and simulated herbivory. — Oecologia 77: 378–381.

— 1990: Herbivory simulations in ecological research. — Trends Ecol. Evol. 3: 91–93.

Baltensweiler, W., Benz, G., Bovey, P. & Delucchi, V. 1977: Dynamics of larch bud moth populations. — Annu. Rew. Entomol. 22: 79–100.

Berenbaum, M. 1983: Effects of tannins on growth and digestion in two species of papilionids. — Ent. Exp. Appl. 34: 245–250.

Bernard-Dagan, C. 1988: Seasonal variations in energy sources and biosynthesis of terpenes in maritime pine. — In: Mattson, W. J., Levieux, J. & Bernard-Dagan, C. (eds.), Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern: 93–115. Springer, New York.

Bernays, E. A., Driver, G. C. & Bilgener, M. 1989: Herbivores and plant tannins. — Adv. Ecol. Res. 19: 263–302.

BMDP statistical software 1988: University of California Press.

Bryant, J. P., Chapin, F. S. III & Klein, D. R. 1983: Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. — Oikos 40: 357–368.

Bryant, J. P., Tuomi, J. & Niemelä, P. 1988: Environmental constraints of constitutive and long-term inducible defenses in woody plants. — In: Spencer, C. K. (ed.), Chemical mediation of coevolution: 376–389. Academic Press, New York.

Clancy, K. M. 1991: Douglas-fir nutrients and terpenes as potential factors influencing western spruce budworm defoliation. — In: Baranchikov, Y. N., Mattson, W. J., Hain, F. P. (eds.), Forest insect guilds: patterns of interaction with host trees. USDA For. Serv. Gen. Tech. Rep. NE-153: 124–134.

— 1992: The role of sugars in western spruce budworm nutritional ecology. — Ecol. Ent. 17: 189–197.

Clancy, K. M., Wagner, M. R. & Tinus, R. W. 1988: Variations in nutrient levels as a defense: identifying key nutritional traits of host plants of the western spruce budworm. — In: Mattson, W. J., Levieux, J. & Bernard-Dagan, C. (eds.), Mechanisms of woody plant defenses against insects: search for pattern: 203–213. Springer, New York.

Ericsson, A., Larsson, S. & Tenow, O. 1980: Effects of early and late season defoliation on growth and carbohydrate dynamics in Scots pine. — J. Appl. Ecol. 17: 747–769.

Feeny, P. 1976: Plant apparency and chemical defense. — Rec. Adv. Phytochem. 10: 1–40.

Fowler, S. V. & Lawton, J. H. 1985: Rapidly induced defences and talking trees: The devil's advocate position. — Am. Nat. 126: 181–95.

Halonen, O., Tulkki, H. & Derome, J. 1983: Nutrient analysis methods. — Metsäntutkimuslaitoksen tiedonantoja 121: 1–28.

Hanski, I. 1987: Pine sawfly population dynamics: patterns, processes, problems. — Oikos 50: 327–335.

Haukioja, E. 1990: Induction of defenses in trees. — Annu. Rev. Entomol. 36: 25–42.

Haukioja, E. & Neuvonen, S. 1985: Induced long-term resistance of birch foliage against defoliators: defensive or incidental? — Ecology 66: 1303–1308.

Haukioja, E. & Neuvonen, S. 1987: Insect population dynamics and induction of plantresistance: the testing of hypothesis. — In: Barbosa, P. & Schultz, J. C. (eds.), Insect outbreaks: 411–432. Academic Press, San Diego.

Haukioja, E. & Niemelä, P. 1979: Birch leaves as a resource for herbivores: seasonal occurrence of increased resistance in foliage after mechanical damage to adjacent leaves. — Oecologia 39: 151–159.

Hartley, S. E. & Firn, R. D. 1989: Phenolic biosynthesis, leaf damage and insect herbivory in birch (Betula pendula). — J. Chem. Ecol. 15: 275–283.

Hartley, S. E. & Lawton, J. H. 1987: The effects of different types of damage on the chemistry of birch foliage and the responses of birch feeding insects. — Oecologia 74: 432–437.

— 1991: Biochemical aspects and significance of the rapidly induced accumulation of phenolics in birch foliage. — In: Tallamy, D. W. & Raupp, M. J. (eds.), Phytochemical induction by herbivores: 105–132. Wiley, New York.

Harvey, G. T. 1974: Nutritional studies of eastern spruce budworm (Lepidoptera: Tortricidae) I. Soluble sugars. — Can. Ent. 106: 353–365.

Hurlbert, S. H. 1984: Pseudoreplication and the design of ecological field experiment. — Ecol. Monog. 54: 187–211.

Karban, R. 1990: Herbivore outbreaks on only young trees: testing hypotheses about aging and induced resistance. — Oikos 59: 27–32.

Karowe, D. N. 1989: Differential effect of tannic acid on two tree-feeding Lepidoptera: implications for theories of plant anti-herbivore chemistry. — Oecologia 80: 507–512.

Larsson, S., Wiren, A., Lundgren, L. & Ericsson, T. 1986: Effects of light and nutrient stress of leaf phenolic chemistry in Salix dasyclados and susceptibility to Galerucella lineola (Coleoptera). — Oikos 47: 205–210.

Leather, S. R., Watt, A. D. & Forrest, G. I. 1987: Insect-induced changes in young lodgepole pine (Pinus contorta): the effect of previous defoliation on oviposition, growth and survival of pine beauty moth, Panolis flammea. — Ecol. Entomol. 12: 275–281.

Lyytikäinen, P. 1992a: Comparison of the effects of artificial and natural defoliation on the growth of diprionid sawflies on Scots pine foliage. — J. Appl. Ent. 114: 57–66.

— 1992b: The influence of damage level in Pinus sylvestris foliage on the performance of diprionid sawflies. — Scand. J. For. Res. 7: 249–257.

Mattson, W. J., Haack, R. A., Lawrence, R. K. & Slocum, S. S. 1991: Considering the nutritional ecology of the spruce budworm in its management. — For. Ecol. Manag. 39: 183–210.

Mattson, W. J. & Scriber, J. M. 1987: Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations. — In: Slansky, F. Jr. & Rodriquez, J. G. (eds.), Nutritional ecology of insects, mites, spiders, and related Invertebrates: 105–146. Wiley, New York.

Myers, J. H. & Bazely, D. 1991: Thorns, spines, prickles, and hairs: are they stimulated by herbivory and do they deter herbivores? — In: Tallamy, D. W. & Raupp, M. J. (eds.), Phytochemical induction by herbivores: 325–344. Wiley, New York.

Neuvonen, S. & Haukioja, E. 1985: How to study induced plant resistance. — Oecologia 66: 456–457.

Neuvonen, S., Haukioja, E. & Molarius, A. 1987: Delayed inducible resistance against a leaf-chewing insect in four deciduous tree species. — Oecologia 74: 363–369.

Neuvonen, S. & Niemelä, P. 1991: Do differences in inducible resistance explain the population dynamics of birch and pine defoliators? — In: Baranchikov, Y. N., Mattson, F. J., Hain, F. P. & Payne, T. L. (eds.), Forest insect guilds: patterns of interaction with host trees. USDA For. Serv. Gen. Tech. Rep. NE-153: 103–112.

Niemelä, P. & Tuomi, J. 1993: Sawflies and inducible resistance of woody plants. — In: Wagner, M. R. & Raffa, K. F. (eds.), Sawfly life history adaptations to woody plants: 391–407. Academic Press, New York.

Niemelä, P., Tuomi, J. & Lojander, T. 1991: Defoliation of the Scots pine and performance of diprionid sawflies. — J. Anim. Ecol. 90: 683–692.

Niemelä, P., Tuomi, J., Mannila, R. & Ojala, P. 1984: The effect of previous damage on the quality of Scots pine foliage as food for diprionid sawflies. — Z. Angew. Entomol. 98: 33–43.

Piene, H. & Percy, K. 1984: Changes in needle morphology, anatomy, and mineral content during the recovery of protected balsam fir trees initially defoliated by the spruce budworm. — Can. J. For. Res. 14: 238–245.

Radford, P. J. 1967: Growth analysis formulae – their use and abuse. — Crop Science 7: 171–175.

Raupp, M. J. & Denno, R. F. 1984: The suitability of damaged willow leaves as food for the leaf beetle, Plagiodera versicolora. — Ecol. Entomol. 9: 443–448.

Schultz, J. C. & Baldwin, I. T. 1982: Oak leaf quality declines in response to defoliation by Gypsy moth larvae. — Science 217: 149–151.

Schwenke, W. (ed.) 1982: Die Forstchädlinge Europas. Bd. 4: Hautflügler und Zweiflügler. — Paul Parey, Hamburg und Berlin.

Sokal, R. R. & Rohlf, F. J. 1981: Biometry. 2 ed. — Freeman and Company, San Francisco.

Thielges, B. A. 1968: Altered polyphenol metabolism in the foliage of Pinus sylvestris associated with European pine sawfly attack. — Can. J. Bot. 46: 724–725.

Tuomi, J., Niemelä, P., Haukioja, E., Sirén, S. & Neuvonen, S. 1984: Nutrient stress. An explanation for plant antiherbivore responses to defoliation. — Oecologia 61: 208–210.

Voipio, R. & Laakso, T. 1992: Chemical composition of the above ground biomass of small-sized trees. — Folia Forestalia 789: 1–22.

Van der Meijden, E., Van Zoelen, A. M. & Soldaat, L. L. 1989: Oviposition by the cinnabar moth, Tyria jacobaeae, in relation to nitrogen, sugars and alkaloids of ragwort, Senecio jacobaea. — Oikos 54: 337–344.

Wagner, M. R. 1988: Induced defenses in ponderosa pine against defoliating insects. — In: Mattson, W. J., Levieux, J. & Bernard-Dagan, C. (eds.), Mechanisms of woody plants defenses against insects: search for pattern: 141–155. Springer, New York.

Wagner, M. R. & Evans, P. D. 1985: Defoliation increases nutritional quality and allelochemicals of pine seedlings. — Oecologia 67: 596–597.

Watt, A. D., Leather, S. R. & Forrest, G. I. 1991: The effect of previous defoliation of pole stage lodgepole pine on plant chemistry, and on the growth and survival of pine beauty moth (Panolis flammea) larvae. — Oecologia 86: 31–35.

West, C. 1985: Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. — Ecol. Entomol. 10: 111–120.