Oecologia (2006) 148: 293–303
DOI 10.1007/s00442-006-0373-8

PLANT ANIMAL INTERACTIONS

Tod L. Osier · Richard L. Lindroth

Genotype and environment determine allocation to and costs of resistance in quaking aspen

Received: 9 March 2005 / Accepted: 18 January 2006 / Published online: 9 February 2006

Abstract Although genetic variability and resource availability both influence plant chemical composition, little is known about how these factors interact to modulate costs of resistance, expressed as negative correlations between growth and defense. We evaluated genotype × environment effects on foliar chemistry and growth of quaking aspen (*Populus tremuloides*) by growing multiple aspen genotypes under variable conditions of light and soil nutrient availability in a common garden. Foliage was analyzed for levels of nitrogen, phenolic glycosides and condensed tannins. Bioassays of leaf quality were conducted with fourth-stadium gypsy moth (*Lymantria dispar*) larvae. Results revealed strong effects of plant genotype, light availability and nutrient availability; the importance of each factor depended upon compound type. For example, tannin concentrations differed little among genotypes and across nutrient regimes under low light conditions, but markedly so under high light conditions. Phenolic glycoside concentrations, in contrast, were largely determined by genotype. Variation in phenolic glycoside concentrations among genotypes was the most important factor affecting gypsy moth performance. Gypsy moth biomass and development time were negatively and positively correlated, respectively, with phenolic glycoside levels. Allocation to phenolic glycosides appeared to be costly in terms of growth, but only under resource-limiting conditions. Context-dependent trade-offs help to explain why costs of allocation to resistance are often difficult to demonstrate.

Communicated by Frederick C. Meinzer

T. L. Osier · R. L. Lindroth
Department of Entomology, University of Wisconsin–Madison, 1630 Linden Drive, Madison, WI, 53706 USA

Present address: T. L. Osier (✉)
Biology Department, Fairfield University, North Benson Road, Fairfield, CT, 06824 USA
E-mail: tosier@mail.fairfield.edu
Fax: 203-254-4253

Keywords Plant–insect interactions · Genotype × environment · Nutrient availability · Light availability · Growth versus defense

Introduction

Genotype and environment determine plant allocation to resistance mechanisms, but the relative importance of, and interactions among, such factors remain poorly understood. Here, we regard resistance as the degree to which insect performance is altered by the experimental treatments (as defined specifically for induced responses by Karban and Myers 1989). Allocation to resistance among genotypes and across environments determines whether resistance is costly, but the role that environment plays is not well understood (Koricheva 2002a). In this study we investigated the relative importance of plant genotype, nutrients and light on resistance traits of aspen and, in turn, how these factors affect costs of allocation to resistance.

Plant genotype has been shown in a large number of experimental systems to be an important determinant of host quality via variation in resistance compounds (e.g., Berenbaum et al. 1986; Bowers and Stamp 1993; Han and Lincoln 1994; Rossi and Stiling 1998). Genotypic variation that impacts herbivores has been well studied in woody plants (Mutikainen et al. 2000; Rousi et al. 1997), including the Salicaceae (Robison and Raffa 1994; Orians and Fritz 1996; Julkunen-Tiitto et al. 1995; Hakulinen et al. 1995; Havill and Raffa 1999; Orians et al. 2003), of which aspen is a member. In quaking aspen, genotypic variation in phenolic glycoside concentrations has repeatedly been found to determine herbivore performance (reviewed in Lindroth and Hwang 1996): high concentrations of phenolic glycosides have strong negative effects on herbivore growth and development (Hwang and Lindroth 1997; Hemming and Lindroth 1995; Hwang and Lindroth 1998; Osier and Lindroth 2001, 2004) and fecundity (Osier et al. 2000). Not only is allocation to phenolic glycosides

widely variable among genotypes of aspen, but also a preliminary study identified production of phenolic glycosides as costly in terms of allocation lost to growth (Hwang and Lindroth 1997). Unlike phenolic glycosides, aspen condensed tannins (Lindroth and Hwang 1996) and condensed tannins in general (Ayres et al. 1997) are not effective resistance compounds against insects. Condensed tannins, however, are highly phenotypically plastic (Osier and Lindroth 2001, 2004), are a large carbon sink in aspen leaves (Lindroth and Hwang 1996), and are a compound class traditionally discussed in association with defensive allocation theory (Herms and Mattson 1992).

Environmental conditions are well known to affect plant allocation to resistance and subsequent insect performance. Light and nutrient availability have been widely studied because of their link to the carbon–nutrient balance of a plant (Bryant et al. 1983). Many of these studies have focused on deciduous trees, including quaking aspen (Larsson et al. 1986; Mutikainen et al. 2000, 2002; Dudt and Shure 1994; Rousi et al. 1996; Ruohomäki et al. 1996; Hemming and Lindroth 1999). To generalize, nutrient addition and shading augment insect growth by suppressing levels of secondary compounds in foliage and enhancing foliar protein (Bryant et al. 1983; Herms 2002; Herms and Mattson 1992; Jones and Hartley 1999). Aspen phytochemistry exhibits those very responses to nutrient and light availability, although the responses of individual compounds vary to some extent (e.g., Hemming and Lindroth 1999; McDonald et al. 1999; Agrell et al. 2000; Osier and Lindroth 2001, 2004). Although the effects of environment on allocation to plant growth and resistance are well known, the role of environment in mediating trade-offs between growth and resistance has not been well studied. If resistance and growth requirements compete for resources, the intensity of this competition would be expected to be most extreme when resources are limiting (Rhoades 1979; Bergelson and Purrington 1996). The notion that resource limitation would reveal trade-offs between growth and defense has some demonstrated support (e.g., Bergelson 1994; Hakulinen et al. 1995; Vrieling and Van Wijk 1994); counterintuitively, however, trade-offs are most often observed under resource-rich conditions (Koricheva 2002a). Although allocation costs are thought to be important constraints in the evolution of plant defense, supporting data have been difficult to produce. One reason, among many, has been the failure to take the environment of the plant into account when considering trade-offs (Bergelson and Purrington 1996; Koricheva 2002a).

To test the roles of plant genotype and environment on allocation to resistance and subsequent costs to growth, we used an experimental system including quaking (= trembling) aspen (*Populus tremuloides*) and the gypsy moth (*Lymantria dispar*). Quaking aspen is the most widely distributed tree species in North America (Dickmann and Stuart 1983) and grows in a variety of environments (Mitton and Grant 1996). Aspens are highly genetically variable; variation can be observed for leaf and bark morphology, leaf phenology, growth rate and susceptibility to disease and herbivores (Barnes 1969; Dickmann and Stuart 1983; Perala 1990; Mitton and Grant 1996). In addition to the effects of genotype on aspen growth and resistance, aspen is likely to respond strongly to nutrient and light availability because of its early successional status and rapid growth rate (Bryant et al. 1983; Herms and Mattson 1992).

Materials and methods

Overview of experimental design

To investigate the relative roles of plant genotype and environment, we used a split-split plot design, with light level as the whole-plot treatment, aspen genotype as the sub-plot treatment and soil nutrient level as the sub-sub-plot treatment. We used two light levels; each light condition was replicated with four shadehouses (a total of eight houses in a fully factorial design). Eight aspen genotypes were grown under the various conditions of light and nutrient availability. We applied two levels of nutrient availability to the experimental saplings. Saplings were divided into two groups: eight genotypes were used for plant growth determinations, and six of the eight genotypes were used for insect bioassays.

Aspen genotypes, propagation and growth conditions

Saplings were propagated from a subset of the genotypes used by Hwang and Lindroth (1997). The genotypes were propagated from potted saplings maintained for several years in a common garden on the University of Wisconsin–Madison campus. Root material was originally collected from several sites in south-central Wisconsin, and one site from west-central Colorado (Hwang and Lindroth 1997). Genotypes from Wisconsin were Dan1 and 2 (from Dane County), Sau2 and 3 (Sauk County) and Wau1 and 2 (Waushara County) (as in Hwang and Lindroth 1997). Genotypes from Colorado were Lar1 and 3 (Larimar County) (as in Hwang and Lindroth 1997).

In summer 1995, aspen material for use in the study was propagated in sand flats from root cuttings as described by Hwang and Lindroth (1997). Individual propagated suckers were planted outside in 1-l pots containing 1:1 sand-to-soil mixture and fertilized at a rate to attain maximum growth. Following leaf drop in autumn 1995, the suckers were bare-rooted and overwintered in refrigerators (4°C). In the spring of 1996, suckers of each genotype were randomly assigned to and planted in their assigned light and soil-fertility treatment conditions.

Saplings were potted individually into 16-l pots containing a 3:2 mixture of sand and local silt-loam field soil. To manipulate nutrient availability, Osmocote 8- to

9-month slow-release fertilizer (18:6:12 N-P-K + micronutrients) was added at a rate of 3.5 g/l to high-nutrient pots; low-nutrient pots received no fertilizer. In the spring of 1997, high-nutrient plants were treated for a second time (top-dressed) with the same dose of fertilizer as used in 1996. We used these soil nutrient levels because they had produced a strong effect on plant growth, without over-fertilization, in a previous study (Hemming and Lindroth 1999).

To alter light availability, aspen were grown within frames covered with light-neutral shade cloth, which blocked 30 and 85% of available sunlight. These levels produced a wide range of aspen growth in a previous study (Hemming and Lindroth 1999).

Saplings were monitored twice a week for insect pests and pathogens throughout the growing period. Insect pests, in general, were rare on the experimental saplings. Aphids were the most common potential pest and were controlled with applications of Talstar (Bifenthrin, FMC Corporation, Baltimore, MD), a synthetic pyrethroid, in summer 1996. Pesticides were not applied, or needed, in 1997 (the season insect bioassays were conducted).

Aspen harvest

To determine the effect of aspen genotype, resource availability and their interactions on aspen growth, saplings were destructively harvested 1.5 seasons after planting (growth period: 11 May 1996–24 June 1997). Three saplings of each treatment combination were dedicated to destructive harvest within each shadehouse (3 saplings × 8 genotypes × 2 nutrient levels × 2 light levels × 4 replicate shadehouses for a total of 384 saplings). Before planting in 1996, we had recorded fresh mass of each experimental sapling. To estimate initial dry mass of these saplings a conversion factor was used. Eight sacrificial saplings of each genotype were weighed, dried and reweighed to obtain fresh-to-dry-mass ratios, and this ratio was used to estimate initial dry mass. To determine final mass of saplings, saplings were harvested (roots, stems and leaves), dried at 70°C to constant mass and weighed. We calculated growth increment (GI) as [(sapling final dry mass)−(sapling estimated initial dry mass)].

Because photosynthetic rates influence the allocation of fixed carbon to growth and defense (Herms and Mattson 1992), we measured photosynthetic rates during midday (10 A.M.–2 P.M.) in the week preceding harvest. One sapling for each shadehouse, nutrient and genotype combination was measured. Photosynthetic measurements were made on the first mature leaf on the terminal shoot. (Foliage that flushed at the initial budbreak, was fully expanded and was deep green was considered mature.) Photosynthesis was measured using an infrared gas analyzer, Li-Cor 6262 (Li-Cor, Lincoln, NE) at a photon flux density of 1,000 μmol m^{-2} s^{-1} provided by a red-light source.

Insect bioassays

To determine the effects of plant genotype, environmental conditions and their interactions on food quality for insect herbivores, we conducted feeding studies with both second- and fourth-stadium gypsy moths. As results from the two sets of bioassays were quite similar, we present data from only the fourth-instar bioassays here.

Fourth-stadium gypsy moth larvae were tested on foliage from six of the eight aspen genotypes under the two light-availability and two soil-fertility treatments. [We used a subset of the genotypes (Dan1 and 2, Sau 2 and 3, Wau1 and Lar3) because the large number of bioassays required for the full complement of treatment combinations was simply impractical.] A set of three saplings for each of the 24 genotypes and resource-availability combinations in each of the four replicate shadehouses was allocated for use in insect bioassays (a total of 96 sets of insect bioassay saplings to allow full replication of the fully factorial design). Foliage for the bioassays was clipped in a disperse pattern from the crown of designated bioassay saplings (terminal leaves were avoided), held in plastic bags over ice for transport to the lab (< 10 min), weighed and immediately placed into waterpiks to maintain leaf turgor. Throughout the duration of the experimental stadium, foliage was changed every 2 days or more frequently as needed.

Gypsy moth egg masses were provided by USDA-APHIS, Otis Air National Guard Base, MA, USA. Egg masses were surface sterilized in a solution of 0.1% sodium hypochlorite solution with 1% Tween 80 as a surfactant. Insect assays were conducted in Percival growth chambers in the University of Wisconsin Biotron because quarantine conditions prohibited the use of gypsy moths outdoors. To simulate temperatures and photoperiod for early summer in Madison, WI, temperature and light conditions used were 23:15°C with a 15L:9D photo regime. Larvae for the bioassay were reared on artificial diet for the first and second stadia and on foliage collected from aspen saplings with low phenolic glycoside levels for the third larval stadium. Subsequently, fourth instars were used to assay the effects of aspen genotype, resource availability and their interactions on growth. Four subsample larvae were tested individually, for a total of 384 individual larvae.

The bioassay began approximately 4 weeks after bud break to match the phenology of the larvae and foliage of the experimental saplings. At this time (23–24 May), the foliage was fully expanded, 3–4 cm in diameter and of appropriate age and toughness for fourth-stadium gypsy moths (Osier, personal observation). Newly molted fourth-stadium larvae were individually weighed and randomly assigned to one of the treatment combinations. Each larva was placed within a 100×15 mm petri dish on an assigned treatment and supplied with foliage ad libitum. The time and date of ecdysis into the fifth instar was recorded, the larvae were frozen, dried at 70°C to constant mass and weighed. All residual foliage

was collected, dried at 70°C to constant mass and weighed.

Larvae used to set up the bioassay were restricted to females. At the start of this experiment, gender was estimated using known mass distributions from previous studies to select only females. This approach was highly successful: of the 384 larvae used in the experiment, all but three were females (determined by inspecting the genital pores of the fifth-stadium larvae at the end of the study). Males were removed from further analysis. The study was restricted to females because if gender is not taken into account, treatment effects can be obscured if, by chance, the distribution of males and females is uneven across treatments. With an experimental design such as this one (with relatively low replication and minimal subsampling) there is a high probability of losing experimental cells because all of the larvae within a particular resource availability × genotype × replicate combination are either male or female. Females were used because the size of gypsy moth females is highly correlated with egg production (Osier et al. 2000).

Insect growth performance, consumption, and food utilization indices were calculated as in Waldbauer (1968), except that relative growth rate (RGR) was modified to use initial biomass rather than average biomass as the relative term (Farrar et al. 1989). For brevity, we present here a reduced data set including growth and development measures only. The full complement of data is provided by Osier (2001).

Chemical analyses

Foliage was collected for phytochemical analyses on 27 May and 24 July 1997 for the fourth-stadium bioassay and sapling harvest data, respectively. For the gypsy moth bioassays and sapling harvest, 15 mature leaves were collected haphazardly from their respective sets of three saplings per shadehouse, genotype and fertility combination (as was done for the foliage collected in the insect bioassays). Foliage was put on ice immediately after collections, weighed, flash frozen in liquid nitrogen, freeze-dried and reweighed.

Concentrations of phenolic glycosides (salicortin and tremulacin) in leaf tissue were determined by high-performance thin-layer chromatography (HPTLC) as in Lindroth et al. (1993). Salicortin and tremulacin purified from aspen leaves served as standards. Condensed tannins were exhaustively extracted from leaf tissue in 70% acetone at 4°C (with 10 mM ascorbic acid as an antioxidant). To quantify condensed tannins in the extract, we used the butanol-HCl method of Porter et al. (1986). As the standard, we used condensed tannins purified from aspen by the method of Hagerman and Butler (1980). We used Kjeldahl analysis to quantify foliar nitrogen. Acid digestions were conducted using the method of Parkinson and Allen (1975), followed by the micro-Nesslerization procedure of Lang (1958). Glycine *p*-toluene-sulfonic acid (5.665% nitrogen) was used as the standard.

Statistical analyses

Quaking aspen phytochemistry and gypsy moth insect bioassay data were analyzed using a split-split plot analysis of variance design [PROC MIXED (Version 8) SAS Institute 1999]. When subsampling was used for the sub-sub-plots (i.e., aspen growth: three subsample saplings; gypsy moth fourth-stadium bioassay: one to four subsample larvae depending upon mortality), a mean was generated among subsamples to provide one datum for a genotype under a particular nutrient availability in each shadehouse. The analysis of variance model was:

$$Y_{ijkl} = \mu + L_i + E_{ij} + G_k + (LG)_{ik} + e_{ijk} + N_l + (LN)_{il} + (GN)_{kl} + (LGN)_{ikl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the average sapling, chemical or insect response to light level i, shadehouse j, genotype k and nutrient level l. Fixed effects were light (L_i), genotype (G_k), light × genotype interaction [$(LG)_{ik}$], nutrient (N_l), light × nutrient interaction [$(LN)_{il}$], genotype × nutrient interaction [$(GN)_{kl}$] and light × genotype × nutrient interaction [$(LGN)_{ikl}$]. Random effects consisted of whole-plot error (E_{ij}), sub-plot error (e_{ijk}) and sub-sub-plot error (ε_{ijkl}). *F*-tests were conducted using degrees of freedom for error assigned by the Satterthwaite approximation (Littell et al. 1996). Because of the highly subsampled nature of this design, variation in mean initial masses of saplings and larvae was low among treatments and thus the covariate did not significantly relate to the dependent variables. Therefore, ANCOVA was not used, or appropriate, according to the model-fitting guidelines of Littell et al. (1996).

To relate gypsy moth growth to aspen phytochemistry, we used stepwise multiple regressions [PROC REG (Version 8) SAS Institute 1999]. Stepwise regressions in SAS use a combination of forward selection and backward elimination ($\alpha = 0.15$) to fit a model. We used group means for each genotype × nutrient × light combination ($n = 24$).

To evaluate allocation costs of resistance in aspen, correlations were calculated as the relationship between phytochemical traits (phenolic glycoside and condensed tannin concentrations) and sapling growth. Correlations were calculated [PROC CORR (Version 8) SAS Institute 1999] for each of the four combinations of resource ability (low light and low nutrients, low light and high nutrients, high light and low nutrients, and high light and high nutrients). To quantify costs of allocation to phenolic glycosides (the only compounds significantly related to growth) in terms of growth equivalents, regression analyses were calculated between phenolic glycosides and sapling growth in each of the four combinations of environmental conditions.

Results

Aspen growth

Aspen growth was strongly affected by both the independent and interactive effects of genotype, nutrients and light (Fig. 1). Growth varied widely among the genotypes, and this variation depended upon nutrient and light availability. The effect of nutrient availability on aspen growth was minimal under light-limited conditions; however, nutrient addition produced a two- to fourfold increase in growth in high light, depending upon genotype (Fig. 1).

Aspen phytochemistry

Phytochemical concentrations depended upon plant genotype, nutrients and light availability; the magnitude and direction of responses to these treatments differed among the different compound types (Fig. 2). Phenolic glycoside concentrations were most strongly dependent upon plant genotype and moderately reduced by nutrient addition and shading (Figs. 2, 3). The effect of light availability depended upon the genotype and nutrient treatment of the saplings. Condensed tannin accumulation was almost completely suppressed by shading, which accounted for nearly 90% of the variation explained by the experimental treatments (Figs. 2, 3). Aspen genotype and the genotype × light interaction were the most important of the numerous other significant (but relatively weak) factors affecting condensed tannin concentrations (Fig. 3). Foliar nitrogen concentrations increased under low light and high nutrient conditions, and varied among genotypes (Fig. 2). Of the variation explained by the experimental factors, light, followed by nutrients, was most important in determining foliar nitrogen concentrations (Fig. 3).

Concentrations of phenolic glycosides and condensed tannins were not significantly correlated across genotypes and nutrient treatments ($r = 0.055$, $P = 0.798$). Levels of tannins were, however, strongly and negatively associated with foliar nitrogen ($r = -0.740$, $P < 0.001$).

Gypsy moth performance

Aspen genotype and nutrient availability independently affected gypsy moth growth and development parameters, whereas light and interactive effects had somewhat less consistent effects (Fig. 4). Gypsy moth relative growth rate (RGR) varied widely among the aspen genotypes and was enhanced by nutrient addition (Fig. 4). Light did not independently affect RGR, but altered the effect of genotype. Aspen genotype and nutrient availability accounted for the majority of the explained variation in RGR; overall, genotype was most important (Fig. 3). As for RGR, final mass of larvae was most strongly determined by genotype and was positively influenced by nutrient addition (Figs. 3, 4). Both genotype and nutrients were important in determining developmental time of gypsy moth larvae (Figs. 3, 4). Although the effect of light was considerably less important than the effect of genotype and the positive effect of nutrients, insect developmental time was the shortest on aspens grown in shade, and the effects of both light and nutrients depended upon genotype. The impacts of genotype and resource availability on larval growth and development were effected primarily through changes in the efficiency with which larvae converted digested food into biomass, rather than by changes in food consumption or efficiency of digestion (data not shown; Osier 2001).

Relationships of gypsy moth performance and aspen phytochemistry

Aspen phytochemicals explained upwards of 80% of the total variation in gypsy moth performance (Table 1). Gypsy moth RGR and final mass related negatively with concentration of phenolic glycosides, which was the first phytochemical variable to enter regression models of the bioassay results. Gypsy moth RGR and final mass related positively to condensed tannin concentrations. Phenolic glycoside concentrations alone explained

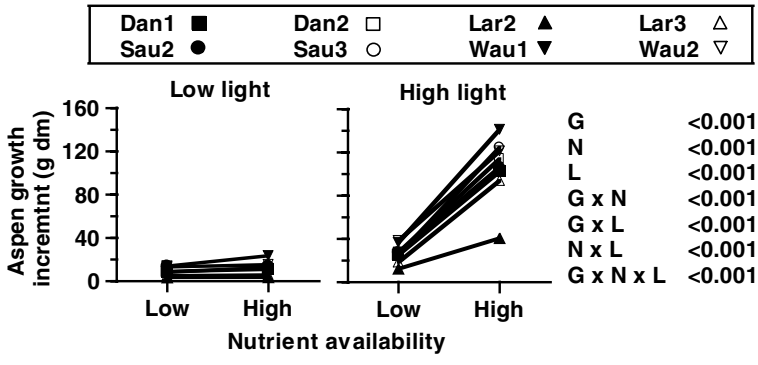

Fig. 1 Norm of reaction plots for aspen growth in response to nutrient and light availability. Each of the eight lines within a *panel* represents a single aspen genotype; each *point* represents the mean response of 12 saplings. *P* values indicate the results of split-split plot ANOVA

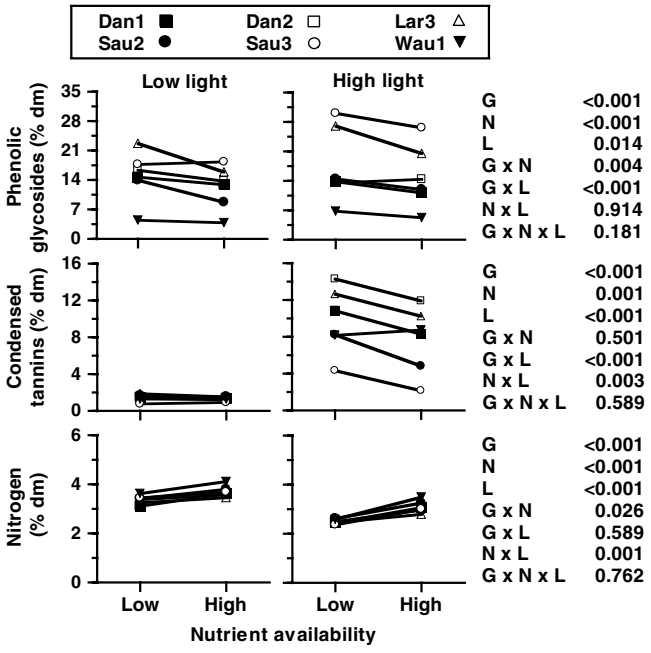

Fig. 2 Norm of reaction plots for primary and secondary metabolites of aspen in response to nutrient and light availability. Foliage was collected during the middle of the fourth-stadium gypsy moth bioassay. Each of the six *lines* within a *panel* represents a single aspen genotype; each *point* represents the mean response of 12 saplings. *P* values indicate the results of split-split plot ANOVA

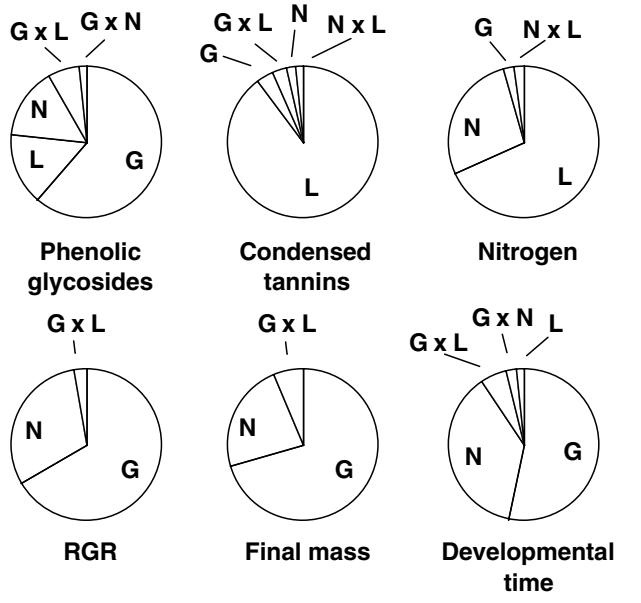

Fig. 3 Proportion of "explained variation" in relation to aspen genotype (*G*), nutrient availability (*N*), light availability (*L*) and their interactions for phytochemistry and insect performance variables associated with the fourth-stadium bioassay. For each variable, experimental treatments and interactions are ranked in decreasing order (clockwise from top) of the proportion of variation explained. Proportion of "explained variation" was calculated as mean square for each treatment/total mean square explained. For the sake of clarity, only variation due to significant parameters and parameters accounting for >1% of the explained variation are shown. Note: For foliar nitrogen the *G*×*N* interaction term accounted for only 0.4% of the explained variation and thus was not included in the figure

approximately 80% of the total variation in insect developmental time.

Relationships between allocation to resistance versus growth in aspen

To investigate relationships between allocation to resistance and growth in aspen, we calculated correlations between phenolic glycosides and sapling growth in each of the four combinations of nutrient and light availability. Phenolic glycosides, but not tannins, were significantly related to aspen growth, so only results for the former are presented. We found extremely strong and negative relationships between allocation to phenolic glycosides and plant growth under the three experimental conditions where either nutrients or light limited plant growth (Fig. 5a–c). However, under the single condition where plant growth was not limited by nutrients and light, no significant relationship was observed (Fig. 5d). Tree growth was not significantly correlated with concentrations of the other phytochemicals measured (including condensed tannin concentrations and the combined concentrations of phenolic glycosides and condensed tannins), or with sapling photosynthesis, under any of the experimental conditions (data for photosynthesis and nonsignificant correlations not shown). Allocation to phenolic glycosides was costly to sapling growth: for every 1% increase in foliar phenolic glycoside levels, growth of aspens was suppressed by 5.4, 4.7 and 2.7% in the low light and low fertility, low light and high fertility, and high light and low fertility combinations, respectively.

Discussion

Aspen growth

Aspen growth was determined by genotype, soil nutrient availability, light availability and interactions among these factors. Based on previous studies with aspen (Hwang and Lindroth 1997; Lindroth et al. 2001; Osier and Lindroth 2004), we expected genotype to play a sizeable role in determining aspen growth. The factorial design resulted in four widely varying growth conditions and, again as expected, low nutrient (Kinney and Lindroth 1997; Hemming and Lindroth 1999) and low light (Hemming and Lindroth 1999; Agrell et al. 2000) availability strongly suppressed aspen growth. The synergistic effect of unlimited light and nutrients on plant growth was the most striking of the sapling responses to the environmental treatments.

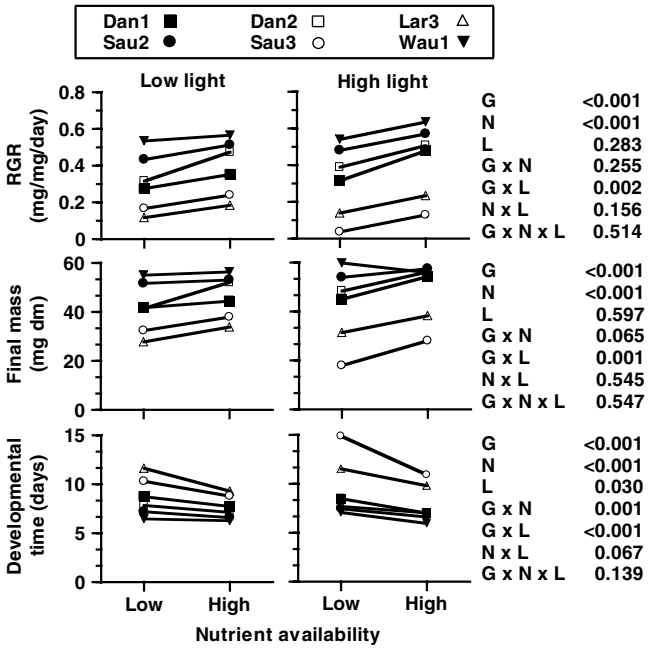

Fig. 4 Norm of reaction plots for fourth-stadium insect performance in response to nutrient and light availability. Each of the sixlines within a *panel* represents a single aspen genotype; each *point* represents the mean response of 12 saplings. *P* values indicate the results of split-split plot ANOVA

Aspen phytochemistry

The main and interactive effects of plant genotype, soil nutrients and light affected concentrations of all phytochemicals were measured; however, the magnitude and direction of responses to these treatments differed widely. Variation in concentrations of phenolic glycosides was driven primarily by plant genotype (Hwang and Lindroth 1998; Osier and Lindroth 2001, 2004). Although nutrients and light had dramatic effects on sapling growth, their role in determining phenolic glycoside concentrations was clearly secondary to that of genotype. The dominance of genotype, over environmental factors, in regulating phenolic glycoside concentrations is consistent with other recent studies with aspen (Lindroth et al. 2001; Osier and Lindroth 2001, 2004). These results support the perspective that production and accumulation of secondary metabolites is under tight genetic control rather than the consequence of simple mass action processes (Hamilton et al. 2001). In contrast to phenolic glycosides, however, condensed tannin concentrations were largely determined by environmental conditions (light availability). The strong impacts of environmental factors on condensed tannin concentrations have been observed in a number of other studies with aspen (Roth et al. 1998; McDonald et al. 1999; Hemming and Lindroth 1999; Kinney et al. 1997; Osier and Lindroth 2001). Thus, phenolic glycosides and condensed tannins exhibit differences in phenotypic plasticity even though these suites of compounds are both products of the shikimic acid pathway. Several papers have shown that multiple products of a single metabolic pathway are often under very different types of biosynthetic control (Koricheva et al. 1998; Keinänen et al. 1999), and this appears to be the case with aspen.

Both phenolic glycosides and condensed tannins responded to the environmental treatments in the *direction* predicted by the carbon–nutrient balance hypothesis (Bryant et al. 1983), growth–differentiation balance hypothesis (Herms and Mattson 1992) and the protein competition model of phenolic allocation (Jones and Hartley 1999). The *magnitude* of the responses, however, was inconsistent with predictions. For example, even under low light conditions where sapling growth was extremely poor, allocation to phenolic glycosides was only slightly less than that by plants growing under high light conditions. In contrast, condensed tannin concentrations were highly environmentally plastic and behaved as if driven largely by the availability of photosynthate to serve as substrate for tannin production. Responses inconsistent with the predictions of the carbon–nutrient balance hypothesis have fueled a vigorous debate regarding its usefulness as a predictive tool (Hamilton et al. 2001; Nitao et al. 2002; Koricheva 2002b; Lerdau and Coley 2002) and its rightful place in the development of a comprehensive theoretical framework of plant defense (Stamp 2003). Apart from the current debate, in this study the carbon–nutrient balance hypothesis proved useful for predicting the direction, but not magnitude, of responses of phenolic glycosides and condensed tannins to experimental treatments.

Table 1 Phytochemical components accounting for variation in gypsy moth performance in the fourth-stadium bioassays (stepwise multiple regressions, $\alpha = 0.15$ was used as the criterion for acceptance to, or rejection from, the model)

Parameter	Stepwise regression model			Partial components		
	Equation	R^2	*P*	Variable	R^2	*P*
RGR	$Y = 0.66 - 0.02(PG) + 0.01(CT)$	0.835	< 0.001	PG	0.800	< 0.001
				CT	0.035	0.049
Final mass	$Y = 64.31 - 1.51(PG) + 0.61(CT)$	0.841	< 0.001	PG	0.786	< 0.001
				CT	0.056	0.013
Dev. time	$Y = 4.18 + 0.28(PG)$	0.832	< 0.001			

CT Condensed tannins, *PG* phenolic glycosides

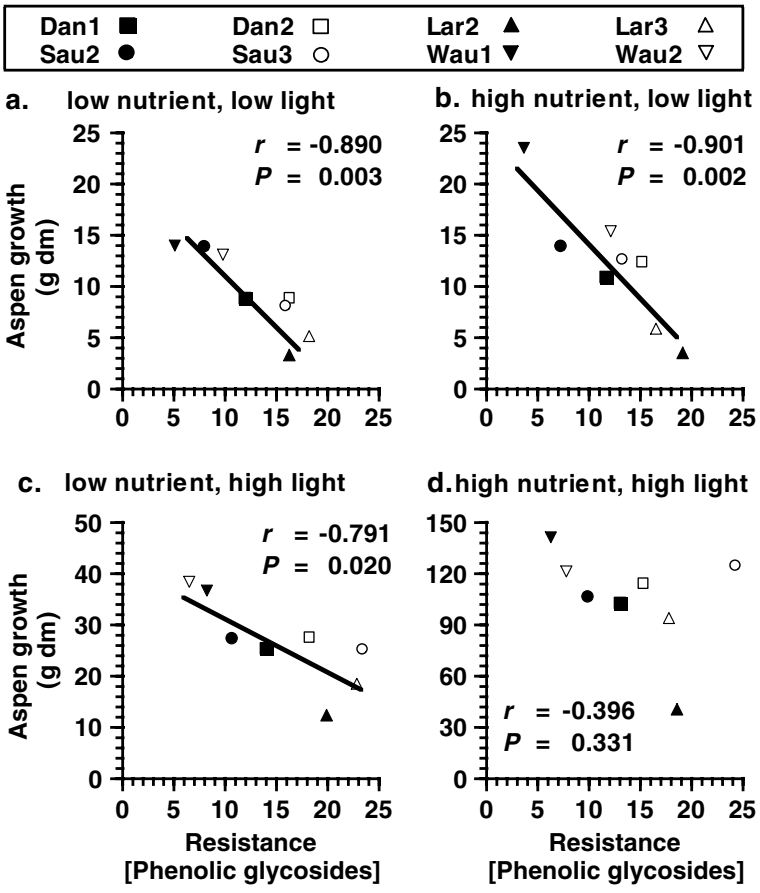

Fig. 5 Correlations between allocation to resistance (phenolic glycosides) and growth in quaking aspen under four combinations of light and nutrient availability. For each light and nutrient availability combination, each *point* within a figure represents a mean of four shadehouses (12 saplings)

Gypsy moth performance

Aspen genotype was consistently the most important factor determining gypsy moth growth and development. As has been routinely observed, host quality for gypsy moths appears to be driven by phenolic glycoside concentrations (Hwang and Lindroth 1997, 1998; Osier et al. 2000; Hemming and Lindroth 1995). Phenolic glycoside concentrations entered and remained in the regression model for every insect performance parameter measured and were the first phytochemical to enter the model. The consistency with which phenolic glycosides enter the model and the large proportion of the variation explained by phenolic glycosides, when in the regression model alone, speak to the overwhelming importance of phenolic glycosides in determining insect performance.

This work ranked the importance of plant genotype, nutrients and light in determining variation in insect performance. Plant genotype was most important, followed by nutrient and finally, light availability. In related studies, the importance of genotype and environment vary, with either genotype (Horner and Abrahamson 1999; Abrahamson et al. 1988; Hakulinen et al. 1995; Orians et al. 2003) or environment (Mutikainen et al. 2000; Orians and Fritz 1996; Rossi and Stiling 1998) implicated as primary causes of variation important for herbivores. A key consideration when attempting to rank the importance of treatments is that the relative strength of each treatment applied is approximately equal. Our goal was to apply levels of each environmental treatment that were biologically realistic and of comparable strength to the others; in all cases we attempted to apply levels of treatment that spanned the known range in aspen. Even when light and nutrient availability were pushed nearly to extremes, environment and genotype by environment interactions were markedly less important than genotype in determining insect performance. Our results suggest that patterns of insect performance among aspen clones in the field are determined much more strongly by genetic variability than by environmental heterogeneity with respect to nutrients or light.

Costs of allocation to resistance in aspen

Why are phenolic glycoside concentrations so variable among genotypes if they are so effective at conferring resistance against insect herbivores? The conventional explanation is that defense is costly, and that because the magnitude of herbivory varies spatially and temporally, the benefits of defense are also variable (Simms 1992). Variable costs and benefits would function to preserve polymorphisms in defensive allocation within plant

populations. Although the notion of trade-offs between growth and defense has been important to the development of theories of plant–herbivore interactions, supporting evidence has been more elusive than logic would suggest (Herms and Mattson 1992; Mole 1994; Koricheva 2002a). One reason, among many, as to why trade-offs may be difficult to discern is that they are context-dependent: expression may depend on resource availability.

In this study, a trade-off between investment in phenolic glycosides and growth was evident only under resource-limited conditions. Under those conditions, variation in allocation to chemical resistance among genotypes explained an extraordinary amount (60–80%) of the variation in growth observed. To our knowledge, these relationships are some of the strongest in the plant defense literature and are much stronger than the average relationship ($r = -0.15$) reported in a comprehensive review by Koricheva (2002a). For each percentage increase in allocation to phenolic glycosides, plant growth decreased dramatically (from almost 3 to over 5%, depending upon environmental conditions). The more severely aspen growth was suppressed by the environmental treatments, the greater was the realized cost of allocation to phenolic glycosides. Our findings suggest that the cost of phenolic glycoside production in young aspen trees and conflicting selection pressures for both growth and defense shaped the evolution of allocation strategies that resulted in the polymorphism of defense evident in natural aspen populations today.

It has been suggested that costs of defense are more likely to be observed under resource-limiting conditions (Rhoades 1979; Fox 1981; Bergelson and Purrington 1996). Koricheva's (2002a) meta-analysis, however, showed limited support for this pattern. She found that the degree to which resource availability plays a role in mediating trade-offs between growth and defense was variable among studies, but that relationships were more often observed under resource-rich than resource-limited conditions. Studies conducted within the Salicaceae, the family to which aspen belongs, exhibit variable results as well. Nitrogen availability appears to mediate the expression of costs of resistance in some willows but not others. Hakulinen et al. (1995) found that biomass of *Salix myrsinifolia* clones was negatively related to foliar concentrations of total phenolics under sub-optimum and optimum fertility conditions, but not under supra-optimum conditions. In contrast, Orians et al. (2003) evaluated correlations between plant growth and phenolic glycosides in *Salix sericea*, and found no cost of allocation to resistance traits under low, medium or high fertility treatments. In that study, however, plant growth and resistance traits exhibited relatively little variation in comparison to what we observed in aspen. The greater degree of genetic variation in aspen growth and phenolic glycoside concentrations likely facilitated detection of a relationship between growth and resistance. Our results are consistent with the notion that trade-offs between resistance and growth can be mediated by environment and that costs are more likely to be detected under resource-limiting conditions. In our nutrient- and light-limited aspen saplings, allocation to growth was redirected in favor of supporting the genetically fixed allocation to resistance.

Conclusions

The maintenance of genetic variation in commitment to chemical defense within plant populations has long intrigued ecologists. The conventional explanation for such variation is that the costs and benefits of chemical defense vary in space and time. Evidence in support of this notion has been slow to accumulate, especially for woody species. The results reported here suggest that costs are more likely to be manifest when studies: (1) target defense compounds that comprise a substantial portion of plant mass, (2) focus on known *defense* compounds rather than secondary metabolites in general and (3) incorporate a range of environments likely to influence costs. Our results also reveal that phenotypic plasticity can vary markedly among classes of secondary metabolites originating from a common biosynthetic (e.g., shikimic acid) pathway. This finding suggests that accumulation of secondary metabolites is determined by divergent regulatory control processes and is not simply a function of mass flow source–sink dynamics (Hamilton et al. 2001). Finally, this work highlights the importance of environmental variation as a driver in the evolutionary trade-offs that have resulted in the expression of resistance polymorphisms in natural plant populations today.

Acknowledgements This research was supported by USDA NRICG grant 95-37302-1810, NSF grant DEB-0074427, and UW Hatch Project no. 3931 to R.L.L. We thank Zach Gillen, Michelle Arnold, Pat Koss, Evan McDonald, Jep Agrell and Shaw-Yhi Hwang for assistance. Lynn Hummel and Laura Van Slyke provided invaluable assistance at the Walnut Street greenhouses, and Tom Sharkey graciously provided the photosynthesis measurements. Thanks to Art Zangerl for advice on genetic correlations; Ken Raffa, Eric Kruger and Jen Klug for thoughtful comments on the manuscript; Rick Nordheim for extensive statistical advice; and Gary Bernon for providing gypsy moth eggs.

References

Abrahamson WG, Anderson SS, McCrea KD (1988) Effects of manipulation of plant carbon nutrient balance on tall goldenrod resistance to a gallmaking herbivore. Oecologia 77:302–306

Agrell J, McDonald EP, Lindroth RL (2000) Effects of CO_2 and light on tree phytochemistry and insect performance. Oikos 88:259–272

Ayres MP, Clausen TP, MacLean SF, Redman AM, Reichardt PB (1997) Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78:1696–1712

Barnes BV (1969) Natural variation and delineation of clones of *Populus tremuloides* and *P. grandidentata* in northern Lower Michigan. Silvae Genet 18:130–142

Berenbaum MR, Zangerl AR, Nitao JK (1986) Constraints on chemical coevoluton: wild parsnips (*Pastinaca sativa*) and the parsnip webworm (*Depressaria pastinacella*). Evolution 40:1215–1228

Bergelson J (1994) The effects of genotype and the environment on costs of resistance in lettuce. Am Nat 143:349–359
Bergelson J, Purrington CB (1996) Surveying patterns in the cost of resistance in plants. Am Nat 148:536–558
Bowers MD, Stamp NE (1993) Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. Ecology 74:1778–1791
Bryant JP, Chapin FS, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368
Dickmann DI, Stuart KW (1983) The culture of poplars in eastern North America. Michigan State University Press, East Lansing
Dudt JF, Shure DJ (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology 75:86–98
Farrar RR, Barbour JD, Kennedy GG (1989) Quantifying food consumption and growth in insects. Ann Ent Soc Am 82:593–598
Fox LR (1981) Defense and dynamics in plant-herbivore systems. Am Zool 21:853–864
Hagerman AE, Butler LG (1980) Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 28:947–952
Hakulinen J, Julkunen-Tiitto R, Tahvanainen J (1995) Does nitrogen fertilization have an impact on the trade-off between willow growth and defensive secondary metabolism? Trees Struct Funct 9:235–240
Hamilton JG, Zangerl AR, DeLucia EH, Berenbaum MR (2001) The carbon-nutrient balance hypothesis: its rise and fall. Ecol Lett 4:86–95
Han K, Lincoln DE (1994) The evolution of carbon allocation to plant secondary metabolites: a genetic analysis of cost in *Diplacus aurantiacus*. Evolution 48:1550–1563
Havill NP, Raffa KF (1999) Effects of elicitation treatment and genotypic variation on induced resistance in *Populus*: impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior. Oecologia 120:295–303
Hemming JDC, Lindroth RL (1995) Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. Oecologia 103:79–88
Hemming JDC, Lindroth RL (1999) Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. J Chem Ecol 25:1687–1714
Herms DA (2002) Effects of fertilization on insect resistance of woody ornamental plants: reassessing an entrenched paradigm. Environ Entomol 31:923–933
Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335
Horner JD, Abrahamson WG (1999) Influence of plant genotype and early season water deficits on oviposition preference and offspring performance in *Eurosta solidaginis* (Diptera: Tephritidae). Am Midl Nat 142:162–172
Hwang S-Y, Lindroth RL (1997) Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. Oecologia 111:99–108
Hwang S-Y, Lindroth RL (1998) Consequences of clonal variation in aspen phytochemistry for late season herbivores. Ecoscience 5:508–516
Jones CG, Hartley SE (1999) A protein competition model of phenolic allocation. Oikos 86:27–44
Julkunen-Tiitto R, Bryant JP, Kuropat P, Roininen H (1995) Slight tissue wounding fails to induce consistent chemical defense in 3 willow (*Salix* spp.) clones. Oecologia 101:467–471
Karban R, Myers JH (1989) Induced plant responses to herbivory. Annu Rev Ecol Syst 20:331–348
Keinänen M, Julkunen-Tiitto R, Mutikainen P, Walls M, Ovaska J, Vapaavuori E (1999) Trade-offs in phenolic metabolism of silver birch: effects of fertilization, defoliation, and genotype. Ecology 80:1970–1986
Kinney KK, Lindroth RL (1997) Responses of three deciduous tree species to atmospheric CO_2 and soil NO_3^- availability. Can J For Res 27:1–10
Kinney KK, Lindroth RL, Jung SM, Nordheim EV (1997) Effects of CO_2 and NO_3^- availability on deciduous trees: phytochemistry and insect performance. Ecology 78:215–230
Koricheva J (2002a) Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. Ecology 83:176–190
Koricheva J (2002b) The carbon-nutrient balance hypothesis is dead: long live the carbon-nutrient balance hypothesis? Oikos 98:536
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
Lang CA (1958) Simple microdetermination of Kjeldahl nitrogen in biological materials. Anal Chem 30:1692–1694
Larsson S, Wiren A, Lundgren L, Ericsson T (1986) Effects of light and nutrient stress on leaf phenolic chemistry in *Salix dasyclados* and susceptibility to *Galerucella lineola* (Coleoptera). Oikos 47:205–210
Lerdau M, Coley PD (2002) Benefits of the carbon-nutrient balance hypothesis. Oikos 98:533
Lindroth RL, Hwang S-Y (1996) Diversity, redundancy, and multiplicity in chemical defense systems of aspen. In: Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York, pp 25–56
Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry, and insect performance. Ecology 74:763–777
Lindroth RL, Roth S, Nordheim EV (2001) Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO_2 enrichment. Oecologia 126:371–379
Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary
McDonald EP, Agrell J, Lindroth RL (1999) CO_2 and light effects on deciduous trees: growth, foliar chemistry, and insect performance. Oecologia 119:389–399
Mitton JB, Grant MC (1996) Genetic variation and the natural history of quaking aspen. Bioscience 46:25–31
Mole S (1994) Trade-offs and constraints in plant-herbivore defense theory: a life-history perspective. Oikos 71:3–12
Mutikainen P, Walls M, Ovaska J, Keinänen M, Julkunen-Tiitto R, Vapaavuori E (2000) Herbivore resistance in *Betula pendula*: effect of fertilization, defoliation, and plant genotype. Ecology 81:49–65
Mutikainen P, Walls M, Ovaska J, Keinänen M, Julkunen-Tiitto R, Vapaavuori E (2002) Costs of herbivore resistance in clonal saplings of *Betula pendula*. Oecologia 133:364–371
Nitao JK, Zangerl AR, Berenbaum MR, Hamilton JG, Delucia EH (2002) CNB: requiescat in pace? Oikos 98:539
Orians CM, Fritz RS (1996) Genetic and soil-nutrient effects on the abundance of herbivores on willow. Oecologia 105:388–396
Orians CM, Lower S, Fritz RS, Roche BM (2003) The effects of plant genetic variation and soil nutrients on secondary chemistry and growth in a shrubby willow, *Salix sericea*: patterns and constraints on the evolution of resistance traits. Biochem Syst Ecol 31:233–247
Osier TL (2001) Genotype and environment as determinants of intraspecific variation in quaking aspen phytochemistry and consequences for an insect herbivore. PhD Thesis, University of Wisconsin, Madison
Osier TL, Lindroth RL (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. J Chem Ecol 27:1289–313
Osier TL, Lindroth RL (2004) Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance. Oecologia 139:55–65
Osier TL, Hwang SY, Lindroth RL (2000) Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. Ecol Entomol 25:197–207

Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Comm Soil Sci Plant Anal 6:1–11

Perala DA (1990) *Populus tremuloides* Michx. Quaking aspen. In: Burns RM, Honkala BH (eds) Silvics of North America. United States Department of Agriculture, Forest Service, Washington, DC

Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223–230

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH (eds) Herbivores, their interaction with secondary plant metabolites. Academic Press, New York, pp 3–54

Robison DJ, Raffa KF (1994) Characterization of hybrid poplar clones for resistance to the forest tent caterpillar. For Sci 40:686–714

Rossi AM, Stiling P (1998) The interactions of plant clone and abiotic factors on a gall-making midge. Oecologia 116:170–176

Roth SK, Lindroth RL, Volin JC, Kruger EL (1998) Enriched atmospheric CO_2 and defoliation: effects on tree chemistry and insect performance. Global Change Biol 4:419–430

Rousi M, Mattson WJ, Tahvanainen J, Koike T, Uotila I (1996) Growth and hare resistance of birches: testing defense theories. Oikos 77:20–30

Rousi M, Tahvanainen J, Henttonen H, Herms DA, Uotila I (1997) Clonal variation in susceptibility of white birches (*Betula* spp.) to mammalian and insect herbivores. For Sci 43:396–402

Ruohomäki K, Chapin FSI, Haukioja E, Neuvonen S, Suomela J (1996) Delayed inducible resistance in mountain birch in response to fertilization and shade. Ecology 77:2302–2311

SAS Institute (1999) SAS user's guide: statistics. SAS Institute, Cary

Simms EL (1992) Costs of plant resistance to herbivory. In: Fritz RS, Simms EL (eds) Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. University of Chicago Press, Chicago, pp 392–425

Stamp NE (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23–55

Vrieling K, Van Wijk CAM (1994) Cost assessment of the production of pyrrolizidine alkaloids in ragwort (*Senecio jacobaea* L.). Oecologia 97:541–546

Waldbauer GP (1968) The consumption and utilization of food by insects. Adv Insect Physiol 5:229–288