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Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory

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Abstract. 1. This study investigated how phytochemical variation among clones of quaking aspen *Populus tremuloides*, growing in a common habitat, affects the growth and fecundity of a model herbivore.

2. Gypsy moth *Lymantria dispar* larvae were reared from egg hatch to pupation on 10 aspen clones in the field or on excised foliage in the laboratory. Foliage was collected from each clone, and concentrations of phenolic glycosides, condensed tannins, nitrogen, and water were determined.

3. Herbivore fitness parameters and aspen phytochemical concentrations varied significantly among clones. In both the field and laboratory, larvae reared on clones containing high concentrations of phenolic glycosides exhibited prolonged developmental times and reduced pupal weights and fecundity. Herbivore performance parameters were also related positively to foliar nitrogen concentrations in the laboratory. Food consumption, but neither growth nor reproductive parameters, were related positively to condensed tannin concentrations.

4. In this study, foliar concentrations of phenolic glycosides were implicated as a significant determinant of food quality for gypsy moths, consistent with results of previous laboratory experiments. Additionally, this study documents a case in which host plant variation at a local level influences the performance and possibly the distribution and abundance of an important herbivore.

Key words. Bottom-up effects, clonal growth habit, intraspecific variation, *Lymantria dispar*, phenolic glycosides, plant–insect interactions, *Populus tremuloides*.

Introduction

Herbivory is not distributed uniformly across available hosts within a population (Krischik & Denno, 1983). Consequently, studies of plant–insect interactions have focused on factors responsible for the observed distribution patterns among and within available hosts. Of these, plant chemistry and its effect on food quality are considered of principal importance (Erlich & Raven, 1964; Schultz, 1988; Bernays & Chapman, 1994; Berenbaum, 1995).

The spatial distribution of both nutritive and defensive compounds within and among individual plants is important as it creates a heterogeneous environment for herbivores feeding on those plants (Krischik & Denno, 1983; Raupp & Denno, 1983). In addition, the spatial scale (i.e. within or among leaves, branches, trees, habitats, etc.) of heterogeneity in food quality is thought to be important for herbivores (Stamp & Bowers, 1990; Bowers & Stamp, 1992; Bingman & Hart, 1993; Suomela & Ayres, 1994; Suomela & Nilson, 1994; Suomela *et al.*, 1995, 1997). The purpose of the study reported here was to investigate the magnitude of among-clone variation in aspen chemistry within a common habitat, and consequences for the performance of a model herbivore.

The clonal scale of variation is important for several reasons. First, the aspen clone, a growth form that produces homogeneous and potentially large masses of individual ramets (Barnes, 1966), is a dominant feature of early successional forests in northern environments around the globe

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(*Populus tremuloides* and *P. grandidentata* in North America; *P. tremula* in Eurasia) (Barnes & Han, 1993). Second, aspen is highly variable among clones and this variation affects herbivore performance (Lindroth & Hwang, 1996b). Finally, during insect population outbreaks, some aspen clones experience nearly complete defoliation while others are only minimally defoliated (T. L. Osier, S.-Y. Hwang and R. L. Lindroth, pers. obs.). Previous research suggests that such interclonal patterns in insect performance result from variation in the phytochemistry of quaking aspen (Lindroth & Hwang, 1996b). This notion, however, has yet to be tested in the field.

Quaking (= trembling) aspen *Populus tremuloides* Michx. is an early successional and rapidly growing tree species, adapted to a variety of habitats (Dickmann & Stuart, 1983; Mitton & Grant, 1996). Quaking aspen is the most widely distributed tree species in North America [northern and western U.S.A. and Canada; Mitton & Grant (1996)] and is attacked by over 100 insect species across its range (Dickmann & Stuart, 1983; Ostry, 1988; Perala, 1990). Aspen reproduces both sexually and asexually, the latter by sprouts (ramets) from lateral roots (Barnes, 1966). Quaking aspen is an extraordinarily variable tree species (Mitton & Grant, 1996); interclonal variation can be observed for leaf and bark morphology, leaf phenology, and growth rate (Barnes, 1969; Dickmann & Stuart, 1983; Perala, 1990; Mitton & Grant, 1996). Moreover, clones vary in susceptibility to disease and herbivores (Barnes, 1969; Dickmann & Stuart, 1983; Perala, 1990).

Aspen phenolic glycosides (salicylates) have been implicated as the primary chemical defence mechanism against insect herbivores in a number of correlative and empirical studies (reviewed in Lindroth & Hwang, 1996b). One such insect is the gypsy moth *Lymantria dispar* L., which causes significant, but not uniform, defoliation to aspen during outbreaks in the Great Lakes region of the U.S.A. To date, however, most bioassays in studies of quaking aspen–gypsy moth interactions have been conducted in laboratory settings, due largely to quarantine restrictions. Hence, the objective of the study reported here was to evaluate the effects of variation in aspen phytochemistry at a local geographical level on the performance of an important herbivore, the gypsy moth. In addition, the study afforded an opportunity to confirm the results of previous studies that used excised foliage in the laboratory by comparing the results of parallel laboratory and field studies. As has been the pattern in laboratory studies, gypsy moth performance was predicted to be related negatively to concentrations of phenolic glycosides among aspen clones.

Materials and methods

Study site

This research was conducted on the Pellston Plain research area of the University of Michigan Biological Station in summer 1996. Pellston Plain, located in the northern lower peninsula of Michigan, U.S.A. (45°33′N, 84°40′W), is a glacial outwash plain with sandy, nutrient-poor soil dominated by lichens, ferns, grasses, and scattered quaking aspen clones (Barnes, 1966). The Pellston Plain research area offers a unique environment to study quaking aspen because individual clones are separated spatially and have well-delineated boundaries (Barnes, 1966).

The gypsy moth recently became established in the Great Lakes region of North America, where aspen is a common component of forests. Although quaking aspen is regarded as a preferred host of the gypsy moth (Leonard, 1981; Lechowicz & Mauffette, 1986), not all individuals are equally susceptible to feeding. Aspens on Pellston Plain experienced no substantial defoliation in 1996; several years previously, the area experienced a population outbreak of the gypsy moth (B. Vande Kopple, resident biologist; pers. comm.).

Ten clones were used in the study. These clones were a subset of 31 clones assayed by Lindroth and Hwang (1996a) for phytochemistry in summer 1995 [clones A, B, C, D, E, F, G, H, I, J correspond to 2, 6, 8, 10, 12, 13, 14, 19, 24, 26, respectively, of Lindroth and Hwang (1996a)]. Well-separated clones with uniform ramet size, full southerly exposure, and absence of signs or symptoms of bark canker were used. To best select clones of a single genetic makeup (i.e. those not consisting of two or more interdigitating individuals), a number of characters including spatial distribution, clone structure, bark and leaf morphology, and phenology of budbreak, were used. Within each clone, six ramets that were matched for size within and among clones were used (clones A, B, C, D, E, F, G, H, I, J had a diameter at breast height of 5.4 ± 0.6, 5.8 ± 1.1, 4.5 ± 1.0, 5.1 ± 0.4, 3.9 ± 0.4, 4.6 ± 0.8, 6.6 ± 1.0, 4.3 ± 0.3, 5.6 ± 0.6, and 6.1 ± 0.5 cm, respectively; ANOVA, d.f. = 9, $F = 1.48$, $P > 0.05$). The ramet served as the unit of replication. In cases where multiple insects were reared per ramet, a mean was generated per ramet and this value used in statistical analyses. Nine clones were distributed along an access road running across Pellston Plain, and one clone (J) was located at the edge of Pellston Plain adjacent to an area of glacial moraine. The minimum distance between clones was 0.1 km, the maximum was 2.0 km.

Gypsy moth bioassays, field study

To assess the effects of aspen phytochemistry on gypsy moth development in the field, two bags, each containing 40 neonate larvae, were secured onto each of six ramets per clone. Bags consisted of light-neutral netting (No-See-Um, Balson-Hercules Group, Pawtucket, Rhode Island; reduces light levels by 15%). Bags containing larvae were moved weekly, or sooner if foliage became limiting, until pupation. Gypsy moth egg masses were provided by the United States Department of Agriculture – APHIS (Otis Air National Guard Base, Massachusetts). Egg masses were surface sterilised in a solution of 0.1% sodium hypochlorite with 1% Tween 80 (Sigma, St Louis, Missouri) as a surfactant, and emerging larvae assigned randomly within and between the field and laboratory components of the study. Neonates were bagged onto clones at leaf-out in synchrony with egg hatch of natural populations of gypsy moths. Larvae were bagged onto clones on two dates (clones A, B, E, I, and J were set up on 22 May

1996; clones C, D, F, G, and H were set up on 24 May 1996). Within each set-up date, all clones began leaf-out on either that or the previous day. Set-up of the study was facilitated by an unusually synchronous leaf-out of aspens in spring 1996, due to a sudden warm period following a very late thaw (ice out in 1996 for nearby South Fishtail Bay of Douglas Lake was the latest date in the 30-year record).

Larvae were reared until the third stadium, then reduced haphazardly to 20 per bag (a total of two bags and 40 larvae per ramet). In mid-June, predatory stinkbugs began to attack larvae through the bags; to prevent further attacks, larvae were double bagged with two layers of No-See-Um mesh (two layers reduce light levels by 30%). Larvae that showed signs of attack by stinkbugs were removed from the study and in no cases were losses due to predation high enough to reduce gypsy moth populations to less than 10 larvae per bag (20 larvae per ramet). Throughout the study, experimental clones had very low populations of natural herbivores; damage as a result of the experimental insects far exceeded that of natural herbivores on each ramet.

At the onset of pupation, bagged gypsy moths were checked every 2 days. Pupae were removed, the date recorded, sex determined (according to Lavenseau, 1982), and pupae were weighed 3 days after removal. Pupae from each ramet were placed in a 200 × 30 mm Petri dish lined with paper towel as ovipositional substrate. The first six females and six males to pupate from each experimental ramet were mated to determine fecundity. Although selection of the first-emerging adults may have overestimated mean fecundity, it nonetheless provides a valid comparative measure among treatments (clones). Dishes were placed on edge to provide a large vertical ovipositional surface and maintained at 24 °C at a LD 15:9 h photoperiod. Scales covering the individual egg masses were removed and the egg masses weighed. Two subsamples of 50 eggs per egg mass were counted and weighed so that the ratio of number of eggs per weight could be used to estimate the number of eggs produced per female.

Gypsy moth bioassays, laboratory study

Larvae were reared in the laboratory to determine the effects of variation in aspen phytochemistry on gypsy moth consumption and survivorship while controlling for microhabitat effects that may be encountered in the field. In parallel with the field study, neonate gypsy moth larvae were reared to pupation in Petri dishes on foliage excised from the experimental ramets. Neonates were put on foliage in dishes on two dates (clones A, B, E, I, and J were set up on 23 May 1996; clones C, D, F, G, and H were set up on 25 May 1996). Neonate larvae were placed 25 per each of two 200 × 30 mm Petri dishes per ramet and supplied with foliage *ad libitum* in floral water piks. Foliage was changed at least every 3 days. Populations of larvae within each dish were reduced randomly to 10 during the fourth stadium; the 10 larvae were then split into two subpopulations and five per dish were reared for the remainder of the experiment (a total of four dishes and 20 larvae per ramet). Dishes containing larvae were maintained on an open-air, roofed porch at the University of Michigan Biological Station to provide natural photoperiod and temperature conditions. Mortality was recorded weekly, date of pupation and pupal weight 3 days after pupation were recorded. As in the field study, the first six female and male pupae from each ramet were mated and fecundity was determined.

Throughout the developmental period of larvae in the laboratory, frass was collected weekly, dried at 70 °C, and weighed. To estimate lifetime consumption, lifetime frass production by study larvae was multiplied by the ratio of foliage consumed to frass produced for fourth-stadium gypsy moths [determined in a separate study for each of the experimental clones (T. L. Osier, unpublished)]. A limitation of this approach is that the ratio of foliage consumed to frass produced may not be consistent throughout the entire developmental period of the larvae. Calculated values should, however, remain useful for relative comparisons among treatments.

Foliar chemistry

Foliage was collected from experimental ramets on five dates that spanned the developmental period of gypsy moths in the field: 27 May (1 week after leaf-out), 10, 17, and 26 June, and 5 July (initiation of pupation). To reduce the effects of shading on foliar chemistry, bags containing larvae were moved weekly. In addition, foliage for chemical assays was collected from a set of bags associated with those containing larvae so that both sets of leaves had similar environmental conditions. To control for the effects of leaf age in the feeding trials and related chemistry collections, new leaves at indeterminately growing shoot tips were avoided. For each of the five harvests, 10–20 leaves were collected per ramet by snipping leaves cleanly at the petioles; removing leaves in this way has been shown not to induce a response from the ramet (Mattson & Palmer, 1988). After removal, the leaves were transported to the laboratory in plastic bags on ice, vacuum-dried at room temperature, ground through 40 mesh in a Wiley Mill (Thomas Company, Philadelphia, Pennsylvania), and stored at −20 °C until analysis. Treatment of leaf material in this manner preserves the labile phenolic compounds in aspen foliage (Lindroth & Koss, 1996).

Per cent leaf water was determined gravimetrically, by the ratio of leaf dry to fresh mass. Micro-Kjeldahl analysis was used to quantify foliar nitrogen using the method of Parkinson and Allen (1975), followed by the micro-Nesslerisation procedure of Lang (1958). Glycine *p*-toluene-sulphonic acid (5.665% nitrogen) was used as the standard. Condensed tannins were extracted exhaustively from leaf tissue in 70% acetone at 4 °C (with 10 mM ascorbic acid as an antioxidant). To quantify condensed tannins in the extract, the butanol-HCl method of Porter *et al.* (1986) was used. As the standard, condensed tannins purified from aspen by the method of Hagerman and Butler (1980) were used. Concentrations of phenolic glycosides in leaf tissue were determined by high-performance thin-layer chromatography (HPTLC) as in Lindroth *et al.* (1993).

Table 1. Correlation matrix of relationships among phytochemicals measured from 10 clones of quaking aspen. Pearson product-moment correlations are based on the mean values for each clone.

Phytochemical	Nitrogen	*P*	Water	*P*	Tannins	*P*
Water	0.02	0.953				
Condensed tannins	−0.33	0.353	−0.50	0.145		
Phenolic glycosides	−0.09	0.813	−0.25	0.487	−0.04	0.916

Salicortin, tremuloidin, and tremulacin standards were purified from aspen leaves; salicin standard was obtained commercially (Sigma, St Louis, Missouri).

Statistical analyses

Analyses were performed similarly for the field and laboratory experiments. For aspen phytochemical concentrations and gypsy moth development, growth, consumption, and fecundity parameters, a mean was calculated per ramet and all analyses were based on the ramet as the unit of replication ($n=6$, except clone H where $n=4$). After leaf-out, it became apparent, based on leaf morphology, that clone H consisted of two interdigitating clones; once identified, two ramets were excluded from the experiment. Fixed-effects ANOVA [Proc GLM (Version 6.12); SAS Institute (1989)] was used to assess clonal effects, sex effects, and their interaction. Critical α was 5% for this study. A cautionary note with respect to ANOVA results is that, due to the clonal nature of quaking aspen, individual experimental units (ramets) may not have been entirely independent. Nevertheless, this was the statistical analysis of choice for ascertaining differences among clones in an undisturbed system (E. V. Nordheim, University of Wisconsin statistician, pers. comm.).

Stepwise multiple regressions [Proc REG (Version 6.12); SAS Institute, 1989] were used to relate gypsy moth growth, consumption, and fecundity parameters to aspen phytochemistry. Stepwise regressions in SAS use a combination of forward selection ($\alpha=0.10$) and backward elimination ($\alpha=0.10$) to fit a model. To present the relative proportion of variance explained by individual independent variables (phytochemicals), the contributions of individual coefficients of determination (partial R^2) to the total (R^2) are provided. This is a valid approach in multiple regression when independent variables in the model are not inter-correlated (Sokal & Rohlf, 1995), as is the case in this study (Table 1). Gypsy moth fitness parameters were related to average concentrations of phytochemicals across the five harvest dates for the 10 clones. Phytochemical concentrations averaged across the season were used in regressions because they consistently yielded the strongest relationships between herbivore performance and phytochemistry. This approach was chosen as a result of an extensive regression-model fitting exercise that included models for all five harvest dates and combinations thereof. For regressions and correlations, mean values per clone ($n=10$ clones) were used for phytochemical and gypsy moth performance variables.

Results

Among-clone variation in foliar chemistry

Concentrations of phytochemicals varied significantly among clones (Fig. 1). Foliar concentrations of phenolic glycosides varied by 2.5-fold; among-clone variation in concentrations was low with one major exception, clone H, which contributed half the total variation (Fig. 1a). Condensed tannins varied by 2.2-fold, and variation among clones was more consistent than for phenolic glycosides. No single clone accounted for a large proportion of the variance, as was the case for phenolic glycosides (Fig. 1b). Concentrations of foliar water and nitrogen differed significantly among clones, but the magnitude of variation was small (1.1- and 1.2-fold, respectively).

Gypsy moth bioassays

Gypsy moth performance varied significantly among aspen clones in both the field and laboratory studies (Figs 2 and 3). Pupal mass of gypsy moths in the field varied by 35 and 29% for females and males, respectively (Fig. 2a). In the laboratory, pupal mass varied by 41% for females and 12% for males (Fig. 2b). Results from the field and laboratory feeding trials were correlated significantly for female and male pupal mass (Table 2).

Larval developmental times varied only slightly, though significantly, for insects reared on different clones in both the field and laboratory (Fig. 2cd). Female developmental time varied by 8% (3.6 days) in both trials, whereas male developmental time varied by 8% (3.8 days) in the field and 11% (4.6 days) in the laboratory. On average, developmental time was slightly (3 days) longer in the field than in the laboratory. Results from the field and laboratory bioassays were not correlated significantly (Table 2), although in all cases the longest developmental times occurred for insects reared on clone H.

In the field, the number of eggs produced per female and the mass of individual eggs varied by 1.5- and 1.1-fold respectively (Fig. 3ac). In the laboratory, the number of eggs produced per female varied 2.0-fold, whereas the mass of individual eggs did not vary significantly (Fig. 3bd). Egg number was correlated highly with female pupal mass in both the field ($r=0.882$, $P<0.001$) and the laboratory ($r=0.925$, $P<0.001$), but field and laboratory results were not correlated significantly (Table 2). In both cases, however, insects reared on clone H produced the fewest eggs.

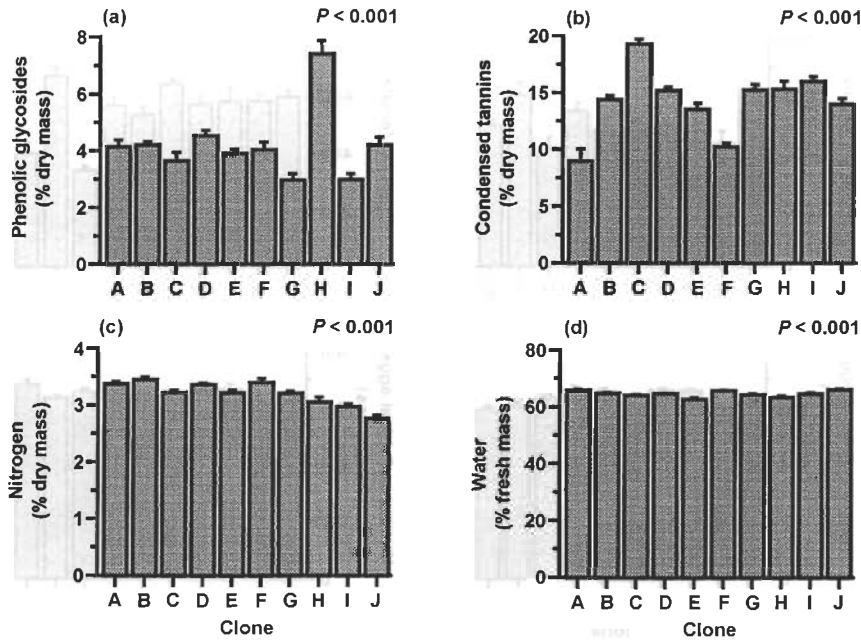

Fig. 1. Clonal variation of quaking aspen phytochemicals averaged over five harvest dates that spanned the developmental period of gypsy moth larvae (means ± 1 SE). (a) Phenolic glycosides, (b) condensed tannins, (c) nitrogen, (d) water. P-values indicate results of one-way ANOVA (clone d.f. = 9). For each clone, $n = 6$ ramets, except clone H where $n = 4$.

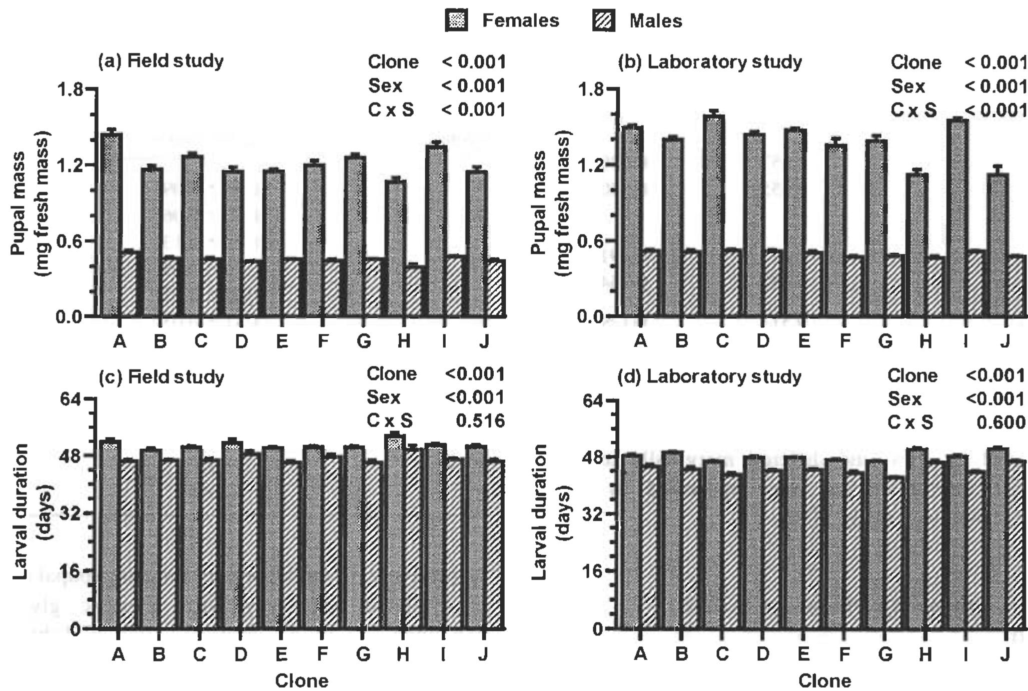

Fig. 2. Variation in pupal mass and developmental time of gypsy moth larvae reared in the field (bagged on ramets) or in the laboratory (excised foliage) on 10 clones of quaking aspen (means ± 1 SE). (a) Pupal mass for the field study, (b) pupal mass for the laboratory study, (c) larval developmental time for the field study, (d) larval developmental time for the laboratory study. P-values indicate results of two-way ANOVA (clone d.f. = 9; sex d.f. = 1; clone × sex d.f. = 9), $n = 6$ ramets, except clone H where $n = 4$.

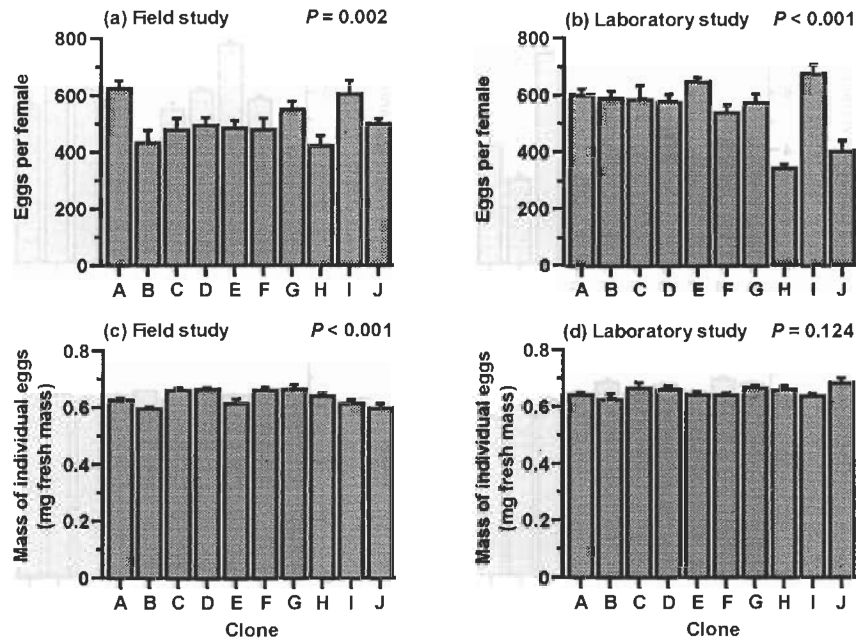

Fig. 3. Variation in egg characteristics of adult gypsy moths reared as larvae in the field (bagged on ramets) or in the laboratory (excised foliage) on 10 clones of quaking aspen (means ± 1 SE). (a) eggs produced per female for the field study, (b) eggs produced per female for the laboratory study, (c) mass of individual eggs for the field study, (d) mass of individual eggs for the laboratory study. *P*-values indicate results of one-way ANOVA (clone d.f. = 9), $n = 6$ ramets, except clone H where $n = 4$.

Table 2. Correlation analyses between the field and laboratory gypsy moth bioassays. Correlations are based on a mean value for each clone. Pearson correlation coefficients are shown.

Parameter	r	P
Pupal mass		
Females	0.657	0.039
Males	0.655	0.040
Larval duration		
Females	0.371	0.291
Males	0.487	0.154
Number of eggs	0.513	0.129
Mass of individual eggs	0.280	0.433

Larval consumption, which was determined only in the laboratory, varied by 29% and differed marginally among clones (Table 3). Larval survival was unaffected by host genotype in the laboratory study (Table 3).

Relationship of gypsy moth performance to aspen phytochemicals

In the field bioassay, pupal mass of both females and males was related negatively to the average concentration of phenolic glycosides across the five sampling dates (Table 4). Quantitative variation in this suite of compounds alone explained 35 and 51% of the variation in female and male pupal mass, respectively. In the laboratory bioassay, pupal mass of females was related negatively with phenolic glycosides, which explained 44% of the observed variation (Table 5).

Table 3. Estimated food consumption and survival for gypsy moth larvae reared from egg hatch to pupation on foliage from 10 clones of quaking aspen in the laboratory (means ± 1 SE are shown). For each clone, $n = 6$ ramets, except clone H where $n = 4$.

Clone	Consumption (g dry mass)	Survival (%)
A	1.58 ± 0.09	97.10 ± 0.02
B	1.62 ± 0.06	97.10 ± 0.02
C	1.78 ± 0.13	97.13 ± 0.04
D	1.76 ± 0.09	97.16 ± 0.03
E	1.68 ± 0.07	97.11 ± 0.02
F	1.51 ± 0.05	97.12 ± 0.03
G	1.65 ± 0.14	97.18 ± 0.04
H	1.67 ± 0.12	97.20 ± 0.05
I	1.90 ± 0.08	97.10 ± 0.03
J	1.47 ± 0.07	97.20 ± 0.03
F	2.010	1.830
P	0.058	0.086

Larval developmental times in the field and laboratory were related positively to the average foliar phenolic glycoside concentrations (Tables 4 and 5). For the field results, phenolic glycosides alone entered and remained in the regression model, whereas for the laboratory results, in addition to phenolic glycosides, nitrogen entered the model.

Table 4. Phytochemical components accounting for variation in gypsy moth fitness parameters for larvae reared on 10 clones of quaking aspen in the field (stepwise multiple regressions, $\alpha=0.10$ was used as the criterion for acceptance to, or rejection from, the model) (PG = phenolic glycosides).

Parameter	Equation	R^2	P
Pupal mass			
Females	$Y=1439.31-52.74(PG)$	0.348	0.073
Males	$Y=584.45-17.36(PG)$	0.506	0.024
Larval duration			
Females	$Y=48.03+0.69(PG)$	0.534	0.016
Males	$Y=43.65+0.81(PG)$	0.722	0.002
Number of eggs	$Y=635.88-30.96(PG)$	0.328	0.084
Mass of individual eggs	No variable met $\alpha=0.10$ entry/elimination criterion		

The number of eggs produced by field- and laboratory-reared gypsy moths was related negatively to concentrations of phenolic glycosides in the foliage consumed as larvae. In addition, the number of eggs produced by laboratory-reared gypsy moths was related positively to foliar nitrogen concentrations (Table 5). In the field and laboratory, variation in individual egg mass was not related to foliar chemistry (Tables 4 and 5).

Food consumption by larvae in the laboratory study was related positively to concentrations of condensed tannins, which explained 42% of the variation in food intake (Table 5). Variation in larval survival was exceedingly small, and not correlated with foliar chemistry (Table 5).

Discussion

Pupal mass, developmental time, egg production, and consumption varied among gypsy moths reared on the 10 aspen clones, as did concentrations of all phytochemicals measured. As predicted, insect performance was poorer on experimental clones containing high concentrations of phenolic glycosides. Results of the laboratory and field studies were generally similar; larvae that fed on foliage containing high concentrations of phenolic glycosides took longer to develop and were smaller and less fecund as adults. These results agree with several other studies in which herbivore performance had demonstrably strong, negative relationships with foliar concentrations of phenolic glycosides (Hemming & Lindroth, 1995; Hwang & Lindroth, 1997, 1998). Empirical studies, in which purified phenolic glycosides were added to either foliage or artificial diet, confirm the role of phenolic glycosides in altering host quality (Lindroth & Hemming, 1990; Hemming & Lindroth, 1995). In the field, herbivore distributions across individuals of another genus in the Salicaceae (*Salix*) are not uniform (Orians & Floyd, 1997; Orians *et al.*, 1997; Roche & Fritz, 1997). This has been attributed, at least in part, to variation in host quality (Roche & Fritz, 1997; Shen & Bach, 1997) and specifically to variability in phenolic glycoside concentrations (Matsuki & MacLean, 1994; Orians *et al.*, 1997).

As expected, the quaking aspen clones displayed minimal variation in foliar concentrations of primary metabolites (nitrogen, water), as has been found previously for individual aspens in a common environment (Lindroth *et al.*, 1987; Hemming & Lindroth, 1995) and across clones (Lindroth & Hwang, 1996a; Hwang & Lindroth, 1997). In contrast, intraspecific variation in concentrations of secondary metabolites (phenolic glycosides and condensed tannins) was found to be large, as has been reported previously (Lindroth *et al.*, 1987; Hemming & Lindroth, 1995, 1999; Hwang & Lindroth, 1997).

Because this study was conducted with undisturbed clones in the field, it was impossible to control for environmental variation among clonal sites. Therefore, the effect of *clone* necessarily includes the effect of environment as well as genotype. Environmental variability can affect aspen foliar chemistry; trees respond to light and fertilisation in accordance with predictions of the carbon/nutrient balance hypothesis (Bryant *et al.*, 1983, 1987; Agrell *et al.*, 1999; Hemming & Lindroth, 1999). Levels of phenolic glycosides, however, are minimally phenotypically plastic (Kinney *et al.*, 1997; Agrell *et al.*, 1999; Hemming & Lindroth, 1999). Thus, at least in regard to phenolic glycosides, the results for clonal differences are most likely due to genetic variation rather than to environmental heterogeneity across the clones.

Phenolic glycosides were strongly implicated as the primary determinant of host quality in both the field and laboratory feeding trials. Nitrogen, however, provided additional explanatory power for several performance indices in the laboratory study. Foliar nitrogen often plays a role in mediating the interactions of plants and their herbivores (Mattson, 1980; Scriber & Slansky, 1981; Slansky, 1993). Among-clone variation in nitrogen in this study, however, was one-tenth that of phenolic glycosides, and thus was probably responsible for a smaller proportion of variation in herbivore growth.

Food consumption by larvae in the laboratory study was related positively to concentrations of condensed tannins. Little evidence exists for condensed tannins functioning as feeding deterrents or toxins for insect herbivores (Lindroth & Hwang, 1996b; Ayres *et al.*, 1997). In this study, high concentrations of condensed tannins may have diluted required nutrients and led to compensatory feeding by the larvae. Similar insect responses have been observed for indigestible diet components such as cellulose (Slansky, 1993).

Table 5. Phytochemical components accounting for variation in gypsy moth fitness parameters for larvae reared on 10 clones of quaking aspen in the laboratory (stepwise multiple regressions, $\alpha=0.10$ was used as the criterion for acceptance to, or rejection from, the model) (CT = condensed tannins, PG = phenolic glycosides, N = nitrogen).

	Regression model			Partial regression components		
Parameter	Equation	R^2	P	Variable	R^2	P
Pupal mass						
Females	$Y=742.13-80.65(\mathrm{PG})$	0.436	0.038			
Males	No variable met $\alpha=0.10$ entry criterion					
Larval duration						
Females	$Y=54.65+0.65(\mathrm{PG})-2.88(\mathrm{N})$	0.619	0.034			
				PG	0.407	0.047
				N	0.212	0.089
Males	$Y=19.55+0.88(\mathrm{PG})-3.23(\mathrm{N})$	0.686	0.014			
				PG	0.470	0.029
				N	0.216	0.064
Number of eggs	$Y=2102.6-67.9(\mathrm{PG})+182.1(\mathrm{N})$	0.733	0.010			
				PG	0.592	0.009
				N	0.141	0.096
Mass of individual eggs	$Y=0.80-0.05(\mathrm{N})$	0.358	0.068			
Consumption	$Y=1254.4+28.64(\mathrm{CT})$	0.422	0.042			
Survivorship	No variable met $\alpha=0.10$ entry/elimination criterion					

Clone H is clearly influential in the analysis of the relationships between phytochemicals, in particular phenolic glycosides, and larval performance. However influential, the inclusion of data for clone H is important for several reasons. First, the clones were selected randomly from the population of clones on Pellston Plain. Second, foliar concentrations of phenolic glycosides for clone H are not exceedingly high in relation to other clones on Pellston Plain (Lindroth & Hwang, 1996a). Finally, the observed phenolic glycoside concentrations and resulting larval performance indices fit within ranges where excellent dose–responses have been observed for gypsy moth larvae feeding on quaking aspen (Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). The contribution of clone H to the overall patterns therefore appears proper and its inclusion in the analyses appropriate.

Although the results of the field and laboratory components of the study were similar (i.e. phenolic glycosides implicated as important defensive compounds), notable differences in results occurred between the two (i.e. the importance of nitrogen in the laboratory compared to the field). Results may have differed simply because of reduced environmental variation in rearing conditions in the laboratory. Defoliation-induced changes in secondary metabolites could also have contributed to variation between the field and laboratory. Such effects, however, were most likely minimal. Foliar damage was extremely light for the experimental trees, did not appear to differ among clones, and the vast majority of damage was inflicted by the experimental larvae. Moreover, although levels of condensed tannins show strong responses to defoliation (Roth *et al.*, 1998; T. L. Osier and R. L. Lindroth, unpublished), levels of phenolic glycosides are only minimally affected, even after severe defoliation (Lindroth & Kinney, 1998; Roth *et al.*, 1998; T. L. Osier and R. L. Lindroth, unpublished).

Clonal variation in chemistry affected the various insect performance parameters differentially. For example, average developmental time varied by less than 4 days for insects fed on the 10 clones. This suggests that the direct effect of clone on duration may not affect herbivore populations greatly (but also see Price *et al.*, 1980). In contrast, female pupal mass in the field varied by 35% and, as a result, egg production of females feeding on the poorest (= high phenolic glycoside) clones was half that of females feeding on the highest quality clones. This suggests that defence by phenolic glycosides in quaking aspen has population-level consequences for the gypsy moth. This is also probably true for a variety of other aspen-feeding Lepidoptera whose performance is influenced by phenolic glycosides (Lindroth & Hwang, 1996b).

Non-uniform spatial distribution of herbivores across clones, as a result of food quality, could have important consequences for the interactions of aspen-feeding herbivores with their natural enemies. The effectiveness of natural enemies may be increased if spatial heterogeneity concentrates herbivores on acceptable hosts (Schultz, 1983). For example, Krause and Raffa (1996) found that predation rate was linked strongly and positively to herbivore cocoon density in a spatially heterogeneous environment. This relationship was attributed to a positive functional response by the predators (Krause & Raffa, 1996). In regard to the spatial scale of clone, resources for herbivores are clumped and herbivores may concentrate on clones where food quality is high. Therefore, searching by arthropod or vertebrate enemies would be localised on such clones and success rate improved. In addition, higher herbivore densities on clones with high food quality would promote the spread of disease among those herbivores. This may occur particularly in species such as the gypsy moth and

forest tent caterpillar *Malacosoma disstria*, which, at high population densities, are susceptible to nuclear polyhedrosis viruses.

Although significant, the magnitude of variation in growth among gypsy moths on different aspen clones was less than that observed in previous studies. The overall fair-to-good food quality of the 10 clones is evidenced by the extraordinarily high and consistent larval survivorship (97%) across the clones. The relatively small amount of variation and overall low concentrations of phenolic glycosides observed can probably explain these results. In this study, variation in phenolic glycosides was only 2.5-fold among clones and the highest concentration was 7%. In contrast, Hemming and Lindroth (1995) found 12.8-fold variation, with the highest concentrations approximately 13% among ramets growing in a common environment. Hwang and Lindroth (1997), using clones of aspen from several locations in a common garden study, found five-fold variation, with the highest concentrations approximately 15%.

The relative lack of variation and overall low concentrations of phenolic glycosides suggest that genetic variation in their accumulation is constrained in the Pellston Plain. Mitton and Grant (1996) suggested that genetic variation in aspen populations is reduced in homogeneous habitats. The xeric, nutrient-poor soils of Pellston Plain may have selected for a growth habit of aspen that is not well defended against insect herbivores. Recent work with quaking aspen has shown that allocation to defence occurs at a cost to growth (*sensu* Rhoades, 1979; Herms & Mattson, 1992), but only when resources are strongly limiting (T. L. Osier, unpublished). On Pellston Plain, clones with fast growth and inherently low defence may have a selective advantage over better-defended, more slowly growing clones.

Conclusion

This study documents a case where the phytochemical profile of a host species at a local level can have important consequences for the fitness of one of its major herbivores. Spatial variation, at the level of clones, in concentrations of a suite of defensive compounds provides a mosaic of host acceptability for gypsy moths and probably other insect herbivores on Pellston Plain. This variation may explain patterns of insect distribution in the field, which are particularly noticeable when herbivores are under outbreak conditions. In addition, this study corroborates results from earlier work in which bioassays were confined to laboratory environments. Finally, this research confirms that phenolic glycosides are of pivotal importance in mediating the interactions of aspen and one of its major enemies.

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