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Within- and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones

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Abstract

We quantified changes in phytochemistry of ten *Populus tremuloides* clones from leaf-out to mid-summer. Significant time, clone and time × clone effects were observed for all phytochemicals measured. Foliar nitrogen and water concentrations declined throughout the period and little inter-clonal variation was observed. Condensed tannin concentrations increased over time and inter-clonal variation was substantial. Patterns for phenolic glycoside concentrations were more complex: depending upon clone, concentrations were highest in the beginning, middle or end of the period monitored. For ten clones sampled in two consecutive years, concentrations of phytochemicals were highly correlated between years, suggesting that the phytochemical profiles of aspen clones are predictable year-to-year. The apparent temporal decline in food quality (e.g., decreases in foliar water and nitrogen and increases in condensed tannins) and diverse temporal patterns in concentrations of phenolic glycosides provide a mosaic of host quality for folivores throughout the spring and early summer. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Populus tremuloides*; Aspen; Phenolic glycosides; Condensed tannins; Intraspecific variation; Temporal variation; Genetic variation; Plant–insect interactions

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1. Introduction

Concentrations of foliar primary and secondary metabolites typically exhibit temporal variation and this may in turn affect the performance of associated herbivores (Scriber and Slansky, 1981; Krischik and Denno, 1983; Mattson and Scriber, 1987; Slansky, 1993). Variability in host quality during leaf expansion is particularly important. The high degree of synchrony between initiation of feeding by many forest insects and budbreak of their hosts is thought to be driven by the rapid temporal decline in food quality during leaf expansion (Feeny, 1970; Raupp et al., 1988; Hunter, 1991; Hunter and Lechowicz, 1992; Van Dongen et al., 1997). Such phenological changes are likely to be of particular importance for folivorous "outbreak" insects, as many if not most feed during spring and early summer (Hunter, 1991).

Quaking aspen (*Populus tremuloides*) is a forest tree species frequently subject to outbreaks of spring-feeding insects. For example, gypsy moth caterpillars (*Lymantria dispar*), forest tent caterpillars (*Malacosoma disstria*), large aspen tortix (*Choristoneura conflictana*) and aspen blotch leafminers (*Phyllonorycter tremuloidiella*) can all cause extensive damage to aspen forests, and feed during the period of leaf expansion and maturation (Dickmann and Stuart, 1983; Perala, 1990). Early seasonal declines in aspen foliar chemistry are likely to influence the performance of such insects, as has been demonstrated for gypsy moths (Hunter and Lechowicz, 1992).

Relatively little is known, however, about temporal changes in specific chemical constituents of aspen leaves during spring and early summer. Roth et al. (1998) documented decreases in water and nitrogen levels, with concomitant increases in tannin and phenolic glycoside levels, in aspen saplings derived from a half-sib seed source that were grown in open-top air pollution chambers. No published information exists, however, on early season temporal variation in chemical composition of aspen in the field.

In contrast, a growing body of literature from common garden studies documents striking chemical variation among aspen clones (Hwang and Lindroth, 1997; Hwang and Lindroth, 1998). This is especially true of aspen's major secondary metabolites: condensed tannins and phenolic glycosides. Moreover, this marked genetic variation in secondary chemistry strongly affects the performance of a variety of aspen-feeding insects (Hwang and Lindroth, 1997,1998). Finally, preliminary evidence suggests that variation among clones in the phenological expression of secondary chemistry differs between trees sampled early and late in a growing season (Hwang and Lindroth, 1998).

Given that phenological changes in aspen chemistry will likely impact herbivore performance, and that genetic differences are known to do so, the interaction of these factors was of interest. The primary objective of this study was to document temporal variation in concentrations of phytochemicals important for spring-feeding herbivores of aspen, and to determine whether such phenological changes differ among clones. A secondary objective was to ascertain the degree to which clonal variation in aspen foliar chemistry is consistent across growing seasons.

Quaking aspen contains two suites of carbon-based phenolic compounds, phenolic glycosides and condensed tannins (Lindroth and Hwang, 1996b). Phenolic glycosides

(salicylates) have been implicated as the primary chemical defense mechanism against generalist insect herbivores in a number of correlative and empirical studies (reviewed in Lindroth and Hwang, 1996b). Quaking aspen contains four such compounds, namely salicin, salicortin, tremuloidin and tremulacin (Lindroth et al., 1987). Of these, salicortin and tremulacin usually occur at higher concentrations, have the greatest toxicity and reduce herbivore performance in a dose-dependent fashion (Lindroth and Hwang, 1996b). Across clones, variation in phenolic glycoside concentration often explains most of the variation in herbivore performance in both common garden (Hwang and Lindroth, 1997,1998) and field (Hemming and Lindroth, 1995; Osier et al., 1999) studies. Condensed tannins, although often present in aspen at high concentrations, have not been found to markedly affect performance of lepidopteran larvae feeding on aspen (Hemming and Lindroth, 1995: Hwang and Lindroth, 1997, 1998; Osier et al., 1999).

2. Materials and methods

2.1. Study site and experimental clones

This research was conducted in the spring and summer of 1996 on property of the University of Michigan Biological Station. The Pellston Plain research area, located in the northern lower peninsula of Michigan, USA, 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 spatially separated, with clearly delineated boundaries.

The ten clones used in this study were a subset of 31 clones assayed by Lindroth and Hwang (1996a) for phytochemistry in the summer of 1995. Our clone designations (A, B, C, D, E, F, G, H, I, J) correspond to (2, 6, 8, 10, 12, 13, 14, 19, 24, 26) of Lindroth and Hwang (1996a). We used clones which only received direct sunlight (i.e., not shaded) and showed no signs or symptoms of bark canker. Within each clone, we chose 6 ramets that were matched for size within and among clones (clones A, B, C, D, E, F, G, H, I, J had a diameter at breast height ± 1 SE 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, df = 9, $F = 1.48$, $P = 0.181$). To control for position effects within the ramet, foliage was collected from the middle third of the ramet. Clones were located along a 2.0 km transect; the minimum distance between clones was 0.1 km. Aspens on Pellston Plain experienced no substantial defoliation in either 1995 or 1996; several years previously, the area experienced a population outbreak of the gypsy moth (B. Vande Kopple, resident biologist; personal communication).

2.2. Early-season foliar chemistry for 1996

Foliage was collected from experimental ramets on five dates (May 27, June 10, June 17, June 26 and July 5) that spanned the developmental period of spring-feeding herbivores, such as the gypsy moth and forest tent caterpillar. For each of the five

collection dates, 10–20 leaves were harvested by cleanly snipping leaves at the petioles. Removing leaves in this way has been shown not to induce a response from the ramet (Mattson and Palmer, 1988). Excised leaves were transported to the laboratory in plastic bags on ice, vacuum dried at room temperature, ground in a Wiley Mill (40 mesh) and stored at $-20°C$ until analyzed. This processing method preserves labile compounds in the foliage; complex phenolic glycosides (e.g., tremulacin, salicortin) do not decompose to simpler forms (e.g., tremuloidin, salicin) (Lindroth and Koss, 1996). Leaf phenology was controlled for during initial set up of the study; leaf-out of the ten clones chosen occurred nearly simultaneously, over a four day period (May 21–May 24, 1996). Unusually synchronous leaf-out of aspens in the spring of 1996 was due to a sudden warm period following a very late thaw. To control for the effects of leaf age in chemistry collections, we collected foliage only from the initial leaf flush (which comprises $> 90\%$ of available leaves), and avoided new leaves at indeterminately growing shoot tips.

For nitrogen determinations, we performed micro-Kjeldahl digestions (Parkinson and Allen, 1975), followed by the micro-Nesslerization procedure of Lang (1958). Glycine *p*-toluene-sulfonic acid (5.665% nitrogen) was digested concurrently and served as the standard. Percent water was determined gravimetrically, by the ratio of leaf fresh-to-dry mass. Condensed tannins were exhaustively extracted from leaf tissue, using 70% acetone (with 10 mM ascorbic acid as an antioxidant) at 4°C. We used the butanol-HCl method of Porter et al. (1986) for quantification of condensed tannins. Condensed tannins purified from aspen by the method of Hagerman and Butler (1980) served as the standard. Concentrations of phenolic glycosides were determined by high performance thin-layer chromatography (HPTLC) as reported by Lindroth et al. (1993). In short, samples were extracted in methanol and applied in duplicate, along with appropriate standards, to HPTLC plates using a Linomat IV applicator (Camag Scientific, NC, USA). Plates were developed, then scanned using a Camag TLC scanner, and resulting chromatograms were analyzed using Camag TLC evaluation software (CATS 3.19). This method of analysis allows precise quantification of phenolic glycosides in samples, and is much faster than HPLC methods. Salicortin, tremuloidin, and tremulacin standards were purified from aspen leaves, and salicin standard was obtained commercially (Sigma, St. Louis, MO, USA). Concentrations of salicin and tremuloidin were exceedingly low for some clones on particular collection dates; a concentration of 0.10% dry mass was established as the low cut-off for measurement and reporting.

2.3. Between-year comparisons of phytochemistry

We compared the phytochemistry of ten clones sampled in 1995 (reported in Lindroth and Hwang, 1996a) with the same clones sampled again in 1996. The survey by Lindroth and Hwang (1996a) was conducted on the 28th June, 1995. For an approximate comparison we used the average phytochemical concentration from our two collection dates (June 26 and July 5) that bracketed theirs. We compared concentrations of all the phytochemicals that were measured in both studies (nitrogen, condensed tannins, salicortin and tremulacin).

2.4. Statistical Analyses

For analysis of variance, the ramet served 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, so two ramets were deleted from the experiment. Repeated measures analysis of variance (Proc GLM, SAS Institute, 1989) was used to assess time effects, clonal effects and their interaction for foliar nitrogen, water, condensed tannins and total phenolic glycosides. Wilks' Lambda P-values are reported for the effect of time and the time × clone interaction (Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root all produced P-values similar to those of Wilks' Lambda).

We originally intended to subject the four individual phenolic glycosides to the same statistical analyses. However, the large number of missing data points, as a result of extremely low levels of salicin and tremuloidin for certain clone and harvest date combinations, precluded conventional analysis. We include those data (Table 1) and interpret trends conservatively and qualitatively in the text.

To relate the results of this study to those of Lindroth and Hwang (1996a) we used correlation analyses (Proc CORR, SAS Institute, 1989). Mean values per clone were used, such that $n = 10$ clones. Means per clone were generated from the four ramets sampled in 1995 and from the six ramets sampled in 1996 (except clone H, where only four ramets were sampled in 1996).

3. Results

3.1. Early-season foliar chemistry for 1996

Concentrations of all phytochemicals varied significantly among the sampling dates and clones (Fig. 1). Quantitative changes in phytochemistry through time were dependent upon clone.

Concentrations of foliar nitrogen declined throughout the sampling period for all clones, and converged toward a common value (Fig. 1). Variation among clones was initially 45%, but by the final collection date was only 15%. Clones with high concentrations of nitrogen early in the season tended to decline more rapidly than did other clones.

Foliar water concentrations also declined throughout the period measured (Fig. 1). Although highly significant, the magnitude of the effects of clone and the time × clone interaction were small. From the first to the final collection date, foliar water concentrations declined, on average, by 21%. Over the collection period, water concentrations varied by only 8% among clones.

Foliar concentrations of condensed tannins were the lowest at leaf-out and peaked or plateaued by late spring (June 10 or 17), depending upon clone (Fig. 1). Absolute variation among clones remained similar for the five collection dates (9–14% leaf dry mass) with a slight peak in mid-June. Although the time × clone effect was statistically significant, the rank order of clones changed little during the study.

Table 1
Summary of individual phenolic glycoside concentrations (% dry mass) for 10 clones of quaking aspen from leaf-out to mid-summer 1996. For each clone $n = 6$ ramets, except clone H where $n = 4$; means $\pm$ 1 S.E. are shown; – indicates concentrations were too low to quantify

Clone	May 27	June 10	June 17	June 26	July 5
A					
Salicin	1.51 ± 0.20	0.84 ± 0.19	1.07 ± 0.23	0.41 ± 0.05	0.88 ± 0.18
Salicortin	0.76 ± 0.09	1.29 ± 0.14	2.46 ± 0.27	2.20 ± 0.36	2.19 ± 0.14
Tremuloidin	0.75 ± 0.13	0.32 ± 0.04	—	—	—
Tremulacin	1.13 ± 0.13	1.08 ± 0.10	1.26 ± 0.10	1.03 ± 0.14	1.64 ± 0.11
B					
Salicin	1.54 ± 0.19	—	—	—	—
Salicortin	0.80 ± 0.10	1.73 ± 0.07	2.64 ± 0.24	1.89 ± 0.10	1.84 ± 0.07
Tremuloidin	0.70 ± 0.04	0.43 ± 0.03	—	—	—
Tremulacin	1.38 ± 0.11	1.78 ± 0.05	2.08 ± 0.12	1.96 ± 0.15	2.08 ± 0.06
C					
Salicin	2.26 ± 0.24	0.34 ± 0.11	0.35 ± 0.05	0.72 ± 0.18	0.47 ± 0.09
Salicortin	0.88 ± 0.12	1.12 ± 0.06	0.92 ± 0.15	1.24 ± 0.29	1.14 ± 0.12
Tremuloidin	1.14 ± 0.08	0.24 ± 0.03	—	—	—
Tremulacin	1.59 ± 0.16	1.33 ± 0.16	1.26 ± 0.27	1.01 ± 0.17	1.13 ± 0.20
D					
Salicin	—	0.71 ± 0.13	0.44 ± 0.07	0.67 ± 0.14	0.74 ± 0.13
Salicortin	2.67 ± 0.17	1.25 ± 0.09	1.91 ± 0.13	2.32 ± 0.41	1.79 ± 0.26
Tremuloidin	0.41 ± 0.11	0.59 ± 0.14	—	—	—
Tremulacin	3.16 ± 0.29	1.16 ± 0.12	0.99 ± 0.13	1.26 ± 0.19	1.80 ± 0.17
E					
Salicin	1.26 ± 0.11	1.92 ± 0.10	1.43 ± 0.09	1.03 ± 0.20	0.53 ± 0.04
Salicortin	0.60 ± 0.19	1.36 ± 0.07	2.19 ± 0.07	1.26 ± 0.11	1.03 ± 0.14
Tremuloidin	0.91 ± 0.15	0.19 ± 0.02	—	—	—
Tremulacin	0.76 ± 0.16	1.19 ± 0.10	1.23 ± 0.18	1.42 ± 0.10	1.05 ± 0.16
F					
Salicin	1.16 ± 0.14	0.19 ± 0.04	—	—	—
Salicortin	0.62 ± 0.09	1.85 ± 0.13	1.73 ± 0.18	2.12 ± 0.04	2.44 ± 0.12
Tremuloidin	0.77 ± 0.08	0.39 ± 0.02	—	—	—
Tremulacin	1.30 ± 0.34	2.06 ± 0.26	1.62 ± 0.15	1.89 ± 0.09	2.08 ± 0.23
G					
Salicin	2.19 ± 0.31	—	—	—	—
Salicortin	0.56 ± 0.09	2.07 ± 0.36	1.23 ± 0.13	1.86 ± 0.22	1.77 ± 0.12
Tremuloidin	0.65 ± 0.10	—	—	—	—
Tremulacin	0.65 ± 0.10	0.76 ± 0.13	0.51 ± 0.06	1.03 ± 0.18	1.12 ± 0.11
H					
Salicin	2.85 ± 0.32	—	—	—	—
Salicortin	2.89 ± 0.54	3.78 ± 1.01	3.66 ± 0.23	2.64 ± 0.36	3.12 ± 0.04
Tremuloidin	1.62 ± 0.26	—	—	—	—
Tremulacin	3.89 ± 1.03	3.46 ± 0.54	2.93 ± 0.30	2.07 ± 0.14	2.12 ± 0.09
I					
Salicin	—	—	—	—	
Salicortin	1.10 ± 0.10	2.77 ± 0.26	1.88 ± 0.12	1.72 ± 0.13	1.49 ± 0.19
Tremuloidin	0.46 ± 0.12	—	—	—	—
Tremulacin	0.81 ± 0.15	1.51 ± 0.38	0.90 ± 0.10	1.24 ± 0.11	1.12 ± 0.26
J					
Salicin	0.99 ± 0.16	0.47 ± 0.12	1.66 ± 0.09	1.40 ± 0.13	1.49 ± 0.11
Salicortin	0.75 ± 0.09	2.32 ± 0.33	1.94 ± 0.22	2.03 ± 0.27	2.29 ± 0.32
Tremuloidin	0.97 ± 0.17	—	—	—	—
Tremulacin	0.72 ± 0.07	1.36 ± 0.20	0.77 ± 0.11	1.01 ± 0.12	1.24 ± 0.12

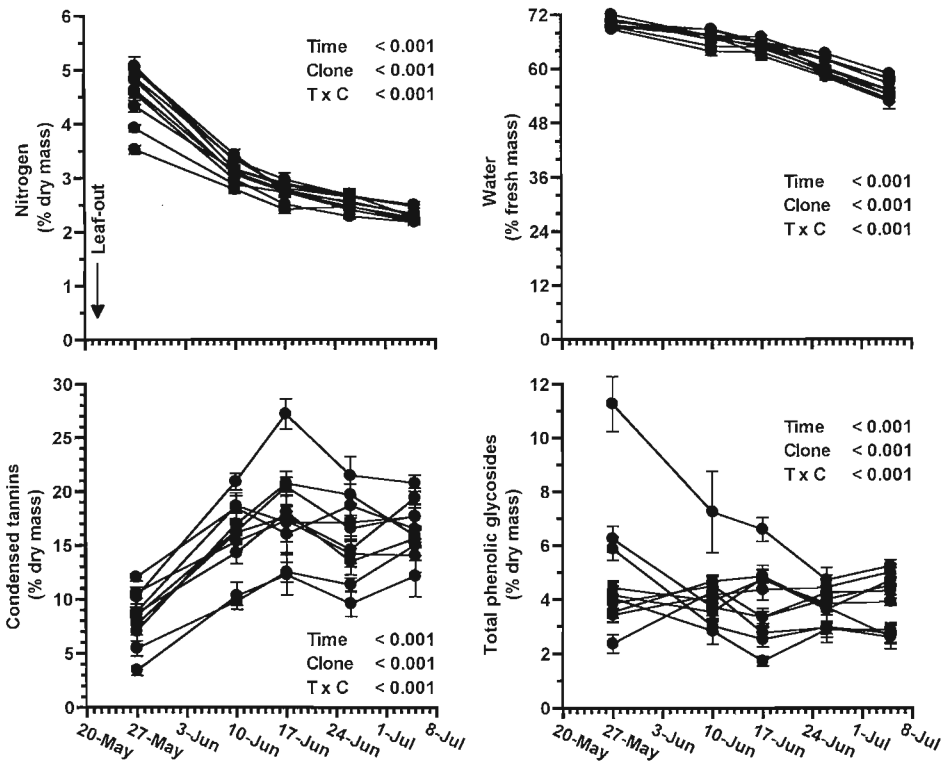

Fig. 1. Early season temporal variation in phytochemistry of ten quaking aspen clones in northern Michigan. *P*-values indicate the results of repeated-measures ANOVA (time df = 4; clone df = 9; time × clone df = 36). For each clone, $n = 6$ ramets, except clone H where $n = 4$.

Foliar concentrations of phenolic glycosides varied significantly over time and among clones (Fig. 1). Moreover, unlike the case for nitrogen, water and condensed tannins, temporal changes in total phenolic glycoside concentrations were strongly dependent upon clone. Two clones (C and H) exhibited the highest concentrations of phenolic glycosides on the first collection date and decreased thereafter (Table 1). In contrast, concentrations in one clone (J) increased throughout the period. For the remaining clones, concentrations of phenolic glycosides were either highest (clone E) or lowest (clones D and G) in the middle of the sampling period (July 17).

The contribution of individual phenolic glycosides (salicin, salicortin, tremuloidin and tremulacin) to the preceding patterns is more difficult to discern (Table 1). Clones C and H had high concentrations of total phenolic glycosides early in the season, due in large part to an abundance of salicin and tremuloidin. Although no single constituent or pair of constituents appears to drive the pattern in total phenolic glycoside concentrations for all clones, salicortin and tremulacin dominate the patterns for many. These two phenolic glycosides occurred in measurable quantities in every clone at every collection date, and made up the majority of the total phenolic glycoside pool.

Table 2
Inter-annual correlations among phytochemicals sampled from ten clones in late June 1995 (Lindroth and Hwang, 1996) and 1996 (this study). Pearson product-moment correlations are based on a mean value for each clone ($n = 10$ clones)

Phytochemical	r	P
Nitrogen	0.690	0.027
Condensed tannins	0.635	0.049
Salicortin	0.881	0.001
Tremulacin	0.684	0.029

Within a clone, concentrations of salicortin and tremulacin were more consistent among collection dates than were concentrations of salicin and tremuloidin (Table 1). For tremuloidin, all ten clones had measurable concentrations on the first or second collection date, but concentrations were not measurable thereafter (Table 1). For salicin, all clones, except clone I, had measurable concentrations on at least one of the sampling dates. Salicin concentrations, on average, were lower late, compared to earlier, in the sampling period (Table 1).

3.2. Between-year comparisons of phytochemistry

Concentrations of all phytochemicals were strongly correlated between years (Table 2). For each of the comparisons, r-values were at least 0.6 and moderately to highly significant.

4. Discussion

Levels of all phytochemicals changed throughout the period of the study, the first half of the growing season. Phytochemical concentrations, as well as temporal changes in such concentrations, also differed among clones. Thus, genetic factors appear to influence not only phytochemical concentrations, but also the temporal dynamics in expression of such traits. In addition, concentrations of nitrogen, condensed tannins and phenolic glycosides in individual clones were highly correlated between years.

Foliar nitrogen concentrations can have strong impacts on herbivores, especially those that feed on trees, for which concentrations are typically quite low (Mattson, 1980; Scriber and Slansky, 1981). The temporal decline in nitrogen availability observed in this as well as other studies may explain the close synchrony of insect feeding stages with budbreak and leaf expansion of their hosts (Raupp et al., 1988; Hunter, 1991; Hunter and Lechowicz, 1992). Temporal declines in nitrogen concentrations were attended by a decrease in inter-clonal variability in concentrations. After the second collection date, inter-clonal variation in foliar nitrogen was small and would likely have little effect on insect herbivores. In a parallel set of studies that tested the effects of inter-clonal variation in phytochemistry on gypsy moths, foliar

nitrogen was implicated as only a marginally important factor influencing insect performance on these ten clones (Osier et al., 1999).

Foliar water concentrations decreased throughout the study period and inter-clonal variation was minimal. Although water concentrations can be important for plant-feeding insects (Scriber and Slansky, 1981; Slansky, 1993), the small amount of inter-clonal variation observed had no detectable effect on gypsy moths in our parallel study (Osier et al., 1999).

Foliar condensed tannins accumulated with time and inter-clonal variation was considerable. Our seasonal patterns are generally similar to those of other published studies of aspen phytochemistry. For example, Roth et al. (1998) found early seasonal increases toward a plateau in June. High concentrations notwithstanding, the efficacy of condensed tannins is thought to be minimal against herbivorous insects in general (Ayres et al., 1997), and aspen-feeding insects in particular (Lindroth and Hwang, 1996b). In our parallel gypsy moth study, condensed tannins appeared to impact larvae only by diluting required nutrients (Osier et al., 1999). If such is the case, seasonal increases in condensed tannins may result in decreased food quality for herbivores.

In contrast to the seasonal pattern exhibited by condensed tannins, levels of phenolic glycosides varied unpredictably over time, and highly among clones, as has been reported previously (Lindroth et al., 1987; Lindroth and Hwang, 1996b). Phenolic glycosides in quaking aspen are the primary resistance mechanism against foliar feeding Lepidoptera (reviewed in Lindroth and Hwang, 1996b). In parallel feeding trials, gypsy moth performance was best predicted by concentrations of phenolic glycosides averaged over the feeding period (Osier et al., 1999). Quantitative variation in phenolic glycosides alone explained 35 and 51% of the variation in female and male pupal mass of field-reared gypsy moths, respectively (Osier et al., 1999).

The ephemerality of salicin and tremuloidin, and trend toward higher concentrations early in the season, invites questions as to their function in quaking aspen foliage. These compounds may serve as precursors to their more complex and biologically active chemical relatives salicortin and/or tremulacin (Pierpoint, 1994), thus accounting for their abundance during leaf expansion.

Foliar concentrations of phytochemicals in ten aspen clones were highly correlated between years. Although leaf chemistry changes through the season, the relative rankings of clones with respect to individual phytochemical components is predictable. This is true especially for phenolic glycosides, for which within-year variation appears, to us, haphazard and unpredictable (among clones), but for which between year variation is highly predictable (within clones). The strong correlation between years for phenolic glycosides is likely due to the facts that levels of the compounds are strongly genetically determined, highly variable among clones, and less responsive to environmental factors such as resource availability and defoliation, than are other aspen constituents (Lindroth and Hwang, 1996b).

Because this study was conducted in the field, the effect of "clone" necessarily includes environmental as well as genotypic factors. Environmental variability does affect aspen foliar chemistry; ramets respond to carbon stresses and surpluses in accordance with predictions of the carbon/nutrient balance hypothesis (Bryant et al.,

1983,1987; Agrell et al., 1998; Hemming and Lindroth, 1999). Phenolic glycoside concentrations are, however, only minimally phenotypically plastic (Kinney et al., 1997; Agrell et al., 1998; Hemming and Lindroth, 1999). Thus, at least for those compounds, the results for clonal differences are most likely due to genetic variation rather than to environmental heterogeneity across the clones.

We recognize that differential herbivory and induction of secondary metabolites could have contributed to clonal and temporal variation in chemistry observed in this study. Such effects, however, were most likely minimal. Foliar damage was extremely light for our experimental trees, and did not appear to differ among clones. Moreover, although levels of condensed tannins show strong responses to defoliation (Roth et al., 1998; Osier and Lindroth, unpubl. data), levels of phenolic glycosides are only minimally affected, even after severe defoliation (Lindroth and Kinney, 1998; Roth et al., 1998; Osier and Lindroth, unpubl. data).

Seasonal changes (from spring to summer) in the rank-ordering of phenolic glycoside concentrations among clones are accompanied by parallel shifts in insect herbivore performance (Hwang and Lindroth, 1998). Given that phenolic glycoside concentrations are genetically determined (Lindroth and Hwang, 1996b), that they are the principal determinants of food quality for aspen-feeding Lepidoptera (Lindroth and Hwang, 1996b), and that seasonal changes in concentrations are paralleled by changes in insect performance (Hwang and Lindroth, 1998), temporal patterns observed among clones that appear haphazard may actually be strongly controlled. The expression of defensive traits on unique schedules may be evolutionarily adaptive (Schultz, 1983) and could account for the variety of seasonal patterns observed in phenolic glycoside concentrations across our ten clones. We suggest that the spatial and temporal mosaic of aspen chemical quality evident in the field represents the culmination of evolutionary processes driven by the costs of chemical defense in terms of reduced growth (Lindroth and Hwang, 1996b; Osier and Lindroth, unpublished data), the benefits of chemical defense in terms of reduced herbivory (Lindroth and Hwang, 1996b), and possibly the benefits of "associational protection" (Tuomi et al., 1994) afforded by proximity to neighboring clones with differing schedules of defense expression.

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