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Effects of genotype and nutrient availability on phytochemistry of trembling aspen (*Populus tremuloides* Michx.) during leaf senescence

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Abstract

We documented temporal patterns in phytochemical composition of *Populus tremuloides* Michx. during leaf senescence, and the influence of genotype and soil nutrient availability on such patterns. Levels of foliar nitrogen, carbohydrates, phenolic glycosides and condensed tannins were quantified for four aspen genotypes grown in a common garden, with low and high levels of soil nutrients. Levels of all compounds tended to decline over time, although the magnitude of change was influenced by plant genotype and nutrient availability. Genetic variation in concentrations of phytochemicals was much greater for phenolic glycosides and tannins than for nitrogen and carbohydrates, and these phenolic signatures generally persisted through leaf abscission. Our results suggest that genotypic and nutrient effects on patterns of chemical change during senescence will likely influence the performance of late-season herbivores on aspen. Moreover, nutrient and especially genotypic variation in phytochemistry of abscised leaves is likely to affect litter decomposition rates.

Keywords: Aaspen; *Populus tremuloides*; Salicaceae; Salicortin; Tremulacin; Phenolic glycosides; Tannins; Genetic variation; Senescence

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1. Introduction

A long-standing interest of chemical ecologists has been identification of the genetic and environmental factors responsible for intraspecific variation in plant chemical composition, and how the importance of such factors — both independently and interactively — changes over time. Such information has been central to improvements in our understanding of the mechanisms that underlie and mediate interactions of plants with herbivores, pathogens and the abiotic environment.

Trembling aspen has proved to be an ideal woody plant species with which to address questions relating to the ecological significance of phytochemical variation. The chemical composition of aspen foliage exhibits striking variation in response to genetic, environmental, and temporal factors (Hemming and Lindroth 1995, 1999; Lindroth and Hwang, 1996a,b; Hwang and Lindroth 1997, 1998; Osier et al., 2000a). Moreover, this variation can markedly alter the performance of herbivores and virulence of pathogens (Lindroth and Hwang, 1996a).

The signature secondary metabolites of aspen are phenolic glycosides (salicylates; Lindroth et al., 1987). Trembling aspen produces a suite of four such compounds, including salicin, tremuloidin, salicortin and tremulacin. Of these, salicortin and tremulacin typically comprise >90% of the phenolic glycoside pool, have the highest toxicity (on a molar basis) and reduce insect performance in a dose-dependent manner (Lindroth and Hwang, 1996a). A second important class of secondary metabolites in aspen is condensed tannins. Although these compounds may occur in very high concentrations, they have not been implicated as defense agents against insect herbivores (Lindroth and Hwang, 1996a; Ayres et al., 1997). Their effects on other organisms are unknown.

To date, most research on the phytochemistry of aspen has focused on chemical variation during leaf expansion and early maturation (Osier et al., 2000a). These are periods of active feeding by a number of outbreak herbivores, such as forest tent caterpillars (*Malacosoma disstria*), gypsy moths (*Lymantria dispar*) and large aspen tortrix (*Choristoneura conflictana*). Little is known, however, about the effects of genetic and environmental factors on foliar chemistry during the stages of late maturation and senescence. Such information is needed to improve our understanding not only of the interactions of aspen with late-season folivores, but of the consequences of chemical variation for leaf litter quality and decomposition (Hättenschwiler and Vitousek, 2000).

In this report we describe patterns in aspen chemical composition during leaf senescence, and how these vary in relation to aspen genotype and nutrient availability. We focus on levels of nitrogen, carbohydrates, phenolic glycosides and condensed tannins, as these constituents are important regulators of herbivory or decomposition (Lindroth and Hwang, 1996a; Hättenschwiler and Vitousek, 2000)

2. Materials and methods

2.1. Experimental design

The experiment employed a 4 × 2 factorial design with repeated measures, including four aspen genotypes, two levels of soil nutrient availability, and three sampling periods. Each combination of aspen genotype and soil fertility was replicated with six independent trees (total of 48).

2.2. Aspen clonal material, propagation, and growth conditions

The four genotypes selected for use in this study represented a range of low to high levels of phenolic glycosides and tannins in early to mid-season foliage (Hwang and Lindroth, 1997). Trees were propagated in 1996 from root cuttings taken from potted saplings, which themselves were propagated from root material collected from aspen clones in south-central Wisconsin (Hwang and Lindroth, 1997). Shoots were grown outside, in individual pots, until leaf drop, whereupon they were harvested, washed, and over-wintered (bare-rooted in moist peat moss) at 4°C. In the spring of 1997, saplings were potted in 36 l pots containing a 7:3 mixture of sand and local field soil (silt loam). To manipulate nutrient availability, Osmocote eight–nine month slow release fertilizer (18:6:12 N–P–K + micro-nutrients) was added at a rate of 3.5 g/l soil to high fertility pots; low fertility pots received no fertilizer. In the spring of 1998, high fertility plants were treated (top dressed) for a second time with the same dose of fertilizer. During 1997–1998, trees were watered with an automatic drip irrigation system.

2.3. Collection and chemical analysis of foliage

We collected leaves three times over a period of eight–nine weeks late in the growing season. Leaves were removed by cleanly snipping at the petiole, a method that has been shown to not induce a response from the tree (Mattson and Palmer, 1988). The first collection ('green leaves') was made on 4 September 1998, when leaves were physiologically active and showed no signs of senescence (yellowing). The second collection was made on 20 October 1998, when trees varied from early- to late-senescent. Following the second collection, we bagged branches with fine nylon mesh screen to facilitate subsequent collection of abscised leaves. For the third harvest ('abscised leaves'), leaf material was collected from mesh bags on 30 October and 6 November, 1998. For all harvests, we collected approximately 10 leaves from each of several branches per tree. The same branches were sampled for all three leaf collections. Due to the size and architecture of the experimental trees, harvested leaves were predominantly 'sun leaves' (i.e. non-shaded). Harvested leaves were immediately placed into crushed ice, then transported to the laboratory and flash-frozen in liquid nitrogen. Leaves were then freeze-dried, ground in a Wiley mill (40 mesh) and stored at −20°C until analyzed.

Nitrogen determinations were made with a LECO FP528 nitrogen analyzer, using

glycine *p*-toluene sulfonic acid as a standard. For carbohydrate analyses, plant material was exhaustively extracted in 80% EtOH (80°C) and centrifuged. Soluble sugars (supernatant) were quantified by a modification of the dinitrosalicylic acid method (Clark, 1964). Because this method measures reducing sugars, extracts were first hydrolyzed in dilute acid (2 N HCl, 100°C, 30 min) to convert sucrose to its reducing monosaccharides. Glucose was used as a standard. Starch (pellet) determinations were made by first adding 0.2 N NaOH to each sample, followed by heating in a boiling water bath (12 min) and neutralization with 1.0 M acetic acid. Samples were then hydrolyzed by addition of amyloglucosidase in Na acetate buffer (0.1 M, pH 4.5) and incubation at 55°C (10 min). Hydrolyzed starch was quantified by the dinitrosalicylic acid method as described previously, using appropriate blanks to account for reaction of the amyloglucosidase solution with dinitrosalicylic acid.

Concentrations of phenolic glycosides were determined by high performance thin-layer chromatography (HPTLC) as reported by Lindroth et al. (1993). Briefly, samples were extracted in methanol and applied, with standards, to HPTLC plates with a Linomat IV applicator (Camag Scientific, NC, USA). Plates were developed in methylene chloride–methanol–tetrahydrofuran (6:1:1), then scanned (274 nm) in a Camag TLC Scanner II. Chromatograms were analyzed with Camag TLC evaluation software (CATS 3.19). Salicortin and tremulacin standards were purified from aspen leaves using flash chromatography (Still et al., 1978).

Finally, condensed tannin concentrations were quantified by the acid butanol method (Porter et al., 1986). Samples were exhaustively extracted in 70% acetone (with 10 mM ascorbic acid as an antioxidant). Condensed tannin standard was purified from aspen leaves using the procedure of Hagerman and Butler (1980).

2.4. Statistical analyses

The effects of aspen genotype and soil nutrient availability on aspen chemistry during leaf senescence were analyzed using repeated measures analysis of variance (ANOVA; PROC GLM, SAS Institute, 1989) with a completely randomized design. In some cases (starch, condensed tannins, salicortin) the symmetry of the covariance matrix did not meet the assumptions of repeated measures ANOVA. In these cases, however, the assumptions of the Huynh–Feldt (H-F) correction were satisfied, so we report H-F corrected *P*-values, as recommended by Potvin and Lechowicz (1990). For simplicity, we provide H-F *P*-values for all variables with a time component, as H-F-corrected and standard *P*-values were similar for parameters that met the assumptions of symmetry.

3. Results

Aspen chemical composition varied substantially among sampling dates, genotypes and nutrient treatments. In addition, plant genotype and nutrient availability interacted over time to alter patterns of chemical changes.

Levels of nitrogen declined during foliar senescence (Fig. 1, Table 1). This decline was steeper for high-nutrient than for low-nutrient trees, such that a difference of 0.8% nitrogen (dry wt.) between nutrient treatments at the first collection date was reduced to 0.2% by the final date. That high-nutrient aspen contained higher levels of nitrogen later into the season is consistent with our observations of leaf color; high-nutrient foliage remained green considerably longer than did low-nutrient foliage. Patterns of decline in nitrogen concentrations appeared to be similar among genotypes, although the significant three-way interaction reveals that some genotypes responded differently over time and between nutrient treatments.

Concentrations of simple sugars declined during senescence, and patterns of change differed among genotype and nutrient treatments (Fig. 1, Table 1). Some genotypes exhibited consistent declines throughout the study period, whereas others showed little change between the first two sampling dates and more rapid change thereafter. Overall, sugar levels declined by 45% in low-nutrient trees, but by only 27% in high-nutrient trees. The significant two-way interaction reveals that during

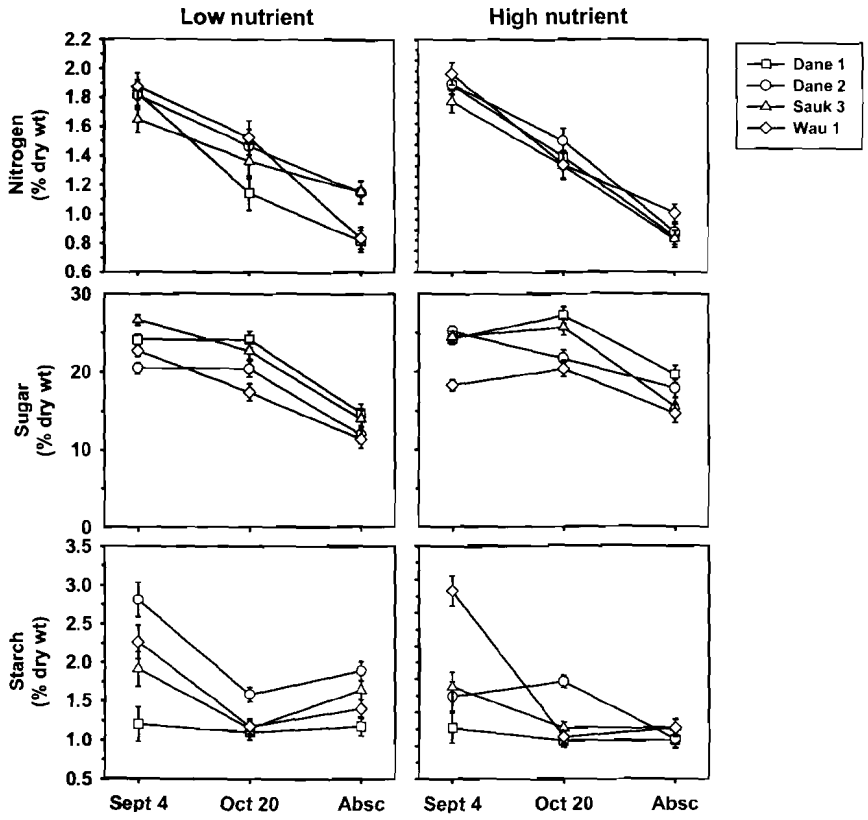

Fig. 1. Effects of aspen genotype and soil nutrient availability on levels of foliar nitrogen and carbohydrates during leaf senescence. Each line represents a different genotype (means ±1 SE, n=6 for each genotype: 'Absc' = abscised leaves.)

Table 1
Summary of *P*-values for the effects of genotype, nutrient availability and time on chemical composition of aspen leaves during senescence. Huynh–Feldt corrected *P*-values are provided for all terms with a time component

Main effects and interactions	Nitrogen	Sugars	Starch	Salicortin	Tremulacin	Condensed tannins
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype	0.167	<0.001	<0.001	<0.001	<0.001	<0.001
Nutrient	<0.001	<0.001	0.234	0.025	0.320	<0.001
Time × genotype	0.068	0.002	<0.001	0.008	<0.001	0.099
Time × nutrient	<0.001	<0.001	0.012	0.003	<0.001	<0.001
Genotype × nutrient	0.514	0.064	0.002	0.601	0.008	0.135
Time × genotype × nutrient	0.011	0.001	<0.001	0.033	0.336	0.133

senescence, patterns of reduction in sugar concentration varied differently among genotypes at different nutrient levels.

Concentrations of starch decreased during senescence, with most of the decline occurring between the first two sampling dates (Fig. 1, Table 1). Genotypes with the highest initial concentrations exhibited the largest declines, such that the variance among clones decreased over time. High nutrient availability differentially affected the aspen genotypes, leading to increases, decreases, and no change in starch concentrations. Moreover, these interactive effects changed over time (significant three-way interaction).

Levels of the phenolic glycoside salicortin declined in most, but not all, genotypes during leaf senescence (Fig. 2, Table 1). Salicortin concentrations were generally lower under high nutrient conditions during the first two, but not the final, collection dates. The interactive effects of nutrient availability and genotype differed over the course of leaf senescence (significant three-way interaction). Temporal changes in levels of the phenolic glycoside tremulacin differed somewhat from those of salicortin (Fig. 2, Table 1). Under low nutrient conditions, tremulacin concentrations markedly declined in one genotype (with the highest initial concentrations), but did change substantially in other genotypes. Under high nutrient conditions, however, tremulacin levels slightly decreased in two genotypes and increased in the two remaining genotypes.

Finally, concentrations of condensed tannins declined during senescence in low-nutrient trees, but did not change appreciably in high-nutrient trees (Fig. 2, Table 1). For every genotype, tannin concentrations were higher under low nutrient conditions than high nutrient conditions in green leaves (first collection date), but not in abscised leaves.

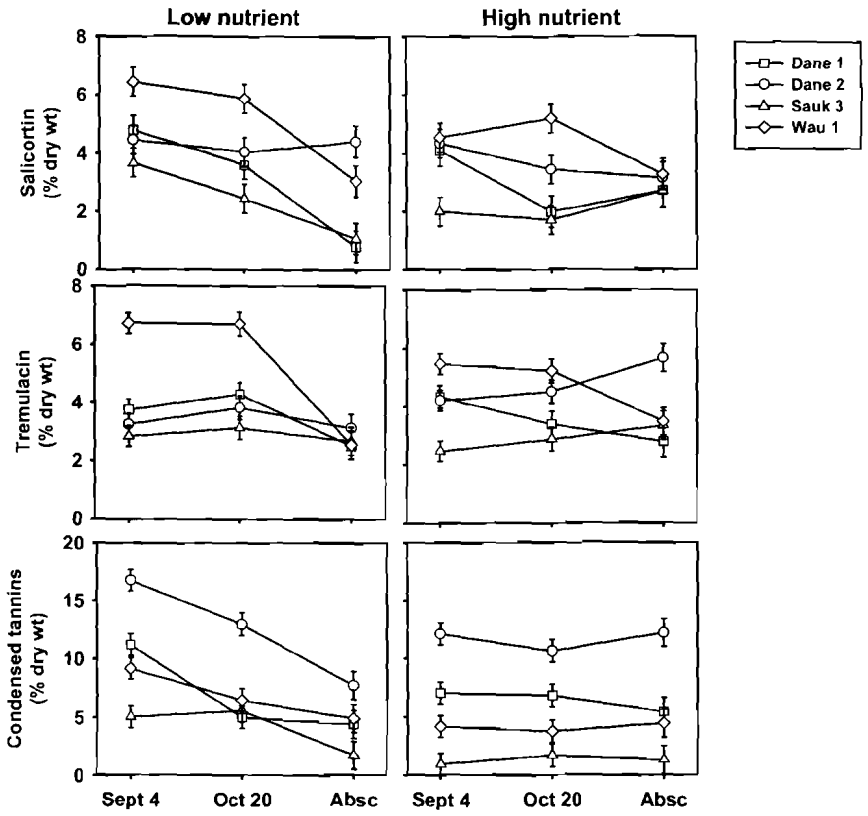

Fig. 2. Effects of aspen genotype and soil nutrient availability on levels of foliar phenolic glycosides and condensed tannins during leaf senescence. Each line represents a different genotype (means ±1 SE, n=6 for each genotype; 'Absc' = abscised leaves.)

4. Discussion

Research on chemical changes that occur during leaf senescence has historically focused on loss of photosynthetic pigments, degradation of protein, and resorption of mineral nutrients. In contrast, changes in the phenolic content of leaves during senescence have received little attention, despite the importance of such constituents for the fate of abscised tissues. Aspen and related *Populus* are a case in point; several studies (Pregitzer et al., 1990; Collier and Thibodeau, 1995; Harvey and van den Driessche, 1999) have assessed the dynamics of foliar nutrients during autumnal senescence, but none has addressed changes in phenolic constituents.

Not surprisingly, levels of every chemical variable changed during leaf senescence. Of greater interest, however, is the fact that temporal patterns of change varied among aspen genotypes (time × genotype interaction), between nutrient levels (time × nutrient interaction), or in relation to the interaction between genotype and nutrients

(time × genotype × nutrient interaction). Moreover, the treatment factor(s) contributing to most of the variance in chemical concentrations differed among compounds.

For nitrogen, most of the variance among means was due to time and nutrient treatment; comparatively little variation was contributed by genotype. Leaves exhibited a typical autumnal decline, due to mobilization and resorption of nitrogen from nitrogen-containing compounds such as ribulose-1,5-bisphosphate carboxylase and chlorophyll. Although leaves of high-nutrient trees had higher initial concentrations of nitrogen, and sustained those levels later into the year, levels of nitrogen were similar in abscised leaves from both nutrient treatments.

Sugar levels showed substantial variation with respect to all treatment variables, and interactions thereof. Overall, time accounted for a large proportion of the total variance, with levels declining due to both translocation to carbohydrate storage tissues (e.g. roots) and continued respiration following the loss of photosynthetic capacity (Collier and Thibodeau, 1995). Leaves of high-nutrient trees lost less soluble sugar than did those of low-nutrient trees, such that sugar concentrations in abscised leaves were 30% greater in the former compared with the latter. The reason for this difference is unclear. One possibility involves differences in the phenology of senescence between the two nutrient treatments. Because leaves of trees in the high nutrient treatment remained green for a longer period of time, translocation of carbohydrates from these leaves may have been cut short by cold temperatures eliciting leaf abscission.

Variance among starch concentrations was due primarily to time and genotype, although significant interactions were observed for all three treatment variables. The rapid decline in the levels of starch between the first and second collection dates suggests that starch mobilization and translocation of sugars occurs early in the process of leaf senescence. Moreover, the various aspen genotypes exhibited the capacity to reduce starch concentrations to comparable levels; although the genotypes differed markedly in concentrations of starch stored in green leaf tissues, they varied only slightly in concentrations remaining in abscised tissues.

For phenolic glycosides, variance in concentrations was due primarily to genotype, with some variance attributable to time and various two- and three-way interactions. Temporal decreases due to degradation or translocation of these compounds are consistent with their nature as 'dynamic' metabolites (Reichardt et al., 1991; Kleiner et al., 1999). Not all genotypes, however, exhibited decreases in phenolic glycoside concentrations. Some showed little change, or slight increases, during leaf senescence. Moreover, no clear pattern emerged as to whether the large genotypic variation in phenolic glycoside levels in green foliage would continue to be expressed in abscised leaves. Such appeared to be the case for salicortin under low-nutrient but not high-nutrient conditions, and for tremulacin under high-nutrient but not low-nutrient conditions.

Finally, for condensed tannins, variance in concentrations was once again due primarily to genotype, with less variance attributable to time and nutrient availability. Interestingly, the temporal decline was exhibited only in low-nutrient, not high-nutrient, trees. Given that condensed tannins are considered to be 'static' metabolites (Reichardt et al., 1991), and that losses of minerals, carbohydrates and proteins from

leaf tissues during senescence result in reduced leaf weight, concentrations of tannins were expected to increase over time. Several explanations exist for the reverse trend. First, apparent decreases could be explained by a change in extractability. For example, tannins may polymerize during senescence; larger polymers are generally less easily extracted. Alternatively, tannins may become more tightly bound to protein or cell wall polysaccharides during the processes of degradation, mobilization and translocation of foliar constituents. Indeed, polyphenol–protein complexes that form during senescence can comprise a substantial portion of the leaf mass (Hättenschwiler and Vitousek, 2000). The possibility also exists that tannins are not as stable as generally considered.

Overall, genotypic variation in levels of aspen phytochemicals was substantially greater for phenolic constituents (glycosides and tannins) than it was for nitrogen and carbohydrates. This difference between 'secondary' and 'primary' metabolite concentrations has been noted previously for green aspen foliage (Lindroth and Hwang, 1996b; Osier et al., 2000a). Indeed, genotypic variation is the major factor explaining differential performance of both spring- and summer-feeding insects on aspen (Hwang and Lindroth 1997, 1998; Osier et al., 2000b). This research suggests that such marked genotypic variation in chemical composition extends through the period of leaf senescence, and may affect the performance of insect herbivores feeding on aspen late into the growing season, as a variety of coleopteran, hymenopteran and lepidopteran folivores have been observed to do (Ashburn and Lindroth, unpublished data).

Results from this research also have implications for the decomposition of aspen leaf litter. Litter quality is one of the most important factors influencing decomposition rates, and is determined by carbon availability (i.e. labile to recalcitrant compounds), mineral (especially nitrogen) content, and chemical modifiers such as tannins (Swift et al., 1979; Anderson, 1991). Tannins and related phenolics alter decomposition by complexing with other litter components (e.g. amino acids) or by inhibiting the degradative activity of enzymes and soil fauna (Horner et al., 1988; Anderson, 1991; Coûteaux et al., 1995; Hättenschwiler and Vitousek, 2000). Because foliar nitrogen levels tended to converge toward similar values, independent of genotypic and environmental factors, our results suggest that this element will likely not alter decomposition rates of aspen litter. For abscised foliage, the greatest difference in nitrogen concentrations between nutrient treatments occurred for genotype Wau 1: 0.83 and 1.36% (dry wt.) for low- and high-nutrient treatments, respectively. These values are nearly identical to those reported by Prescott et al. (1999) for aspen leaf litter from control and fertilized treatments, for which no difference in decomposition rate occurred over a four-year period.

In contrast to our results with nitrogen, phenolic (glycoside and tannin) 'signatures' of the different aspen genotypes generally (but not invariably) extended to abscised litter. Such genotypic variation in litter phenolic composition would likely influence decomposition rates. Schimel et al. (1996) showed that over a 30-day assay period, simple phenolics from *Populus balsamifera* stimulated nitrogen uptake by microbes (increasing immobilization) whereas tannins inhibited microbial respiration

(reducing mineralization). Both processes would reduce soil nitrogen availability, at least over the short term.

In conclusion, both genotypic and environmental factors, and their interactions, influence the chemical composition of aspen during leaf senescence. Such phytochemical variation is likely to influence the interactions of aspen with herbivores during this period, as well as to carry over to affect leaf litter decomposition and nutrient cycling.

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References

Anderson, J.M., 1991. The effects of climate change on decomposition processes in grassland and coniferous forests. Ecol. Appl. 1, 326–347.

Ayres, M.P., Clausen, T.P., MacLean, S.F. Jr., Redman, A.M., Reichardt, P.B., 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78, 1696–1712.

Clark, J.M. Jr., 1964. Experimental Biochemistry. Freeman, San Francisco, CA.

Collier, D.E., Thibodeau, B.A., 1995. Changes in respiration and chemical content during autumnal senescence of *Populus tremuloides* and *Quercus rubra* leaves. Tree Physiol. 15, 759–764.

Coûteaux, M.-M., Bottner, P., Berg, B., 1995. Litter decomposition, climate and litter quality. Trends Ecol. Evol. 10, 63–66.

Hagerman, A.E., Butler, L.G., 1980. Condensed tannin purification and characterization of tannin-associated proteins. J. Agric. Food Chem. 28, 947–952.

Harvey, H.P., van den Driessche, R., 1999. Poplar nutrient resorption in fall or drought: influence of nutrient status and clone. Can. J. For. Res. 29, 1916–1925.

Hättenschwiler, S., Vitousek, P.M., 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol. Evol. 15, 238–243.

Hemming, J.D.C., Lindroth, R.L., 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. Oecologia 103, 79–88.

Hemming, J.D.C., Lindroth, R.L., 1999. Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. J. Chem. Ecol. 25, 1687–1714.

Horner, J.D., Gosz, J.R., Cates, R.G., 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. Am. Nat. 132, 869–883.

Hwang, S.-Y., Lindroth, R.L., 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. Oecologia 111, 99–108.

Hwang, S.-Y., Lindroth, R.L., 1998. Consequences of clonal variation in aspen phytochemistry for late season folivores. Ecoscience 5, 508–516.

Kleiner, K.W., Raffa, K.F., Dickson, R.E., 1999. Partitioning of ^{14}C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: evidence for secondary metabolite turnover. Oecologia 119, 408–418.

Lindroth, R.L., Hsia, M.T.S., Scriber, J.M., 1987. Characterization of phenolic glycosides from quaking aspen (*Populus tremuloides*). Biochem. Syst. Ecol. 15, 677–680.

Lindroth, R.L., Hwang, S.-Y., 1996a. Diversity, redundancy and multiplicity in chemical defense systems of aspen. Recent Adv. Phytochem. 30, 25–56.

Lindroth, R.L., Hwang, S.-Y., 1996b. Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). Biochem. Syst. Ecol. 24, 357–364.

Lindroth, R.L., Kinney, K.K., Platz, C.L., 1993. Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry and insect performance. Ecology 74, 763–777.

Mattson, W.J., Palmer, S.R., 1988. Changes in foliar minerals and phenolics in trembling aspen, *Populus tremuloides*, in response to artificial defoliation. In: Mattson, W.J., Levieux, J., Bernard-Dagan, C. (Eds.), Mechanisms of Woody Plant Defenses Against Insects. Search for Pattern. Springer, New York, NY, pp. 157–169.

Osier, T.L., Hwang, S.-Y., Lindroth, R.L., 2000a. Within- and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones in northern Michigan, USA. Biochem. Syst. Ecol. 28, 197–208.

Osier, T.L., Hwang, S.-Y., Lindroth, R.L., 2000b. 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.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.

Potvin, C., Lechowicz, M.J., 1990. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 71, 1389–1400.

Pregitzer, K.S., Dickmann, D.I., Hendrick, R., Nguyen, P.V., 1990. Whole-tree carbon and nitrogen partitioning in young hybrid poplars. Tree Physiol. 7, 79–93.

Prescott, C.E., Kabzems, R., Zabek, L.M., 1999. Effects of fertilization on decomposition rate of *Populus tremuloides* foliar litter in a boreal forest. Can. J. For. Res. 29, 393–397.

Reichardt, P.B., Clausen, T.P., Bryant, J.P., 1991. Role of phenol glycosides in plant-herbivore interactions. In: Keeler, R.F., Tu, A.T. (Eds.), Handbook of natural toxins, vol. 6. Marcel Dekker, New York, pp. 313–333.

SAS Institute, Inc., 1989. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC.

Schimel, J.P., Van Cleve, K., Cates, R.G., Clausen, T.P., Reichardt, P.B., 1996. Effects of balsam poplar (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Can. J. Bot. 74, 84–90.

Still, W.C., Kahn, M., Mitra, A., 1978. Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. 43, 2923–2925.

Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. Blackwell Publications, Oxford, UK.