Effects of variable phytochemistry and budbreak phenology on defoliation of aspen during a forest tent caterpillar outbreak

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Abstract 1 The present study assessed the relationship between clonally variable rates of defoliation in trembling aspen (Populus tremuloides Michx.) and two potential resistance traits: defensive chemistry and leaf phenology. 2 In 2001, coincident with a major outbreak of the forest tent caterpillar (Malacosoma disstria Hubner) in the northcentral U.S.A., we monitored defoliation rates, phytochemical composition, and foliar development in 30 clones of trembling aspen. Leaf chemistry was also assessed in re-flushed leaves and 2 years post-outbreak. 3 Early in the season, differences in defoliation among clones were substantial but, by mid-June, all clones were completely defoliated. Leaf nitrogen, condensed tannins, and phenolic glycosides varied among clones but did not relate to defoliation levels. Budbreak phenology differed by 3 weeks among clones and clones that broke bud early or late relative to forest tent caterpillar eclosion experienced reduced rates of defoliation. 4 Defoliation led to increased tannins and slight decreases in phenolic glycoside concentrations in damaged leaf remnants, but to moderately decreased tannins and a six-fold increase in phenolic glycosides in reflushed leaves. This shift in chemical composition may significantly affect late season herbivores. 5 These results suggest that aspen chemical resistance mechanisms are ineffective during intense episodic eruptions of outbreak folivores such as the forest tent caterpillar. Variable budbreak phenology may lead to differential susceptibility during less intense outbreak years and, at peak forest tent caterpillar population densities, mechanisms affording tolerance are probably more important than chemical defences.

Keywords Chemical defence, condensed tannins, defoliation estimation, Malacosoma disstria, phenolic glycosides, Populus tremuloides.

Introduction
Plants have evolved diverse strategies to escape the negative effects of herbivory. Toxic chemicals (Schultz, 1988), physical barriers (Myers & Bazely, 1991), variable phenology (Aizen & Patterson, 1995) and tolerance (Strauss & Agrawal, 1999) have all been documented as important defensive mechanisms. General patterns of herbivore feeding preferences among and within species relate most closely to the distributions of plant allelochemicals (Cates & Rhodeas, 1977; Krischik & Denno, 1983; Cornell & Hawkins, 2003).

Within a species, allocation to secondary metabolites can vary considerably, depending on proximate factors such as genotype (Berenbaum & Zangerl, 1992), resource availability (Bryant et al., 1983; Herms & Mattson, 1992), ontogeny (Donaldson et al., 2006) and herbivore-mediated induction (Karban & Baldwin, 1997). Understanding the evolutionary factors that maintain phytochemical variation within a species requires a careful examination of the consequences of that variation.

Trembling aspen (Populus tremuloides Michx.) produces insect resistance factors (phenolic glycosides), the expression of which is strongly genetically determined (Osier & Lindroth, 2001, 2004, 2006). Because aspen reproduces asexually via root suckering, clonal stands of trees (ranging
in size from several to several thousand individuals) exhibit similar phenotypes, including resistance to defoliation. Field surveys under non-outbreak conditions have shown that aspen clones display considerable quantitative variation in phenolic glycoside concentrations (Hemming & Lindroth, 1995; Lindroth & Hwang, 1996b; Osier et al., 2000a). Moreover, experimental work with young aspen trees has revealed that genotypic variation in chemical composition strongly influences insect performance and rates of defoliation (Osier & Lindroth, 2006; Donaldson & Lindroth, 2007). Whether variation in aspen chemistry leads to heterogeneous rates of defoliation among mature aspen clones is unknown. That such may be the case, however, is suggested by the fact that, during outbreaks of defoliating Lepidoptera, damage rates can vary markedly among adjacent clones (J. R. Donaldson & R. L. Lindroth, personal observation).

An alternative explanation for clonal variation in defoliation rates is differences in tree phenology. Budbreak and leafout can vary by several weeks among aspen clones in a common location. Spring-feeding insects typically experience narrow windows of opportunity, comprising periods of time during which the availability and quality of foliage are optimal for insect fitness (Chilcote et al., 1992; Hunter, 1993; Hunter & Elkinton, 2000). Thus, variable synchrony of leafout and egg hatch may also contribute to clonal variation in defoliation rates, due to impacts on larval mortality and dispersal (Witter & Waisanen, 1978; Chilcote et al., 1992; Parry et al., 1998; Jones & Despland, 2006).

This field study took advantage of an outbreak of forest tent caterpillars (Malacosoma disstria) in northern Wisconsin. Our primary goal was to evaluate whether clonal variation in defoliation was related to plant chemistry or phenology. We predicted that: (i) the relative magnitude of defoliation among clones would be inversely proportional to leaf phenolic glycoside concentrations, and (ii) that early- and late-flushing clones (i.e. relative to forest tent caterpillar eclosion) would experience lower rates of defoliation due to decreased availability or quality of foliage. We emphasize that this study aimed to elucidate causes of spatial (clonal) variation in defoliation of aspen during an outbreak, and not the causes of temporal variation in defoliation linked to the decadal forest tent caterpillar population cycle.

A secondary goal was to track the response of aspen chemistry both during defoliation in partially damaged leaves (rapid induced resistance) and after defoliation in reflushed leaves (delayed induced resistance). Based on previous work by Osier and Lindroth (2001, 2004), we predicted that condensed tannin concentrations would increase in damaged leaves during the outbreak and would also be higher in reflushed leaves. Because phenolic glycosides are less plastic in response to resource availability and have shown only slight inducibility in previous studies (Roth et al., 1998; Hemming & Lindroth, 1999; Osier & Lindroth, 2001; for the special case of young, indeterminately growing aspen, see also Stevens and Lindroth, 2005), only minimal changes in their concentrations were expected in response to defoliation.

Study system

Aspen is an early successional tree species with wide distribution in North America (Perala, 1990; Mitton & Grant, 1996). It exhibits extraordinary genetic variation in leaf and stem morphology, bark texture, growth rate, phenology (flowering, budbreak, senescence), chemistry and susceptibility to herbivores and pathogens (Cheliak & Dancik, 1982; Dickmann & Stuart, 1983; Perala, 1990; Lindroth & Hwang, 1996a; Mitton & Grant, 1996). Aspen is host to a diverse array of both specialist and generalist herbivores (Lindroth & Hwang, 1996a) including the forest tent caterpillar. The forest tent caterpillar is a native generalist folivore and trembling aspen is a primary host of this species in the Great Lakes region. Aspen chemical defences and aspen-insect interactions are fairly well characterized (Lindroth & Hwang, 1996a) but the extent to which aspen chemical defences affect larval distributions and rates of defoliation during outbreaks of defoliating insects is unknown.

In the Great Lakes region, forest tent caterpillar populations fluctuate with cycles of approximately 7–12 years (Fitzgerald, 1995). Outbreak populations are typically sustained for several years, after which disease and parasitism lead to population collapses (Parry, 1995; Roland & Kaupp, 1995). Severe defoliation over multiple years markedly suppresses aspen growth (Duncan & Hodson, 1958; Churchill, et al., 1964; Fitzgerald, 1995) and moderately increases mortality in understory trees, but generally causes little mortality in otherwise healthy upper canopy trees (Duncan & Hodson, 1958; Churchill et al., 1964). Defoliation also leads to marked reductions in sexual reproduction in natural populations (J. R. Donaldson personal observation, 2001–2004) and in experimentally defoliated trees (Stevens et al., 2007). An indirect consequence of severe defoliation during outbreaks includes increased susceptibility to Hypoxylon (Anderson & Martin, 1981) and Cytospora cankers (Guyon et al., 1996), both significant causes of mortality in mature aspen trees.

Materials and methods

Forest tent caterpillar outbreak

From 1999 to 2003, a severe outbreak of forest tent caterpillars occurred throughout much of the northern Great Lakes region. In 2001, at peak population density, almost six million hectares of aspen-dominated forest were severely defoliated in northern Minnesota, Wisconsin, and Michigan’s Upper Peninsula. By June 2001, significant forest tent caterpillar larval mortality occurred due to nucleo-polyhedrosis virus and starvation. This mortality, combined with high rates of parasitism and a late and cool spring in 2002, resulted in a population crash in much of northern Wisconsin. The study sites of the present investigation were among those where populations crashed; thus, they experienced no detectable defoliation after 2001.

Study site and aspen clone selections

In 1999 and 2000, substantial, and differential, levels of defoliation were observed in aspen dominated forests near
Rhinelander, Wisconsin. In 2000, 40 aspen clones were identified that incurred defoliation levels in the range 0–100%. Clones were identified based on characteristics of leaf morphology, bark texture (Osier et al., 2000b) and stand structure. Care was taken to select clones in areas with similar forest structure (e.g., associated tree species, stand ages, etc.). In spring 2001, clone identities were reassessed based on budbreak phenology (see below) and characteristics mentioned above. Several of the previously identified clones appeared to be of mixed genotypes (e.g. asynchronous budbreak, branching architecture); consequently, 30 of the original 40 clones were selected for study. For each clone, we haphazardly selected and marked five canopy dominant ramets (individual trees). Those five trees in each clone were assessed in subsequent surveys.

### Egg mass surveys

Egg mass distribution surveys were conducted to assess potential differences in forest tent caterpillar densities among clones. According to Batzer et al. (1995), egg mass surveys are a reliable predictor of population density. In early April 2000, we sampled ten of the 30 study clones (selected randomly from clones we were able to get permission to cut trees from). Avoiding edge trees, three healthy (or two for one of the clones), canopy dominant, ramets with a diameter at breast height (dbh) of 12–16 cm were felled and the numbers of forest tent caterpillar egg masses were recorded. Tree heights and dbh were measured using a clinometer and dbh tape, respectively. Egg mass ‘densities’ were calculated relative to tree diameter² x height (a common metric of tree size) and compared among the 10 clones.

### Leaf phenology

Budbreak and leaf expansion phenology were monitored throughout the study. On 6 May 2001, average phenological stage was recorded for each of the 30 clones. Several ramets (3–4) from each clone were observed and each clone was assigned to a phenological class within one of six categories, with category ‘1’ clones having completely dormant buds and category ‘6’ clones having fully expanded leaves (Table 1). Ramets within clones had highly synchronous budbreak and leaf expansion phenologies.

<table>
<thead>
<tr>
<th>Class</th>
<th>Leaf phenological stage*</th>
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<tbody>
<tr>
<td>1</td>
<td>Buds dormant</td>
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<tr>
<td>2</td>
<td>Buds green and expanding</td>
</tr>
<tr>
<td>3</td>
<td>Leaves &lt; 1 cm</td>
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<tr>
<td>4</td>
<td>Leaves 1-2 cm</td>
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<tr>
<td>5</td>
<td>Leaves 2-3 cm</td>
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<tr>
<td>6</td>
<td>Fully expanded</td>
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*Leaf dimensions were measured as width.

### Defoliation estimates

Whole tree visual estimates were used to assess defoliation. Estimates were made on three separate occasions (24–25 May, 30–31 May and 7 June 2001), generally corresponding to third, fourth and early fifth forest tent caterpillar larval stadia, respectively. Three observers used binoculars to make independent whole tree defoliation estimates for the five haphazardly selected trees in each clone. Independent estimates were averaged and defoliation was assessed as one of seven defoliation classes (0–5%, 6–15%, 16–30%, 31–50%, 51–70%, 91–100%). These defoliation classes followed similar methods as reported by Maclean and Lidstone (1982), with modifications to better suit field conditions and patterns of aspen defoliation. Previous studies indicate that whole tree estimates are 83–90% accurate compared with a shoot count method (Maclean & Lidstone, 1982) and comprise the most appropriate method for nondestructive assessments of defoliation (Landsberg & Ohmart, 1989). Average defoliation for a clone was calculated by averaging the midpoints of the defoliation class recorded for each of the five trees assessed.

### Leaf collections

Six different leaf collections were made, four in 2001, and two in 2003. In spring 2001, leaf samples were collected on 18 May, 29 May and June 8 from the same five trees per clone for which defoliation estimates were made. On 29 May, however, some trees could not be sampled because no leaves remained, and by 8 June, only 11 clones had ramets with sufficient leaves remaining for collection. Reflushed leaves were collected from all 30 clones in August 2001. On 19 June and 16 August 2003, we resampled a randomly selected subset (13) of the original 30 clones. Our protocol for sampling ramets was to collect five to ten leaves from each of three haphazardly selected locations in the canopy using a shotgun or a pole pruner. Leaf samples were immediately placed under crushed ice and then transferred to a vacuum dryer within 8 h of collection. Samples were vacuum dried (2–4 days at 50–100 millitorr), ground in a Wiley mill (#40 screen; Arthur H. Thomas Company, Philadelphia, Pennsylvania) and stored at –20°C in sealed vials.

### Chemical analyses

Total leaf nitrogen was determined with a LECO elemental analyzer (St Joseph, Michigan) using glycine p-toluene-sulfonate as a standard. Total condensed tannin concentrations were quantified by a modified acid butanol method (Porter et al., 1986) using purified aspen tannins as a standard. Salicin, salicortin, tremuloidin and tremulacin concentrations were determined by high-performance thin layer chromatography using the respective purified aspen phenolic glycosides as standards (Lindroth et al., 1993). Chemical concentrations were calculated on a percent dry weight basis.

### Statistical analysis

All analyses were done using JMP IN, version 4.04 (SAS Institute Inc., 2001). Statistical comparisons among clones
were made using the individual ramet as the experimental unit of replication (n=5/clone). Defoliation values were highly skewed (i.e. most clones were heavily defoliated) and could not be transformed to meet the assumptions of parametric statistical tests. Therefore, differences in defoliation among clones were assessed using the Kruskall–Wallis nonparametric chi-square test (Zar, 1999). Differences in nitrogen, condensed tannins and total phenolic glycosides among the 30 clones were assessed using analysis of variance. Correlation analyses and multiple regression were used to ascertain relationships between leaf chemistry concentrations and defoliation.

To assess the potential effect of budbreak phenology on susceptibility to forest tent caterpillar defoliation, we compared average defoliation (on 25 May) among the six phenological classes (Table 1). Defoliation was compared as described previously. Because data were nonparametric and unbalanced, a Kruskal–Wallace rank sums test was employed and post-hoc mean comparisons were made using Dunn’s Q-test (Sokal & Rohlf, 1995; Zar, 1999). Changes in aspen chemistry over time were assessed in two ways. First, average (per clone) nitrogen, condensed tannins and phenolic glycosides were calculated for each of six collection times over 2001 and 2003. Statistical analysis of these data was complicated by the large number of missing data due to complete defoliation on the third collection date. Therefore, the mean ± SE are presented for all available clones by sampling date and general trends are described, but without statistical interpretation. Next, a subset of clones (nos. 5, 7, 10, 24, 25, 30) for which data from each collection date were available (with sufficient replication) was compared using analysis of variance, with the six collection dates as repeated measures and clone as a main effect (Zar, 1999). Where the assumption of sphericity was met (i.e. Mauchly criterion test; P>0.05), we used unadjusted univariate F-tests for within subject effect tests (Zar, 1999). For tests in which the assumption of sphericity was not met, Greenhouse–Geisser epsilon adjusted P-values were used as suggested by Maxwell and Delaney (1990).

Results

Among-clone variation in egg mass density

Egg mass density varied markedly from ramet to ramet, in the range 8–41 egg masses, with an overall average of 24 egg masses per tree. However, egg mass density did not vary among the ten clones surveyed (F=1.47, d.f.=9, P = 0.25), nor were densities on these clones related to early measures of defoliation (P=0.28).

Among-clone variation in defoliation

Percent defoliation varied among clones on all three dates but any differences were most apparent early in the season (Fig. 1). On 25 May, defoliation was in the range <10 to almost 100% among clones (χ²=113.76, d.f. = 29, P<0.001). By 30 May, almost half of the clones had been completely defoliated (χ²=121.73, d.f. = 29, P<0.001) and, by 7 June, all but eight clones were more than 90% defoliated (χ²=93.32, d.f. = 29, P<0.001). After 7 June, defoliation continued until all except clone 25 were completely stripped of foliage (data not shown).

Among-clone variation in leaf chemistry

Leaf chemistry results from the 18 May collection revealed significant among-clone variation (Fig. 2). Total nitrogen concentrations were in the range 2.8–4.9% dry weight (F=8.35, d.f.=29, P<0.001). Condensed tannin concentrations were in the range 14% to >24% dry weight among the clones (F=4.029, d.f.=29, P<0.001). Concentrations of phenolic glycosides (total phenolic glycosides = salicin + salicortin + tremuloidin + tremulacin) varied more than eight-fold among clones, from as low as 0.8% to almost 7% dry weight (F=11.48, d.f.=29, P<0.001). Individual phenolic glycosides showed identical patterns of variability among clones (data not shown). As is typically the case, tremulacin and salicortin were present at substantially higher concentrations than salicin and tremuloidin (Lindroth & Hwang, 1996a).

Correlation and regression analyses indicated that percent defoliation on 24/25 May was not related to leaf concentrations of nitrogen (r=0.032, P=0.704), condensed tannin (r=0.101, P=0.224) or phenolic glycosides (r=0.053, P=0.543) as measured on 18 May. Multiple regression analysis for the same date, using the composite set of phytochemical variables, also revealed no significant relationship between defoliation and leaf chemistry (r²=0.012, P=0.478). Because phytochemical concentrations can change as leaves mature after budbreak (Osier et al., 2000a), an additional assessment was made for clones that remained available for sampling throughout the study period (for each of the first three sample dates). For this subset of clones, there was also no indication of a relationship between leaf chemistry and defoliation.

Among-clone variation in budbreak phenology

Timing of budbreak varied among clones by greater than three weeks, with some clones breaking bud coincident with forest tent caterpillar emergence, some prior to forest tent caterpillar emergence, and other clones more than 10 days later. To assess the effects of budbreak phenology on defoliation, we determined average defoliation rates for clones in each phenological category (Fig. 3). Clones categorized as intermediate in their budbreak phenology (classes 4 and 5) broke buds (began leaf-out) approximately coincident with caterpillar eclosion phenology, which occurred over a period of less than a week within all clones. On 25 May, clones having intermediate phenology exhibited greater defoliation than those that broke bud relatively early and late (χ²=38.04, d.f.=5, P<0.001). The lightest defoliation occurred in clones that broke bud more than 1 week after forest tent caterpillar emergence (class 1). By 30 May, many clones had already become fully defoliated and dispersing larvae had reduced much of the earlier variation among phenological stages (χ²=21.71, d.f. = 5, P<0.001). By 7 June, almost all clones

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were completely defoliated (Fig. 1) and, although an overall chi-square test suggested that means were significantly different ($\chi^2 = 18.69$, d.f. = 5, $P < 0.002$), the more conservative Dunn’s Q-test showed no differences in defoliation among phenological classes (Fig. 3).

**Short- and long-term effects of defoliation on chemistry**

Phytochemical concentrations changed during the course of the outbreak and in reflushed leaves after the outbreak (Fig. 4). Nitrogen concentrations decreased as defoliation progressed (i.e. as leaves expanded). A 25% reduction in average nitrogen concentration occurred from early to late in the season (May to August 2001). Reflushed leaves (August) were fully expanded and hardened and thus of comparable ‘age’ to leaves in the 8 June collection. Nitrogen concentrations decreased similarly from June to August in both outbreak (2001) and non-outbreak (2003) years. Tannin concentrations increased by over 30% during the 2001 outbreak, and then returned to early season levels in reflushed leaves. By contrast, during 2003, tannin concentrations did

Figure 1 Defoliation of 30 aspen clones by forest tent caterpillars near Rhinelander, Wisconsin, on three dates in 2001. Bar heights are clone averages ± SE ($n = 5$ ramets per clone).
not change between June and August. Average phenolic glycoside concentrations decreased slightly during the outbreak in 2001 whereas, in reflooded leaves, concentrations increased six-fold. The marked increase in concentration in reflooded foliage occurred for each of the four phenolic glycosides produced in aspen (data not shown). In 2003, no such increase occurred in late season foliage.

A repeated measures analysis of variance among clones 5, 7, 10, 24, 25 and 30 confirmed the general patterns illustrated by Fig. 4. Concentrations of nitrogen, tannins, and phenolic glycosides each changed over time (Fig. 5; Table 2). Clones also differed notably in their response over time for all three phytochemicals measured (clone × time interaction).

**Discussion**

**Aspen leaf chemistry and defoliation**

Although defoliation was variable early in the 2001 season, of the 30 clones surveyed, all but one was completely...
Defoliation of aspen was so high that many clones were completely defoliated before larvae had completed their development. As a result, dispersing caterpillars eventually consumed lightly or previously nondefoliated clones, as well as less-preferred host species. The single clone that escaped complete defoliation was not unusual with respect to any of the variables measured. Both chemical composition and timing of leaf-out were average relative to the other clones surveyed. Even though aspen leaf chemistry varied significantly among the 30 clones in the present study, we found no relationship between chemistry and early season defoliation. Clearly, phenolic glycosides, previously shown to be highly biologically active (Hemming & Lindroth, 1995; Lindroth & Hwang, 1996a, Donaldson & Lindroth, 2007), did not play a defensive role against forest tent caterpillar defoliation during the outbreak of 2001.
Two reasons potentially explain why chemistry had no effect on defoliation in this study. First, density-driven changes in forest tent caterpillar dispersal behaviour and host acceptability may account for a lack of chemical-based resistance to defoliation during extreme forest tent caterpillar outbreaks. When outbreak populations are extremely large, preferred hosts are completely defoliated before larvae complete their development. As with gypsy moths (Rossiter, 1987), forest tent caterpillar larvae respond to such conditions by shifting to less preferred clones and even species. Second, mature aspen ramets may not rely on chemical defence to protect from defoliation against recurring cyclical outbreak species such as the forest tent caterpillar. For example, Mattson et al. (1991) suggest that expansive outbreak herbivores such as forest tent caterpillars may actually select against high levels of chemical defences because dense herbivore populations eventually defoliate regardless of level of allocation to chemical defences. Furthermore, phenolic glycosides exhibit biological activity against forest tent caterpillars at concentrations starting at approximately 5% dry weight (Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). In spring 2001 and 2003, most clones in the present study had total phenolic glycoside concentrations below 4%. Other recent studies (e.g. Donaldson et al., 2006) in Wisconsin have also found that canopy-dominant aspen have somewhat lower concentrations of phenolic glycosides than recorded in previous studies from northern Michigan (Lindroth & Hwang, 1996b) and much lower concentrations than documented for aspen in the western United States (R.L. Lindroth, unpublished data). Given the findings of the present study, it is unlikely that phenolic glycosides play a significant defensive role during forest tent caterpillar outbreaks in mature aspen clones in this region. By contrast, phenolic glycosides do provide protection against insect defoliation in young aspen (Donaldson & Lindroth, 2007) and against other herbivores (e.g. porcupines; B. Diner, D. Berteaux & R.L. Lindroth, unpublished data; Lindroth & Hwang, 1996a) in mature aspen stands.

Increasing evidence suggests that aspen utilizes alternative defensive strategies at different ontogenetic stages of development. Young aspen ramets have markedly higher concentrations of phenolic glycosides compared with mature ramets (Donaldson et al., 2006). Concentrations gradually decrease as ramets mature (over approximately 15 years). This pattern may be driven by strong selection pressure from mammalian browsers when trees are young (Swihart & Bryant, 2001). As suggested by Mattson et al. (1991), aspen is probably adapted to the cyclical nature of forest tent caterpillar outbreaks, and tolerance may be more important than chemical defence as trees mature. Tolerance is thought to increase in mature ramets because of their relatively small leaf weight to total plant weight ratio and their ability to store reserves in large stems and extensive root systems (Marquis, 1984; Byington et al., 1994).

**Budbreak phenology and defoliation**

Budbreak phenology had a significant, but temporary, effect on aspen susceptibility to defoliation. Clones that broke bud early and late relative to caterpillar eclosion experienced less early defoliation. Although these differences in defoliation diminished as larvae began to disperse en masse, under less severe outbreak conditions differences in defoliation among clones would probably persist. Studies in other systems have demonstrated that leaf phenology affects host susceptibility to early season herbivores (Floate et al., 1993; Hunter, 1993;
Mopper & Simberloff, 1995; Hunter & Elkinton, 2000; Chen et al., 2001) and several studies have examined the effects of budbreak phenology in aspen (see Witter & Waisanen, 1978; Chilcote et al., 1992; Parry et al., 1998; Jones & Despland, 2006). For example, Parry et al. (1998) manipulated the timing of forest tent caterpillar eclosion and deployed neonate larvae on aspen trees in the field at various stages of leaf phenology (post-budbreak). Development was slowed for asynchronously deployed larvae, leading to markedly higher rates of depredation of forest tent caterpillar larvae. Jones and Despland (2006) found the same pattern for neonate forest tent caterpillars developing on aspen foliage at varying phenological stages. Similarly, Chilcote et al. (1992) described a ‘window of susceptibility’ determined by budbreak phenology after which gypsy moth development is slowed.

Larval survival may also be significantly reduced when neonates eclose prior to leaf emergence. For example, in 2002, larvae emerged 2 weeks before aspen clones broke bud. This led to very high mortality rates (i.e. larvae desiccated or starved) and contributed to the widespread population crash of forest tent caterpillars (W.M. Mattson & K. Scanlon, personal communication, 2002). Our data suggest that variations in budbreak phenology will explain a higher proportion of variation in defoliation among clones in non-outbreak years.

If budbreak phenology has lasting effects on defoliation levels in moderate outbreak years, forest tent caterpillars may impose significant disruptive selection pressure on aspen budbreak phenology. In the present study, clones varied in the timing of first budbreak by approximately 3 weeks (20% of the growing season), even among adjacent stands. By contrast, forest tent caterpillar emergence occurs over a much shorter time period (several days). As suggested by Yu et al. (2001), much of the phenological variation in budbreak timing is probably genetically-based and inheritable. Defoliation damage significantly reduces growth and delays reproduction in young common garden aspen (Stevens et al., 2007) and reproductive buds were essentially absent from aspen in previously defoliated field sites in 2001–2003 (post-defoliation). Therefore, in a spatially and temporally variable environment, occasional benefits of early or late budbreak (when there is a moderate risk of herbivory) may counter the stabilizing selection pressure imposed by the stochastic risks of frost damage for early flushing clones or the opportunity costs in productivity for late-flushing clones.

### Short- and long-term changes in aspen chemistry

Previous studies with aspen have examined the temporal changes that occur in leaf chemistry during the growing season (Osier et al., 2000a; Lindroth et al., 2002). These seasonal changes are most pronounced during leaf expansion as nitrogen and phenolic glycoside concentrations decrease and condensed tannin concentrations increase (Osier et al., 2000a). The decreasing nitrogen concentrations observed in early 2001 are probably due to normal seasonal changes. The fact that nitrogen concentrations did not differ between June 2001 and June 2003, when no forest tent caterpillar damage occurred, supports this conclusion.

In contrast to nitrogen, condensed tannin concentrations were clearly elevated in June 2001 relative to June 2003, presumably as a rapid induced response (Tuomi et al., 1994) to defoliation damage. Roth et al. (1998), Osier and Lindroth (2001) and Stevens and Lindroth (2005) reported similar rapidly induced increases in tannins in defoliated aspen trees. Tannins do not appear to have anti-herbivore properties in this system but induced increases in their concentrations may play other important roles. For example, tannins inhibit microbial activity and thus may protect damaged leaves against microbial pathogens (Scalbert, 1991; Field & Lettinga, 1992; Schultz et al., 1992). Another speculative role for induced tannins may be to protect damaged leaf remnants against photo-oxidative stress in a thinned canopy (Close & McArthur, 2002). Regardless, rapidly induced tannins are unlikely to significantly affect forest tent caterpillar performance (Hemming & Lindroth, 1995, 2000) and, therefore, are unlikely to protect against further forest tent caterpillar defoliation.

Phenolic glycoside concentrations exhibited a delayed induced response (Haukioja, 1990) to forest tent caterpillar damage: concentrations were six-fold higher in fully expanded reflushed leaves than in fully expanded first-flush leaves. This response is similar to that documented for young,
indeterminately-growing aspen after heavy defoliation (Stevens & Lindroth, 2005). The mechanism for such a striking change in allocation is unclear. One possibility is that activation of dormant meristems results in a rejuvenation of newly formed tissues. For example, Chapin et al. (1985, 1986) showed that herbivory-induced activation of dormant buds in willow produces shoots with juvenile morphological characteristics. In aspen, clonal root sprouts contain much higher phenolic glycoside concentrations than do mature ramets within a clone (Donaldson et al., 2006). Similar physiological and ontogenetic mechanisms may be responsible for increased phenolic glycoside concentrations in both reflushed leaves and new ramets produced from root sprouts. Alternatively, the mechanism may be an actively induced response that reflects the increased costs of late season herbivory in previously defoliated trees. Regardless, the ecological consequences of such marked increases in phenolic glycosides are likely to be significant for late season herbivores such as the big poplar sphinx moth (Hwang & Lindroth, 1998).

Conclusions

The present study was conducted to further our understanding of the roles of genetically-linked chemical defence and phenological traits in mediating rates of defoliation during an insect outbreak in natural aspen populations. The results suggest that, in spite of significant phytochemical variation among aspen clones, chemical resistance appears to be ineffective, at least during extreme eruptions of outbreak foliviore such as forest tent caterpillars in the Great Lakes Region. Even early in the study when clear differences in defoliation occurred among clones, leaf chemistry was not correlated with that variation. Budbreak phenology appeared to have a significant effect on early season defoliation rates; however, this effect was short-lived in 2001, probably because of the high forest tent caterpillar population density. Interestingly, all 30 clones had fairly low levels of phenolic glycosides. The ubiquitously low concentrations of chemical defences in these advanced stands may reflect an evolutionary scenario that selects against high allocations to defence. For example, where aspen are subject to intense, recurrent outbreaks of defoliating lepidopteran, larger ramets may rely more heavily on tolerance to defoliation rather than investing in chemical defence (Mattson et al., 1991).

During less intense outbreaks or in years leading up to peak population densities, defoliation is highly variable among clones. Budbreak phenology is probably important in explaining this variation and forest tent caterpillar herbivory may present a disruptive selection pressure on this trait. Finally, increased allocation to phenolic glycosides in reflushed leaves may not directly affect forest tent caterpillars, but is likely to have significant consequences for late season herbivores.

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