Variable extrafloral nectary expression and its consequences in quaking aspen

Patricia Doak, Diane Wagner, and Adam Watson

Abstract: Extrafloral nectaries (EFNs) are secretory glands most commonly linked to defensive mutualisms. Both a plant's need for defense and the strength of defense provided by mutualists will vary with plant condition and local insect community. Thus, the benefit of EFNs may vary spatially and temporally. However, little attention has been paid to natural variation in the presence and abundance of EFNs within and among individuals of the same species. Quaking aspen, *Populus tremuloides* Michx., bear EFNs on a subset of their leaves. Here, we describe patterns of EFN expression on shoots within ramets, among ramets, and among putative clones in interior Alaska. We also examine the relationship between EFN presence and herbivory by both the very abundant aspen leaf miner, *Phyllocnistis populiella* Chambers, and less common chewing herbivores. The proportion of leaves bearing EFNs varied from 33% to 87% among distinct aspen stands. Within stands, short (1–2 m height) ramets had higher EFN frequency than their taller (>4 m) neighbors. Patterns of herbivory also differed between short and tall ramets. Compared with leaves without EFNs, those with EFNs suffered less mining damage on short ramets but slightly higher damage on tall ramets. Tall ramets suffered more chewing damage than short ramets, but this damage was unrelated to the presence of EFNs. Our results suggest that variable EFN expression may be explained by variation in the benefits of EFNs. Leaves with EFNs on short ramets benefit through reduction in leaf mining, but this benefit does not extend to tall ramets or other forms of herbivory.

Key words: mutualism, extrafloral nectaries, *Populus tremuloides*, *Phyllocnistis populiella*.

Résumé : Les nectaires extrafloraux (EFNs) sont des glandes sécrétrices, le plus souvent associées à des mutualismes de défense. À la fois le besoin de défense et la force de défense, fournis par les mutualistes, varient selon la condition de la plante et la communauté d'insectes locaux. Ainsi, le bénéfice des EFNs peut varier dans l'espace et dans le temps. Cependant, on a accordé peu d'attention à la variation naturelle de la présence et de l'abondance des EFNs, sur et entre les individus de la même espèce. Le tremble, *Populus tremuloides* Michx., porte des EFNs sous un sous-ensemble de feuilles. Les auteurs décrivent les patrons d'expression des EFNs sur les tiges, sur les ramets, entre les ramets, et entre des clones présumés, à l'intérieur de l'Alaska. Les auteurs examinent également la relation entre la présence d'EFNs et l'herbivorie par la très abondante mineuse des feuilles, le *Phyllocnistis populiella* Chambers, ainsi que des herbivores phyllophages moins fréquents. La proportion des feuilles portant des EFNs varie de 33 % à 87 %, entre divers peuplements de tremble. À l'intérieur des peuplements, les ramets courts (1–2 m de hauteur) montrent des fréquences plus grandes d' EFNs que les ramets voisins plus longs (>4 m). Les patrons d'herbivorie diffèrent également entre les ramets courts et longs. Comparativement aux feuilles sans EFNs, celles qui portent des EFNs montrent des dommages moindres par la mineuse sur les ramets courts, mais des dommages légèrement supérieurs sur les ramets longs. Les phyllophages endommagent plus fortement les ramets longs, mais ces dommages ne sont pas reliés à la présence d'EFNs. Les résultats suggèrent que la variation des EFNs pourrait s'expliquer par la variation des bénéfices des EFNs. Les feuilles possédant des EFNs sur leurs ramets courts bénéficient d'une réduction des dommages par la mineuse, mais ce bénéfice ne s'étend pas aux ramets longs ou autres formes d'herbivorie.

Mots clés : mutualisme, nectaires extrafloraux, *Populus tremuloides*, *Phyllocnistis populiella*.

[Traduit par la Rédaction]

Introduction

Extrafloral nectaries (EFNs) are secretory glands most commonly linked to defensive mutualisms (Bronstein 1998). While some defensive mutualisms are obligate, most are facultative (Hoeksema and Bruna 2000; Koptur 1992). Both

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a plant's need for defense and the strength of defense provided by mutualists will vary with plant condition and local insect community. For instance, in a low-herbivore environment, there may be little benefit in provisioning body guards. Moreover, if defending mutualists are absent or ineffective, EFNs may become a liability not just in energy expenditure (Rutter and Rausher 2004) but also by attracting herbivores (Henneberry et al. 1977; Adjei-Maafo et al. 1983; Beach et al. 1985). Most research examining variability in a plant's contribution to EFN defensive mutualisms has focused on nectar traits (e.g., volume, composition) (see Agrawal and Rutter 1998 for a review; Heil et al. 2000, 2001; Wackers et al. 2001; Ness 2003). Variation in nectar traits can impact the strength and effectiveness of defensive

mutualisms (Bentley 1977; Veena et al. 1989; Ness 2003; Kost and Heil 2005). A few studies have examined the heritability of nectar traits and found both strong genetic and environmental contributions to trait variation (see Mitchell 2004 for a review).

Natural variation in the presence and abundance of EFNs within and between plants has received much less attention because this variation is either absent in many species or has been overlooked. Yet the study of this natural variation has great potential to improve our understanding of the evolution of EFNs in particular and mutualism and plant defense more generally. First, by relating EFN expression to herbivory in natural systems, we can begin to understand current selection on the trait. In addition, the existence of leaves with and without EFNs on the same plant provides the opportunity to study the impact of EFNs while controlling for plant-level sources of variation. Variation in EFN frequency is reported for a few species. *Vicia faba* L. rapidly increased the development of EFNs after artificial leaf damage (Mondor and Addicott 2003), and *Acacia drepanolobium* (Harms) Sjostedt. more slowly decreased the numbers of EFNs on new tissue when herbivores were excluded (Huntzinger et al. 2004). Rudgers (2004) identified heritable variation in the proportion of leaves bearing EFNs in wild cotton and demonstrated that variability in multispecies interactions led to different selection regimes at three study sites (Rudgers and Strauss 2004).

Quaking aspen, *Populus tremuloides* Michx., bears EFNs on a subset of its leaves. EFNs have been reported in 25 of the 35 species of *Populus* (Curtis and Lersten 1978; Keeler 1979; Pemberton 1990) and occur on 35 million year old fossilized leaves of the extinct *Populus crassa* (Pemberton 1992). Variability in EFN expression in *Populus* has long been recognized (Trelease 1881), although, to our knowledge, the current function of *Populus* EFNs remains undocumented. We use this variability to examine the influence of EFNs on herbivore damage at the scale of individual leaves. Visitors to EFNs may reduce herbivory on leaves bearing EFNs through predatory and antagonistic interactions with major herbivores. On the other hand, EFN variability at the leaf scale might be unimportant if defending mutualists patrol areas of a plant more distant from the nectar source.

The costs and benefits of EFNs may change as plants grow. Plant architecture impacts arthropod community composition and plants of differing height can host quite distinct species assemblages (Strong et al. 1984). High foliage will tend to experience higher solar radiation and wind speed, factors that may limit activity of both aspen herbivores and their natural enemies. For flightless, ground-nesting insects such as most ant species in the temperate zone, the energetic costs of reaching high foliage (Lipp et al. 2005; Yanoviak et al. 2005) may outweigh the benefits of foraging at EFNs. Potential mutualists such as ants may drop out of species assemblages on even moderately tall trees. Therefore, the benefits of EFNs may differ between short and tall trees, and variable expression of EFNs might allow plants to adjust allocation to biotic defense in response to changes in the net benefit provided by EFNs.

We use the aspen system to explore patterns of natural variation in EFN expression and address three specific questions: (*i*) does the frequency of EFNs differ on short versus tall ramets, (*ii*) does herbivore damage differ between leaves with and without EFNs, and (*iii*) does the relationship between herbivory and EFN presence differ with ramet height?

Materials and methods

Study system

Aspen and aspen EFNs

In interior Alaska, quaking aspen is most abundant on south- and southwest-facing slopes, hill tops, and ridge lines. Much of its reproduction is asexual, resulting in the formation of clonal stands, which in our area do not appear to exceed a few hectares in extent.

Aspen EFNs occur where the petiole meets the leaf (Fig. 1*a*). Nectary number per leaf ranges from 0 to 6 with 0 and 2 being most common. A typical aspen plant expresses EFNs on only a subset of its leaves, and this expression varies with leaf type. In aspen, the first five to eight leaves that appear in spring are preformed leaves that overwintered as leaf primordia. Some plants are heterophyllous, growing additional "neoformed" leaves during the summer. EFNs are visited by many hymenopteran species. We commonly observe four ant species on aspen: *Formica* sp. 1, *Formica* sp. 2, *Camponotus herculeanus* (Linnaeus, 1758), and *Myrmica* sp. (vouchers submitted to the California Academy of Sciences for identification) (D. Wagner and P. Doak, unpublished data). Ants are typically present on trees at low abundance; for example, it is rare to find more than five ants on a small tree. None of the ant species that visit aspen appear to be highly aggressive (personal observation). Parasitoid wasps are known to visit the nectaries in our region (S. Armbruster[3], personal communication, 2005). In addition, adult moths of a major herbivore, the aspen leaf miner *Phyllocnistis populiella* Chambers, also commonly feed at EFNs.
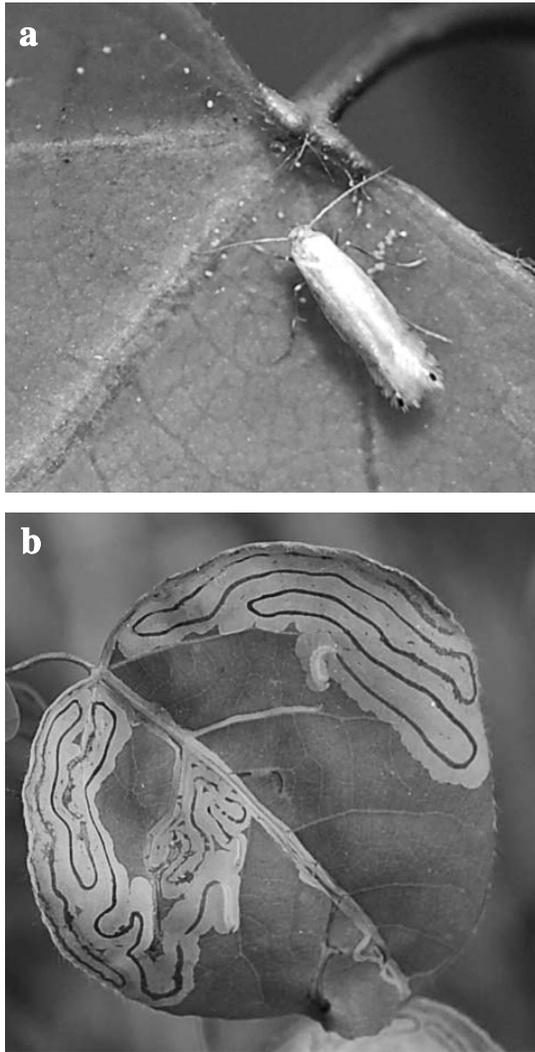
Aspen leaf miner

The aspen leaf miner *Phyllocnistis populiella* (Lepidoptera: Gracillariidae) is a widespread herbivore of aspen occurring through much or all of aspen's range. The leaf miner normally occurs at very low population densities. High populations were reported in aspen stands throughout much of British Columbia and the Yukon Territories from 1955 to 1962 (Condrashoff 1964); however, records suggest that population densities during this outbreak were lower than those currently observed. Condrashoff (1964) also noted that localized outbreaks occurred in the 1950*s* in Alberta, Wyoming, Idaho, and Colorado.

The aspen leaf miner population in interior Alaska has been steadily increasing since 1996 and has become noticeable and widespread since 2001. In 2005, it was at outbreak levels on 266 900 ha of Alaska lands (U.S. Forest Service 2005) and extended into the Yukon Territory of Canada (Natural Resources Canada 2004). It does not appear to discriminate between individual aspen trees or clones and thus results in most trees within an outbreak being heavily impacted. On average, in the Fairbanks area, trees surveyed

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Fig. 1. (*a*) Aspen leaf miner moth beside two EFNs near the petiole-leaf junction of an aspen leaf. (*b*) Leaf with aspen leaf miner damage; this leaf does not have EFNs.

from 2002 to 2005 had over 50% of their leaf area mined. Related work demonstrates the negative impact of leaf mining damage on aspen photosynthetic rate, water balance, and growth (D. Wagner et al., unpublished data.). While *Phyllocnistis populiella* was largely restricted to aspen early in the current outbreak, it has increased its use of *Populus balsamifera* L. over the last few years (P. Doak, personal observation).

Adult moths overwinter at the base of trees and in leaf litter. They become active as soon as spring temperatures allow, usually early May in our region, and are often seen in large numbers before aspen has broken bud. Mating occurs in the spring and oviposition begins as soon as aspen leaf tissue is exposed. Moths also commonly drink nectar from aspen EFNs. Eggs are laid singly on both upper and lower leaf surfaces, and there is a strong preference to oviposit on the youngest leaf tissue available. After oviposition, the eggs gradually sink into the leaf tissue. About 1 week after oviposition, larvae hatch directly into the subepidermal layer.

Larvae move and consume tissue along a horizontal plane, creating a shallow serpentine mine (Fig. 1*b*). The for-

ward orientation of their mouthparts appears to restrict them to feeding in a narrow layer just beneath the epidermis, and they consume only the single layer of epidermal cells, leaving the photosynthetic tissue intact (Condrashoff 1962). Thus, miners on the upper and lower leaf surfaces do not come into contact by eating through leaves, nor do they cross from one side to the other at the edges of leaves. Because only the epidermal cells are eaten, the two-dimensional measure of mine area provides an excellent estimate of tissue loss.

After approximately 2 weeks of development through four instars, larvae create pupal chambers in small leaf folds, usually at leaf edges. By early to mid-June, most larvae have pupated. Adults emerge approximately 10–14 days later but do not mate until the following spring.

We have identified three groups of natural enemies. We have observed mites consuming eggs. Ants appear to rip open mines and pupal chambers to remove larvae. In our dissections and rearings from 2004, we found a single parasitoid, *Closterocerus trifasciatus* Westwood (Hymenoptera, Eulophidae); however, in 2006, we reared at least five species of chalcid parasitoids from *Phyllocnistis populiella* larvae and pupae (species determinations are in progress).

Other aspen herbivores

During the research presented here, the aspen leaf miner was the only abundant herbivore on aspen. Small amounts of chewing damage were inflicted by a variety of insects—primarily lepidopteran larvae and chrysomelid beetles. While aspen is subject to periodic defoliation by the large aspen tortrix, *Choristoneura conflictana* Walker, the last recorded outbreak in our region ended in 1990 (R. Werner, unpublished data, http://www.lter.uaf.edu).

Field methods

All field work was conducted in interior Alaska near Fairbanks (64°48′N, 147°42′W). Sites were at approximately 300 m elevation with the exception of the ED site at 720 m.

In the summer of 2004, we conducted a large survey to examine patterns of EFN expression, aspen leaf miner damage, and tissue loss from chewing herbivores. Surveys were conducted after leaf miner pupation so that all leaf mining was complete. Survey dates for the four sites were 10 June (BNZ and RP sites), 19 June (WR site), and 30 June (ED site). The sites were separated by a minimum of 10 km.

At each site, we haphazardly picked one shoot on each of 40 short ramets (0.5–2 m height). A shoot comprises the leaves extending from current-year growth. For each leaf, we recorded the position along the shoot, the presence of EFNs, length and width to the nearest millimetre, the percentage of leaf area mined on the tops and bottoms of leaves, and the percentage of leaf area consumed by other chewing herbivores. At the BNZ and ED sites, we also collected the same data for 40 shoots from tall ramets (>5 m height).

We assessed percent herbivore damage using visual estimation. We checked our visual estimates of percent leaf area mined by first estimating mining for the tops and bottoms of 150 leaves in the field. Leaves were then scanned on a flatbed scanner and percent mining measured using image analysis software (Scion Image, Fredrick, Maryland).

We found that our visual estimates were accurate ($R^2 > 0.90$ for both leaf surfaces) and used these estimates throughout.

We determined the relationship between linear measurements of leaf size and leaf area using linear regression. In the field, we measured the length and width of 20 leaves at each of three study sites (BNZ, ED, and WR). Leaves were then collected in plastic bags and returned to the laboratory where they were scanned and leaf area was measured using image analysis. Both width alone and the product of width and length explained ≥90% of the variance in fresh leaf area. Therefore, we use leaf width as a proxy for leaf area in many of our analyses.

In the spring of 2005, we collected preliminary data to identify genetic and environmental sources of variance in EFN expression. At each of 11 geographically distinct sites (separated by a minimum 0.5 km), we chose 12 ramets that because of size and physical proximity appeared likely to belong to a single clone. We scored EFN expression on all leaves on each of six shoots per ramet. We only used shoots with at least five leaves and the five basal leaves present. This allowed us to examine the contribution of within-ramet, between-ramet within putative clone, and between-clone components of variance. Because each clone occupies a distinct location, the effect of clone cannot be separated from possible environmental effects on EFN expression.

Data analysis

Where appropriate, we used normal probability plots and scatter diagrams of residuals to verify parametric assumptions. Proportional response data were arcsine square root transformed and reported predicted values are back-transformed.

Patterns of EFN expression

We used the 2004 data from short ramets to examine the relationship between the presence of EFNs and leaf position using a nonlinear mixed-model logistic regression (PROC GLIMMIX; SAS Institute Inc., Cary, North Carolina). EFN presence was the dichotomous response variable, and leaf position and its square were included as fixed effects. Ramet was included as a random effect.

The 2005 data were used to examine variation in EFN frequency among ramets and clones. We used PROC MIXED to estimate the impacts of putative clone and individual ramet. The unit of measure was the shoot and the response variable was the proportion of leaves with EFNs.

Does EFN frequency differ with ramet height?

We used nonlinear logistic regression (PROC GLIMMIX) to compare the frequency of EFN expression on leaves from short and tall ramets. Tall ramets rarely produce neoformed leaves; therefore, we restricted this comparison to preformed leaves at leaf positions 1–5. The unit of analysis was the individual leaf. Fixed effects included site (BNZ and ED), leaf position, the square of leaf position, ramet height (dichotomous: short versus tall), and the interactions of height with position and with position squared. Ramet within site was included as a random effect.

Does herbivore damage differ between leaves with and without EFNs?

To examine the relationship between the proportion of leaf area mined and the presence of EFNs, we used our large data set from short trees at the four study sites. We used linear mixed-model analyses (PROC MIXED) with EFN presence (dichotomous), leaf position, and leaf width as fixed effects. Site and ramet within site were included as random factors. We restricted our analyses to leaf positions 3–7 for the following reasons: Owing to the nonrandom association between leaf position and EFN presence (see Results, Patterns of EFN expression), almost all leaves at positions 1 and 2 had EFNs, making unbiased comparison at these positions impossible. In addition, most leaves at positions greater than 7 expanded after the peak period of leaf miner oviposition. We performed separate analyses for mining on leaf tops and bottoms.

We also performed an analysis (PROC MIXED) at the level of shoots to examine the impact of the proportion of leaves bearing EFNs on total mining damage.

Because the majority of leaves did not suffer any tissue loss from other chewing herbivores, we used a mixed-model logistic regression (PROC GLIMMIX) to examine the effect of EFNs on the probability that a leaf experienced chewing damage. In all other ways, the analysis was designed as described above for leaf mining.

Does the relationship between herbivory and EFN presence differ with ramet height?

We used the data from the BNZ and ED sites to examine the impact of both EFNs and ramet height on herbivore damage. The models for both leaf mining and chewing damage were as above with the inclusion of height and the interaction of height and EFN presence as additional fixed effects.

Results

Patterns of EFN expression

EFN expression displayed a quadratic relationship with leaf position (position: $\chi^2 = 173.39$, $P < 0.0001$; position^2: $\chi^2 = 162.76$, $P < 0.0001$) (Fig. 2). The ratio of the generalized χ^2 statistic and its degrees of freedom was 0.99; values close to 1 indicate little residual overdispersion. The first one or two preformed leaves to expand usually had EFNs, and EFN frequency declined on the more distal preformed leaves (Fig. 2). Neoformed leaves had high frequencies of EFN expression, although there was variation in the presence and number of EFNs. Because the number of preformed leaves differed between trees, leaf positions 4–7 included both pre- and neo-formed leaves with the balance shifting as leaf position increased (Fig. 2). To confirm that these data were not distorted by leaf loss before the census date, we also examined patterns in a variety of other data sets including those taken just after spring leaf expansion (P. Doak and D. Wagner, unpublished data). The pattern of EFN expression on preformed leaves was confirmed.

In a survey of EFN presence on 12 aspen ramets within each of 11 putative clones, we found strong evidence of developmental and (or) environmental plasticity for EFN expression. The mean frequency of leaves with EFNs per shoot varied from 33% to 87% across the 11 putative clones. We found that 27% of the total variance among shoots was attributable to differences among ramets within clones,

Fig. 2. Mean (±SE) proportion of leaves bearing EFNs by leaf position from survey of small ramets (1–2 m height) at four sites. The line is predicted from a nonlinear mixed-model logistic regression. Leaf positions to the left of the shaded area are almost exclusively preformed, those to the right are neoformed, and the others represent a mixture of both leaf types.
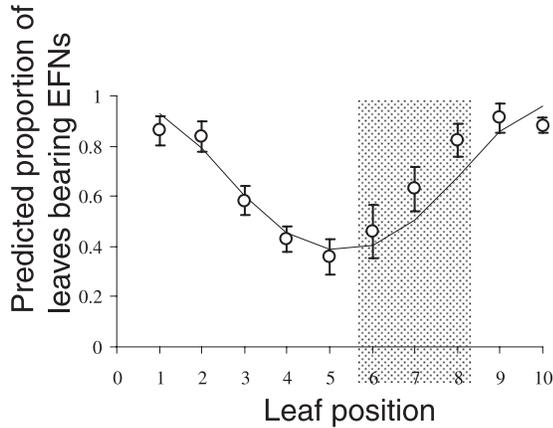
while 39% was attributable to differences among clones. The clone effect includes both genetic and environmental variance.

Does EFN frequency vary with ramet height?

EFN frequency varied with site ($\chi^2 = 58.36$, $P < 0.0001$), leaf position ($\chi^2 = 44.56$, $P < 0.0001$), and position^2 ($\chi^2 = 17.77$, $P < 0.0001$) (Fig. 3). The interactions of height with leaf position ($\chi^2 = 9.24$, $P = 0.0025$) and position^2 ($\chi^2 = 8.49$, $P = 0.0037$) were also significant, while the main effect of height was not ($\chi^2 = 2.19$, $P = 0.14$) (Fig. 3). The ratio of the generalized χ^2 statistic and its degrees of freedom was 0.71, indicating little residual overdispersion. The interactions of height and leaf position were largely driven by the fact that the most basal leaves almost always bore EFNs regardless of height, while EFN frequency was significantly higher on low ramets for positions 2–4 (Fig. 3). A model excluding leaf position 1 resulted in no significant interactions of height and leaf position and significantly higher EFN frequency on short ramets ($\chi^2 = 50.37$, $P < 0.0001$). EFN expression dropped off more steeply in tall than in shorter ramets. In addition, tall ramets almost never added neoformed leaves to their shoots; thus, for tall ramets, all leaves were preformed.

Does herbivore damage differ between leaves with and without EFNs?

In our survey of short ramets at four sites, mining damage was widespread with 70% of leaf tops and 69% of leaf bottoms experiencing some mining. Considering leaf positions 3–7, 87% and 82% of leaves suffered mining on the top and bottom surfaces, respectively, and leaves without EFNs suffered more mining damage on both their top ($F_{1,579} = 16.38$, $P < 0.0001$) and bottom ($F_{1,579} = 16.27$, $P < 0.0001$) surfaces than did leaves with EFNs (Fig. 4). The proportion of leaf surface mined decreased distally along the shoot (top: $F_{1,579} = 18.83$, $P < 0.0001$; bottom: $F_{1,579} = 11.65$, $P < 0.001$), and percent mining on the bottom surface also declined with leaf size ($F_{1,579} = 16.29$, $P < 0.0001$).

Fig. 3. Proportion of leaves with EFNs by leaf position within site and ramet height. (*a*) BNZ site; (*b*) ED site. Sample size per data point varies from 39 to 41. Best predicted lines (solid, tall ramets; broken, short ramets) are from nonlinear mixed-model logistic regression.
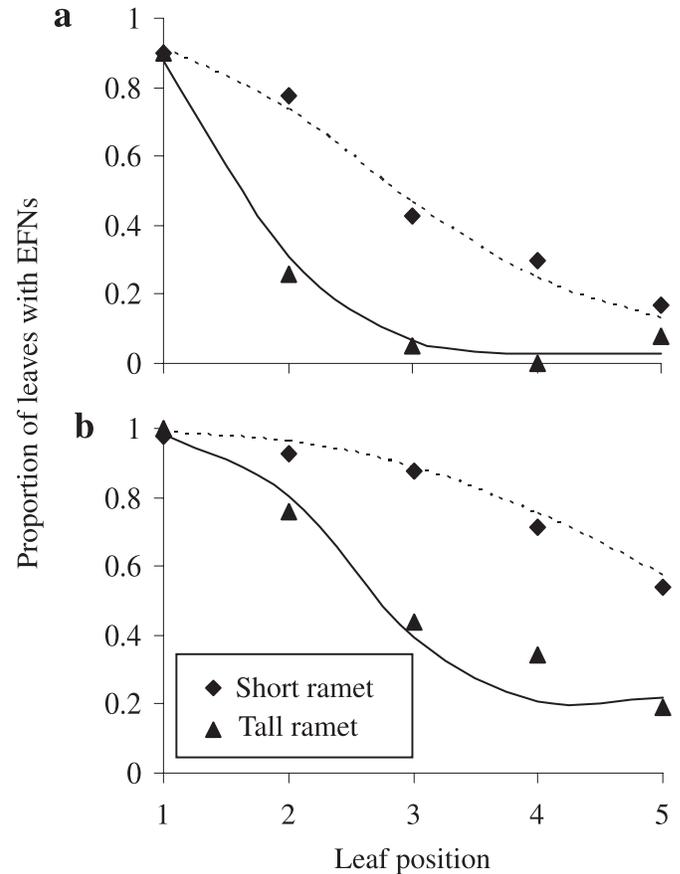
Leaf-level effects of EFN presence scale up to shoot-level impacts. The proportion of total leaf area mined on a shoot declined with increasing frequency of EFNs (top: $F_{1,158} = 23.62$, $P < 0.0001$; bottom: $F_{1,158} = 7.01$, $P = 0.009$) (Fig. 5).

Whether leaves were damaged by other chewing herbivores was not significantly related to EFN presence ($\chi^2 = 0.05$, $P = 0.82$; back-transformed least squares mean ± SE = 31±8% for both EFN and no-EFN leaves). Chewing damage appeared unrelated to leaf position or width.
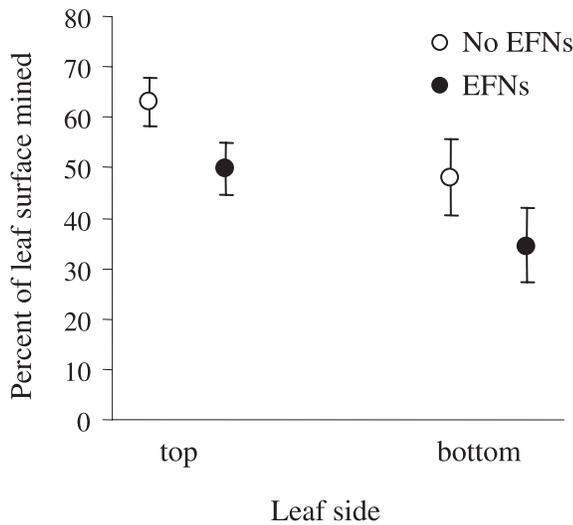
Does the relationship between herbivory and EFN presence differ with ramet height?

The relationship between mining damage, EFN presence, and plant height was complex and differed for the two leaf surfaces. The frequency of mining was somewhat higher on tall ramets. At the BNZ and ED sites, on short ramets, 85% of leaf tops and 80% of leaf bottoms were mined, while on tall ramets, 92% and 90% of tops and bottoms, respectively, were mined.

Mining on leaf top

There was a significant interaction of plant height and EFN presence on mining of leaf tops ($F_{1,650} = 18.34$, $P < 0.0001$). For tall ramets, mining on leaf tops was signifi-

Fig. 4. Back-transformed least squares means of percentage (±SE) of leaf surface mined by *Phyllocnistis populiella* on leaves with and without EFNs on short aspen ramets (1–2 m height).

Fig. 5. Proportion of total leaf surface mined ((*a*) leaf tops, (*b*) leaf bottoms) as a function of the proportion of leaves on the shoot that bear EFNs. Data points are for short ramets and include all leaf positions on a shoot. Predicted lines are back-transformed from linear mixed models on arcsine square root transformed data.

cantly higher on leaves with EFNs (contrast: $F_{1,650} = 4.89$, $P = 0.0273$), contrary to the opposite and significant pattern on short ramets (contrast: $F_{1,650} = 16.75$, $P < 0.0001$) (Fig. 6).

Mining on leaf bottom

There was a marginally significant interaction of plant height and EFN presence for mining on leaf bottoms ($F_{1,650} = 4.24$, $P = 0.040$). Leaves with EFNs had significantly less mining than leaves lacking EFNs on short ramets (contrast: $F_{1,650} = 6.12$, $P = 0.014$), but the difference was not significant for tall ramets (contrast: $F_{1,650} = 0.52$, $P = 0.470$) (Fig. 6).
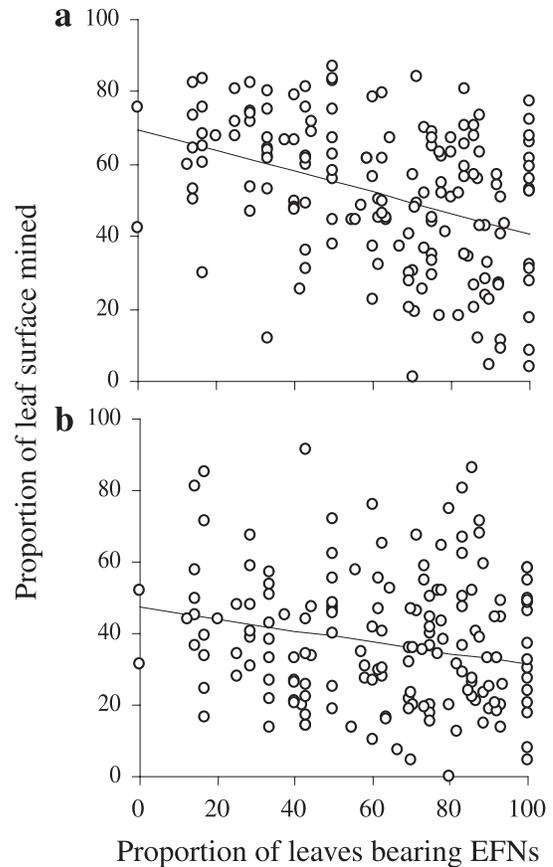
Other herbivory

The probability of damage from other chewing herbivores was higher for leaves on tall plants (back-transformed least squares mean ± SE = 0.62 ± 0.07) than on short plants (0.45 ± 0.07) ($\chi^2 = 12.65$, $P < 0.001$), but was unrelated to either EFNs or the interaction of EFNs with height. Considering only those leaves that sustained chewing damage, the loss of leaf tissue was slightly greater for leaves on tall ramets (16%) versus short ramets (11%) ($F_{1,302} = 8.83$, $P = 0.003$).

Discussion

In aspen, variability in EFN expression exists at many scales: along shoots, between pre- and neo-formed leaves, among shoots within ramets, among ramets within clones, and among putative clones. In other species, EFN traits, including expression, are often under strong environmental influence (Mondor and Addicott 2003; Mitchell 2004). Variability within ramets and putative clones suggests that there are also strong environmental effects on EFN expression in aspen.

Variable selection pressures may contribute to the maintenance of variation in EFN expression and plasticity for this trait (Bronstein 1994). EF nectar secretion is inducible by herbivory in a variety of plant species (Heil et al. 2001;

Wackers et al. 2001; Ness 2003; Heil et al. 2004), and for some species, EFN expression appears to be similarly responsive to herbivory (Mondor and Addicott 2003; Huntzinger et al. 2004). Not surprisingly, the relationship between aspen EFNs and herbivore damage depends on the herbivore species in question. Of greater interest is that the relationship between EFNs and mining varies with tree height. Variation in the efficacy of mutualisms is well known (Thompson 1988; Bronstein 1994, 1998; Alonso 1998; Kersch and Fonseca 2005), and our results suggest that the benefits of EFN expression to aspen may differ between short and tall ramets of the same clone.

The relationship between EFN presence and herbivory suggests that EFNs may be more beneficial for leaves on short compared with tall ramets and could actually be a liability on tall ramets. While EFNs are associated with reduced mining on short trees, the opposite is true on tall ramets where EFNs are either unrelated (leaf bottoms) or positively related (leaf tops) to mining damage. Related research (D. Wagner et al., unpublished data) demonstrates that leaf mining impacts aspen performance and suggests that mining is likely to exert selective pressure on aspen. In contrast, the presence and amount of tissue loss caused by other chewing herbivores were unrelated to EFN expression.

Fig. 6. Back-transformed least squares means of percentage (±SE) of leaf surface mined by *Phyllocnistis populiella* on short (1–2 m) versus tall (>4 m) ramets at the BNZ and ED sites. (*a*) Leaf top; (*b*) leaf bottom.
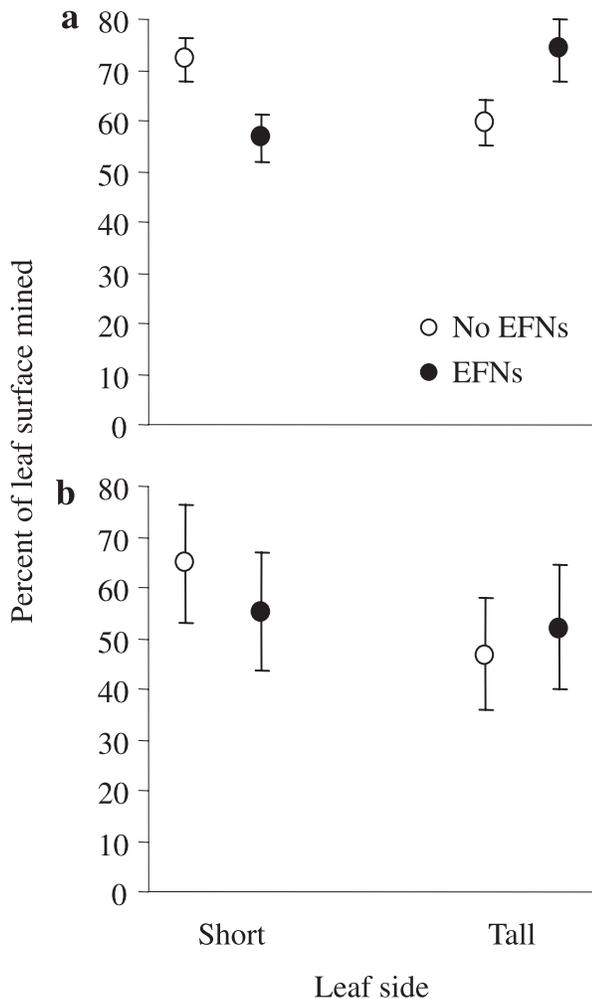
This type of herbivory was also more frequent on tall versus short ramets and resulted in greater damage on tall versus short ramets, further suggesting that there may be little benefit from EFNs on tall trees. Relatively low EFN frequency on tall ramets may represent adaptive developmental or environmental plasticity in response to height-dependent variation in the benefits of EFNs. Fiala and Maschwitz (1991) reported height-dependent expression of EFNs in a number of species of *Macaranga* (Euphorbiaceae) trees with only saplings of some species having EFNs.

The mechanism behind the height effect on herbivory is unknown, but our results suggest that EFN-mediated mutualisms may not function in the canopy. EFN defensive mutualisms most commonly involve ants (Bronstein 1998). The ants that we find at aspen EFNs are ground nesters, and the potential energetic gain from EF nectar may not be great enough to offset the additional energy needed to climb trees. On the other hand, a variety of research has indicated that mound-forming ants of the *Formica rufa* Linnaeus, 1761 group can influence populations of herbivores on trees (for early reviews, see Adlung 1966; Way and Khoo 1992). However, much of this research has been restricted to work

on trees <4 m in height (e.g., Laine and Niemela 1980; Mahdi and Whittaker 1993; Koricheva et al. 1995; Karhu and Neuvonen 1998; Bishop and Bristow 2001; Riihimaki et al. 2005) or the lower foliage of larger trees (e.g., Halaj et al. 1997). Skinner and Whittaker (1981) did find lower herbivore numbers at both 2 and 8 m heights in sycamore trees foraged by ants. Interestingly, during early-season sampling, they found lower herbivory on the 2 versus 8 m branches of foraged trees but no difference in unforaged trees, suggesting that ants had a greater impact on herbivory lower in the canopy (Skinner and Whittaker 1981). Warrington and Whittaker (1985) also found reductions in herbivory associated with ant presence at heights of up to 10 m in sycamore canopies. Review of the literature concerning the impacts of ground-nesting ants on forest herbivory highlights the need for more research into possible height effects on ant foraging.

Life in the canopy may also be harsher for the aspen leaf miners themselves. Insect behavior may be altered in the canopy because of different environmental effects such as wind. For instance, increased wind speed may make movement risky for the tiny adult aspen leaf miners, and they may be more prone to ovipositing in close proximity to EFN feeding sites. If EFN mutualisms are weak or nonexistent on tall ramets, energetic and ecological costs associated with EFN expression (Henneberry et al. 1977; Adjei-Maafo et al. 1983; Beach et al. 1985; Bronstein 1994; Rutter and Rausher 2004) may outweigh any benefits.

Aspen EFN presence and number are correlated with some types of herbivory at the scale of individual leaves, suggesting that a plant could "fine-tune" its allocation to EFN-mediated defense. While we did not measure secretion of EF nectar, it is likely that variation in nectar production also impacts insect visitation (Heil et al. 2001; Ness 2003) by both leaf miner adults and potential mutualists. We found that leaf-level impacts of EFNs result in lower tissue loss to miners at the scale of whole shoots, suggesting that herbivore load is not merely shifted from leaves with EFNs to those without. Ness (2003) found that increased nectar production of *Catalpa bignonioides* Walt. EFNs was related to increased ant protection at the scale of whole plants. We do not yet know if the benefits of EFN expression at the scale of individual aspen leaves lead to nonadditive effects of high EFN expression at the scale of whole ramets. In addition, as with other studies (Ness 2003), we cannot rule out the possibility that lower herbivory on leaves bearing EFNs is influenced by correlated leaf traits (e.g., chemistry).

Our results suggest that there may be much learned from examining the influence of EFNs at the within-plant scale, selection for plasticity in EFN expression over a plant's lifetime, and variable selection for EFN expression or other EFN traits at small spatial scales.

Acknowledgements

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