

# Long-term impact of a leaf miner outbreak on the performance of quaking aspen

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**Abstract:** The aspen leaf miner, *Phyllocnistis populiella* Cham., has caused widespread and severe damage to aspen in the boreal forests of western North America for over a decade. We suppressed *P. populiella* on individual small aspen ramets using insecticide at two sites near Fairbanks, Alaska, annually for 7 years and compared plant performance with controls. Insecticide treatment successfully reduced leaf damage by *P. populiella* during most years and had little effect on herbivory by externally feeding invertebrates. By the end of the study, control ramets had suffered a reduction in height and girth relative to treated ramets and to the original, pretreatment size. Control ramets produced smaller leaves during some years and, after 7 years, produced fewer total shoots and leaves than ramets sprayed with insecticide. Treatment did not affect mortality, but at the warmer of the two sites, ramets sustaining ambient levels of leaf mining were significantly more likely to die back to basal sprouts than those treated with insecticide. We conclude that a decade of *P. populiella* outbreak has caused strongly negative effects on aspen development and the production of aboveground tissues.

**Résumé :** La mineuse serpentine du tremble (*Phyllocnistis populiella* Cham.) cause des dommages sévères et très répandus dans les forêts boréales de peuplier faux-tremble de l'ouest de l'Amérique du Nord depuis plus de 10 ans. À chaque année pendant 7 ans, nous avons éliminé la mineuse sur de petits ramets de peuplier faux-tremble à l'aide d'un insecticide dans deux stations près de Fairbanks, en Alaska, et nous avons comparé la performance des plants traités à celle de plants témoins. L'insecticide a réussi à réduire les dommages foliaires causés par la mineuse durant la plupart des années et a eu peu d'effet sur l'herbivorisme par les invertébrés qui se nourrissent des feuilles. À la fin de l'étude, les ramets témoins avaient subi une réduction en hauteur et en circonférence relativement aux ramets traités et à leur dimension originale avant le traitement. Les ramets témoins ont produit de plus petites feuilles durant certaines années. Après sept ans, ils avaient produit au total moins de pousses et moins de feuilles que les ramets traités avec un insecticide. Le traitement n'a pas eu d'effet sur la mortalité mais, dans la station la plus chaude, les ramets qui subissaient les attaques régulières de la mineuse étaient significativement plus susceptibles de dépérir jusqu'au rejet basal que ceux qui avaient été traités avec un insecticide. Nous concluons qu'une dizaine d'années d'épidémie de mineuse a eu des effets négatifs importants sur le développement du peuplier faux-tremble et sur la production de biomasse aérienne. [Traduit par la Rédaction]

## Introduction

Performance has declined and mortality increased for many tree species across western North America during the past several decades (van Mantgem et al. 2009). Recent regional growth declines and dieback of quaking aspen (*Populus tremuloides* Michx.) are a particular source of concern among biologists and land managers (Huang and Anderegg 2012). Although much attention has focused on arid regions of the southwestern US, declines in growth and survival have been documented in the boreal forest as well (Michaelian et al. 2011). Evidence from western Colorado indicates that dieback is the result of water stress, which causes hydraulic failure in roots and branches (Anderegg et al. 2012). However, dendroecological studies of aspen in the Canadian boreal forest indicate that attack by phytophagous insects may also play a role in dieback (Frey et al. 2004).

Outbreaks of insect herbivores can have significant negative effects on plant performance (Yang 2012). However, some plant populations have considerable tolerance for herbivory (Strauss and Agrawal 1999), even during insect outbreaks (Kessler et al. 2012). The vast majority of our current understanding of the effect of insect outbreaks on plant performance comes from the study of defoliating insects. Population outbreaks of leaf-mining insects, while less common and less well-studied than defoliator outbreaks, can also have negative effects on growth and reproduction of the host (e.g., Raimondo et al. 2003). Feeding by leaf miners is

often restricted to one or a few tissues within leaves (Hering 1951); consequently, injury caused by leaf mining may be less harmful to the physiology of the plant than wholesale leaf removal. On the other hand, defoliation may provide opportunities for growth compensation that leaf mining damage does not. Studies of aspen and the tropical palm *Chamaedorea elegans* Mart., for example, indicate that defoliated plants can benefit from a rapid increase in light penetration through the canopy, increasing photosynthesis by the remaining leaves (Kruger et al. 1998; Anten and Ackerly 2008). In contrast, a rapid increase in light penetration is not typical of leaf-mining damage. While early abscission of mined leaves is common across several broadleaf tree species, mined leaves typically remain on the plant during the height of the growing season and abscise late in the summer (e.g., Pritchard and James 1984; Stiling and Simberloff 1989; Salleo et al. 2003; Wagner et al. 2008), providing relatively little opportunity for photosynthetic compensation due to an increase in light availability.

As of 2012, aspen in the boreal forests of Alaska and the Yukon Territories had sustained high levels of foliar damage by the epidermal leaf miner, *Phyllocnistis populiella* Cham., for more than a decade (USDA Forest Service 2012; Yukon Energy, Mines and Resources, Forest Management Branch 2012). At the peak of the outbreak in 2007, *P. populiella* infested over 300 000 ha of Alaskan forest. During the same year, 100% of the aspen trees surveyed near Fairbanks, Alaska, sustained leaf-mining damage, which

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**Table 1.** Average ( $\pm$  SE) climate characteristics recorded near the two study sites in interior Alaska, Bonanza Creek Long Term Ecological Research Area (BNZ) and Ester Dome (ED), between 2005 and 2011.

Month	Temperature ( $^{\circ}$ C)		Precipitation (mm)		Max snow depth (mm)	
	BNZ	ED	BNZ	ED	BNZ	ED
Apr.	1.8 $\pm$ 0.8	0.0 $\pm$ 0.9	6.1 $\pm$ 4.0	8.4 $\pm$ 1.6		
May	11.3 $\pm$ 0.5	9.2 $\pm$ 0.6	15.1 $\pm$ 4.9	19.2 $\pm$ 5.5		
June	14.7 $\pm$ 0.3	13.3 $\pm$ 0.3	53.9 $\pm$ 6.1	54.8 $\pm$ 8.2		
July	16.2 $\pm$ 0.6	14.6 $\pm$ 0.7	63.3 $\pm$ 11.8	69.9 $\pm$ 13.2		
Aug.	12.9 $\pm$ 0.6	12.0 $\pm$ 0.9	59.7 $\pm$ 10.8	70.0 $\pm$ 13.9		
<b>Growing season average</b>	11.5 $\pm$ 0.5a	9.8 $\pm$ 0.5b	40.9 $\pm$ 2.9a	42.1 $\pm$ 2.2a		
<b>Winter average</b>					505 $\pm$ 50a	718 $\pm$ 89b

**Note:** Measurements were made at Bonanza Creek Long Term Research Station LTER1, 3.8 km from the BNZ study site (elevation 352 m) and climate station USC00502868 near the apex of Ester Dome, 0.9 km from the ED study site (elevation 664 m). Data were obtained from the Bonanza Creek Long Term Ecological Research Program and the Alaska Climate Research Center. Growing season average temperature, average precipitation, and maximum snow depth were compared between sites with paired *t* tests; different letters indicate significant differences ( $n = 7$  years,  $P < 0.05$ ).

extended over an average 58% of the total leaf surface area (SD 31,  $n = 743$  leaves on 124 trees across 4 sites; unpublished data). Previous work demonstrated that leaf mining by *P. populiella* impairs photosynthesis and causes early leaf abscission, thereby slowing aspen growth rates as measured over 3 years (Wagner et al. 2008). The goal of the present study was to assess the effects of the *P. populiella* outbreak on aspen mortality, dieback, and above-ground tissue production over a longer period of time.

## Materials and methods

### Natural history of the herbivore

*Phyllocnistis populiella* is a univoltine, gracillariid moth native to North America. A specialist on the genus *Populus*, it has been collected throughout much of the US and Canada (Condrashoff 1964). In Alaska, both larvae and adults feed on *Populus tremuloides*, the larvae consuming leaves and adults consuming extrafloral nectar. Adults overwinter and emerge in late April or May, before bud break (Wagner et al. 2012). Females begin to lay eggs just as aspen leaves unfurl from buds. Eggs are laid on both the adaxial and abaxial leaf surfaces. Larval feeding is restricted to the epidermal cell layer on the side of the leaf on which the egg is laid, forming a pale, serpentine mine. Larvae pupate within the leaf and eclose in June. Natural enemies of *P. populiella* in Alaska include ants and predacious mites, which will attack eggs and larvae, and several species of parasitoid wasp in the family Eulophidae, which predominately attack late-instar larvae and pupae.

### Study sites

The study was conducted at two sites within the boreal forest near Fairbanks, Alaska, one within the Bonanza Creek Long Term Ecological Research Area (64.7087 $^{\circ}$ , -148.3243, elev. 214 m, and hereafter referred to as BNZ) and another at higher elevation on the summit of Ester Dome (64.8763 $^{\circ}$ , -148.0634 $^{\circ}$ , elev. 714 m, and hereafter referred to as ED). Both were located on south-facing slopes. Experimental aspen ramets at site BNZ were contained within an area of 1.0 ha characterized by mixed aspen, white spruce (*Picea glauca* (Moench) Voss), and birch (*Betula neoalaskana* Sarg.) with a primarily aspen overstory; the density of aspen ramets >20 cm tall in 2005 was 10 850 per ha. Site ED was 1.1 ha in area and characterized by mixed white spruce, alder, and aspen. The vegetation at this site was more diffuse than at BNZ, with little overstory and widely distributed mature aspen trees; the density of aspen ramets >20 cm tall was 2345 per ha. During the growing season, the climate of BNZ was warmer than that of ED but the sites received equivalent amounts of precipitation (Table 1). During the winter, BNZ accumulated less snow than ED (Table 1).

Aspen reproduce asexually, as well as sexually, and can form clonal stands. We did not analyze the genetic identity of the focal ramets in this study. There were no obvious breaks in aspen density or morphology within sites that would suggest clonal boundaries, and recent genetic work indicates that identification of aspen clones based on morphology or proximity to other trees is inaccurate (Wyman et al. 2003). Aspen stands usually contain more than one genotype, and the median size of monoclonal stands is estimated to be much smaller than the area of our study sites (Namroud et al. 2006; Mock et al. 2008; De Woody et al. 2009).

### Experimental design

To test the effects of the leaf miner *P. populiella* on long-term aspen performance, we sprayed aspen ramets with insecticide annually for 7 years and compared survivorship, prevalence of dieback, and aboveground tissue production with controls. In May of 2005, we chose 40 aspen ramets 0.5–2 m tall at each site (One ramet at ED was destroyed during the first year, leaving  $n = 39$  at this site). Within each study site, experimental ramets were dispersed throughout the 1 ha area, to avoid artifacts due to insecticide drift onto control ramets. The diameters of the ramets, measured at 20 cm height (a convenient height for observers and about half the height of the smallest ramets), ranged from 6 to 33 mm. Half of the ramets at each site were assigned at random to receive annual insecticide treatments, a process that resulted in intermixing of treatment and control ramets in space. The focal ramets were initially taller at site BNZ than at ED (BNZ 146 cm  $\pm$  3 SE; ED 113 cm  $\pm$  5 SE;  $F_{1,75} = 32.28$ ,  $P < 0.0001$ ), but treatment and control groups did not differ ( $F_{1,75} = 0.54$ ,  $P = 0.46$ ).

Our goal in the experiment was to reduce *P. populiella* mining damage without suppressing other species of arthropod herbivores or predators. Since *P. populiella* is one of the earliest arthropods to emerge in the spring, we reduced nontarget impacts by applying insecticide as early as possible following bud break and *P. populiella* oviposition. We chose the nonsynthetic, broad-spectrum insecticide Conserve (Dow AgroSciences, Indianapolis, Indiana) because the active ingredient, spinosad, is effective against lepidopteran leaf miner larvae, breaks down rapidly in sunlight, and has low acute toxicity to humans (USDA National Organic Standards Board 2002). We sprayed plants each year from 2005 to through 2011 between the dates of 26 May and 2 June, when small *P. populiella* leaf mines were observed and the rate of oviposition had slowed. Using a hand-powered pump sprayer, we applied insecticide at a concentration of 1.56 mL L $^{-1}$ , which delivered approximately 11 mg of spinosad per ramet. In 2005 and 2006, the insecticide was reapplied 3–7 days later; from 2007 on, ramets received only a single application of insecticide per year to further minimize nontarget effects. Ants were the only arthro-

Pods noted on the aspen during insecticide application. When treated aspen ramets were sprayed with insecticide, control ramets were sprayed with an equal volume of water.

Between mid-July and mid-August of each year, we observed each plant and scored as dead those ramets with no living foliage present. Rarely, ramets scored as dead in one year produced leaves the next, and in these cases the earlier assessment of mortality was corrected. In this study we differentiate between the outright mortality of a ramet and dieback, which we define as the loss of the canopy tissues accompanied by the formation of sprouts from below 20 cm (generally from the stem–root collar).

During the annual midsummer surveys, we also measured leaf size and herbivory for 2–3 shoots per ramet. We chose shoots from all heights and aspects without regard to mining damage, and collected data from the seven most proximal leaves on each shoot. Measurements were made nondestructively to avoid impacts on growth. For each sampled shoot, we visually estimated herbivory as the percentage of the leaf area damaged. Leaf mining damage caused by *P. populiella* was assessed to the nearest 1% on the adaxial and abaxial leaf surfaces separately, and these two values were averaged for an estimate of total mining damage per leaf. Prior to data collection, all observers were tested to ensure that their visual estimates of mining damage correlated well with measurements of leaf damage made using image analysis software ( $R^2 > 0.90$  for all observers,  $n > 55$  leaves; ImageJ software, National Institutes of Health). The percentage of the leaf missing or skeletonized by other herbivore species was estimated as well. We measured the maximum width of each leaf to the nearest millimetre. Before beginning the study, we had established that leaf width, measured at the widest point to the nearest millimetre, predicted leaf area with high precision ( $R^2 > 0.94$ ,  $n = 42$  leaves from 42 trees across both study sites; leaf area ( $\text{cm}^2$ ) =  $-4.5901 + 0.3770 \times$  leaf width (mm) +  $0.0015 \times$  leaf width<sup>2</sup>). We therefore chose to measure leaf width only, rather than both width and length, to reduce labor.

In June 2012, approximately 1 year following the last treatment with insecticide, we again surveyed all aspen ramets and recorded mortality and dieback. For all live ramets, we measured the maximum height of living leaf tissue, the diameter at 20 cm height, the total number of live shoots, and the number of leaves on three shoots chosen haphazardly from all available heights and aspects. Because some ramets had experienced dieback during the study, the main stem in 2012 was not always the same main stem measured at the onset of the study in 2005. To assess the likelihood of browsing damage on insecticide-treated and control ramets, we also recorded, for each ramet, the presence or absence of freshly cut twigs indicative of browsing by mammals during the previous winter. To avoid confusing mortality with delayed phenology, all ramets were revisited in August 2012 to confirm the earlier observations.

### Data analysis

To verify the effectiveness of the insecticide treatment at decreasing leaf mining across sites and over time, we used a univariate repeated measures ANOVA. The dependent variable was the percentage of leaf area mined, averaged within each ramet. Fixed effects in the model were treatment, site, year, and their interactions. The random effect of individual ramet nested within treatment and site served as the error term for the tests of treatment and site effects.

To investigate possible nontarget effects of insecticide treatment, we tested the effect of annual insecticide treatment on the leaf area damaged by chewing insects that fed externally, calculated as the average sum of percentage leaf area missing and skeletonized within each ramet. These data violated the assumptions of ANOVA, so treatment and control medians were compared within sites and years using nonparametric Wilcoxon tests.

We calculated the effect of the insecticide treatment on the probability of mortality and dieback between 2005 and 2012 using separate Kaplan–Meier procedures (Kalbfleisch and Prentice 1980). The dependent variable for the mortality analysis was the number of years each ramet remained alive; ramets that survived throughout the 7 years were censored from the analysis. The set of ramets that suffered dieback included some that later died and others that survived in a smaller state. The dependent variable for the dieback analysis was the number of years prior to the first incidence of dieback. Ramets that maintained a canopy throughout the study were censored and ramets that suffered mortality without prior dieback were excluded from the analysis. Data from the two sites were analyzed separately. Probabilities of mortality and dieback by insecticide-treated versus control ramets were compared using Wilcoxon tests.

To investigate the influence of leaf mining on leaf expansion, we tested the effect of insecticide treatment on leaf width using an ANOVA model similar to that applied to leaf-mining data. Leaf size varies predictably with the leaf's positional order along the shoot, so we first averaged leaf width at each leaf position (positions 1–7, with position 1 being proximal to the main stem) across shoots within ramets and years. These data were then square-root-transformed to meet model assumptions. Fixed effects in the statistical model included insecticide treatment, site, year, their interactions, and leaf position. The random effect of individual ramet nested within treatment and site was included to serve as the error term for significance tests of these effects. In the case of a significant interaction between treatment and site or year, a priori contrasts were used to compare treatment and control means.

The effect of experimental treatment, site, and their interaction on the final (2012) measurements of surviving ramet height, diameter of the main stem at 20 cm height, total shoot number, average leaves per shoot, and estimated total number of leaves (calculated as the product of shoot number and average leaves per shoot) were analyzed with two-way ANOVA. In the case of a significant interaction, treatment and control means were contrasted within site. To investigate changes in the size of treated and control ramets between the start and end of the study, we tested whether ramet height and main stem diameter in 2012 differed from the 2005 pretreatment values using paired *t* tests.

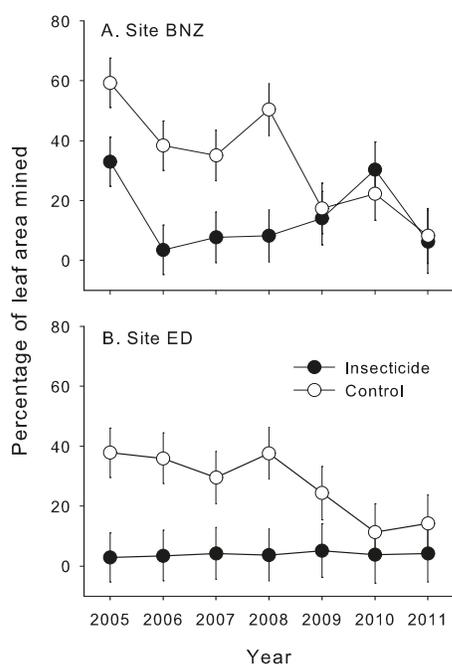
Mammalian browsing can cause mortality and reduce growth of small aspen (Kauffman et al. 2010). If experimental reduction in leaf-mining damage inadvertently altered the palatability of aspen as a source of winter browse, then mammalian browsing might confound the effects of insecticide treatment on aspen mortality and dieback. We therefore investigated the effect of treatment, site, and their interaction on the presence of 2012 browsing damage using a logistic analysis.

## Results

### Patterns of herbivory

The application of insecticide successfully reduced leaf-mining damage during most years of the study (Fig. 1). The average percentage of leaf area mined varied over space, with significantly greater damage at BNZ than at ED (Table 2). The percentage of leaf area mined also varied from year to year (Table 2), with particularly high levels of damage between the years of 2005 and 2008; during this period unsprayed ramets sustained mining damage to 30%–60% of the total leaf surface on average (Fig. 1). Ambient levels of mining damage dropped in 2009 as the outbreak waned and remained around 10%–30% thereafter (Fig. 1). Insecticide treatment significantly reduced leaf-mining damage overall (average percentage of mining damage across all years  $\pm$  SE; insecticide treatment,  $9.3\% \pm 1.5$ ; and control,  $23.9\% \pm 1.5$ ), and was more effective at ED than at BNZ (Fig. 1 and Table 2). Overall treatment effectiveness varied over time, becoming less effective in the later

**Fig. 1.** Effect of annual insecticide treatment on the percentage of leaf area mined by *Phyllocnistis populiella* at two sites: (A) Bonanza Creek Long Term Ecological Research Area (BNZ) and (B) Ester Dome ED ( $n = 40$  and  $39$  ramets, respectively). Symbols indicate least-squares means; error bars are 95% confidence intervals.



**Table 2.** Analysis of variance testing the effect of insecticide treatment on aspen leaf mining by *Phyllocnistis populiella* across sites and years ( $n = 79$  ramets).

Source of variation	df	F	P
Insecticide	1, 74	93.3	<0.0001
Site	1, 74	14.76	0.0003
Site × Insecticide	1, 74	1.4	0.23
Year	6, 397	13.9	<0.0001
Insecticide × Year	6, 397	12.1	<0.0001
Site × Year	6, 397	6.3	<0.0001
Insecticide × Site × Year	6, 397	1.3	0.27

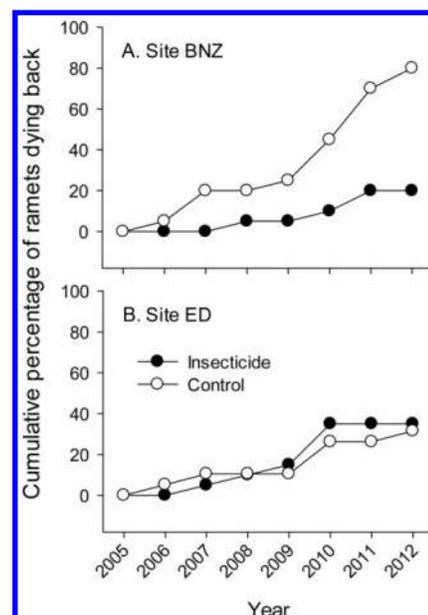
years of the study, as ambient levels of leaf mining declined, leading to a significant interaction between treatment and year; this decline in treatment effectiveness was observed at both sites (Fig. 1 and Table 2).

In contrast to its effect on leaf mining, early-season application of insecticide had little impact on leaf damage caused by externally feeding chewing and skeletonizing insects. External feeding damage was surprisingly low across sites and years; on average, <1% of the leaf area was damaged each year, and median damage was 0% for all combinations of treatment and year. The two study sites did not differ in median percent leaf damage sustained by external feeders ( $P > 0.1$ ), so data from the two sites were combined to test the effect of treatment. Differences in percent damage between insecticide-treated and control aspen were not significant except during 2006 and 2007, when insecticide reduced average percent damage from 0.10% (control mean) to 0.01% (treatment mean) and from 0.02% to 0.00%, respectively ( $\chi^2 = 4.2$  and  $P = 0.04$  for both comparisons).

#### Mortality and top dieback

During the 7 years of the study, 13 of 40 aspen ramets at BNZ (32%) and 12 of 39 ramets at ED (31%) died outright. Experimental reduction in leaf-mining damage did not alter the probability of

**Fig. 2.** The percentage of ramets subject to experimentally reduced (solid symbols) and ambient (open symbols) leaf mining that died back annually at two study sites ( $n = 40$  at (A) Bonanza Creek Long Term Ecological Research Area (BNZ) and  $n = 39$  at (B) Ester Dome (ED)).



mortality at either study site ( $\chi^2 < 2.6$ ,  $df = 1$ ,  $P > 0.11$ ). The cause of mortality was obvious for only one of these ramets, which was severed at the root crown. For the remaining ramets, there was no evidence of pathogen attack and the cause of death was unidentified.

Aspen dieback was affected by insecticide treatment at one of the two sites. At BNZ, aspen ramets that sustained ambient levels of leaf mining were significantly more likely to die back than ramets treated with insecticide (Fig. 2A;  $\chi^2 = 9.33$ ,  $df = 1$ ,  $P = 0.002$ ). By 2012, 16 of the 20 control ramets at BNZ had died back during the previous 7 years; in contrast, only 4 of the 20 insecticide-treated plants had died back (Fig. 2A). At the higher elevation site ED, insecticide treatment had no effect on dieback (Fig. 2B,  $\chi^2 = 0.51$ ,  $df = 1$ ,  $P = 0.41$ ). There was no evidence that patterns of browsing caused the observed patterns of dieback. Aspen ramets in the treatment and control groups were equally likely to have been browsed during the winter of 2011–2012 ( $\chi^2 = 0.07$ ,  $df = 1$ ,  $P = 0.5$ ), and this effect did not differ significantly across sites (treatment × site interaction  $\chi^2 = 3.63$ ,  $P = 0.06$ ).

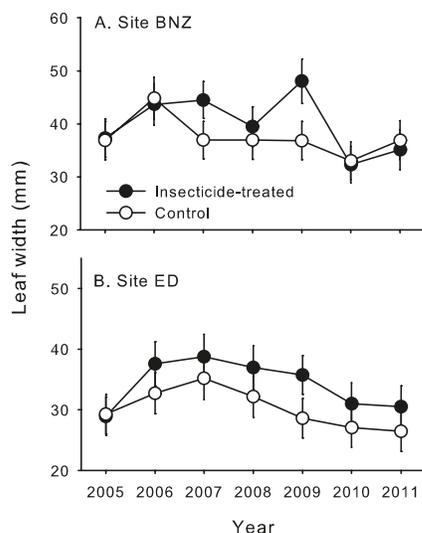
#### Leaf size

Experimental reduction of leaf-mining damage had a positive effect on leaf size in general, but the magnitude of the effect varied across sites and years. The average leaf width of aspen ramets treated with insecticide was 9.8% greater than the leaves of control ramets (back-transformed least-squares means  $\pm$  SE; insecticide-treated, 36.9 mm  $\pm$  1.1; and control, 33.6  $\pm$  1.1; Table 3). Average leaf width strongly varied between sites, with aspen at BNZ having larger leaves than those at ED (Table 3 and Fig. 3). The effect of treatment on leaf width varied over time, and varied differently over time at the two study sites (Table 3). Contrasts between treatment and control within years and sites revealed that insecticide treatment had a statistically significant ( $P < 0.05$ ) positive effect on leaf width during 2007 at BNZ, and during 2009 at both sites (Fig. 3). To investigate whether the negative effect of leaf mining on leaf width was local or systemic, we re-ran the analysis on the subset of leaves with no leaf-mining damage. Insecticide treatment had the a significant positive effect on the width

**Table 3.** Analysis of variance testing the effect of insecticide treatment on aspen leaf width across sites and years ( $n = 3272$  leaves on 79 ramets).

Source of variation	df	F	P
Insecticide	1, 74	4.3	0.04
Site	1, 74	17.2	<0.0001
Insecticide × Site	1, 74	0.3	0.57
Year	6, 3175	52.8	<0.0001
Insecticide × Year	6, 3175	10.6	<0.0001
Site × Year	6, 3175	9.1	<0.0001
Insecticide × Site × Year	6, 3175	4.5	<0.0001
Leaf position	6, 3162	58.8	<0.0001

**Fig. 3.** Effect of annual treatment with insecticide to reduce leaf mining on the average width of aspen leaves at two sites ( $n = 40$  at (A) Bonanza Creek Long Term Ecological Research Area (BNZ) and  $n = 39$  at (B) Ester Dome (ED)). Values are back-transformed least-squares means; error bars are 95% confidence intervals. Asterisks denote pairs of treatment and control that are significantly different ( $P < 0.05$ ).

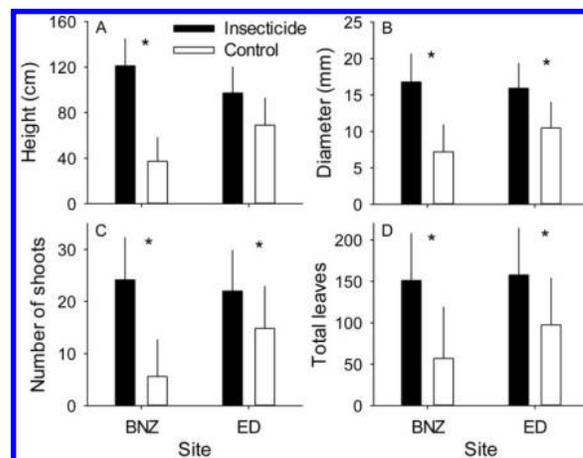


of unmined leaves relative to controls during the same combinations of site and year as for the larger data set (data not shown).

### Final aboveground tissue production

Seven years of experimental treatment to reduce leaf mining had strong positive effects on final aspen size and aboveground tissue production. By 2012, aspen ramets that had been treated with insecticide were, on average, 113% taller than ramets that had sustained ambient levels of leaf mining (Fig. 4A), a statistically significant difference (Table 4). The effect of insecticide treatment on height was stronger at BNZ than at ED (Fig. 4A), leading to a significant treatment-by-site interaction (Table 4), and post hoc tests supported a significant treatment effect on height at BNZ but not at ED. Across sites, treatment with insecticide increased the diameter of the main stem by 83% and the total number of shoots by 126% (Figs. 4B and 4C and Table 4). The average number of leaves per shoot did not respond to treatment (Table 4) and, although there was a statistically significant interaction between treatment and site, post hoc comparison of means revealed no significant differences between any combination of site and treatment ( $P > 0.05$ ; data not shown). Total leaf production, estimated as the product of shoots and leaves per shoot, was on average 100% greater for insecticide-treated ramets than for control ramets (Fig. 4D and Table 4).

**Fig. 4.** Average size and tissue production by surviving aspen ramets after 7 years of experimental leaf miner suppression ( $n = 27$  at each of two sites). (A) Maximum height of living foliage. (B) Diameter of the main stem at 20 cm in height. (C) Number of living shoots. (D) Total number of leaves. Error bars indicate 95% confidence intervals. Asterisks indicate significant differences between treatment and control ( $P < 0.05$ ). BNZ, Bonanza Creek Long Term Ecological Research Area; ED, Ester Dome.



**Table 4.** Analysis of the effects of annual insecticide treatment to reduce leaf mining damage on final aspen size and tissue production at two sites ( $n = 54$  ramets surviving until 2012).

Dependent variable	df	F value		
		Treatment	Site	Treatment × Site
Height	1, 49	<b>24.22</b>	0.12	<b>5.94</b>
Diameter main stem	1, 46	<b>17.25</b>	0.44	1.29
Number of shoots	1, 49	<b>10.98</b>	0.82	2.15
Leaves per shoot	1, 42	0.14	0.01	<b>5.07</b>
<b>Total leaves</b>	1, 42	<b>7.13</b>	0.67	0.35

Note: F values in boldface type are statistically significant at  $P < 0.05$ .

Treatment-related differences in aspen ramet size were a consequence of shrinkage by ramets exposed to ambient levels of leaf mining rather than high growth rates by ramets treated with insecticide. Across 7 years of study, aspen ramets exposed to ambient levels of leaf mining lost, on average, 74% and 20% of their 2005 pretreatment height at sites BNZ and ED, respectively, a statistically significant change at both sites (Table 5; BNZ:  $t = -10.7$ ,  $df = 15$ ,  $P < 0.0001$ ; ED:  $t = -3.4$ ,  $df = 11$ ,  $P = 0.006$ ). Moreover, ramets experiencing top dieback replaced the original main stem with a new sprout from the base, and this replacement resulted in a significant 54% decrease in average trunk diameter by ramets experiencing ambient leaf mining at BNZ (Table 5;  $t = -4.2$ ,  $df = 11$ ,  $P = 0.002$ ); this was not the case at ED ( $P > 0.05$ ;  $t = -1.5$ ,  $df = 12$ ,  $P = 0.2$ ), where dieback was less common. In contrast to the more highly damaged control ramets, ramets with experimentally reduced levels of leaf mining maintained (but did not increase) their height and trunk diameter over the 7 years of the study ( $|t| < 0.4$ ,  $df = 10-13$ ,  $P > 0.1$ ).

### Discussion

The results of this experiment indicate that a multi-year outbreak of the leaf miner *P. populiella* had strong negative impacts on aboveground tissue production by aspen. By the seventh year of the study, ramets subject to ambient levels of leaf mining possessed, on average, half the leaves and fewer than half the shoots than ramets on which leaf-mining damage had been reduced. However, the response of aspen trees to leaf mining was delayed.

**Table 5.** Change in the height and stem diameter of surviving aspen ramets from the pretreatment state in 2005 to the post-treatment state in 2012.

Measurement	Site	Control	Insecticide
Height change (cm)	BNZ	-105±21* (16)	-35±40 (11)
	ED	-37±24* (12)	-15±18 (13)
Diameter change (mm)	BNZ	-10±5* (12)	0±2 (11)
	ED	-3±4 (12)	0±5 (13)

Note: BNZ, Bonanza Creek Long Term Ecological Research Area; ED, Ester Dome. Ramets subject to ambient ("control") and reduced ("insecticide") leaf mining are listed separately. Stem diameters were measured at 20 cm in height; ramets shorter than this in 2012 could not be measured. Values are averages ± 95% confidence intervals (n). An asterisk indicates a statistically significant shift to smaller size over the 7 years (paired t test,  $P < 0.05$ ).

For example, during the first 3 years of the study, when leaf-mining damage was intense and the effectiveness of the insecticide treatment was high, the growth of ramets sustaining ambient levels of leaf mining was lower than that of ramets treated with insecticide, but growth rates were still positive and no leaf-miner-related dieback was detected (Wagner et al. 2008). During the subsequent 4 years of experimental manipulation, both the average ambient leaf-mining damage and the effectiveness of the treatment declined, yet during this period ramets exposed to ambient leaf mining declined in size relative both to control ramets and to their original size. Similarly, the strongest effect of treatment on leaf width occurred after 4 years of herbivore reduction and during a year (2009) in which the insecticide treatment was not particularly effective. The results indicate that the responses of these small aspen trees to leaf-mining damage were complex and lagged behind the receipt of the most severe damage. Leaf mining by *P. populiella* reduces photosynthesis in aspen leaves, apparently by preventing the stomata from opening normally (Wagner et al. 2008). Aspen tolerance to herbivory appears to be related to the amount of biomass stored in the stems (Stevens et al. 2008). Over many years, low rates of carbon assimilation may degrade aspen tolerance, leading to losses of above-ground tissue production such as those documented here. Our results underscore the need for long-term studies to capture the overall effects of insect outbreaks.

The most severe effects of leaf mining were seen at the lower elevation BNZ site where the incidence of dieback was higher in aspen ramets exposed to ambient levels of leaf mining. Although frequently associated with disturbances such as fire and drought, dieback to basal sprouts has also been described for hybrid poplar in response to high levels of defoliation by the gypsy moth (Kosola et al. 2001). Partly as a result of the high incidence of dieback, by the end of the study, control ramets at BNZ were less than a third the height of insecticide-treated ramets and had lost over a meter of their original height. Leaf-mining-related loss of canopy height was less extreme at ED than at BNZ, but control ramets at ED also lost a significant portion of their original height over the 7 years. Although there was no effect of insecticide treatment on ramet mortality, a reduction in average ramet size has implications for the ability of aspen to compete and persist. Aspen is shade-intolerant and a reduction in foliage height may decrease a ramet's ability to compete for light. Potential competitors of small aspen at our study sites include white spruce and Alaska birch, as well as understory shrubs. Moreover, height reduction extends the duration of an aspen ramet's vulnerability to mammalian browsers such as moose and snowshoe hare, and browsing pressure can affect the ability to recruit into the adult population (e.g., Kauffman et al. 2010). The effects of leaf mining on small aspen ramets at BNZ suggest that long term *P. populiella* outbreaks may impact not only aspen performance but plant community structure in some areas.

Because the experiment was conducted at only two sites, the cause of response differences between sites cannot be ascertained. The higher incidence of leaf-mining-related dieback at BNZ might reflect the higher average leaf-mining intensity at this site, but differences in dieback might also be related to climate and aspen genetics. BNZ had higher average growing season temperature, and plants at this site may have experienced more frequent water stress, despite similar precipitation at the sites. However, there is little evidence that water stress increases the impact of herbivory on woody plant species (Hawkes and Sullivan 2001). Aspen genetic composition and diversity may also have affected the results. If genetic diversity within sites was very low, then differences in responses among sites might reflect the responses of a small number of genotypes. While variation in genetic composition and diversity between sites may have influenced the results, it is very unlikely that all study ramets at a site were a single genet. A large proportion of genets within natural aspen stands appear to be represented by a single ramet (estimates range from 48% to >75%), and where monoclonal stands occur, the median area of coverage appears to be much less than the 1 ha area of our plots (De Woody et al. 2009; Mock et al. 2008; Namroud et al. 2006). Thus, whereas the number of ramets sampled may have exceeded the number of genets, differences between sites are almost certainly more general than a comparison of only two genotypes.

Studies that quantify the effect of herbivory on leaf size typically focus on direct reductions in leaf area due to tissue consumption. Unlike insect defoliators, *P. populiella* feeds internally and thus does not directly remove leaf area. Nonetheless, our results indicate that feeding by *P. populiella* can have a systemic negative effect on aspen leaf size, by reducing leaf expansion. Two mechanisms may explain this effect. First, reduced photosynthesis due to leaf mining, combined with the cost of mounting an induced defense in response to herbivore damage, might compete for nutrients with leaf expansion (Dale 1988). Several defensive compounds are inducible by foliar damage in aspen, including phenolic glycosides (Clausen et al. 1991; Young et al. 2010), tannins (Stevens and Lindroth 2005), and trypsin-inhibiting compounds (Haruta et al. 2001). Second, reduction in leaf size may be a direct consequence of induced defense. For example, induction of defense in *Rumex obtusifolius* L. by insect herbivory or the application of jasmonic acid leads to a systemic increase in cell-wall-bound peroxidase activity, increasing cell wall toughness and reducing leaf expansion and final size (Moore et al. 2003). Similarly, application of jasmonic acid to young hybrid poplars (*Populus nigra* × *Populus deltoides*) reduces the rate of leaf expansion (Arnold et al. 2004).

Aspen is an ecologically important species. Relative to other habitat types, aspen-dominated forest supports high plant and bird diversity (Turchi et al. 1995; Stohlgren et al. 1997), and aspen trees provide a source of high-quality forage for wildlife (Bryant and Kuropat 1980; Beck et al. 2006). Declines in aspen performance in recent decades have therefore become a focus of scientific inquiry and concern (e.g., Anderegg et al. 2012). Our study indicates that a widespread and long-term outbreak of the leaf-mining insect *P. populiella* has contributed to performance declines of small aspen ramets in Alaska. A dendroecological study conducted during the same Alaskan *P. populiella* outbreak also reported evidence of strong performance declines in large aspen trees (Juday et al. 2012). Changes in boreal fire severity due to climate warming have led some researchers to predict that communities currently dominated by conifers may shift toward deciduous tree species such as aspen and birch (Johnstone et al. 2010). However, the risk of heavy herbivory is also predicted to increase as the climate warms (Ayres and Lombardero 2000). Severe long-term outbreaks of herbivorous insects such as *P. populiella* complicate predictions of how boreal forest communities will respond to global change (Volney and Fleming 2000).

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