

HOW ASPEN TREE HEIGHT INFLUENCES ASPEN LEAF MINER (*PHYLLOCNISTIS POPULIELLA*) OVIPOSITION  
AND PERFORMANCE

By

Giovanni Tundo, B.S.

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in

Biological Sciences

University of Alaska Fairbanks

May 2021

© 2021 Giovanni Tundo

APPROVED:

Pat Doak, Committee Chair

Diane Wagner, Committee Co-Chair

Greg Breed, Committee Member

Diane Wagner, Chair

*Department of Biology and Wildlife*

Kinchel Doerner, Dean

*College of Natural Science & Mathematics*

Richard Collins, *Director of the Graduate School*

## Abstract

Under the optimal oviposition theory, insects are expected to lay eggs on hosts that maximize the success of their offspring. Tree height is known to be an important factor influencing the distribution of phytophagous insects because some species perform better at a distinct range of heights. This difference in performance could lead to incorrect estimates of population parameters if surveys are only conducted on one host plant height. Aspen leaf miners (*Phyllocnistis populiella*) have undergone a major outbreak in interior Alaska over the last two decades. We quantified patterns of aspen leaf miner oviposition and juvenile survival over 2 years and found that aspen leaf miners were approximately 1.5 times more likely to survive on tall trees than short trees. Parasitism and both egg and larval predation were lower on tall trees. Aspen leaf miners on tall trees also had larger pupal masses than those on short trees. Although aspen leaf miners performed better on tall trees, the number of eggs laid per leaf did not significantly differ by tree height. There were no significant differences in leaf foliar nitrogen between tall and short trees. We also found little differences in wind speed between tall and short trees that could explain ovipositional patterns. Ovipositional patterns may partially reflect the difference in phenology between tall and short aspen trees. Aspen leaf miners only lay eggs on new leaves. Tall aspen trees leafed out 7 days earlier on average than short aspen trees, and tall trees, unlike short trees, ceased to produce new leaves after budburst. Consequently, there was little overlap in the availability of tall and short aspen trees for oviposition, so even if aspen leaf miners have a preference for laying more eggs on tall than short trees, they can only act on it during the short time period when tall trees are available for oviposition. The results suggest that population projections based on data collected from only short trees may underestimate future aspen leaf miner population growth due to lower juvenile survival rates and pupal masses on short trees. More broadly, the results highlight the importance of examining multiple tree heights when studying the performance and population dynamics of phytophagous insects. They also suggest that phenological differences between plants may constrain insects from using higher quality hosts.



Table of Contents

	Page
Title Page.....	i
Abstract.....	iii
Table of Contents.....	v
List of Figures.....	vii
List of Tables.....	vii
Acknowledgements.....	viii
1 Introduction.....	1
2 Materials and Methods.....	6
2.1 Natural History.....	6
2.2 Surveys.....	7
2.2.1 Early Leaf Survey.....	7
2.2.2 Late Leaf Survey.....	8
2.3 Leaf Nitrogen.....	8
2.4 Wind Speed.....	9
2.5 Aspen Phenology.....	9
2.6 Data Analyses.....	10
2.6.1 Oviposition.....	10
2.6.2 Performance.....	11
2.6.3 Damage.....	11
2.6.4 Leaf Characteristics.....	12
2.6.5 Wind Speed.....	12
2.6.6 Phenology.....	12
3 Results.....	13
3.1 Oviposition.....	13
3.2 Performance.....	14
3.3 Damage.....	22
3.4 Leaf Characteristics.....	23
3.5 Wind Speed.....	23
3.6 Phenology.....	24
4 Discussion.....	26
4.1 Mortality Factors.....	26
4.2 Performance.....	28
4.3 Constraints on Oviposition.....	29
5 Conclusion.....	30
6 References.....	31
Appendix.....	39



List of Figures

	Page
Figure 1: Geographical area over which trees in Alaska damaged by the aspen leaf miner .....	2
Figure 2: A leaf that has been mined by the aspen leaf miner .....	7
Figure 3: Oviposition by aspen leaf miners by leaf side and tree height.....	13
Figure 4: Probabilities of different mortality sources.....	14
Figure 5: Distribution of the outcomes of all aspen leaf miners .....	17
Figure 6: Predictions based on GLMMs of the outcomes of all juvenile aspen leaf miners.....	18
Figure 7: Distribution of mortality events of aspen leaf miners.....	19
Figure 8: Juvenile survival probability of aspen leaf miners by tree height and leaf side.....	20
Figure 9: Predictions of the probability of juvenile aspen leaf miner (ALM) survival.....	20
Figure 10: Aspen leaf miner pupal mass by tree height and leaf side .....	21
Figure 11: Aspen leaf miner mining damage by tree height and leaf side .....	23
Figure 12: Mean phenology index values for tall in short aspen trees.....	25

List of Tables

	Page
Table 1: Description and location of study sites .....	7
Table 2: The locations of the four sites used to measure wind.....	9
Table 3: Index of shoot phenology.....	10
Table 4: Results of the models examining the probabilities of egg predation .....	15
Table 5: Results for the model examining aspen leaf miner pupal masses.....	21
Table 6: Results for the models testing probability of mining damage and percent mining .....	22
Table 7: Results for wind and gust speed .....	24
Table 8: Results for the leaf-out phenology model .....	25

## Acknowledgements

I intend to publish in a peer-reviewed journal with Pat Doak, Diane Wagner, and Greg Breed as co-authors. I would like to thank Jennifer Wheeler for tremendous help with the surveys, and the University of Alaska Fairbanks Forest Soils lab for help with the nitrogen analysis. I would also like to thank numerous sources of funding that helped make this thesis possible, include the Ted McHenry Biology Field Research Fund, summer fellowships from the Institute of Arctic Biology, teaching assistant funding from the Department of Biology and Wildlife, and funding from Bonanza Creek Long Term Ecological Research.

## 1 Introduction

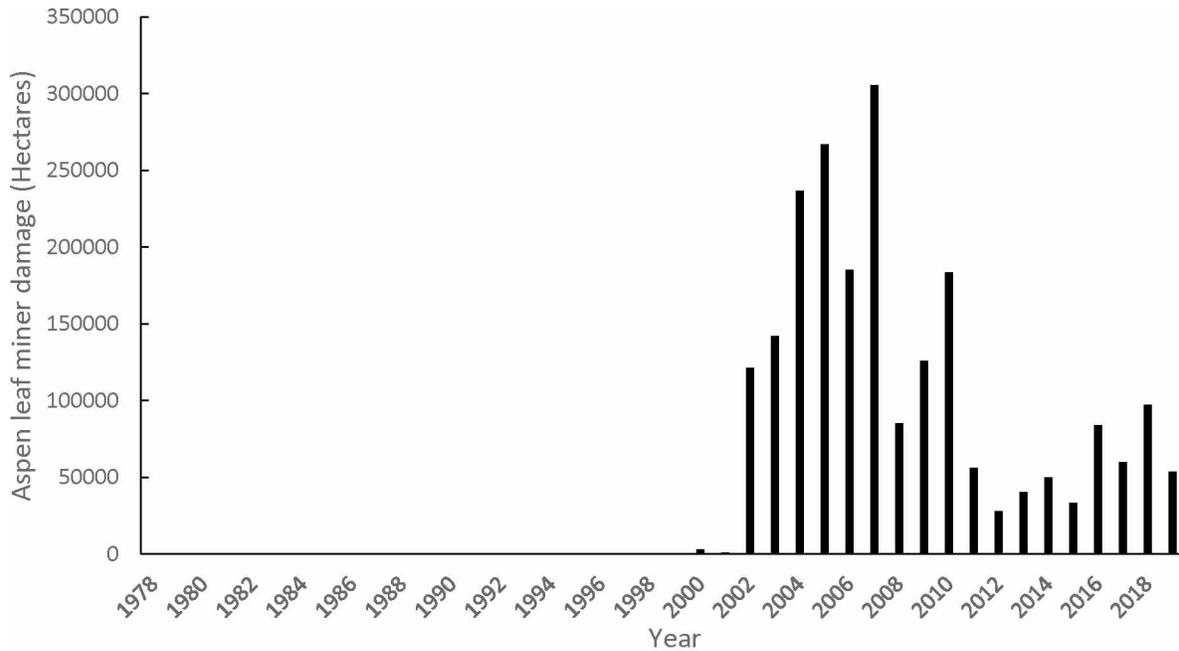
The optimal oviposition theory states that insects will lay eggs on hosts that maximize the success of their offspring (Jaenicke 1978). Optimal oviposition theory assumes that host plants, or even locations within a plant, vary in quality for offspring, that adults can detect these differences in quality, that the adult is constantly making eggs, and that adult preference for sites correlates to offspring success (i.e. 'mother knows best') (Levins and MacArthur 1969, Thompson 1988). Therefore, when plant height influences offspring success, adult female insects are expected to prefer one plant height over another. Optimal oviposition theory is similar to optimal foraging theory, with the main difference being that under optimal oviposition theory, adults are trying to maximize their lifetime fitness by having the largest number of offspring survive to adulthood to reproduce, while optimal foraging theory focuses more on the direct needs of the adult (Scheirs and De Bruyn 2002). Optimal oviposition theory may be especially important for understanding the distribution and population dynamics of phytophagous insects with limited mobility in the larval stage, because their distribution and success is highly dependent on where the adults lay eggs.

Plant height (or age) is an important factor in the distribution of phytophagous insects because it is likely to affect offspring success (Price 1991). Some insects may perform better on one tree height over another, potentially resulting in an uneven distribution of that insect over the height of the trees (Jaenicke 1978). Similarly, there could be an uneven distribution within trees if insects perform better in the upper or lower canopy. Studies examining insect performance or damage from insect pests often only investigate at one tree height, which may result in an incomplete story (e.g. Fritz and Nobel 1990, Auerbach and Alberts 1992, Watt et al. 1997, McMillin et al. 1998, Kagata and Ohgushi 2001, Goane et al. 2008, Mustafa et al. 2014, Arshad et al. 2019, Bellone et al. 2020). Several of these papers extrapolated their results to say how their insect study species performed on the plant species overall without regard to height (e.g. Auerbach and Alberts 1992, McMillin et al. 1998), which could be problematic if insect performance varies by height. Population estimates extrapolated from research from a single tree height may be inaccurate if the performance and/or abundance of that insect varies by tree height, which is why investigations at multiple tree heights can be crucial for understanding the population dynamics of an insect herbivore.

While many phytophagous insects are evenly distributed within the tree canopy, some are more abundant in the upper canopy and others are more abundant in the lower canopy (Basset et al. 2003, Grimbacher and Stork 2007, Ulyshen 2011). Numerous factors, such as the quality and quantity of resources (Basset et al. 1992, Novotny et al. 2003), ability to disperse (Neves et al. 2013), interspecific

interactions such as parasitism (Chaij et al. 2016), predation, and competition (Davis et al. 1997), and the microclimate (e.g. light, temperature, wind, and humidity) (Geiger et al. 2003) may vary by foliage height and could influence the distribution of insects.

The aspen leaf miner (ALM; *Phyllocnistis populiella*) is a serpentine leaf mining moth whose larvae feed on quaking aspen trees (aspen; *Populus tremuloides*). ALMs occur across most or all of the range of *P. tremuloides* at low-density endemic levels but recently, ALMs have recently reached population levels in interior Alaska unprecedented in the existing record (Fig. 1). There have also been reports of outbreaks in the past, notably in western Canada in the 1950s and 1960s (see Condrashoff 1964). Other annual surveys measuring ALM survival, abundance, and damage in interior Alaska have been conducted over the last 16 years, but they have almost exclusively been conducted on short (<2 m in height) aspen ramets (Doak and Wagner 2015).



**Figure 1:** Geographical area over which trees in Alaska damaged by the aspen leaf miner were observed. The data were compiled from annual forest health conditions reports in Alaska (US Forest Service 1978:2019) where the US Forest Service performed aerial surveys of tree damage. The aerial surveys began in 1978, but aspen leaf miners were first recorded in 2000. Note that the total area surveyed varies annually – though the regions surveyed are the same - and surveys only represent about 20% of the total forested area of Alaska.

The ALM provides a good system for studying ovipositional patterns at different plant heights, because ALM larvae are restricted to the leaf surface on which they hatch until they emerge as adults. ALM females also continuously produce eggs during the oviposition period (Condrashoff 1964), which is an assumption of the optimal oviposition theory. Previous research suggests that adults lay eggs optimally with respect to certain factors for example they tend to avoid leaf surfaces that already have conspecific eggs on them (Dennis et al. 2015), and they avoid leaves that would likely make their offspring more vulnerable to predators (Mortensen et al. 2013, Dennis et al. 2015). There may also be differences between tall and short aspen trees that could influence ALM oviposition, such as natural enemies (Basset et al. 2003), plant defenses (Donaldson et al. 2006a, Young et al. 2010), nutritional quality (Donaldson et al. 2006a), quantity of resources (Spasojevic et al. 2014), phenology (Augspurger and Bartlett 2003), and microclimate (Geiger et al. 2003).

One potential advantage for ALM larvae on leaves of tall trees is that tall trees tend to have lower effective chemical defenses than short (<2 m) trees. Previous research on aspen trees found that the leaves of taller, mature trees contained a lower concentration of phenolic glycosides and a higher percentage of condensed tannins than shorter, younger trees (Donaldson et al. 2006a, Young et al. 2010, Smith et al. 2011, Cope et al. 2019). Phenolic glycosides can serve as effective chemical defenses against insect herbivores (Lindroth 2001, Donaldson et al. 2006a, Osier and Lindroth 2006, Stevens et al. 2007, Holeski et al. 2009). In contrast, condensed tannins are inconsistent as effective defenses against insects (Faeth and Bultman 1986, Hemming and Lindroth 1995, Ayres et al. 1997).

Along with chemical defenses, nutritional quality, may also vary by tree height and influence ALM oviposition and performance. Foliage quality can correlate with distributional patterns of phytophagous arthropods in temperate forests (Ulyshen 2011). Plants and animals are typically growth limited by the scarcest resource available, which is often a usable source of nitrogen for insects (Mattson 1980). Insect leaf-feeders tend to perform better on plants that have higher levels of nitrogen (Galway et al. 2004). Across many species of deciduous trees, leaf nitrogen tends to increase with tree height (Kenzo et al. 2015). Previous research has found mixed results for how foliar nitrogen varies with aspen age and size. One study found that foliar nitrogen slightly increases with age (Donaldson et al. 2006a), another found that it slightly decreases with age (Cope et al. 2019), and a third found no significant effect of age (Smith et al. 2011). Aspen foliar nitrogen varies strongly with light levels and soil quality (Donaldson et al. 2006b), which could contribute to variation among studies.

The natural enemies of ALMs may also vary by tree height. Parasitoids and predators, such as ants and mites, forage on aspen leaves for nectar from extrafloral nectaries (EFNs). EFNs are sugar-

secreting glands located on the top side of some leaves of aspen trees. EFNs attract predators of insect herbivores (Bentley 1977). ALMs on short trees may be more exposed to some natural enemies than ALMs on tall trees for several reasons. The leaves of short trees have a higher frequency of EFNs than the leaves of tall trees (Doak et al. 2007, Young et al. 2010). There is potentially an energetic cost to forage on tall trees compared to short trees for some natural enemies. For example, ant nests in our study system are located in the ground, so there would be higher energetic and time costs for ants to forage on tall aspen trees (Holt and Askew 2012). This energetic cost could apply to some other predators and parasitoids as well, if they may spend more time near the ground.

Larval performance may also be related to leaf size, which could vary by tree height. Because ALMs feed on only the epidermal cell layer, they are restricted to one leaf side during development through the pupal stage. Therefore, larger leaves could potentially support more ALMs. This pattern could influence the number of eggs laid on each leaf, the survival probability of ALMs, and the size of the surviving individuals. Among some woody plant species in a temperate forest, leaf size was found to generally be higher in saplings than adults (Spasojevic et al. 2014). If leaf size differs between tall and short aspen trees, then that pattern could result in differences in ALM performance by tree height.

Differences in the microclimate of tall and short trees could affect oviposition and performance. In forests, the wind speed, light exposure, and temperature tend to be lower, and relative humidity higher, in the lower canopy than the upper canopy (Parker 1995, Geiger et al. 2003), although this varies between forest types and locations within a forest (e.g. edge vs center) (Geiger et al. 2003). Climatic gradients, especially wind, may play an important role in the vertical distribution of some insect species because small insects may have trouble with directional flight in windy conditions (Brown et al. 1997, Basset et al. 2003, Grimbacher and Stork 2007, Ulyshen 2011). Without directed flight, it would be more difficult for ALMs to fly to a leaf to oviposit.

Another factor that may constrain the oviposition of leaf miners is tree leaf-out times (Faeth et al. 1981, Brown et al. 1997, Miller and Ware 2014, Falk et al. 2018, Ekholm et al. 2020). Some studies have found a strong relationship between spring leaf-out times and leaf miner damage though the direction of the relationship varies (Condrashoff 1958, Condrashoff 1964, Miller and Ware 2014, Ekholm et al. 2020). This suggests that the phenology of leaf-out might affect patterns of oviposition, which in turn affect leaf damage. The oviposition period of a population of ALMs lasts for about 6 weeks. An aspen leaf is only attractive for ALM egg-laying for the first 7-10 days after it begins expanding (Condrashoff 1964), so ALMs may need to travel to new stands of aspen to maximize their oviposition. Research in temperate forests has found that in numerous deciduous tree species, there are consistent

intraspecific phenological differences in leaf-out times between tall and short trees with short trees leafing-out earlier than tall trees (Augspurger and Bartlett 2003, Osada and Hiura 2019). In contrast, previous observations at our study sites suggest that tall aspen trees leaf out before short trees. Unlike some short trees which continuously produce new leaves during the summer, tall trees cease to produce new leaves after initial budburst. Consequently, ALMs might have a greater opportunity to lay eggs on tall trees at the beginning of the season and on short trees later, but short trees will have a longer time period when their leaves are suitable for oviposition.

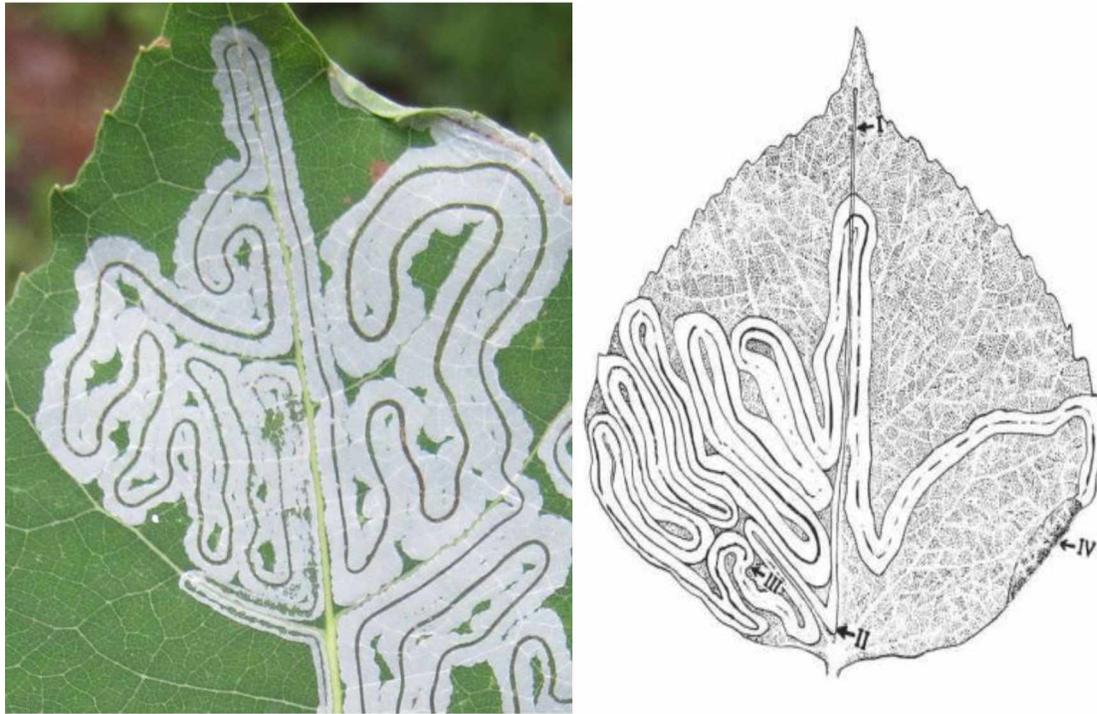
Tree height is an often overlooked yet potentially crucial factor for understanding insect herbivore performance and population dynamics. Here we address three main questions: 1. Do ALM lay more eggs on tall or short trees? 2. Do ALM perform better on tall or short trees? 3. With respect to tree height, does ALM oviposition match their performance. Performance here is measured as juvenile survival probability and pupal mass, because larger pupal mass is usually correlated with higher overwinter survival (Sinclair 2015) and higher reproductive success for female insects (Honěk 1993, Moreau et al. 2006). Because of potentially lower mortality from natural enemies and chemical defenses on tall trees, ALM performance is likely to be higher on tall trees than short trees. If female oviposition site preference is shaped by offspring performance, we would expect more oviposition on tall trees than short trees. However, there are other height-related factors we examine that may impact oviposition and performance such as microclimate, larval nutrition, and the availability of ovipositional substrate due to differences in phenology.

## 2 Materials and methods

### 2.1 Natural History

The aspen leaf miner (*Phyllocnistis populiella*) is an univoltine moth that feeds on quaking aspen trees (*Populus tremuloides*) (Fig. 2) (Condrashoff 1964). ALM moths overwinter as adults and emerge in early spring. As soon as aspen trees begin to leaf out, ALMs mate and then oviposit on the surfaces of new aspen leaves over a period of about 6 weeks (Condrashoff 1964). The adults feed on EFNs, which may impact their oviposition behavior. They oviposit on both the leaf top and bottom, which is uncommon for leaf-mining microlepidoptera (Reavey and Gaston 1991). The eggs are attached onto the leaf surface and hatch in 3-6 days (Condrashoff 1964). If the egg is eaten by a predator, a scar is usually left on the leaf, unless the egg is removed shortly after oviposition. Larvae go through four instars before pupating. They spend 4-5 days in each of the first three instars. In their 4<sup>th</sup> instar, the mouthparts

atrophy and they cannot feed. They create a fold in the leaf using silk and then pupate within two days (Condrashoff 1964). The pupae emerge as adults about two weeks later (Condrashoff 1964).



2A

2B

**Figure 2:** (A) A leaf that has been mined by the aspen leaf miner. (B) A diagram of an aspen leaf miner mine with the numbers representing the instar number. I, first instar; II, second instar; III third instar; IV, fourth instar (from Condrashoff 1964). The leaf miner creates a fold in the leaf and pupates after the fourth instar.

ALMs encounter many sources of juvenile mortality including natural enemies and intraspecific interference competition (Doak and Wagner 2015). At our study sites near Fairbanks, Alaska, at least 12 species of solitary, idiobiont parasitoids attack the ALMs with many acting as both primary and hyperparasitoids (unpublished data). These parasitoids lay one egg per host and kill the host when they lay the egg preventing further growth. Because of this, the parasitoids mostly attack late instar (3<sup>rd</sup> and 4<sup>th</sup>) larvae and pupae (no egg parasitoids have been found).

## 2.2 Surveys

The surveys for this study took place in 2018 and 2019. We performed two surveys each year: an early leaf survey (late May/early June) to examine whether ALM oviposition and egg predation differed between tall and short trees, and a late leaf survey (late June/early July) to determine if juvenile (larval and pupal) ALM performed better on leaves of tall trees than short trees. We surveyed at 4 different sites near Fairbanks, Alaska (Table 1; these sites have been used for annual surveys of aspen herbivory since 2004; see Doak and Wagner 2015). Short trees were defined as 1-2 meters in height, while tall trees were defined as 7-15 meters in height from which we collected shoots that were 5-6 meters from the ground. At each site, we haphazardly chose 30 short and either 20 (2018) or 30 (2019) tall aspen ramets and collected one shoot from each ramet. Aspen trees are clonal and some individuals within a site may be genetically identical, but genetic identity was not determined in this study.

**Table 1.** Description and location of study sites. BNZ, ED, RP, and WR were used for the early and late leaf surveys. ED and WR were used for the leaf nitrogen analysis. MF, RP, and WR were used for the phenology survey.

Site	Latitude (°)	Longitude (°)	Elevation (m)
Bonanza Creek (BNZ)	64.71N	148.33W	231
Ester Dome (ED)	64.88N	148.06W	720
Mayo Field (MF)	64.53N	147.54W	167
Ridgepointe (RP)	64.82N	147.97W	258
West Ridge (WR)	64.86N	147.86W	180

### 2.2.1 Early Leaf Survey

On each leaf side of every leaf of the collected shoots, we counted the number of ALM eggs, egg scars, and newly initiated mines to estimate the total oviposition and probability of egg predation. We conducted the survey after the majority of oviposition had occurred but before the mines coalesced. Our values of oviposition and egg predation represent lower bounds because eggs removed shortly after oviposition may not leave a scar, possibly resulting in underestimates for total oviposition and egg predation, and some oviposition may have occurred after the survey.

### 2.2.2 Late Leaf Survey

On each leaf side of every leaf of the collected shoots, we counted the number of ALMs and recorded the ALM life stage as early larva (1<sup>st</sup> and 2<sup>nd</sup> instar), late larva (3<sup>rd</sup> and 4<sup>th</sup> instar), or pupa. We collected all live ALM pupae, determined their sex, freeze-killed them, dried them in an oven (50 °C) for three days, and then weighed them individually. The average number of larvae counted per leaf in the late leaf survey is usually lower than the average number of eggs counted per leaf in the early leaf survey. Some eggs never hatch, and some larvae die early in development and are hard to detect. We refer to this difference between the number of eggs and larvae across surveys as “undetected loss”. For each individual ALM, we also recorded whether it was alive or dead, and the cause of death when possible.

We recorded a mortality event as parasitism if a parasitoid wasp (egg, larva, or pupa) was present in or near the ALM. We recorded an event as predation if the mine or fold was broken open and the ALM was missing. Several sources of mortality simply leave larvae dead in the mine and are therefore more difficult to identify. For example, intraspecific interference competition and predation by predators that pierce the mine to consume ALM hemolymph both leave the ALM carcass largely intact. No cause of mortality other than parasitism and predation is easy to identify, so we defined any ALM larva or pupa dead within the mine and with no parasitoid nearby as “other mortality”.

We measured the width of each leaf as a proxy for leaf area because leaf width is highly correlated with leaf area ( $r^2 > 0.90$ ). We visually estimated the percent of mining damage on each leaf side. Everyone working on this survey had accurate visual estimates ( $r^2 > 0.90$ ; see Doak et al. 2007).

### 2.3 Leaf Nitrogen

We tested whether leaf percent nitrogen was higher in tall or short aspen trees at two sites near Fairbanks (ED and WR; Table 1). We collected shoots from WR on 12 June 2019 and from ED on 18 June 2019 after the majority of ALM feeding had taken place. At each site, we haphazardly chose 15 short and 15 tall trees. For each tree, we collected three shoots that had at least seven leaves. The shoots were immediately stored in a cooler and then in a refrigerator at 4 °C.

Subsequent measurements were made on the first 7 (starting from the bottom of the shoot) intact leaves on each shoot. We removed the petioles, pooled the leaves from each shoot resulting in three samples for each tree, dried them in a drying oven (50 °C) for 3 days, and then ground them to a fine powder. Percent leaf nitrogen was measured for a single 0.100 mg sample per shoot, using a LECO 2000 CNS analyzer (Laboratory Equipment Company, St. Joseph, Michigan). We also took four additional

0.100 mg samples from 4 shoots (2 from tall trees and 2 from short trees) to estimate within-sample variation.

## 2.4 Wind Speed

We examined if wind speed was higher in the canopy of tall trees than short trees. Wind speed and gust speed were measured using Davis® Wind Speed and Direction Smart Sensors (S-WCF-M003) (one at each height) over 30 second intervals. Wind speed was measured as the average speed over that interval while gust speed was measured as the highest 3-second gust of wind. The sensors were placed sequentially for at least 15 days at each of four sites (see Table 2) within the canopy of tall and short trees at the University of Alaska Fairbanks campus in the summer of 2019. Using only two sensors, we were unable to monitor sites simultaneously. The bases of the tall and short tree at each site were located within 3 m of one another. We placed the short sensor 1 m from the ground on a pole positioned next to the short tree and placed the tall sensor approximately 5 m from the ground attached to a tall tree and extended outwards by approximately 0.5m.

**Table 2.** The locations of the four sites used to measure wind and the dates at each site (all in 2019). Canopy cover was measured using a spherical convex densiometer using 4 compass directions per location at 4 locations around the focal trees, and then calculating the mean and standard error (SE).

Site	Dates (MM/DD)	Latitude	Longitude	Elevation (m)	Mean canopy cover (%)	SE canopy cover
1	05/16-06/03	64.85748N	147.861592W	180	73.3	5.3
2	06/03-06/22	64.85797N	147.863464W	176	75.2	7.4
3	06/27-07/24	64.85681N	147.839766W	174	67.1	3.6
4	07/24-08/20	64.85651N	147.839669W	167	61.4	1.4

## 2.5 Aspen Phenology

We conducted a survey to compare phenological differences between tall and short trees. The phenology survey was performed to determine if there was a difference in leaf-out times between tall and short aspen trees at the same site and quantify the difference. We quantified the phenology of budburst and leaf expansion on tall and short trees at common sites. We surveyed trees at three sites (ranging in area from 1000-5000 m<sup>2</sup>) near Fairbanks, Alaska (MF, RP, and WR: Table 1) and recorded

aspen phenological stage daily during early May 2019. To quantify the phenological stage, we used an index in which 1 indicated that the overwintering buds were completely closed and brown, and 5 indicated that the leaves of the shoot were completely unfurled (Table 3). We scored 10 tall and 10 short trees every day at each site by recording the most common index value across the tree. The trees were chosen haphazardly daily with a minimum distance of 5 m between trees; some trees may have been censused multiple times throughout the survey period. If shoots on an individual tree were about equally divided between two index values, we recorded the average.

**Table 3.** Index of shoot phenology.

Index number	Description
1	Bud is completely brown
2	Bud has some green on it
3	The leaves of the shoot have begun to separate from each other, but every leaf is still furled
4	At least one of the leaves of the shoot is completely unfurled
5	All leaves of the shoot are completely unfurled

## 2.6 Data Analyses

For the egg and larval surveys, any leaf that was missing more than 50% of its area (~1.5% of all leaves collected) was excluded from the analysis. We also excluded all leaves beyond leaf position 7. Leaves beyond this position tend to be unavailable at the time of the early leaf survey. All statistical analyses were conducted using R 1.2.5033 (R Core Team 2019).

### 2.6.1 Oviposition

In order to test the effect of tree height on oviposition, we used a generalized linear mixed model (GLMM, R-package *lme4*; Bates et al. 2015) with a Poisson distribution and a log link function (see Appendix 1 for detailed descriptions of all statistical models). The fixed effects were height, leaf side, and the interaction of height and leaf side. Due to the non-independence of data taken from two sides of the same leaf and multiple leaves from the same tree, leaves nested within tree and trees within site and year were treated as random effects. Site and year were also treated as random effects.

### 2.6.2 Performance

In order to examine the relationship between tree height and different measures of ALM performance, we executed a series of generalized linear mixed models (GLMMs) with a binomial distribution (response = survival) and a logit link. The different models examined egg predation, each source of larval or pupal mortality (parasitism, predation, and other mortality), and overall juvenile (larval and pupal) survival. The replicates in each analysis were individual ALM and the dependent variables were binary: survived or died. Height, leaf side, and their interaction were included as fixed effects in all models. The number of ALMs on the same leaf side and the interaction between the number of ALMs and tree height were included as fixed effects for the juvenile parasitism, predation, other mortality, and overall survival models because ALMs are known to compete with each other in the mine (Doak and Wagner 2015). Mortality from interspecific competition can happen early in larval development, which could decrease the probabilities of other sources of mortality. The random effects were the same as for the oviposition model. The GLMMs on overall juvenile survival and mortality failed to converge when the Laplace approximation (the default method in R-package *lme4*) was used for parameter estimates, so we used penalized iteratively reweighted least squares (PIRLS; Bates et al. 2015) instead in order to keep the same random effects structure across models. Although PIRLS resulted in larger standard errors, this did not affect the significance of the fixed effects in these models.

We examined ALM pupal mass as another measure of ALM performance using a linear mixed effects model (R-package *lme4*; Bates et al. 2015). The fixed effects were the same as the oviposition model, but leaf width was also included. The random effects were site, year, and tree nested within site and year.

### 2.6.3 Damage

To test how ALM mining damage varies by tree height, we used GLMMs. The data used were from the late leaf survey. One issue was that the percent mining data had many zeros in the data set (about 30% of leaf sides had zero mining). To account for the zero-inflated data, first, we examined the probability of a leaf side being mined using a GLMM assuming a binomial distribution and a logit link function. We treated this as a binomial variable describing the leaf side as mined (1) or not mined (0). Then, for the subset of leaves with mining, we used a mixed effects model having proportion mined (which was logit transformed) as the dependent variable. The fixed effects and random effects for both models were the same as the oviposition model.

#### 2.6.4 Leaf Characteristics

We examined leaf width and percent nitrogen in leaves using linear mixed effects models to determine if they varied by tree height because they could be correlated to ALM performance. Leaf width was measured during the late survey while percent nitrogen was measured during the leaf nitrogen survey. The only fixed effect for both models was height. The random effects for the leaf width model were site, year, and tree nested within site within year. For the percent nitrogen model, the random effects were site, tree, and shoot nested within tree within site.

#### 2.6.5 Wind Speed

To assess whether wind speed varied by tree height, we used linear models. We examined the data for time periods when ALMs are most active: 6:00 am – 10:00 am and 6:00 pm – 12:00 am (personal observation). We averaged the data for each time period within each day to account for autocorrelation within the data. The fixed effects were height, time of day (morning or evening), and their interaction. We ran separate models for each site because sampling dates differed.

#### 2.6.6 Phenology

For the phenology survey, we used an accelerated failure time model as a time-to-event analysis (R-package *survival*; Therneau 2020). The event was leafing out, defined as a phenology index value of 5 (Table 3). Each day we sampled 10 trees per height class per site for a total of 60 subjects. Although they were not necessarily the same trees every day, we treated them as if they were for the analysis. For example, if we recorded that no tall trees at a site were leafed-out on day 2, but three were leafed-out on day 3, then we would have 3/10 subjects coded as having leafed-out on day 3 even though some of those trees may have leafed-out earlier. We used a log-likelihood test to choose the best distribution and frailty for the model, which were the Weibull distribution and gamma frailty. A z-test was used to test for the significance of height.

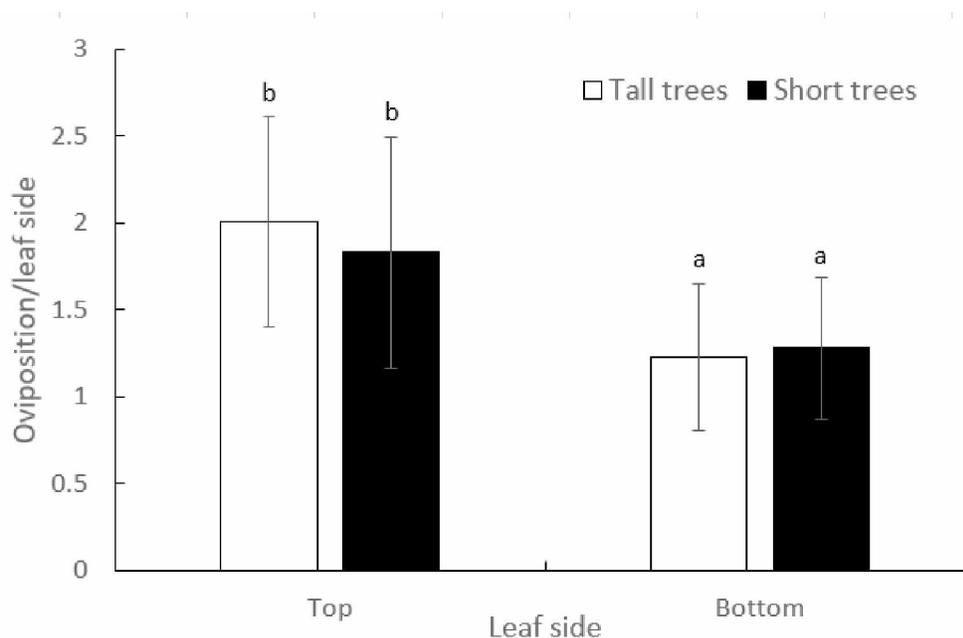
To test for significance in the models, we conducted Wald Chi-squared tests by using the ‘Anova’ function in the *car* package to test the fixed effects for each response variable (Fox and Weisberg 2019). Least square means (R-package *lsmeans*; Lenth 2016) and Tukey comparisons (R-package *multcompView*; Graves et al. 2019) for all pairwise comparisons were performed *post-hoc* from most models and used in figures. We also used R-package *lmerTest* to extract degrees of freedom for some mixed models (Kuznetsova et al. 2017).

We conducted diagnostics of the linear models to test for normality and homoscedasticity of variances. We used the Lilliefors test (R-package *nortest*; Gross and Ligges 2015) for normality and Levene’s test (R-package *lawstat*; Gatswith 2020) for equality of error variances. We also visually examined the residuals (R-package *broom*; Robinson and Hayes 2020).

### 3 Results

#### 3.1 Oviposition

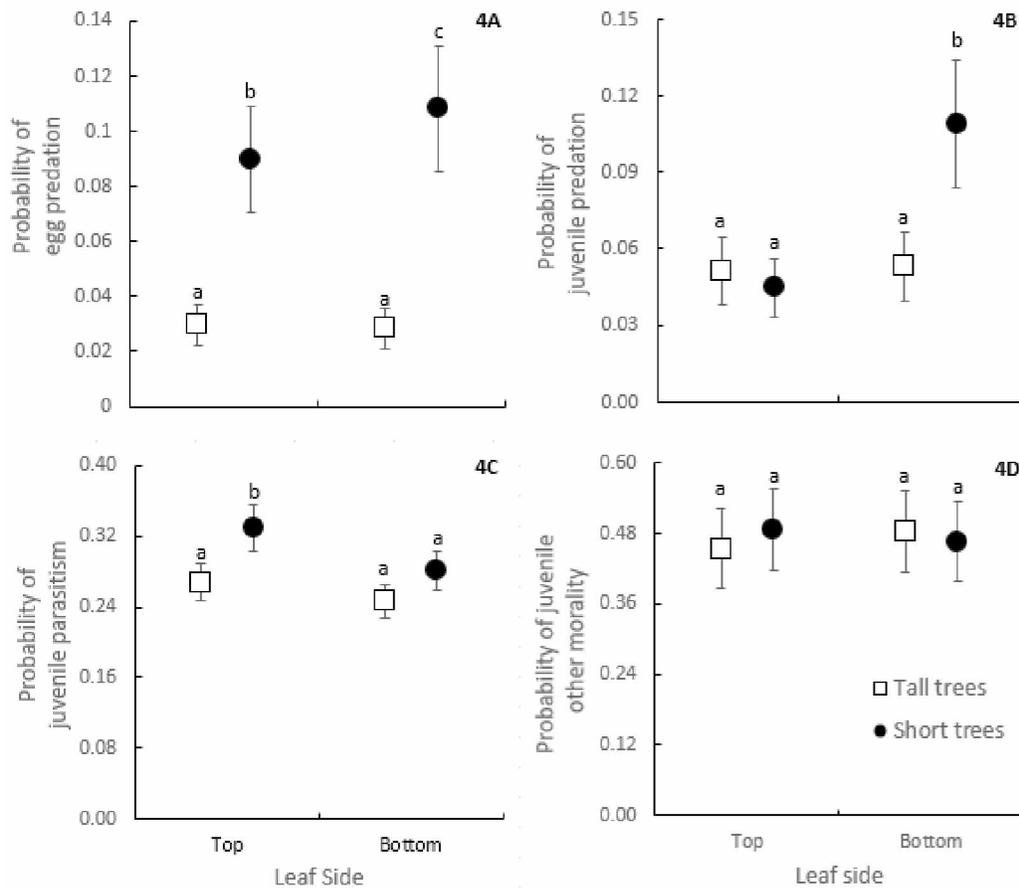
We found that overall, ALMs did not lay more eggs per leaf on tall trees than short trees. There was no significant effect of height on the mean number of eggs laid per leaf side, and ALMs laid 1.5 times more eggs on the top side of the leaf than the bottom side of the leaf ( $\chi^2$ : 515,  $P < 0.0001$ , d.f. = 1, Fig. 3). The interaction between tree height and leaf side was also significant ( $\chi^2$ : 13.3,  $P = 0.00027$ , d.f. = 1), apparently because the magnitude of the difference between leaf sides was larger for tall than short trees. However, Tukey-Kramer post-hoc comparisons of means did not support or explain the interaction (Fig. 3).



**Figure 3:** Oviposition by aspen leaf miners by leaf side and tree height. The bars represent back-transformed least square means of the total number of aspen leaf miner initiations (eggs, scars, and mines) per leaf side. Error bars represent  $\pm 1$  standard error. Letters represent Tukey-Kramer groupings.

### 3.2 Performance

The probability of egg predation was over 3 times higher in short trees than tall trees (Table 4, Fig. 4A). There was also a smaller, but significant effect of leaf side with the probability of egg predation being higher on the bottom side of leaves than the top side (Table 4). This pattern was only observed on short trees (Fig. 4A). We observed no egg parasitism.



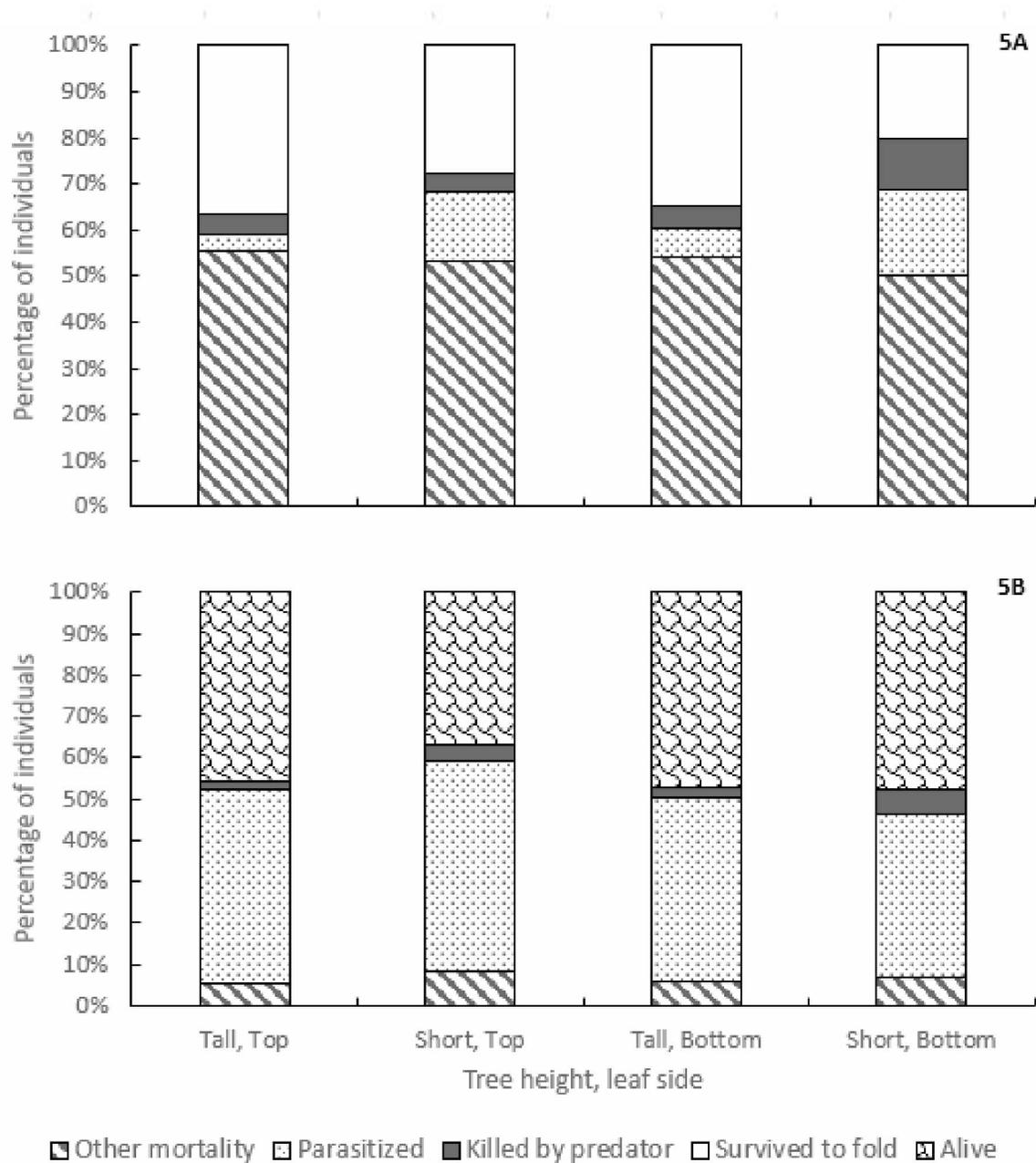
**Figure 4:** Probabilities of different mortality sources at the egg and juvenile (larval and pupal) stages for ALM by tree height and leaf side. The points represent back-transformed least square means and the error bars represent standard error. Letters represent Tukey-Kramer groupings. Note differences in the scale of the y-axis among panels.

**Table 4.** Results of the models examining the probabilities of egg predation and juvenile (larval and pupal) predation, parasitism, other mortality, and survival of the aspen leaf miner. “ALM” refers to the total number of aspen leaf miners on the same leaf surface. The \* indicates an interaction between two effects. P-values and significance levels were calculated using Wald Chi-Squared tests. Bold P-values indicate a significant effect at  $P < 0.05$ .

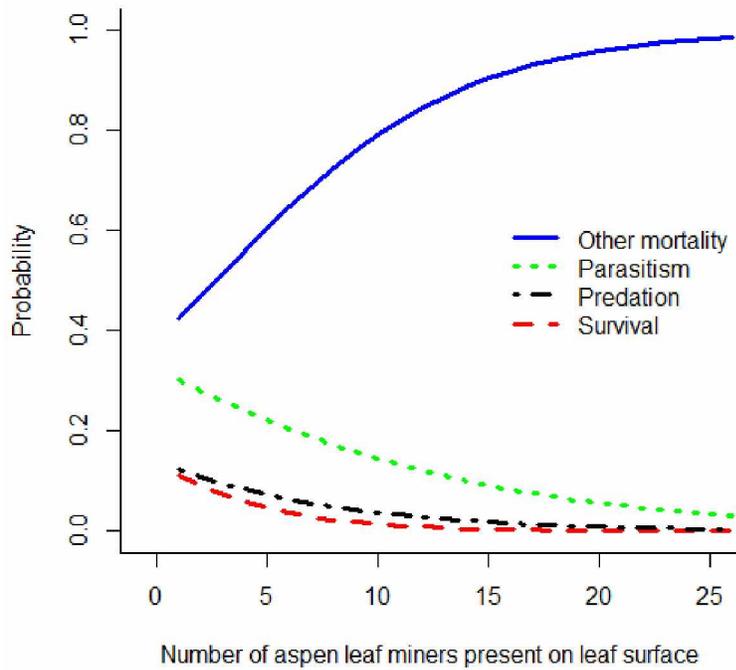
Response	Fixed Effect	$\chi^2$	Df	P-value
Egg predation	Height	81.3	1	<b>&lt;0.0001</b>
	Leafside	7.17	1	<b>0.0074</b>
	Height*Leafside	2.72	1	0.0989
Juvenile predation	Height	5.39	1	<b>0.0202</b>
	Leafside	29.6	1	<b>&lt;0.0001</b>
	ALM	21.0	1	<b>&lt;0.0001</b>
	Height*Leafside	22.2	1	<b>&lt;0.0001</b>
	Height*ALM	0	1	0.999
Juvenile parasitism	Height	18.6	1	<b>&lt;0.0001</b>
	Leafside	9.55	1	<b>0.00199</b>
	ALM	79.5	1	<b>&lt;0.0001</b>
	Height*Leafside	1.07	1	0.301
	Height*ALM	3.24	1	0.0719
Juvenile other mortality	Height	0.0569	1	0.811
	Leafside	0.155	1	0.694
	ALM	219	1	<b>&lt;0.0001</b>
	Height*Leafside	4.24	1	<b>0.0394</b>
	Height*ALM	1.78	1	0.182
Juvenile survival	Height	28.9	1	<b>&lt;0.0001</b>
	Leafside	0.436	1	0.509
	ALM	59.2	1	<b>&lt;0.0001</b>
	Height*Leafside	0.0327	1	0.857
	Height*ALM	5.06	1	<b>0.0245</b>

We counted a total of 8278 juvenile (larval and pupal) ALMs in the late leaf survey over the two study years. Of the total, 86% had died: 69% in the mine and 17% in the fold. 60% of ALMs were found on the top side of leaves while 40% were found on the bottom side of leaves. The largest source of juvenile ALM mortality was mortality that could not be definitively assigned to predation or parasitism ("other mortality", 56% of all ALMs; Fig. 5), followed by parasitism (24% of all ALMs), and predation (7% of ALMs). The remainder of ALMs (14%) survived (Fig. 5).

The probability of juvenile predation, which was defined as ALMs that were removed from the leaf, was significantly higher on short trees than tall trees, but only for the bottom side of the leaves (Table 4, Fig. 4B). The probability of predation was more than twice as high on the bottom side of leaves of short trees (11% (SE  $\pm$  3%)) than the top side of leaves of short trees (4% (SE  $\pm$  1%)), but it did not differ between the leaf sides on tall trees. The probability of juvenile predation decreased when more ALMs were present on the same leaf side (Fig. 6; estimate from GLMM: -0.142, z-value: -3.419,  $P < 0.0001$ ).

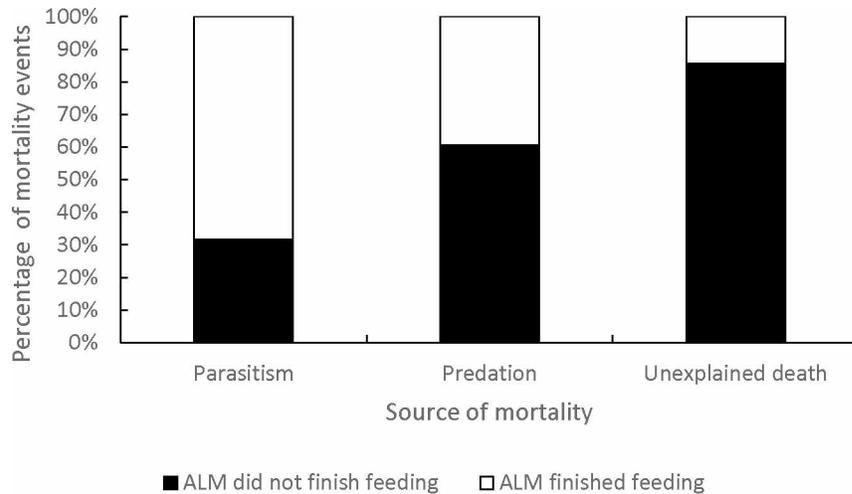


**Figure 5:** Distribution of the outcomes of all aspen leaf miners by tree height and leaf side. Outcomes include other mortality, parasitism, killed by predator, or survived. (A) mine, and (B) fold.



**Figure 6.** Predictions based on GLMMs of the outcomes of all juvenile aspen leaf miners (ALMs) by the number of ALMs on the same leaf side.

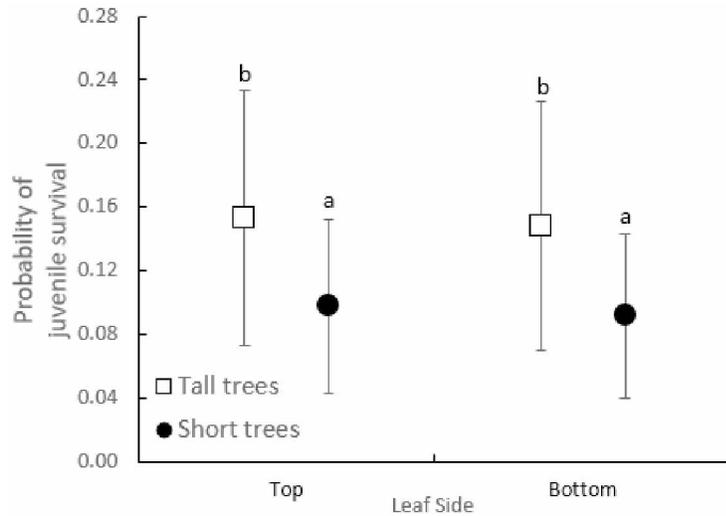
The probability of juvenile parasitism was significantly higher on short trees than tall trees (Table 4, Fig. 4C). The probability of parasitism also decreased when more individuals were present on the same leaf side (Fig. 6; estimate from GLMM: -0.104, z-value: -6.22,  $P < 0.0001$ ). Most (68%) parasitism events occurred after the ALMs had finished feeding (Fig. 7).



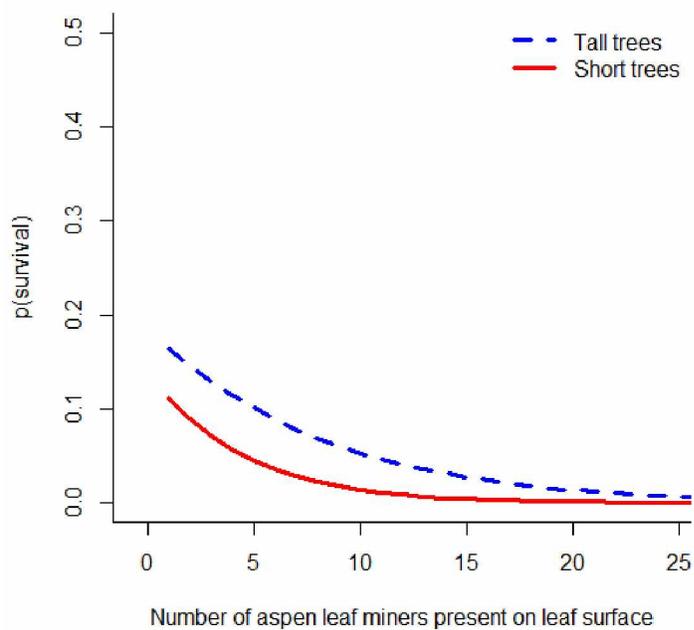
**Figure 7.** Distribution of mortality events of aspen leaf miners (ALMs) by whether the ALMs had finished feeding or not.

The probability of juvenile mortality not definitively attributable to predator and parasites (other mortality) did not vary significantly by tree height or leaf side (Fig 4D, Table 4). When more ALMs were present on the same leaf side, the probability of other mortality increased (Fig. 6; estimate from GLMM: 0.182, z-value: 10.62,  $P < 0.0001$ ). The overall probability other mortality was about 40% even when it was the only ALM on the leaf surface for both tall and short trees (Fig. 6). Other mortality was the largest source of mortality in the leaf mine, but it accounted for a very small proportion of mortality in the fold (Fig. 5).

Overall, the probability of juvenile survival of ALMs was about 1.5 times higher on tall trees than short trees (Table 4, Fig. 8). The probability of survival decreased when more individuals were present on the same leaf side (Fig. 9, estimate from GLMM: -0.242, z-value: -6.63,  $P < 0.0001$ ), and this effect was more notable in short trees than tall trees (Table 4, Fig. 9). Leaf side itself had no significant effect on survival (Table 4).



**Figure 8:** Juvenile survival probability of aspen leaf miners by tree height and leaf side. The points represent back-transformed least square means of survival probability by tree height and leaf side. Error bars represent standard error. Letters represent Tukey-Kramer groupings.

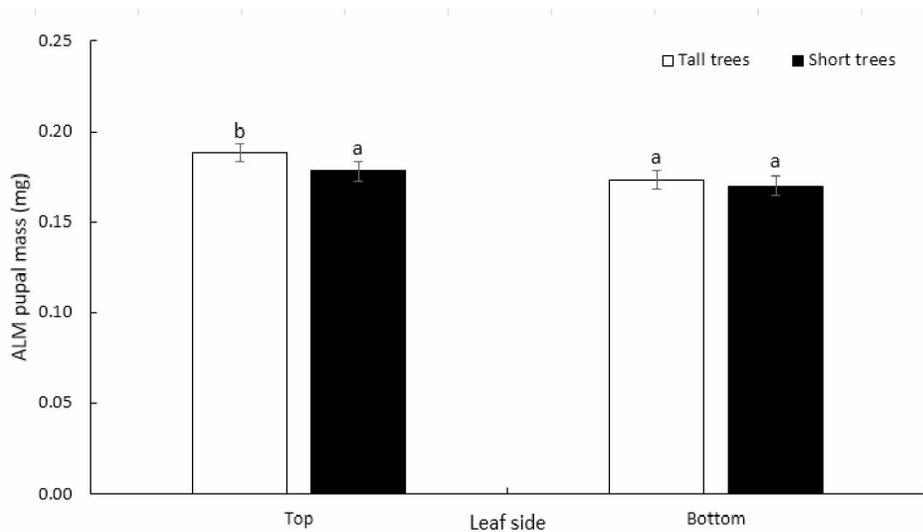


**Figure 9.** Predictions of the probability of juvenile aspen leaf miner (ALM) survival for tall and short aspen trees by the number of ALMs present on leaf surface.

ALM pupal mass was larger on the tall trees than the short trees, and larger on the top side of leaves than the bottom side of leaves (Table 5, Fig. 10). The interaction between tree height and leaf side was marginally significant because the difference in pupal mass between ALM on the top and bottom side of leaves was higher on tall trees than short trees (Fig. 10). ALM pupal mass was also positively correlated with leaf width (estimate from mixed model: 0.000723, t-value: 7.82, p-value < 0.0001, d.f. = 729).

**Table 5.** Results for the model examining aspen leaf miner pupal masses by tree height and leaf side. The \* indicates an interaction between two effects. P-values and significance levels were calculated using Wald Chi-Squared tests. Bold P-values indicate a significant effect at  $P < 0.05$ .

Fixed Effect	$\chi^2$	Df	P-value
Height	4.22	1	<b>0.0399</b>
Leafside	46.6	1	<b>&lt;0.0001</b>
Leaf width	61.2	1	<b>&lt;0.0001</b>
Height*Leafside	2.97	1	0.08505



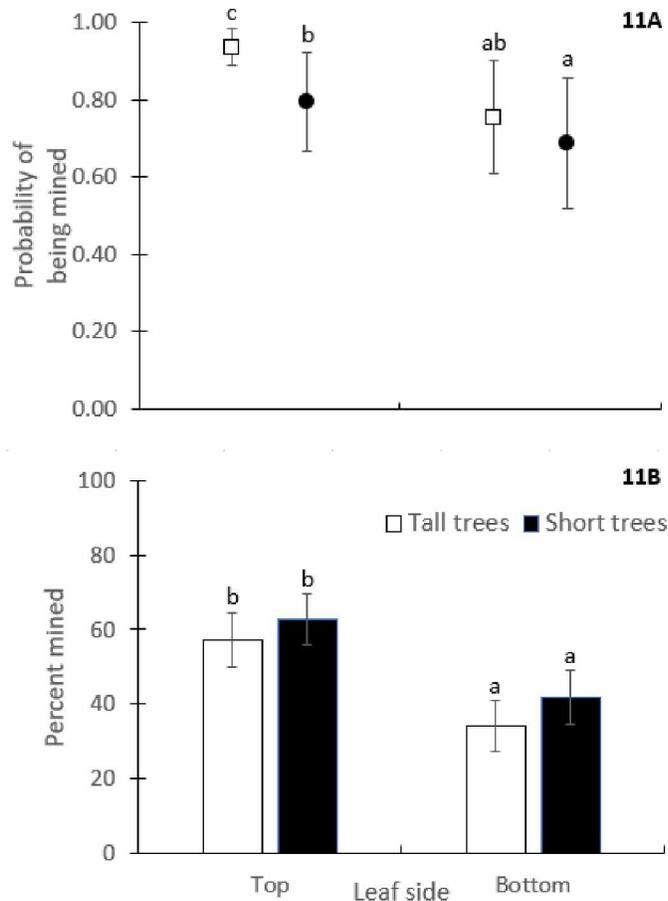
**Figure 10:** Aspen leaf miner pupal mass by tree height and leaf side. The points represent back-transformed least square means and the error bars represent standard error. Letters represent Tukey-Kramer groupings.

### 3.3 Damage

Mining damage varied by tree height and leaf side. The probability of a leaf side being mined was higher for tall trees than short trees, and higher for the top side of leaves than the bottom side of leaves (Table 6, Fig. 11A). There was also a significant interaction, with the top side of leaves on tall trees having a higher probability of being mined relative to the other categories (Table 6, Fig. 11A). The overall probability of a leaf side being mined was high ( $0.82 \pm 0.12$ ). Considering only the subset of trees that were mined to some extent, percent mining damage was significantly higher on the top side of leaves than the bottom side of leaves, and short trees had slightly higher percent mining damage than tall trees (Table 6), but this result was not supported by the post-hoc tests (Fig. 11B).

**Table 6.** Results for the models testing probability of mining damage and percent mining. P-values and significance levels were calculated using Wald Chi-Squared tests. Bold P-values indicate a significant effect at  $P < 0.05$ .

Response	Fixed Effect	$\chi^2$	Df	P-value
Probability of mining	Height	16.7	1	<b>&lt;0.0001</b>
	Leafside	112	1	<b>&lt;0.0001</b>
	Height*Leafside	35.2	1	<b>&lt;0.0001</b>
Percent mining	Height	4.55	1	<b>0.0330</b>
	Leafside	256	1	<b>&lt;0.0001</b>
	Height*Leafside	0.647	1	0.421



**Figure 11:** Aspen leaf miner mining damage by tree height and leaf side. (A) shows the probability of an aspen leaf side having mining damage by tree height and leaf side while (B) shows the percent of leaf area mine. The points represent back-transformed least square means and the error bars represent standard error. Letters represent Tukey-Kramer groupings.

### 3.4 Leaf Characteristics

Leaves of tall trees ( $50.7 \pm 4.0$  mm) were on average about 25% wider than leaves of short ( $39.5 \pm 4.0$  mm) trees ( $\chi^2$ : 114.08,  $P < 0.0001$ , d.f. = 1). Percent nitrogen was marginally higher in the leaves of short ( $2.71 \pm 0.14\%$ ) trees compared to tall ( $2.59 \pm 0.14\%$ ) trees ( $\chi^2$ : 3.52,  $P = 0.0566$ , d.f. = 1).

### 3.5 Wind Speed

Height had very little effect on wind and gust speed (Table 7). At site 1, wind speed was higher for tall trees than short trees in the morning, but there were no other significant differences. Wind and

gust speed were also significantly higher in the evening than the morning (Table 7). The interaction between height and time of day was not significant.

**Table 7.** Results for wind and gust speed showing the least square means with standard errors in parentheses, and the Tukey-Kramer comparisons. Wind speed was the average wind speed recorded in a 30 second period. Gust speed was the highest value in that thirty second period.

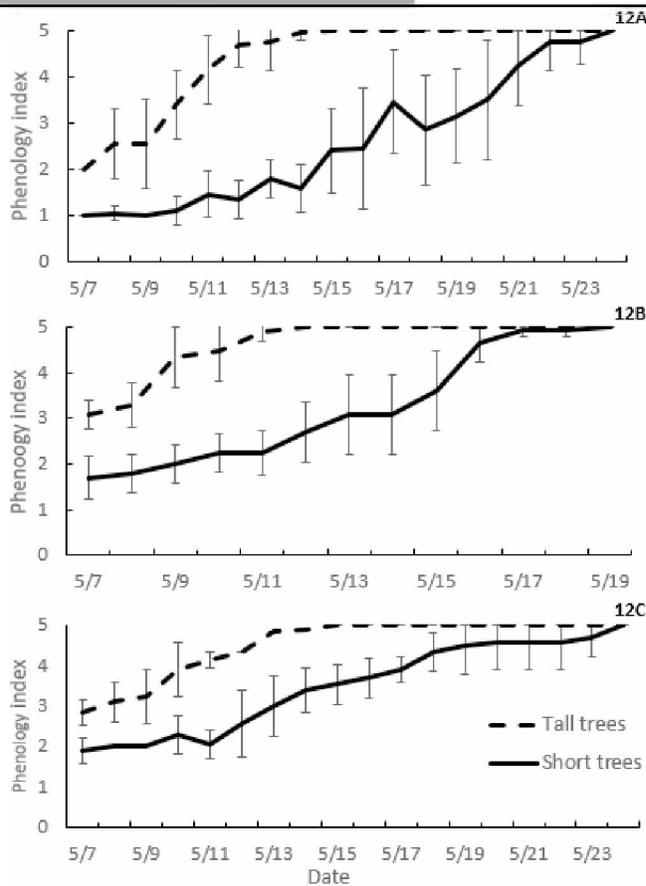
Site	Wind Speed (m/s)				Gust Speed (m/s)			
	Short, morning	Tall, morning	Short, evening	Tall, evening	Short, morning	Tall, morning	Short, evening	Tall, evening
Site 1	0.523 (0.067)	0.787 (0.067)	0.884 (0.067)	1.03 (0.067)	1.04 (0.0864)	1.33 (0.0864)	1.51 (0.0864)	1.73 (0.0864)
	a	b	b	b	a	ab	bc	c
Site 2	0.586 (0.067)	0.564 (0.067)	0.907 (0.067)	0.861 (0.067)	0.912 (0.087)	0.916 (0.087)	1.28 (0.087)	1.30 (0.087)
	a	a	b	b	a	a	b	b
Site 3	0.646 (0.143)	0.677 (0.143)	1.41 (0.143)	1.59 (0.143)	1.19 (0.17)	1.26 (0.17)	2.11 (0.17)	2.32 (0.17)
	a	a	b	b	a	a	b	b
Site 4	0.872 (0.176)	0.898 (0.176)	1.21 (0.176)	1.25 (0.176)	1.33 (0.19)	1.39 (0.19)	1.77 (0.19)	1.76 (0.19)
	a	a	a	a	a	a	a	a

### 3.6 Phenology

Tall trees fully leafed-out about 7 days earlier than short trees on average (Table 8). A similar pattern was observed across all sites (Fig. 12).

**Table 8.** Results for the leaf-out phenology model testing how long it takes for tall and short aspen trees to leaf-out. The model was an accelerated failure time model with a Weibull distribution and gamma frailty. Bold P-values indicate a significant effect at  $P < 0.05$ .

Response	Effect	Value	exp(value)	Std. Error	z value	P-value
<b>Phenology: Leaf-out times</b>	<b>(Intercept)</b>	2.70	14.9	0.49	5.55	<b>&lt;0.0001</b>
	<b>Height:Tall</b>	-0.691	0.501	0.036	-19.5	<b>&lt;0.0001</b>
	<b>Log(scale)</b>	-2.00	0.135	0.100	-20.0	<b>&lt;0.0001</b>



**Figure 12:** Mean phenology index values for tall in short aspen trees recorded in May 2019 at three sites. (A) Represents points from site MF, (B) represents points from site RP, and (C) represents data from site WR. Error bars represent standard deviation.

## 4 Discussion

According to optimal oviposition theory, we would expect ALMs to lay more eggs on hosts on which their offspring perform the best. Our results showed that there was no significant difference in ALM oviposition per leaf surface between tall and short aspen trees even though ALMs performed better on tall trees. In both the egg and juvenile stages, ALMs were more likely to survive on leaves of tall trees than short trees because fewer individuals were killed by natural enemies on tall trees. ALM pupae on tall trees were also larger than those on short trees, possibly because tall trees have larger leaves. While leaf side had no effect on overall juvenile survival, egg predation was higher on the bottom side of leaves. ALMs on the top side of leaves were larger than those on the bottom side of leaves, and ALMs laid more eggs on the top side of leaves than the bottom side. Thus, patterns of oviposition matched performance with respect to leaf side, but not tree height. The apparent mismatch between oviposition and survival for tree height, but not leaf side, suggests that there could be factor(s) constraining ALM oviposition on tall trees. One such factor may be the differences in phenology of leaf-out times between tall and short trees.

### 4.1 Mortality Factors

Explanations for the lower survival of ALMs on short trees include higher egg and juvenile predation. One of the common predators in this system is ants. Ants visit aspen trees for their sugar secreting EFNs, and leaves of short aspen trees are more likely to have EFNs than leaves of tall aspen trees (Doak et al. 2007, Wooley et al. 2007, Young et al. 2010). Defoliation induces EFN sugar secretion in aspen (Newman and Wagner 2013). Ants visit some aspen genets more frequently following an increase in nectar availability suggesting that an increase in nectar production in response to insect herbivory may increase predation pressure (Newman et al. 2015). ALMs are less likely to lay eggs on leaves with EFNs than leaves without EFNs, which is consistent with the hypothesis that they are minimizing the risk of natural enemy attack on their offspring (Mortensen et al. 2013, Dennis et al. 2015). Some species of EFN-visiting desert ants are more likely to nest near trees that have EFNs (Wagner and Nicklen 2010). Ant nests in our system are in the ground. If they are closer to the foliage of short trees than tall trees, then there should be higher energetic and time costs for foraging on tall trees than short trees (Holt and Askew 2012). Not only is the distance longer, but while climbing, the energetic cost for ant locomotion increases and their speed decreases relative to movement on a horizontal plane (Holt and Askew 2012). Therefore, ants may be more abundant on the foliage of short trees than tall trees, which may explain the higher ALM predation probability on short trees. Although

there was a large difference in the probability of predation between tree heights and leaf sides, it was still a relatively small contributor to mortality overall.

Another reason for lower ALM survival on short trees was the higher probability of parasitism. In our study, approximately 25% of all ALM larvae and pupae were parasitized. One potential reason for the lower probabilities of parasitism on tall trees compared to short trees is that these differences could be the result of temporal asynchrony in the foraging period of the parasitoids and the availability of hosts (Godfray et al. 1994). Because the leaf-out times of tall and short trees differ, ALMs on tall and short trees could be at different stages of development at any one time. If parasitoids are not equally abundant throughout the development of ALMs, then this pattern could explain the different probabilities of parasitism. Most parasitoid oviposition may occur after the ALMs on tall trees have finished feeding but before the ALMs on short trees have finished feeding. Another potential reason for the higher parasitism on short trees is that parasitoid adults use EFNs, located on the top side of aspen leaves, as a food source. Parasitoids that use EFNs have increased encounters with insect herbivores near EFNs (Heil 2015). Leaves of short trees are more likely to have EFNs than leaves of tall trees, which could explain the higher parasitism on short trees.

EFNs may act as an indirect defense by attracting natural enemies of insect herbivores, but most ALMs are parasitized after they finished feeding (Fig. 7). There would likely be less of an immediate benefit to the plant, but late parasitism could negatively affect ALM populations over the long term. ALMs can experience negative density-dependence during their early instars because of heavy intraspecific competition (Doak and Wagner 2015). The effects of parasitism on a host population are much more detrimental if the parasitism occurs after density-dependent stages (Mills and Getz 1996). If the parasitism occurs before any interference competition, then it is likely compensatory mortality and will have little to no effect on the overall ALM population size. However, if the parasitism occurs after the interference competition, then it is likely additive mortality and will have a much stronger effect on the overall ALM population. For example, say there are 10 ALM eggs laid on a leaf surface which is large enough to only support 3 ALM. If no ALMs were parasitized, then at least 7 would die due to competition and at most 3 would survive to pupate. If 3 ALMs were parasitized early on, then there would still be at least 4 that die due to competition and at most 3 that survive to pupate. On the other hand, if 3 ALMs were parasitized after the competition has occurred, then no ALMs will survive to pupate. Therefore, the timing of the parasitism can have a large impact on how many ALMs survive even if the number of parasitism events remains the same; an issue that warrants further study.

Mortality that could not be definitively assigned to predation or parasitism did not vary by tree height. The overall proportion of this "other mortality" was approximately 56%. Possible causes of other mortality may include piercing predators, interference competition, plant defenses, disease, and malnutrition. Other mortality increased when more ALMs occurred on the same leaf surface (Fig. 6) suggesting that much of this lower survival may be from interference competition, which is consistent with previous research (Doak and Wagner 2015). However, when only one ALM was present on a leaf side, other mortality was still about 40% for both tall and short trees (Fig. 6), so a notable proportion of other mortality was attributable to some other factor such as malnutrition. This factor is likely not predation because a previous experiment on ALM found that other mortality was still high even when natural enemies were excluded (Doak and Wagner 2015).

#### 4.2 Performance

ALMs performed better on tall trees than short trees in terms of survival and pupal mass, and they performed better on the top side of leaves than the bottom side in terms of pupal mass only. The higher survival in tall trees in our survey is due to a lower probability of parasitism and predation. Tall trees have larger leaves, and larger leaves also give ALMs more food to consume which could explain the differences in pupal mass between ALMs on tall and short trees (Fig. 10). While it may seem like the reason for higher performance in tall trees is that larger leaves reduce mortality due to interference competition (Doak and Wagner 2015) and provide more food resources, this pattern is inconsistent with our results. Mortality from interference competition did not appear to vary by tree height because "other mortality" did not vary by tree height. Pupal mass was larger on the top side of leaves too, not just on tall trees.

We had expected that ALMs would perform better on the tree height that had higher nutritional quality (i.e. percent foliar nitrogen). We found that percent nitrogen was slightly, but not significantly, higher in the leaves of short (2.71 (SE  $\pm$  0.14%)) compared to tall (2.59 (SE  $\pm$  0.14%)) trees. Previous research has found mixed results for how foliar nitrogen varies with aspen age (Donaldson et al. 2006a, Smith et al. 2011, Cope et al. 2019). In European aspen (*Populus tremula*) leaves, leaf foliar nitrogen concentration increases with leaf size (Hajek et al. 2013), but even though we found that leaves of tall aspen trees are larger, they did not have higher concentrations of nitrogen. The small difference in nitrogen we found may not be biologically significant for insect herbivores (Mattson 1980). Previous research on the aspen blotch leaf miner (*Phyllonorycter salicifoliella*) found that a 0.4% difference in percent nitrogen did not impact pupal mass (Kopper and Lindroth 2003).

Phenolic glycosides are more concentrated in the leaves of short aspen trees than tall trees (Donaldson et al. 2006a, Young et al. 2010, Smith et al. 2011, Cope et al. 2019). Phenolic glycosides in the diet can reduce the performance of some insect herbivores by decreasing survival and growth (Lindroth 2001, Donaldson et al. 2006a, Osier and Lindroth 2006, Stevens et al. 2007, Holeski et al. 2009). We found that mortality not definitively attributable to predators and parasitoids did not vary by tree height (Table 4), so it is unlikely that phenolic glycosides had any effect on survival. It is possible that the lower pupal mass on short trees is partly due to the higher concentration of phenolic glycosides. However, we cannot attribute the differences in pupal mass between leaf sides to phenolic glycosides because we do not know whether phenolic glycosides in epidermal cells vary with leaf side.

While leaf side had no effect on overall survival, the pupal mass of ALMs on the top side was larger than the pupal mass of ALMs on the bottom side (Fig. 10). This may help to explain why ALMs also preferred laying eggs on the top side over the bottom side (Fig. 3), a pattern consistent with previous research (Condrashoff 1964, Dennis et al. 2015). It is still unclear why ALMs have larger pupal masses on the top side of leaves.

Performance is related to leaf damage because higher survival and pupal mass should translate into more mining damage, but the inverse is not necessarily true. Leaves with high mining damage do not necessarily have higher ALM survival or larger ALM. Percent mining damage overall was much higher on the top side of leaves than the bottom side of leaves, and slightly higher on leaves of short trees than tall trees (Table 6, Fig. 11). The difference between the leaf sides is expected because more eggs are laid on the top side of leaves, which is consistent with previous research (Condrashoff 1964), and leaf side had no effect on survival.

#### 4.3 Constraints on Oviposition

ALMs perform better on tall trees than short trees, but overall, their oviposition does not match their performance with respect to tree height. Previous research has found that ALMs tend to lay eggs on leaves that reduce their offspring's vulnerability to predators (Mortensen et al. 2013, Dennis et al. 2015), but we did not find this result with respect to tree height. There may be factor(s) that prevent ALMs from laying more eggs on tall trees where they perform better. Two potential constraints we examined were wind speed and phenology.

Wind speed can impact small flying insects because they cannot perform directed flight if their maximum flight speed is lower than the wind speed (Compton 2002). Even at windspeeds slightly below this threshold, directed flight can be costly (Dudley 2000). Maximum flight speed is strongly correlated

with body length and wingspan (Dudley 2000). There is currently no data on the flight speeds of ALMs; however, insects with a body length of 2 mm (about the size of ALMs) would be expected to have a maximum flight speed between 0.6-1.0 m/s (Dudley 2000), which was in the same range as the average wind speed in our study, and much lower than gust speed. This suggests that ALMs could frequently have trouble with directed flight in general. However, the average wind and gust speed did not vary by tree height at most sites (Table 7), so wind speed is likely not constraining ALMs from laying more eggs on leaves of tall trees.

Tree phenology possibly constrains ALMs from laying more eggs on tall trees than short trees. The window for oviposition is very short especially for tall trees, and we found that tall and short aspen trees may not completely overlap in availability for oviposition. Tall trees were fully leafed-out earlier than short trees by about 7 days on average across 3 sites (Table 8, Fig. 12). This may be a biologically significant difference because ALMs only lay eggs on new leaves and stop laying eggs about 7-10 days after leaves begin expanding (Condrashoff 1964). Tall trees stop producing new leaves following bud burst, meaning that there would be few available leaves from tall trees for ALM oviposition at a site about 10 days after the leaves of those trees begin expanding, while many short trees continuously produce new leaves throughout the ovipositional period of ALMs. Consequently, ALMs may not always have a choice between two heights at one time. A choice between two host types is necessary for optimal oviposition. It is possible that if there was a complete overlap in leaf-out times between tall and short trees at one site that ALMs may lay more eggs per leaf surface on tall trees than short trees at that site if they have a preference for laying eggs on tall trees over short trees. Additionally, a short timeframe of availability is also a constraint, because ALMs produce relatively large eggs for their size (Condrashoff 1964), and so may not be able to produce and lay many eggs in a 7-10-day window. Therefore, tree phenology may impact why ALMs do not lay more eggs per leaf surface on tall trees than short trees. A similar pattern was observed in a study on the aspen leaf blotch miner wherein phenological differences between host trees partly explained the lower rates of oviposition on the host on which they performed best (Auerbach and Alberts 1992). The researchers found that phenological differences between different host species can impact oviposition while we found that phenological differences between different life stages of a single host species may also impact oviposition.

## 5 Conclusion

Although we found no difference in ALM oviposition between tall and short trees, there is a clear difference in ALM performance with ALM performing better in leaves of tall trees than short trees.

This appears to contradict the optimal oviposition theory, but upon closer examination, we found that there was a significant difference in spring leaf-out times such that the foliage of tall and short trees may not be available for oviposition at the same time. Even if ALMs prefer laying eggs on tall trees over short trees, they cannot act on it at all times. This result supports that phenological differences associated with plant developmental stages can constrain insects from using higher quality hosts. We also found evidence supporting the optimal oviposition theory because ALMs performed better on the top side of the leaf than the bottom side of the leaf, and they laid more eggs on the top side of the leaf. The results highlight how small differences within a habitat, such as differences in leaf side or tree height, can have significant impacts on insect performance. Population predictions for any species are partially based on the abundance and survival probabilities of individuals; thus, incorrect estimates of survival can lead to incorrect population predictions. Population estimates of any insect herbivore based on surveys from only one tree height may be incorrect indicating the importance of testing tree height as a factor when studying the oviposition or performance of any phytophagous insect.

## 6 References

- Arshad, Muhammad, Muhammad Irfan Ullah, Muhammad Afzal, Mian Anjum Murtaza, Ejaz Ahraf, Zahoor Hussain, Syed Muhammad Ali Zahid, and Maryam Riaz. 2019. "Image Analysis Estimate of Leaf Area Damage Caused by Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) Larvae on Different Citrus Cultivars." *Sarhad Journal of Agriculture* 35 (3): 948–54. <https://doi.org/10.17582/journal.sja/2019/35.3.948.954>.
- Augspurger, Carol K., and Elizabeth A. Bartlett. 2003. "Differences in Leaf Phenology between Juvenile and Adult Trees in a Temperate Deciduous Forest." *Tree Physiology* 23 (8): 517–25. <https://doi.org/10.1093/treephys/23.8.517>.
- Auerbach, Michael, and Jeffrey D. Alberts. 1992. "Occurrence and Performance of the Aspen Blotch Miner, *Phyllonorycter salicifoliella*, on Three Host-Tree Species." *Oecologia* <https://doi.org/10.1007/BF00319008>.
- Ayres, Matthew P., Thomas P. Clausen, Stephen F. MacLean, Ahnya M. Redman, and Paul B. Reichardt. 1997. "Diversity of Structure and Antiherbivore Activity in Condensed Tannins." *Ecology* 78 (6): 1696–1712. [https://doi.org/https://doi.org/10.1890/0012-9658\(1997\)078\[1696:DOSAAA\]2.0.CO;2](https://doi.org/https://doi.org/10.1890/0012-9658(1997)078[1696:DOSAAA]2.0.CO;2).
- Basset, Yves, Peter M. Hammond, Héctor Barrios, Jeremy D. Holloway, and Scott E. Miller. 2003. *Vertical Stratification of Arthropod Assemblages*. Edited by Yves Basset, Vojtech Novotny, and R.L. Kitching. *Arthropods of Tropical Forests: Spatio-Temporal Dynamics and Resource Use in the Canopy*. Cambridge, UK: Cambridge University Press.
- Basset, Yves, Aberlenc Henri-Pierre, and Gérard Delvare. 1992. "Abundance and Stratification of Foliage Arthropods in a Lowland Rain Forest of Cameroon." *Ecological Entomology* 17 (4). <https://doi.org/10.1111/j.1365-2311.1992.tb01063.x>.
- Bates, Douglas, Martin Mächler, Benjamin M. Bolker, and Steven C. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67 (1): 1–48. <https://doi.org/10.18637/jss.v067.i01>.

- Bellone, Davide, Christer Björkman, Axel Schmidt, Jonathan Gershenson, and Maartje J. Klapwijk. 2020. "Effect of Forest Stand Type on Host Plant Quality and Direct and Indirect Effects on Pine Sawfly Performance." *Agricultural and Forest Entomology* <https://doi.org/10.1111/afe.12415>.
- Bentley, Barbara L. 1977. "Extrafloral Nectaries and Protection by Pugnacious Bodyguards." *Annual Review of Ecology and Systematics* 8 (1): 407–27. <https://doi.org/10.1146/annurev.es.08.110177.002203>.
- Brown, Jessi L., Steve Vargo, Edward F. Connor, and Michael S. Nuckols. 1997. "Causes of Vertical Stratification in the Density of *Cameraria hamadryadella*." *Ecological Entomology* 22 (1): 16–25. <https://doi.org/10.1046/j.1365-2311.1997.00046.x>.
- Chaij, Jaquelina, Mariano Devoto, Marina Oleiro, Enrique J. Chaneton, and Noemi Mazia. 2016. "Complexity of Leaf Miner–Parasitoid Food Webs Declines with Canopy Height in Patagonian Beech Forests." *Ecological Entomology* 41 (5): 599–610. <https://doi.org/10.1111/een.12332>.
- Compton, Steve G. 2002. *Sailing with the Wind: Dispersal by Small Flying Insects*. Edited by J.M. Bullock, R.E. Kenward, and R.S. Hails. *Dispersal Ecology*. Oxford: Blackwell Publishing.
- Condrashoff, S. F. 1958. "Differences in Aspen Phenology and Survival of Immature Stages of *Phyllocnistis populiella* Chamb." *Bi-Monthly Progress Report Division Forest Biology Department Agriculture Canada* 14 (6): 3–4.
- Condrashoff, S. F. 1964. "Bionomics of the Aspen Leaf Miner, *Phyllocnistis populiella* Cham. (Lepidoptera: Gracillariidae)." *The Canadian Entomologist* 96 (6): 857–74. <https://doi.org/10.4039/Ent96857-6>.
- Cope, Olivia L., Eric L. Kruger, Kennedy F. Rubert-Nason, and Richard L. Lindroth. 2019. "Chemical Defense over Decadal Scales: Ontogenetic Allocation Trajectories and Consequences for Fitness in a Foundation Tree Species." *Functional Ecology* 33 (11): 2105–15. <https://doi.org/10.1111/1365-2435.13425>.
- Davis, Andrew J., Johannes Huijbregts, Ashley H. Kirk-Spriggs, J. Krikken, and Steven L. Sutton. 1997. "The Ecology and Behaviour of Arboreal Dung Beetles in Borneo." In *Canopy Arthropods*, 417–32.
- Dennis, Russell, Patricia Doak, and Diane Wagner. 2015. "Aspen Leaf Miner (*Phyllocnistis populiella*) Oviposition Site Selection Mediated by Aspen (*Populus tremuloides*) Extrafloral Nectaries." *Arthropod-Plant Interactions* 9 (4): 405–13. <https://doi.org/10.1007/s11829-015-9380-1>.
- Doak, Patricia, and Diane Wagner. 2015. "The Role of Interference Competition in a Sustained Population Outbreak of the Aspen Leaf Miner in Alaska." *Basic and Applied Ecology* 16 (5): 434–42. <https://doi.org/10.1016/j.baae.2015.04.001>.
- Doak, Patricia, Diane Wagner, and Adam Watson. 2007. "Variable Extrafloral Nectary Expression and Its Consequences in Quaking Aspen." *Canadian Journal of Botany* 85 (1): 1–9. <https://doi.org/10.1139/b06-137>.
- Donaldson, Jack R., Eric L. Kruger, and Richard L. Lindroth. 2006a. "Competition- and Resource-Mediated Tradeoffs between Growth and Defensive Chemistry in Trembling Aspen (*Populus tremuloides*)." *New Phytologist* 169 (3): 561–70. <https://doi.org/10.1111/j.1469-8137.2005.01613.x>.
- Donaldson, Jack R., Michael T. Stevens, Heidi R. Barnhill, and Richard L. Lindroth. 2006b. "Age-Related Shifts in Leaf Chemistry of Clonal Aspen (*Populus tremuloides*)." *Journal of Chemical Ecology* 32 (7): 1415–29. <https://doi.org/10.1007/s10886-006-9059-2>.
- Dudley, Robert. 2000. *The Biomechanics of Insect Flight: Form, Function, Evolution*. Princeton, NJ: Princeton University Press.
- Ekhholm, Adam, Ayco J.M. Tack, Pertti Pulkkinen, and Tomas Roslin. 2020. "Host Plant Phenology, Insect Outbreaks and Herbivore Communities – The Importance of Timing." *Journal of Animal Ecology*. 89 (3): 829–41. <https://doi.org/10.1111/1365-2656.13151>.

- Faeth, Stanley H., Susan Mopper, and Daniel Simberloff. 1981. "Abundances and Diversity of Leaf-Mining Insects on Three Oak Host Species: Effect of Host Plant Phenology and Nitrogen Content of Leaves." *Oikos (Denmark)* 37: 238–51. <http://agris.fao.org/agris-search/search.do?recordID=XE8121410>.
- Faeth, Stanley H., and Thomas L. Bultman. 1986. "Interacting Effects of Increased Tannin Levels on Leaf-Mining Insects." *Entomologia Experimentalis et Applicata* 40 (3): 297–301. <https://doi.org/10.1111/j.1570-7458.1986.tb00515.x>.
- Falk, Michael A., Richard L. Lindroth, Ken Keefover-Ring, and Kenneth F. Raffa. 2018. "Genetic Variation in Aspen Phytochemical Patterns Structures Windows of Opportunity for Gypsy Moth Larvae." *Oecologia* 187 (2): 471–82. <https://doi.org/10.1007/s00442-018-4160-0>.
- Fox, John, and Weisberg Sandford. 2019. *An R Companion to Applied Regression Third Edition*. SAGE Publications, Inc.
- Fritz, Robert S., and Jennifer Nobel. 1990. "Host Plant Variation in Mortality of the Leaf-folding Sawfly on the Arroyo Willow." *Ecological Entomology* 15 (1): 25–35. <https://doi.org/10.1111/j.1365-2311.1990.tb00780.x>.
- Galway, Kylie E., Richard P. Duncan, P. Syrett, R. M. Emberson, and A. W. Sheppard. 2004. "Insect Performance and Host-Plant Stress: A Review from a Biological Control Perspective." In *Proceedings of the XI International Symposium on Biological Control of Weeds*, edited by J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morrin, and J.K. Scott, 394–99. Canberra, Australia: CSIRO Entomology.
- Gastwith, Joseph L., Yulia R. Gel, W. L. Wallace Hui, Vyacheslav Lyubchich, Weiwen Miao, and Kimihiro Noguchi. 2020. "Lawstat: An R Package for Biostatistics, Public Policy, and Law." <https://cran.r-project.org/package=lawstat>.
- Geiger, Rudolf, Robert H. Aron, and Paul Todhunter. 2003. *The Climate near the Ground*. Lanham, MD: Rowman & Littlefield Publishers. Inc.
- Goane, Lucía, Graciela Valladares, and Eduardo Willink. 2008. "Preference and Performance of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on Three Citrus Hosts: Laboratory and Field Assessment." *Environmental Entomology* 37 (4): 1025–34. [https://doi.org/10.1603/0046-225X\(2008\)37\[1025:PAPOPC\]2.0.CO;2](https://doi.org/10.1603/0046-225X(2008)37[1025:PAPOPC]2.0.CO;2).
- Godfray, Hugh C.J., Michael P. Hassell, and Robert D. Holt. 1994. "The Population Dynamic Consequences of Phenological Asynchrony between Parasitoids and Their Hosts." *The Journal of Animal Ecology* 63 (1): 1–10. <https://doi.org/10.2307/5577>.
- Graves, Spencer, Hans-Peter Piepho, Selzer Luciano, and With Help From Sundar Dorai-Raj. 2015. "Package 'multcompView' Visualizations of Paired Comparisons." *R Package* [Http://CRAN.R-Project.Org/Package=multcompView](http://CRAN.R-Project.Org/Package=multcompView). <https://cran.r-project.org/package=multcompView>.
- Grimbacher, Peter S., and Nigel E. Stork. 2007. "Vertical Stratification of Feeding Guilds and Body Size in Beetle Assemblages from an Australian Tropical Rainforest." *Austral Ecology* 32 (1): 77–85. <https://doi.org/10.1111/j.1442-9993.2007.01735.x>.
- Gross, Juergen, and Uwe Ligges. 2015. "Nortest: Tests for Normality. R Package Version 1.0-4." URL: <Http://CRAN.R-Project.Org/Package=Nortest>.
- Hajek, Peter, Dietrich Hertel, and Christoph Leuschner. 2013. "Intraspecific Variation in Root and Leaf Traits and Leaf-Root Trait Linkages in Eight Aspen Demes (*Populus tremula* and *P. tremuloides*)." *Frontiers in Plant Science* 4: 415. <https://doi.org/10.3389/fpls.2013.00415>.
- Heil, Martin. 2015. "Extrafloral Nectar at the Plant-Insect Interface: A Spotlight on Chemical Ecology, Phenotypic Plasticity, and Food Webs." *Annual Review of Entomology* 60: 213–32. <https://doi.org/10.1146/annurev-ento-010814-020753>.

- Hemming, Jocelyn D.C., and Richard L. Lindroth. 1995. "Intraspecific Variation in Aspen Phytochemistry: Effects on Performance of Gypsy Moths and Forest Tent Caterpillars." *Oecologia* 103 (1): 79–88. <https://doi.org/10.1007/BF00328428>.
- Holeski, Liza M., Michael J. C. Kearsley, and Thomas G. Whitham. 2009. "Separating Ontogenetic and Environmental Determination of Resistance to Herbivory in Cottonwood." *Ecology* 90 (11): 2969–73. <https://doi.org/10.1890/08-2378.1>.
- Holt, Natalie C., and Graham N. Askew. 2012. "Locomotion on a Slope in Leaf-Cutter Ants: Metabolic Energy Use, Behavioural Adaptations and the Implications for Route Selection on Hilly Terrain." *Journal of Experimental Biology* 215 (15): 2545–50. <https://doi.org/10.1242/jeb.057695>.
- Honěk, Alois. 1993. "Intraspecific Variation in Body Size and Fecundity in Insects: A General Relationship." *Oikos* 66 (3): 483–92. <https://doi.org/10.2307/3544943>.
- Jaenike, John. 1978. "On Optimal Oviposition Behavior in Phytophagous Insects." *Theoretical Population Biology* 14 (3): 350–56. [https://doi.org/10.1016/0040-5809\(78\)90012-6](https://doi.org/10.1016/0040-5809(78)90012-6).
- Kagata, Hideki, and Takayuki Ohgushi. 2001. "Resource Partitioning among Three Willow Leaf Miners: Consequence of Host Plant Phenology." *Entomological Science* 4 (2): 257–63. <https://www.ecology.kyoto-u.ac.jp/~ohgushi/old/public/PDF/Ohgushi79.pdf>.
- Kenzo, Tanaka, Yuta Inoue, Mitsunori Yoshimura, Megumi Yamashita, Ayumi Tanaka-Oda, and Tomoaki Ichie. 2015. "Height-Related Changes in Leaf Photosynthetic Traits in Diverse Bornean Tropical Rain Forest Trees." *Oecologia* 177 (1): 191–202. <https://doi.org/10.1007/s00442-014-3126-0>.
- Kopper, Brian J., and Richard L. Lindroth. 2003. "Responses of Trembling Aspen (*Populus tremuloides*) Phytochemistry and Aspen Blotch Leafminer (*Phyllonorycter tremuloidiella*) Performance to Elevated Levels of Atmospheric CO<sub>2</sub> and O<sub>3</sub>." *Agricultural and Forest Entomology* 5 (1): 17–26. <https://doi.org/10.1046/j.1461-9563.2003.00158.x>.
- Kuznetsova, Alexandra, Per B. Brockhoff, and Rune H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82 (13): 1–26. <https://doi.org/10.18637/jss.v082.i13>.
- Lenth, Russell V. 2016. "Least-Squares Means: The R Package lsmeans." *Journal of Statistical Software* 69 (1): 1–33. <https://doi.org/10.18637/jss.v069.i01>.
- Levins, Richard, and Robert MacArthur. 1969. "An Hypothesis to Explain the Incidence of Monophagy." *Ecology* 50 (5): 910–11. <https://doi.org/10.2307/1933709>.
- Lindroth, Richard L. 2001. "Adaptations of Quaking Aspen for Defense against Damage by Herbivores and Related Environmental Agents." [https://www.fs.fed.us/rm/pubs/rmrs\\_p018/rmrs\\_p018\\_273\\_284.pdf](https://www.fs.fed.us/rm/pubs/rmrs_p018/rmrs_p018_273_284.pdf).
- Mattson Jr., William J. 1980. "Herbivory in Relation to Plant Nitrogen Content." *Annual Review of Ecology and Systematics* 11 (1): 119–61. <https://doi.org/10.1146/annurev.es.11.110180.001003>.
- McMillin, Joel D., Michael J. Anderson, Elizabeth E. Butin, and Elwood R. Hart. 1998. "Phenology and Infestation Patterns of the Cottonwood Twig Borer (Lepidoptera: Tortricidae) in Iowa." *Great Lakes Entomologist* 31 (3&4): 1–10.
- Miller, Fredric, and George Ware. 2014. "Seasonal Activity, Within-Tree Distribution, and Ovipositional and Feeding Preference of the Elm Leafminer, *Fenusa ulmi* (Hymenoptera: Tenthredinidae)." *Journal of Environmental Horticulture* 32 (1): 39–48. <https://doi.org/10.24266/0738-2898.32.1.39>.
- Mills, Nicholas J., and Wayne M. Getz. 1996. "Modelling the Biological Control of Insect Pests: A Review of Host-Parasitoid Models." *Ecological Modelling* 92 (2–3): 121–43. [https://doi.org/10.1016/0304-3800\(95\)00177-8](https://doi.org/10.1016/0304-3800(95)00177-8).
- Moreau, Jérôme, Betty Benrey, and Denis Thiery. 2006. "Assessing Larval Food Quality for Phytophagous Insects: Are the Facts as Simple as They Appear?" *Functional Ecology* 20 (4): 692–600. <https://doi.org/10.1111/j.1365-2435.2006.01145.x>.

- Mortensen, Brent, Diane Wagner, and Patricia Doak. 2013. "Parental Resource and Offspring Liability: The Influence of Extrafloral Nectar on Oviposition by a Leaf-Mining Moth." *Oecologia* 172 (3): 767–77. <https://doi.org/10.1007/s00442-012-2525-3>.
- Mustafa, Irfan, Muhammad Arshad, Abdul Ghani, Iftikhar Ahmad, Abu Bakar Muhammad Raza, Farzana Saddique, Saira Asif, Mobushir Riaz Khan, and Haroon Ahmed. 2014. "Population Dynamics of Citrus Leaf Miner on Different Varieties of Citrus in Correlation with Abiotic Environmental Factors in Sargodha District, Punjab, Pakistan." *Phytoparasitica* 42 (3): 341–48. <https://doi.org/10.1007/s12600-013-0371-4>.
- Neves, Frederico de Siqueira, Carlos Frankl Sperber, Ricardo Ildefonso de Campos, and Janaina Pizzatti Soares. 2013. "Contrasting Effects of Sampling Scale on Insect Herbivores Distribution in Response to Canopy Structure." *Revista de Biologia Tropical* 61 (1): 125–37.
- Newman, Jonathon R., and Diane Wagner. 2013. "The Influence of Water Availability and Defoliation on Extrafloral Nectar Secretion in Quaking Aspen (*Populus tremuloides*)." *Botany* 91 (11): 761–67. <https://doi.org/10.1139/cjb-2013-0147>.
- Newman, Jonathon R., Diane Wagner, and Patricia Doak. 2015. "Impact of Extrafloral Nectar Availability and Plant Genotype on Ant (Hymenoptera: Formicidae) Visitation to Quaking Aspen (Salicaceae)." *Canadian Entomologist* 148 (1): 36–42. <https://doi.org/10.4039/tce.2015.38>.
- Novotny, Vojtech, Yves Basset, and Roger L. Kitching. 2003. "Herbivore Assemblages and Their Food Resources." In *Arthropods of Tropical Forests Spatio-Temporal Dynamics and Resource Use in the Canopy*, edited by Y. Basset, V. Novotny, S. Miller, and R. Kitching, 40–53. Cambridge: Cambridge University Press.
- Osada, Noriyuki, and Tsutomu Hiura. 2019. "Intraspecific Differences in Spring Leaf Phenology in Relation to Tree Size in Temperate Deciduous Trees." *Tree Physiology* 39 (5): 782–91. <https://doi.org/10.1093/treephys/tpz011>.
- Osier, Tod L., and Richard L. Lindroth. 2006. "Genotype and Environment Determine Allocation to and Costs of Resistance in Quaking Aspen." *Oecologia* 148 (2): 293–303. <https://doi.org/10.1007/s00442-006-0373-8>.
- Parker, Geoffrey. 1995. *Structure and Microclimate of Forest Canopies*. Edited by M.D. Lowman and N.M. Nadkarni. *Forest Canopies*. San Diego, CA: Academic Press. <https://doi.org/10.1177/193758671400700404>.
- Price, Peter W. 1991. "The Plant Vigor Hypothesis and Herbivore Attack." *Oikos* 62 (2): 244–51. <https://doi.org/10.2307/3545270>.
- Reavey, Duncan, and Kevin J. Gaston. 1991. "The Importance of Leaf Structure in Oviposition by Leaf-Mining Microlepidoptera." *Oikos* 61 (1): 19–28. <https://doi.org/10.2307/3545403>.
- Robinson, David, and Alex Hayes. 2020. "Broom: Convert Statistical Analysis Objects into Tidy Tibbles." <https://cran.r-project.org/package=broom>.
- Scheirs, Jan, and Luc De Bruyn. 2002. "Integrating Optimal Foraging and Optimal Oviposition Theory in Plant-Insect Research." *Oikos* 96 (1): 187–91. <https://doi.org/10.1034/j.1600-0706.2002.960121.x>.
- Sinclair, Brent J. 2015. "Linking Energetics and Overwintering in Temperate Insects." *Journal of Thermal Biology* 54: 5–11. <https://doi.org/10.1016/j.jtherbio.2014.07.007>.
- Smith, Eric A., Sean B. Collette, Thomas A. Boynton, Tiffany Lillrose, Mikel R. Stevens, Matthew F. Bekker, Dennis Eggett, and Samuel B. St Clair. 2011. "Developmental Contributions to Phenotypic Variation in Functional Leaf Traits within Quaking Aspen Clones." *Tree Physiology* 31 (1): 68–77. <https://doi.org/10.1093/treephys/tpq100>.
- Spasojevic, Marko J., Elizabeth A. Yablon, Brad Oberle, and Jonathan A. Myers. 2014. "Ontogenetic Trait Variation Influences Tree Community Assembly across Environmental Gradients." *Ecosphere* 5 (10): 1–20. <https://doi.org/10.1890/ES14-000159.1>.

- Stevens, Michael T., Donald M. Waller, and Richard L. Lindroth. 2007. "Resistance and Tolerance in *Populus tremuloides*: Genetic Variation, Costs, and Environmental Dependency." *Evolutionary Ecology* 21 (6): 829–47. <https://doi.org/10.1007/s10682-006-9154-4>.
- Therneau, Terry. 2020. "A Package for Survival Analysis in R. R Package Version 3.1-12." <https://cran.r-project.org/package=survival>.
- Thompson, John N. 1988. "Evolutionary Ecology of the Relationship between Oviposition Preference and Performance of Offspring in Phytophagous Insects." *Entomologia Experimentalis et Applicata* 47 (1): 3–14. <https://doi.org/10.1111/j.1570-7458.1988.tb02275.x>.
- U.S. Forest Service. 1978:2019 (compiled). "Forest Health Conditions in Alaska" Anchorage, AK.
- Ulyshen, Michael D. 2011. "Arthropod Vertical Stratification in Temperate Deciduous Forests: Implications for Conservation-Oriented Management." *Forest Ecology and Management* 261 (9): 1479–89. <https://doi.org/10.1016/J.FORECO.2011.01.033>.
- Wagner, Diane, and E. Fleur Nicklen. 2010. "Ant Nest Location, Soil Nutrients and Nutrient Uptake by Ant-Associated Plants: Does Extrafloral Nectar Attract Ant Nests and Thereby Enhance Plant Nutrition?" *Journal of Ecology* 98 (3): 614–24. <https://doi.org/10.1111/j.1365-2745.2010.01640.x>.
- Watt, Allan D., Nigel E. Stork, Colin McBeath, and Gerald L. Lawson. 1997. "Impact of Forest Management on Insect Abundance and Damage in a Lowland Tropical Forest in Southern Cameroon." *The Journal of Applied Ecology* 34 (4): 985–98. <https://doi.org/10.2307/2405288>.
- Wooley, Stuart C., Jack R. Donaldson, Adam C. Gusse, Richard L. Lindroth, and Michael T. Stevens. 2007. "Extrafloral Nectaries in Aspen (*Populus tremuloides*): Heritable Genetic Variation and Herbivore-Induced Expression." *Annals of Botany* 100 (6): 1337–46. <https://doi.org/10.1093/aob/mcm220>.
- Young, Brian, Diane Wagner, Patricia Doak, and Thomas Clausen. 2010. "Within-Plant Distribution of Phenolic Glycosides and Extrafloral Nectaries in Trembling Aspen (*Populus tremuloides*; Salicaceae)." *American Journal of Botany* 97 (4): 601–10. <https://doi.org/10.3732/ajb.0900281>.

**Appendix:** Description of the statistical models used for each response variable used in R. GLMM stands for generalized linear mixed model. The log link function was used for GLMMs with a Poisson distribution and the logit link function was used for GLMMs with a binomial distribution. ALM stands for aspen leaf miner. Leaf position was a random effect nested within tree, which was also nested within site and year. “#ALM” refers to the total number of ALM on the same leaf side.

Response	Distribution	Fixed effects	Random effects
Oviposition	Poisson	Height Leaf side Height*leaf side	Site Year Tree Leaf position
Egg predation	Binomial	Height Leaf side Height*leaf side	Site Year Tree Leaf position
Parasitism	Binomial	Height Leaf side Height*leaf side #ALM #ALM*Height	Site Year Tree Leaf position
Predation	Binomial	Height Leaf side Height*leaf side #ALM #ALM*Height	Site Year Tree Leaf Position
Other mortality	Binomial	Height Leaf side Height*leaf side #ALM #ALM*Height	Site Year Tree Leaf Position
Survival	Binomial	Height Leaf side Height*leaf side #ALM #ALM*Height	Site Year Tree Leaf position

<b>Pupal masses</b>	Gaussian	Height Leaf side Height*leaf side	Site Year Tree
<b>Probability of mining</b>	Binomial	Height Leaf side Height*leaf side	Site Year Tree Leaf position
<b>Percent mining</b>	Gaussian	Height Leaf side Height*leaf side	Site Year Tree Leaf position
<b>Leaf width</b>	Gaussian	Height	Site Year Tree
<b>Percent nitrogen</b>	Gaussian	Height	Site Tree Shoot
<b>Wind speed</b>	Gaussian	Height Time of day Height*Time of day	None
<b>Gust speed</b>	Gaussian	Height Time of day Height*Time of day	None
<b>Phenology: leaf-out times</b>	Weibull	Height	Site