**The role of interference competition in a sustained population outbreak of the aspen leaf
miner in Alaska**

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Abstract

Direct density-dependence through intraspecific competition may be an important mechanism permitting sustained herbivore outbreaks. In theory, interference competition could allow a relatively stable number of herbivore individuals to survive while moderating host plant damage. This research examined the potential role of intraspecific competition in permitting a decade-long outbreak of the aspen leaf miner, *Phyllocnistis populiella*, on *Populus tremuloides* in interior Alaska. A combination of observational and experimental studies examined larval food requirements, food resources, and the impacts of *P. populiella* larval density on survival, mass, and leaf mining damage. These results were then compared to those from nine years of survey data examining the density of eggs and pupal chambers, as well as leaf mining damage. The number of *P. populiella* eggs per leaf surface often exceeded the number that could be supported through larval development. Consistent with the expectations of interference competition, the probability of larval survival displayed a decelerating decline with increasing density. Pupal mass of surviving individuals was not related to larval density suggesting little impact of exploitative competition. Mean percent of leaf area mined saturated between 65 and 75%. Taken together these results suggest that strong interference competition largely precludes exploitative competition in *P. populiella* larvae thereby allowing some individuals to survive and attain normal pupal size even when densities far surpass the carrying capacity of the resource. Interference competition also limits host plant damage thereby contributing to the preservation of a healthy resource base. By constraining both larval survival and host plant damage, interference competition may foster the maintenance of sustained outbreaks of *P. populiella*.

Zusammenfassung

Direkte Dichteabhängigkeit durch intraspezifische Konkurrenz kann ein wichtiger Mechanismus

38 sein, der dauerhafte Massenentwicklung von Herbivoren ermöglicht. Theoretisch könnte
39 Interferenz einer relativ stabilen Zahl von Herbivoren erlauben zu überleben, während der
40 Schaden an der Wirtspflanze gemildert wird. Diese Studie untersuchte die potentielle Rolle der
41 intraspezifischen Konkurrenz für die Ermöglichung einer 10-jährigen Massenentwicklung des
42 Gemeinen Pappelblattminierers, *Phyllocnistis populiella*, auf *Populus tremuloides* in
43 Zentralalaska. In einer Kombination von Beobachtungen und Experimenten untersuchten wir die
44 Nahrungsansprüche der Larven, die Nahrungsressourcen und den Einfluss der Larvenabundanz
45 des Minierers auf die Überlebensrate, Masse und Blattschaden. Diese Ergebnisse wurden dann
46 verglichen mit denen einer neunjährigen Erfassung der Abundanzen von Eiern und
47 Puppenkammern sowie des Blattschadens. Die Zahl der *P. populiella*-Eier pro Blatt überstieg oft
48 die Zahl der Larven, die ihre Entwicklung dort vollenden konnten. In Übereinstimmung mit den
49 Erwartungen für die Interferenz, zeigte die Überlebenswahrscheinlichkeit der Larven einen
50 Abfall mit steigender Larvendichte. Die Puppenmasse der überlebenden Individuen zeigte keinen
51 Bezug zur Larvendichte, was auf einen geringen Einfluss der exploitativen Konkurrenz
52 hindeutet. Bis zu 65 bis 75% der Blattfläche wurden miniert. Zusammengenommen legen diese
53 Ergebnisse nahe, dass Interferenz weitgehend exploitative Konkurrenz unter *P. populiella*-Larven
54 ausschließt, wodurch einige Individuen überleben und die normale Puppengröße erreichen
55 können, sogar wenn die Individuendichten die Kapazität der Nahrungsressource bei weitem
56 überschreiten. Interferenz begrenzt auch den Schaden für die Wirtspflanze, was zum Erhalt einer
57 funktionstüchtigen Ressourcenbasis beiträgt. Indem sowohl die Überlebensrate der Larven als
58 auch der Schaden an der Wirtspflanze begrenzt wird, kann Interferenz die
59 Aufrechterhaltung von dauerhaften Massenentwicklungen von *P. populiella* unterstützen.

60 **Keywords:** boreal forest; contest competition; herbivory; plant-insect interactions; population
61 regulation

62

Introduction

Intraspecific competition can have important feedbacks on population dynamics and interspecific interactions (Haukioja et al. 1983, Berryman 1987, Myers 1988, Abbott & Dwyer 2007).

Berryman (1987) proposes that sustained herbivore outbreaks result when direct negative density dependence occurs at high population density thereby allowing the maintenance of insect and host populations. Interference competition has the potential to cap the number of surviving individuals and provide this density dependent feedback. If interference reduces herbivore densities prior to strong exploitative competition, it may limit host plant damage and allow surviving herbivores to acquire adequate resources to maintain quality. These feedbacks may allow the maintenance of healthy herbivore and host plant populations across multiple years of high herbivore density.

We were interested in whether intraspecific interference competition was moderating feedbacks in an herbivore that experiences sustained outbreaks. *Phyllocnistis populiella* Chambers (Lepidoptera: Gracillariidae), the aspen leaf miner, is a widespread herbivore of quaking aspen, *Populus tremuloides* Michx. The leaf miner normally occurs at low population densities (<1 mine/tree; personal observation). However, spatially stable *P. populiella* outbreaks can persist for a decade or more over large regions (Condrashoff 1964, USDA Forest Service 2012, Yukon Energy, Mines and Resources 2012). The palearctic congener, *Phyllocnistis labyrinthella*, also persists at high population densities on European aspen, *Populus tremula*, for extended periods of time (Sundby 1953, Albrechtsen et al. 2010). *P. populiella* caused extensive damage to interior Alaska aspen stands from 2002 to 2012 (USDA Forest Service 2012). During the outbreak *P. populiella* displayed little if any discrimination among host plant individuals resulting in heavy damage to all trees. On average, aspen saplings surveyed in 2004-2011 had 45% of their leaf area

mined. Mature aspen trees suffered even greater mining damage; in 2006 saplings and mature trees experienced 49 and 70% leaf area mined, respectively.

Leaf mining species often experience strong intraspecific larval competition because larval movement restricted to the immediate vicinity of the oviposition site limits the reduction of competition through dispersal (Connor & Taverner 1997). While most leaf miner populations seldom if ever reach densities where intraspecific competition becomes important, a quarter of the species considered by Auerbach, Connor and Mopper (1995) either experienced population outbreaks or persisted at high densities. In outbreaking populations of leaf miners, only intraspecific competition impacting larval survival and/or pupal mass commonly acts in a negative density-dependent manner and thus has the potential to regulate populations (Auerbach, Connor & Mopper 1995).

P. populiella provides a good system for examining the impacts of intraspecific competition on herbivores and their host plants. Larvae are restricted to feeding on a single leaf surface, and during outbreaks, the number of *P. populiella* eggs laid per leaf surface may exceed the number of larvae that can be supported through development. Interference competition occurring early in development has the potential to reduce overall plant damage and thereby ensure adequate food for the growth of surviving individuals.

We hypothesized that intraspecific interference competition had the potential to regulate *P. populiella* populations and moderate host plant damage thereby fostering sustained outbreaks. We used a combination of field experiments, observational studies, and broader surveys during an outbreak of *P. populiella* to address the following questions:

- 1) Do *P. populiella* larvae experience intraspecific competition as indicated by initial densities that exceed food availability and a negative effect of density on larval performance?

- 109
- 110 2) Do *P. populiella* larvae experience interference competition as indicated by a decelerating
- 111 decline in survival probability with increasing density?
- 112 3) Do *P. populiella* larvae experience exploitative competition as indicated by a negative
- 113 relationship between density and the mass of surviving *P. populiella* individuals?
- 114 4) Does leaf mining damage asymptote below 100% with increasing *P. populiella* density?
- 115

116 **Materials and methods**

117 *Natural history*

118 *Phyllocnistis populiella* is univoltine with adult moths overwintering in leaf litter (Wagner et al.

119 2012). Mating occurs in the early spring (May in Interior Alaska), and eggs are laid singly on

120 both upper and lower leaf surfaces of young, expanding leaves. After oviposition, eggs gradually

121 sink into the leaf epidermis and hatch directly into the epidermal cell layer after approximately

122 one week. Once eggs have begun to sink into leaves, egg predation results in a clearly

123 discernable scar (hereafter: egg scar) that can be used to better estimate total oviposition.

124 Members of the genus *Phyllocnistis* form epidermal leaf mines (Condrashoff 1964). Their

125 mouthparts constrain them to chewing in a forward direction, thus restricting them to the leaf

126 surface in which they hatch and to consumption of a single cell layer. While in some systems

127 epidermal miners can use the petiole as a route between leaf surfaces, the flattened petioles of

128 *Populus tremuloides* appear to preclude this. Because only the epidermal cells are eaten, the two

129 dimensional measure of mine area provides an excellent estimate of tissue consumption. Mine

130 development follows a stereotypical pattern: newly hatched larvae mine until encountering a leaf

131 vein; mines then follow these veins, usually, but not always, towards the midrib and then towards

the leaf base (Fig. 1A). Later in development, mines depart from the veins and wind back on themselves (Fig. 1B); if a larva runs out of available tissue in its immediate path, it will cross earlier mines in search of unconsumed epidermal cells (Fig. 1C). They do not appear to avoid any parts of the leaf (personal observation). After approximately 2 weeks of development through four instars (Condrashoff 1964) and while remaining within the mine, larvae create pupal chambers in small leaf folds (hereafter: pupal folds). Adults emerge approximately 10–14 days later in late-June to early-July. These adults will then overwinter to reproduce the following spring.

Larvae in mines are preyed on by ants, which remove the prey from mines, and mites and adult parasitoids, which puncture and feed on the hemolymph of prey but do not remove the carcass. They are also parasitized by a suite of generalist idiobiont Eulophid parasitoids that kill their hosts at the time of oviposition. When multiple eggs are laid on a leaf surface it is common for mines to coalesce and for larvae to engage in antagonistic encounters that can result in death of one or both larvae (Fig. 1) (Condrashoff 1964). While Condrashoff (1964) refers to these larval interactions as “cannibalism”, he provides no further comments on whether he observed intraspecific consumption of killed individuals. We also lack observations or data to clarify whether these antagonistic interactions are truly cannibalistic.

Research was conducted from 2004 to 2012 at four sites in interior Alaska near Fairbanks (site abbreviations BNZ, ED, RP, WR; see Appendix A: Table S1 for site descriptions).

Leaf consumption

Methods: We estimated the leaf area required for the development of *P. populiella* larvae without competitors. In 2006 at the RP site we marked 6 to 15 leaf surfaces on each of 30 small aspen ramets ($n = 312$ leaf surfaces), and when necessary we removed eggs to reduce *P. populiella*

densities to 1 individual per leaf surface. We collected the 120 leaves containing individuals that survived to form pupal folds and scanned each leaf while still fresh. Total leaf area and area mined were then measured using image analysis software (Scion Image, Fredrick, MD, USA). Combining estimates of the leaf area needed to support an individual and survey data on leaf size allowed us to assess the potential for intraspecific competition. As part of a larger effort to track *P. populiella* population dynamics (see Oviposition and Miner Surveys, below), we monitored the maximum width of mature aspen leaves in 2006 – 2012 at all four study sites. Leaf width is a good predictor of leaf area (Doak, Wagner & Watson 2007). Because the survey data used leaf width as a proxy for leaf size, a conversion was required. In 2004 we measured fresh leaf width and fresh leaf areas (Scion Image) for leaves from short aspen ramets (< 2m) at two sites (BS $n = 20$; ED $n = 22$) and used regression analysis to derive the relationship between width and area.

Intraspecific competition

Methods: To test the effects of larval density on *P. populiella* survival and performance as well as leaf damage, we performed a manipulative field experiment. In 2004 at the BNZ site, on each of 2 branches of each of 30 small aspen trees (1-2 m tall), we selected 4 leaves bearing 5 or more eggs on the upper leaf surface. Each leaf was randomly assigned to one of 4 treatment densities: 1, 2, 3, or 5 larvae/leaf top; this resulted in 60 leaves per treatment group. We removed all eggs and larvae from the bottom surface and reduced numbers on the top surface to match assigned treatments. The initial treatments were established on 17 and 18 May. On June 7, we measured leaf width, visually estimated the percent of leaf area mined (our visual estimates of mining are quite accurate; $r^2 > 0.90$; Doak, Wagner & Watson 2007), and counted the number of pupal folds. Each fold was dissected and all surviving *P. populiella* pupae were weighed to the nearest microgram. After eclosion, adult moths were dried and weighed.

During some years, maximum leaf miner egg densities can far exceed 5 eggs per leaf surface (unpublished data). Therefore, we conducted an additional, observational study in order to examine the impacts of a wider range of densities than was feasible in the competition experiment. In 2006 we marked four leaves on each of two shoots on each of 10 short ramets at the WR site ($n=80$ leaves). Leaves were initially surveyed on 6 June when the majority of eggs had hatched but before mines coalesced. This allowed a count of the number of mines that were initiated. On 21 June, after larvae had pupated but prior to eclosion, we collected all leaves and recorded leaf width, the percent of each leaf surface mined, and the number of pupal folds.

Analysis: Separate but parallel statistical models were used to analyze data from 2004 and 2006. Given our focus on intraspecific competition, we examined survival through the stages when larvae can directly interact. The possibility of direct larval interaction ceases at the time that the pupal fold is formed; therefore we use survival to the formation of the pupal fold as our response variable. *P. populiella* larvae occurring on the same leaf surface have the potential to compete regardless of the size of the leaf; therefore, we used the number of individuals per leaf surface as our measure of density and included a covariate to account for leaf size. We used logistic models (SAS PROC NLMIXED) to examine the relationship between the probability of larval survival and larval density. We compared models with three different density functions: a linear function and two decelerating functions consistent with the expectations of interference competition, the negative exponential and $1/(1+\text{density})$. All models included leaf width as a covariate and the random effect of ramet. AICc and Akaike weights were used to compare model fits. To determine if leaf mining damage saturated below 100%, we examined whether percent mining damage was better explained by linear or Michaelis-Menten functions of density with leaf width included as a covariate and the random effect of ramet. AICc was used to compare model fits. To

assess the occurrence of exploitative competition, we examined the impact of density on pupal weight using a linear mixed model with mean pupal weight (per leaf surface) as the response, density and leaf width as fixed effects, and ramet as a random effect.

Predator exclusion experiment

Methods: We used a predator exclusion experiment to disentangle the contributions of intraspecific competition and predation to larval mortality, thereby allowing us to estimate the importance of competition to overall mortality. While some larval mortality can be definitively assigned to the action of predators and parasitoids, many larvae die of unidentifiable causes. This “unexplained” mortality may be due to intraspecific competition, predation that does not remove the larva, adult parasitoid feeding, disease, or poor plant quality. By excluding natural enemies we were able to largely eliminate their contributions to unexplained mortality. In 2007 at the ED site we haphazardly selected 30 aspen ramets (<2.5 m height). On 1 June 2007 after the majority of eggs were laid, we bagged one branch with a polyester plant sleeve (Reemay Inc., Old Hickory, TN, USA) fastened with twist-ties to exclude natural enemies and marked a second branch for use as a control. On 21 June, we collected three haphazardly selected shoots from each bagged and control branch. We recorded data for all leaves from 1-2 shoots per branch. We measured leaf width, counted the number of pupal folds and dissected mines to find dead larvae. Larval mortality was classified as parasitism (parasitoid egg, larva, or pupa present), predation (mine ripped open and larva missing), or unexplained (no identifiable cause of death).

Analysis: Logistic generalized linear mixed models examined the effect of predator exclusion on the probability of unexplained larval mortality in the mine and the probability of survival to the formation of the pupal fold. Treatment (exclusion or control), larval density (number of mines detected during dissections) and their interaction were fixed effects in the models, leaf width was

included as a covariate, and ramet was included as a random effect. To determine if the probability of unexplained death reached an asymptote, we compared models with a linear versus Michaelis-Menten function of density. For larval survival we compared the same functions of density used in the competition analyses. AICc and Akaike weights were used to compare model fits.

Oviposition and miner surveys

Methods: We used long-term surveys to better understand how the mechanisms identified in the focused density and predator exclusion studies may relate to *P. populiella* population dynamics. We conducted twice yearly surveys to quantify *P. populiella* population numbers, leaf mining damage and larval survival. From 2004 to 2012, we conducted both early season surveys of *P. populiella* egg numbers and late season surveys of larval survival and mining damage. During each survey we sampled a single shoot on each of approximately 30 haphazardly chosen ramets (<2 m height) at each of 4 sites (see Appendix A: Tables S1 and S2 for details). Sampled ramets differed between early and late season surveys and among years. We conducted oviposition surveys near the end of the oviposition period each year; the number of *P. populiella* eggs and newly initiated mines per leaf surface were counted. Given that eggs preyed upon shortly after oviposition may not leave a scar and some oviposition occurred after the surveys, these estimates represent lower bounds on egg density. After the majority of larvae had formed pupal folds but prior to most eclosion, we conducted a second survey. We estimated the percent leaf area mined, counted the number of pupal folds, and measured leaf width.

Analysis: To better understand the influence of *P. populiella* density on aspen damage and possible feedbacks on the herbivore's population dynamics, we examined the relationships of both the number of pupal folds and mining damage with initial eggs numbers. Because the same

leaves were not tracked through a season, we relied on site-level means calculated each year. Separately for leaf tops and bottoms, we calculated means for initial eggs and mines, number of pupal folds per leaf surface, and percent of leaf surface mined (for leaf positions 1-7). We then used AICc to compare the fit of linear and Michaelis-Menten models of initial density with mean leaf width and surface (top/bottom) included as covariates.

Results

Leaf consumption

P. populiella larvae without any competitors on their leaf surface ate an average of 6.05 ± 0.10 cm² of epidermal tissue. The relationship between fresh leaf area (cm²) and fresh leaf width (cm) was: $\text{area} = -7.755 + 5.236 \text{width}$ ($r^2 = 0.94$). Using this conversion, a leaf of fresh width 2.64 cm provides 6.05 cm² of fresh leaf area on both the top and bottom surfaces.

Considering all leaves in positions 1-7 surveyed in 2006-2012: 21% were less than 2.64 cm and thus not likely to support more than one larva per leaf surface, and an additional 68% were 2.64-5.27 cm and large enough to support 1 - 2 individuals per leaf surface (see Appendix A: Fig. S1). From 2004 to 2012, 20-54% of leaf surfaces had more than 2 eggs and mines during oviposition surveys (Fig. 2).

Intraspecific competition

In both the 2004 experimental and 2006 observational studies, the probability of surviving to the formation of a pupal fold displayed a non-linear decrease with the initial number of individuals per leaf surface and was best explained by the $1/(1+\text{density})$ function (Figs 3A and B; see Appendix A: Tables S3 and S4) (Akaike weights: 0.81, 0.38 for 2004 and 2006 respectively).

Mining damage displayed a decelerating increase with density, reaching an average of 65% of the leaf area mined at the highest initial density of 5 egg per leaf surface in the 2004 experiment and 70-75% at densities of 10-15 in the 2006 observational study (Figs 3C and D). The saturating Michaelis-Menten function provided a much better fit to the data than did the linear function of density (Akaike weights 0.93, 0.99, respectively for 2004 and 2006; see Appendix A: Tables S5 and S6). Percent mining decreased with increasing leaf width in both studies.

Pupal mass did not vary with density (Figs 3E and F) in 2004 ($F_{1,172} = 0.08$, $P = 0.78$) or 2006 ($F_{1,7} = 1.98$, $P = 0.203$). It increased with leaf width in 2004 ($F_{1,40} = 7.98$, $P = 0.007$) but not 2006 ($F_{1,7} = 0.91$, $P = 0.373$; $n=52$). Pupal mass was a strong predictor of adult dry mass ($r^2 = 0.73$, $n = 22$, $F_{1,21} = 54.30$, $P < 0.0001$).

Predator exclusion experiment

The total number of living and dead *P. populiella* per leaf surface discovered at the time of dissection varied from 0 to 14 and did not significantly differ between the predator exclusion and control treatments (Exclusion: 3.18 ± 0.26 , mean $\pm$ se; Control: 3.33 ± 0.26 ; $F_{1,73} = 0.55$, $P = 0.46$) indicating that both treatments had the potential to experience similar levels of intraspecific competition. Both parasitism and predation were almost completely eliminated by the predator exclusion treatment (1% parasitism, 0.1% predation). This experiment was conducted at moderate to high *P. populiella* densities with a mean of 3.45 (± 0.20 SE) larvae found per leaf surface (see Appendix A: Fig. S2 to compare to long term data). In the control treatment, parasitism rates of larvae in mines (0.09 ± 0.01) were close to the long term mean (0.11 ± 0.01) while the rate at which mines were ripped open and predated (0.01 ± 0.003) was lower than the long term mean (0.05 ± 0.01) (unpublished data; long term means calculated for 7 years of survey data across 4 sites).

The probability of unexplained larval death in the mine displayed an asymptotic increase with the number of larvae per leaf surface (Fig. 4A; see Appendix A: Tables S5 and S7) that was better explained by the Michaelis-Menten than linear function of density ($\Delta\text{AICc} = 30$). While at low numbers per leaf surface, unexplained larval death was greater on control compared to predator exclusion branches, it became indistinguishable as density increased (Fig. 4A). The probability of larval survival to the formation of the pupal fold was higher on predator-exclusion compared to control shoots but also displayed a significant treatment by density effect (Fig. 4B; see Appendix A: Tables 5 and 8). The probability of survival decreased with density and was best described by the model containing the $1/(1+\text{density})$ function (Akaike weight = 0.68; see Appendix A: Table S6). Treatment differences were greatest at low densities (Fig. 4B).

Survey patterns

The survey provided data on temporal patterns of *P. populiella* population size and leaf mining damage, and these are provided in the supplemental material (see Appendix A: Fig. S2). Both the mean number of pupal folds and mean mining damage per leaf surface were better modelled by the saturating Michaelis-Menten function of egg density than by a linear function (Fig. 5; see Appendix A: Tables S5 and S9). The relationship between percent mining and density (Fig. 5B) was similar to that observed in the 2006 density study (Fig. 3D), and leaf area damaged reached a mean maximum of approximately 70%.

Discussion

We found evidence for negative density dependent effects of intraspecific interference competition among *P. populiella* larvae. Given their food requirements for development and the size of aspen leaves, 89% of surveyed leaf surfaces could support two or fewer larvae. Yet in the

years 2004 to 2012, 20-54% of leaf surfaces had >2 eggs and mines during egg surveys. Our results indicate that the probability of individual survival declined with increasing *P. populiella* density. While some species of leaf miner seldom reach densities where intraspecific competition strongly impacts survival (Tack et al. 2009), *P. populiella* clearly does reach and remain at these densities for extended periods. While survival through the feeding stages varied between our 2004 and 2006 density studies, the impact of density was similar with individual larval survival probability decreasing by 19-24% with 2 and by 57-58% with 5 individuals per leaf surface (as compared to 1 per leaf surface).

Negative density dependent survival was maintained when predators were excluded, indicating that either intraspecific or host plant interactions must be responsible. Aspen chemical defenses include condensed tannins and phenolic glycosides (Lindroth & St. Clair 2013), but neither likely imposed strong negative feedbacks on *P. populiella*. Condensed tannins are unlikely to provide strong defense against insect herbivores (Lindroth & St. Clair 2013). While *P. populiella* mining induces aspen phenolic glycosides (Young et al. 2010a), percent mining is negatively correlated with phenolic glycoside concentration (Young et al. 2010b), perhaps because moths avoid laying eggs on leaves with high levels of chemical defense. Thus, leaves with higher larval densities and greater mining tend to have lower levels of phenolic glycoside defense, negating the possibility that it is responsible for the observed negative density-dependent survival. Therefore it appears most likely that intraspecific interactions are responsible for the observed patterns of density dependent survival.

Intraspecific competition can occur through exploitation and/or interference. Leaf mining species vary in intraspecific aggression, but leaf level impacts of intraspecific competition are often strong (Auerbach, Connor & Mopper 1995, Eber 2004). *P. populiella*'s serpentine mine and the

tendency of early instars to mine to and then follow major veins (Condrashoff 1964) increases the probability of encounters between young larvae (Fig. 1A; see Appendix A: Fig. S3). Because mines tend to coalesce early in larval development, larval numbers are reduced prior to inflicting much damage to the leaf. To quantify this, in 2004 we repeatedly photographed leaves and tracked early larval development and mining damage on 120 leaf surfaces (unpublished data). Considering all leaf surfaces where 7 or more larvae (9.5 ± 0.6 SE; range 7 to 15) initiated mines ($n = 17$), an average of $60 \pm 2\%$ of larvae had died when an average of only $16 \pm 2\%$ of the leaf surface was mined.

Pupal weights did not vary with density indicating that on average individuals are not strongly impacted by the amount of leaf tissue consumed by their competitors. These findings suggest that interference effectively precludes exploitative competition for epidermal tissue by removing competitors early in development when consumption is still low. Similarly, Quiring and McNeil (1984, 1985) concluded that intraspecific interference and cannibalism in the dipteran leaf miner, *Agromyza frontella*, most strongly impacted surviving individuals through removal of a future exploitative competitor. Interference competition, in conjunction with the number and size of leaves available, appears to effectively cap *P. populiella* population size. Additionally, interference competition allows for a segment of the population to survive competitive interactions, in contrast to purely exploitative competition that can lead to 100% mortality if available food does not allow any individuals to complete development (Miller 1967). It appears that interference competition has the potential to regulate population density by creating a density ceiling (Nicholson 1954), and at high population density, it provides the direct density-dependent feedback associated with sustained insect outbreaks (Berryman 1987). Even at high population densities individual larvae receive adequate nutrition to complete development at a

normal size. Although not studied in *P. populiella*, pupal size and subsequent adult size are likely to impact adult overwinter survival, mating success, and fecundity (Leather 1988; Tammaru, Esperk & Castellanos 2002). The maintenance of pupal weight even under conditions of strong intraspecific competition should aid in sustaining high population densities.

Further, our results suggest that when *P. populiella* population densities are moderate to high, the ability of natural enemies to reduce larval survival may be limited. While the scope of our predator exclusion experiment is limited, we found that differences in survival probabilities between the predator exclusion and control treatments were greatest at the lowest densities per leaf surface. This suggests that at low densities when intraspecific competition is absent or weak, natural enemies (especially parasitoids) may have a significant influence on larval survival rates. With increasing density the impact of natural enemies became increasingly compensatory to the effects of intraspecific competition, leading to similar larval survival rates with or without these additional mortality factors. This pattern could restrict the ability of natural enemies to control *P. populiella* and allow sustained outbreaks.

Negative density-dependent survival of larvae through the feeding stages is also evident in our 7 years of survey data. The mean number of pupal folds per leaf surface only exceeded 1.0 in two cases and never exceeded 1.5. Thus even at outbreak densities when 10 or more eggs may be laid per leaf surface, the number of larvae surviving is constrained by the number of available leaves. Because a great deal of this larval mortality occurs through interference at early instars, mean mining damage also asymptotes and is kept below 75%. This cap on *P. populiella* damage combined with aspen's high tolerance to herbivore damage (Lindroth & St. Clair 2013, Wagner & Doak 2013) likely results in a productive resource base even after many years of high *P. populiella* numbers and may foster sustained outbreaks.

Finally, the asymptotic relationship between mining damage and *P. populiella* density also results in poor correspondence between measures of mining damage and *P. populiella* population size. The population dynamics of insect pests are often inferred from damage estimates, such that static damage levels may be used to infer static insect population dynamics. However, in many cases including that of *P. populiella*, this can be misleading as the insect population may be increasing or decreasing without resulting in noticeable changes in damage levels. Only careful monitoring of insect populations and not only plant damage will provide critical insight into the drivers of population dynamics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXXX.

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461

Figure captions

Fig. 1. Patterns of *P. populiella* leaf mining. Early coalescence of *P. populiella* mines on aspen leaf (A; oviposition sites circled; dead *P. populiella* larvae marked with Xs; living larvae marked with arrows; these larvae hatched approximately 5-7 days prior to being photographed). After leaving leaf veins, mines tend to wind tightly (B; two living larvae marked with arrows and one recently killed larva marked with X) and later, if larva run out of food in their immediate path, they will cross earlier mines in search of unconsumed epidermal tissue (C; areas where mines cross circled; pupal chambers indicated by arrows).

Fig. 2. Proportion of leaf surfaces (leaf positions 1 -7) with 0, 1, 2, or >2 eggs and mines in early season surveys.

Fig. 3. Impact of number of *P. populiella* larvae per leaf surface on the probability of larval survival to the formation of a pupal fold (A, B), percent leaf surface mined (C, D), and mean pupal mass (E, F) from 2004 (A, C, E) and 2006 (B, D, F) studies. Predicted lines from GLMM analyses are displayed with observed percent surviving (A, B) or raw data (C-F). Note that the scales of the x-axes differ between left and right hand columns. Calculated at mean leaf widths of 5.65 and 5.00 cm for 2004 and 2006, respectively. In panels B and E, some data points are overlaid and obscured. In panels A and B predicted lines are backtransformed from the logit.

Fig. 4. Impacts of predator exclusion (solid line, closed dots) vs control (dashed line, open dots) on the probability of individual unexplained death (A) and survival to formation of the pupal fold (B) relative to density. Dots are raw data. Predicted lines are from logistic GLMM and calculated at the mean leaf width = 3.59 cm. Predicted lines are backtransformed from the logit.

484 Fig. 5. Mean pupal folds per leaf surface (A) and mean percent mining (B) relative to mean eggs
485 and mines. Data are site by year means from survey data with Michaelis-Menten estimated lines
486 for top (filled, solid) and bottom (open, dashed) leaf surfaces.

487

488
489 Fig. 1.

Fig. 2.

Fig. 3.

497 Fig. 4.

Fig. 5.