Leafminer Impacts on Photosynthetic Ability of *Populus tremuloides*

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Abstract In recent years, one of the dominant tree species of the boreal forests of North America, *Populus tremuloides*, has experienced increased leaf mining herbivory. On a landscape scale this leaf damage manifests as a change of the canopy from green to silver in late spring. The catalyst for this change is predation of the mesophyll tissue of these leaves by the newly hatched cohort or generation of leaf "mining" insect larvae. While no studies have been published investigating the effects of this mining on leaf function it has been assumed that damage caused by these miners inhibits the plants overall ability to photosynthesize. Historically, outbreaks of these larvae have not been frequent enough to cause significant die back of *P. tremuloides*. However, a recent series of warm winters has allowed more adult insects to overwinter, leading to a number of high intensity outbreaks. I found a reduction in photosynthetic ability of mined leaves of *P. tremuloides* in aspen stands surrounding Fairbanks, Alaska. A loss of up to 75% of leaf photosynthetic ability was detected for highly damaged leaves. Mining on undersides of leaves was found to have a more detrimental impact than mining on the top surface. This is attributed to the tissue structure of leaves and possible damage to stomata and changes to volume of interstitial space in spongy mesophyll tissue in lower parts of the leaf that may increase photorespiration. The impact of sustained leaf damage on stand health has yet to be determined. However, prolonged high intensity herbivory may contribute to tree death, and climate projections for continued warming suggest this stress may intensify in the future.

Introduction

Populus tremuloides Michx (quaking aspen) is a deciduous tree distributed widely across most of North America from Mexico to the Arctic (Perala 2003). A common observation of the foliage of *P. tremuloides* a few weeks after leaf out in early to late spring is a change of color from bright green to silver. This change in leaf color results from a type of defoliation inflicted by the larvae of various insects of the order *Lepidoptera*, most prevalently *Phyllocnistis populiella* (Hennon *et al*. 2001). Commonly known as leafminers, these larvae burrow between the epidermal tissue layers of leaves "mining" chlorophyll rich mesophyll tissue within the leaf (Borror and DeLong 1971). This mining creates the visible paths across the leaf surfaces, which are wider than the burrowing insects themselves and cover much more area than the leaf cells that were actually consumed. This impact has not historically been observed to be a significant direct cause of mortality for affected *P. tremuloides*, but it has been assumed to affect photosynthetic ability of mined leaves (DeFoliart 2003, pers. comm.), may make mined leaves more susceptible to subsequent attack by other insects or pathogens, and require the trees to alter their uptake and allocation of nutrients to compensate (Kaitaniemi *et al.*1999, Zangerl *et al.* 2002, Ruess 2003, pers. comm.).

Throughout the cold northern regions of the *P. tremuloides* range, the intensity of leaf miner and other insect defoliator attacks has been observed to be increasing drastically in recent years (DeFoliart 2003, pers. comm.). Temperatures in interior Alaska have increased by as much as 5˚C over the past three decades (Lachenbruch 1994, Chapin et al. 2000), and the common assumption is that this allows more adult insects to survive the winters than was typical previously (DeFoliart 2003, pers. comm.). It is also suspected that consistent defoliation of trees can lead to increased tree mortality (Kaitaniemi *et al.*1999). To date, however, there have not been any studies published estimating the actual loss of photosynthetic ability of *P. tremuloides* when damaged by leafminer herbivory. I have attempted to quantify the impact of mining on photosynthetic ability of individual leaves of *Populus tremuloides* in relation to the surface area affected by mining activity. These results may be useful in projecting the cost of global warming to the fitness of *P. tremuloides* in this biome.

Methods

Site Selection Three stands of *P. tremuloides* were located in the greater area of Fairbanks, Alaska. These sites were selected to be representative of different stand densities and severity of attack. The first site selected was in a very open, isolated stand and its individuals appeared to exhibit the least amount of foliar damage. The second site selected was a narrow strip of higher density that ran adjacent to a black spruce stand. This site exhibited a high amount of foliar damage. The third site contained the highest density of trees, also along the periphery of a mixed species stand, and also appeared to exhibit the most foliar damage.

Tree Selection Within each stand three trees were selected for sampling. Each of these individuals was chosen to belong to different size/age groups. One tree from each site was under 1.5m tall and young, one was between 2m and 3m, and one was a fairly mature tree, greater than 3m in height. This selection process was somewhat randomized by sampling the third tree found that fit into each size category at each site.

Leaf Selection Between four and six leaves were selected for analysis from each of these trees. Leaves were selected in pairs from opposite sides of the same branch to ensure that they were also of the same cohort (grown at the same time under very similar biological conditions). These pairs were selected to meet two criteria: that both members of the pair be free of visible non-miner related damage or necrosis, and that differing amounts of surface area covered by miner trails be evident.

Data Collection Measurements of photosynthesis, stomatal conductance, intra-stomatal CO_2 concentration, and changes in leaf and surrounding temperature were taken with a LI-COR LI 6400 Portable Photosynthesis System (LI-COR, inc., 4421 Superior Street, Lincoln, NE 68504) using a $6cm^2$ leaf chamber attachment. The LI 6400 calculates photosynthesis and conductance in real time using an IRGA (Infrared Gas Analyzer) located immediately adjacent to the leaf chamber. This IRGA converts changes in CO_2 and H_2O concentrations in an air stream flowing through the chamber/IRGA system to net CO_2 and H_2O fluxes between the enclosed portion of leaf and the surrounding air. These measurements were taken in response to increasing intensity of photosynthetically active radiation (PAR) and were recorded as light curve responses.

After initial test trials, it was determined PAR settings of 0, 150, 300, 600, 1000, and 1600 μmol photon (quanta) $flux{\cdot}m^{-2}{\cdot}s^{-1}$ were appropriate for the light curve. 1600 $\mu mol{\cdot}\ m^{-2}{\cdot}s^{-1}$ is considered about the maximum natural level of PAR encountered at the latitude of Fairbanks

(Reuss 2003, pers. comm.). Damage inflicted to leaves by mining was measured as the fraction of each surface of the leaf (top and bottom) showing discoloration due to separation of the epidermis. Leaves were then given a score representing the total damage from both sides. These scores were used subsequently in the data analysis. No estimations of actual photosynthetic tissue loss to predation by the miners were made as leaf dissections indicated this loss was very small. All of the leaves sampled were characterized as sun leaves, so they should have developed under similar, high exposure, light conditions. Measurements were taken for leaves growing on higher branches by first cutting the branch on which they were growing down from the tree, placing the cut end in a bucket with water, and then re-cutting that end under water. This method is commonly used for analyzing photosynthesis of canopy leaves (Ruess 2003, pers. comm.).

Data Analysis Multivariate regression analysis was used to characterize the relative significance of damage on the bottom leaf surface versus the top leaf surface. A single damage score was then assigned to each leaf that appropriately weighted (by coefficients) the effects of the damage on each of the surfaces such that these damages were combined as

$$\text{Damage Score} = \frac{(\text{coefficient}_{\text{top}})\text{fraction damaged}_{\text{top}}+(\text{coefficient}_{\text{bottom}})\text{fraction damaged}_{\text{bottom}}}{\text{coefficient}_{\text{top}}+\text{coefficient}_{\text{bottom}}}.$$

The difference between damage scores of members of the same pair was then plotted against percent differences in photosynthetic ability. This pairing was performed to eliminate variables other than miner damage from the relationship, such as leaf age, location in tree, and nutrients available during leaf development.

Results

A strong relationship is evident between photosynthetic ability and percent area of leaves affected by mining (Fig. 1). This effect was expressed across the conditions of PAR leaves were exposed to (Fig. 2). However, leaf dark respiration, or production of CO_2 in the absence of light, which can be assessed as –(photosynthesis) when PAR equals zero, did not vary with leaf damage (Fig. 3). A multivariate regression analysis performed for the purposes of assigning leaves a single damage score that included both top surface and bottom surface mining, revealed that mining on the bottom surface of the leaves actually affects the photosynthetic ability more significantly than mining on the top surface by a factor of 1.27. A plot of the weighted damage score of each leaf against maximum photosynthesis as measured at 1600 μmol photon (quanta) flux PAR·m^{-2}·s^{-1} is presented in Fig. 4.

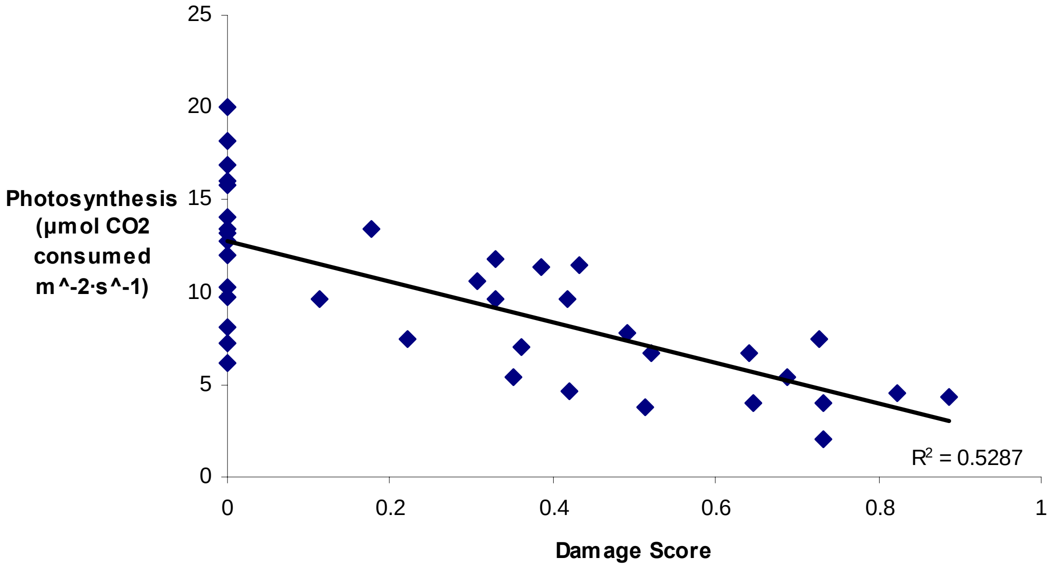

Fig. 1 Measured Maximum Photosynthesis (At PAR=1600μmol m^-2·s^-1)

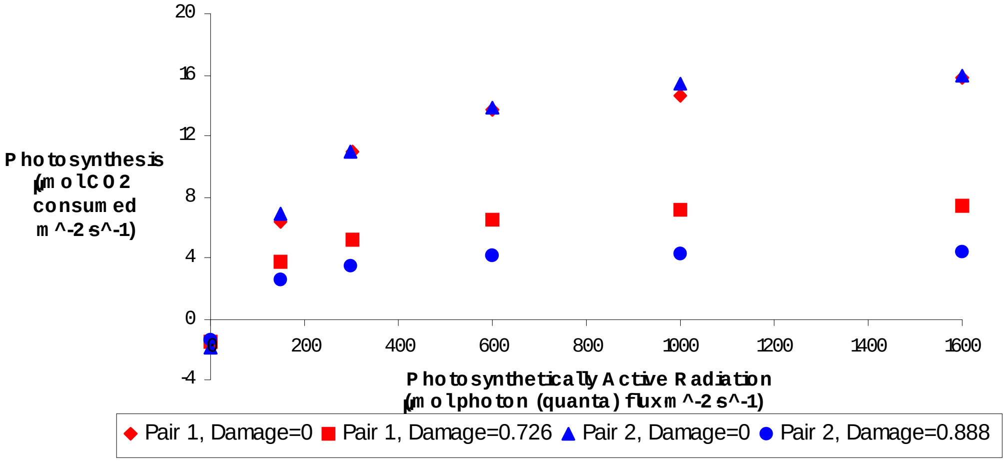

Fig. 2 Light Curves Demonstrating Differences Between Members of Two Pairs of Leaves

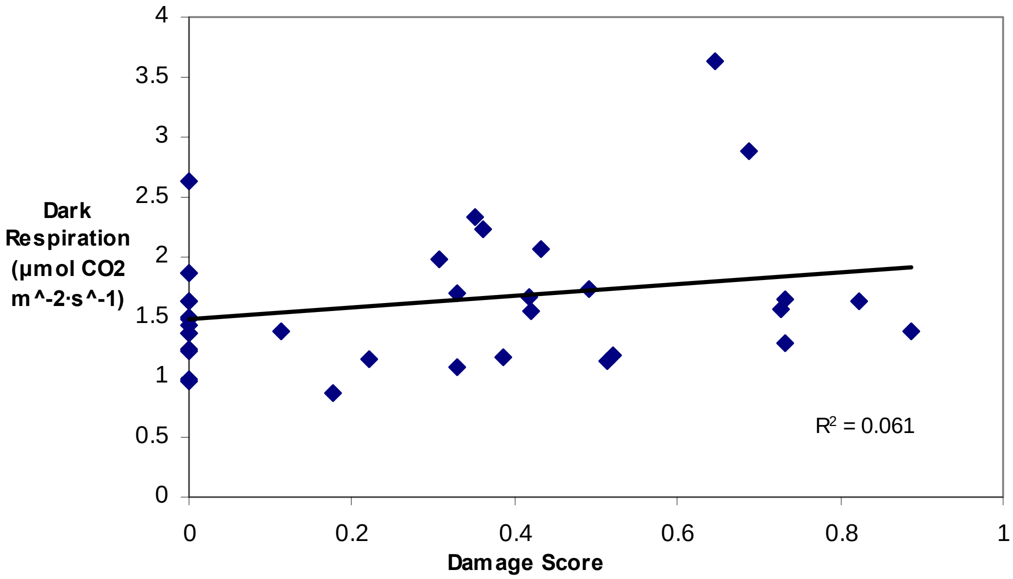

Fig. 3 Dark Respiration and Damage Score

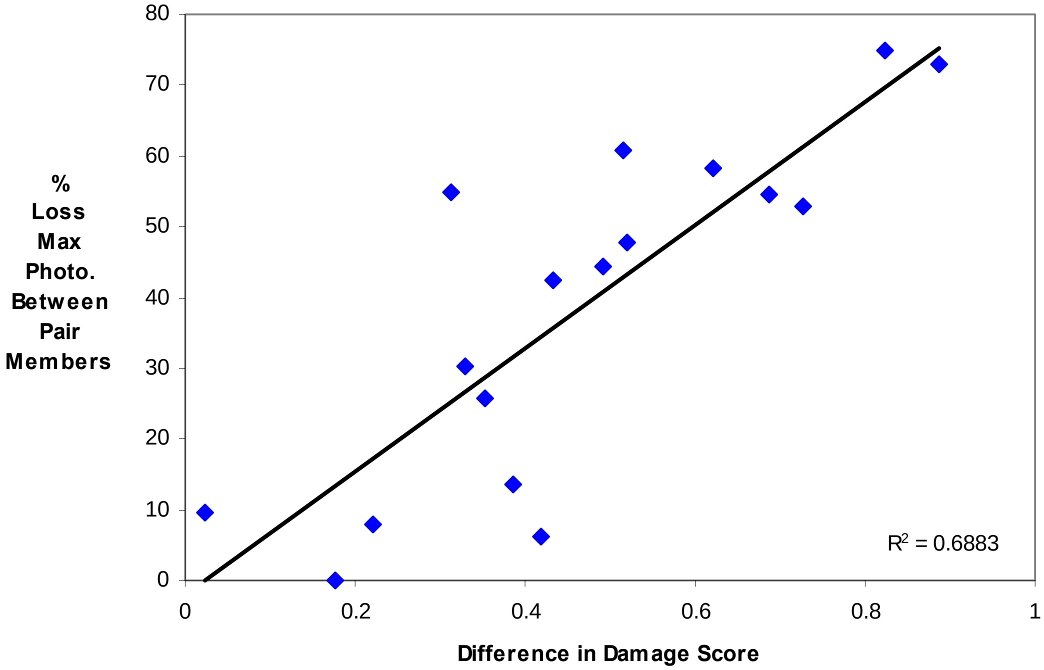

Fig 4 Plot of Calculated Paired Differences

Discussion

A strong relationship between leafminer damage and photosynthetic ability of leaves was identified. To better define this relationship and its dynamics, it would be ideal to expand my data set to include a larger sample size, and track changes in the photosynthesis of individual leaves over the course of the season, which I was not able to do here. The most surprising detail of the relationship detected between leaf damage and photosynthetic ability is that damage more immediately beneath the bottom (abaxial) surface of the leaves seems to be more detrimental to photosynthesis than top (adaxial) surface damage. This result was unexpected considering that the highest C fixation occurs in the leaf tissue nearer the dorsal side of the leaf (Evans 1995 in Lambers *et al.* 1998) where the higher concentration of chloroplasts is contained (Hopkins 1995). Also, the adaxial surface is the surface that generally receives most direct solar radiation, and the trails of leafminers (which are whitish in color) increase the albedo, or reflectance of incident radiation, of this surface, decreasing the amount of energy received by chloroplasts.

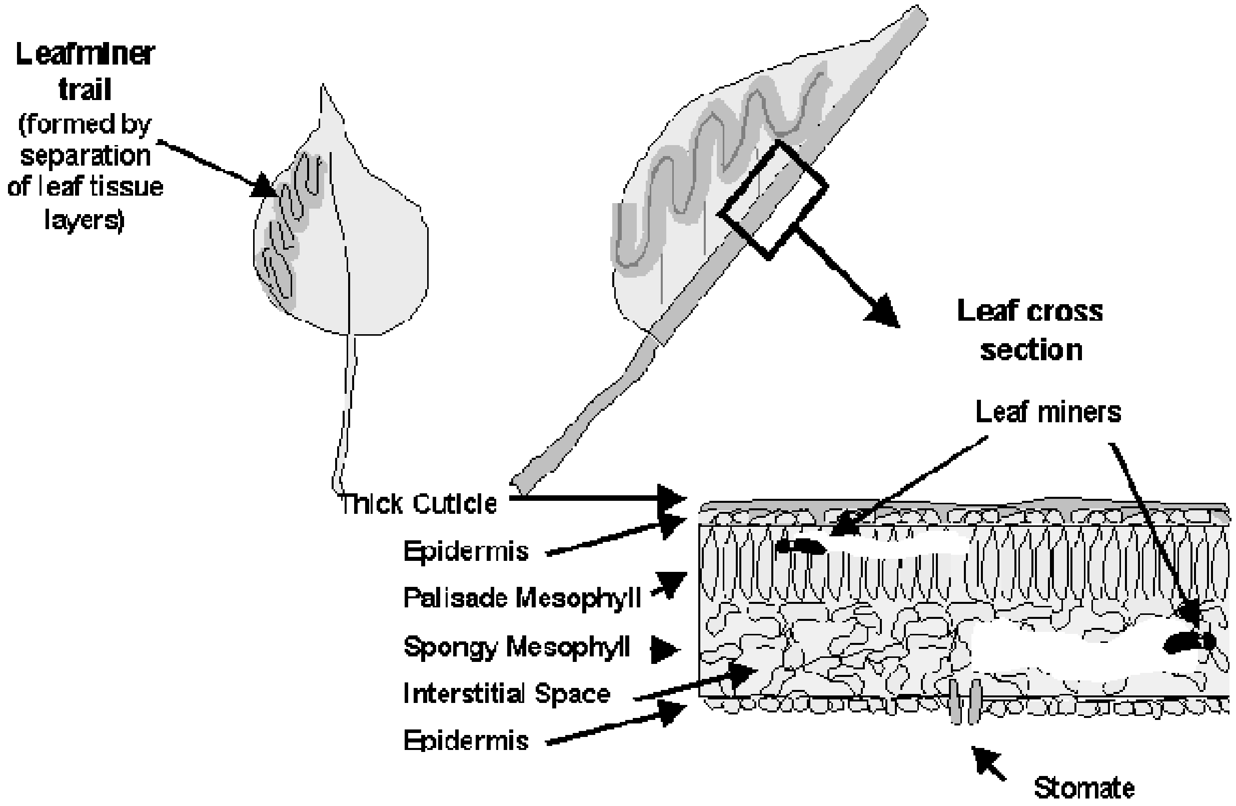

Fig. 5 (Provided by Myers-Smith 2003)

The internal structure of the leaves of *P. tremuloides* may be responsible for this phenomenon. *P. tremuloides* leaves are of fairly typical dicotyledonous form. Stomata that regulate the flow of gasses between the leaf and external environment are located on the bottom surface. The top surface is covered by a more substantial protective waxy cuticle than the bottom surface. The palisade parenchyma, which contains most of the leaf's chlorophyll, forms the upper layers of mesophyll, while spongy layer mesophyll, which has a much lower density of chlorophyll, constitutes the lower layer along with interstitial space (see Fig. 5).

The thick cuticle and the structural integrity of palisade mesophyll may enable miners of the adaxial surface to remain very close to the exterior of leaf, inadvertently damaging less of the leaf's deeper tissue. This assumption matches visual observations of top surface mines that actually raise a ridge along the trails. In contrast, the bottom surface mines appear deeper in the leaf tissue. Lower-surface mining may also adversely influence the effectiveness of stomata and increase the volume of the interstitial space; this would alter regulation of internal CO2 concentrations due to a lower internal resistance. It is also likely that miners in the spongy layer mesophyll are required to eat more of the leaf tissue than those in the palisade layer to compensate for the lower concentration of chloroplasts (and therefore nitrogen) in the spongy layer.

A significant portion of net photosynthetic loss could also be reflective of increased photorespiration. Photorespiration is photorespiratory carbon oxidation, PCO, and occurs in plants with the photosynthetic pathway of *P. tremuloides*. It results from competition between molecules of O_2 and CO_2 to react with the enzyme that fixes CO_2 in the chloroplasts to make sugars, but has equal affinity for both oxygen and carbon dioxide. Photorespiration is the competitive oxygenation by O_2 of the initial carboxylation enzyme RuBP carboxylase/ oxygenase (Chollet 1977). The cycle involves the reaction between oxygen and RuBP carboxylase/oxygenase to form phosphoglycolate. Phosphoglycolate is subsequently metabolized in a series of reactions that result in the release of a molecule of CO_2 (Hopkins 1995). Photorespiration is a function of temperature, oxygen and CO_2 concentrations and the rate of photorespiration increases with higher O_2, lower CO_2 and higher temperature (Lambers *et al.* 1998). The spaces opened within the leaf as miners pass through may allow higher concentrations of O_2 to interact with the RuBP carboxylase/ oxygenase, increasing the amount of photorespiration. This may be more pronounced in the spongy mesophyll than the palisade mesophyll because of the amount of open interstitial space.

The effects of leafminer defoliation on fecundity of *P. tremuloides* are very hard to predict. Responses of plants to herbivory can be very complicated and much research has been completed investigating these interactions. Some research indicates that certain kinds of herbivore attack might not have particularly negative impacts on the plant species involved due to compensation at the level of individual plants or plant populations (Crawley 1983, Karban *et al.* 1997, Oleksyn *et al.* 1998). Populations can sometimes benefit if herbivory alleviates crowding through removing certain individuals, which in turn allows others to grow more rapidly; individuals may benefit if herbivore feeding removes shaded leaves or causes a shift in distribution of carbohydrates that maintains the rate of net photosynthesis (Crawley 1983). As leafminers do not seem to discriminate when attacking trees, and do not cause removal of leaves or even decreased dark respiration, it does not appear either of these processes is demonstrated in this interaction. Oleksyn *et al.* (1998) reported that primary host species for *Agelastica alni,* possess leaf level physiological adaptations and defense mechanism that can attenuate the negative effects of its herbivory. Despite *P. tremuloides* role as the primary host of such species as *P. populiella,* its loss of photosynthetic ability when attacked does not indicate this effect. Karban *et al.* (1997) suggest more consideration needs to be given to the possible benefits of facultative resistance.

Zangerl *et al.* (2002) demonstrated that the photosynthetic ability of attacked leaves of *Pastinaca sativa* was compromised across an area about six times the size of the area directly affected by folivores, and that estimating photosynthetic losses based solely on area of leaf removed could underestimate impacts by a factor of three. They also asserted production of chemical defenses could be costly both in terms of seed production and respiration. Attack by insect herbivores removing 25-50% of foliage were found to greatly suppress tree productivity of two tree species for long periods of time (Morrow and LaMarche 1978). Effects of miner damage may extend well beyond the leaf area they actually injure, and be costly in term of tree production, seed production, and respiration.

Marquis (1992) reported evidence that resource movement is restricted in woody plants even under pressures of herbivory, and suggested that relatively small amounts of folivory can produce considerable detrimental effects to these plants depending on details of damage pattern and level. In deciduous woody plants, the effects of leaf herbivory are likely to be most damaging when defoliation occurs in spring, as is the case with *P. tremuloides* leafminers, because nutrients invested in spring growth are primarily derived from previously stored resources (Kaitaniemi *et al.*1999). Kaitaniemi *et al.* (1999) found that spring defoliation may also inhibit the ability of a plant to replenish its reserves as photosynthetic area available to support nutrient uptake by roots is reduced. They suggested there would be increased mortality risk due to exhaustion of stores following several subsequent herbivore attacks. In their research, mountain birch maintained leaf area at the expense of growth and reproduction, which may be as important a tolerance strategy as induced resistance (production of chemical defenses) to a long-lived plant. More leaf production at the expense of growth and reproduction in response to loss of productivity of leaf area due to leafminer folivory may be expected of *P. tremuloides*.

I conclude that if winter temperatures continue to warm as projected, outbreaks of leafminers will remain at least as frequent and severe as they have been in recent years. This may contribute to considerable changes in the population dynamics of *P. tremuloides* and possible widespread die back of this tree in the relatively near future. The impacts this will have on ecosystems containing this species are very difficult to predict. Trlica and Rittenhouse (1993) assert that herbivory has the potential to affect ecosystem structure and function both above and below ground. Aside from the direct interactions of this aspen tree with the other species of its ecosystems, alterations to soil nutrient cycling in *P. tremuloides* stands may result as trees have higher demand for such elements as nitrogen to incorporate into defense compounds. This could affect the balance of limiting nutrients and species present in the soil ecosystem beneath these stands. Also, aspen are prolific in forest types dominated by wildfire. They are both very fire resistant and good at colonizing after fire (Perala 2003). With pressures that reduce their growing and reproductive abilities, dynamics of recovery of these forests after fires will be altered as well.

The longer-term future of the interaction between *P. tremuloides* and its leafminers is even more uncertain as dramatic changes in the atmospheric concentration of CO_2 enter the equation. CO_2 concentration is increasing by 1.5 ppm/yr, and is expected to double by the end of the century (increasing from 360 ppm to 700 ppm) relative to present levels (Wigley and Raper 1992, Stiling *et al.* 1999). Working in a scrub oak community in Florida, Stiling *et al.* (1999) found that doubling atmospheric CO_2 concentrations reduces plant foliar nitrogen concentrations, decreases abundance of leaf mining insect herbivores, increases per capita leaf consumption by leafminers, and increases leafminer mortality. While reduced foliar quality contributed directly to the decrease in herbivore survival, the major factor increasing mortality was the effects of changes in herbivore feeding (bigger, more obvious mines, delayed growth of leafminers in a somewhat weakened state). This elicited greater top-down pressure from natural enemies such as parasitoids (Stiling *et al.* 1999). However, it is unclear if this same response should be expected for species such as *Phyllocnistis populiella* in sub-arctic environments, especially given the time frame and ability of insects to rapidly adapt. By the turn of the century, these species will have gone through 97 generations as this atmospheric change takes place. Given this consideration, it is also unclear if such an effect would outweigh higher overwintering successes expected of leaf miner adults with warmer winters.

Tjoelker *et al.* (1998) found that the relative growth rate of *P. tremuloides* also increased markedly growing in CO_2 concentrations of 580 ppm, though this effect decreased as the trees matured. This might suggest that some of the growing and reproductive ability loss to leaf miner defoliation might be compensated for as well.

In summary, a strong relationship between leaf area damaged by trails of leaf miners and leaf photosynthetic ability was identified. Because leaf miners actually consume very little foliar material, and damage on the abaxial side of leaves weighs more heavily than top surface damage, it is proposed that a significant portion of this loss of photosynthetic ability is due to alterations in gas exchange control of the leaf, which may elevate photorespiration. The consequences of continued consecutive outbreaks of leaf miners may contribute to widespread die off of *P. tremuloides* in its current range, though long-term projections of ecosystem feedbacks and compensation are very complicated and have considerable uncertainties.

Acknowledgements

I would like to express special thanks to University of Alaska, Fairbanks graduate student Isla Myers-Smith for her help and suggestions throughout this project, UAF Professor Roger Ruess for his instruction and use of expensive equipment, Linda DeFoliart for her helpful collaboration, and ES 196 instructors John Latto and Donna Green for their suggestions and assistance in writing this report.

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