# A perspective on the importance of within-tree variation in mortality risk for a leaf-mining insect

**C. Low**[1] **and D. Hanley**[2]

[1]Cornell University, Ithaca, NY, USA
[2]University of Guelph, Guelph, ON, Canada

*Correspondence to:* C. Low (cl754@cornell.edu)

**Abstract.** Within-tree variation in abiotic conditions can create a mosaic of fitness gradients for herbivorous insects. To explore these effects, we quantified the patterns of mortality of the solitary oak leafminer, *Cameraria hamadryadella* (Lepidoptera: Gracillariidae), which lives within leaves of white oak, *Quercus alba*. We found differential patterns of survival and larval feeding rate within the tree and in association with several abiotic factors: light levels, leaf nitrogen content, and canopy height. We suggest that the leaf scale microhabitat conditions are fundamental to plant-herbivore-enemy interactions because of the differential fitness effects on herbivores. Such effects would be missed by studies that average effects by whole plants. Our study population of *C. hamadryadella* is located within the Orland E. White State Arboretum of Virginia in Boyce, Virginia, USA.

## 1 Introduction

Common to all herbivorous insects, survival and reproductive success depend critically on the quality of the host plant upon which they feed. Moreover, the microhabitat conditions that surround an herbivore while it feeds can directly influence its performance, growth, and survival through differences in temperature, humidity, and exposure to natural enemies and pathogens (Alonso, 1997; Cornelissen and Stiling, 2006; Kang et al., 2009). Therefore, the decision of where to feed for an herbivore can be critical for both survival and fecundity, since the plant serves as both a shelter and a food resource (Connor and Taverner, 1997). This has led numerous studies to investigate the correlations between female oviposition decisions and offspring fitness (Connor, 2006, 1991; Craig and Abrahamson, 1999; Courtney and Kibota, 1990; Craig et al., 1989; Sadek, 2011). The aim of our study was to characterize the mosaic of survival and performance patterns for insect herbivores against the within-tree variation in abiotic conditions. The importance of such patterns have been strongly supported by recent evidence (Pincebourde et al., 2007; Pincebourde and Casas, 2005, 2006a, b; van Loon et al., 2005).

Herbivore fitness should vary at the leaf-level because the abiotic conditions surrounding a leaf can affect not only leaf quality, but also the physiology, mobility, and safety of an insect herbivore which often remains sedentary while feeding (Collinge and Louda, 1988; Connor, 1988; Stamp and Wilkens, 1993). For example, the amount of sunlight that reaches a leaf affects temperature and humidity levels, which together affect photosynthetic efficiency and the leaf nutritional quality. However, these factors can also contribute to the risk of desiccation for eggs, larvae or pupae. In addition, because insects are ectothermic, temperature differences can alter the ability of an insect to flee attacks by predators and to thermoregulate (Chai and Srygley, 1990; Fordyce and Shapiro, 2003; Pincebourde and Casas, 2006a). Lastly, the ambient light habitat can play a direct role in predation risk by affecting the visual conspicuousness of the herbivore to its enemies, which has been rarely considered (but see, Low, 2008).

Here, we present the results from a descriptive study in which we quantified several ecological variables that we hypothesized to be correlates of survival in a population of a leaf-mining moth, *Cameraria hamadryadella* (Lepidoptera: Gracillariidae). These are ambient light intensity,
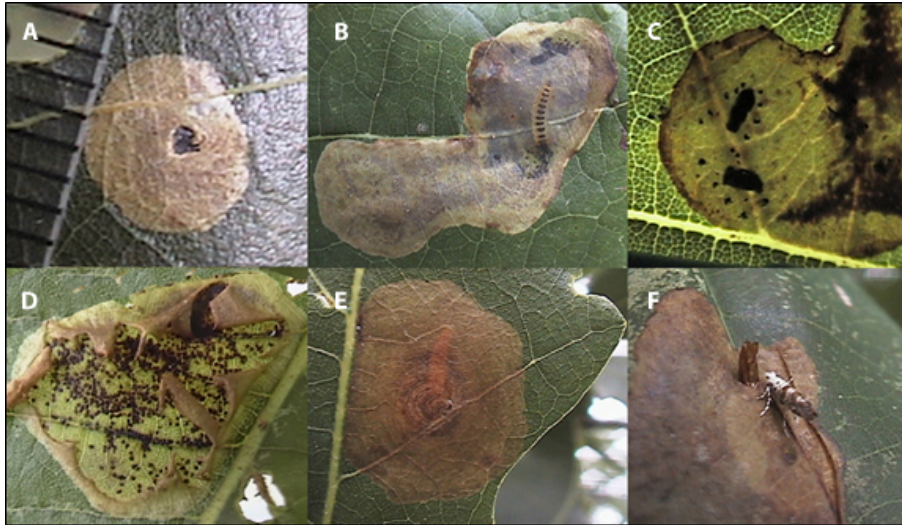
**Figure 1.** Physical cues used to diagnose the fates of *Cameraria hamadryadella*. **(A)** Parasitioid emergence hole; **(B)** parasitism indicated by the black bands in the host larva; **(C)** pupae of an endoparasitoid after consuming host and pupating within its mine; **(D)** predation indicated by a broken mine with larva removed; **(E)** intact mine and visible larva showing no obvious signs of parasitism or disease; **(F)** newly emerged adult and from pupal exuvia at mine surface.

leaf quality, and canopy height. Because *C. hamadryadella* larvae remain within a single leaf throughout their development from egg stage to pupation, the immediate conditions surrounding them and their natal leaf are likely to be critical influences on their fitness. We present some general patterns of larval mortality and performance against the variability of leaf-level abiotic conditions.

## 2   Material and methods

### 2.1   Study system

We studied a population of the solitary oak leafminer, *Cameraria hamadryadella* (Lepidoptera: Gracillariidae) within the oak grove at the Orland E. White State Arboretum of Virginia in Boyce, VA, which has been researched previously (Connor and Cargain, 1994; Connor, 1991; Connor and Beck, 1993; Connor et al., 1999). *Cameraria hamadryadella* is a bivoltine leaf-mining moth that feeds on oaks (*Quercus* spp.) (Needham et al., 1928). Eggs are laid singly onto the upper surfaces of leaves, and when larvae hatch, they burrow into the mesophyll layer of the leaf. Each larva creates a solitary mine by excavating the leaf mesophyll as it feeds, which creates a visually conspicuous mining scar on the upper surface of the leaf (Fig. 1). Mating, oviposition, and egg hatch of *C. hamadryadella* typically occur between mid-May and early June, and larval development is completed between mid-July and mid-August. A second generation typically occurs during the fall months, which overwinters as pupae within the fallen leaves. Our study was restricted to the first generation of *C. hamadryadella*.

We focused our sampling effort to a single large oak tree that was between 100–150 yr in age because of the low densities of larvae in other trees and general logistical difficulties of accessing those leaves. Therefore, we would like to make the clear caveat that that the results from this work are descriptive, but nevertheless can help to shape ideas for future studies.

### 2.2   Larval mortality and performance

Between 28 and 30 June 2002, we monitored selected *Q. alba* leaves with a single leaf mine each from 3 strata (mean height in cm ± 1 SD): (1) ground level ($n = 18$, $126 \pm 27$ cm), (2) middle ($n = 26$, $409 \pm 67$ cm), and (3) canopy ($n = 24$, $591 \pm 65$ cm). In total, we scored and monitored 68 individual leaf-mining larvae, which were accessed using a hydraulic cherry picker operated with a tractor and free climbing techniques. We scored survival, parasitism, or predation using physical cues such as the pupal exuviae of *C. hamadryadella*, parasitoid emergence holes, or predation scars, respectively (Fig. 1). "Unknown" was assigned to those larvae that had ceased to develop but the source of death could not be determined with visual indicators. These mortality types may be caused by host plant defences, inadequate biotic or abiotic conditions for larval growth, failed parasitoid development after killing the host, or diseases caused by fungi, bacteria, viruses, or other pathogens (Downum, 1992).

Because mine size corresponds directly to the amount of leaf tissue consumed by an individual leafminer, we used the change in the area of a mine over time as a proxy for feeding rate and larval performance. To estimate mine expansion rate, we measured mine area using digital images taken of mines
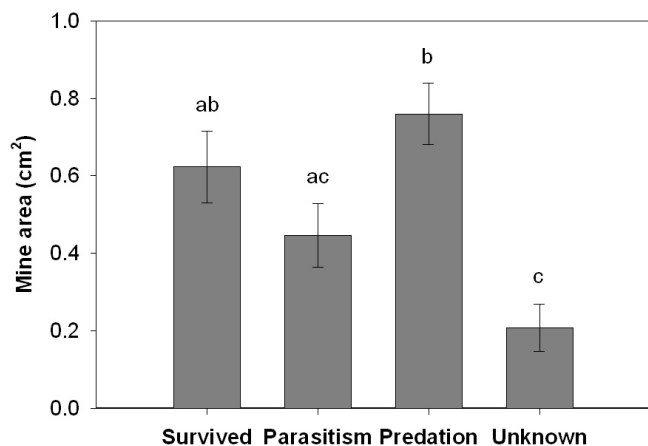
**Figure 2.** Mine sizes of larvae according to each fate category (mean ± 1 se). Letters above error bars indicate significant differences ($p < 0.05$) detected from pairwise tests using Bonferroni method of multiple comparisons.



**Figure 3.** Light intensity at each leaf associated with each fate class under clear and overcast lighting conditions (mean ± 1 se).

every 5–7 days, from 1 July to 7 August 2004. We quantified mine area using image analysis software (Sigma Scan 5.0). We calculated larval feeding rate as the difference between initial and final mine sizes divided this by the total number of days. We also used these data to determine mortality of larva as "unknown" if a mine had not increased in size.

## 2.3 Micro-environmental variables

We calculated the absolute brightness of the ambient light as the total irradiance ($\mu$mol photons per m$^2$ per sec) summed across the visible light spectrum (300 to 700 nm). Irradiance spectra were measured approximately 10 cm above each leaf using a portable spectroradiometer (USB2000 from Ocean Optics, Inc.) and a cosine corrected sensor (CC-3-UV), calibrated with a standard light source (Licor 1800-02). We sampled the solar irradiance on 28 July and 6 August 2002, which were characterized by overcast and clear conditions, respectively. We restricted our sampling between 11:00–15:00 hours hours for both dates, and only when the sun was overhead and unobstructed by clouds on the "clear day". On the overcast day, sunlight was diffuse and indirect for all measurements. Although the amount of light received by a leaf can change with shifts in the angle of the sun, we quantified light levels when the sun was directly overhead so that the maximum brightness was available, which allowed us to have consistent measurements with maximal solar incidence. We expected that by measuring maximum range of lighting conditions between clear skies and diffuse sunlight, our data would capture the typical range of within-tree variation in ambient light conditions.

As leaf quality indicators, we measured leaf nitrogen content (percent of leaf mass) and specific leaf area (cm$^2$ per g). For leaf nitrogen content, all leaves were collected at the end of the study and dried at 60 °C for 48 h. Leaves were
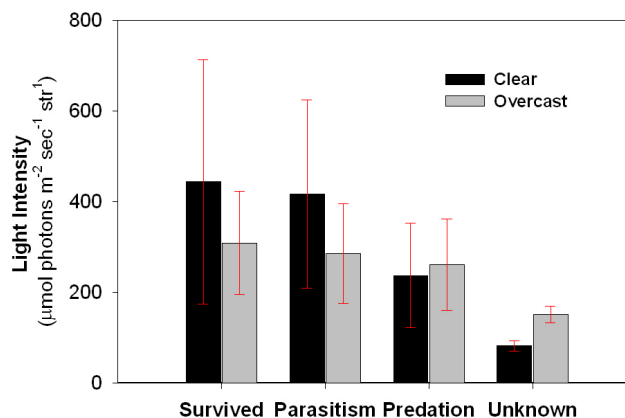
then weighed and scanned into digital images to measure leaf area. They were then ground into a homogenous powder to pass through a 40-mesh sieve, and sent the samples to the Department of Air and Natural Resources (DANR) Analytical Lab, University of California, Davis for analysis of total percent nitrogen using a combustion method. We measured specific leaf area after cutting out the mines and petioles before digital measurements of the remaining leaf area and dry weight. Increases in specific leaf area are often correlated with increased light interception and the rate of photosynthesis per unit nitrogen, or "nitrogen-use efficiency" (Poorter and Evans, 1998; Reich et al., 1998; Lei et al., 1996; Schieving and Poorter, 1999). Thus, if specific leaf area, nitrogen, and light are critical variables that can predict leaf quality, then the performance of herbivorous insects should also be positively correlated with each of these variables (Suomela et al., 1995; Awmack and Leather, 2002).

## 3 Results

### 3.1 Larval mortality

In total, we determined the fates of 64 larvae. Eleven survived to pupation (17 %), 16 died from parasitism (25 %), 22 died from predation (34 %), and 15 died from unknown causes (23 %). We were unable to recover 4 of the marked leaves. The average mine area (cm$^2$) differed between fate classes ($F_{3,60} = 9.5$, $P < 0.001$, Fig. 2), and post-hoc comparison tests detected significant differences in mine size between survival and unknown ($P = 0.01$), parasitized and predation ($P = 0.04$), and predation and unknown ($P < 0.001$) (Fig. 2). Those mines that survived were intermediate in size overall. Larvae that died from unknown causes had the smallest mines, which indicated lack of development soon after egg hatch. Post-hoc analysis showed that the average height at which unknown causes of mortality occurred was significantly greater than the heights of the other fates ($\chi^2 = 8.8$,

**Table 1.** Results from stepwise multiple regression analysis of feeding rate (ln-transformed) and three predictor variables: light intensity (μmol photons), leaf nitrogen content (percent), and canopy height (m). The statistics evaluate the changes in variance explained with the addition of each predictor variable; hence three basic models are evaluated. Light was excluded when using the light intensity data from the overcast day.

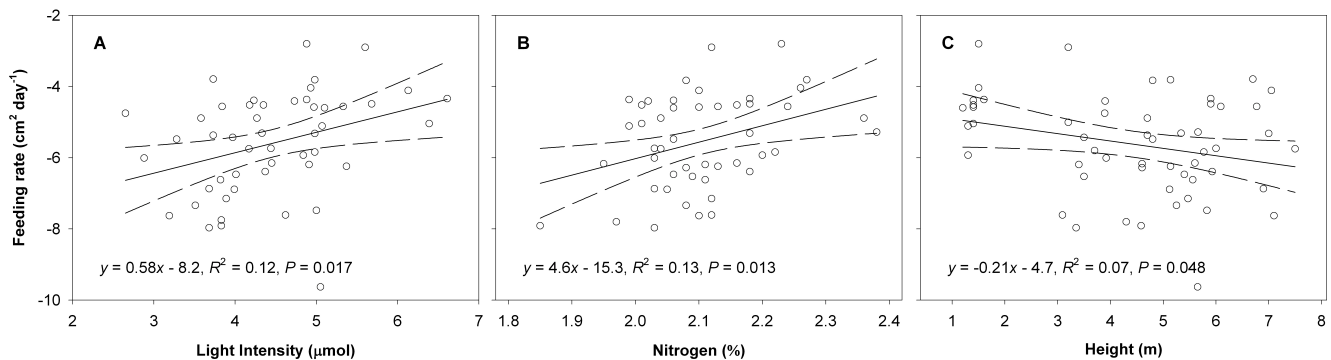| Model | $R^2$ | $R^2\Delta$ | $F\Delta$ | $P(F\Delta)$ | SS | $F$ | d$f$ | $P$ |
|---|---|---|---|---|---|---|---|---|
| Clear day | | | | | | | | |
| Light | 0.16 | 0.16 | 7.66 | 0.009 | 11.4 | 7.66 | 1,39 | 0.009 |
| Light, nitrogen | 0.26 | 0.10 | 4.92 | 0.033 | 58.0 | 6.67 | 2,38 | 0.003 |
| Light, nitrogen, height | 0.35 | 0.09 | 5.05 | 0.031 | 24.2 | 6.60 | 3,37 | 0.001 |
| Overcast day (light excluded) | | | | | | | | |
| Nitrogen | 0.17 | 0.17 | 4.92 | 0.007 | 11.6 | 8.18 | 1,39 | 0.007 |
| Nitrogen, height | 0.29 | 0.12 | 5.05 | 0.016 | 19.5 | 7.83 | 2,38 | 0.001 |



**Figure 4.** Univariate regression analyses of feeding rate to light intensity from clear day (**A**), percent leaf nitrogen (**B**), and canopy height (**C**). Dashed lines represent 95 % confidence intervals. Please note that a log-scale was used for feeding rate and light intensity. (* $P < 0.05$)

d$f = 1$, $P = 0.003$). The average brightness of areas where the unknown category of mortality occurred tended to be lower on average but this was not statistically significant ($F_{3,50} = 1.1$, $P = 0.34$, Fig. 3).

### 3.2   Larval performance

Larval performance, measured as feeding rate, could be predicted by light intensity from the clear day only, canopy height, and leaf nitrogen according to a stepwise multiple regression analysis (Table 1, Fig. 4). When using the overcast day light data, light intensity was excluded from the model, and only nitrogen and height were significant predictors (Table 1). In the clear day model, 35 percent of the variance in larval feeding rate ($y$) could be explained by light intensity ($x_I$), nitrogen content ($x_N$), and canopy height ($x_H$) ($y = 0.003x_I + 5.6x_N - 0.2x_H - 16.7$; $F_{3,37} = 6.6$, $P = 0.001$). After light was excluded from the overcast day model, nitrogen and height explained 32 percent of the variance in larval feeding rate ($y = 6.6x_N - 0.2x_H - 18.5$; $F_{3,38} = 5.7$, $P = 0.003$). The exclusion of light as an explanatory variable from the overcast day model is likely due to the low variability of over-

cast conditions, where the lighting conditions can be characterized as white and diffuse (Supplement Fig. S1).

### 4   Discussion

Our work aimed to capture the details of plant-insect interactions at the microhabitat scale and to assess the relevance of within-tree variation of several environmental variables on the survival of the solitary leafminer, *C. hamadryadella* on white oak, *Q. alba*. First, we found that mortality occurred most commonly with small and large mines, leaving the intermediate-sized mines as the safest size overall. Because the leaf mine is the amount of leaf material that has been consumed, this result suggests that the upper end of herbivory damage may be limited by natural enemies in terms of the amount of damage that a single leafminer could do. In general, mine size might also be a reliable cue for natural enemies to detect a suitable host or prey since it corresponds to the size of a larva. In the case with leaf-mining larvae, avian predators, except for chickadees, may not prefer small leaf-mining larvae because they are difficult to excavate and especially if alternate prey are available (Low and Connor, 2003; Connor et al., 1999).

Given that there is differential mortality risk associated with mine size, then feeding rate is likely to play a role in how a leafminer might negotiate these stage-specific risks. Especially for those species that have restricted mobility, microhabitat conditions are likely to directly affect larval performance and growth (Fortin and Mauffette, 2002; Levesque et al., 2002; Mattson, 1980). In particular, our result that both light intensity and leaf nitrogen content were significant predictors of feeding rate supports the potential importance of both direct and indirect effects on larval performance. Because light is correlated with temperature and humidity, it can affect both larval metabolism and feeding activity. On the other hand, light also affects the photosynthetic efficiency of the leaf, and as a result, larvae are indirectly affected by subsequent changes in leaf nutritional quality. Light and nitrogen together can have synergistic effects on larval fitness by affecting their performance and the time of exposure to mortality risks (Mattson, 1980; Benrey and Denno, 1997).

Lastly, we found that greater canopy heights were associated with slower feeding rates and a greater incidence of the unknown category of mortality. Previous research has found that tree architecture and surrounding landscape can influence microhabitat temperatures and, as a consequence, leafminer growth rate (Pincebourde et al., 2007). Because we found no differences in the nitrogen content of leaves with height, we suggest that there are other factors related to tree architecture and microclimate that are limiting the growth and development of larvae in the upper canopy. The association between height and unknown mortality could reflect the within-tree variability in defence against herbivory or other agents, such as pathogens, that could cause larval death at early stages of development. In general, the clear patterns of mortality risks found within a tree are likely to represent a complex landscape for herbivorous insects and leaf-level plant defence strategies (Gripenberg et al., 2007; Suomela and Ayres, 1994). From the herbivore's perspective, variation in abiotic conditions at the microhabitat scale is critical for survival because these conditions can regulate their exposure to different mortality agents.

The association between the abiotic environment, leaf quality, and mortality risk factors points to the complexity of trophic interactions that can occur within a tree. We suggest that future research investigating interactions in plant-insect-enemy systems should consider the interactions between natural enemy behaviour, host plant characters, and environmental conditions at the leaf-level (Suomela, 1996; Suomela and Ayres, 1994). In addition, more research is needed to understand the mechanistic links between the abiotic environment and herbivore survival (e.g., Pincebourde et al., 2007; Pincebourde and Casas, 2005, 2006a, b; van Loon et al., 2005). Understanding optimization patterns and trade-offs within an individual tree can provide key insights into the importance of within-tree variation, which can be masked when averaging effects between entire plants or trees.

**Supplementary material related to this article is available online at: http://www.web-ecol.net/12/27/2012/ we-12-27-2012-supplement.pdf**.

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