Hanging in the treetops: an *in situ* experiment in ancient hemlock assessing outbreak defoliator performance among crown levels

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Abstract. 1. Past studies have shown that both egg lay and larval feeding of a generalist defoliator, pale-winged gray (*Iridopsis ephyraria* Walker) (Lepidoptera: Geometridae), are concentrated in the mid-lower crown of eastern hemlock [*Tsuga canadensis* (L.) Carr.].

2. Using tree-climbing techniques, we carried out *in situ* bioassays in large hemlock trees (∼25 m) to determine whether the observed foraging preferences are adaptive and how they reflect associated intra-tree variations in microhabitat quality associated with temperature and foliage nutritional quality.

3. In 1 of 2 years, larval survival was significantly higher in the shaded lower versus sunlit upper crown; however, in both years, groups that fed in the sunlit upper crown branches had larger male and female moths and more female-biased sex ratios.

4. Differences in pale-winged gray performance among crown levels were somewhat supported by trends in foliage nutritional chemistry but not well-correlated with variation in temperature.

5. The present study is one of only a few to carry out bioassays on large mature trees and results reinforce the idea that foraging patterns of herbivores are likely to reflect trade-offs among several factors that vary between sunlit and shaded branches within forest canopies.

Key words. Foliage quality, foraging behavior, *Iridopsis ephyraria*, microclimate, tree canopy ecology.

Introduction

Mature tree crowns, owing to their large size and structural complexity, offer a complex mosaic of food and protection for many organisms, including a broad variety of herbivorous insects. Although foliage may be found throughout the crown, the branch tips and upper crown are the most open to sunlight and, compared with the crown interior, tend to provide warmer microclimates and more nutritious foliage for herbivores (White, 1984; Dudt & Shure, 1994; Fortin & Mauffette, 2002; Osier &

Jennings, 2007). However, insect herbivores foraging in open parts of the crown may also be more exposed to natural enemies (Eikenbary & Fox, 1968; Weseloh, 1972; Kemp & Simmons, 1978; Jennings & Houseweart, 1983; Aikens & Buddle, 2012) or extreme weather (Quiring, 1994; Bento *et al.*, 2001; Johns *et al.*, 2012). These competing selective pressures are likely to play a central role in shaping how, when, and where both predators and herbivores forage within trees (Denno & McClure, 1983).

While there is considerable evidence showing that herbivore distribution and abundance varies within trees (e.g. Pinault *et al.*, 2007; Fry *et al.*, 2009; Eveleigh & Johns, 2014), comparatively less work has gone into examining the underlying mechanisms influencing observed patterns. To a large extent, this knowledge gap reflects the inherent challenges of accessing the crowns of

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large trees for experimentation. Experiments for defoliators can be particularly daunting as much of the foliage produced by trees occurs at the edge of the crown, on thin branches far up in the tree and many meters from the main trunk. Circumventing this challenge, several field experiments in recent decades have instead studied small trees (1–3 m tall), focusing on moth and sawfly herbivores that tend to outbreak on regenerating trees (e.g. Quiring, 1993; Carroll & Quiring, 1994; McKinnon *et al.*, 1999; Johns *et al.*, 2006, 2010). Although substantial variation in defoliation has also been reported to occur in mature trees (e.g. Pinault *et al.*, 2007; Fry *et al.*, 2009; Eveleigh & Johns, 2014), the difficulty in accessing the foliated parts of their crowns (i.e. the branch tips) has generally precluded detailed field experiments. Indeed, past experiments in mature trees have relied almost exclusively on bioassays conducted under laboratory conditions using cut shoots or leaves (Carisey & Bauce, 1997; Fortin & Mauffette, 2002, but see Larrivée and Buddle 2009).

In the present study, we used tree-climbing techniques to study *in situ* the effects of heterogeneity in microhabitat quality within crowns of mature eastern hemlock [*Tsuga canadensis* (L.) Carr.] on the performance of a generalist insect herbivore, the pale-winged gray (*Iridopsis ephyraria* Walker) (Lepidoptera: Geometridae). Pale-winged gray is a widely distributed moth that persists at relatively low population densities across much of Canada and the U.S.A. (Rindge, 1966). However, in 2002, there was an unprecedented outbreak of pale-winged gray in numerous hemlock groves throughout Nova Scotia, Canada, including Kejimkujik National Park (Pinault *et al.*, 2007). After only a single year of defoliation associated with larval feeding, nearly 90% of understory hemlock saplings were killed; in the following year, nearly 40% of the mature hemlock were killed (Pinault *et al.*, 2007; Pinault & Quiring, 2008). The rapid and dramatic impact of the larval feeding on trees is associated, at least in part, with their capacity to eat multiple age-classes of foliage, although the developing foliage is preferred (Pinault *et al.*, 2009). Other studies also indicated that defoliation was concentrated in mid-lower crown levels (Pinault *et al.*, 2009), owing, at least in part, to the apparent oviposition preference of egg-laying moths and feeding preference of larvae for mid-lower crown levels (Hervieux *et al.*, 2012). This foraging pattern contrasts with that of other conifer-feeding insects that have been studied in the past, the majority of which feed predominantly in the upper crown of their host (e.g. Wallin & Raffa, 1998; Quiring, 1993; Johns *et al.*, 2006; Eveleigh & Johns, 2014; but see Anstey *et al.*, 2002).

We tested the hypothesis that the observed foraging preference of a pale-winged gray for mid and lower crown foliage was adaptive (i.e. promoted higher larval performance than potential alternative strategies). Experiments were conducted on large, mature hemlock trees to assess the performance of larvae in shaded and sunlit parts of the upper crown and the shaded lower crown. To gather some additional information on microhabitat quality and the possible mechanisms influencing the observed intra-crown distribution, we also assessed temperature and foliage nutritional chemistry near each of our treatment branches.

Material and methods

Experimental design

A manipulative field study was carried out in 2009 and 2010, to evaluate the effects of foliage quality and temperature on the performance of pale-winged gray developing within crowns of large mature hemlock. Field studies were carried out in southwest Nova Scotia, Canada in 2009 and 2010. Sites were located at Moose Hill (44°7′25.17″N/64°47′44.34″W) in 2009–2010 and Milton (44°4′40.64″N/64°49′10.44″W) in 2010. One mature eastern hemlock dominated stand was selected at each site. Mature trees were approximately 25 m tall, with a mean diameter at breast height of 162.06 ± 8.48 cm and 162.00 ± 6.74 cm at Moose Hill ($n = 9$ trees) and Milton ($n = 9$ trees), with new trees selected each year. The selected stands did not contain any noticeable defoliation, and few naturally occurring pale-winged gray larvae were observed during the 2-year study. First-instar pale-winged gray larvae used in manipulative experiments (described below) were collected from other unmanaged hemlock stands in Queens County, Nova Scotia. Larvae were reared on branches in sleeve cages at locations on mature trees that varied in their exposure to sunlight and nutrient qualities.

We haphazardly selected two branches per tree from each of three canopy 'zones': (i) sunlit upper crown; (ii) shaded upper crown; and (iii) shaded lower crown. To minimize the chance that branches in the shaded upper crown and shaded lower crown were exposed to sunlight, we selected shaded branches in the upper and lower crown that never extended >1 m from the tree bole. To access the upper crown of trees, we employed a modified single-rope climbing technique (e.g. Laman, 1995; Ter Steege, 1998), which allowed us to enter safely the vegetative crown and to access the tips of branches.

After budburst, one open vial containing five first-instar larvae was attached to each selected branch with twist ties. Each branch was then enclosed in a sleeve cage, as described in Pinault *et al.* (2009). Previous studies have demonstrated that sleeve cages protect caterpillars from parasitoids and predators but do not significantly influence caterpillar development, branch growth, or leaf chemistry (Rossiter *et al.*, 1988; Quiring, 1993; Carroll & Quiring, 1994; Parsons *et al.*, 2005). Each sleeve cage contained multiple foliage age-classes, although because of the feeding preference of pale-winged gray larvae (Pinault *et al.*, 2009), mainly developing foliage was eaten. Relatively low levels of defoliation (<30% for any age-class) in sleeve cages indicated that foliage was not limiting for larvae.

In August, after most larvae had pupated, branches were cut (with sleeve cages still attached) and transported to a laboratory at the University of New Brunswick for processing. Surviving juveniles (late-instar larvae or pupae) found in sleeve cages were collected and reared in plastic containers containing moistened vermiculite. Water and current-year to 2-year-old foliage were provided on a daily basis to mature late-instar larvae until they pupated. Emerged adults were sexed based on antennal morphology (Rindge, 1966). The length of the subcostal vein on the forewing of emerged adults was measured as a fitness correlate as realised fecundity is positively related to subcostal vein length for pale-winged gray under laboratory conditions (D.Q., unpublished data). Similar relationships between female

Table 1. Akaike's information criteria (AICs) of generalized linear mixed models evaluating the effects of Treatment (A), Year (B), Site (C), and Tree (D) on larval and total survival of the pale-winged gray moth.

Larval survival					Total survival			
#	Model	AICc	Δ_i	W_i	Model	AICc	Δ_i	W_i
1	**A + B + A:B + (1|C/D)**	**60 0.86**	**0.00**	**0.58**	**A + B + A:B + (1|C/D)**	**495.39**	**0.02**	**0.41**
2	A + B + (1|C/D)	604.45	3.59	0.10	**A + B + (1|C/D)**	**497.31**	**1.94**	**0.16**
3	A + (1|C/D)	606.31	5.45	0.04	A + (1|C/D)	501.45	6.08	0.02
4	**B + (1|C/D)**	**602.33**	**1.47**	**0.28**	**B + (1|C/D)**	**495.37**	**0.00**	**0.41**

A total of 163 sleeve cages were retained for analyses.
A = Treatment = categorical variable with three levels: Shade upper crown, Shade lower crown and Sun upper crown, B = Year = categorical variable with two levels: 2009 and 2010 and (1|C/D) = Random effect of Tree ID nested in Site. Larvae in the upper crown were located either in the partial shade (Upper shade) or fully exposed to sunlight (Upper sun), whereas all larvae in the lower crown were located on fully shaded branches (Lower shade). Δ_i = Delta AIC and W_i = Akaike weights. Significant effects ($P < 0.05$) of treatment are represented in bold.

moth wing length and fecundity have been established for moths in the past (e.g. Carroll & Quiring, 1993).

To assess variation in temperature between crown locations, one DS1923 temperature iButtons logger (Dallas Semiconductor, San Jose, California) was placed inside one sleeve cage in each of the three crown positions on two trees at Moose Hill and on two trees at Milton. The iButtons recorded temperature (°C) once per hour and remained in the sleeve-cages throughout the season. Temperature data were collected only in 2010.

To assess foliage nutritional chemistry, as it related to foliage water content, other nutrients (nitrogen, sugars, P, Ca, and Mg) and secondary compounds (phenol and monoterpenes), in 2010 we collected shoots from branches adjacent to branches bearing sleeve cages from each crown position on three trees per site. Foliage for biochemical analyses was collected at the beginning (early June) and end (late July) of larval development, immediately frozen and stored at −20 °C. The current-year foliage

was processed and analyzed at Laval University. For a complete description of the techniques used for biochemical analysis of the foliage, refer to Bauce *et al.* (1994). Briefly, mineral contents were assessed using a full injection analyzer and soluble sugars were analyzed by performing a methanol–chloroform–water extraction (Dubois *et al.*, 1956). An acetone extraction plus the Folin–Denis method (Swain & Hillis, 1959) was used to obtain total phenolic content. On the foliage, a methanol–hexane extraction was performed to assess monoterpene concentrations. Extracts were analyzed with a Varian GC3900 gas chromatograph (Varian, Palo Alto, California).

Statistical analyses

The influence of treatment (i.e. the three crown locations) and year on pale-winged gray performance (Tables 1 and 2), foliage

Table 2. Akaike's information criteria (AICs) of the generalized linear mixed model of subcostal vein length of the pale-winged gray moth according to Treatment (A), Year (B), Sex (C), Site (D), and Tree (E) variables.

#	Model	AICc	Δ_i	W_i
	Subcostal vein length[a]			
1	A + B + C + A:B + A:C + B:C + A:B:C + (1|D/E)	294.29	10.97	0.00
2	A + B + C + A:B + A:C + B:C + (1|D/E)	290.08	6.77	0.01
3	A + B + C + A:B + A:C + (1|D/E)	289.18	5.87	0.02
4	A + B + C + A:B + B:C + (1|D/E)	285.56	2.25	0.10
5	A + B + C + A:C + B:C + (1|D/E)	291.26	7.95	0.01
6	**A + B + C + A:B + (1|D/E)**	**284.78**	**1.47**	**0.15**
7	A + B + C + B:C + (1|D/E)	286.85	3.54	0.05
8	A + B + C + A:C + (1|D/E)	289.68	6.37	0.01
9	**A + B + C + (1|D/E)**	**285.35**	**2.03**	**0.11**
10	A + B + (1|D/E)	300.45	17.14	0.00
11	**A + C + (1|D/E)**	**283.31**	**0.00**	**0.30**
12	B + C + (1|D/E)	286.28	2.97	0.07
13	A + (1|D/E)	298.39	15.08	0.00
14	**C + (1|D/E)**	**284.46**	**1.15**	**0.17**
15	B + (1|D/E)	298.45	15.14	0.00

A total of 187 individuals were retained for analyses.
A = Treatment = categorical variable with three levels: Shade upper crown, Shade lower crown and Sun upper crown, B = Year = categorical variable with two levels: 2009 and 2010, C = Sex = categorical variable with two levels: Male and Female and (1|D/E) = Random effect of Tree ID nested in Site. Larvae in the upper crown were located either in the partial shade (Upper shade) or fully exposed to sunlight (Upper sun), whereas all larvae in the lower crown were located on fully shaded branches (Lower shade). Δ_i = Delta AIC and W_i = Akaike weights.
[a]Significant effects ($P < 0.05$) of treatment are represented in bold.

Table 3. Influence of crown location, sun exposure, and month on the nutritional quality of current-year foliage in terms of nutrients [nitrogen, water, sugar (mean ± SE, in %), minerals (P, Mg and Ca (mean ± SE, in ppm)], and allelochemicals [monoterpenes (mean ± SE, in ng/mg of dry foliage) and phenolics (mean ± SE, in %)] in mature eastern hemlock during the summer of 2010.

Constituent	Month[a]					
	June			July		
	Lower shade	Upper shade	Sun	Lower shade	Upper shade	Sun
Nutrients						
Nitrogen	2.54 ± 0.11	2.48 ± 0.09	2.42 ± 0.13	**1.99 ± 0.13b**	**1.72 ± 0.20a**	**1.77 ± 0.02b**
Sugar	**11.85 ± 1.31a**	**13.52 ± 1.40a**	**18.55 ± 1.81b**	14.09 ± 4.61	10.63 ± 0.80	14.07 ± 0.72
Water	**20.21 ± 0.40a**	**22.61 ± 0.26b**	**25.48 ± 0.51c**	39.65 ± 0.80	39.96 ± 0.31	41.27 ± 0.30
P	**612.92 ± 58.04a**	**1013.75 ± 218.89b**	**879.17 ± 125.16a**	974.58 ± 299.33	541.67 ± 56.60	774.00 ± 138.20
Ca	289.33 ± 32.93	245.00 ± 13.19	253.67 ± 19.21	507.33 ± 34.00	590.17 ± 65.44	416.00 ± 40.28
Mg	**328.00 ± 30.66a**	**484.81 ± 124.20a**	**603.58 ± 82.83b**	549.03 ± 106.12	349.08 ± 40.44	418.49 ± 152.10
Allelochemicals						
Phenolics	0.46 ± 0.03	0.69 ± 0.06	0.93 ± 0.39	1.08 ± 0.47	0.80 ± 0.37	0.29 ± 0.07
α-Pinene	359.87 ± 30.21	382.01 ± 36.45	348.44 ± 20.00	377.26 ± 18.61	353.32 ± 25.74	377.46 ± 33.20
Camphene	**272.12 ± 15.80a**	**276.58 ± 23.71a**	**208.97 ± 9.15b**	364.22 ± 16.17	332.37 ± 21.64	350.09 ± 25.66
β-Pinene	106.41 ± 11.01	113.72 ± 16.80	79.61 ± 2.55	90.50 ± 6.72	80.23 ± 8.72	90.64 ± 10.52
Myrcene	235.22 ± 22.50	254.78 ± 18.63	213.40 ± 20.22	**143.40 ± 6.12a**	**119.77 ± 10.63b**	**131.94 ± 10.77a**
α-Phellandrene	**0a**	**44.43 ± 29.79b**	**11.84 ± 11.84a**	91.32 ± 9.59	79.45 ± 10.43	92.37 ± 11.83
Limonene	**88.94 ± 9.38a**	**95.25 ± 13.98a**	**63.37 ± 2.07b**	88.95 ± 6.68	87.42 ± 9.83	87.69 ± 10.33
Bornyl acetate	**446.34 ± 20.45a**	**441.10 ± 27.33a**	**365.16 ± 14.02b**	600.63 ± 63.26	604.43 ± 39.79	635.21 ± 51.73

[a]Significant effects ($P < 0.05$) of treatment are represented in bold, and significant differences among treatments are represented by different letters.

chemistry (Table 3), and temperature was evaluated using a generalized linear mixed model (glmm) with the glmer or the lmer functions in the lme4 package in the R statistical program (Bates *et al.*, 2015; R Core Team, 2015). Model selection was carried out using the Akaike's Information Criterion (AIC) using procedures specified in Mazerolle (2006). For larval survival throughout larval development (i.e. first instar to pupation) and overall development (i.e. first instar to adult emergence), we used a binomial distribution for a discrete variable with two possible outcomes (survival or death). To assess treatment, year, and sex effects on wing length (i.e. subcostal vein length), a Gaussian distribution for a continuous variable was used. A Gaussian distribution was also used to test treatment effects on foliar chemical constituents (i.e. nitrogen content, sugar, P, Ca, Mg, monoterpenes, and phenolics), water content, and temperature. Trees nested in the site were treated as a random effect in all models. The best models for explaining differences in response variables among treatments were selected based on the smallest differences in AIC (Δi). In a few instances, more than one model produced an Δi < 2 and thus multiple models were selected (Mazerolle, 2006). Although some studies have suggested that one might also consider models with Δi up to 7 (e.g. Burnham *et al.*, 2011), we have chosen to use the conservative cut-off of Δi < 2 to select models that have, in our opinion, the greatest biological significance. When more than one model was selected for a parameter of interest, we obtained a model-average estimate and an unconditional standard error from a set of candidate models. To determine the effects of treatment and year on the adult sex ratio, we used Pearson's chi-squared test.

Results

In 2009, neither larval survival (i.e. from the first instar to pupation) nor total survival (i.e. from the first instar to adult emergence) were significantly influenced by treatment (Fig. 1a). In 2010, however, larval survival was highest in the shaded lower crown and lowest in the sunlit upper crown (Fig. 1b); however, this trend did not persist through adult emergence (i.e. total survival, Fig. 1b).

In both years, the sex ratios of adults were female biased in the sunlit upper crown branches compared with those in the shaded upper crown or lower crown ($\chi^2 = 6.01$, P < 0.05) (Fig. 2). In general, the wing lengths of females were consistently longer than those of males (Fig. 3). During both years, males and females that developed on sunlit upper crown branches had longer wings than those that developed on shaded branches in the upper or lower crown (Fig. 3).

Average temperatures in the shaded upper crown ($\bar{x} = 17.97\,°C \pm 0.06$) were similar to those in the sunlit upper crown ($\bar{x} = 17.89\,°C \pm 0.06$) but significantly higher than those in the shaded lower crown ($\bar{x} = 17.44\,°C \pm 0.06$, P < 0.001). For the duration of the experiment, the minimum/maximum temperatures for each treatment were: shaded upper crown 6.12/32.62 °C; sunlit upper crown 6.12/31.64 °C; and shaded lower crown 5.63/30.68 °C.

In June, some nutrient concentrations varied between treatments. Sugar, water, and magnesium concentrations were higher in the foliage located in the upper crown that was directly exposed to sunlight. Phosphorus concentrations also varied between treatments and were higher in the upper shaded crown than in the lower shaded crown (Table 3). In July, nitrogen and phosphorus concentrations were not significantly lower in the shaded foliage located in the upper crown (Table 3).

Some allelochemicals also varied in concentration between treatments. In June, camphene, β-pinene, limonene, and bornyl acetate concentrations were lower in the sunlit foliage located in the upper crown whereas α-phellandrene concentrations were higher in the shaded foliage located in the upper crown (Table 3).
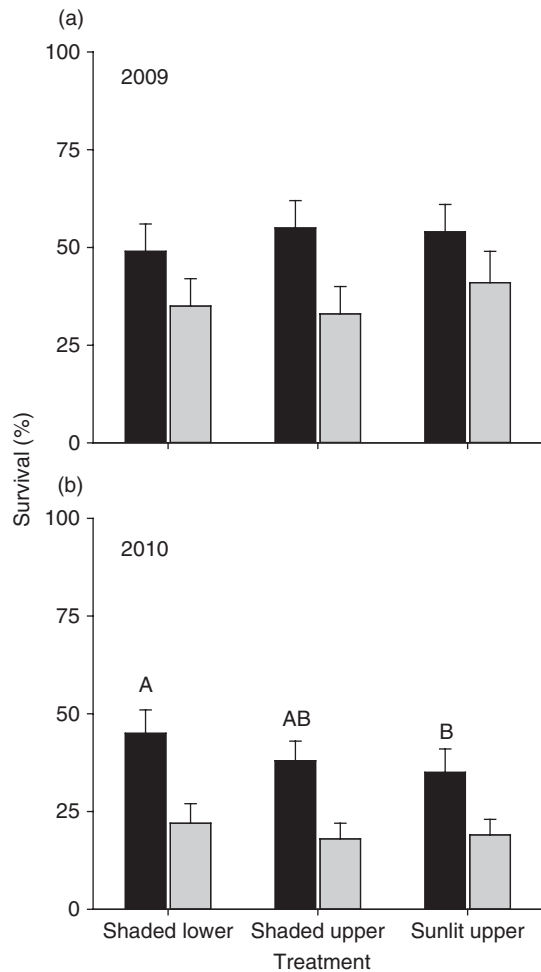
Fig. 1. Influence of crown location (crown level and sun exposure) in (a) 2009 and (b) 2010 on larval (i.e. first instar to pupation, dark bars) and total (i.e., first instar to adult emergence, gray bars) survival. Reported data are model-average predictions ($\pm$ unconditional SE). Larvae in the upper crown were located either in the partial shade (Shaded upper) or fully exposed to sunlight (Sunlit upper), whereas all larvae in the lower crown were located on fully shaded branches (Shaded lower). Bars with different letters represent significantly different results ($\Delta i < 2$).

In July, myrcene concentrations were smaller in the shaded foliage located in the upper crown. Differences in phenolics concentrations were marginally significant and revealed that their concentrations were smaller in the sunlit foliage located in the upper crown.

Discussion

Results from our study indicate that feeding on shaded lower versus sunlit upper crown branches of mature hemlock may in some years confer survival benefits to pale-wing gray larvae. However, we found there to be a much more consistent benefit to avoiding shaded foliage, as indicated by the more female-biased sex ratios and larger male and female moths that developed in groups feeding on sunlit upper crown branches. Collectively, these results
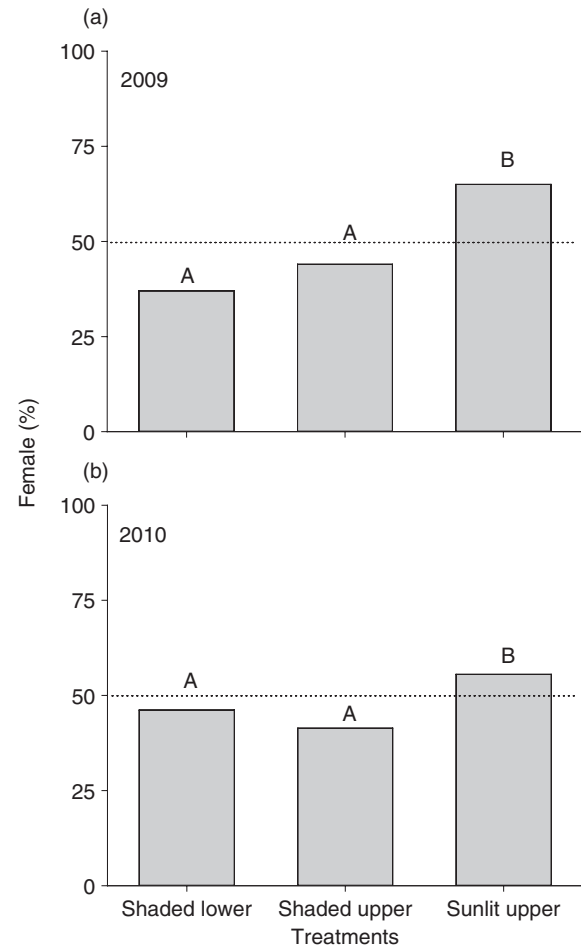
Fig. 2. Influence of crown location (crown level and sun exposure) in (a) 2009 and (b) 2010 on the percentage of survivors that were female (i.e. after pupation). The dotted horizontal line marks where sex ratios would be balanced at 1 : 1 (i.e. 50%). Groups in the upper crown were on branches in partial shade (Shaded upper) or fully exposed to sunlight (Sunlit upper), whereas those in the lower crown were on fully shaded branches (Shaded lower). Bars with different letters represent significantly different results ($P < 0.05$).

seem indicative of performance trade-offs associated with feeding in different parts of the tree crown, and perhaps related to the growth benefits of feeding on sunlit foliage versus occasional survival costs caused by exposure to weather extremes (e.g. hygrothermal stress).

Previous studies in young conifers indicate several key factors that can influence foraging patterns of lepidopteran and sawfly herbivores within trees. In several studies, foliage on sunlit upper crown branches is favored and tends to yield the highest performance, at least for the later instar larvae. For example, studies of spruce bud moth (*Zeiraphera canadensis* Mut. & Free.) in young white spruce [*Picea glauca* (Moench) Voss] found that larvae initiate feeding in the mid-crown but later disperse acropetally (sensu Quiring, 1993), from the mid to upper crown before they complete development (Carroll & Quiring, 1994). This foraging strategy was attributed to acropetal patterns
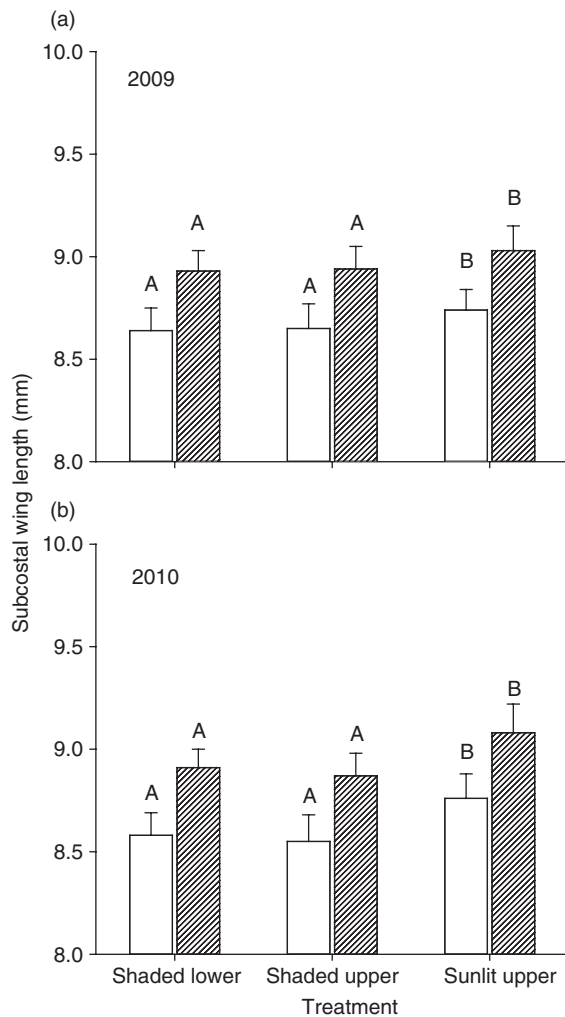
(a)

(b)

Fig. 3. Influence of crown location (crown level and sun exposure) in (a) 2009 and (b) 2010 on the subcostal vein length of adult males (white bars) and females (diagonal bars). Reported data are model-average predictions (± unconditional SE). Larvae in the upper crown were located either in the partial shade (Shaded upper) or fully exposed to sunlight (Sunlit upper), whereas all larvae in the lower crown were located on fully shaded branches (Shaded lower). Bars with different letters represent significantly different results ($\Delta i < 2$).

in shoot development and allowed larvae to enhance survival and size through feeding on newly burst, highly nutritious buds immediately after breaking hibernation and a second time after dispersing (Carroll & Quiring, 1994). Similar intra-tree foraging patterns in yellowheaded spruce sawfly (*Pikonema alaskensis* Roh.) feeding on young spruce (*Picea* spp.) produced a higher larval survival and more female-biased sex ratios than alternative strategies, owing to the superior nutritional quality of the relatively large shoots of the upper crown (Johns *et al.* 2009, Johns *et al.*, 2010). For this sawfly, the cost of feeding in the upper crown associated with exposure to microclimatic extremes was muted by their tendency to feed on the underside of shoots (Johns *et al.*, 2012). However, in contrast to both of these systems, pale-winged gray appear to move very little

among crown levels after egg lay, and densities tend to be highest in the lower crown throughout larval development (Hervieux *et al.*, 2012). The seasonal distribution of pale-winged gray is more akin to that of another generalist caterpillar, the elm spanworm [*Ennomos subsignaria* (Hübner)], which tends to prefer and accrue higher survival through feeding in the lower crown of its maple host (Fry *et al.*, 2009). Our bioassays, however, suggest a more complex response for pale-winged gray to variations among crown levels. Although there may be survival advantages to feeding on shaded branches in some years, for both years the sex ratio was more female biased and male and female size (and perhaps fecundity; Carroll & Quiring, 1993) was higher for larvae that fed on sunlit branches in the upper crown. The performance benefits observed for larvae developing on sunlit foliage suggests that sunlit foliage may have superior nutritional quality.

Our foliage chemical analyses provided some indications of why moths were larger and more female biased on sunlit upper crown foliage. In June, when most larvae were still in their early to mid-instars, upper crown, sunlit foliage tended to have higher concentrations of nutrients such as sugars, water and Mg, and fewer harmful allelochemicals such as β-pinene, camphene, limonene, and bornyl acetate. However, these same effects were not detected in foliage collected in July, which may have been, at least partially, because of changes associated with needle maturation. Because early instar larvae tend to be more sensitive than older instars to variation in foliage nutritional quality (Hochuli, 2001), we expect that the nutritional state of foliage from our June collection may have the greatest bearing on pale-winged gray performance. In general, our bioassay results are also consistent with past studies of tree foliar chemistry, which have found sunlit foliage to have a lower surface area, greater thickness, and more nitrogen and sugars than shaded foliage (e.g. White, 1984; Fortin & Mauffette, 2002; Oishi *et al.*, 2006; Osier & Jennings, 2007).

The lower survival of larvae in the sunlit upper crown in year 2 may be indicative of hygrothermal stress associated with exposure; however, these results were not consistent between years, and our results suggested only subtle differences in microclimate among crown levels. Part of the reason for this may be that microclimatic differences between sunlit and shaded branches in the upper crown were relatively subtle, especially when compared with differences among crown levels, making it difficult to detect their influence. However, other unmeasured parameters such as solar insolation may also have an important influence on pale-winged gray performance and distribution. It is also difficult to rule out the possible dampening influence the sleeve cage might have had on microclimatic exposure in the upper crown, although these effects would have, at least, been consistent among treatments (Johns *et al.*, 2010). Field or laboratory studies that control for differences in microclimate would be better suited to teasing out the relative influence of these different microclimatic factors (e.g. Levesque *et al.*, 2002).

Although not tested in our study, pressure from natural enemies could explain why pale-winged gray in nature tend to avoid the relatively exposed sunlit foliage of their host – past studies have shown higher parasitism and predation rates for insect herbivores foraging in the upper versus the lower crown of trees. For example, Eveleigh and Johns (2014) reported

that several parasitoid species that attack spruce budworm (*Choristoneura fumiferanae* Clemens) have a disproportionately high impact on larvae overwintering in the upper compared with the lower crown of mature balsam fir. Many predators are also known to forage preferentially on insects feeding on more exposed parts of the forest canopy (Šipoš & Kindlmann, 2013). In our study, the possible complementary role natural enemies play in shaping the foraging behaviors of pale-winged gray cannot be discounted as our sleeve cages prevented both parasitism and predation.

Conclusions

Few field studies have been carried out in large trees to examine how and why herbivores lay eggs or feed where they do, and whether those foraging behaviors are adaptive. Our study highlights some of the benefits of carrying out *in situ* experiments in large trees to answer some of these questions, despite obvious challenges such as the difficulty accessing the vegetative crowns of mature trees. Our results suggest an interesting herbivore–plant dynamic perhaps arising as a consequence of foraging trade-offs in response to bottom-up and (potentially) top-down factors, and to some extent microclimate. We should note that field studies such as ours complement rather than replace more controlled laboratory work–indeed, future laboratory research would greatly complement our study by examining under more controlled conditions the factors governing feeding preferences and performance of insect herbivores in trees. However, taken together, laboratory and field studies should enhance the understanding of herbivore foraging within individual tree crowns and more generally how these interactions contribute to ecosystem function in mature forest canopies.

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