

Performance of the invasive weevil *Polydrusus sericeus* is influenced by atmospheric CO₂ and host species

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- Abstract**
- 1 Natural forest systems constitute a major portion of the world's land area, and are subject to the potentially negative effects of both global climate change and invasion by exotic insects. A suite of invasive weevils has become established in the northern hardwood forests of North America. How these insects will respond to increasing CO₂ or O₃ is unknown.
 - 2 The present study examined the effects of elevated atmospheric CO₂ and O₃ on the invasive weevil *Polydrusus sericeus* Schaller at the Aspen Free Air CO₂ Enrichment (FACE) site near Rhinelander, Wisconsin. A performance assay was conducted in the laboratory during the summer of 2007 using mated pairs of *P. sericeus* fed a combination of aspen, birch and maple foliage. We recorded leaf area consumption, oviposition and adult longevity. We also conducted visual abundance surveys in the field from 2004 to 2007 on aspen and birch at Aspen FACE.
 - 3 Elevated CO₂, but not O₃, significantly affected *P. sericeus* performance. Female, but not male, longevity was reduced under elevated CO₂. *Polydrusus sericeus* also produced fewer eggs under elevated CO₂ conditions compared with ambient conditions. Adult *P. sericeus* strongly preferred birch over both aspen and maple, regardless of fumigation treatment.
 - 4 The effects of elevated CO₂ on *P. sericeus* populations at Aspen FACE were minimal, and varied among years and host tree species. *Polydrusus sericeus* abundance was significantly greater on birch than aspen. Over the long term, elevated CO₂ may reduce adult female longevity and fecundity of *P. sericeus*. Further studies are needed to evaluate how this information may scale to ecosystem impacts.

Keywords *Acer saccharum*, *Betula papyrifera*, carbon dioxide, coleoptera, curculionidae, feeding trials, global change, invasive species, ozone, *Polydrusus sericeus*, *Populus tremuloides*.

Introduction

Invasive insects and increasing concentrations of tropospheric CO₂ and O₃ are joint threats to forest ecosystems. Elevated CO₂ tends to increase photosynthesis and overall plant biomass (Will & Ceulemans, 1997; Isebrands *et al.*, 2001; King *et al.*, 2001), at the same time as reducing host plant quality for herbivores as a result of an increase in total foliar carbon, including some carbon-based secondary compounds (Zvereva & Kozlov, 2006; Stiling & Cornelissen, 2007), and a decrease in nitrogen (Lawler *et al.*, 1997; Norby *et al.*, 1999; Kopper & Lindroth, 2003a, b). Altered phytochemistry typically results in increased

plant consumption by herbivores to compensate for lower food quality (Lindroth & Dearing, 2005; Stiling & Cornelissen, 2007). Tropospheric O₃ generally decreases plant health by reducing photosynthesis and biomass production (Heath, 1994; Coleman *et al.*, 1995) and tends to increase the production of carbon-based secondary compounds (Koricheva *et al.*, 1998). As a result of the stimulation of carbon-based secondary compounds, elevated O₃ may decrease host plant palatability to insect herbivores. Nonetheless, a range of responses has been observed, depending on the particular insect, host species and chemical group (Valkama *et al.*, 2007).

As globalization increases, so does the risk of new invasive species becoming established (Jenkins, 1996). Biological invasions are a major threat to the biodiversity of native flora

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and fauna (Gurevitch & Padilla, 2004; Hendrix *et al.*, 2006) and cost the U.S.A. billions of dollars annually (Pimentel *et al.*, 2005). Invasive species may persist for years before their discovery, especially in forest systems (Liebhold *et al.*, 1995; Mattson *et al.*, 2007). Over 400 documented invasive forest insect species exist in North America (Mattson *et al.*, 1994), and their invasions can alter natural trophic interactions or lead to changes in tree species composition, forest structure and ecosystem processes (Orwig & Foster, 1998; Hoebeke *et al.*, 2005).

Understanding how elevated CO₂ and O₃ will influence herbivore performance and abundance is critical to predicting the future state of forest ecosystems. Most research on the effects of elevated CO₂ and/or O₃ on insect herbivores has focused on Lepidoptera. Few studies have monitored coleopteran performance under elevated CO₂ and O₃ conditions (Coviella & Trumble, 1999; Valkama *et al.*, 2007; Vigue & Lindroth, 2010), even though almost 30% of invasive species are beetles (Mattson *et al.*, 1994). A complex of invasive weevils (Coleoptera: Curculionidae) of European origin has established in northern hardwood forest systems of North America (Coyle *et al.*, 2008). This complex is the most common above- and below-ground arthropod mesofauna in this ecosystem (Pinski *et al.*, 2005a). *Polydrusus sericeus* Schaller is the second most abundant invasive weevil species in northern hardwood forests and its distribution range spans much of eastern North America (Coyle *et al.*, 2008). Adults are polyphagous folivores, emerging from the soil in May. Mating occurs after an obligatory feeding period, and eggs are deposited in or near the soil. Larvae feed on fine roots of various woody plants from July to April, at which time pupation occurs. All of the invasive weevils, including *P. sericeus*, are univoltine. Currently, little is known regarding the effect of *P. sericeus* on northern forest ecosystems, although their impacts are suspected to be substantial (Coyle *et al.*, 2008).

Given the potentially large impact of invasive species, it is important to understand how global change may influence their ecology. The first objective of the present study was to assess *P. sericeus* performance on paper birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh.) foliage (comprising three ecologically and economically important tree species in northern hardwood forests) produced under elevated CO₂ and O₃ conditions. Because *P. sericeus* is a generalist feeder and foliar quality typically declines slightly under both elevated CO₂ and O₃ concentrations (e.g. reduced nitrogen, increased phenolics), we hypothesized that individual weevil performance would decrease in both treatments. We also expected weevils to perform better on birch than aspen or maple, based on prior studies (Pinski *et al.*, 2005a, b). We also examined the abundance of weevils at the Aspen Free Air CO₂ Enrichment (FACE) site to determine whether the effects of elevated CO₂ and O₃ on weevil consumption and performance in the laboratory would match population responses to elevated CO₂ and O₃. We hypothesized that reduced *P. sericeus* performance on trees receiving elevated CO₂ and O₃ concentrations would decrease weevil abundance compared with trees receiving ambient CO₂ and O₃ concentrations. We hypothesized that

P. sericeus would be more abundant on birch compared with aspen or maple.

Materials and methods

Study location and design

The study was conducted at the Aspen FACE experimental site (89.5°W, 45.7°N) located in Oneida Co., Wisconsin. Soils are Pandus sandy loam with occasional clay layers approximately 30 cm deep (Dickson *et al.*, 2000). Twelve treatment rings (diameter 30 m), each separated by a minimum of 100 m, were arranged in a split-plot randomized complete block design. Rings were divided into three blocks from north to south, and received one of four treatments ($n = 3$ per treatment): control, +CO₂ (target concentration = 560 p.p.m.); +O₃ (target concentration = 1.5 × ambient); and +CO₂+O₃ (measured CO₂ and O₃ levels are shown in Table 1) (<http://aspenface.mtu.edu/results.htm>). Gases were dispensed between 07.00 h and 19.00 h from budbreak to budset (average growing season length from 2004 to 2007 was 139 days). Unfortunately, the ozone generator malfunctioned for approximately 2 weeks at the beginning of the study, although the residual effects of elevated O₃ on tree chemistry persisted through this period (J. Couture and R. L. Lindroth, unpublished data). One half of each ring was planted with a combination of five aspen genotypes (clones 8 L, 42E, 216, 259, 271), one fourth of each ring was planted with a mixture of birch and aspen, and the remaining fourth with a mixture of maple and aspen. Additional details about the design and fumigation processes of the Aspen FACE are provided in Dickson *et al.* (2000).

Laboratory assay

We monitored weevil emergence visually, and began collecting weevils in the field as emergence began. We hand collected 200 mating pairs of *P. sericeus* from birch trees growing on the perimeter of the FACE site on 21 June 2007. Each weevil pair was placed into a separate vial and starved for 24 h prior to the experiment.

The next day, three trees each of birch, aspen (clone 216), and maple were marked in each FACE ring for foliage collection (three sets of three trees per ring). We collected several sun-exposed leaves from each tree, placed them into a zip lock bag, and transported them to the laboratory in a cooler. Sun leaves were chosen because visual surveys revealed that weevils were most commonly found on the present year's growth exposed to high sunlight (Pinski *et al.*, 2005a; M. L. Hillstrom, personal observation). One or two leaves of each species were grouped together, with petioles wrapped in wet cotton, and remoistened every 3–4 days to prevent desiccation. Leaves were placed into a single Petri dish (15 × 1.5 cm) lined with Whatman #1 filter paper (Whatman, U.K.). Leaves were replaced every 7 days to maintain a consistent leaf appearance (green) and quality (moisture). One pair of mating *P. sericeus* was added to each dish along with one half of a Kimwipe tissue (Kimberly-Clark, Roswell, Georgia), which served as an

Table 1 May, June and July mean air temperatures, precipitation, and CO₂ and O₃ levels (daytime p.p.m.) when *Polydrusus sericeus* adults were present from 2004 to 2007 at the Aspen Free Air Carbon dioxide Enrichment site near Rhineland, Wisconsin

Year	Month	Average maximum temperature (°C)	Average minimum temperature (°C)	Total precipitation (cm)	Ambient CO ₂ (p.p.m.)	Elevated CO ₂ (p.p.m.)	Ambient O ₃ (p.p.b.)	Elevated O ₃ (p.p.b.)
2004	May	16	3	3.2	378	516	36	38
	June	21	8	2.7	376	518	34	47
	July	24	11	3.6	368	533	35	43
2005	May	16	4	1.1	383	531	34	45
	June	26	13	1.0	374	524	40	53
	July	27	13	1.8	365	528	37	53
2006	May	18	6	4.5	385	500	45	52
	June	24	9	0.6	377	540	42	51
	July	29	13	4.4	379	545	40	49
2007	May	20	6	2.0	393	520	48	57
	June	25	10	3.6	402	520	45	50
	July	26	13	4.2	390	493	34	39

oviposition site (Pinski *et al.*, 2005b). Kimwipes tissues with egg masses were kept moist and replaced every 3–4 days, and stored in separate Petri dishes until eggs could be counted. All Petri dishes were kept on a two-tiered plant rack in front of an open sliding door. Weevils were exposed to outdoor day/night conditions (Table 1) for the duration of the experiment and were rotated daily to prevent differences in sunlight exposure. In total, we used five Petri dishes for each of three sets of three trees (birch, aspen, maple) per treatment ring, for a total of 45 experimental units per control, +CO₂, +O₃, and +CO₂+O₃ treatment.

Leaf area consumed for each dish was divided by the number of weevil days for that dish. One weevil day was equivalent to each 24-h period that an individual weevil survived. To calculate leaf area consumed, leaves were scanned (Winfolia, Regent Instruments, Inc., Canada) after removal from Petri dishes to determine leaf area remaining after beetle feeding. We then digitally filled in the missing leaf portions and calculated the original leaf area (Hamilton *et al.*, 2005).

Weevil mortality was recorded every 3–4 days. In every pairing, the female was larger than the male, allowing when the male and female had died to be determined. Eggs were counted approximately twice weekly. Eggs collected on 7, 10 and 14 July were kept at approximately 20°C, sprayed with water every 3–4 days to keep them moist, and allowed to hatch. We recorded the proportion of eggs that hatched to determine differences in hatching rate among treatments. We did not measure initial female size, which may or may not influence adult fecundity (Leather, 1988), but suggest that female size may not have a strong effect on the number of eggs produced by leaf feeding beetles that feed extensively as adults and oviposit over a long period (Coyle *et al.*, 1999).

Field visual surveys

Aspen and birch trees were visually inspected for *P. sericeus* in mid-July from 2004 to 2007. Forty-eight trees per species were sampled each year. In 2004 and 2005, birch and aspen (genotype 216) were sampled in the inter-planted aspen-birch section of each ring. In 2006 and 2007, we sampled the same

birch trees but aspen (genotype 216) trees were sampled in the mixed aspen genotype section. Each tree was visually inspected for 10 min and the number of weevils recorded. To account for differences in tree size (number and size of branches), we surveyed random branches for approximately 3.3 min at the bottom, middle and top of each tree without inspecting any branch or leaf more than once. Surveying visually also allowed us to account for any differences in leaf area as a result of species or treatments because inspection takes the same amount of time per unit leaf area (i.e. trees with more leaves per branch take longer to inspect, so fewer total branches, but the same total leaf area per tree, is inspected).

Statistical analysis

Leaf area consumed per Petri dish was summed for the duration of the experiment. Mean leaf area consumed across dishes and trees was calculated for each tree species in each ring (unit of replication), log transformed to satisfy assumptions of normality and equal variance, and analysed as a two-way crossed split plot analysis of variance (ANOVA). The main effects were block, CO₂ and O₃, with a CO₂ × O₃ interaction term. Subplot effects were tree species and tree species by main effects. We recognize that measuring the amount of leaf area consumed does not account for potential differences in leaf mass per area as a result of elevated CO₂ or O₃. We examined data from neighbouring aspen and birch trees from July 2007 and found that elevated CO₂ did not affect leaf mass per area. Elevated O₃ increased mass per area by 10%, although this effect was not significant (J. Couture and R. L. Lindroth, unpublished data). Accounting for the increased mass per area resulted in no significant change to our estimates of leaf consumption under elevated O₃. Thus, we will refer to leaf area consumed, rather than mass consumed.

Longevity data met normality and equal variance assumptions, and were analysed as a two-way crossed split plot ANOVA with block, CO₂ and O₃ main effects and the CO₂ × O₃ interaction term. Subplot effects included sex and sex by main effects.

Table 2 Effects of ambient and elevated CO₂ and O₃ on leaf consumption by *P. sericeus*

Tree species	Treatment				Species means
	Control	+CO ₂	+O ₃	+CO ₂ +O ₃	
Birch	0.472 ± 0.044	0.450 ± 0.040	0.495 ± 0.144	0.450 ± 0.062	0.467 ± 0.003a
Aspen	0.038 ± 0.006	0.056 ± 0.008	0.041 ± 0.006	0.038 ± 0.004	0.043 ± 0.003b
Maple	0.019 ± 0.004	0.023 ± 0.004	0.020 ± 0.004	0.020 ± 0.006	0.020 ± 0.002c

Data represent the mean ± SE leaf area consumed (cm²) per weevil per day, for *n* = 3 FACE rings per fumigation treatment. Tree species means sharing a letter are not significantly different at α = 0.05 (Tukey's honestly significant difference).

Egg production rate (i.e. the number of eggs produced per female per day) and total egg production (i.e. the number of eggs produced by a mating pair of weevils throughout the duration of the experiment) were log transformed to meet assumptions of normality and equal variance. Both eggs per day and total number of eggs produced were analysed using a two-way crossed ANOVA with block, CO₂, O₃ and CO₂ × O₃. Egg hatch rate was analysed using the same model as egg production.

Weevil abundance data were log transformed and analysed as a two-way, crossed, split-split plot ANOVA with block, CO₂ and O₃ as the main effects and an interaction term (CO₂ × O₃). Tree species and tree species crossed with each main effect were the first subplot factors, and the second subplot factors were year and year crossed with each main effect and first subplot effects. Significant terms were examined in more detail using Tukey's honestly significant difference.

Results

Leaf area consumption

Elevated CO₂ and O₃ did not significantly affect the leaf area consumed by *P. sericeus* (all P > 0.431). Weevils strongly preferred birch foliage regardless of fumigation treatment, and also preferred aspen over maple ($F_{2,16}$ = 385.39, P < 0.001) (Table 2). Across fumigation treatments, the average leaf area consumption per dish was 90% birch, 7% aspen and 3% maple.

Longevity

Feeding on enriched CO₂ foliage reduced *P. sericeus* female longevity by 19% but male longevity was unaffected (sex × CO₂: $F_{1,8}$ = 7.94, P = 0.023; Fig. 1). Elevated O₃ and the CO₂ × O₃ interaction did not significantly affect *P. sericeus* longevity (all P > 0.535).

Fecundity

Polydrusus sericeus egg production rate declined by 23% (F_1 = 7.46, P = 0.034) and total egg production declined by 29% (F_1 = 6.08, P = 0.049) for females fed foliage produced under elevated CO₂ compared with ambient CO₂ (Fig. 2). We observed no significant O₃ or CO₂ × O₃ interaction for either fecundity parameter (all P > 0.788). Eight female *P. sericeus* laid more than 1000 eggs over their lifetime, with a maximum

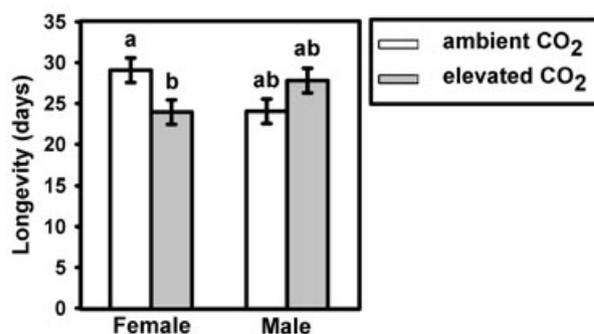


Figure 1 Effects of ambient and elevated CO₂ on female and male *Polydrusus sericeus* longevity. Means ± SE sharing the same letter are not significantly different at α = 0.05 (Tukey's honestly significant difference).

production of 1342 eggs by a single female. Females continued to oviposit after males had died. Egg hatch rate was consistent among treatments (model: P = 0.671, CO₂: P = 0.611, O₃: P = 0.445), in the range 93–96%.

Abundance

Although the main effects of elevated CO₂ and O₃ on *P. sericeus* abundance were not significant (all P > 0.897), we did observe a significant CO₂ × tree species × year interaction ($F_{3,280}$ = 3.28, P = 0.022). In summary, CO₂ had little effect on weevil abundance in 2004 and 2006 but increased the abundance of weevils on aspen and decreased the abundance of weevils on birch in 2005. The pattern for 2005 was reversed for 2007. We found no significant CO₂ × O₃ interaction (P = 0.574). *Polydrusus sericeus* was 3.9-fold more abundant on birch than on aspen across the 4 years of this study ($F_{1,280}$ = 265.28, P < 0.001; Fig. 3). Weevil abundance varied 2.1-fold among years ($F_{3,280}$ = 16.06, P < 0.001; Fig. 3).

Discussion

Fumigation effects

Performance. Elevated CO₂ did not alter foliar consumption by *P. sericeus* but did reduce female longevity and oviposition rate. Birch leaf chemistry data from a concurrent study indicate that elevated CO₂ had minimal effects on nitrogen and condensed tannin concentrations (relative increases of only

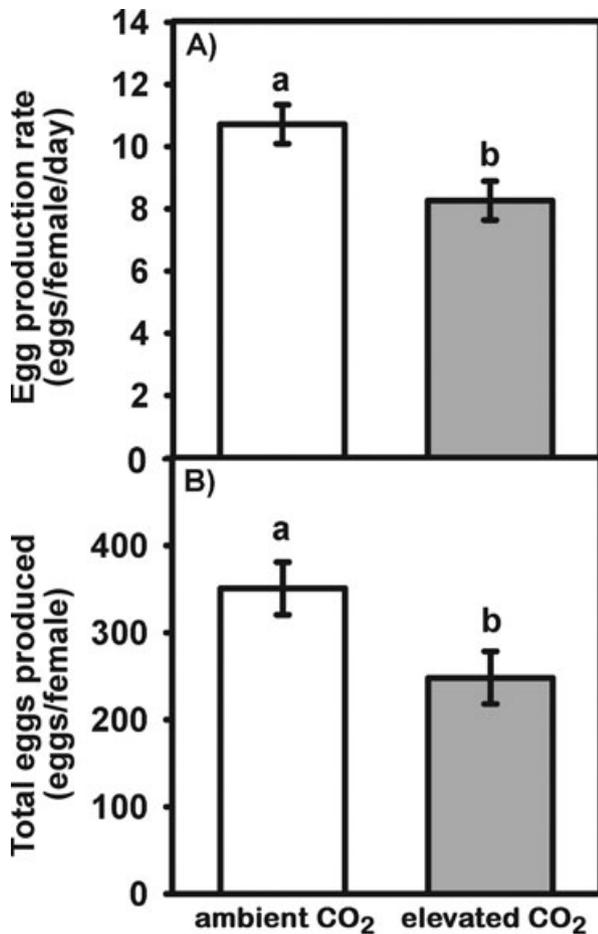


Figure 2 Effects of CO₂-treated foliage on (A) egg production rate and (B) total eggs produced. Means ± SE within a figure sharing the same letter are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference).

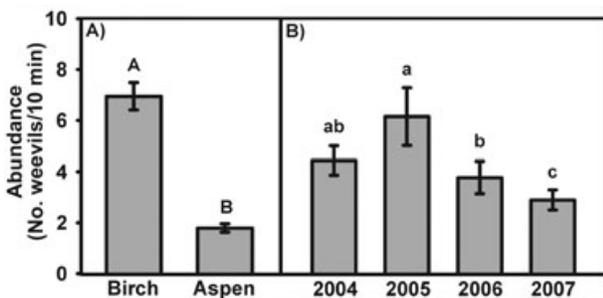


Figure 3 Mean ± SE abundance of *Polydrusus sericeus* (A) for birch and aspen, pooled across fumigation treatments and years, and (B) for each year of the study, pooled across fumigation treatments and tree species. Means sharing the same letter are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference).

1% and 6%, respectively; J. Couture and R. L. Lindroth, unpublished data), which are comparable with previous changes in birch chemistry at Aspen FACE (Agrell *et al.*, 2005). Such small increases appear unlikely to alter feeding and

performance. Likewise, elevated CO₂ does not increase the toughness of birch leaves at Aspen FACE (Oksanen *et al.*, 2001). Thus, the cause of reduced female longevity and fecundity remains unknown. Elevated CO₂ reduced oviposition of *Phyllonorycter tremuloidiella* Braun on aspen trees at Aspen FACE (Kopper & Lindroth, 2003b) but *Popillia japonica* Newman at SoyFACE lived 8–25% longer and laid approximately two-fold more eggs on soybean plants exposed to elevated CO₂ compared with ambient conditions (O'Neill *et al.*, 2008).

No significant elevated O₃ effects were evident on *P. sericeus* performance. Elevated O₃ had minimal effects on nitrogen and condensed tannin concentrations (relative reduction of 10% and 22%, respectively) in birch at Aspen FACE in July 2007 (J. Couture and R. L. Lindroth, unpublished data). Friewald *et al.* (2008) found that *Phyllobius pyri* L. weevils feeding on hybrid aspen preferred younger (later budburst), less thick elevated O₃ leaves. Trees at Aspen FACE experience later budburst when exposed to elevated O₃ (Karnosky *et al.*, 2003). Therefore, the increased quality associated with younger leaves may offset or surmount the reduced quality as a result of decreased nitrogen and increased condensed tannins. Changes in foliar chemistry under elevated O₃ in the present study agree with previous results at Aspen FACE (Kopper *et al.*, 2001; Agrell *et al.*, 2005). Other studies have found no change in insect performance on elevated O₃ foliage (Trumble *et al.*, 1987; Whittaker *et al.*, 1989; Costa *et al.*, 2001; Kopper & Lindroth, 2003b; Awmack *et al.*, 2004; O'Neill *et al.*, 2008). Additional studies, however, show that elevated O₃ may increase insect preference (Jeffords & Endress, 1984; Jones & Coleman, 1988; Endress *et al.*, 1991; Fortin *et al.*, 1997; Kopper & Lindroth, 2003a; Agrell *et al.*, 2005; Friewald *et al.*, 2008) and performance (Coleman & Jones, 1988; Whittaker *et al.*, 1989; Holton *et al.*, 2003). Differences in fumigation setup (FACE, open top chamber, etc.), insect and plant species, plant age and O₃ concentration probably contribute to this diversity of responses.

Abundance. Elevated CO₂ increased the abundance of weevils on aspen and decreased the abundance of weevils on birch in 2005, decreased the abundance of weevils on aspen and increased the abundance of weevils on birch in 2007, and had little effect on weevil abundance in 2004 and 2006. Yearly variability (1997–2003) in tree growth responses to elevated CO₂ at Aspen FACE has been linked to the amount of photosynthetically active radiation in July and temperature in October (Kubiske *et al.*, 2006). Similarly, variability in abiotic conditions probably affected weevil abundance. Previous work suggests that elevated CO₂ may increase weevil abundance (Altermatt, 2003; Hillstrom & Lindroth, 2008) but decrease general folivore abundance (Bezemer & Jones, 1998; Stiling & Cornelissen, 2007). Differences in the taxonomy or feeding guild of the insect, experimental design, fumigation concentrations, or plant species and plant age may explain the contrasting abundance responses of chewing herbivores to elevated CO₂ across studies.

Elevated O₃ had no effect on *P. sericeus* abundance, in agreement with the lack of change in foliar consumption or

insect performance. Over the last decade, elevated O₃ has reduced the abundance of most insect species examined at Aspen FACE (Loranger *et al.*, 2004; Hillstrom & Lindroth, 2008). Elevated O₃ may, however, increase the abundance of species, such as bark beetles, that preferentially attack O₃-stressed host plants (Cobb *et al.*, 1968; Grodzki *et al.*, 2004). More studies are necessary to determine whether responses differ among insect orders or feeding guilds.

Host species

Polydrusus sericeus were substantially more abundant on birch than aspen, probably because birch is their preferred food (Pinski *et al.*, 2005b). Previous research at Aspen FACE has shown that control birch and aspen leaves have almost equal nitrogen content but birch foliage has approximately half the concentration of condensed tannins relative to aspen (Agrell *et al.*, 2005). Fewer structural compounds (e.g. 25% less fibre in birch leaves compared with aspen leaves in July 2006) (J. Couture and R. L. Lindroth, unpublished data) could also make birch more palatable than aspen or maple.

Conclusions

As documented for other herbivores (Hillstrom & Lindroth, 2008), CO₂ and O₃ acted independently on performance and abundance of *P. sericeus*. Concentrations of elevated CO₂ above 500 p.p.m. have the potential to decrease *P. sericeus* populations by reducing female longevity and fecundity. By contrast, elevated O₃ alone or in combination with elevated CO₂ did not alter *P. sericeus* performance or abundance. Atmospheric CO₂ concentrations interact with weather factors to produce variable plant responses among years; it remains unknown whether such effects may also influence abundance and performance of herbivorous insects. Finally, the results obtained in the present study also support the findings of Pinski *et al.* (2005b) indicating that *P. sericeus* prefer birch foliage to aspen or maple foliage.

Damage to trees by adult *P. sericeus* may not change with the concentrations of CO₂ and O₃ predicted for 2060. If concentrations continue to rise to those predicted for 2100, however, the changes we found in longevity and fecundity at elevated CO₂ could be exacerbated, leading to significant impacts on non-indigenous weevil populations. Reductions in *P. sericeus* abundance could also change competitive interactions, leading to increased populations of other weevils in the complex or further invasion of northern hardwood forests by other insects. Future studies should consider the effects of atmospheric change on other invasive weevil species, both individually and as a guild, in northern hardwood forests.

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