Agricultural and Forest Entomology

Agricultural and Forest Entomology (2010), **12**, 267–276 DOI: 10.1111/j.1461-9563.2010.00475.x

Effects of genotype, elevated CO_2 and elevated O_3 on aspen phytochemistry and aspen leaf beetle *Chrysomela crotchi* performance

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Abstract

1 Trembling aspen *Populus tremuloides* Michaux is an important forest species in the Great Lakes region and displays tremendous genetic variation in foliar chemistry. Elevated carbon dioxide (CO_2) and ozone (O_3) may also influence phytochemistry and thereby alter the performance of insect herbivores such as the aspen leaf beetle *Chrysomela crotchi* Brown.

2 The present study aimed to relate genetic- and atmospheric-based variation in aspen phytochemistry to *C. crotchi* performance (larval development time, adult mass, survivorship). The experiment was conducted at the Aspen Free-Air CO_2 Enrichment (FACE) site in northern Wisconsin. Beetles were reared on three aspen genotypes under elevated CO_2 and/or O_3. Leaves were collected to determine chemical characteristics.

3 The foliage exhibited significant variation in nitrogen, condensed tannins and phenolic glycosides among genotypes. CO_2 and O_3, however, had little effect on phytochemistry. Nonetheless, elevated CO_2 decreased beetle performance on one aspen genotype and had inconsistent effects on beetles reared on two other genotypes. Elevated O_3 decreased beetle performance, especially for beetles reared on an O_3-sensitive genotype. Regression analyses indicated that phenolic glycosides and nitrogen explain a substantial amount (27–45%) of the variation in herbivore performance.

4 By contrast to the negative effects that are typically observed with generalist herbivores, aspen leaf beetles appear to benefit from phenolic glycosides, chemical components that are largely genetically-determined in aspen. The results obtained in the present study indicate that host genetic variation and atmospheric concentrations of greenhouse gases will be important factors in the performance of specialist herbivores, such as *C. crotchi*, in future climates.

Keywords Carbon dioxide, FACE, herbivore performance, ozone, ozone-tolerance, phenolic glycosides, plant–insect interactions, *Populus tremuloides*, specialist, trembling aspen.

Introduction

Trembling aspen *Populus tremuloides* Michaux is the most widely distributed tree in North America and an important woody crop in the Great Lakes region (Piva, 1996; Coyle *et al*., 2001; Isebrands *et al*., 2001). Aspen is also one of the most genetically variable tree species in North America (Perala, 1990). Substantial clonal variation is present in foliar chemical attributes such as condensed tannin and phenolic glycoside concentrations (Hwang & Lindroth, 1997; Osier & Lindroth, 2001, 2004; Donaldson & Lindroth, 2007). Some aspen secondary compounds, such as phenolic glycosides, can negatively alter insect herbivore performance (Hemming & Lindroth, 1995; Hwang & Lindroth, 1998; Donaldson & Lindroth, 2007).

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Intraspecific genetic variation in the concentrations of these constituents contributes to differential performance of insect herbivores on various aspen genotypes (Hwang & Lindroth, 1997; Osier *et al*., 2000; Osier & Lindroth, 2006; Donaldson & Lindroth, 2007). Such changes in herbivore performance are likely to influence the success of specific aspen genotypes during herbivore outbreaks (Gruppe *et al*., 1999; Donaldson & Lindroth, 2007).

Herbivore performance may also be influenced by atmosphere-induced alterations in plant phytochemistry. Concentrations of atmospheric CO_2 and O_3 have been increasing subsequent to the industrial revolution (Solomon *et al*., 2007) and are expected to continue rising in the future (Fowler *et al*., 1999; Sitch *et al*., 2007). Previous research has shown that elevated concentrations of CO_2 and O_3 may significantly affect plant physiology and foliar chemistry, thereby affecting plant–herbivore interactions. For example, foliar concentrations of nitrogen are typically reduced under elevated CO_2 (Lawler *et al*., 1997; Roth *et al*., 1997; Norby *et al*., 1999; Kopper & Lindroth, 2003a). Elevated CO_2 also generally causes an increase in plant carbon uptake, thereby increasing carbon-based secondary compounds such as condensed tannins and phenolic glycosides (Roth *et al*., 1997; Saxe *et al*., 1998; Lindroth *et al*., 2002). Leaf-chewing insects reared on plants exposed to elevated CO_2 typically exhibit increased consumption rates, longer development times and reduced pupal masses (Lindroth & Dearing, 2005). The effects of elevated O_3 on foliar nitrogen are somewhat inconsistent in the literature (Koricheva *et al*., 1998; Holton *et al*., 2003; Kopper & Lindroth, 2003a, b; Agrell *et al*., 2005). However, research indicates that O_3 often increases carbon-based secondary compounds such as phenolics (Runeckles & Krupa, 1994; Koricheva *et al*., 1998). Herbivorous insects reared on plants grown under elevated O_3 show variable responses in performance, depending on insect species and host species or genotype (Agrell *et al*., 2000; Holton *et al*., 2003; Kopper & Lindroth, 2003a, b; Agrell *et al*., 2005). Variation in plant chemistry and herbivore responses as a result of a changing climate may have considerable impacts on future aspen populations.

Most previous research on the effects of elevated CO_2 and O_3 on aspen phytochemistry and chewing insect performance has focused on Lepidoptera herbivores. Surprisingly, few studies have addressed the effects of elevated CO_2 (Coviella & Trumble, 1999; Johns *et al*., 2003) or elevated O_3 (Valkama *et al*., 2007; Freiwald *et al*., 2008) on Coleoptera herbivores. Several Coleoptera species are well-adapted to host plants, such as aspen, that contain high concentrations of salicylates (phenolic glycosides). The larvae of these specialists typically employ plant-derived salicylaldehyde in their own defence secretions (Rowell-Rahier & Pasteels, 1982; Pasteels *et al*., 1983). Indeed, many studies have addressed the effects of poplar phytochemistry on the feeding preferences of chrysomelids and have found that phenolic glycoside concentrations influence host specificity (Tahvanainen *et al*., 1985; Matsuki & MacLean, 1994; Orians *et al*., 1997; Ikonen *et al*., 2001). Although phenolic glycosides may negatively affect the performance of generalist chrysomelids, they generally have little to no negative impact, and may improve the performance of specialist chrysomelids (Matsuki & MacLean, 1994; Orians *et al*., 1997; Rank *et al*., 1998). Condensed tannins, however, negatively affect the performance (e.g. growth) of chrysomelids (Donaldson & Lindroth, 2004).

The aspen leaf beetle *Chrysomela crotchi* Brown is a common chrysomelid beetle that specializes on poplars. Females lay eggs during June and July and the larvae can persist from July to September. One generation is produced per year (Smereka, 1965). Larvae feed on the lower surface of the leaf, skelotonizing the structure and leaving only the veins behind. Adults also feed on foliage, leaving only major veins uneaten. In outbreak years, these beetles can defoliate aspen trees in localized areas (U.S. Department of Agriculture, 2000).

The present study aimed to relate *C. crotchi* performance to genetic-, CO_2- and O_3-based variation in *Populus tremuloides* phytochemistry *in situ*. Specifically, we examined the relationships of beetle larval development time, adult mass and survivorship to foliar nitrogen, condensed tannin and phenolic glycoside concentrations. We predicted that aspen phytochemistry and beetle performance would vary among aspen genotypes. In particular, we expected that beetle performance would improve as foliar nitrogen and phenolic glycoside concentrations increased and tannin concentrations decreased. We expected CO_2 and O_3 to decrease foliar quality (e.g. decrease nitrogen, increase tannins) and subsequently decrease beetle performance as well.

Materials and methods

This research was conducted during the 2003 growing season at the Aspen Free Air CO_2 Enrichment (FACE) site (89.5°W, 45.7°N) near Rhinelander, Wisconsin. Aspen FACE is a 32-ha site consisting of twelve 30-m diameter rings. The overall experimental design at Aspen FACE is a split-plot randomized complete block. The Aspen FACE site is divided into three blocks, north to south, and each block contains four FACE rings, one each of four experimental treatments: ambient CO_2 and O_3 (control); elevated CO_2, ambient O_3 ($+CO_2$); ambient CO_2, elevated O_3 ($+O_3$); and elevated CO_2 and O_3 ($+CO_2+O_3$). The whole-plot treatments include the CO_2 and O_3 (2×2) factors; the subplot factor is aspen genotype (see below). Ambient levels of CO_2 averaged 384 μL/L and 32 nL/L for O_3. Elevated target levels for CO_2 were 560 μL/L and $1.5 \times$ ambient for O_3. These levels were chosen based on predictions of CO_2 and O_3 concentrations in the northern Great Lakes Region for the year 2060 (Dickson *et al*., 2000). Actual levels of elevated CO_2 averaged 537 ± 77 μL/L, and 51 ± 22 nL/L for elevated O_3, in 2003 (J. Sober, personal communication). Fumigants were applied during daylight hours of the growing season (21 May to 12 October). As a result of the photochemical properties of O_3 production and to mimic actual O_3 fluctuations, target concentrations of O_3 were adjusted daily according to current weather conditions. No O_3 treatment was administered on cool (<15°C) days or when leaf surfaces were wet due to precipitation or condensation. Additional details about the design and operation of the Aspen FACE are provided in Dickson *et al*. (2000).

One section of each Aspen FACE ring contains a mix of five trembling aspen genotypes. At the time of this experiment, the

trees were 7 years old and in their sixth year of fumigation. For the present study, we chose to work with aspen genotypes 216, 271 and 42E because of the range of O_3 sensitivity demonstrated by these trees. Genotypes 216 and 271 are relatively O_3-tolerant, whereas 42E is O_3-sensitive (Karnosky *et al*., 1996). Three aspen trees per genotype per ring were used for this experiment (108 total trees).

Approximately 40 female beetles were collected from the Aspen FACE site (outside of FACE rings) and allowed to mate with a similar number of males in cages containing fresh aspen shoots in water picks. Each female produces multiple clusters of eggs, with the number of eggs per cluster in the range 1–150. Egg clusters were removed daily and stored in a refrigerator (4°C) until enough eggs had been collected to proceed with the experiment. A typical egg cluster contained approximately 20–30 eggs. We created additional clusters of 20–30 eggs by grouping small clusters together or dividing large clusters, until we had a total of 108 clusters (one for each experimental tree). The eggs were transferred to a 25°C environmental growth chamber (Percival Scientific Inc., Boone, Iowa) until eclosion. Upon eclosion, egg clusters were randomly assigned across genotypes and fumigation treatments and were placed on fresh, detached Aspen FACE leaves. Once larvae had crawled onto the leaves, the leaves were paper-clipped onto the FACE trees from which they had derived. The branch including the larvae was enclosed in a fine, double mesh sleeve (No-See-Um insect netting, approximately 100 holes/cm^2; Quest Outfitters, Sarasota, Florida) to exclude predators and parasitoids. We placed larvae onto each of three trees per aspen genotype, in each of three FACE rings per CO_2 and O_3 treatment, resulting in 180–270 insects used per genotype × fumigation treatment combination.

The beetle larvae were monitored daily and moved to new branches (on the same tree) as the food source was depleted. Upon pupation, larval development time and beetle survivorship were recorded. Pupae were removed from the tree and placed in a Percival environmental chamber set at 25/21°C (day/night) under an LD 15 : 9 h photocycle to monitor development from pupae to adults. Sex and adult weight were determined within 24 h after adults emerged.

Because Aspen FACE is an open-air facility, trees are exposed to endemic levels of insect feeding. Although induction of aspen defensive chemistry, especially tannins (Osier & Lindroth, 2001), could have been possible, it was unlikely given the minimal damage (well below 10% at the time of this study), and so was not addressed in the present study.

Approximately 15 leaves (not fed on by aspen leaf beetles) were collected from each experimental tree midway through the larval development period. Leaves were collected from multiple branches and canopy heights to ensure an accurate representation of leaf chemistry for each tree crown. Leaves were clipped at the petiole and placed on ice for transport to the laboratory. In the laboratory, leaves were flash frozen in liquid nitrogen, freeze-dried (at −20°C), ground through a no. 40 mesh in a Wiley Mill (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, Pennsylvania), and stored at −20°C until analysed. Leaves were analysed for nitrogen, condensed tannins and phenolic glycosides. Nitrogen concentration was determined with a LECO nitrogen analyser (LECO Corporation, St. Joseph, Michigan), using glycine *p*-toluenesulfonate (Hach Company, Loveland, Colorado) as the reference standard. Condensed tannins were extracted and quantified with the butanol-HCl method (Porter *et al*., 1986), using purified aspen condensed tannins as the reference standard. Salicortin and tremulacin concentrations were determined via high-performance thin layer chromatography, using purified aspen salicortin and tremulacin reference standards (Lindroth *et al*., 1993).

Analysis of variance (PROC Mixed; SAS, version 8.0; SAS Institute, Cary, North Carolina) was used for statistical analysis of treatment effects on phytochemistry and beetle performance. The statistical model employed was:

$$Y_{ijkl} = \mu + B_i + C_j + O_k + CO_{jk} + e_{ijk} + G_l + CG_{jl} + OG_{jl} + COG_{jkl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the average response of block i, CO_2 level j, O_3 level k and genotype l. Fixed effects were CO_2 level (C_j), O_3 level (O_k), genotype (G_l) and their interaction terms [(CO_{jk}), (CG_{jl}), (OG_{jl}), (COG_{jkl})]. Random effects were block (B_i), whole plot error (e_{ijk}) and subplot error (ε_{ijkl}). Simple correlation and regression analysis were used to relate beetle performance to quantitative phytochemistry variables (R: A Language and Environment for Statistical Computing, version 2.4.1; R Foundation for Statistical Computing, Austria). Concentrations of salicortin and tremulacin were summed and presented in the regression analysis as 'total phenolic glycosides' as a result of their chemical similarities and parallel results when analysed separately. Partial correlation coefficients were calculated using the equation:

$r^2_{Y.X/T} = R^2_{Y.XT} - r^2_{Y.T}$ where r is the coefficient of correlation, Y is the dependent (beetle performance) variable, X and T are independent (phytochemistry) variables, and R^2 is the multiple coefficient of correlation (Abdi, 2007). The notation '$_{Y.X}$' signifies the correlation coefficient between variables Y and X; the notation '$_{/T}$' signifies that variable T is included in the regression model. The term $R^2_{Y.XT}$ was determined with regression analysis, and $r^2_{Y.T}$ was determined with simple correlation. Outliers identified using Bonferroni corrected p-values were removed from the analyses. Also, beetles that did not survive to pupation were excluded from the analyses of development time and adult mass.

Low statistical power is inherent to FACE experimental designs. Thus, when a conventional significance level of 0.05 is employed, the probability of type II errors increases, even when potentially important treatment effects occur (Filion *et al*., 2000). We therefore report significant values in the range $0.05 < P < 0.10$ as 'marginally significant.'

Results

Foliar chemistry

CO_2 and O_3 had little to no effect on aspen chemistry (Fig. 1 and Table 1). Nitrogen concentrations varied among genotypes, but the magnitude of variation was very small (1.7–1.8% dry mass, averaged across fumigation treatments). No significant CO_2 or O_3 effect on nitrogen was detected.

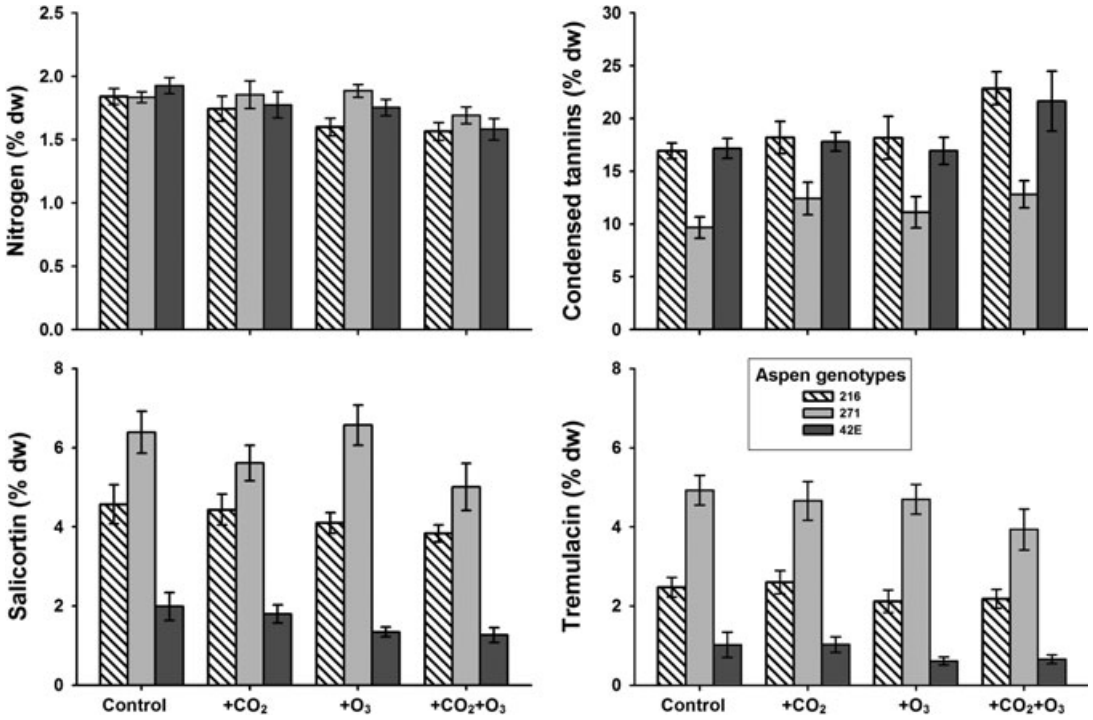

Figure 1 Effects of CO_2 and O_3 on foliar chemistry in aspen genotypes 216, 271 and 42E. Bars and vertical lines indicate the mean ± SE.

Table 1 Summary of *F* values, degrees of freedom and *P* values for the effects of genotype, CO_2 and O_3 on foliar chemistry of aspen leaves

	Nitrogen		Condensed tannins		Salicortin		Tremulacin	
Main effects and interactions	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*
Genotype	4.51 (2.88)	0.014	52.82 (2.88)	<0.001	127.83 (2.88)	<0.001	146.26 (2.87)	<0.001
CO_2	1.23 (1.6)	0.310	4.73 (1.6)	0.073	3.21 (1.6)	0.123	0.26 (1.6)	0.631
O_3	2.54 (1.6)	0.162	2.46 (1.6)	0.168	2.53 (1.6)	0.163	2.50 (1.6)	0.166
Genotype × CO_2	0.67 (2.88)	0.517	0.11 (2.88)	0.896	2.28 (2.88)	0.108	1.13 (2.87)	0.329
Genotype × O_3	1.77 (2.88)	0.176	0.78 (2.88)	0.462	0.29 (2.88)	0.750	0.02 (2.87)	0.976
CO_2 × O_3	0.09 (1.6)	0.770	0.79 (1.6)	0.409	0.23 (1.6)	0.648	0.11 (1.6)	0.750
Genotype × CO_2 × O_3	1.32 (2.88)	0.273	1.48 (2.88)	0.234	0.36 (2.88)	0.696	0.20 (2.87)	0.817

Condensed tannin concentrations ranged from 11% to 19% among genotypes. Genotypes 216 and 42E had similar condensed tannin concentrations, whereas levels in genotype 271 were 38% lower. Elevated CO_2 increased condensed tannin concentrations by 17% (marginally significant) across all genotypes.

Aspen genotypes exhibited striking variation in concentrations of phenolic glycosides, with concentrations highest in genotype 271 and lowest in 42E (Fig. 1). Elevated CO_2 and O_3 had no significant independent or interactive effects on salicortin and tremulacin concentrations.

Beetle performance

Aspen genotype, CO_2 and O_3 influenced the performance of aspen leaf beetles (Fig. 2 and Table 2). Female larval development time averaged 31, 30 and 36 days when reared on genotypes 216, 271 and 42E, respectively (pooled across fumigation treatments). Similarly, male larval development time averaged 31, 29 and 35 days when reared on genotypes 216, 271 and 42E, respectively. Female larvae reared on genotype 271 experienced a slight (8%) increase in development time under elevated CO_2 conditions, whereas females on genotypes 216 and 42E experienced no change or minor decreases in development times under elevated CO_2 conditions (significant genotype × CO_2 interaction). No significant change in development time was observed for male larvae under elevated CO_2 conditions. Both female and male larvae experienced increases in development time across genotypes under elevated O_3, although more so for females reared on 42E (23%) than those on 216 (7%) or 271 (6%) (significant genotype × O_3 interaction). No significant CO_2 × O_3 interaction was detected for larval development time. We observed a 15% and 10% increase in development time for female and male larvae, respectively (pooled across genotypes), under the combined elevated CO_2 and elevated O_3 plots compared with ambient treatments.

Genotype, CO_2 and O_3 independently or interactively influenced adult beetle mass. Females reared on genotypes 216,

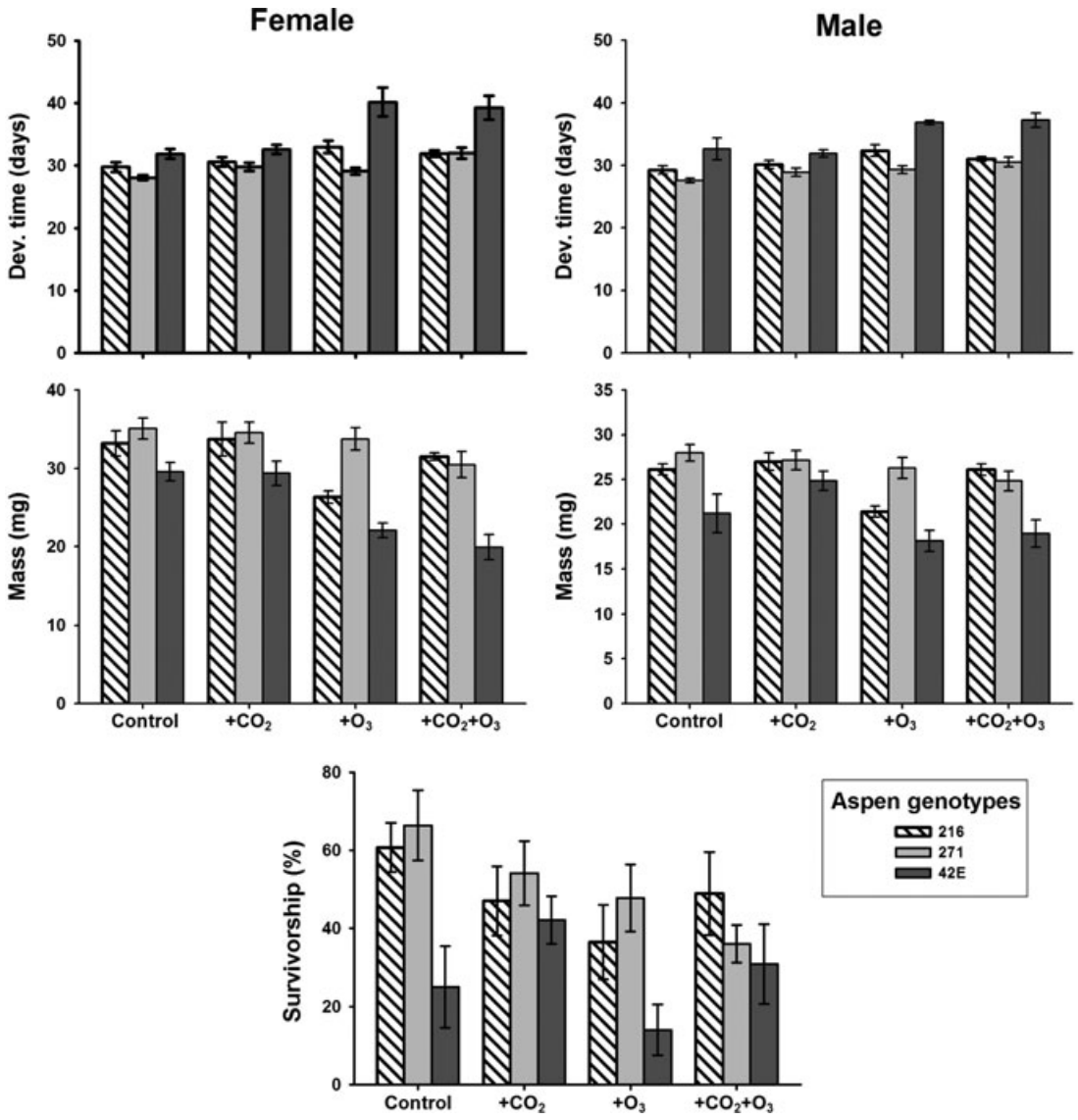

Figure 2 Effects of CO_2 and O_3 on performance of beetles reared on aspen genotypes 216, 271 and 42E. Bars and vertical lines indicate the mean ± SE. Dev. time, development time.

Table 2 Summary of *F* values, degrees of freedom and *P* values for the effects of genotype, CO_2 and O_3 on aspen leaf beetle performance

	Development time				Adult mass					
	Female		Male		Female		Male		Survivorship	
Main effects and interactions	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*
Genotype	67.01 (2.70)	<0.001	52.66 (2.70)	<0.001	33.28 (2.73)	<0.001	28.42 (2.72)	<0.001	8.47 (2.87)	<0.001
CO_2	1.06 (1.6)	0.344	0.21 (1.6)	0.663	0.00 (1.6)	0.989	3.58 (1.6)	0.106	0.25 (1.6)	0.632
O_3	15.20 (1.6)	0.008	21.55 (1.6)	0.003	23.30 (1.6)	0.003	16.06 (1.6)	0.007	15.03 (1.6)	0.008
Genotype × CO_2	5.21 (2.70)	0.008	1.67 (2.70)	0.195	3.52 (2.73)	0.035	4.62 (2.72)	0.013	2.73 (2.87)	0.071
Genotype × O_3	10.82 (2.70)	<0.001	4.79 (2.70)	0.011	3.96 (2.73)	0.023	1.41 (2.72)	0.252	0.18 (2.87)	0.837
CO_2 × O_3	0.17 (1.6)	0.698	0.27 (1.6)	0.624	0.00 (1.6)	0.955	0.08 (1.6)	0.786	1.45 (1.6)	0.275
Genotype × CO_2 × O_3	1.11 (2.70)	0.335	0.81 (2.70)	0.451	2.15 (2.73)	0.123	2.16 (2.72)	0.123	0.82 (2.87)	0.443

Table 3 Phytochemicals accounting for variation in beetle performance (multiple regressions using backward elimination; the selection criterion for remaining in the model was $\alpha = 0.10$)

	Regression model				Partial coefficients			
Parameter	Equation	Adjusted R^2	F (d.f.)	P	Variable	r^2	t-value	$P(t)$
Development time								
Females	$Y = 45.09 - 0.56$ (PG) $- 5.35$ (N)	0.445	35.88 (2.85)	<0.001	PG	0.321	7.149	<0.001
					N	0.118	4.230	<0.001
Males	$Y = 39.98 - 0.50$ (PG) $- 3.13$ (N)	0.416	31.23 (2.83)	<0.001	PG	0.349	−7.121	<0.001
					N	0.057	−2.887	0.005
Adult mass								
Females	$Y = 10.64 + 0.76$ (PG) $+ 8.36$ (N)	0.367	26.82 (2.87)	<0.001	PG	0.253	5.904	<0.001
					N	0.114	4.007	<0.001
Males	$Y = 15.95 + 0.52$ (PG) $+ 2.95$ (N)	0.271	17.14 (2.85)	<0.001	PG	0.241	5.314	<0.001
					N	0.032	1.960	0.053
Survivorship	$Y = 27.67 + 2.21$ (PG)	0.088	11.02 (1.103)	0.001	PG	0.097	3.320	0.001

PG, total phenolic glycoside; N, nitrogen

271 and 42E averaged 31, 33 and 25 mg, respectively (pooled across fumigation treatments). Males reared on genotypes 216, 271 and 42E averaged 25, 26 and 21 mg, respectively. Elevated CO_2 increased beetle mass for females on genotype 216 (10%), but decreased mass for females on genotypes 271 (6%) and 42E (5%) (significant genotype × CO_2 interaction). Elevated CO_2 increased beetle mass for males on genotypes 216 (12%) and 42E (11%) but decreased mass for those on genotype 271 (4%) (significant genotype × CO_2 interaction). Elevated O_3 decreased beetle mass across all aspen genotypes (16% and 12% decreases for females and males, respectively, pooled across genotypes). Elevated O_3 decreased mass by 14%, 8% and 29% for female beetles reared on genotypes 216, 271 and 42E, respectively (significant genotype × O_3 interaction). By contrast, the effect of elevated O_3 on male mass was consistent across genotypes. No significant CO_2 × O_3 interaction was detected for beetle mass. We observed a 17% decrease in female mass and 7% decrease in male mass under the combined fumigation treatment compared with ambient (pooled across genotypes).

Aspen genotype strongly affected beetle survivorship. Averaged across all treatments, 48%, 51% and 28% of beetles survived on genotypes 216, 271 and 42E, respectively. Elevated CO_2 did not affect the survivorship of beetles reared on genotype 216, decreased survivorship on genotype 271 (21%) and increased survivorship on 42E (87%) (marginally significant genotype × CO_2 interaction). By contrast, elevated O_3 decreased survivorship consistently (27%) across all genotypes. No significant CO_2 × O_3 interaction was found. The combined fumigation treatment decreased survivorship by 19% and 46% for beetles reared on genotypes 216 and 271, respectively, and increased survivorship by 23% for beetles on genotype 42E compared with ambient conditions.

In summary, all measures of beetle performance (larval development time, adult mass and survivorship) were best on aspen genotype 271 and worst on genotype 42E. Elevated CO_2 negatively affected beetles reared on 271 but had inconsistent effects on beetles reared on 216 or 42E. Overall, elevated O_3 decreased beetle performance across genotypes; however, elevated O_3 had the most negative impact on beetles reared on 42E.

Beetle performance in relation to foliar chemistry

Aspen phytochemistry explained a substantial amount (9–45%) of the variation in leaf beetle performance (Table 3). Total foliar phenolic glycosides and nitrogen concentration were inversely related to larval development time for both sexes, indicating that higher concentrations of foliar phenolic glycosides and nitrogen are correlated with shorter development times. Furthermore, total phenolic glycosides and nitrogen concentration positively affected adult mass of both males and females (Table 3). Total phenolic glycoside concentrations explained a minimal amount (9%) of the variation in beetle survivorship, having a positive influence on survivorship.

Discussion

As predicted, aspen genotype had a significant impact on foliar chemistry and beetle performance. Nitrogen and phenolic glycosides, in particular, were the most influential with respect to beetle performance, with both having a positive influence on the specialist herbivore. Aspen leaf beetles performed best on aspen genotype 271 and worst on genotype 42E. Although elevated CO_2 and O_3 had minimal effects on the phytochemical constituents analysed in the present study, the gases influenced beetle performance nonetheless. Elevated CO_2 negatively affected the performance of beetles on genotype 271 but had inconsistent effects across performance variables for beetles reared on genotypes 216 and 42E. Elevated O_3 negatively affected beetle performance across all genotypes, and most strongly for beetles reared on aspen genotype 42E, a relatively O_3-sensitive genotype.

Foliar chemistry

As predicted, phytochemistry varied significantly among aspen genotypes. Similar to the results of several previous studies (Hemming & Lindroth, 1995; Lindroth *et al*., 2002; Osier & Lindroth, 2004), aspen genotype had a significant effect on foliar nitrogen concentration. A large genetic effect was observed for condensed tannin concentrations, consistent with previous research (Hwang & Lindroth, 1998; Kopper &

Lindroth, 2003b; Donaldson & Lindroth, 2004). We also observed notable variation in salicortin and tremulacin concentrations among aspen genotypes, consistent with the findings reported by Hwang and Lindroth (1997) and Osier and Lindroth (2004).

Elevated CO_2 and O_3 had minimal effects on aspen phytochemistry, in contrast to our prediction and the results obtained in several previous Aspen FACE studies (Agrell *et al*., 2000; Holton *et al*., 2003; Kopper & Lindroth, 2003b). Nonetheless, elevated CO_2 increased condensed tannins, consistent with the findings of Peltonen *et al*. (2005) for birch foliage under elevated CO_2 conditions. Although previous studies have reported an increase in phenolic concentrations under elevated O_3 (Yamaji *et al*., 2003; Peltonen *et al*., 2005; Valkama *et al*., 2007), these effects are not typically observed when O_3 levels are 1.5 × ambient or lower (Valkama *et al*., 2007). Because Aspen FACE ozone levels are 1.5 × ambient, the absence of significant O_3 effects on phenolic concentrations is not surprising. Furthermore, the meta-analysis of Valkama *et al*. (2007) revealed no significant effects of elevated O_3 on tannins or combined elevated CO_2 and O_3 on overall phenolic concentrations.

Several factors may help explain the minimal effects of CO_2 and O_3 observed in the present study, relative to earlier work at Aspen FACE. First, the effects of CO_2 and O_3 on foliar chemistry can shift temporally through a season (Gifford *et al*., 2000; Kopper & Lindroth, 2003b; Marinari *et al*., 2007). Kopper and Lindroth (2003b) found that the major impact of fumigation treatment on nitrogen concentrations, for example, occurred earlier in the growing season compared with when the present study was conducted. Second, the diminution of fumigant impact in the present study could possibly reflect ontogenetic variation in tree responsiveness to CO_2 and O_3: younger, rapidly-growing trees in open stands may be more responsive than older trees in closed-canopy stands.

Beetle performance

Effects of genetic variation in aspen. As predicted, genetic variation in aspen significantly influenced *C. crotchi* performance in the present study. *Chrysomela crotchi* larvae developed fastest on the genotype (271) with the lowest concentrations of condensed tannins and the highest concentrations of phenolic glycosides, as predicted, which supports the notion that specialist herbivores on *Populus* avoid condensed tannins and prefer high concentrations of phenolic glycosides. Phenolic glycosides are probably a valuable resource for specialist leaf beetles such as *C. crotchi*, perhaps serving as a precursor for a defence chemical, such as salicylaldehyde (Rowell-Rahier & Pasteels, 1982), or providing glucose for energy metabolism (Rowell-Rahier & Pasteels, 1986).

Adult beetle mass was also influenced by aspen genotype in our study. The results obtained in the present study are in contrast to several studies (Orians *et al*., 1997; Coyle *et al*., 2001; Donaldson & Lindroth, 2004) that report no significant differences in leaf beetle mass among multiple host genotypes. However, Augustin *et al*. (1997) and Glynn *et al*. (2004) did find genetic effects on *Chrysomela scripta* and *Phratora vulgatissima* pupal mass, respectively.

Finally, aspen genotype also significantly influenced *C. crotchi* survivorship. In a study by Coyle *et al*. (2001) that examined the effects of eight *Populus* clones on *C. scripta* performance, larval survivorship was differentially influenced by *Populus* genotype when assayed over multiple beetle generations. Similarly, Augustin *et al*. (1997) reported that *C. scripta* survival varied among five *Populus* clones. The results of the present study suggest that *C. crotchi* survivorship increases with increasing phenolic glycoside concentrations, which are predominantly genetically-determined.

Aspen leaf beetles may use salicylates to synthesize their own defence secretions to combat predation, an adaptation that is common among salicylate-feeding chrysomelids (Rowell-Rahier & Pasteels, 1982; Pasteels *et al*., 1983). The results obtained in the present study are consistent with this hypothesis because beetle survivorship increased with increasing phenolic glycoside concentrations. Although we had intended to prevent predation with mesh enclosures, we occasionally found that stink bugs had successfully pierced through the mesh enclosures and killed beetle larvae. Less frequently, we observed that cryptic syrphid fly larvae had been accidentally enclosed with the larvae, resulting in additional beetle predation. Predation by stink bugs and syrphid fly larvae across genotypes and fumigation treatments accounted for 0–33% of the total beetle mortality (data not shown). Beetles feeding on genotype 42E grown in control and $+O_3$ FACE rings experienced the highest predation (33% of mortality was a result of predation). These patterns of mortality suggest that leaf beetles feeding on hosts with higher phenolic glycoside concentrations (i.e. genotypes 216 and 271) may have experienced reduced predation because more salicylates were available for defence.

Host plant quality explains many of the observed differences in beetle performance among aspen genotypes in the present study. In particular, *C. crotchi* appear to perform better on aspen genotypes with higher nitrogen and phenolic glycoside concentrations, as we predicted. These results are similar to those of previous studies on *Populus* and *Salix* leaf beetle specialists. Rowell-Rahier (1984) found that willow containing phenolic glycosides were often fed upon by specialists and avoided by generalist feeders. Rank (1992) found that larvae and adult *Chrysomela aeneicollis* preferred feeding on salicylate-rich willows rather than salicylate-poor willows. Rank *et al*. (1998) showed that larvae of the leaf beetle *Phratora vitellinae* developed faster on salicylate-rich willows than salicylate-poor willows. Moreover, previous studies have shown that salicylates may stimulate feeding by leaf beetles (Rank, 1992; Orians *et al*., 1997), which may explain the shortened development time and increased beetle mass observed for those larvae reared on aspen genotypes with higher phenolic glycoside concentrations.

Effects of elevated CO_2. We expected to observe a decrease in plant quality and, subsequently, beetle performance under elevated CO_2; however, we observed no significant change in plant quality. We did observe a change in beetle performance, and these changes differed among aspen genotypes. In particular, we observed a decrease in beetle performance on genotype 271 under elevated CO_2. In a study on beetle larvae (*Phratora vitellinae*) reared on willow, Veteli *et al*. (2002) found that elevated

CO_2 reduced relative growth rates, probably as a result of a decrease in food quality (nitrogen content). Furthermore, Johns and Hughes (2002) observed that elevated CO_2 and temperature decreased survivorship of leaf miners. However, O'Neill *et al*. (2008) observed increased longevity and fecundity of Japanese beetles fed soybeans grown under elevated CO_2. In the present study, beetles performed best on genotype 271 under ambient CO_2, causing this genotype to be more susceptible to aspen leaf beetle damage than the other genotypes tested. However, this genotype may be less susceptible to leaf beetle damage in future high-CO_2 environments (as indicated by decreased beetle performance), and thus better able to compete with other genotypes. Because elevated CO_2 had little effect on aspen phytochemistry in the present study, the factors contributing to changes in beetle performance remain unresolved. Nonetheless, the trends in our data indicate that elevated CO_2 may lower the concentration of salicortin in some genotypes (e.g. genotype 271), and this effect may negatively influence the performance of specialist leaf beetles.

Effects of elevated O_3. As we predicted, elevated O_3 generally decreased beetle performance. Previous research (Kopper & Lindroth, 2003a) documented an increase in development time of male blotch leafminers under elevated O_3. By contrast, the effects of elevated O_3 on the forest tent caterpillar have been somewhat inconsistent, causing development time to decrease (Kopper & Lindroth 2003b), increase or remain the same (Fortin *et al*., 1997). However, the aspen blotch leafminer and *C. crotchi* are specialist herbivores that are likely adapted to species (such as aspen) rich in phenolic glycosides, whereas the forest tent caterpillar is a generalist herbivore that is typically deterred by high concentrations of phenolic glycosides (Lindroth & Bloomer, 1991; Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). Thus, the influence of atmospheric pollutants on herbivore performance may be dependent on the herbivore's feeding behaviour (generalist or specialist) and the specific chemical fingerprint of each host genotype.

Elevated O_3 typically decreased adult mass, and the magnitude of change was genotype-specific in females. Lyytikäinen *et al*. (1996) and Fortin *et al*. (1997) observed a decrease in larval mass of sawflies and male pupal mass of forest tent caterpillars, respectively, when reared on hosts produced under elevated O_3. However, Kopper and Lindroth (2003b) reported an O_3-induced increase in pupal mass of forest tent caterpillars, probably as a result of decreased foliar concentrations of phenolic glycosides and slightly increased concentrations of nitrogen. By contrast, elevated O_3 did not affect pupal mass of aspen blotch leafminers (Kopper & Lindroth, 2003a). In the present study, a combination of factors, including a decrease in nitrogen and a decrease in phenolic glycoside concentrations (although not significant), may have contributed to the decline in aspen leaf beetle mass under elevated O_3. Freiwald *et al*. (2008) documented the importance of phenolic glycosides to leaf beetles that feed on salicaceous species such as aspen. These authors suggest that *Phyllobius pyri* preferred feeding on O_3-exposed leaves because these leaves were physiologically younger (O_3 delays bud burst), and therefore had lower leaf mass per area and higher concentrations of phenolic glycosides than control leaves. Thus, fumigation treatments such as O_3 can influence leaf structure as well as composition, which may have contributed to the differences in beetle performance observed in the present study.

Elevated O_3 reduced survivorship across all genotypes. Few studies have researched the effects of elevated O_3 on herbivore survivorship on perennial plants. No significant O_3 effects were observed by Lyytikäinen *et al*. (1996), Fortin *et al*. (1997), or Kopper and Lindroth (2003a).

Aspen genotypes 216 and 271 are relatively O_3-tolerant, whereas genotype 42E is relatively O_3-sensitive (Karnosky *et al*., 2003). Under elevated O_3, beetles performed better on less sensitive genotypes (216 and 271) than on a more sensitive genotype (42E). In high O_3 environments, O_3-tolerant aspen may be more susceptible to outbreaks by specialist herbivores and thus be less successful than predicted based on physiological responses to O_3 alone. Conversely, when herbivore interactions are considered, O_3-sensitive aspen genotypes may be less compromised under future atmospheric conditions than previously predicted.

Conclusions

Substantial genetic variation in trembling aspen may differentially influence the performance of specialist herbivores, such as the aspen leaf beetle, under both ambient and altered atmospheric conditions. Indeed, aspen genotype affected all aspen phytochemistry and leaf beetle performance parameters measured in the present study. Not surprisingly, the most important chemical factors that influenced beetle performance (i.e. phenolic glycosides and nitrogen) are largely genetically-determined.

Many previous studies have shown that phenolic glycosides negatively impact the performance of insect herbivores. The present study, however, shows that phenolic glycoside concentrations positively influence the performance of aspen leaf beetles. This result is congruent with the specialized adaptation of *C. crotchi* to feeding on salicylate-rich species, such as aspen, although the adaptive advantage of this specialization (defence or improved nutrition) remains unexplored.

Despite the minimal atmospheric effects on foliar chemistry, elevated CO_2 and O_3 did alter the performance of aspen leaf beetles. Elevated CO_2 influenced development time (females only), mass and survivorship differently depending on genotype. In general, elevated O_3 negatively influenced beetle performance. However, beetles performed better on O_3-tolerant genotypes than on the O_3-sensitive genotype, which has important implications for the future genetic structure of aspen populations. Indeed, aspen are likely to show differential susceptibility to leaf beetle specialists (e.g. *C. crotchi*) both among and within genotypes under future atmospheric conditions. Coupled with changes in clonal feeding preference that may occur under elevated CO_2 and/or O_3 (Agrell *et al*., 2005), aspen populations will experience changing impacts by insect herbivores under future atmospheric conditions.

Acknowledgements

Aspen FACE is principally supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG02-95ER62125 to Michigan Technological University,

and Contract No. DE-AC02-98CH10886 to Brookhaven National Laboratory, the U.S. Forest Service Northern Global Change Program and North Central Research Station, Michigan Technological University, and Natural Resources Canada–Canadian Forest Service. This work was also supported by National Science Foundation grant DEB-0129123 and Department of Energy (Office of Science, BER) grant DE-FG02-06ER64232 to R.L. Lindroth. L. Vigue was supported by the Ford Foundation (Pre-doctoral Fellowship) and the University of Wisconsin-Madison (Advanced Opportunity Fellowship). We thank three anonymous reviewers for their constructive comments and suggestions. We thank Michelle Tremblay and Ed Mondor for their assistance with beetle collection, Jun Zhu for her assistance with the statistical analyses and Matthias Jaime and Brian Miranda for their assistance with the artwork.

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Accepted 12 January 2010
First published online 12 April 2010