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Response of quaking aspen genotypes to enriched CO_2: foliar chemistry and tussock moth performance

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Abstract 1 Genetic variation in the phytochemical responses of plants to CO_2 enrichment is likely to alter trophic dynamics, and to shift intraspecific selection pressures on plant populations. We evaluated the independent and interactive effects of atmospheric CO_2 and quaking aspen (*Populus tremuloides* Michx.) genotype on chemical composition of foliage and performance of the whitemarked tussock moth (*Orgyia leucostigma* J. E. Sm.).

2 This research was conducted at the Aspen FACE (Free Air CO_2 Enrichment) site in northern Wisconsin, U.S.A. Leaf samples were collected periodically from each of three genetically variable aspen genotypes growing under ambient and elevated CO_2, and analysed for levels of primary and secondary metabolites. Tussock moth larvae were reared *in situ* on experimental trees, and development times and pupal masses were recorded.

3 Foliar chemical composition varied among aspen genotypes and in response to CO_2 enrichment. However, chemical responses of trees to elevated CO_2 were generally consistent across genotypes.

4 Larval development times varied among host genotypes and increased slightly for insects on high-CO_2 plants. Enriched CO_2 tended to reduce insect pupal masses, particularly for females on one of the three aspen genotypes.

5 $CO_2 \times$ genotype interactions observed for plant chemistry and insect performance in this study with a small number of genotypes are probably too few, and too weak, to shift selection pressures in aspen populations. These results differ, however, from earlier work in which more substantial $CO_2 \times$ genotype interactions were observed for plant chemistry.

Keywords Aspen, CO_2, FACE, feeding trials, genetic variation, plant–insect interactions, secondary metabolites.

Introduction

The fitness of insect herbivores is strongly determined by the chemical composition of their food plants, which in turn is influenced by environment, genetics and interactions between environment and genetics (Fritz & Simms, 1992; Herms & Mattson, 1992; Karban & Baldwin, 1997). Certain environmental conditions are now undergoing change at a global scale, and these changes can be expected to alter not only the productivity of plants, but also the performance of organisms associated with those plants. Atmospheric CO_2 is of particular interest, as concentrations are expected to increase throughout this century (Houghton *et al.*, 1996). Enriched CO_2 alters the quantity and quality of plant biomass (Ceulemans & Mousseau, 1994; Saxe *et al.*, 1998) as well as the fitness of herbivorous insects (Lincoln *et al.*, 1993; Watt *et al.*, 1995; Lindroth, 1996a,b; Bezemer & Jones, 1998). Moreover, the magnitude and direction of herbivore responses vary among both plant and insect species (Lindroth, 1996a,b; Bezemer & Jones, 1998).

In contrast to the many studies that have investigated interspecific variation in plant responses to enriched CO_2, few have addressed intraspecific variation in responses. These are of pivotal importance for assessing the potential of plants for evolutionary adaptation to high CO_2 environments (Geber & Dawson, 1993; Curtis *et al.*, 1994; Bazzaz *et al.*, 1995; Ward & Strain, 1999). Even fewer studies have evaluated such variation in relation to plant primary and

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secondary metabolites (e.g. Fajer *et al.*, 1992; Goverde *et al.*, 1999; Mansfield *et al.*, 1999; Lindroth *et al.*, 2001), and only two have addressed the consequences of such changes for insect herbivores (Mansfield *et al.*, 1999; Goverde *et al.*, 1999).

Particularly during insect population outbreaks, a substantial proportion of foliar biomass can be transferred to the forest floor in the form of insect frass. As for leaf litter, the decomposition of insect frass is likely to be influenced by its nitrogen and tannin concentrations (Anderson, 1991; Lovett & Ruesink, 1995; Hättenschwiler & Vitousek, 2000). Nothing has been reported, however, with respect to the impact of CO_2 enrichment on the chemical composition of insect frass.

The purpose of the research reported here was to evaluate the independent and interactive effects of atmospheric CO_2 and plant genotype on foliar chemical composition and herbivorous insect performance. Our experimental system consisted of quaking aspen (*Populus tremuloides*) and the whitemarked tussock moth (*Orgyia leucostigma*). Quaking aspen is one of the most abundant and genetically variable tree species in the Great Lakes region of North America (Dickmann & Stuart, 1983; Mitton & Grant, 1996). The whitemarked tussock moth is a polyphagous, tree-feeding lepidopteran. Although generally not considered a pest species, populations have been known to reach outbreak levels in both urban and forest settings (Rose & Lindquist, 1982). Specific objectives of our research were to assess: (1) the effects of enriched CO_2 on foliar chemistry of aspen under natural, unchambered conditions; (2) whether such effects vary among aspen genotypes; (3) the consequences of CO_2- and genotype-mediated variation in foliar chemistry for long-term performance of tussock moths; and (4) the consequences of CO_2- and genotype-mediated variation in foliar chemistry for nitrogen and tannin levels in insect frass.

Materials and methods

Experimental site

This study was conducted in the summer of 1999 at the Aspen Free Air CO_2 Enrichment (FACE) facility in northern Wisconsin, U.S.A (89.7° W, 45.7° N). This facility contains 12 FACE rings (30 m diameter), designed to assess the independent and interactive effects of CO_2 and O_3 on the structure and function of northern hardwood stands. Seedling quaking aspen (five clones), paper birch and sugar maple were planted (1×1 m spacing) in the rings in 1997. A stand of mixed aspen genotypes is located in the eastern half of each ring. Fumigation commenced in spring 1998, and is conducted only during daylight hours of the growing season. The three aspen genotypes selected for our study were approximately 1.5–3 m in height in spring 1999. These genotypes differ with respect to intrinsic growth rates as well as growth responses to both elevated CO_2 and O_3 (Dickson *et al.*, 2001; Isebrands *et al.*, 2001). Further details about the Aspen FACE facility are provided by Dickson *et al.* (2001).

Experimental design

As the focus of this experiment was on CO_2, only six of the 12 FACE rings were used. The overall experimental design was a split-plot, with CO_2 level (ambient and 560 μL/L) as the whole plot treatment and aspen genotype (clones 216, 259, 271) as the subplot treatment. We restricted our study to three genotypes because trees from only these genotypes were large enough to support long-term insect bioassays. The FACE site is divided into three blocks on a north–south gradient, with each block containing one ring of each treatment. Within each ring, we selected three individual trees from each of the three aspen genotypes for use in this study.

Phytochemical analyses

Leaves to be used for phytochemical analyses were collected three times during the course of the study (3 June, 15 June and 29 June). So as to accurately represent leaves fed upon by tussock moths, branches used for foliar collection were enclosed in the same mesh material as used to contain insects in the bioassays (see 'Insect bioassays'). Relative branch position and sun exposure were similar for foliage used for chemical and insect assays.

Leaves (2–3 g fresh mass) were removed by cleanly snipping at the petiole, a method that has been shown to not induce a chemical response from the tree (Mattson & Palmer, 1988). Samples were stored under crushed ice and transported to the laboratory (a maximum of 4 h from first leaf excision), where they were flash-frozen with liquid nitrogen and freeze-dried. Dried leaf material was ground and stored at −20 °C prior to chemical analysis.

We conducted chemical analyses for leaf constituents known to be responsive to atmospheric CO_2 levels and likely to influence insect growth performance. These included nitrogen, soluble sugars and starch, phenolic glycosides and condensed tannins. Nitrogen determinations were made with a LECO FP528 nitrogen analyser, using glycine *p*-toluene sulphonate as a standard. Soluble sugar and starch concentrations were measured using a modification of the dinitrosalicylic acid method as described by Lindroth *et al.* (2002). Concentrations of the phenolic glycosides salicortin and tremulacin were measured by high performance thin-layer chromatography (HPTLC) as reported by Lindroth *et al.* (1993). Salicortin and tremulacin standards were purified from aspen leaves using flash chromatography (Still *et al.*, 1978). Finally, condensed tannin concentrations were quantified by the butanol-HCl method of Porter *et al.* (1986), which hydrolytically converts proanthocyanidins to anthocyanidins. Condensed tannins for use as reference standards were purified from aspen leaves by adsorption chromatography (Hagerman & Butler, 1980).

Insect bioassays

Tussock moth egg masses were provided by the Forest Pest Management Institute, Canadian Forest Service (Sault St. Marie, Ontario, Canada). Egg masses were surface-sterilized in a solution of 0.1% sodium hypochlorite and

1% Tween 80, then placed into a Percival® growth chamber (26 : 18 °C and LD 16 : 8 h cycle) until hatch.

Upon hatching (27 May 1999), we randomly assigned 60 larvae to each of three aspen trees per genotype, per FACE ring. To reduce mortality associated with transfer and establishment of small larvae, we retained larvae in ventilated 2.5 × 15 cm Petri dishes for the duration of the first stadium. Larvae were fed leaves excised from their assigned tree, and rearing containers were maintained within the FACE rings, so larvae were exposed to the fumigation treatments. Upon moulting to second instars, larvae assigned to each tree were apportioned to two mesh bags per tree (30–40 larvae per bag). A second mesh bag was secured around the outside of each bioassay bag to reduce predation by hemipteran predators. To prevent excessive defoliation of the trees, the number of larvae in each bag was reduced to 10 randomly selected individuals during the third larval stadium. When enclosed branches became heavily defoliated, bags and insects were moved to new locations on each tree. As indices of insect performance, we recorded duration of the larval development period and pupal mass for all insects that successfully pupated. Pupal mass was determined 3 days after pupation.

To assess the impact of CO_2 enrichment and genotype on frass chemistry, we filled 25 mL vials (one vial per tree) with frass from fifth stadium insects, removed leaf particles, then freeze-dried and ground the material. Chemical analyses for nitrogen and tannins were conducted as described previously for foliar samples.

Statistics

Analysis of variance (ANOVA; PROC MIXED, Littell *et al.*, 1996) was used for statistical analysis. For analysis of phytochemical data we used a blocked split-plot design with repeated measures over time. The a priori statistical model employed was:

$$Y_{ijkl} = \mu + B_i + C_j + e_{ij} + G_k + T_l + CG_{jk} + CT_{jl} + GT_{kl} + CGT_{jkl} + \varepsilon_{ijkl}$$

where Y_{ijkl} was the average response of block i, CO_2 level j, genotype k, and time l. CO_2 level (C_j), genotype (G_k), time (T_l), and their interaction terms (CG_{jk}, CT_{jl}, GT_{kl} and CGT_{jkl}) represent fixed effects. Block (B_i), whole plot error (e_{ijk}) and subplot error (ε_{ijkl}) represent random effects. Use of the aforementioned model for inference relies on the assumption that treatment effects are the same for each block (i.e. block and treatment effects are additive). We found, however, that this assumption was not met. Here we describe the procedure by which lack of fit was determined and the corresponding changes required for analysis.

To explore the assumption, we considered the previous model augmented by terms representing the interaction between each fixed effect and block:

$$Y_{ijkl} = \mu + C_j + B_i + BC_{ij} + G_k + T_l + CG_{jk} + CT_{jl} + GT_{kl} + CGT_{jkl} + (BG_{ik} + BT_{il} + BCG_{ijk} + BCT_{ijl} + BGT_{ikl} + BCGT_{ijkl}).$$

Thus, e_{ijk} was partitioned into block × CO_2 (BC_{ij}), block × genotype (BG_{ik}) and block × CO_2 × genotype (BCG_{ijk}), whereas ε_{ijkl} was partitioned into block × time (BT_{il}), block × CO_2 × time (BCT_{ijl}), block × genotype × time (BGT_{ikl}) and block × CO_2 × genotype × time ($BCGT_{ijkl}$). By using likelihood methods integral to PROC MIXED, we determined that one or more of these interaction terms was significant for all response variables (Littell *et al.*, 1996). Therefore, *F*-tests were conducted for all main effects with degrees of freedom for error assigned using the Satterthwaite approximation (Milliken & Johnson, 1984; Littell *et al.*, 1996). Means and standard errors were calculated using the LSMEANS procedure and are reported for each CO_2 × genotype × time combination.

Data for insect performance and frass chemistry were analysed using a modification of the model used for analysis of phytochemical data. For insect performance, the time factor was removed, and sex was added to the model (tussock moths are sexually dimorphic). Frass chemical data were analysed similarly, after removal of the sex term. *F*-tests were performed with degrees of freedom for error assigned using the Satterthwaite approximation (Milliken & Johnson, 1984; Littell *et al.*, 1996), as described for analysis of phytochemical data. Means and standard errors were calculated using the LSMEANS procedure statement and are reported for each CO_2 × genotype × sex combination (insect performance) or CO_2 × genotype (frass chemistry) combination.

Due to the low number of replicates ($n = 3$), we report *P*-values <0.10 as 'significant', thereby reducing the probability of type II statistical errors (Filion *et al.*, 2000). For readers requiring a more stringent α, we included exact *P*-values and degrees of freedom for all main effects and interactions (Tables 1–3).

Results

Foliar chemistry

The chemical composition of aspen leaves was influenced by CO_2, genotype, time and interactions among those factors. Enriched CO_2 reduced foliar nitrogen levels by 5% relative to controls, and this effect did not differ significantly among genotypes or over time (Fig. 1, Table 1). Nitrogen levels were lower in genotype 216 than in genotypes 259 and 271, especially early in the season (genotype × time interaction). Concentrations of simple sugars were unaffected by CO_2 treatment, and were higher in genotype 216 than in genotypes 259 and 271 (Fig. 1, Table 1). Levels were dynamic over time (differentially so among genotypes), and tended to peak in the second collection period (15 June). Similarly, foliar starch concentrations were not affected by CO_2 environment, but differed among genotypes, and the difference varied through time (genotype × time interaction; Fig. 1, Table 1).

Concentrations of the major carbon-based secondary metabolites of aspen were also influenced by CO_2, genotype, time and their interactions. Enriched CO_2 increased

Table 1 Summary of statistical analysis of the effects of CO_2 and genotype on chemical composition of aspen leaves. Degrees of freedom were estimated by the Satterthwaite approximation (Milliken & Johnson, 1984; Littell *et al.*, 1996)

Main effects and interactions		Nitrogen	Sugars	Starch	Salicortin	Tremulacin	Condensed tannins
CO_2	*F*(d.f.)	7.3 (1,7.3)	<0.1 (1,13.3)	<0.1 (1,11.8)	9.8 (1,4.6)	15.7 (1,6)	0.34 (1,3.5)
	P	0.064	0.984	0.980	0.029	0.008	0.595
Genotype	*F*(d.f.)	15.8 (2,5)	8.3 (2,9.1)	17.6 (2,10.3)	5.0 (2,4)	7.0 (2,4)	51.2 (2,8.5)
	P	0.007	0.009	<0.001	0.081	0.049	<0.001
Time	*F*(d.f.)	81.8 (2,5.2)	5.6 (2,9.8)	40.7 (2,5.5)	5.4 (2,7.9)	2.6 (2,24)	21.9 (2,5.5)
	P	<0.001	0.024	<0.001	0.033	0.098	0.002
CO_2 × genotype	*F*(d.f.)	4.4 (2,3.8)	1.6 (2,8.3)	0.6 (2,10.3)	0.3 (2,5.8)	1.6 (2,6)	9.9 (2,5.6)
	P	0.104	0.255	0.565	0.746	0.282	0.014
CO_2 × time	*F*(d.f.)	0.3 (2,3.2)	0.7 (2,9.8)	1.19 (2,4.3)	<0.1 (2,6.7)	0.3 (2,24)	5.0 (2,2.8)
	P	0.752	0.511	0.387	0.961	0.742	0.118
Genotype × time	*F*(d.f.)	6.3 (4,6.9)	3.5 (4,8.9)	11.5 (4,15.7)	4.6 (4,9.9)	2.9 (4,24)	6.5 (4,8.9)
	P	0.018	0.056	<0.001	0.024	0.044	0.010
CO_2 × genotype × time	*F*(d.f.)	2.5 (4,8.5)	1.5 (4,7.9)	1.3 (4,15.7)	0.6 (4,7.4)	0.4 (4,24)	1.4 (4,8.6)
	P	0.123	0.280	0.321	0.662	0.844	0.316

Table 2 Summary of statistical analysis of the effects of CO_2 and genotype on tussock moth performance. Degrees of freedom were estimated by the Satterthwaite approximation (Milliken & Johnson, 1984; Littell *et al.*, 1996)

Main effects and interactions		Development time	Pupal mass
CO_2	*F* (d.f.)	11.9 (1,8.2)	8.2 (1,6.1)
	P	0.008	0.029
Genotype	*F* (d.f.)	18.6 (2,11.6)	1.5 (2,4.1)
	P	<0.001	0.325
Sex	*F* (d.f.)	37.5 (1,3.5)	394.3 (1,5.5)
	P	0.006	<0.001
CO_2 × genotype	*F* (d.f.)	1.7 (2,8.2)	1.8 (2,6.1)
	P	0.235	0.237
CO_2 × sex	*F* (d.f.)	7.3 (1,5.8)	3.0 (1,6.4)
	P	0.037	0.130
Genotype × sex	*F* (d.f.)	0.6 (2,5.1)	0.68 (2,5.5)
	P	0.574	0.546
CO_2 × genotype × sex	*F* (d.f.)	1.2 (2,5.8)	3.3 (2,6.4)
	P	0.380	0.106

Table 3 Summary of statistical analysis of the effects of CO_2 and genotype on chemical composition of tussock moth frass. Degrees of freedom were estimated by the Satterthwaite approximation (Milliken & Johnson, 1984; Littell *et al.*, 1996)

Main effects and interactions		Condensed tannins	Nitrogen
CO_2	*F*(d.f)	5.2 (1,3.2)	0.23 (1,3.8)
	P	0.102	0.660
Genotype	*F*(d.f)	8.7 (2,5.0)	3.4 (2,7.4)
	P	0.024	0.091
CO_2 × genotype	*F*(d.f)	1.4 (2,3.5)	0.21 (2,7.4)
	P	0.361	0.817

levels of phenolic glycosides in all genotypes, an average of 15 and 32%, respectively, for salicortin and tremulacin (Fig. 2, Table 1). This effect of CO_2 did not differ significantly among genotypes or over time. During the 26-day period spanning the foliar collections, levels of phenolic glycosides increased slightly in genotype 216, remained generally stable in genotype 259, and increased markedly in genotype 271. Responses of condensed tannins to CO_2 enrichment varied strongly among aspen genotypes; levels decreased in genotype 216 but increased in genotypes 259 and 271 (Fig. 2, Table 1). Levels increased strongly over time in genotypes 216 and 259, but only slightly in genotype 271, which contained the lowest overall concentrations.

Tussock moth performance

CO_2 enrichment and aspen genotype influenced the long-term development and growth of whitemarked tussock moths, although the magnitude of effects was generally small. High CO_2 prolonged larval development times by an average of 1.3 days for females and 2.9 days for males (Fig. 3, Table 2). Development times were shorter for insects reared on genotype 259 than for those reared on genotypes 216 or 271. Enriched CO_2 exhibited a slight but significant impact on insect pupal mass, when averaged across genotypes and sex (Fig. 3, Table 2). The most pronounced effect occurred in females reared on genotype 259, for which high CO_2 reduced pupal masses by 21%. Finally, although development times averaged only 12% longer for females than for males, pupal masses averaged 127% greater for females than for males.

The chemical composition of tussock moth frass varied in relation to CO_2 treatment and host genotype. Nitrogen concentrations averaged 7.6% lower in the frass of insects from enriched CO_2 environments, relative to that of insects in ambient CO_2 environments, a marginally significant effect (Fig. 4, Table 3). Nitrogen concentrations also varied (slightly but significantly) among frass samples from insects reared on different aspen genotypes. Condensed tannin concentrations were highly variable both within and among treatments (Fig. 4, Table 3). Tannin levels were not affected by CO_2 treatment, but differed among host genotypes (a marginally significant response).

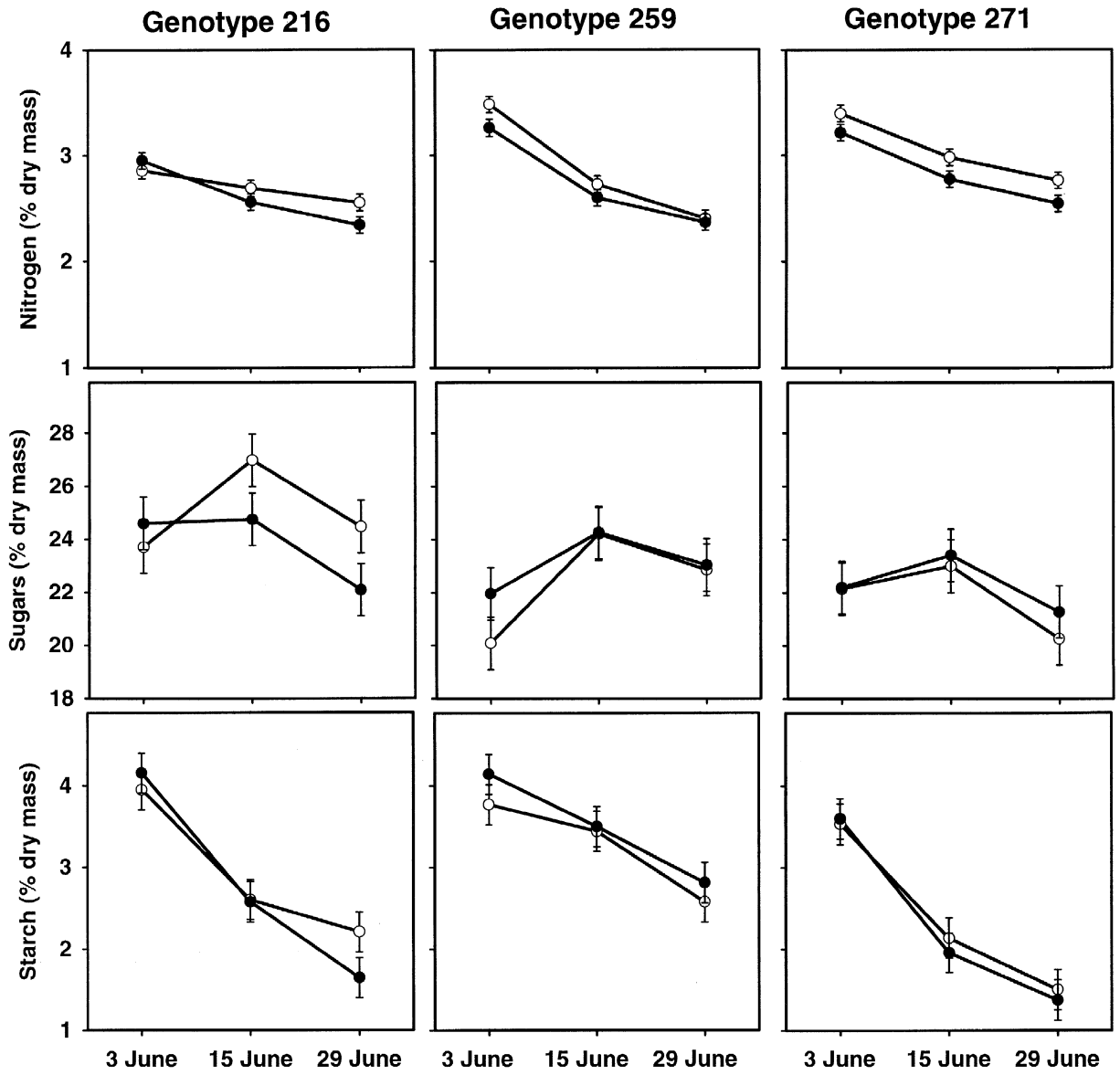

Figure 1 Effects of CO_2 and genotype on nitrogen and carbohydrate levels in quaking aspen foliage. ○ and ● represent ambient and elevated CO_2, respectively. Error bars indicate ±1 standard error of least squares means.

Discussion

Investigation of genetic variation in the effects of enriched CO_2 on both the chemical composition of plants, and the consequences of such changes for herbivore performance, is important for understanding the evolutionary responses of plants to global environmental change. Overall, this study revealed numerous independent effects of CO_2 and genotype on aspen chemistry and tussock moth performance, but relatively few interactive effects. These latter effects are the ones of primary interest, as they illustrate the potential for evolutionary responses of aspen populations to enriched CO_2.

Considering primary metabolites, nitrogen levels declined in response to CO_2 enrichment, and both nitrogen and carbohydrate levels varied among genotypes and over time. Responses of these constituents to high CO_2, however, were relatively uniform among genotypes. In a similar study with potted aspen genotypes grown in a greenhouse, Lindroth *et al.* (2001) also found no significant $CO_2 \times$ genotype interactions for foliar nitrogen and starch concentrations. Julkunen-Tiitto *et al.* (1993) reported the same results for nitrogen and sugar levels in clones of *Salix myrsinifolia*.

Phenolic glycosides and condensed tannins comprise a substantial proportion of the leaf mass of aspen, and levels of these compounds vary among genotypes as well as in response to resource availability (Lindroth & Hwang, 1996; Hwang & Lindroth, 1997; Hemming & Lindroth, 1999; Lindroth *et al.*, 2001). Previous studies have shown variable

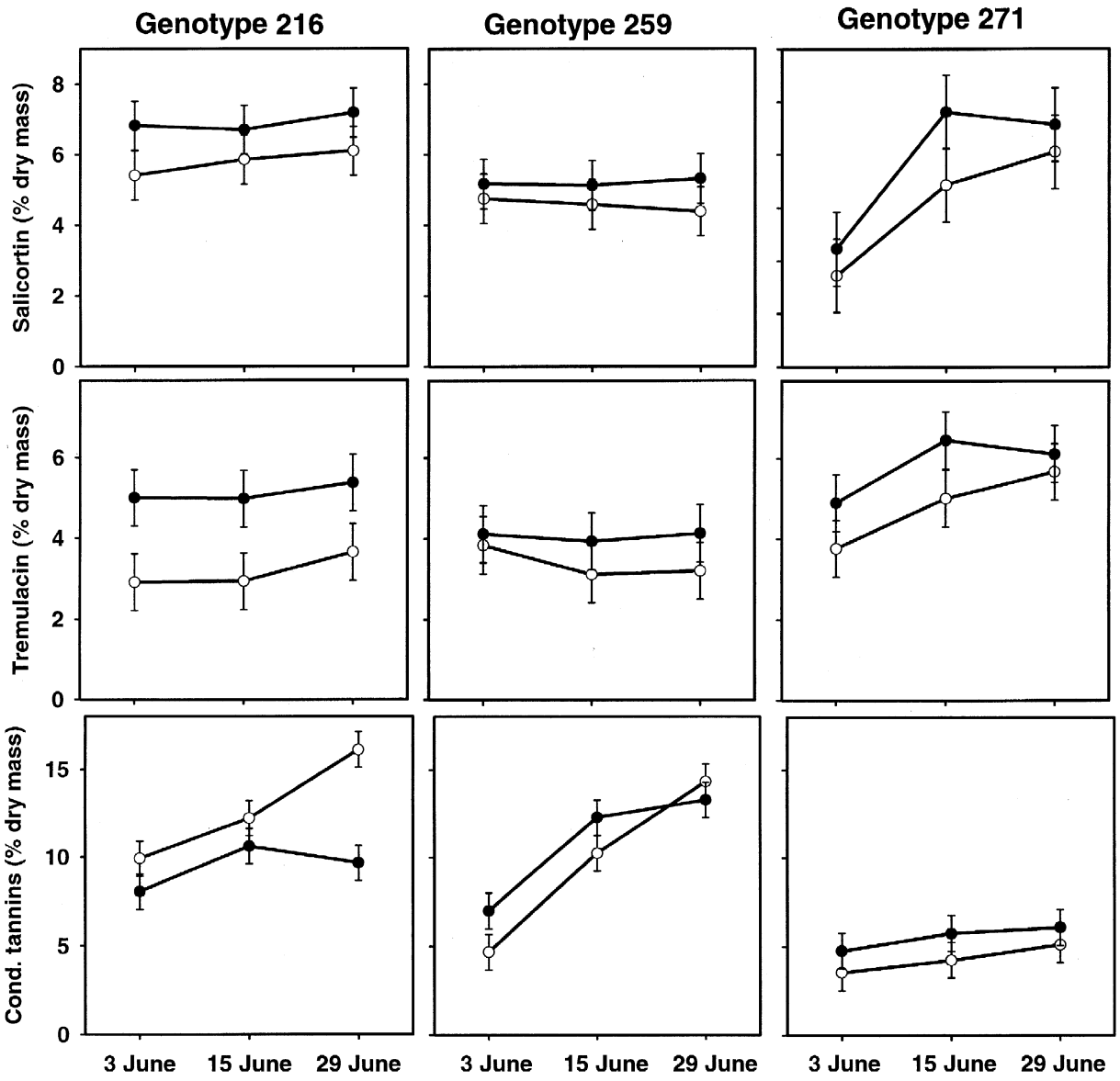

Figure 2 Effects of CO_2 and genotype on phenolic glycoside and condensed tannin levels in quaking aspen foliage. ○ and ● represent ambient and elevated CO_2, respectively. Error bars indicate ±1 standard error of least squares means.

responses of aspen secondary metabolites to enriched CO_2, ranging from slight declines to significant increases (Lindroth *et al.*, 1993; Lindroth & Kinney, 1998; Lindroth *et al.*, 2001). In this study, high CO_2 concentrations led to increased phenolic glycoside levels in all genotypes, whereas tannin levels increased in two genotypes and declined in a third. Thus, a significant $CO_2 \times$ genotype interaction was observed only for condensed tannins. These results differ from our earlier research (Lindroth *et al.*, 2001), in that significant $CO_2 \times$ genotype interactions were previously identified for both phenolic glycosides and tannins. In that study, however, a larger number of genotypes was investigated. In related research, Julkunen-Tiitto *et al.* (1993) reported significant $CO_2 \times$ genotype interactions for phenolic glycoside concentrations in *Salix myrsinifolia*, and Mansfield *et al.* (1999) had similar results for condensed tannin concentrations in quaking aspen.

We caution that conclusions regarding the relative lack of $CO_2 \times$ genotype interactions affecting foliar chemistry should be drawn with recognition of the context and constraints of this study. First, we used only three aspen genotypes, a minimal number for assessment of genetic variation. Still, the absence of genotype $\times$ CO_2 interactions cannot be attributed to genetic uniformity among these aspen clones. The genotypes vary considerably with respect to chemical composition (Table 1), tolerance to O_3 (Dickson *et al.*, 2001), and growth response under enriched CO_2 (Isebrands *et al.*, 2001). Genetic variation in growth response to CO_2 is not, however, mirrored by variation in phytochemical response to CO_2. Second, the magnitude of

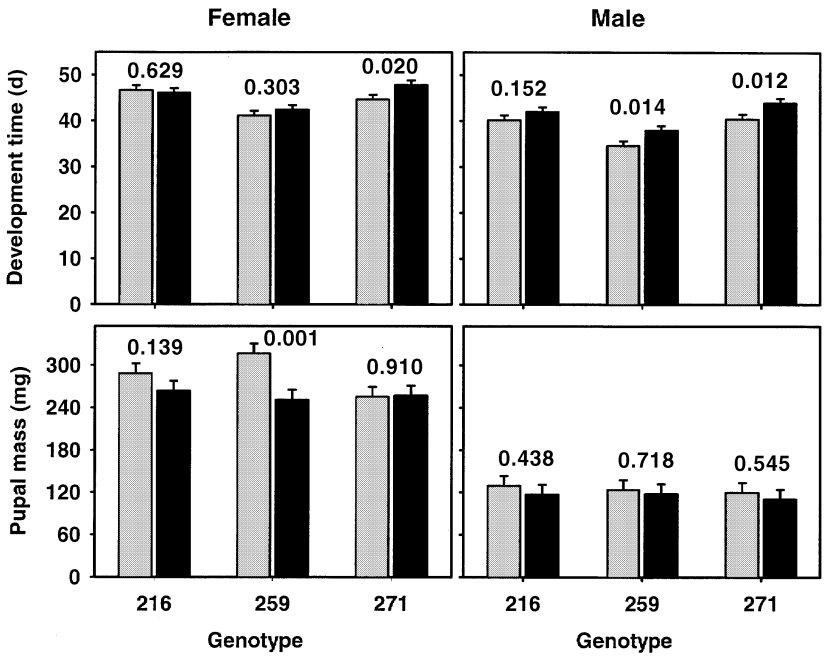

Figure 3 Effects of CO_2 and aspen genotype on larval development time and pupal mass of whitemarked tussock moths. Light and dark shading represent ambient and elevated CO_2, respectively. Error bars indicate + 1 standard error of least squares means. Numbers above bars represent *P*-values for tests of significance (PROC MIXED, differences of least squares means) between CO_2 treatments within a genotype.

the CO_2 treatment employed in this study (560 μL/L) was small compared with that used in earlier, related studies (typically 650–700 μL/L). A stronger environmental factor should elicit stronger gene × environment effects. Third, the magnitude of any particular CO_2 × genotype interaction may itself be influenced by other environmental factors. For example, interactive effects of CO_2 and genotype on aspen tannin concentrations tend to be reduced under high nutrient conditions (Lindroth *et al.*, 2001), which is the case for soil at the Aspen FACE site (Dickson *et al.*, 2001).

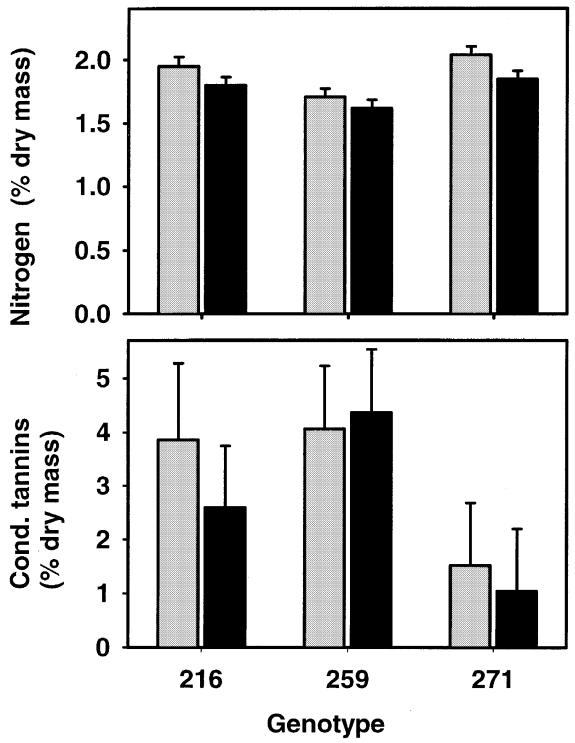

Figure 4 Effects of CO_2 and aspen genotype on nitrogen and condensed tannin levels in whitemarked tussock moth frass. Light and dark shading represent ambient and elevated CO_2, respectively. Error bars indicate + 1 standard error of least squares means.

Given the relatively small independent and interactive effects of CO_2 on plant chemistry, that such effects on insect performance were equally small is not surprising. Enriched CO_2 slightly prolonged development times, especially of males, and reduced pupal masses, especially of females on genotype 259. Decreased female pupal mass is likely to result in lowered fecundity. The particularly large difference in performance of females on genotype 259 under ambient and elevated CO_2 is, however, difficult to explain. Survival and growth of tussock moths decline when larvae are reared on aspen containing high levels of phenolic glycosides (Agrell *et al.*, 2000; Lindroth, unpublished data). Large pupal masses of females reared on genotype 259 under ambient CO_2 may reflect the especially low levels of phenolic glycosides in those trees. However, the increase in levels of phenolic glycosides under high CO_2 was greater for genotype 216 than for 259, whereas corresponding changes in female pupal masses were less. Overall, results from this study agree with those from Agrell *et al.* (2000), conducted with aspen grown in a greenhouse. They found slight to no effects of CO_2 treatment on tussock moth performance (survival, development, pupal mass) under low light conditions, but large effects under high light conditions. Potential CO_2 effects in our study may have been ameliorated by shading due to canopy architecture and enclosure of branches in double mesh bags. Overall, these results suggest that under atmospheric conditions predicted for the future, performance of tussock moths will be at most moderately and negatively affected by CO_2, and that the magnitude of

such effects may vary among plant genotypes and in relation to resource availability.

Very few studies have evaluated the implications of genotypic variation in plant response to CO_2 for plant–insect interactions. In the most comprehensive study to date, Goverde *et al.* (1999) showed that genotypic variation in response of *Lotus corniculatus* to CO_2 enrichment carried over to affect performance of the lycaenid butterfly *Polyommatus icarus*. Moreover, different maternal lines of *P. icarus* responded differently to CO_2 treatments. More recently, Agrell & Lindroth (unpublished data) found that relative preferences of forest tent caterpillars for aspen genotypes 216 and 259 shifted markedly under high CO_2. Similarly, Holton (2001) showed that performance of a dipteran parasitoid (*Compsilura concinnata*) of tent caterpillars was influenced by interactions between CO_2 and aspen genotype. In short, results from several studies suggest that $CO_2 \times$ genotype interactions have the potential to affect herbivorous insects via both bottom-up and top-down processes.

Leaf-chewing insects modify the structure and composition of leaf material transferred to the forest floor via frass, and can thereby alter ecosystem nutrient dynamics (Schowalter *et al.*, 1986; Lovett & Ruesink, 1995). Given that the efficiencies of conversion of tree foliage to lepidopteran biomass are typically in the range 2–31% (Slansky & Scriber, 1985), much more leaf material enters the forest litter layer in the form of frass than in the form of insect tissues. We are aware of no studies, however, that have evaluated the effects of CO_2 enrichment on levels of chemical constituents likely to influence rates of frass decomposition. In this study, the relative difference (7.6%) between levels of nitrogen in low- and high-CO_2 frass was comparable to the relative difference (6.0%) between low- and high-CO_2 foliage near the time of frass collection. Thus, the CO_2 'signature' in foliage was carried over to insect frass. Similarly, condensed tannin profiles in frass reflected CO_2 and genotype differences in tannin levels of third-collection foliage. Debate exists over whether CO_2-mediated changes in green leaf chemistry will persist in leaf litter (Norby *et al.*, 2000), but this work suggests that such changes may persist when leaf tissue is converted into insect frass. Indeed, insects may even amplify CO_2-mediated reductions in substrate nitrogen if they respond to low-nitrogen foliage by increasing nitrogen utilization efficiencies (Williams *et al.*, 1994).

In conclusion, this study demonstrates that long-term CO_2 enrichment produces minor to moderate changes in aspen foliar chemical composition, and that such changes are generally consistent across at least a small sample of genetically variable aspen genotypes. Moreover, these changes produce at best only modest changes in insect performance. If the $CO_2 \times$ genotype interactions observed for plant chemistry and insect performance in this study are representative of those occurring in the field (and our previous research suggests that this may not be the case), then they are unlikely to serve as forcing factors in the evolution of aspen populations. Aspen chemistry has been shown to respond more strongly to other genotype $\times$ environment interactions (e.g. light, soil nutrient availability), with correspondingly larger impacts on leaf-feeding insects (Osier & Lindroth, 2001; unpublished data). Given the importance of trophic interactions to the evolutionary dynamics of both plants and animals, assessments of genetic variation in plant/herbivore response to CO_2 enrichment deserve continued attention by the global change research community.

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