Oecologia (2003) 134:95–103
DOI 10.1007/s00442-002-1090-6

PLANT ANIMAL INTERACTIONS

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Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore

Received: 16 April 2002 / Accepted: 16 September 2002 / Published online: 22 October 2002

Abstract The purpose of this study was to assess the independent and interactive effects of CO_2, O_3, and plant genotype on the foliar quality of a deciduous tree and the performance of a herbivorous insect. Two trembling aspen (*Populus tremuloides* Michaux) genotypes differing in response to CO_2 and O_3 were grown at the Aspen FACE (Free Air CO_2 Enrichment) site located in northern Wisconsin, USA. Trees were exposed to one of four atmospheric treatments: ambient air (control), elevated carbon dioxide ($+CO_2$; 560 µl/l), elevated ozone ($+O_3$; ambient ×1.5), and elevated CO_2+O_3. We measured the effects of CO_2 and O_3 on aspen phytochemistry and on performance of forest tent caterpillar (*Malacosoma disstria* Hübner) larvae. CO_2 and O_3 treatments influenced foliar quality for both genotypes, with the most notable effects being that elevated CO_2 reduced nitrogen and increased tremulacin levels, whereas elevated O_3 increased early season nitrogen and reduced tremulacin levels, relative to controls. With respect to insects, the $+CO_2$ treatment had little or no effect on larval performance. Larval performance improved in the $+O_3$ treatment, but this response was negated by the addition of elevated CO_2 (i.e., $+CO_2$+O_3 treatment). We conclude that tent caterpillars will have the greatest impact on aspen under current CO_2 and high O_3 levels, due to increases in insect performance and decreases in tree growth, whereas tent caterpillars will have the least impact on aspen under high CO_2 and low O_3 levels, due to moderate changes in insect performance and increases in tree growth.

Keywords Free-Air CO_2 Enrichment · *Populus tremuloides* · Genetic variation · *Malacosoma disstria* · Plant–insect interactions

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Introduction

Atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) levels are expected to have marked impacts on plant–insect interactions in the future, with CO_2 concentrations projected to double during this century (Houghton et al. 1996) and O_3 concentrations projected to triple within the next 40 years (Chameides et al. 1994). Both of these pollutants can alter concentrations of foliar constituents in trees (Riemer and Whittaker 1989; Watt et al. 1995; Lindroth 1996a, b; Koricheva et al. 1998; Norby et al. 1999), and such changes can have positive, negative, or no effect on the performance of leaf-chewing insects (e.g., Trumble et al 1987; Chappelka et al. 1988; Coleman and Jones 1988; Lindroth 1996a, b; Fortin et al. 1997; Bezemer and Jones 1998; Coviella and Trumble 1999; Kopper et al. 2001; Kopper and Lindroth 2002).

Compared with the number of studies that have investigated the individual effects of CO_2 or O_3 on plant–insect interactions, few have investigated the effects of co-exposure. This paucity of information is striking given that approximately half of the world's forests are expected to experience increased co-exposure to CO_2 and O_3 by 2100 (Fowler et al. 1999). Studies have shown that elevated CO_2 can potentially reduce the deleterious effects of O_3 stress on some physiological processes in deciduous trees (Volin and Reich 1996; Volin et al. 1998; Grams et al. 1999; but see Kull et al. 1996; Karnosky et al. 1999). With respect to insect performance, evidence indicates that these pollutants may affect plant–insect interactions differently depending on whether they are administered alone or in combination (Kopper et al. 2001; Kopper and Lindroth 2002).

Understanding the intraspecific response of plants to these atmospheric pollutants is important for evaluating the potential evolutionary response of species to future atmospheric conditions. For example, foliar chemistry can vary among plant genotypes, which can alter the performance of herbivorous insects (e.g., Bingaman and Hart 1993; Suomela and Nilson 1994; Lindroth and Hwang 1996; Mutikainen et al. 2000; Osier et al. 2000). To date,

however, few studies have investigated genotypic variation in phytochemistry in response to CO_2 enrichment (Fajer et al. 1992; Mansfield et al. 1999; Goverde et al. 1999; Holton 2001; Lindroth et al. 2001, 2002b) and even fewer studies have investigated the consequences of such variation for herbivorous insects (Mansfield et al. 1999; Goverde et al. 1999; Lindroth et al. 2002b). We are aware of only one other study (Holton 2001) that evaluated genotypic variation in foliar quality in response to atmospheres amended with elevated levels of O_3, or CO_2 and O_3, and consequences thereof for insect herbivores.

The purpose of this study was to assess the independent and interactive effects of CO_2 and O_3 on the phytochemistry of two trembling aspen (*Populus tremuloides*) genotypes, and to evaluate the consequences of CO_2- and O_3-mediated changes in phytochemistry for the performance of forest tent caterpillar (*Malacosoma disstria*) larvae. Trembling aspen and forest tent caterpillars were selected for use because they are common species in forests of the north-central USA. Trembling aspen is one of the most genetically variable and widely distributed tree species in North America (Dickmann and Stuart 1983; Mitton and Grant 1996), with secondary chemistry consisting mainly of phenolic glycosides and condensed tannins. The forest tent caterpillar, which is native throughout most of the United States and southern Canada (Stehr and Cook 1968; Fitzgerald 1995), is an eruptive herbivore that periodically defoliates aspen forests. Previous work in our laboratory has shown that aspen is not uniformly susceptible to forest tent caterpillar damage, with chemical defensive compounds, namely phenolic glycosides, mediating insect performance on different aspen genotypes (Hemming and Lindroth 1995; Hwang and Lindroth 1997).

Materials and methods

Experimental design

This experiment was conducted in north-central Wisconsin, USA (W 89.5°, N 45.7°) at the Aspen Free-air CO_2 Enrichment (Aspen FACE) site. The 32 ha site contains twelve FACE rings (30 m diameter) set up as a blocked full factorial design. The FACE site is divided into three blocks on a north-south gradient, with each block containing each of the following treatments: ambient air, supplemental CO_2, supplemental O_3, and supplemental CO_2 and O_3. Dickson et al. (2000) provide further information on the experimental design, set-up, and operation of the FACE site.

Fumigation treatments are administered during daylight hours of the growing season. Ambient air is blown into the control rings to account for any blower effects. For elevated CO_2, the target level is 560 µl/l, based on concentrations predicted for the year 2050 (Houghton et al. 1996). For elevated O_3, target concentrations are the same as levels currently experienced in urban areas of the southwestern Great Lakes region of the USA (Pinkerton and Lefohn 1987). Because O_3 production is dictated by light and temperature, sunny days receive 90–100 nl/l, cloudy days receive 50–60 nl/l, and cool (<15°C) days receive no O_3 treatment. In addition, O_3 fumigation is not applied when leaves are wet due to dew or precipitation.

A portion of each FACE ring contains a mix of five aspen genotypes, vegetatively propagated from greenwood cuttings (Karnosky et al. 1996). We chose to work with genotypes 216 and 271 because both are responsive to CO_2 enrichment, and genotype 271 is more sensitive than 216 to O_3 exposure (Karnosky et al. 1996, 1999). We had no knowledge of phytochemical levels or insect performance for these aspen genotypes prior to planting the trees in the FACE array.

Aspen seedlings were planted in the rings in 1997 and exposed to the pollutant treatments starting in spring 1998. At the time of this study, aspen trees were 4 years old. Within each ring, three aspen trees per genotype were used for both foliar collections and insect bioassays.

Phytochemical analysis

For phytochemical analyses, leaves were collected on four dates (24 May, 6 June, 14 June, and 23 June) during larval development. In order to equalize light levels, branches used for foliar collection were bagged with the same mesh material (no-see-um, Balson-Hercules Group, Pawtucket, R.I., USA) as used for insect bioassays. For all four collection dates, foliage was selected from random branches at the same relative position as the foliage used for insect bioassays. 2–3 g (fresh mass) of foliage was excised at the petiole from each tree and stored on ice. Upon return to the laboratory (<4 h from field collection), leaves were flash frozen in liquid nitrogen, freeze-dried, ground, and stored at –20°C prior to analysis. Analyses were conducted to determine concentrations of nitrogen, starch, phenolic glycosides, and condensed tannins. Nitrogen levels were determined with a LECO FP528 nitrogen analyzer (St. Joseph, Mich., USA), using glycine *p*-toluenesulfonate as the reference standard. Starch levels were determined by first separating starch from soluble sugars. Starch was then enzymatically hydrolyzed to glucose using amyloglucosidase (Prado et al. 1998). Glucose concentrations were quantified using a modification of the dinitrosalicylic acid method (Lindroth et al. 2002a). Levels of the phenolic glycosides salicortin and tremulacin were measured using high performance thin layer chromatography (HPTLC) with purified aspen salicortin and tremulacin used as reference standards (Lindroth et al. 1987). Condensed tannin concentrations were quantified using a modification of the butanol-HCl method of Porter et al. (1986) with purified aspen condensed tannins used as the reference standard.

Insect bioassays

Bioassays were conducted to determine the effects of CO_2 and O_3 on several performance parameters, including larval consumption (4th instar to pupation), duration of larval development, and pupal mass. Forest tent caterpillars were obtained as egg masses from the Forest Pest Management Institute, Canadian Forest Service (Sault Ste. Marie, Ontario, Canada). Each of our study trees received one egg mass, which was tied to a branch and covered with no-see-um mesh to protect insects from predators and parasitoids. Upon hatching (16–17 May 2000), larvae were allowed to establish on newly emerging leaves. When larvae became second instars, the total number per tree was randomly reduced to 40 (which were divided equally between two bags) to prevent substantial defoliation of the trees. Later, five early fourth instars per tree were randomly selected and placed into individual 'consumption' bags to assess foliar consumption from the fourth instar to pupation. Measuring consumption for only fourth and fifth instars provides a valid estimate of total consumption because approximately 95% of the aspen foliage consumed by forest tent caterpillars is eaten during these two instars (Hodson 1941).

Initially, each 'consumption' bag contained 10 individually measured (length and width) leaves. Once a larva consumed most of the leaves, additional leaves were measured and the larva was moved to the new set of leaves. This procedure was repeated until the larva pupated. Care was taken to use only first-flush leaves.

Leaves not consumed by the larva were removed, oven dried (60°C), and weighed.

To estimate the actual amount consumed per larva, remaining leaf mass was subtracted from initial leaf mass, which was determined on the basis of leaf area/mass calculations. Leaf length and width were measured in the field, providing an index (length×width) of leaf area. Area: mass conversion formulas were determined via linear regression, using leaf area indices and dry weights of a subset of twelve leaves collected from each genotype in each fumigation treatment (r^2>0.80 for all equations). These formulas were then used to convert our field leaf area indices to dry mass for each fumigant treatment×genotype combination.

In addition to foliar consumption, larval development time and pupal mass were measured for each larva in the individual 'consumption' bags that successfully pupated. Pupae were sexed and weighed 3 days after pupation.

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. The statistical model employed was:

$$Y_{ijklm} = \mu + B_i + C_j + O_k + CO_{jk} + e_{ijk} + G_l + CG_{jl} + OG_{kl} + COG_{jkl} + T_m + CT_{jm} + OT_{km} + COT_{jkm} + GT_{lm} + CGT_{jlm} + OGT_{klm} + COGT_{jklm} + \varepsilon_{ijklm} \quad (1)$$

where Y_{ijklm} was the average response of block i, CO_2 level j, O_3 level k, genotype l, and time m. Fixed effects included CO_2 level (C_j), O_3 level (O_k), genotype (G_l), time (T_m), and their interaction terms [(CO_{jk}), (CG_{jl}), (CT_{jm}), (OG_{kl}), (OT_{km}), (COG_{jkl}), (CGT_{jlm}), (OGT_{klm}), and ($COGT_{jklm}$)]. Random effects included block (B_i), whole plot error (ε_{ijk}), and subplot error (ε_{ijklm}). *F*-tests were conducted for all main effects with degrees of freedom for error assigned using the Satterthwaite approximation (Milliken et al. 1984; Littell et al. 1996). Means and standard errors (based on the pooled variance) are reported for each CO_2×O_3×genotype×time combination.

For analysis of insect performance data, time was omitted and sex was added to the model (to account for sexual dimorphism) as a sub-subplot. CO_2 level (C_j), O_3 level (O_k), genotype (G_l), sex (S_n), and their interaction terms [(CO_{jk}), (CG_{jl}), (CS_{jn}), (OG_{kl}), (OS_{kn}), (COG_{jkl}), (CGS_{jln}), (OGS_{kln}), and ($COGS_{jkln}$)] represented fixed effects and block (B_i), whole plot error (ε_{ijk}), subplot error (ε_{ijkl}), and sub-subplot error (ε_{ijkln}) represented random effects. F-tests were performed and degrees of freedom for error were assigned in the same manner as for the phytochemical data analysis (Littell et al. 1996). Means and standard errors (based on the pooled variance) are reported for each CO_2×O_3×genotype×sex combination.

The low number of replicates (n=3 at the whole plot level) in this experiment increases the probability of type II errors. Recognizing the trade-off between committing type I and type II errors, we report *P*-values <0.10 as "significant" (Filion et al. 2000). For those desiring a more stringent a, exact *P*-values for all main effects and interactions are provided in Tables 1 and 2.

Table 1 Summary of *P* values for the effects of CO_2, O_3, genotype, and time on phytochemistry

Main effects and interactions	Nitrogen	Starch	Salicortin	Tremulacin	Condensed tannins
CO_2	0.010	<0.001	0.855	0.011	0.134
O_3	0.760	<0.001	0.029	0.042	0.004
CO_2×O_3	0.127	0.091	0.435	0.917	0.101
Genotype	<0.001	<0.001	0.015	<0.001	<0.001
CO_2×genotype	0.139	0.841	0.878	0.347	0.143
O_3×genotype	0.001	0.447	0.117	0.921	0.186
CO_2×O_3×genotype	0.576	0.668	0.429	0.474	0.104
Time	<0.001	0.016	<0.001	<0.001	<0.001
CO_2×time	0.013	0.067	0.339	0.605	0.181
O_3×time	<0.001	0.107	0.534	0.271	<0.001
CO_2×O_3×time	0.310	0.903	0.206	0.521	0.174
Genotype×time	<0.001	0.300	0.002	0.226	<0.001
CO_2×genotype×time	0.264	0.666	0.677	0.471	0.417
O_3×genotype×time	0.128	0.942	0.652	0.858	0.484
4-way interaction	0.298	0.588	0.830	0.801	0.468

Table 2 Summary of *P* values for the effects of CO_2, O_3, genotype, and sex on insect performance

Main effects and interactions	Development time	Consumption	Pupal mass
CO_2	0.012	0.265	0.023
O_3	0.014	0.357	0.040
CO_2×O_3	0.095	0.948	0.208
Genotype	0.002	0.390	0.126
CO_2×genotype	0.247	0.412	0.952
O_3×genotype	0.003	0.207	0.031
CO_2×O_3×genotype	0.009	0.011	0.065
Sex	0.011	<0.001	<0.001
CO_2×sex	0.147	0.409	0.024
O_3×sex	0.710	0.240	0.029
CO_2×O_3×sex	0.443	0.307	0.023
Genotype×sex	0.123	0.685	0.457
CO_2×genotype×sex	0.658	0.866	0.836
O_3×genotype×sex	0.359	0.886	0.161
4-way interaction	0.055	0.597	0.395

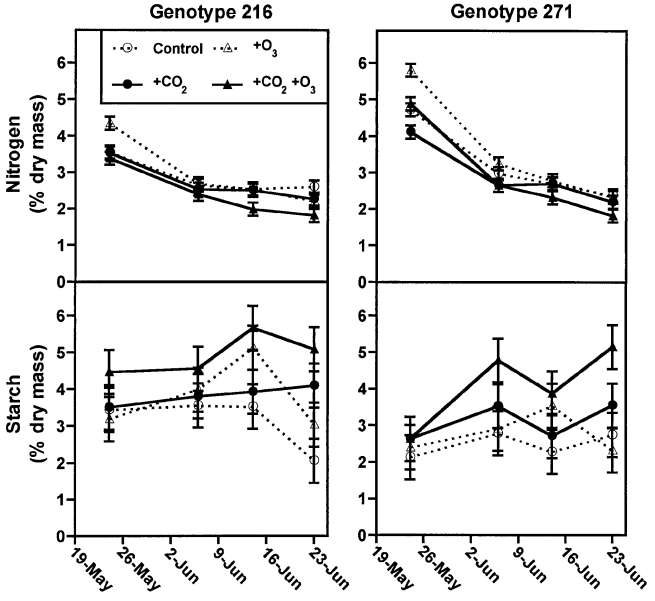

Fig. 1 Concentrations of nitrogen and starch under control, $+CO_2$, $+O_3$, and $+CO_2+O_3$ fumigation treatments. *Vertical lines* represent ±1 SE (calculated from the pooled variance)

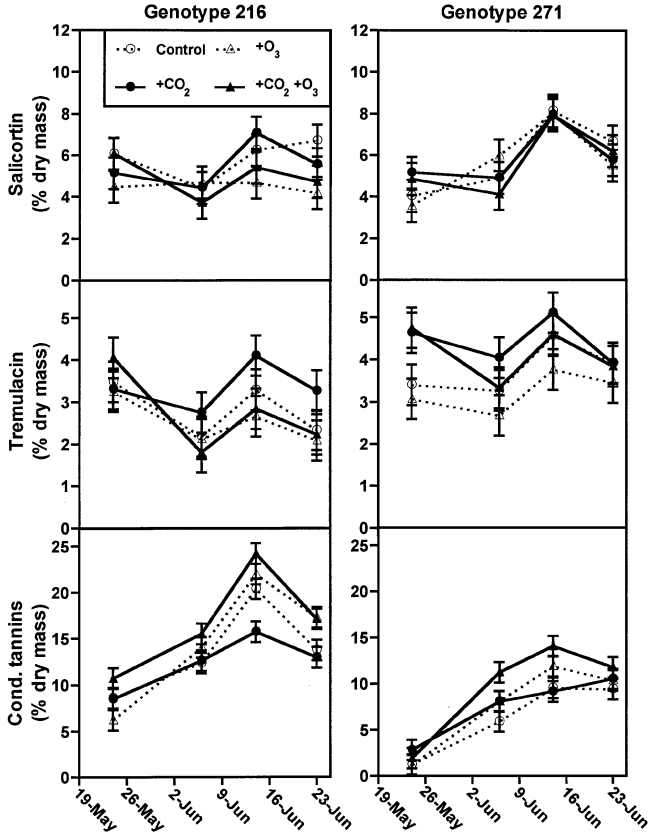

Fig. 2 Concentrations of salicortin, tremulacin and condensed tannins under control, $+CO_2$, $+O_3$, and $+CO_2+O_3$ fumigation treatments. *Cond. tannins*, condensed tannins. *Vertical lines* represent ±1 SE (calculated from the pooled variance)

Results

Phytochemistry

Aspen primary metabolites were influenced by CO_2, O_3, genotype, time, and interactions among the factors (Table 1, Fig. 1). Elevated CO_2 reduced nitrogen levels by 13% for both genotypes, relative to ambient CO_2. The effect of elevated CO_2 on nitrogen levels also depended on time, with the magnitude of variation between CO_2-fumigated and nonfumigated trees tending to decrease as the season progressed (CO_2×time interaction). Elevated O_3 affected nitrogen levels but the response differed between genotypes, with levels decreasing by 4% in genotype 216 but increasing by 6% in genotype 271, relative to ambient O_3 (Fig. 1). As the season progressed, the amount of variation between O_3-fumigated and nonfumigated trees tended to decrease, contributing to a significant O_3×time interaction. Genotype 271 had higher concentrations of nitrogen than did genotype 216, especially early in the growing season (genotype×time interaction). For starch, the effects of CO_2 and O_3 depended on whether the pollutants were administered alone or in combination, with concentrations in the $+CO_2+O_3$ treatment more than double the increases experienced when CO_2 and O_3 were administered alone (+19% in $+CO_2$, +15% in $+O_3$, and +38% in $+CO_2+O_3$ treatments relative to the control treatment). The effect of CO_2 on starch levels also varied over time, becoming more pronounced in older leaves. Although the response to pollutants was the same for both genotypes, genotype 216 averaged 32% more starch than did genotype 271.

Levels of carbon-based secondary metabolites were also influenced by CO_2, O_3, genotype, time, and their interactions (Table 1, Fig. 2). CO_2 enrichment did not affect salicortin, but increased tremulacin levels by 18% relative to ambient conditions. Elevated O_3 decreased both salicortin and tremulacin levels by approximately 11%, relative to ambient air. Phenolic glycoside levels varied between genotypes, with levels of salicortin and tremulacin higher (11 and 26%, respectively) in 271 than in 216. The effect of CO_2 on condensed tannin levels depended on O_3 and genotype. For both genotypes, tannin levels in the $+CO_2$ and $+O_3$ treatments were not significantly different from those in the control treatment. When these pollutants were administered in combination, however, tannin levels were 14 and 49% higher in genotypes 216 and 271, respectively, than under control conditions, contributing to a marginal CO_2×O_3×genotype interaction. The effect of O_3 on condensed tannin concentrations varied over time, with levels tending to increase after the first leaf collection date (O_3×time interaction). With respect to genotypic differences in tannin levels, genotype 216 had higher concentrations of tannins than did genotype 271, but the magnitude of difference varied over time (genotype×time interaction).

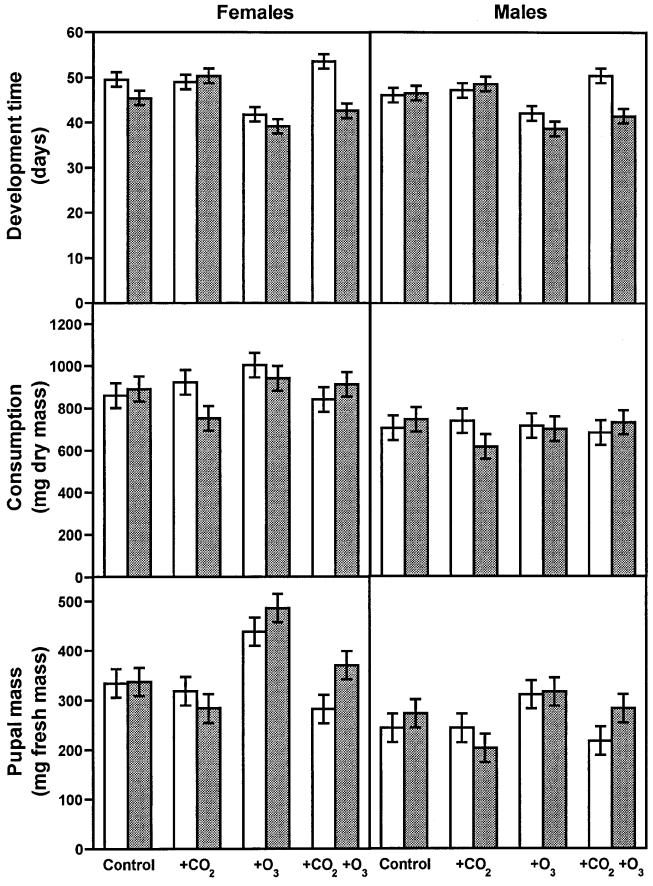

Fig. 3 Forest tent caterpillar performance on genotype 216 (*light bars*) and genotype 271 (*dark bars*) under control, $+CO_2$, $+O_3$, and $+CO_2+O_3$ treatments. *Vertical lines* represent ±1 SE (calculated from the pooled variance)

Insect performance

CO_2, O_3, genotype, sex, and interactions among the factors influenced tent caterpillar performance (Table 2, Fig. 3). For development time, larvae on both genotypes developed similarly between the $+CO_2$ and the control treatment, except that females reared on genotype 271 took 11% longer to develop under high CO_2. The $+O_3$ treatment decreased development times for larvae on genotypes 216 and 271 by 12% and 15%, respectively, relative to their respective control treatment. Larvae performed differently between the CO_2 and O_3 treatments administered alone versus in combination, and these responses varied still further depending on genotype and sex. Elevated CO_2 negated the decrease in development time observed under the high O_3 treatment, resulting in development times 9% longer than the control treatment for larvae reared on genotype 216 and similar to the control treatment for females on genotype 271. For males on genotype 271, however, elevated CO_2 did not alter the effect of high O_3, with development times remaining similar to those in the $+O_3$ treatment. This variation in response to CO_2 and O_3 between genotypes and sex contributed to a significant $CO_2\times O_3\times$genotype×sex interaction.

Foliar consumption by tent caterpillars was influenced by CO_2, O_3, and genotype, although differences among treatments were relatively small (Table 2, Fig. 3). Consumption in the pollutant-amended and control treatments was similar for insects on genotype 216. However, consumption decreased 16% in the $+CO_2$ treatment (relative to the control treatment) for larvae on genotype 271. This CO_2-mediated decrease was negated by O_3, resulting in similar amounts consumed between the $+CO_2+O_3$ and control treatments. The variation in response between genotypes to the fumigation treatments contributed to a significant $CO_2\times O_3\times$genotype interaction.

The effect of CO_2 on pupal mass was influenced by interactions with O_3, genotype, and sex (Table 2, Fig. 3). Pupal mass did not differ between larvae reared in the $+CO_2$ and control treatments. In the $+O_3$ treatment, however, larvae were 31% larger on both aspen genotypes, compared with their respective control treatment. Elevated CO_2 negated the O_3-mediated increase in pupal mass, and the magnitude of amelioration was greater for genotype 216 than for genotype 271 ($CO_2\times O_3\times$genotype interaction). Larval response to the fumigation treatments was also influenced by sex, with females responding more strongly than males to the fumigation treatments ($CO_2\times O_3\times$sex interaction).

Discussion

CO_2 and O_3 altered concentrations of foliar metabolites, and these effects were influenced further by aspen genotype. Elevated CO_2 reduced foliar nitrogen by 13% in both genotypes, which is within the range typically exhibited by trees under enriched CO_2 (McGuire et al. 1995; Curtis and Wang 1998; Cotrufo et al. 1998; Norby et al. 1999). The CO_2-mediated reduction in nitrogen levels in this study could be the result of accelerated plant growth and increased levels of carbon-based metabolites resulting from an increased carbon economy, thereby reducing nitrogen levels on a per mass basis (i.e., carbon dilution effect; Coleman et al. 1993; Curtis 1996). Alternatively, foliar nitrogen levels may have declined if ribulose bisphosphate carboxylase/oxygenase (rubisco) levels were reduced due to trees acclimating to CO_2 enrichment (reviewed by Saxe et al. 1998). Genotype 271 had higher early season nitrogen levels under elevated O_3 than did genotype 216, a result we attribute to O_3-mediated differences in leaf expansion rates among genotypes. Foliage on genotype 271 was phenologically younger than genotype 216 for the first collection date but not for the second, by which time the difference in nitrogen concentrations had disappeared.

Elevated CO_2 and O_3 also affected levels of carbon-based metabolites, with the magnitude and direction of response depending on genotype. Starch and tannin concentrations were highest in the $+CO_2+O_3$ treatment,

and although the response to elevated CO_2 and O_3 was the same for both genotypes, genotype 216 had higher starch and tannin levels than did genotype 271. The increase in starch and tannin levels in the $+CO_2+O_3$ treatment likely resulted from O_3 limiting growth (i.e., reduced carbon sink) more than photosynthesis under high CO_2, as has been documented for aspen at the FACE site (Isebrands et al. 2001; Noormets et al. 2001). These results are therefore consistent with predictions of the carbon/nutrient balance hypothesis (Bryant et al. 1983; Tuomi et al. 1988). With respect to phenolic glycosides, elevated CO_2 increased tremulacin levels, whereas O_3 reduced salicortin and tremulacin levels, for both genotypes. The CO_2-mediated increase in tremulacin levels is probably the result of increased carbon availability and is consistent with results from other CO_2-aspen studies (e.g., McDonald et al. 1999; Agrell et al. 2000; Lindroth et al. 2002b) The O_3-mediated reduction in salicortin and tremulacin levels is also consistent with previous research (Kopper and Lindroth 2002; Holton 2001), and suggests that O_3 may either accelerate turnover rates of phenolic glycosides or interfere with their production by decreasing the availability of photosynthate or activity of enzymes in the shikimic acid pathway.

CO_2 and O_3 influenced forest tent caterpillars, but responses depended on whether the pollutants were administered alone or in combination, and varied between sexes and aspen genotypes. The $+CO_2$ treatment did not greatly alter insect performance, with the only effects being that females took longer to develop and larvae consumed less (on genotype 271) than did those reared in the control treatment. The modest effect of the $+CO_2$ treatment on insect performance was rather surprising given that previous studies have generally reported more dramatic changes in tent caterpillar response to CO_2-mediated changes in foliar quality (Lindroth et al. 1993; Roth et al. 1997). Our study, however, was conducted at 560 µl/l whereas most previous research used CO_2 levels around 700 µl/l. The moderate effect of CO_2 on insect performance in this study was likely due to the subtle differences in foliar chemistry between the $+CO_2$ and control treatments. Changes in insect performance can be attributed to changes in foliar quality, because genotype 271 had lower nitrogen and higher tremulacin levels when grown under elevated CO_2 than did this genotype grown under ambient CO_2. These CO_2-mediated changes in foliar quality are important because a reduction in protein and increase in phenolic glycosides have been shown to negatively affect the performance of forest tent caterpillars (Lindroth and Bloomer 1991). We suspect that the reason larvae on genotype 216 were unaffected by the $+CO_2$ treatment was because tremulacin levels were not as high as those in genotype 271. Why larvae reared on genotype 271 in the $+CO_2$ treatment consumed less foliage than those in the control treatment remains unclear, especially because previous research has shown that tent caterpillars reared on CO_2-exposed aspen increased consumption rates by 30–75% (Lindroth et al. 1993; Roth et al. 1997). A possible explanation is that the CO_2-mediated increase in tremulacin levels made aspen less palatable, causing larvae to consume the minimum amount necessary to complete development.

Larvae performed best in the $+O_3$ treatment, developing faster and growing larger than did larvae in the control treatment. Our results are consistent with the research of Fortin et al. (1997) as well as Awmack and Lindroth (unpublished data). The former study reported that forest tent caterpillars developed faster and preferred to feed on sugar maple foliage exposed to 3× ambient O_3 levels, although the degree of significance varied among years. The latter study, which was conducted at the Aspen FACE site, documented that forest tent caterpillars developed faster, grew larger, and had higher fecundity on paper birch exposed to the $+O_3$ treatment relative to larvae in the control treatment. Herms et al. (1995) demonstrated that the reason forest tent caterpillars had improved performance when fed O_3-exposed aspen foliage was because they were more efficient at converting digested food into biomass, resulting in increased growth rates. In the present study, the increase in insect performance in the $+O_3$ treatment can be explained by pollutant-mediated changes in foliar quality, with the O_3-exposed trees having lower levels of tremulacin and salicortin and higher early-season levels of nitrogen than did the control trees. Moreover, the higher early-season nitrogen levels for genotype 271 may explain why larvae on genotype 271 developed slightly faster than did those on genotype 216.

Our most striking result was that the improvement in insect performance observed under $+O_3$ was nullified by CO_2 enrichment. Moreover, the magnitude of change was influenced by aspen genotype. These results for CO_2 and O_3 contrast with those from a growth chamber study by Herms et al. (1995) who found that the pollutants did not interact to affect the performance of first or fourth instar tent caterpillars reared on aspen. Results from our study provide further evidence that short-term studies conducted over one instar do not necessarily provide an accurate measure of the effects of these pollutants over the entire larval development period. In our study, larvae performed either less well (those reared on genotype 216) or were unaffected (those reared on genotype 271) in the $+CO_2+O_3$ treatment, relative to larvae in the control treatment. Results from this study are similar to previous research conducted at the Aspen FACE site (Holton 2001; Kopper et al. 2001; Kopper and Lindroth 2002) in that the $+CO_2+O_3$ treatment had a modest effect on larval development time and pupal mass relative to the controls. In this study, the most likely cause for CO_2 negating the benefits of O_3 is that early-season nitrogen levels in the $+CO_2+O_3$ treatment were similar to those in the control, and lower than in the $+O_3$ treatment. Foliar nitrogen levels can also explain the difference in larval performance between the two genotypes in the $+CO_2+O_3$ treatment because nitrogen levels were 18% lower in genotype 216 than in genotype 271.

The effects of plant genotype on insect performance have been well documented (e.g., Fritz and Simms 1992),

including studies conducted with aspen (Hemming and Lindroth 1995; Hwang and Lindroth 1997; Osier et al. 2000). To date, however, few studies have investigated the role of plant genotype in plant–insect interactions under atmospheres enriched with CO_2 (Mansfield et al. 1999; Goverde et al. 1999; Holton 2001; Lindroth et al. 2002b). Research has shown that plant genotype can be important in determining the effects of CO_2 on plant–insect interactions. For example, Goverde et al. (1999) demonstrated that the foliar quality of *Lotus corniculatus* responded differently to elevated CO_2 depending on genotype and that these genotype-specific changes in foliar quality had implications for the performance of the herbivorous insect, *Polyommatus icarus*. With respect to the role of genotype in O_3- and CO_2+O_3-plant–insect interactions, Holton (2001) found that aspen genotypes responded differently to O_3 and CO_2+O_3 fumigation and that these genotype-specific changes in response to the fumigation treatments differentially affected the performance of tent caterpillar larvae and their parasitoid, *Compsilura concinnata*. The differences in plant genotypic response to CO_2 and O_3 and the resultant effects on insect herbivores may favor certain genotypes under impending atmospheric conditions.

Over the last decade, global change scientists, including ourselves, have called for increased biological realism in global change research (e.g., Körner 2000). Saxe et al. (1998) argued for long-term studies of trees under natural conditions (e.g., FACE environments), that include assessments of genetic variation in tree response to multiple and interacting global change and environmental factors (e.g., CO_2, O_3, resource availability). A drawback of such an approach, as evidenced in our study, is the difficulty of drawing general conclusions about main effects. Much of the experimental variance resides in higher order interactions, thus precluding the formation of inferences with broad applicability. This state of affairs is, admittedly, intellectually dissatisfying. Yet it is an accurate reflection of the complexity of the natural world. Moreover, we view it as incentive for additional, biologically realistic studies that, corporately, will facilitate formation of broad generalizations about the consequences of global environmental change.

From the plant perspective, a key question is whether CO_2 and O_3 exacerbate or ameliorate the effects of tent caterpillars on aspen. Isebrands et al. (2001) estimated aboveground biomass of aspen at the FACE site. They found that genotype 271 grows faster than does genotype 216, and that the relative effect of the fumigation treatments on growth were similar for both genotypes. Tree growth was enhanced in the +CO_2 treatment, reduced in the +O_3 treatment, and unaltered in the +CO_2+O_3 treatment, relative to controls. Based on these growth estimates and our insect performance data, we suggest that forest tent caterpillars will have the greatest impact on aspen under current CO_2 levels in areas of high O_3 pollution. Because tent caterpillars had the largest pupal mass (and probably greater fecundity) in the +O_3 treatment, population densities of the caterpillars may increase under atmospheric conditions that hinder tree growth, at least to the point where food availability becomes limiting. Moreover, forest tent caterpillar defoliation may increase the susceptibility of aspen to the pathogenic fungi, *Hypoxylon mammatum* (Anderson and Martin 1981). Under elevated CO_2, the impact of tent caterpillars on aspen will vary in relation to O_3 levels. In areas of low O_3 concentrations, tent caterpillars will likely have less of an impact on aspen because tree growth is enhanced. In areas of high O_3 concentrations, however, tent caterpillars likely will not impact aspen differently than what is currently observed under ambient conditions because tree growth was similar between the +CO_2+O_3 and control treatments. Overall, aspen is likely to be more negatively affected by tent caterpillars in areas that experience high, rather than minimal, O_3 pollution.

In conclusion, this research shows that aspen foliar quality can change, and does so differentially among genotypes, in response to elevated levels of CO_2 and O_3. These genotype-specific changes in foliar chemistry differentially altered insect performance, although the overall difference in insect performance between genotypes was modest. Results from this study suggest that tent caterpillars will have the greatest impact on aspen under current CO_2 and high O_3 levels due to increases in insect performance and decreases in tree growth. Conversely, tent caterpillars will have the least impact on aspen under high CO_2 and low O_3 levels, due to decreases in insect performance and increases in tree growth. Future studies that investigate a wider range of genetic variation in trees, focusing on species that have important or unique ecological roles, and the consequences of this genetic variation for herbivorous insects, will be particularly useful in improving our ability to predict ecosystem-level changes to atmospheric pollutants.

Acknowledgements This project would not have been possible without the help of K. Krein, A. Weldon, K. Holton, K. Zachman, T. Osier, and C. Awmack. We also thank the Canadian Forest Service (Sault St. Marie, Ontario, Canada) for graciously providing forest tent caterpillar egg masses, H. Barnhill for help with chemical analyses, E. Nordheim for statistical assistance, and J. Sober for FACE technical assistance. Finally, we thank E. Kruger, R. Jeanne, K. Raffa, and D. Young for comments on the manuscript. This work was funded by National Science Foundation grant DEB-9707263 and University of Wisconsin McIntire-Stennis project WIS04457. Site support for the FACE facility was provided by the U.S. Forest Service and U.S. Department of Energy (OBER grant DE-FG02–95ER62125).

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