Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO₂ and O₃

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Abstract   1 This research was conducted at the Aspen FACE (Free Air CO₂ Enrichment) site located in northern Wisconsin, U.S.A. where trembling aspen (*Populus tremuloides* Michaux) trees were exposed to one of four atmospheric treatments: elevated carbon dioxide (CO₂; 560 µL/L), elevated ozone (O₃; ambient × 1.5), elevated CO₂ and O₃, or ambient air. We evaluated the effects of these fumigants on aspen foliar quality and the performance of aspen blotch leafminer (*Phyllonorycter tremuloidiella* Braun).

2 CO₂ and O₃ each affected foliar quality, with the major changes consisting of an 11% reduction in nitrogen under elevated CO₂ and a 20% reduction in tremulacin under elevated O₃. In the CO₂ + O₃ treatment, nitrogen levels were reduced by 15% and CO₂ ameliorated the O₃-mediated reduction in tremulacin levels.

3 *Phyllonorycter tremuloidiella* were allowed to colonize trees naturally. Elevated CO₂ and O₃ reduced colonization rates by 42 and 49% relative to ambient CO₂ and O₃, respectively. The only effect of fumigation treatments on larval performance occurred under elevated O₃, where male development time and larval consumption increased by 8 and 28%, respectively, over insects reared under ambient O₃.

4 These data demonstrate that the individual and combined effects of CO₂ and O₃ can alter aspen foliar chemistry and that these alterations in foliar chemistry produce little to no change in larval performance. However, both CO₂ and O₃ greatly reduced oviposition. In order to ascertain the full effects of CO₂ and O₃ on insect performance, future studies should address both population- and individual-level characteristics.

Keywords CO₂, FACE, foliar quality, insect performance, O₃, *Phyllonorycter tremuloidiella* (aspen blotch leafminer), plant–insect interactions, *Populus tremuloides* (trembling aspen).

Introduction

Approximately half of the world's forests are expected to experience increased co-exposure of CO₂ and O₃ by 2100 (Fowler *et al.*, 1999). Atmospheric CO₂ concentrations are projected to double during this century (Houghton *et al.*, 1996) and tropospheric O₃ concentrations are projected to triple within the next 40 years (Chameides *et al.*, 1994). Consequently, research has begun to focus on the combined effects of these pollutants on tree species (e.g. Kull *et al.*, 1996; Volin & Reich, 1996; Volin *et al.*, 1998; Grams *et al.*, 1999; Karnosky *et al.*, 1999). Few studies, however, have focused on the interactive effects of these pollutants on biotic processes, such as herbivory. Both elevated CO₂ and O₃ can alter plant phytochemistry (Koricheva *et al.*, 1998) and in turn, insect performance (e.g. Bezemer & Jones,

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1998; Jackson *et al.*, 2000), but their effects when administered together are poorly known.

CO_2 enrichment alters tree–insect interactions largely through changes in foliar quality (Lindroth, 1996a,b; Bezemer & Jones, 1998). Trees grown in a CO_2-enriched environment typically exhibit increased concentrations of carbon-based metabolites and decreased concentrations of nitrogen in foliage (Watt *et al.*, 1995; Saxe *et al.*, 1998; Norby *et al.*, 1999). Leaf-chewing insects generally respond to these changes by increased development time and consumption but decreased pupal mass. To date, only a few CO_2–plant–insect studies have been conducted with leafminers, which exhibited responses similar to those of externally feeding folivores (Salt *et al.*, 1995; Docherty *et al.*, 1996; Smith & Jones, 1998; Stiling *et al.*, 1999).

Like elevated CO_2, O_3 is known to alter foliar quality (Riemer & Whittaker, 1989; Koricheva *et al.*, 1998) and these changes probably influence plant–insect interactions. In contrast to CO_2 research, relatively few studies have been conducted on the effects of O_3 exposure on foliar chemistry and, in turn, insect performance. Leaf-chewing folivores have shown positive, negative and no response to O_3-mediated changes in foliar quality (Trumble *et al.*, 1987; Chappelka *et al.*, 1988; Coleman & Jones, 1988; Jackson *et al.*, 2000; Kopper & Lindroth, 2001). Research is lacking, however, on the effects of O_3-exposed foliage on the leafminer feeding guild.

The purpose of this study was to determine the effects of elevated CO_2 and O_3 (both alone and in combination) on a common tree and on a specialist, leafmining herbivore. The specific objectives were to determine the extent to which foliar quality of trembling aspen (*Populus tremuloides* Michaux) changes when exposed to elevated CO_2, O_3 and $CO_2 + O_3$ environments, and the impact of these changes on performance of the aspen blotch leafminer (*Phyllonorycter tremuloidiella* Braun). Leafminers may perform differently than free-feeding folivores because the latter are mobile and can choose optimal foliage for development, whereas leafminers must remain on the leaf selected by the mother. Furthermore, leafmining insects provide an excellent opportunity to study insect performance because they are apparent and leave behind a record of their feeding, making estimations of consumption rates possible.

Trembling aspen and the aspen blotch leafminer were selected for use because they are common species in the forests of the north-central U.S.A. Trembling aspen is an early successional tree species with secondary metabolites originating from the shikimic acid pathway and consisting primarily of phenolic glycosides and condensed tannins (Palo, 1984; Lindroth *et al.*, 1987). In central Canada and western Great Lake states, trembling aspen is the preferred host for *P. tremuloidiella* (Martin, 1956). This leafminer is univoltine and can occur in epidemic numbers (Auerbach, 1991). Larvae feed on the leaf mesophyll and palisade layers of various species of *Populus* and *Salix* (Martin (1956). Adults are crepuscular, with females ovipositing on expanding leaves (Auerbach, 1991; Auerbach & Alberts, 1992). Eggs are large enough to be seen without magnification (~0.30 mm). Egg hatch occurs in late May and the larvae

undergo five instars, with development completed by mid-July. Mines are oval and approximately 10 mm in diameter when larvae pupate.

Methods

Experimental design and set-up

This experiment was conducted at the Aspen Free-air CO_2 Enrichment (Aspen FACE) site located in north-central Wisconsin, U.S.A. (89.7° W, 45.7° N). The site contains 12 FACE rings (30 m diameter) set up as a blocked full factorial design, with two levels of CO_2 (ambient and 560 µL/L) and two levels of O_3 (ambient and elevated). Each treatment is replicated three times. The elevated CO_2 concentrations employed are based on levels predicted for 50–60 years in the future. Target O_3 levels are modelled to match seasonal and diurnal profiles of levels currently realized in urban areas in the western Great Lakes region (Pinkerton & Lefohn, 1987; Karnosky *et al.*, 1996). To account for the photochemical nature of O_3 production, target levels are modified depending on the weather. For example, daily O_3 levels on hot and sunny days reached a peak concentration of 90–100 nL/L and on cloudy days reached a peak concentration of 50–60 nL/L. No ozone fumigation occurred when the temperature was below 15 °C or when the leaf surfaces were wetted from fog, dew or rain events. For the control treatment, ambient air was blown into the rings. Fumigation occurred only during daylight hours of the growing season.

Each ring was divided into three sections: the eastern half, south-western quarter and north-western quarter. The eastern half of the ring contained a stand of mixed aspen genotypes (five clones), the south-western quarter was alternately planted with an aspen clone (216) and paper birch, and the north-western quarter was alternately planted with an aspen clone (216) and sugar maple. This study was conducted in the aspen-birch quarter with trees planted 1 m apart. For a complete description of the experimental design, set-up and operation of the FACE site, consult Dickson *et al.* (2000).

Trembling aspen saplings were vegetatively propagated from greenwood cuttings (Karnosky *et al.*, 1996; Dickson *et al.*, 2000). 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 3 years old. Five aspen trees within each ring were used for both foliar collections and insect bioassays.

Phytochemical analysis

For phytochemical analyses, leaves were collected on three dates (9 June, 23 June and 7 July 1999) 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, Rhode Island, U.S.A.; reduces light levels by 15%) as used for insect bioassays. Foliage was selected at the same relative position and sun exposure as the foliage used for insect

bioassays. For each collection date, 2–3 g (fresh mass) of foliage was excised at the petiole from each tree, weighed and stored on ice. Upon returning 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 water, nitrogen, starch, phenolic glycosides and condensed tannins. Water concentrations were determined gravimetrically. Nitrogen concentrations were determined with a LECO FP528 nitrogen analyser (St. Joseph, MI, U.S.A.), using glycine *p*-toluenesulphonate as the reference standard (Hach Co., Loveland CO, U.S.A.). For determination of starch, we separated starch from soluble sugars and enzymatically (amyloglucosidase) hydrolysed it to glucose (Prado *et al.*, 1998). To quantify glucose concentrations we used a modification of the dinitrosalicylic acid method (Lindroth *et al.*, 2002a). Concentrations of the phenolic glycosides salicortin and tremulacin were quantified using high performance thin layer chromatography (HPTLC), with purified aspen salicortin and tremulacin as reference standards. Condensed tannin concentrations were measured using a modification of the butanol-HCl method of Porter *et al.* (1986), with purified aspen condensed tannins as the reference standard.

Insect bioassays

Adults of *Phyllonorycter tremuloidiella* were allowed to colonize trees naturally. On each tree, 150 leaves were surveyed for *P. tremuloidiella* eggs to determine colonization rates. Branches used for measuring colonization rates were 1 m above the ground and faced west for all trees. In two instances, trees had fewer than 150 leaves on one branch. For these trees, we continued to count leaves on the next branch to the south. For insect survivorship, 15 mines per tree were randomly selected (from the 150) and monitored from egg deposition to larval pupation to estimate mortality rates.

To assess larval performance, five mines were randomly selected on each tree and covered with No-See-Um mesh to protect them from predation (25 mines/ring). Development time, pupal mass and consumption were recorded for each larva that pupated. Due to the small size of the pupae, a microbalance (Mettler-Toledo, Greifensee, Switzerland) was used to obtain pupal mass. Consumption estimates were based on aerial determinations of mines. Each mine was cut out of the leaf along with a leaf disk of similar size. Frass and exuvia were removed from the mine and both the mine and leaf disk were weighed and scanned using a bench-top leaf area meter (LI-3100, Licor, Lincoln, NB, U.S.A.). Mines and leaf disks were then oven-dried (60 °C) and reweighed. We used the formula as presented by Mansfield *et al.* (1999) to estimate the leaf dry mass consumed (DM$_c$) by each larva:

$$DM_c = A_m(LMA_m - LMA_m)$$

where A$_m$ is the leafmine area, LMA$_u$ is the dry mass per area of the unmined leaf (obtained from a corresponding leaf disk) and LMA$_m$ is the dry mass per area of the mined leaf.

Statistics

We used analysis of variance (ANOVA; PROC MIXED, Littell *et al.*, 1996) for statistical analysis. For analysis of phytochemical data we employed a full factorial design with repeated measures. The a priori statistical model employed was:

$$Y_{ijkl} = \mu + B_i + C_j + O_k + CO_{jk} + e_{ijk} + T_l + CT_{jl} + OT_{kl} + COT_{jkl} + \varepsilon_{ijkl}$$

where Y$_{ijkl}$ was the average response of block *i*, CO$_2$ level *j*, O$_3$ level *k* and time *l*. Fixed effects included CO$_2$ level (C$_j$), O$_3$ level (O$_k$), time (T$_l$) and their interaction terms (CO$_{jk}$), (CT$_{jl}$), (OT$_{kl}$) and (COT$_{jkl}$). Random effects included block (B$_i$), whole plot error (e$_{ijk}$) and the subplot error (ε_{ijkl}). This a priori model assumes that the block and all other treatments are additive (i.e. that the treatment effects are the same for each block). Through the course of our analysis, we found that this assumption was not met. Therefore, the previous model was augmented with terms representing the interaction between each fixed effect and block:

$$Y_{ijkl} = \mu + B_i + C_j + O_k + CO_{jk} + [BC_{ij} + BO_{ik} + BCO_{ijk}] + T_l + CT_{jl} + OT_{kl} + COT_{jkl} + [BT_{il} + BCT_{ijl} + BOT_{ikl} + BCOT_{ijkl}]$$

where e$_{ijk}$ was partitioned into block × CO$_2$ (BC$_{ij}$), block × O$_3$ (BO$_{ik}$) and block × CO$_2$ × O$_3$ (BCO$_{ijk}$) and ε_{ijkl} was partitioned into block × time (BT$_{il}$), block × CO$_2$ × time (BCT$_{ijl}$), block × O$_3$ × time (BOT$_{ikl}$) and block × CO$_2$ × O$_3$ × time (BCOT$_{ijkl}$). By using likelihood methods incorporated in PROC MIXED, we determined that one or more of these interaction terms were significant for all response variables (Littell *et al.*, 1996). Thus, *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 are reported for each CO$_2$ × O$_3$ × time combination.

For analysis of insect performance data, time was omitted and sex was added to the model to account for potential sexual dimorphism. For insect colonization and survivorship rates, sex was omitted from the model because we were unable to determine the sex of eggs or dead larvae. *F*-tests were performed and degrees of freedom for error were assigned in the same manner as for phytochemical data analysis (Littell *et al.*, 1996). Means and standard errors are reported for each CO$_2$ × O$_3$ × sex or CO$_2$ × O$_3$ combination.

The low number of replicates ($n = 3$) in this experiment increases the probability of type II errors. We report *P*-values < 0.10 as 'significant' (Filion *et al.*, 2000), but for readers requiring a more stringent α, we include exact *P*-values for all main effects and interactions (Tables 1 and 2).

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

Main effect and interactions	Water	Nitrogen	Starch	Salicortin	Tremulacin	Condensed tannins
CO_2	0.227	0.039	0.895	0.780	0.568	0.642
O_3	0.356	0.107	0.345	0.127	0.074	0.988
$CO_2 \times O_3$	0.958	0.066	0.146	0.664	0.407	0.958
Time	0.008	<0.001	0.005	0.010	0.135	0.452
$CO_2 \times$ time	0.988	0.762	0.115	0.389	0.392	0.279
$O_3 \times$ time	0.819	0.101	0.790	0.369	0.731	0.034
$CO_2 \times O_3 \times$ time	0.702	0.317	0.062	0.396	0.831	0.007

Results

Phytochemistry

Aspen phytochemistry changed in response to CO_2 and O_3 treatments. Phytochemical levels also varied over time, sometimes differently among fumigation treatments (i.e. significant fumigation $\times$ time interaction, Table 1). Foliar water concentrations decreased 10% from the first to the last collection regardless of the fumigation treatment (Fig. 1). Elevated CO_2 reduced foliar nitrogen levels by 11% relative to ambient CO_2. Furthermore, CO_2 interacted with O_3 to reduce nitrogen levels by 15%, relative to trees grown in the control treatment (Table 1, Fig. 1). Nitrogen concentrations decreased 13% from the first to the last collection, regardless of the fumigation treatment (Table 1, Fig. 1). The effect of the CO_2 and O_3 fumigation on starch levels varied over time, with levels tending to increase over those in the ambient treatment for the second collection but decreasing below ambient levels by the third collection (Fig. 1). Salicortin levels were not responsive to any of the fumigation treatments but declined slightly over time. Tremulacin levels were not affected by elevated CO_2 but were reduced (20%) by elevated O_3 (Table 1, Fig. 2). Condensed tannin concentrations were affected by the interaction of CO_2, O_3 and time (Table 1). In the elevated O_3 treatment, tannin concentrations increased 10% from the first to the last foliar collection. In the $CO_2 + O_3$ treatment, however, tannin concentrations decreased 12% from the first to the last foliar collection, with most of this decline occurring between the first and second collections (Fig. 2).

Insect performance

CO_2 and O_3 independently and interactively influenced insect performance, but the magnitude of the response depended upon sex and differed among the variables studied (Table 2). Consequences for colonization rates were the same whether the insects were exposed to elevated CO_2 or O_3, with colonization rates reduced by 42 and 49%, respectively, relative to ambient CO_2 and O_3 (Fig. 3). Colonization rates under the combined pollutant treatment were similar to those under the individual pollutants (Table 2, Fig. 3). Survivorship (egg deposition to pupation), however, was not affected by fumigation treatment (Table 2, Fig. 4). Development times of female larvae were unresponsive to CO_2 and O_3 (Table 2, Fig. 5). Those of males, however, increased 8% for insects in the elevated O_3 and $CO_2 + O_3$ treatments, relative to controls (Fig. 5). Consumption was not affected by elevated CO_2. Larvae reared under elevated O_3, however, consumed 28% more food than did larvae reared under ambient O_3 (Table 2, Fig. 5). Pupal mass was not affected by any of the fumigation treatments. Females, however, were 20% larger than males.

Discussion

Phytochemistry

Elevated CO_2 independently reduced nitrogen levels and had no effect on carbon-based metabolites. The CO_2-mediated decline in nitrogen levels was consistent with previous CO_2–aspen studies (e.g. Roth *et al.*, 1997, 1998;

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

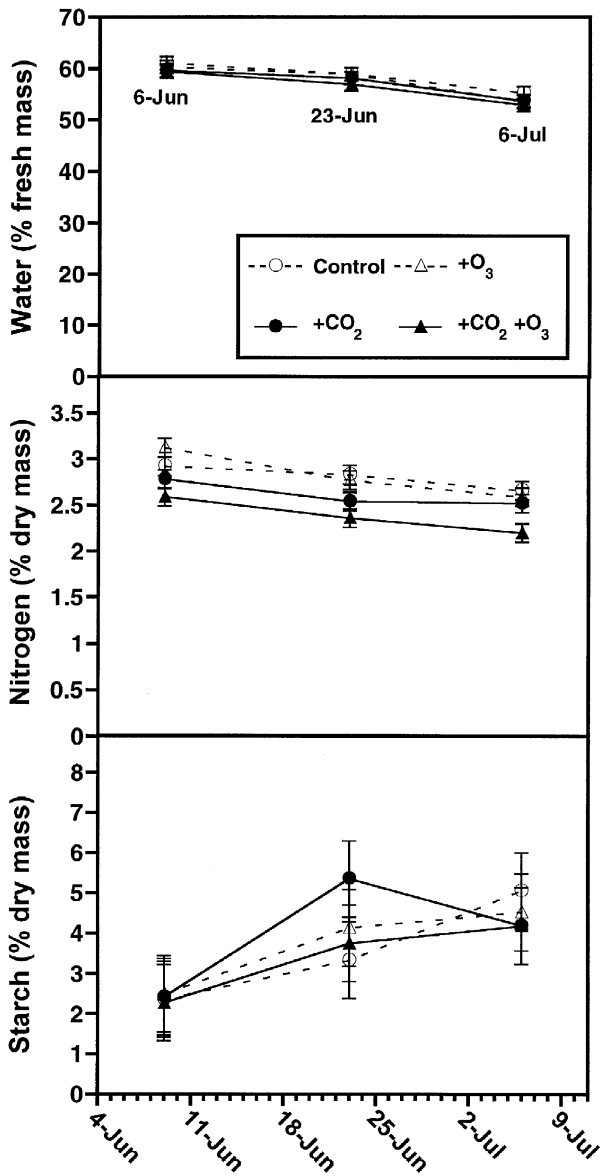| Main effects and interactions	Colonization	Survivorship	Development time	Consumption	Pupal mass
CO_2	0.058	0.850	0.108	0.478	0.125
O_3	0.026	0.690	0.232	0.085	0.346
$CO_2 \times O_3$	0.103	0.371	0.182	0.810	0.292
Sex	–	–	0.561	0.828	0.004
$CO_2 \times$ sex	–	–	0.242	0.755	0.382
$O_3 \times$ sex	–	–	0.060	0.270	0.897
$CO_2 \times O_3 \times$ sex	–	–	0.028	0.358	0.180

Figure 1 Concentrations of water, nitrogen, and starch under control, elevated CO$_2$, elevated O$_3$, and elevated CO$_2$ + O$_3$ fumigation treatments. Error bars indicate ±1 standard error (calculated from the pooled variance).

Figure 2 Concentrations of secondary metabolites under control, elevated CO$_2$, elevated O$_3$, and elevated CO$_2$ + O$_3$ fumigation treatments. Error bars indicate ±1 standard error (calculated from the pooled variance).

Lindroth & Kinney, 1998). Generally, CO$_2$ enrichment has been shown to increase levels of starch, phenolic glycosides and condensed tannins in trembling aspen, although not all metabolites may be uniformly affected (e.g. Roth *et al.*, 1997, 1998; Lindroth & Kinney, 1998; Lindroth *et al.*, 2002b). Several explanations exist for the modest response in carbon-based metabolite levels observed in this study. First, levels of carbon-based metabolites have been shown to be less responsive to elevated CO$_2$ under conditions of high nutrient availability, such as exist at the FACE site (Dickson *et al.*, 2000), than under conditions of low nutrient availability (Kinney *et al.*, 1997; Mansfield *et al.*, 1999; Lindroth *et al.*, 2001b). Second, most CO$_2$–tree studies use
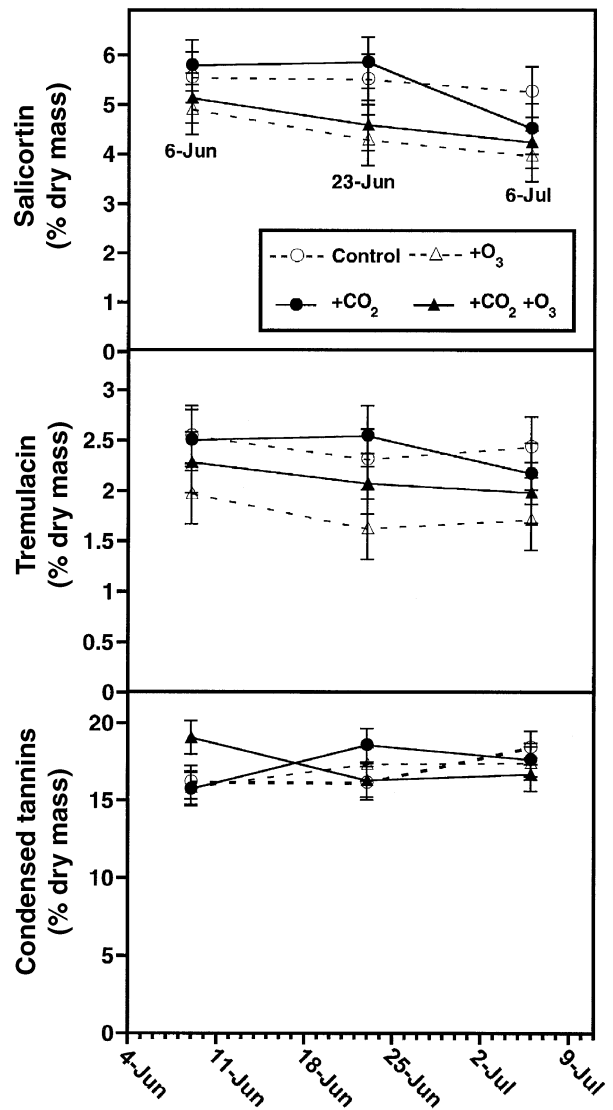
a CO$_2$ concentration higher than that used in this study (700–650 μL/L vs. 560 μL/L) (Roth & Lindroth, 1994; Lindroth *et al.*, 1995; Agrell *et al.*, 1999, 2000; McDonald *et al.*, 1999). Finally, CO$_2$-mediated accumulation of carbon-based metabolites varies among aspen genotypes (Mansfield *et al.*, 1999; Lindroth *et al.*, 2001a, 2002b) and the genotype used in this study may be particularly unresponsive. We must point out, however, that in a concurrent study using a different set of aspen (clone 216), trees responded to elevated levels of CO$_2$ by increasing levels of salicortin and tremulacin and decreasing levels of condensed tannins (Lindroth *et al.*, 2002b). Why the two sets of trees responded differently between the two studies is unclear, given that the only differences between the studies were the position of the trees within the ring and the tree
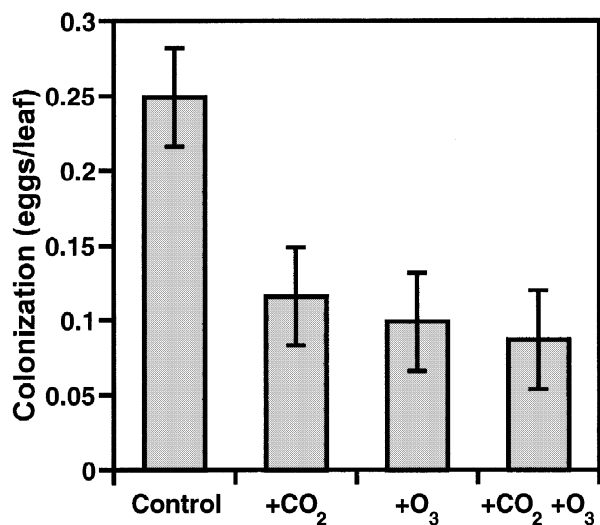
Figure 3 Colonization of aspen by blotch leafminers under control, elevated CO_2, elevated O_3, and elevated $CO_2 + O_3$ fumigation treatments. Error bars indicate ± 1 standard error (calculated from the pooled variance).
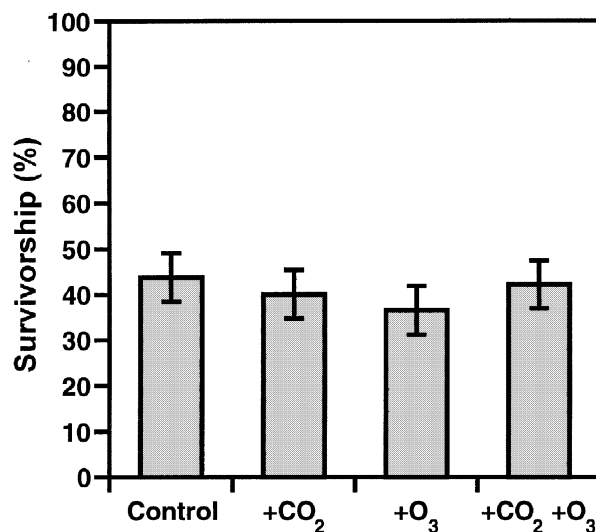

Figure 4 Survivorship of aspen blotch leafminers under control, elevated CO_2, elevated O_3, and elevated $CO_2 + O_3$ fumigation treatments. Error bars indicate ± 1 standard error (calculated from the pooled variance).

species with which they were interplanted (aspen–birch vs. mixed aspen genotypes).

Elevated O_3, like elevated CO_2, altered concentrations of some foliar constituents, and these effects were further modified by interactions with CO_2 and time. The exacerbated decrease in nitrogen levels under the $CO_2 + O_3$ treatment may be due to the effect of each pollutant on ribulose bisphosphate carboxylase (Rubisco) concentrations. Elevated CO_2 can reduce Rubisco levels (reviewed by Saxe *et al.*, 1998), a response that has been demonstrated for aspen at the FACE site (Takeuchi *et al.*, 2001). O_3 can inhibit the synthesis of Rubisco (Pell *et al.*, 1994; Bortier *et al.*, 2000). Overall, identifying general patterns of the effect of O_3-exposure on foliar nitrogen levels is difficult because previous research has demonstrated that O_3 can cause foliage to have higher, lower, or unaltered levels of nitrogen (Koricheva *et al.*, 1998). With respect to starch, research typically reports an O_3-mediated decrease in concentrations (Bücker & Ballach, 1992; Friend & Tomlinson, 1992; Lavola *et al.*, 1994), which is attributed to the conversion of starch into soluble sugars used to repair O_3 injury (Lavola *et al.*, 1994). In this study, aspen trees exhibited symptoms (e.g. reduced growth, leaf necrosis) characteristic of O_3 exposure but this damage did not reduce starch levels. With respect to secondary metabolites, O_3 exposure tended to reduce tremulacin concentrations. The reason for this decrease is unknown but may include a reduction in biosynthesis due to decreased photosynthate availability or enzyme activity. Alternatively, O_3 may accelerate turnover rates of tremulacin. Tremulacin levels under the $CO_2 + O_3$ treatment were similar to those in the control treatment, signifying that CO_2 enrichment can ameliorate the O_3-mediated reduction of some metabolites. O_3 interacted with CO_2 and time to affect tannin levels. Tannin levels under the $CO_2 + O_3$ treatment were significantly higher

than those in the other fumigation treatments for the first collection, but did not differ for the remaining collection dates. We are uncertain of the cause of this response, although one reason may be developmental changes in susceptibility to CO_2 and O_3 exposure, with early season foliage more strongly affected by co-exposure than late season foliage. Our results indicate that when applied in combination, CO_2 and O_3 can exacerbate reductions in concentrations of some phytochemicals (e.g. nitrogen) while negating the effects of either pollutant acting alone for others (e.g. tremulacin).

Insect performance

Colonization rates were dramatically reduced by elevated CO_2 and O_3. This suppression, however, tended to be ameliorated when pollutants were administered in combination, resulting in colonization rates similar to those observed when administered alone. Other researchers have investigated the effects of CO_2 and O_3 on insect oviposition (Jones & Coleman, 1988; Stange *et al.*, 1995; Stange, 1997; Jackson *et al.*, 2000) but this study is the first to assess the combined effects of these pollutants on oviposition. Previous research typically reported that both elevated CO_2 and O_3 reduce oviposition (e.g. Jones & Coleman, 1988; Thompson & Drake, 1994; Stange, 1997; but see Jackson *et al.*, 1999). For example, a pyralid moth (*Cactoblastis cactorum*) reduced oviposition rates when exposed to $720\,\mu L/L$ of CO_2 (Stange, 1997). Similarly, a chrysomelid beetle (*Plagiodera versicolora*) preferred to oviposit on charcoal-filtered, opposed to O_3-exposed, cottonwood leaves (Jones & Coleman, 1988). In our study, ovipositing females were not directly exposed to either pollutant because oviposition occurs during the evening (M. Auerbach, pers. comm.), several hours after cessation of fumigation.
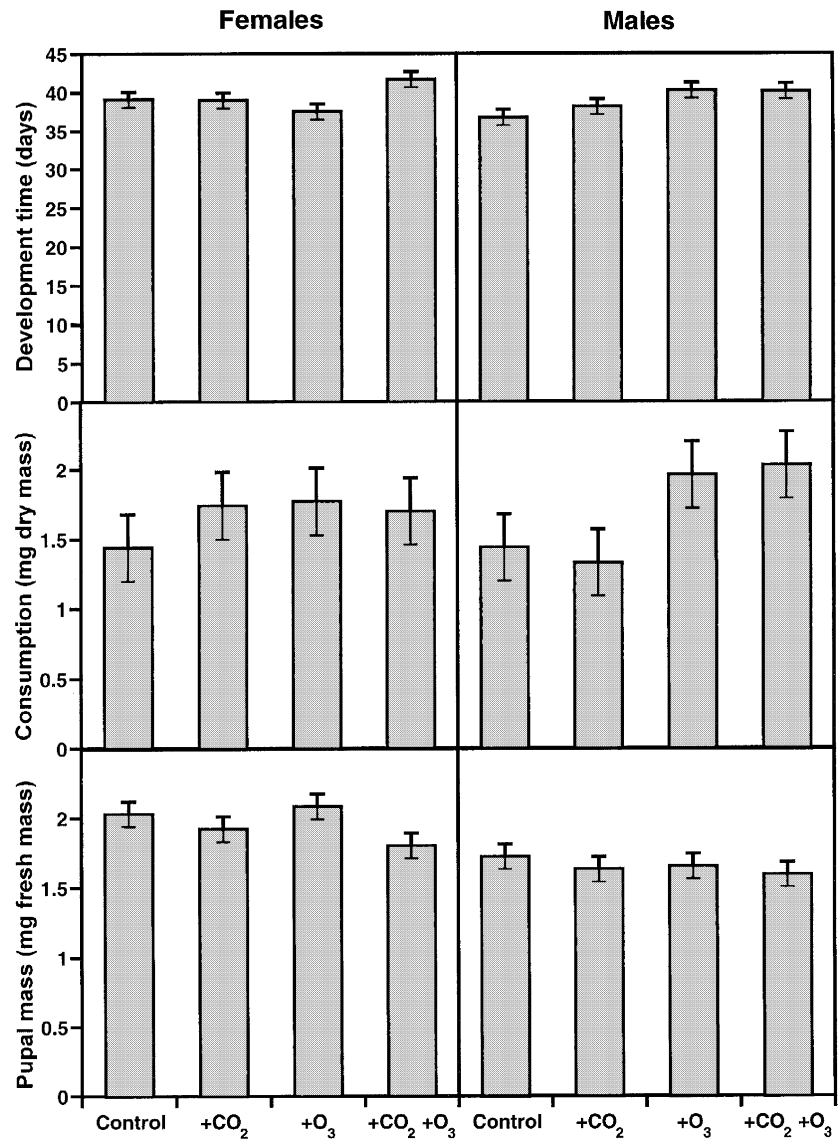
Figure 5 Aspen blotch leafminer performance under control, elevated CO_2, elevated O_3 and elevated $CO_2 + O_3$ fumigation treatments. Error bars indicate ± 1 standard error (calculated from the pooled variance).

Furthermore, leaf age, a factor known to influence *P. tremuloidiella* colonization (Auerbach, 1991; Auerbach & Alberts, 1992), was similar among all four treatments. A more likely explanation for the reduced colonization rates is changes to the leaf surface. Elevated levels of CO_2 and O_3 were shown by other researchers at the FACE site to alter the molecular composition and production of, and to degrade, aspen epicuticular waxes (Karnosky *et al.*, 1999; K. Percy, pers. comm.). Epicuticular waxes are known to be important oviposition stimulants for some insect species (Eigenbrode & Espelie, 1995). Alternatively, reduced colonization could also be due to pollutant-mediated alterations in other oviposition stimulants or deterrents.

Elevated CO_2 and O_3 treatments had relatively minor effects on larval performance, and the magnitude of these depended on treatment, performance variable and sex. Survivorship (egg and larval) was not affected by either CO_2 or O_3. Elevated CO_2 also did not independently influence

insect development, feeding or pupal mass. These results differ from earlier research in that previous studies conducted with leafminers and free-feeding folivores typically report changes in development time, consumption or pupal mass (Lincoln *et al.*, 1993; Salt *et al.*, 1995; Watt *et al.*, 1995; Docherty *et al.*, 1996; Lindroth, 1996a,b; Bezemer & Jones, 1998; Smith & Jones, 1998; Coviella & Trumble, 1999; Stiling *et al.*, 1999). Our results are similar to another study with *P. tremuloidiella* where no difference in consumption and only a marginal difference in pupal mass was found between insects in ambient and enriched CO_2 (Mansfield *et al.*, 1999). In our study, the lack of a CO_2 effect on larval performance is probably a consequence of similar foliar chemistry between the CO_2 and control treatments.

In contrast to CO_2, O_3 independently and interactively affected larval performance, and these results varied between males and females. Larvae tended to consume

more leaf tissue under elevated O_3 than did those reared under ambient O_3, a response also demonstrated in other studies. For example, Coleman & Jones (1988) found that imported willow leaf beetle (*Plagiodera versicolora*) increased consumption when reared on willow foliage fumigated with O_3. In our study the increase in consumption, at least in the $CO_2 + O_3$ treatment, could be due to a decrease in foliar nitrogen, which is the most limiting nutrient for herbivorous insects (e.g. Mattson, 1980). Regarding development time and pupal mass, we found a moderate increase in male development time in both the O_3 and $CO_2 + O_3$ treatments, relative to those reared in control rings. Male pupal mass along with female development time and pupal mass, however, were unresponsive to elevated O_3. Why male development time increased and female development time did not in response to O_3 exposure remains unclear, although the effect on males was small and only marginally significant.

To conclude, elevated levels of CO_2 and O_3, alone and in combination, had modest effects on foliar chemistry and these changes produced at most only slight changes in larval performance. Our most striking result was that CO_2 and O_3 reduced colonization rates by nearly half, relative to the respective ambient treatments, demonstrating that these pollutants can markedly affect *P. tremuloidiella* oviposition. Because leaf age is an important determinant of *P. tremuloidiella* oviposition (Auerbach, 1991; Auerbach & Alberts, 1992) and because elevated levels of CO_2 and O_3 can alter the leaf phenology of some tree species (Gunthardt-Goerg *et al.*, 1993; Ceulemans & Mousseau, 1994; Saxe *et al.*, 1998; Norby *et al.*, 1999), we suggest that CO_2- and O_3-mediated changes in leaf phenology may influence colonization rates beyond the changes due simply to altered oviposition stimulants or deterrents. If the leaf phenology of some aspen genotypes is affected more by these pollutants than is that of others, then CO_2 and O_3 sensitivity may ultimately influence host preference in this system. Additional research is required to investigate the cause of the CO_2- and O_3-mediated reduction in colonization and to determine if these results vary among genotypes and across environments. Finally, this research emphasizes the need for studies to investigate both population- and individual-level parameters to determine the full effects of CO_2 and O_3 on insect performance.

Acknowledgements

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