Responses of trembling aspen (Populus tremuloides) phytochemistry and aspen blotch leafminer (Phyllophaga 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 (Phyllophaga 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 Phyllophaga 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, Phyllophaga 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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CO₂ enrichment alters tree-insect interactions largely through changes in foliar quality (Lindroth, 1996a,b; Bezemer & Jones, 1998). Trees grown in a CO₂-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 CO₂-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 CO₂, O₃ is known to alter foliar quality (Riemer & Whittaker, 1989; Koricheva et al., 1998) and these changes probably influence plant-insect interactions. In contrast to CO₂ research, relatively few studies have been conducted on the effects of O₃ exposure on foliar chemistry and, in turn, insect performance. Leaf-chewing folivores have shown positive, negative and no response to O₃-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 O₃-exposed foliage on the leafminer feeding guild.

The purpose of this study was to determine the effects of elevated CO₂ and O₃ (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 CO₂, O₃ and CO₂ + O₃ environments, and the impact of these changes on performance of the aspen blotch leafminer (Phyllonorycter tremuloidella 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. tremuloidella (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 larval pupate.

**Methods**

**Experimental design and set-up**

This experiment was conducted at the Aspen Free-air CO₂ 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 CO₂ (ambient and 560 μL/L) and two levels of O₃ (ambient and elevated). Each treatment is replicated three times. The elevated CO₂ concentrations employed are based on levels predicted for 50–60 years in the future. Target O₃ 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 O₃ production, target levels are modified depending on the weather. For example, daily O₃ 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-toluensulphonate 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 Phyllophaga 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 aerolations 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 benchtop 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 (DMc) by each larva:

\[
DMc = A_m (LMA_m - LMA_m)
\]

where \(A_m\) is the leafmine area, \(LMA_m\) 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:

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

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 \((e_{jkl})\). 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:

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

where \(e_{jk}\) was partitioned into block \(\times CO_2\) \((BC_{ij})\), block \(\times O_3\) \((BO_{ik})\) and block \(\times CO_2 \times O_3\) \((BCO_{jk})\) and \(e_{jkl}\) was partitioned into block \(\times time\) \((BT_{il})\), block \(\times CO_2 \times time\) \((BCT_{jil})\), block \(\times O_3 \times time\) \((BOT_{kil})\) and block \(\times CO_2 \times O_3 \times time\) \((BCOT_{ijkl})\). 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 \(CO_2 \times O_3 \times time\) 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 \(CO_2 \times O_3 \times sex\) or \(CO_2 \times O_3\) 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 \(\alpha\), 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 CO₂, O₃ and time on aspen phytochemistry

<table>
<thead>
<tr>
<th>Main effect and interactions</th>
<th>Water</th>
<th>Nitrogen</th>
<th>Starch</th>
<th>Salicortin</th>
<th>Tremulacin</th>
<th>Condensed tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>0.227</td>
<td>0.039</td>
<td>0.895</td>
<td>0.780</td>
<td>0.568</td>
<td>0.642</td>
</tr>
<tr>
<td>O₃</td>
<td>0.356</td>
<td>0.107</td>
<td>0.345</td>
<td>0.127</td>
<td>0.074</td>
<td>0.988</td>
</tr>
<tr>
<td>CO₂ × O₃</td>
<td>0.956</td>
<td>0.066</td>
<td>0.146</td>
<td>0.064</td>
<td>0.135</td>
<td>0.958</td>
</tr>
<tr>
<td>Time</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.010</td>
<td>0.392</td>
<td>0.279</td>
</tr>
<tr>
<td>CO₂ × time</td>
<td>0.988</td>
<td>0.762</td>
<td>0.115</td>
<td>0.389</td>
<td>0.731</td>
<td>0.034</td>
</tr>
<tr>
<td>O₃ × time</td>
<td>0.819</td>
<td>0.101</td>
<td>0.790</td>
<td>0.369</td>
<td>0.831</td>
<td>0.007</td>
</tr>
<tr>
<td>CO₂ × O₃ × time</td>
<td>0.702</td>
<td>0.317</td>
<td>0.062</td>
<td>0.396</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

Phytochemistry

Aspen phytochemistry changed in response to CO₂ and O₃ treatments. Phytochemical levels also varied over time, sometimes differently among fumigation treatments (i.e. significant fumigation × time interaction, Table 1). Foliar water concentrations decreased 10% from the first to the last collection regardless of the fumigation treatment (Fig. 1). Elevated CO₂ reduced foliar nitrogen levels by 11% relative to ambient CO₂. Furthermore, CO₂ interacted with O₃ 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 CO₂ and O₃ 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 CO₂ but were reduced (20%) by elevated O₃ (Table 1, Fig. 2). Condensed tannin concentrations were affected by the interaction of CO₂, O₃ and time (Table 1). In the elevated O₃ treatment, tannin concentrations increased 10% from the first to the last foliar collection. In the CO₂ + O₃ 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

CO₂ and O₃ 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 CO₂ or O₃, with colonization rates reduced by 42 and 49%, respectively, relative to ambient CO₂ and O₃ (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 CO₂ and O₃ (Table 2, Fig. 5). Those of males, however, increased 8% for insects in the elevated O₃ and CO₂ + O₃ treatments, relative to controls (Fig. 5). Consumption was not affected by elevated CO₂. Larvae reared under elevated O₃, however, consumed 28% more food than did larvae reared under ambient O₃ (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 CO₂ independently reduced nitrogen levels and had no effect on carbon-based metabolites. The CO₂-mediated decline in nitrogen levels was consistent with previous CO₂–aspen studies (e.g. Roth et al., 1997, 1998;
Lindroth & Kinney, 1998). Generally, CO₂ 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 CO₂ 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 CO₂-tree studies use a CO₂ 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, CO₂-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 CO₂ 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 1 Concentrations of water, nitrogen, and starch under control, elevated CO₂, elevated O₃, and elevated CO₂ + O₃ fumigation treatments. Error bars indicate ±1 standard error (calculated from the pooled variance).

Figure 2 Concentrations of secondary metabolites under control, elevated CO₂, elevated O₃, and elevated CO₂ + O₃ 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 O3, like elevated CO2, altered concentrations of some foliar constituents, and these effects were further modified by interactions with CO2 and time. The exacerbated decrease in nitrogen levels under the CO2 + O3 treatment may be due to the effect of each pollutant on ribulose bisphosphate carboxylase (Rubisco) concentrations. Elevated CO2 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). O3 can inhibit the synthesis of Rubisco (Pell et al., 1994; Bortier et al., 2000). Overall, identifying general patterns of the effect of O3-exposure on foliar nitrogen levels is difficult because previous research has demonstrated that O3 can cause foliage to have higher, lower, or unaltered levels of nitrogen (Koricheva et al., 1998). With respect to starch, research typically reports an O3-mediated decrease in concentrations (Bäcker & Ballach, 1992; Friend & Tomlinson, 1992; Lavola et al., 1994), which is attributed to the conversion of starch into soluble sugars used to repair O3 injury (Lavola et al., 1994). In this study, aspen trees exhibited symptoms (e.g. reduced growth, leaf necrosis) characteristic of O3 exposure but this damage did not reduce starch levels. With respect to secondary metabolites, O3 exposure tended to reduce tremulacin concentrations. The reason for this decrease is unknown but may include a reduction in biosynthesis due to decreased photosynthetic availability or enzyme activity. Alternatively, O3 may accelerate turnover rates of tremulacin. Tremulacin levels under the CO2 + O3 treatment were similar to those in the control treatment, signifying that CO2 enrichment can ameliorate the O3-mediated reduction of some metabolites. O3 interacted with CO2 and time to affect tannin levels. Tannin levels under the CO2 + O3 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 CO2 and O3 exposure, with early season foliage more strongly affected by co-exposure than late season foliage. Our results indicate that when applied in combination, CO2 and O3 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 CO2 and O3. 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 CO2 and O3 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 CO2 and O3 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 µL/L of CO2 (Stange, 1997). Similarly, a chrysomelid beetle (Plagiostema versicolora) preferred to oviposit on charcoal-filtered, opposed to O3-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.
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 CO₂ and O₃ 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 CO₂ and O₃ 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 CO₂ or O₃. Elevated CO₂ 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 folioves 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; Bezem & 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 CO₂ (Mansfield et al., 1999). In our study, the lack of a CO₂ effect on larval performance is probably a consequence of similar foliar chemistry between the CO₂ and control treatments.

In contrast to CO₂, O₃ independently and interactively affected larval performance, and these results varied between males and females. Larvae tended to consume...
more leaf tissue under elevated O₃ than did those reared under ambient O₃, a response also demonstrated in other studies. For example, Coleman & Jones (1988) found that imported willow leaf beetle (Plagiopertha versicolora) increased consumption when reared on willow foliage fumigated with O₃. In our study the increase in consumption, at least in the CO₂ + O₃ 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 O₃ and CO₂ + O₃ treatments, relative to those reared in control rings. Male pupal mass along with female development time and pupal mass, however, were unresponsive to elevated O₃. Why male development time increased and female development time did not in response to O₃ exposure remains unclear, although the effect on males was small and only marginally significant.

To conclude, elevated levels of CO₂ and O₃ 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 CO₂ and O₃ reduced colonization rates by nearly half, relative to the respective ambient treatments, demonstrating that these pollutants can markedly affect *P. tremuloides* oviposition. Because leaf age is an important determinant of *P. tremuloides* oviposition (Auerbach, 1991; Auerbach & Alberts, 1992) and because elevated levels of CO₂ and O₃ can alter the leaf phenology of some tree species (Gunther-Stoer et al., 1993; Ceulemans & Mousseau, 1994; Saxe et al., 1998; Norby et al., 1999), we suggest that CO₂- and O₃-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 that of others, then CO₂ and O₃ sensitivity may ultimately influence host preference in this system. Additional research is required to investigate the cause of the CO₂- and O₃-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 CO₂ and O₃ on insect performance.

**Acknowledgements**

This project would not have been possible without the help of V. Jakobi and T. Osier. We thank M. Auerbach for advice on the natural history of *P. tremuloides* and E. Nordheim and P. Crump for statistical assistance. This research was supported by National Science Foundation (grant DEB-9707263) and the FACE facility was funded by the US Department of Energy, Office of Biological and Environmental Research (Grant DE-FG02-98ER62125).

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Accepted 8 June 2002