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CO_2 and O_3 effects on host plant preferences of the forest tent caterpillar (*Malacosoma disstria*)

JEP AGRELL*, BRIAN KOPPER†,[1], EVAN P. MCDONALD‡ and RICHARD L. LINDROTH§
*Department of Animal Ecology, Ecology Building, Lund University, SE-223 62 Lund, Sweden, †Department of Entomology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA, ‡USDA Forest Service, Forest Sciences Laboratory, 5985 highway K, Rhinelander, WI 54501 USA, §Department of Entomology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706 USA

Abstract

Elevated levels of CO_2 and O_3 affect plant growth and phytochemistry, which in turn can alter physiological performance of associated herbivores. Little is known, however, about how generalist insect herbivores respond *behaviorally* to CO_2- and O_3-mediated changes in their host plants. This research examined the effects of elevated CO_2 and O_3 levels on host plant preferences and consumption of forest tent caterpillar (FTC, *Malacosoma disstria* Hbn.) larvae. Dual choice feeding assays were performed with foliage from birch (*Betula papyrifera* Marsh.) and aspen (*Populus tremuloides* Michx., genotypes 216 and 259). Trees were grown at the Aspen Free Air CO_2 Enrichment (FACE) facility near Rhinelander, WI, USA, and had been exposed to ambient or elevated concentrations of CO_2 and/or O_3. Levels of nutritional and secondary compounds were quantified through phytochemical analyses. The results showed that elevated O_3 levels increased FTC larval preferences for birch compared with aspen, whereas elevated CO_2 levels had the opposite effect. In assays with the two aspen genotypes, addition of both CO_2 and O_3 caused a shift in feeding preferences from genotype 259 to genotype 216. Consumption was unaffected by experimental treatments in assays comparing aspen and birch, but were increased for larvae given high O_3 foliage in the aspen genotype assays. Elevated levels of CO_2 and O_3 altered tree phytochemistry, but did not explain shifts in feeding preferences. The results demonstrate that increased levels of CO_2 and O_3 can alter insect host plant preferences both between and within tree species. Also, consequences of altered host quality (e.g., compensatory consumption) may be buffered by partial host shifts in situations when alternative plant species are available. Environmentally induced changes in host plant preferences may have the potential to alter the distribution of herbivory across plant genotypes and species, as well as competitive interactions among them.

Key words: CO_2 and O_3 exposure, deciduous trees, FACE, forest tent caterpillar, host plant preferences, plant–insect interactions.

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Introduction

Plants grown under enriched atmospheric CO_2 typically show decreases in levels of nitrogen (McGuire *et al*., 1995) and increases in levels of carbon-based metabolites such as carbohydrates and phenolics (Lincoln *et al*., 1993; Poorter *et al*., 1997; Peñuelas & Estiarte 1998; Veteli *et al*., 2002), although responses vary among species (Koricheva *et al*., 1998). These phytochemical changes, in turn, may affect performance of associated insect herbivores (e.g., Whittaker 1979; Trumble *et al*., 1987; Fajer *et al*., 1989; Lindroth *et al*., 1993a; Lindroth 1996; Agrell *et al*., 2000; Hartley *et al*., 2000; Percy *et al*., 2002). Thus, ecosystem changes may occur through interactive effects of shifted competitive balance among plant species and altered

Correspondence: J. Agrell, tel. + 46 46 2228852, fax + 46 46 2224716, e-mail: jep.agrell@zooekol.lu.se
[1]Present address: USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine Building 1, 1 Gifford Pinchot Drive, Madison, WI 53726 USA.

impact of important herbivorous insect species (e.g., Lindroth *et al*., 1993a; Saxe *et al*., 1998).

The effects of elevated CO_2 levels are frequently modulated, however, by other environmental variables, such as resource availability (Bazzaz & Miao, 1993; Kinney *et al*., 1997; Lawler *et al*., 1997; Pritchard *et al*., 1997; Roth *et al*., 1997; Agrell *et al*., 2000). Another potentially influential factor, which has been increasingly recognized in recent years, is ozone (O_3). Tropospheric O_3 levels are on the rise (Chameides *et al*., 1994), with elevated levels commonly affecting plants by decreasing photosynthesis and growth (e.g., Dickson *et al*., 1998; Utriainen *et al*., 2000; Noormets *et al*., 2001). O_3 exposure can also affect phytochemistry by altering levels of carbohydrates (e.g., Bolsinger *et al*., 1991; Pääkkönen *et al*., 1998; Saleem *et al*., 2001) and phenolic compounds (Runeckles & Krupa, 1994). In general, plant responses to O_3 exposure are opposite to those observed under CO_2 enrichment, resulting in the notion that elevated CO_2 may ameliorate ecological effects of elevated O_3 (Allen 1990; Runeckles & Krupa 1994; Dickson *et al*., 1998; Grams *et al*., 1999; Karnosky *et al*., 1999) One important aspect of plant–insect interactions, that has largely been ignored in previous global change studies, is how changes in atmospheric composition may alter insect host plant preferences. The few studies conducted to date have shown that the effects of CO_2 on host plant species preferences of invertebrates range from nil to substantial (Arnone *et al*., 1995; Traw *et al*., 1996; Lederberger *et al*., 1997, 1998; Díaz *et al*., 1998; Peters *et al*., 2000; Goverde & Erhardt, 2003). Even fewer studies have addressed the relationship between O_3 pollution and herbivore feeding preferences, with existing data typically suggesting increased preference for O_3-exposed plants (Jones & Coleman, 1988; Lin *et al*.,1990; Bolsinger *et al*., 1992; Fortin *et al*., 1997). The potential for ecosystem consequences through host plant shifts in future environments is substantial, since plant responses to atmospheric changes exhibit great inter- and intraspecific variation (CO_2: Bazzaz *et al*., 1990; Lindroth *et al*., 1993a; Kinney *et al*., 1997; Peñuelas & Estiarte, 1998; Körner 2000, O_3: Jordan *et al*., 1991; Volin *et al*.,1993; Karnosky *et al*., 1999; Saleem *et al*., 2001). Variation in plant responses to CO_2, coupled with behavioral adjustments of insect herbivores, may alter plant competitive interactions and species composition.

The need for further research in this area has repeatedly been emphasized (e.g., Lincoln *et al*., 1993; Körner 1996; Bezemer & Jones, 1998), for several reasons. First, environmentally induced changes in food plant quality may affect physiological performance of insect herbivores (Runeckles & Krupa, 1994; Bezemer & Jones, 1998), and the rate at which insects are able to track these changes depends on their behavioral adjustments. Second, a strong behavioral response to environmentally induced changes in plant quality (e.g., a shift to a relatively more palatable host plant) may minimize reductions in herbivore growth and reproduction, and thereby buffer against pronounced changes in herbivore population densities. Finally, altered host preferences may be an important factor influencing herbivore impacts on plant production. Insects feeding on CO_2-enriched foliage commonly exhibit increased consumption, presumably as a response to reduced food quality (compensatory consumption, see Fajer *et al*., 1989; Kinney *et al*., 1997; Agrell *et al*., 2000). However, Peters *et al*. (2000) demonstrated that elevated CO_2 levels did not lead to increased consumption if the herbivores had several food plants available.

We examined the effects of elevated CO_2 and O_3 on host plant preferences of forest tent caterpillar (FTC, *Malacosoma disstria* Hbn.) larvae. The FTC is an eruptive generalist defoliator in North American hardwood forests, causing extensive damage during outbreak years (Fitzgerald, 1995). FTC has been widely used in global change research, giving the advantage that considerable data on growth and performance in altered environments are available (e.g., Lindroth *et al*., 1993a; Fortin *et al*., 1997; Roth *et al*., 1997; Kopper & Lindroth, 2003a). Its food consumption behavior, however, remains poorly understood. Other research (Robison & Raffa, 1997) has demonstrated that these larvae exhibit clear host preferences even as first and second instars, and that their growth is enhanced by this host selectivity. Through two-choice bioassays we determined the relative preferences of fourth instar FTC for paper birch (*Betula papyrifera* Marsh.) and two genotypes of quaking aspen (*Populus tremuloides* Michx.) grown under different CO_2 and O_3 conditions. Previous studies found that with respect to growth and phytochemistry, responses of aspen were slightly stronger than those of birch to elevated CO_2 (e.g., Roth & Lindroth, 1995; McDonald *et al*., 1999; Agrell *et al*., 1999, 2000). The two aspen genotypes selected for use in these O_3/CO_2 experiments were initially chosen for use at Aspen FACE because they differ in O_3 sensitivity, with genotype 259 being more sensitive (i.e., showing more visible leaf damage, epicuticular wax degradation, and stomatal occlusion) to O_3 exposure than genotype 216 (Karnosky *et al*., 1996, 1999). In order to investigate how availability of an alternative host plant affected consumption, we also determined consumption rates for larvae with either only aspen available, or with both aspen and birch as potential food sources. Finally, we analyzed levels of foliar nutrients and secondary compounds to examine relationships between host tree quality and larval preferences.

The following hypotheses were examined:

(1) Elevated levels of CO_2 and O_3 alter FTC host plant preferences, both within and between plant species.
(2) Elevated levels of CO_2 ameliorate O_3 effects on plants and insects.
(3) Shifts in FTC host plant preferences are determined by CO_2- and O_3-induced changes in levels of nutrients and secondary substances of the host plants.
(4) CO_2 and O_3 effects on consumption are more pronounced when FTC has access to only one plant species than when an alternative host species is available.

Methods

Experimental site

The study was performed near Rhinelander, Wisconsin (89.5° W, 45.7° N), within the Aspen Free Air CO_2 Enrichment (Aspen FACE) site. Details about the experimental design and operation of this FACE site are available in Karnosky *et al*. (1999). In short, the experimental site is 32 ha with 12 FACE rings (30 m diameter), in a 2×2 factorial design. During this study three of the rings received ambient air (control, CO_2: 360 $\mu L\,L^{-1}$; O_3: 35–60 $nL\,L^{-1}$), three received supplemental CO_2 ($+CO_2$: 560 $\mu L\,L^{-1}$), three received supplemental O_3 ($+O_3$: 1.5 × ambient cumulative exposure), and three received CO_2 and O_3 ($+CO_2+O_3$). To control for potential within-site differences (e.g., soil and moisture), the site is divided into three blocks along a north–south gradient, each containing one ring of each treatment. Concentrations of CO_2 and O_3 are continuously monitored, with elevated CO_2 and O_3 treatments applied during daylight hours of the growing season (May–September). Experimental O_3 levels simulated dynamic profiles realized in urban areas in the western Great Lakes region (Pinkerton & Lefohn, 1987; Karnosky *et al*., 1996). Because of the photochemical nature of tropospheric O_3 formation, daily O_3 applications were adjusted according to weather conditions, with target concentrations being 90–100 $nL\,L^{-1}$ on sunny days and 50–60 $nL\,L^{-1}$ on cloudy days. No O_3 treatment was applied on cool (<15 °C) days or when leaves were wet from rain or condensation.

Plant material

This study utilized two genotypes of quaking aspen (216, originating from Bayfield, WI, USA, and 259, originating from Porter, IN, USA) and paper birch (originating from Houghton county, MI, USA). Leaves for bioassays were taken at approximately 2/3 of tree height, at the midpoint of representative branches. Sampling occurred between 09:00 hours and noon. Leaves were cut at the base of the petiole, immediately placed in plastic bags, and put on ice for transport to the laboratory. Each leaf was weighed, its area measured to the nearest 0.01 cm^2 using a leaf area meter (LI-3000A, LI-COR, Lincoln, NE, USA), and the petiole inserted into a 1.5 mL plastic microfuge tube containing tap water to maintain hydration during the bioassay.

Bioassays

Bioassays examined effects of CO_2 and O_3 exposure on food preferences of fourth instar FTC. Larvae were collected during late second or early third instar (i.e. while still feeding gregariously and before showing extensive dispersal). All larvae were collected from large solitary red oaks (*Quercus rubra*) outside Rhinelander, WI, USA, to reduce the likelihood that they had experienced birch or aspen prior to the experiment. Larvae were placed in ventilated plastic boxes (20 cm × 15 cm × 15 cm) and reared on oak leaves until used in bioassays during mid–fourth instar. Bioassays were conducted between May 24 and June 3. Experimental larvae were starved for 2 h prior to the onset of the assay. One mid-fourth instar larva was weighed, placed into a Petri dish (15 cm × 2.5 cm), and provided with one leaf from each of two different tree species, genotypes or fumigation treatments. The larva was allowed to feed for 20–24 h. To ensure that differences in leaf consumption indeed mirrored leaf preferences rather than, for example, compensatory consumption on a nutritionally inferior leaf, observations of each larva were made during each bioassay. Direct observations were made during the first hour, to ensure that the larva encountered both leaves at least once. In addition, the position of the larva was noted after 6, 12, and 18 h, and when the test was terminated. Only tests where the larva (a) had encountered both leaves within the first hour, (b) were known to have shifted between the two leaves at least once more, and (c) had fed on both leaves, were included in the analyses. After removal of the larva, remaining leaf material was collected, dried at 60 °C for 72 h, and weighed for estimates of consumption. The initial dry mass of leaves was calculated from area : mass ratios determined at the onset of the bioassay. To calibrate the relationship between area and dry mass for bioassay leaves, we collected an additional similar-sized leaf at the same time, from the same tree and branch, as the bioassay leaf. The leaf was weighed fresh and its area measured.

It was then dried at 60 °C and reweighed. Thereby, we also obtained an estimate of leaf water content (% fresh mass). We favored using area instead of wet mass to calculate initial dry mass of experimental leaves because variation in weather among days (e.g., precipitation and humidity) made wet masses of leaves more prone to measurement error. Relative preferences for bioassay leaves were calculated as dry mass leaf consumed (initial mass–final mass) divided by the total dry mass consumed during the bioassay. Consumption during these bioassays was calculated as relative consumption rate (i.e. total leaf dry mass (mg) consumed per mg larva (initial mass) per 24 h).

Three different bioassays with FTC larvae were conducted. The first bioassay, investigating potential host shifts between species (species comparison), assessed effects of CO_2 and O_3 on the relative preference for aspen (genotype 216) vs. birch. The second bioassay, investigating potential shifts between genotypes (genotype comparison), assessed effects of CO_2 and O_3 on the relative preference for aspen genotypes 216 vs. 259. The third bioassay (fumigation comparison) was performed to investigate treatment effects on preferences within species/clones, and thereby, the causes behind potential preference shifts between species/clones observed in the first two assays. This bioassay examined preferences for control vs. $+CO_2$ or $+O_3$ foliage within each tree type.

For the species and genotype comparisons we performed ten assays (subsamples) with different trees per true replicate (each of three FACE rings) of each CO_2 and O_3 combination (four treatments). This resulted in 120 species comparison assays (aspen vs. birch), and 120 genotype comparison assays (aspen 216 vs. aspen 259). For the fumigation comparison six assays with different trees were performed for each contrast (control vs. $+CO_2$ and control vs. $+O_3$) and tree type, resulting in 108 within species/genotype assays (six assays × two fumigation treatment combinations × three replicates × three tree types). No tree was included more than once in any of the three bioassays.

Foliar chemistry

Leaves for foliar chemistry were collected in the same way and at the same time as those used in the bioassays. Samples of four to six leaves were taken from each of three different trees for each species/genotype, fumigation treatment and replicate (ring). Leaves were cut at the base of the petiole, put in plastic bags, transported to the lab on ice, and flash frozen in liquid nitrogen. After freeze-drying, the leaf material was ground in a Wiley mill (40-mesh screen; Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA, USA), and stored at −20 °C. Nutritional analyses included nitrogen and starch. Major secondary metabolites in aspen and birch are phenolic compounds (Palo, 1984; Lindroth *et al*., 1987), and we analyzed for levels of condensed tannins in both species, as well as, for the phenolic glycosides salicortin and tremulacin in aspen.

We determined nitrogen concentrations by high-temperature combustion, followed by thermoconductometric detection (LECO FP528 nitrogen analyzer, St Joseph, MI, USA), with glycine *P*-toluenesulfonate used as the reference standard (5.667% N, Hach Co., Loveland, CO, USA). To determine starch concentrations, we first separated starch from soluble sugars and enzymatically hydrolysed starch to glucose using the method of Prado *et al*. (1998). We then quantified glucose concentrations using a modification of the dinitrosalicylic acid method as described by Lindroth *et al*. (2002). Concentrations of condensed tannins were analyzed using a modification of the butanol-HCl method of Porter *et al*. (1986). We used condensed tannins purified from birch and aspen via adsorption chromatography as the reference standard for the respective tree species. Finally, we determined levels of salicortin and tremulacin, the most abundant phenolic glycosides in aspen (Lindroth *et al*., 1987), using high performance thin layer chromatography (HPTLC), with purified salicortin and tremulacin as standards.

Statistical analyses

The overall experimental set-up is a randomized complete block design, with three replications of each atmospheric treatment combination. Atmospheric treatments are CO_2 and O_3 exposure, with the factor levels being ambient and a single elevated concentration of each, making these treatments fixed effects within analysis of variance (ANOVA). Block effects and block × treatment effects are considered random within this design. Therefore, a mixed model analysis was required. We used the PROC Mixed component of SAS software (SAS Institute, 1988–2000), a linear mixed effect (LME) model procedure that uses likelihood functions to generate ANOVA statistics. For analysis of main effects of CO_2 and O_3, the block × treatment error terms can be pooled or partitioned, depending on patterns of variation. To determine whether error partitioning was required (i.e., significant block × CO_2 or block × O_3 effects), likelihood ratio statistics were computed from differences in −2 restricted maximum likelihood indices for models with pooled and partitioned error terms (Littell *et al*., 1996). Pooled error terms were appropriate

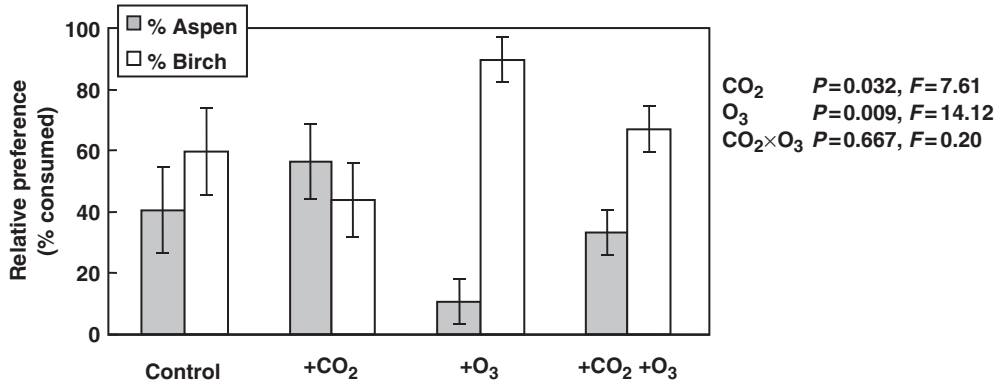

Fig. 1 Feeding preferences (% of total dry mass consumption) of forest tent caterpillars provided a choice between quaking aspen (genotype 216) and paper birch grown under ambient or elevated levels of CO_2 and O_3. Vertical lines represent $\pm$ 1 SE. *P* values from two-way ANOVA with CO_2 and O_3 level as independent variables. The ANOVA model used one value per bioassay (% aspen 216 consumed), but to aid visualization data are presented graphically showing relative preference for both leaf categories. Appropriate denominator degrees of freedom for *F*-tests were determined by Satterthwaite's approximation (df = 1, 6 for CO_2, O_3 and $CO_2 \times O_3$).

for all analyses (i.e. there were no significant block × treatment effects). Within PROC Mixed, appropriate denominator degrees of freedom for F-tests were determined by Satterthwaite's approximation.

For both the species comparison and genotype comparisons, assays represent subsamples within the true replicates (rings). Although bioassays gave two values (% leaf tissue consumed for two choices) per assay, only one value (% consumption for one of the paired leaves) was used in the ANOVA analysis. Analyses of effects of host species availability on consumption (i.e. differences in consumption between aspen–birch (species comparison) assays and aspen–aspen (genotype comparison) assays) required addition of a split-plot factor to the mixed-model described above. Atmospheric treatments then represent the whole-plot level, and the species availability effect and its interactions are evaluated as sub-plot factors.

Data from the fumigation comparison assays (i.e. control (ambient) leaves vs. + CO_2 or + O_3 leaves) were analyzed separately with paired *t*-tests. Data calculated as proportions (%) were arcsine-square root transformed prior to statistical analyses to correct for heterogeneity of variances. Throughout the statistical analyses *P*-values <0.05 are considered as significant, whereas *P*-values between 0.05 and 0.10 are denoted marginally significant.

Results

Bioassays

Species comparison. Under ambient CO_2 and O_3 conditions FTC larvae consumed slightly less aspen 216 than birch (42% vs. 58%, SE = 14%). Host plant preferences were significantly altered, however, by both elevated CO_2 and O_3 (Fig. 1). Overall, elevated CO_2 levels increased larval preference for aspen relative to birch, whereas elevated O_3 levels had the opposite effect. Indeed, the most pronounced difference compared with control (ambient) conditions was observed for caterpillars fed foliage from the + O_3 treatment, where birch was consumed almost exclusively (88% of the total dry mass consumed, SE = 8%, Fig. 1). The effects of CO_2 and O_3 on larval feeding preferences were independent of one another (no significant interaction).

Genotype comparison. The CO_2 and O_3 treatments also influenced larval preferences for the two aspen genotypes, 216 and 259 (Fig. 2). Under ambient CO_2 and O_3 conditions larvae showed a small, but nonsignificant preference for aspen 216 over 259 (57% vs. 43%, SE = 5%), whereas under elevated levels of either CO_2 or O_3 the preference for aspen 216 increased. Effects of elevated O_3 levels were somewhat stronger than were CO_2 effects, which were only marginally significant. No interactive effects of CO_2 and O_3 on larval preferences were detected.

Consumption. Larval consumption differed between the species comparison assays (when larvae had access to both birch and aspen) and the genotype comparison assays (when larvae had access to only aspen). With both birch and aspen available consumption varied between 0.254 and 0.300 mg (mg larvae^{-1}) day^{-1}, and was unaffected by experimental treatment (Fig. 3). In assays with only aspen available, however, larvae increased consumption if the foliage was from elevated O_3 treatments: consumption was on average 34% higher in elevated than in ambient O_3 treatments

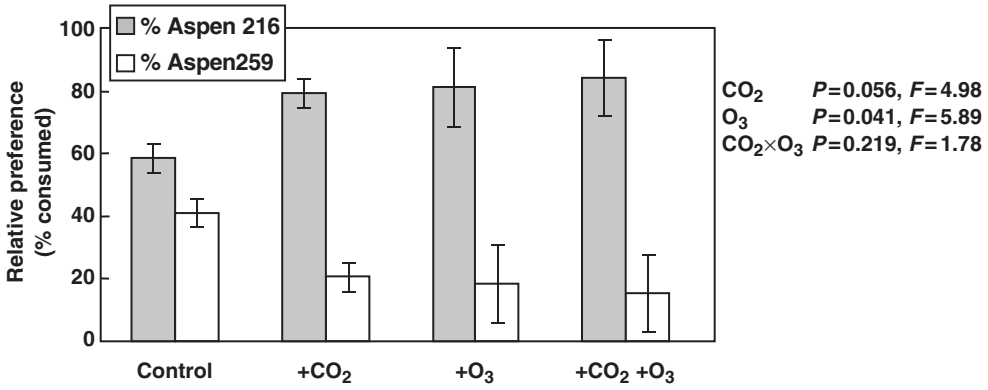

Fig. 2 Feeding preferences (% of total dry mass consumption) of forest tent caterpillars provided a choice between two quaking aspen genotypes (216 and 259) grown under ambient or elevated levels of CO_2 and O_3. Vertical lines represent $\pm$ 1 SE. *P*-values from two-way ANOVA with CO_2 and O_3 level as independent variables. The ANOVA model used one value per bioassay (% aspen 216 consumed), but to aid visualization data are presented graphically showing relative preference for both leaf categories. Appropriate denominator degrees of freedom for F-tests were determined by Satterthwaite's approximation (df = 1, 8 for CO_2, O_3 and $CO_2 \times O_3$).

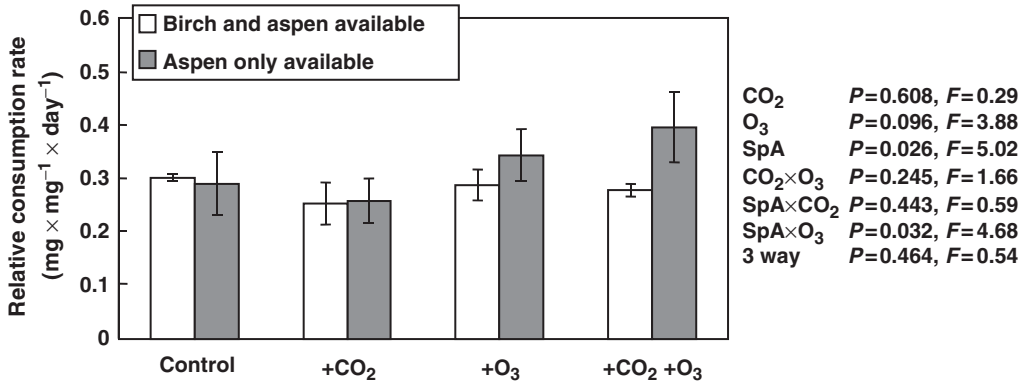

Fig. 3 Combined consumption rate (mg leaf drymass $\times$ mg larva drymass$^{-1} \times$ day^{-1}) of forest tent caterpillars in two-choice feeding assays with either paper birch and quaking aspen genotype 216 (two species available), or two quaking aspen genotypes (216 and 259, one species available), grown under ambient or elevated levels of CO_2 and O_3. Consumption rate is calculated from total dry mass consumed of the two leaves available. Vertical lines represent $\pm$ 1 SE. *P*-values from split-plot analysis with CO_2 and O_3 level as independent variables, and species availability (SpA) as sub-plot factor. Appropriate denominator degrees of freedom for F-tests were determined by Satterthwaite's approximation (df = 1, 6.01 for CO_2 and $CO_2 \times O_3$; df = 1, 6.02 for O_3; df = 1, 211 for SpA, SpA $\times$ CO_2, SpA $\times$ O_3, and 3 way interaction).

(0.367 and 0.274 mg (mg larvae^{-1}) day^{-1}, respectively). The level of CO_2 did not alter consumption in the aspen–aspen assays. The significant interactive effect of plant species availability and O_3 level ($P = 0.032$) reflects the effect of O_3 in the aspen–aspen, but not in the aspen–birch, bioassays (Fig. 3).

Fumigation comparison. The within species/genotype assays, with control vs. + CO_2 or + O_3 leaves, revealed that larvae did not distinguish between birch leaves from different experimental treatments (i.e., larvae consumed equal amounts of birch leaves from control and fumigated treatments, Fig. 4). Corresponding data for aspen 216 showed that elevated CO_2 did not affect feeding preferences, but that larvae tended to avoid foliage from elevated O_3 treatments compared with control foliage (29% vs. 71%, SE = 7%, of total dry mass consumed, respectively). The overall strongest fumigation effects were observed with aspen 259, for which control foliage was preferred over elevated O_3 foliage, and tended to be preferred over elevated CO_2 foliage (Fig. 4).

Foliar chemistry

The effects of CO_2 and O_3 on concentrations of nutrients and carbon based secondary compounds in birch and the two aspen genotypes were variable (Table 1). For

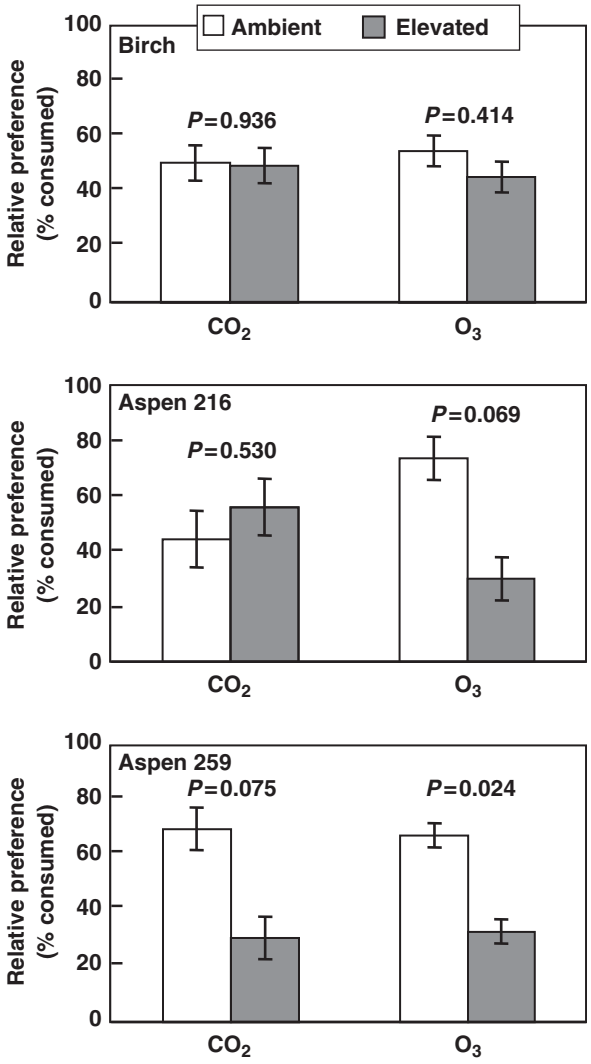

Fig. 4 Feeding preferences (% of total dry mass consumption) of forest tent caterpillars provided a choice between foliage from trees grown under ambient (control) and elevated levels of CO_2 or O_3. Data presented for paper birch and two genotypes of quaking aspen (216 and 259). Vertical lines represent $\pm$ 1 SE. *P*-values from paired *t*-tests for control vs. + CO_2 and control vs. + O_3 treatments, respectively. *t*-values from paired *t*-tests, Birch: $t_2 = 0.09$ and 1.02; Aspen 216: $t_2 = 0.75$ and 3.61; Aspen 259: $t_2 = 3.45$ and 6.30, for CO_2 and O_3, respectively.

birch, elevated levels of O_3 slightly decreased water content, whereas CO_2 availability had no detectable influence. Nitrogen was affected only in the + CO_2 + O_3 treatment, where levels decreased 22% compared with the other treatments, demonstrating a significant interaction effect. Interactive effects of CO_2 and O_3 on starch and condensed tannin levels were marginally significant: in low CO_2 treatments exposure to O_3 had little effect, whereas, in high CO_2 treatments exposure to O_3 increased levels of starch and condensed tannins by 63% and 44%, respectively (Table 1).

For aspen genotype 216, elevated CO_2 levels caused a marginally significant decrease in foliar water content (Table 1). However, both nitrogen and starch contents were unaffected by the experimental treatments. Condensed tannin concentrations increased on average 18% under elevated levels of O_3. Regarding phenolic glycosides, levels of salicortin did not differ between treatments, whereas concentrations of tremulacin were influenced by the interactive effects of CO_2 and O_3. High O_3 levels decreased tremulacin levels 34% in elevated CO_2 treatments, but this effect was not detected in ambient CO_2 treatments (Table 1).

Aspen genotype 259 showed no response to experimental treatments for water and nitrogen concentrations. Starch content increased significantly in both elevated CO_2 and O_3, by 26% and 51%, respectively. Condensed tannin levels also tended to increase in high CO_2 treatments. Concentrations of salicortin showed substantial variation between treatments, but differences were not statistically significant because of large error terms. Similar to genotype 216, tremulacin concentrations varied with CO_2 and O_3 levels. Elevated O_3 decreased tremulacin content, particularly under enriched CO_2 conditions ($CO_2 \times O_3$ interaction; Table 1).

Discussion

Larval host plant preferences

This study demonstrates that changes in the atmospheric environment, through effects on plants, can significantly alter host preference behavior of an important herbivore. Previous studies, investigating the influence of elevated CO_2 on host plant preferences, primarily reported negligible effects (e.g.,Arnone *et al*., 1995; Traw *et al*., 1996, Lederberger *et al*., 1997, 1998; Díaz *et al*., 1998). To date, only two studies have demonstrated CO_2 induced changes in herbivore preferences among host plants. Peters *et al*. (2000) found that a generalist herbivore slug (*Deroceras reticulatum*) tended to increase preference for legumes over nonlegumes in response to CO_2 enrichment. Similarly, Goverde and Erhardt (2003) showed that *Coenonympha pamphilus* larvae altered their preference for different grass species when these had been grown in elevated CO_2. Interestingly, in the present study we were able to establish altered host plant preferences not only *between* species (and genus), but also *within* a species (Fig. 2). Increased knowledge about genotypic variation will improve our understanding of how atmospheric changes affect plants (Peñuelas & Estiarte, 1998; Saxe *et al*., 1998), and this study demonstrates that

Table 1 Foliar chemistry of paper birch and two genotypes of quaking aspen (216 and 259) under control, elevated CO_2 ($+CO_2$), elevated O_3 ($+O_3$), and elevated CO_2 and O_3 ($+CO_2+O_3$) fumigation treatments

Species/ genotype	Chemical constituent	Fumigation treatment				df	*P*-values (*F*-values)		
		Control	$+CO_2$	$+O_3$	$+CO_2+O_3$		CO_2	O_3	$CO_2 \times O_3$
Birch	Water	68.2 ± 1.0	68.6 ± 1.8	66.7 ± 1.1	65.7 ± 0.1	1, 8	0.998 (0.00)	0.033 (6.58)	0.689 (0.17)
	Nitrogen	2.6 ± 0.2	2.8 ± 0.2	2.8 ± 0.1	2.1 ± 0.1	1, 8	0.085 (3.86)	0.088 (3.77)	0.022 (8.04)
	Starch	11.3 ± 0.6	9.6 ± 2.3	11.1 ± 1.5	15.6 ± 1.6	1, 8	0.358 (0.95)	0.064 (4.60)	0.051 (5.27)
	Condensed tannins	6.8 ± 0.6	5.6 ± 0.3	6.8 ± 0.4	8.0 ± 0.8	1, 30	0.971 (0.00)	0.041 (4.55)	0.059 (3.86)
Aspen 216	Water	71.9 ± 1.5	70.7 ± 1.1	72.3 ± 0.4	69.9 ± 0.5	1, 8	0.078 (4.07)	0.890 (0.02)	0.584 (0.33)
	Nitrogen	2.6 ± 0.3	2.5 ± 0.2	2.7 ± 0.2	2.4 ± 0.1	1, 6	0.128 (3.11)	0.676 (0.19)	0.460 (0.62)
	Starch	3.6 ± 0.9	3.8 ± 1.3	4.0 ± 0.9	4.5 ± 0.3	1, 6	0.280 (1.41)	0.136 (2.96)	0.669 (0.20)
	Condensed tannins	12.4 ± 1.1	12.6 ± 0.3	14.0 ± 0.6	15.5 ± 1.3	1, 30	0.417 (0.68)	0.034 (4.93)	0.563 (0.34)
	Salicortin	4.4 ± 0.4	4.4 ± 0.3	4.7 ± 2.2	3.7 ± 0.6	1, 6	0.633 (0.25)	0.820 (0.06)	0.627 (0.26)
	Tremulacin	2.2 ± 0.3	2.7 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	1, 30	0.513 (0.44)	0.011 (7.24)	0.022 (5.81)
Aspen 259	Water	74.8 ± 0.5	73.1 ± 1.4	75.0 ± 0.4	74.0 ± 1.0	1, 8.4	0.105 (3.31)	0.597 (0.30)	0.844 (0.04)
	Nitrogen	3.5 ± 0.1	3.3 ± 0.1	3.2 ± 0.2	3.1 ± 0.1	1, 6	0.166 (2.48)	0.132 (3.04)	0.489 (0.54)
	Starch	2.2 ± 0.5	2.8 ± 0.5	3.3 ± 1.6	4.1 ± 0.5	1, 30	0.008 (8.18)	0.001 (24.4)	0.554 (0.36)
	Condensed tannins	7.0 ± 3.0	10.9 ± 1.3	10.1 ± 0.6	11.0 ± 1.7	1, 7.8	0.059 (4.88)	0.180 (2.17)	0.210 (1.87)
	Salicortin	3.5 ± 0.5	5.9 ± 1.7	5.0 ± 0.2	3.9 ± 2.6	1, 8	0.622 (0.26)	0.822 (0.05)	0.243 (1.59)
	Tremulacin	2.2 ± 0.4	2.8 ± 0.1	2.2 ± 0.3	2.0 ± 0.2	1, 32	0.121 (2.54)	0.024 (5.61)	0.035 (4.83)

Data are presented as % dry mass (mean + 1 SE), except for water, which is presented as % fresh mass. *P*- and *F*-values from two-way ANOVA with CO_2 and O_3 level as independent variables. Appropriate denominator degrees of freedom (df) for *F*-tests were determined by Satterthwaite's approximation.

such variation can also significantly affect associated herbivores.

Overall, elevated O_3 levels altered FTC host plant preferences more than did elevated CO_2 levels. Larvae avoided O_3-exposed foliage especially in aspen. These results contrast with those of some previous studies, where plants exposed to high levels of O_3 were preferred over control plants (Jeffords & Endress, 1984; Jones & Coleman, 1988; Lin *et al*., 1990; Endress *et al*., 1991, Fortin *et al*., 1997). However, our results show some agreement with Bolsinger *et al*. (1992), who reported decreased preference of monarch butterfly larvae (*Danaus plexippus*) for the common milkweed (*Asclepias syriaca*) exposed to elevated O_3, although they observed the opposite pattern for bloodflower (*Asclepius curassavica*). Our data, together with that of previous studies, thus demonstrate that the effects of O_3 may vary among different plant-insect systems, thus contributing to differential changes in relative host plant preferences of insects.

Causes of altered host preferences

What caused FTC larvae to change their host plant preferences in response to altered levels of CO_2 and O_3? Reduced preference for aspen relative to birch in elevated O_3 seems to have resulted from how FTC larvae in these treatments altered their response to aspen, but not to birch. The fumigation comparison revealed that larvae did not distinguish between birch from different fumigation treatments, whereas they clearly avoided aspen from the $+O_3$ treatment (Fig. 4). The reason why elevated CO_2 levels increased preference for aspen over birch remains unclear, because larvae did not significantly increase preference for $+CO_2$ aspen (Fig. 4). The reduced preference for aspen 259 compared with aspen 216 in high CO_2 treatments is explained by the palatability of aspen 259, which was affected by CO_2 fumigation (Fig. 4). Elevated O_3 levels, on the other hand, caused larvae to avoid aspen 259 and prefer 216, which was surprising considering that for both aspen genotypes larvae fed more on control compared with $+O_3$ leaves. However, it is possible that negative O_3 effects on palatability were somewhat stronger for aspen 259, as suggested by the statistical analyses (Fig. 4). Overall, the pattern that palatability of aspen was more affected than that of birch, and that aspen 259 was affected more than aspen 216, is consistent with expectations from previous studies. Aspen often show more pronounced growth and phytochemical responses to manipulations of the atmospheric environment than does birch (Roth & Lindroth 1995; McDonald *et al*., 1999; Agrell *et al*., 1999, 2000; but see Lindroth *et al* 2001). Correspondingly, of the two aspen genotypes, aspen 259 is more sensitive to O_3 exposure than is aspen 216 (Karnosky *et al*., 1999).

Levels of important nutrients and secondary compounds were examined in an effort to identify the mechanisms underlying shifts in FTC host plant preferences. CO_2 effects were smaller than expected from previous studies on birch and aspen, although in general agreement with those studies (e.g., Lindroth *et al*., 1993a; Roth & Lindroth, 1995; Agrell *et al*., 2000; Lindroth *et al*., 2001; Kopper *et al*., 2001; Kopper & Lindroth 2003a, b). However, as could be expected from the bioassays, tree responses were generally stronger to elevated O_3 than to elevated CO_2 levels. This included also levels of phenolic compounds, which commonly increase in high O_3 (e.g., Bolsinger *et al*.,1991; Jordan *et al*., 1991; Pääkkönen *et al*., 1998; Saleem *et al*., 2001), although we found the phenolic constituents to be differently affected by O_3 exposure (cf. Jordan *et al*., 1991; Saleem *et al*., 2001; Kopper & Lindroth, 2003a). We also found some interactive effects of O_3 and CO_2 on phytochemistry, but since O_3 effects were in general strongest in elevated CO_2, our data seem to contradict the idea of an ameliorating effect of CO_2 on O_3 exposed plants (e.g., Allen 1990; Dickson *et al*., 1998; Grams *et al*., 1999; Karnosky *et al*., 1999).

We could thus establish that fumigation treatments caused changes in the phytochemical composition of trees, but how well did these data explain observed changes in host tree preferences of FTC larvae? The only chemical component showing a somewhat consistent covariation with larval preferences was condensed tannins. The tree becoming relatively less preferred as a result of CO_2 or O_3 treatment was in general also the one for which average levels of condensed tannins were most positively (or least negatively) affected by that treatment. However, changes in our phytochemical measures could not always be confirmed statistically and could only partially explain the observed effects on FTC host plant preferences. Thus, treatment effects on larval feeding behavior were possibly also affected by foliar characteristics not examined, e.g. sugar levels (Wu *et al*., 1990) or structural changes that may have occurred in the leaves (Karnosky *et al*., 1999; Percy *et al*., 2002). The exact causes of altered host plant preferences in response to elevated levels of CO_2 and/or O_3 require further investigation.

Larval feeding behavior and ecosystem effects

Insects commonly show increased (compensatory) consumption when feeding on foliage that has been exposed to high levels of either CO_2 (e.g., Fajer *et al*., 1989; Roth & Lindroth, 1995; Lindroth 1996; Kinney *et al*., 1997; Agrell, 2000) or O_3 (e.g., Jones & Coleman, 1988; Endress *et al*., 1991; Bolsinger *et al*., 1992). Although our data on consumption during the bioassays suffer from the fact that they were collected over a short term, they nonetheless allow us to draw some interesting conclusions. Overall, consumption varied depending on the combination of foliage available during the bioassays. When birch and aspen were available (species comparison assays), consumption was unaffected by the experimental treatments. In contrast, larvae in the genotype comparison assays exhibited increased consumption in the high O_3 treatment (Fig. 3). This result is in agreement with observed changes in feeding preferences. Larvae in birch–aspen assays avoided aspen and fed primarily on birch in the high O_3 treatment (Fig. 1), and because palatability of birch was little affected by the experimental treatments (Fig. 4), the larvae did not need to increase consumption to compensate for reductions in foliage quality. For larvae in aspen–aspen assays, however, both leaf types available were less attractive if coming from high O_3 treatments. Therefore, the increased consumption observed for larvae tested on high O_3 aspen foliage was presumably because of reduced palatability of all available leaves, and no access to an alternative (unaffected) food source.

We caution that relative shifts in larval food preferences are not necessarily indicative of ultimate insect performance (growth, survival and reproduction) on any particular host plant. As a case in point, results from this study suggest that ozonated aspen foliage is less preferred than control foliage. Yet, Kopper & Lindroth, (2003a) found that FTC restricted to feed on aspen in the elevated O_3 treatment consumed similar amounts of food to, and grew larger than, FTC in the control treatment. Future research assessing the impacts of CO_2 or O_3 on herbivory should account for shifts in both feeding preference and performance of insect individuals, as well as changes in the dynamics of insect populations.

Our finding that changes in consumption depend on the plant species available relates directly to the potential problems associated with obtaining relevant consumption estimates (e.g., Körner, 1996; Peters *et al*., 2000). Peters *et al*. (2000) showed that elevated CO_2 levels did not increase consumption if the herbivore had several food plants available, and proposed caution regarding the use of estimates from single species feeding assays when making predictions about changes in consumption in future plant-herbivore systems. Our data support that view. Although assays with only aspen demonstrated increased consumption with increasing O_3 levels, no such effect could be detected when foliage from an alternative host tree (birch) was available. Environmentally induced changes in food quality may thus cause shifts to relatively more

palatable host plants, thereby offsetting trends toward increased consumption.

Increasing levels of atmospheric pollutants may result in ecosystem changes through interactive effects of shifted competitive balance among plant species and altered impact of important herbivorous insect species (e.g., Lindroth *et al*., 1993a; Saxe *et al*., 1998). Of course, data from laboratory conditions cannot be directly translated to the ecosystem level, since many other environmental variables are excluded and the specific experimental conditions may to some extent influence the results (see Goverde & Erhardt, 2003). Nevertheless, detailed analyses of herbivore responses (e.g., shifts in host utilization) in an altered environment will advance our understanding of processes underlying ecosystem change. Our data suggest that elevated levels of O_3 will shift preferences away from aspen and towards birch. However, because high CO_2 levels caused the opposite effect (i.e. increased preference for aspen compared with birch) parallel increases of these two environmental pollutants may counteract each other, resulting in a relatively minor impact on FTC feeding preferences (Fig. 1). This combined influence of O_3 and CO_2 represents the only example in our study where the effects of O_3 were ameliorated by elevated levels of CO_2 (cf. Allen, 1990, Dickson *et al*., 1998, Grams *et al*., 1999, Karnosky *et al*., 1999). In summary, our results show that generalist insect herbivores can detect and respond to environmentally induced changes in their food plants by increased exploitation of alternative hosts. Such host shifts have the potential to influence population changes normally associated with altered host quality, but this buffering effect will, of course, depend on the quality of the alternative host plants (cf. Arnone *et al*., 1995; Peters *et al*., 2000).

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