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PLANT ANIMAL INTERACTIONS

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Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype

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Abstract This study examined the effects of carbon dioxide (CO_2)-, ozone (O_3)-, and genotype-mediated changes in quaking aspen (*Populus tremuloides*) chemistry on performance of the forest tent caterpillar (*Malacosoma disstria*) and its dipteran parasitoid (*Compsilura concinnata*) at the Aspen Free-Air CO_2 Enrichment (FACE) site. Parasitized and non-parasitized forest tent caterpillars were reared on two aspen genotypes under elevated levels of CO_2 and O_3, alone and in combination. Foliage was collected for determination of the chemical composition of leaves fed upon by forest tent caterpillars during the period of endoparasitoid larval development. Elevated CO_2 decreased nitrogen levels but had no effect on concentrations of carbon-based compounds. In contrast, elevated O_3 decreased nitrogen and phenolic glycoside levels, but increased concentrations of starch and condensed tannins. Foliar chemistry also differed between aspen genotypes. CO_2, O_3, genotype, and their interactions altered forest tent caterpillar performance, and differentially so between sexes. In general, enriched CO_2 had little effect on forest tent caterpillar performance under ambient O_3, but reduced performance (for insects on one aspen genotype) under elevated O_3. Conversely, elevated O_3 improved forest tent caterpillar performance under ambient, but not elevated, CO_2. Parasitoid larval survivorship decreased under elevated O_3, depending upon levels of CO_2 and aspen genotype. Additionally, larval performance and masses of mature female parasitoids differed between aspen genotypes. These results suggest that host-parasitoid interactions in forest systems may be altered by atmospheric conditions anticipated for the future, and that the degree of change may be influenced by plant genotype.

Keywords Free-air CO_2 enrichment · Forest tent caterpillar · *Malacosoma disstria* · Quaking aspen · Tritrophic interactions

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Introduction

Elevated levels of atmospheric CO_2 and O_3 significantly affect tree physiology, growth, and phytochemistry, as well as interactions between trees and insects (Riemer and Whittaker 1989; Bezemer and Jones 1998; Ceulemans et al. 1999; Coviella and Trumble 1999). Yet relatively little is known about the effects of these pollutants beyond the second trophic level. Moreover, these gaseous pollutants may interact to modify the impacts of either pollutant alone (Ceulemans et al. 1999; Fowler et al. 1999) and the consequences of these interactions are poorly understood for all trophic levels.

Foliage of trees grown under elevated CO_2 commonly exhibits increased levels of starch and carbon-based secondary compounds (especially phenolics), coupled with decreased levels of nitrogen (Lindroth 1996a; Koricheva et al. 1998). These changes, in turn, can alter the fitness of herbivorous insects. Leaf-chewing insects typically respond to CO_2-induced changes in foliar quality through increased consumption, slower development, and reduced pupal mass (Lindroth 1996a; Bezemer and Jones 1998; Coviella and Trumble 1999). The responses of both trees and leaf-chewing insects vary, however, among species (Lindroth et al. 1993a; Bezemer and Jones 1998; Williams et al. 2000), as well as among plant genotypes (Goverde et al. 1999; Mansfield et al. 1999; Lindroth et al. 2001a). Such variation could have considerable implications for future community structure

and dynamics. Finally, few studies have assessed how CO_2-mediated changes in plant quality influence the performance of natural enemies of leaf-chewing insects. Early studies of such effects on individual organisms found the influence of elevated CO_2 to be weak or non-existent on the third trophic level, despite significant changes in herbivore performance (Roth and Lindroth 1995; Lindroth et al. 1997).

Relative to CO_2, even less is known about the effects of elevated O_3 on tree chemistry or trophic interactions. General responses of trees to O_3 exposure include increased levels of foliar carbon-based compounds (e.g., phenolics) and decreased nitrogen concentrations (Riemer and Whittaker 1989; Koricheva et al. 1998). For herbivorous insects, responses to O_3-mediated changes in tree foliar composition have been highly variable across the limited number of studies conducted. Performance of leaf-chewers increased, decreased, or did not change (Coleman and Jones 1988; Kopper and Lindroth 2003a, 2003b), and responses were species-specific (Cannon 1993; Lindroth et al. 1993b). To date, no published work has assessed the potential effects of O_3-mediated changes in foliar quality on interactions between insect herbivores and their natural enemies.

Elevated CO_2 and O_3 have the potential to alter parasitoid fitness through numerous means. First, the pollutants could directly affect adult parasitoids by altering searching efficiencies. Under elevated O_3, searching efficiency of a hymenopteran parasitoid declined, as did the proportion of hosts parasitized (Gate et al. 1995). Second, prolonged development time of, and increased consumption by, leaf-chewing hosts may increase exposure of hosts to natural enemies (e.g., Parry et al. 1998). In fact, Stiling et al. (1999) found that leafminers consumed more foliage and suffered increased parasitoid attack under elevated CO_2. Third, both pollutants tend to increase phenolics and decrease nitrogen concentrations in foliage. These changes in host diet could reduce parasitoid survival, development, size, sex ratio, fecundity, or success of parasitism (Barbosa et al. 1982; Vinson and Barbosa 1987). The effects of host diet may be direct, via exposure to allelochemicals (Greenblatt et al. 1982; Karowe and Schoonhoven 1992; English-Loeb et al. 1993), or indirect, by degradation of host quality (Greenblatt and Barbosa 1981; Warren et al. 1992; Roth et al. 1997). Fourth, reduced quality of the host's diet could improve the fitness of parasitoids by weakening the host's physiological defenses (e.g., encapsulation) against parasitoids (Turlings and Benrey 1998). In conclusion, the net effect of CO_2 or O_3 on parasitoid performance could be positive, negative, or nil. The magnitude, direction, and underlying mechanism of change will likely depend on the biology of the host-parasitoid complex considered.

The purpose of this study was to evaluate the effects of CO_2-, O_3-, and genotype-mediated changes in tree chemistry on insect-parasitoid interactions. Specific objectives were to examine the effects of such changes in the foliar quality of quaking aspen (*Populus tremuloides*) on performance of the forest tent caterpillar (*Malacosoma disstria*) and its dipteran endoparasitoid, *Compsilura concinnata*. We tested the following predictions:

1. Nitrogen will decrease and starch and carbon-based based secondary metabolites will increase in CO_2- and O_3-enriched foliage.
2. The magnitude of change in foliar chemistry will be greater under elevated CO_2+O_3 than under elevated CO_2 or O_3 alone.
3. *C. concinnata* will exhibit prolonged larval development times and reductions in growth and survival when reared in forest tent caterpillars feeding on CO_2- or O_3-enriched foliage.
4. The magnitude of change in the fitness of *C. concinnata* will be greater under elevated CO_2+O_3 than under elevated CO_2 or O_3 alone.

Materials and methods

Experimental system

Quaking aspen is a predominant forest type in the Great Lakes region and is a preferred host of forest tent caterpillars (Hodson 1941). The major foliar secondary metabolites of aspen are phenolic compounds, including phenolic glycosides and condensed tannins (Palo 1984). Levels of these phytochemicals can vary greatly among aspen genotypes and, in turn, affect herbivore performance (Lindroth and Hwang 1996). High levels of phenolic glycosides are toxic to forest tent caterpillars, reducing growth and prolonging development times (Lindroth and Bloomer 1991; Hemming and Lindroth 1995; Hwang and Lindroth 1997).

The forest tent caterpillar is a polyphagous herbivore found throughout most of the United States (Stehr and Cook 1968). It is a cyclical outbreak species known to cause severe defoliation to quaking aspen during outbreaks in the north-central United States. *Compsilura concinnata* (Tachinidae) is an introduced, generalist endoparasitoid that attacks over 140 species of Lepidoptera, including forest tent caterpillars (Arnaud 1978). *C. concinnata* most commonly attacks third through fifth instar larvae. The female deposits a larva into the host, where it develops between the peritrophic membrane and midgut wall. With its anal hooks, the larva attaches to the peritrophic membrane in a position allowing for alignment of its spiracles with the host trachea (Bourchier 1991). In this location, *C. concinnata* is likely to avoid cellular defense reactions of its host, but simultaneously increases its potential for exposure to toxins in the host's diet. Earlier studies showed dietary tannins to have negative (Bourchier 1991) or nondetectable (Mallampalli et al. 1996) effects on fitness of *C. concinnata*. As a fully developed third instar, the maggot burrows through the integument, emerges from the deceased host, and pupates.

Experimental design and set-up

This research was conducted during the summer of 2000 at the Aspen Free Air CO_2 Enrichment (FACE) site in northern Wisconsin, USA. The 32 ha site consists of twelve 30 m diameter rings in a 2×2 factorial design. Three rings are allotted for each treatment: ambient, elevated CO_2 (+CO_2), elevated O_3 (+O_3), and elevated CO_2 plus O_3 (+CO_2+O_3). Replicates are blocked from north to south across the site.

One section of each ring is planted with five quaking aspen (*Populus tremuloides*) genotypes differing in O_3 sensitivity. At the time of this experiment, aspen trees ranged from 4 to 5 m in height

and were in their third year of fumigation. We used two genotypes: genotype 216 is O_3-tolerant (little impact on growth) whereas genotype 259 is O_3-sensitive (substantial impact on growth) (Karnosky et al. 1996). Fumigation treatments were administered during daylight hours of the growing season. The targeted CO_2 gas concentration was 560 µl l^{-1}, about 200 µl l^{-1} above current average levels. Ozone concentrations were targeted to approximately 1.5× ambient. The experimental levels of CO_2 and O_3 were based on predicted concentrations for the northern Great Lakes region in 2050 (Dickson et al. 2000; Karnosky et al. 2003). Due to the photochemical nature of tropospheric O_3 formation, concentrations varied daily depending upon weather conditions. On average, target O_3 concentrations were 90–100 nl l^{-1} on sunny days, 50–60 nl l^{-1} on cloudy days, and no exposure was provided during periods of cold weather (<15°C), or when leaf surfaces were wet due to fog, dew, or rain. Ambient air was blown into control rings. Additional details of site description, design, and operation of the FACE facility are provided by Dickson et al. (2000).

Foliar chemistry

Midway through the larval development period of *C. concinnata*, we collected approximately 20 leaves from each experimental tree (three trees per genotype per FACE ring). Foliage was collected from a bagged branch on each tree to ensure that leaves collected for chemical analysis and those being fed upon by larvae were under similar environmental (shade) conditions. Leaves were removed by cleanly snipping at the petiole so as to not induce a chemical response from the tree (Mattson and Palmer 1988). The collected foliage was transported to the laboratory in plastic bags on ice, flash frozen in liquid nitrogen, freeze-dried, ground through a no. 40 mesh in a Wiley Mill, and stored at −20°C until chemical analysis.

Foliage samples (n=72) were analyzed for primary and secondary metabolites of quaking aspen likely to influence forest tent caterpillar performance (Arteel and Lindroth 1992). Foliar nitrogen was quantified by high-temperature combustion, followed by thermoconductometric detection (LECO FP 528 nitrogen analyzer). The nitrogen analyzer was calibrated against glycine *p*-toluenesulfonate (Hach, Loveland, Colo.) as a standard. Starch content was quantified by enzymatic conversion to glucose (Prado et al. 1998), followed by colorimetric detection of liberated glucose via a modified dinitrosalicyclic acid method (Lindroth et al. 2002a). Concentrations of phenolic glycosides (salicortin and tremulacin) were determined by high performance thin-layer chromatography (Lindroth et al. 1993a) with standards purified from aspen trees. Condensed tannins were extracted from leaf tissue in 70% acetone (with 10 mM ascorbic acid) and quantified colorimetrically, using the butanol-HCl method of Porter et al. (1986). As a standard, we used condensed tannins purified from aspen by adsorption chromatography (Hagerman and Butler 1980).

Bioassays

Forest tent caterpillar performance

The Canadian Forest Service (Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada) provided forest tent caterpillar egg masses. Egg masses were surface sterilized in a solution of 0.1% sodium hypochlorite with 1% Tween 80 (Sigma Chemical, St. Louis, Mo.) as a surfactant.

Forest tent caterpillars were reared from egg hatch until death or pupation in fine-mesh bags covering a single branch. We tied an egg mass to a single branch on each of the 72 trees and double-bagged it to reduce exposure of larvae to natural enemies, such as pentatomids. When larvae reached the second stadium, we reduced the number of caterpillars to approximately 100 per tree and divided them between two bags per tree. As larvae entered the fourth stadium, we removed one bag of caterpillars for parasitization (see below) and reduced the number in the remaining bag to 10 individuals. Larvae were moved to new branches as food was depleted within the bags (approximately every 2 days once larvae reached the third stadium). Upon pupation, pupae were removed and larval development time recorded. Pupae were weighed 4 days after pupation and sex was determined according to Muggli (1974).

C. concinnata performance

The Canadian Forest Service provided *C. concinnata* puparia. Puparia were reared to adults in a Percival environmental chamber at 25°C, 16L: 8D. Adults were fed berry tea with honey and misted daily with water.

For each tree (three per genotype, two genotypes per ring), one bag of early fourth-instar forest tent caterpillars was visually assessed, and ten caterpillars of intermediate size were removed. We weighed the ten larvae as a group (to obtain an average host mass) and then parasitized the larvae individually. Each caterpillar was introduced into a sleeve cage containing gravid *C. concinnata* females and remained exposed until a single act of larviposition was observed.

Larviposition was defined as the act of a fly landing on the caterpillar, and the posterior of the abdomen contacting the caterpillar. A droplet of hemolymph would often appear at the location of larviposition. Such an event was not necessary, however, to indicate successful parasitization. In previous laboratory trials we found *C. concinnata* larvae to emerge from caterpillars extruding a hemolymph droplet as well as from those that did not. In addition, we tested our criteria, based on female behavior, as an accurate indicator of successful parasitism and found 95% larval emergence from hosts. (Gypsy moth larvae, reared on artificial diet, were used in those trials.) Nonetheless, we recognize that measures of parasitoid larval mortality may be overestimated in this study due to instances in which females appeared to larviposit but in fact did not.

We recorded the date of larviposition and returned the ten parasitized caterpillars, in one bag, to the appropriate tree. We checked host larvae daily for mortality. Upon death, each caterpillar was removed from the mesh bag and placed into a 10 ml vial with a mesh top. The vials were kept on trees in a separate mesh bag to maintain existing environmental conditions under which the parasitoids were developing.

We removed parasitoid puparia from the vials as they emerged from the host larvae and recorded larval development times. If no parasitoid larva had emerged within 5 days of host pupation, we dissected the cocoon of the host to check for the presence of a parasitoid puparium. Parasitoid mortality was assumed if no puparium was found. Larval survivorship of the parasitoid was calculated on a per tree basis by dividing the number of larvae that emerged and successfully pupated by the number of hosts. In only one case did more than one parasitoid (two) emerge from a single host. These data were discarded given the potential confounding effects of intraspecific competition on parasitoid performance. Puparia were reared to adults in a Percival environmental chamber at 23°C, 16L: 8D. Flies were frozen upon emergence, sexed (according to Culver 1919), freeze-dried, and weighed.

Statistical analyses

This study utilized a three factor (CO_2, O_3, and genotype), split-plot design with blocking. The 12 rings were divided into three blocks. Whole plot treatments consisted of two, crossed factors (CO_2 and O_3). The subplot consisted of two aspen genotypes (216 and 259). Means for each subplot value were derived by averaging over three trees per subplot. We used analysis of variance (ANOVA; PROC MIXED; Littell et al. 1996) for statistical analysis. The blocked split-plot model was:

$$Y_{ijkl} = \mu + B_i + C_j + O_k + CO_{jk} + e_{ijk} + G_l + CG_{jl} + OG_{jl} + COG_{jkl} + \varepsilon_{ijkl} \quad (1)$$

where Y_{ijkl} represents the average response of block i, CO_2 level j, O_3 level k, and genotype l. Fixed effects were CO_2 level (C_j), O_3 level (O_k), genotype (G_l), and their interaction terms [(CO_{jk}), (CG_{jl}), (OG_{jl}), and (COG_{jkl})]. Random effects included block (B_i), whole plot error (e_{ijk}), and subplot error (ε_{ijkl}).

Forest tent caterpillar and *C. concinnata* performance data were analyzed separately by sex to account for sexual dimorphism, except for parasitoid larval survivorship, for which determination of sex was not possible. Survivorship data were represented as proportions and transformed by arcsine [square root (proportion)] so resulting data had an underlying distribution that was nearly normal with equal variance (Zar 1984). Again, data were aggregated to result in a single value per subplot. Host mass data were log-transformed to correct for unequal variance.

For all models, *F*-tests were conducted for fixed effects with degrees of freedom for error assigned using the Satterthwaite approximation (Littell et al. 1996). Means and standard errors were calculated using the LSMEANS (least-squares means) procedure and are reported for each $CO_2 \times O_3 \times$ genotype combination. Due to the low number of replicates for CO_2 and O_3 (n=3 FACE rings) and the related risk of type II statistical errors, we report *P* values in the range 0.05–0.10 as marginally significant (Filion et al. 2000).

We used multiple regressions (backward elimination) (PROC REG; SAS 1989) to relate herbivore performance to aspen phytochemicals, and parasitoid performance to both aspen phytochemicals and host mass. Mean values per tree were used for herbivore and parasitoid development time and pupal or adult mass. The criterion for a variable to remain in the model was α=0.10.

Results

Foliar chemistry

CO_2, O_3, aspen genotype, or their interactions significantly affected the chemical composition of aspen foliage (Fig. 1, Table 1). Elevated CO_2 and O_3 both slightly decreased foliar nitrogen concentrations, whereas in combination the pollutants reduced nitrogen concentrations by an average of 20% relative to controls (nearly significant $CO_2 \times O_3$ interaction). CO_2 had no effect on starch, but high levels of O_3 increased starch concentrations. Concentrations of salicortin tended to increase (although not significantly so), while those of tremulacin were not altered, under enriched CO_2. Enriched O_3 decreased concentrations of both phenolic glycosides. In combination, concentrations of salicortin and tremulacin in genotype 216 decreased an average of 25% under elevated levels of O_3, whereas concentrations in genotype 259 decreased by only 15% (marginally significant $O_3 \times$ genotype interaction). Levels of condensed tannins increased in response to elevated O_3, particularly so in the presence of elevated CO_2 (averaging 27% over control values for the two genotypes).

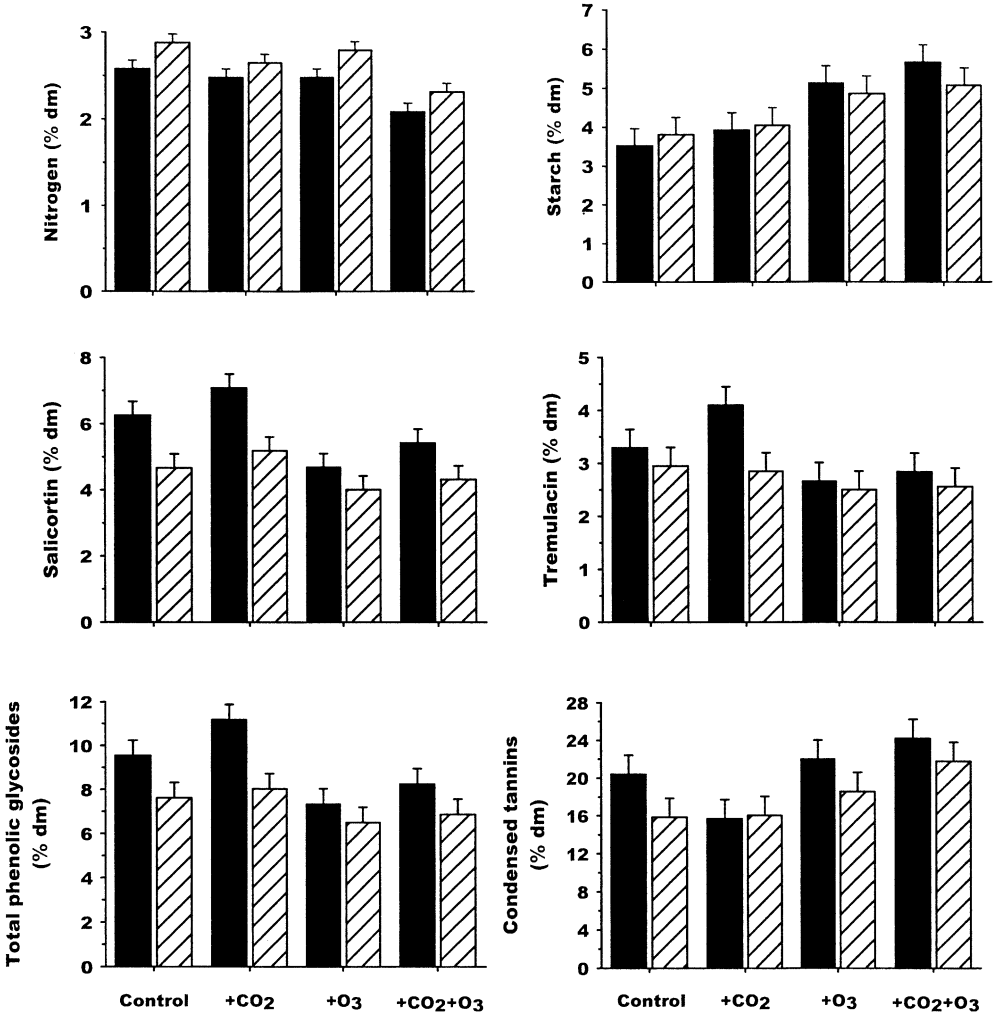

Fig. 1 Effects of CO_2 and O_3 fumigation on foliar chemistry in aspen genotype 216 (*solid bars*) and 259 (*hatched bars*). *Vertical lines* indicate +1 SE calculated from the pooled variance

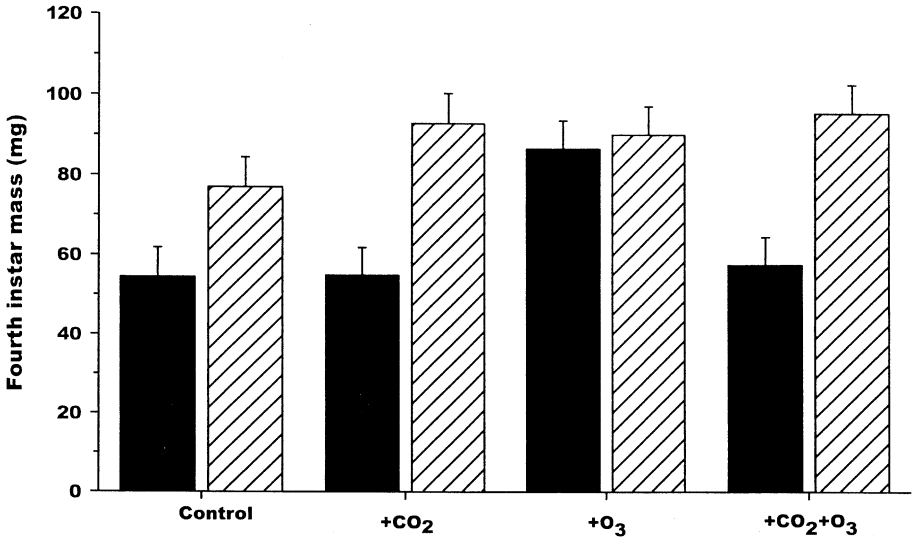

Fig. 2 Masses of fourth instar forest tent caterpillars, subsequently used as hosts for *Compsilura concinnata*, reared on aspen genotype 216 (*solid bars*) and 259 (*hatched bars*) under elevated CO_2 and O_3. Nontransformed data are presented. *Vertical lines* indicate +1 SE calculated from the pooled variance

Table 1 Summary of *P* values for the effects of CO_2, O_3, and genotype on chemical composition of aspen leaves

Main effects and interactions	Nitrogen	Starch	Salicortin	Tremulacin	Total phenolic glycosides[a]	Condensed tannins
CO_2	<0.001	0.276	0.109	0.347	0.157	0.849
O_3	0.005	0.004	0.007	0.030	0.011	0.007
$CO_2 \times O_3$	0.070	0.934	0.822	0.635	0.721	0.077
Genotype	0.002	0.709	0.001	0.017	<0.001	0.008
$CO_2 \times$ genotype	0.455	0.675	0.493	0.220	0.286	0.115
$O_3 \times$ genotype	0.797	0.280	0.138	0.170	0.087	0.654
$CO_2 \times O_3 \times$ genotype	0.870	0.883	0.914	0.341	0.680	0.293

[a] Total phenolic glycosides = salicortin + tremulacin

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

Main effects and interactions	Fourth instar mass	Development time		Pupal mass	
		Female	Male	Female	Male
CO_2	0.513	0.163	0.002	0.347	0.177
O_3	0.007	0.118	0.656	0.287	0.125
$CO_2 \times O_3$	0.024	0.071	0.109	0.202	0.288
Genotype	<0.001	<0.001	<0.001	0.002	<0.001
$CO_2 \times$ genotype	0.017	0.013	<0.001	0.220	0.777
$O_3 \times$ genotype	0.200	0.539	0.018	0.714	0.146
$CO_2 \times O_3 \times$ genotype	0.278	0.002	0.023	0.251	0.819

Foliar composition differed appreciably between the aspen genotypes for all metabolites quantified except starch. Genotype 259 had, on average, 11% more nitrogen than did genotype 216, but lower levels of salicortin (29%), tremulacin (19%), and condensed tannins (14%). The two genotypes responded similarly to CO_2- and O_3-enrichment, except for minor differences with respect to the responses of phenolic glycosides to high levels of O_3.

Herbivore performance

Two sets of data were used for assessment of forest tent caterpillar performance. One set consisted of responses to treatments by fourth instar larvae subsequently used as hosts for *Compsilura concinnata*, while the second consisted of responses to treatments by larvae reared from egg hatch to pupation.

Fourth instar mass

CO_2, O_3, aspen genotype, or their interactions altered the masses of larvae used as hosts (Fig. 2, Table 2). Masses of larvae reared on the two aspen genotypes were similar under ambient CO_2 (control, $+O_3$), but divergent under elevated CO_2, contributing to a significant $CO_2 \times$ genotype interaction. Elevated O_3 increased masses of larvae on both aspen genotypes, and more so at ambient than elevated CO_2 ($CO_2 \times O_3$ interaction). Larvae feeding on genotype 259 weighed an average of 40% more than did those feeding on genotype 216.

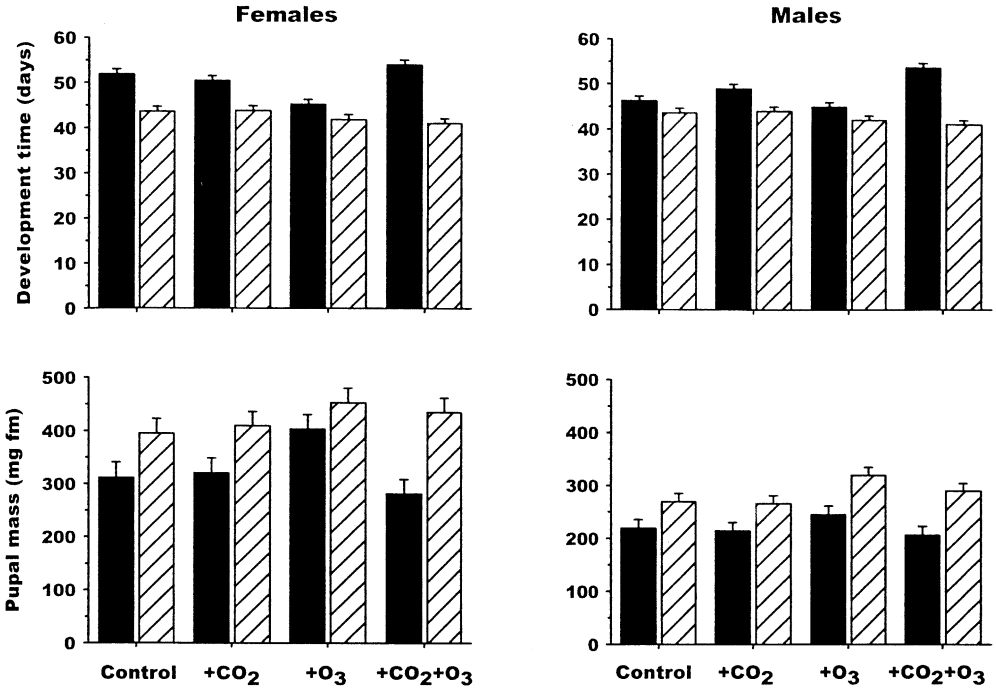

Fig. 3 Larval development times and pupal masses (fresh) of forest tent caterpillars reared on aspen genotype 216 (*solid bars*) and 259 (*hatched bars*) under elevated CO_2 and O_3. *Vertical lines* indicate +1 SE calculated from the pooled variance

Table 3 Phytochemicals accounting for variation in herbivore performance (multiple regressions using backward elimination, α =0.10 was the criterion for remaining in the model) (*CT* condensed tannins, *N* nitrogen, *PG* phenolic glycosides[a])

Parameter	Regression model			Partial regression components[b]		
	Equation	R^2	*P*	Variable	R^2	*P*
Larval development time						
Females	53.84+0.98(PG)–6.12(N)	0.315	<0.001	PG	0.187	<0.001
				N	0.138	<0.001
Males	41.32+1.01(PG) +0.24(CT)–3.39(N)	0.282	<0.001	PG	0.163	<0.001
				CT	0.035	0.084
				N	0.032	0.098
Pupal mass						
Females	253.29–15.10(PG)+94.85(N)	0.212	<0.001	PG	0.125	0.003
				N	0.093	0.010
Males	462.80–14.48(PG)–4.57(CT)	0.282	<0.001	PG	0.262	<0.001
				CT	0.134	<0.001

[a] For regression analyses, phenolic glycosides (salicortin and tremulacin) were pooled
[b] Due to correlations among independent variables, values depend on order and partial R^2 values do not accurately represent the proportion of variance explained by each variable in the model

Larval development time and pupal mass

Air treatments, aspen genotype, and their interactions influenced the performance of larvae reared to pupation, and differentially so between sexes (Fig. 3, Table 2). Independently, enriched CO_2 and O_3 did not affect female larval development times. However, elevated O_3, in relation to ambient air, reduced the development times of female larvae reared on genotype 216 an average of 6.5 days (CO_2 × O_3 × genotype interaction). For males, elevated CO_2 slowed larval development for those reared on genotype 216, and more so when in combination with elevated O_3. Conversely, CO_2 and O_3, in combination, slightly reduced the development time of males reared on genotype 259 (CO_2 × O_3 × genotype interaction). Female and male larvae reared on genotype 259 developed faster than did larvae reared on genotype 216, an average of 8 and 6 days, respectively. CO_2 and O_3 treatments did not significantly alter pupal masses. Pupae of both sexes responded similarly to the two aspen genotypes, with those reared on genotype 259 weighing an average of 29% more than those on genotype 216.

Relationship of herbivore performance to aspen phytochemicals

Overall, foliar chemical composition of aspen explained relatively little (21–32%) of the variation in forest tent caterpillar performance (Table 3). Larval development

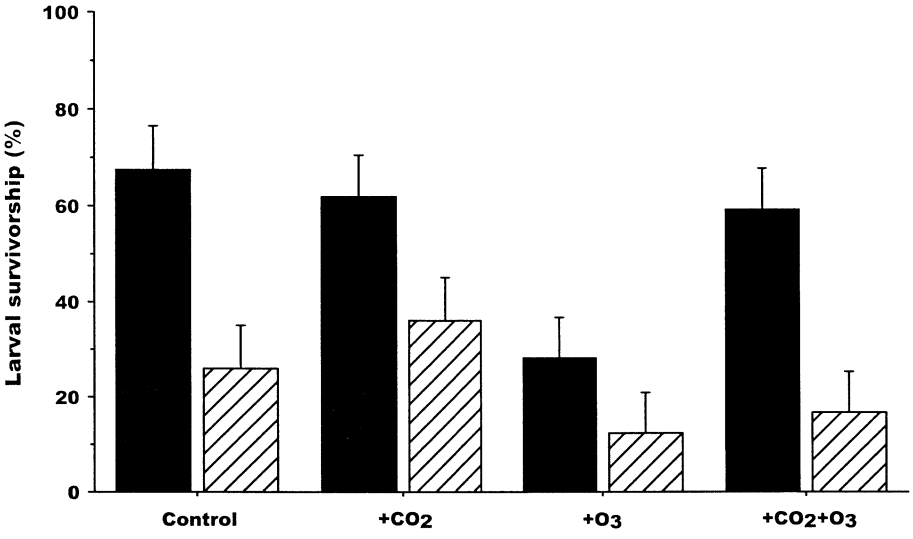

Fig. 4 Survivorship of *C. concinnata* associated with aspen genotype 216 (*solid bars*) and 259 (*hatched bars*) under elevated CO_2 and O_3. Non-transformed data are presented. *Vertical lines* indicate +1 SE calculated from the pooled variance

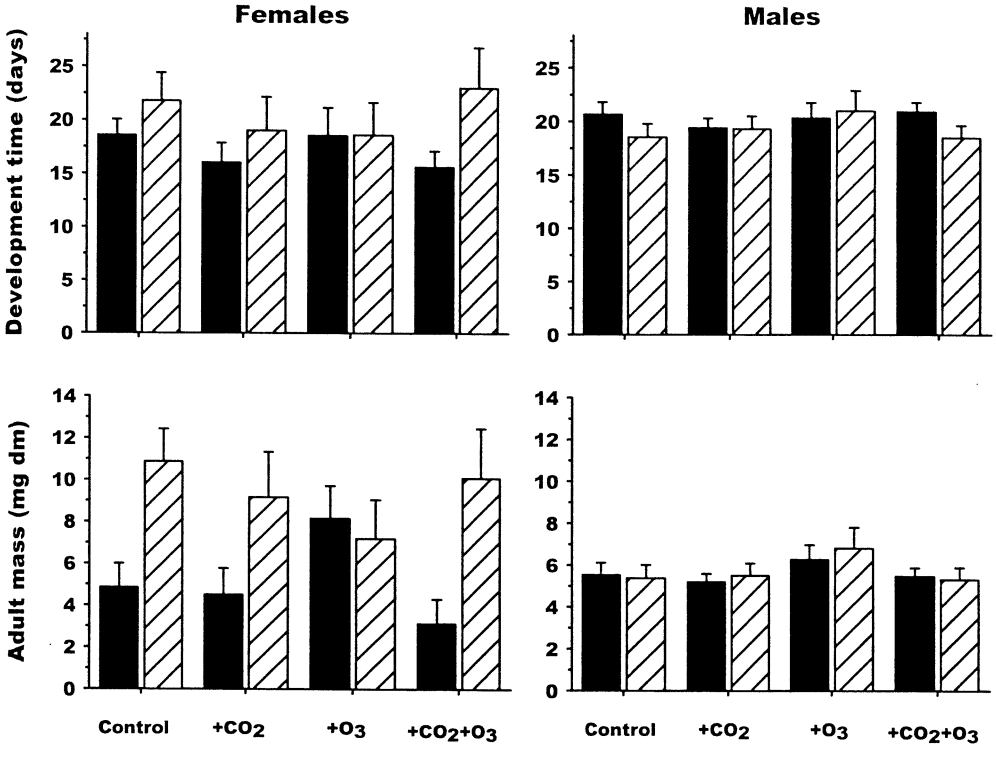

Fig. 5 Larval development times and adult masses (dry) of *C. concinnata* associated with aspen genotype 216 (*solid bars*) and 259 (*hatched bars*) under elevated CO_2 and O_3. *Vertical lines* indicate +1 SE calculated from the pooled variance

times were correlated with dietary concentrations of phenolic glycosides, nitrogen, and (for males) tannins. Pupal masses were correlated with concentrations of phenolic glycosides, nitrogen (females) and tannins (males).

Parasitoid performance

CO_2, O_3, aspen genotype, or their interactions altered larval survivorship as well as performance of female *C. concinnata* (Figs. 4, 5, Table 4). In general, enriched CO_2 had little effect on larval survivorship except in the presence of elevated O_3, where survivorship of insects on genotype 259 declined, relative to those in the $+CO_2$ treatment (marginally significant $CO_2 \times O_3 \times$ genotype interaction). Elevated O_3 alone markedly decreased larval survivorship, but this decline was offset in the presence of enriched CO_2 for parasitoids associated with genotype 216. Overall, survivorship of parasitoid larvae associated with genotype 259 was only half that of larvae associated with genotype 216. Neither CO_2 nor O_3 affected parasitoid larval development times. Female larvae developed faster (average of 3.4 days) in hosts reared on genotype 216 compared to those on genotype 259. CO_2 and O_3 interacted to affect female adult masses, and differentially

Table 4 Summary of *P* values for the effects of CO_2, O_3, and genotype on parasitoid performance

Main effects and interactions	Larval survivorship	Development time		Adult mass	
		Female	Male	Female	Male
CO_2	0.073	0.607	0.565	0.400	0.173
O_3	0.001	0.962	0.509	0.863	0.222
$CO_2 \times O_3$	0.204	0.371	0.724	0.987	0.250
Genotype	<.001	0.083	0.234	0.004	0.773
$CO_2 \times$ genotype	0.637	0.350	0.748	0.194	0.895
$O_3 \times$ genotype	0.784	0.864	0.887	0.347	0.903
$CO_2 \times O_3 \times$ genotype	0.087	0.321	0.125	0.074	0.527

Table 5 Phytochemicals and host mass accounting for variation in parasitoid performance (multiple regressions using backward elimination, $\alpha = 0.10$ was the criterion for remaining in the model) (*CT* condensed tannins, *HM* host mass, *PG* phenolic glycosides[a])

Parameter	Regression model			Partial regression components[b]		
	Equation	R^2	*P*	Variable	R^2	*P*
Larval survivorship	−0.03+0.07(PG)−0.01(HM)+0.02(CT)	0.225	<0.001	PG	0.101	0.005
				HM	0.068	0.020
				CT	0.044	0.060
Larval development time						
Females	No variable met α =0.1 criterion for remaining in model					
Males	No variable met α =0.1 criterion for remaining in model					
Adult mass						
Females	24.27–1.20(PG)–0.53(CT)+0.04(HM)	0.621	<0.001	PG	0.373	<0.001
				CT	0.333	<0.001
				HM	0.063	0.048
Males	No variable met α =0.1 criterion for remaining in model					

[a] For regression analyses, phenolic glycosides (salicortin and tremulacin) were pooled.
[b] Due to correlations among independent variables, partial R^2 values do not accurately represent the proportion of variance explained by each variable in the model.

so for parasitoids associated with the two aspen genotypes. Under high levels of O_3 but ambient CO_2, female adult masses tended to increase for parasitoids associated with genotype 216, and decrease for those associated with genotype 259 (marginally significant $CO_2 \times O_3 \times$ genotype interaction). Overall, female flies from hosts reared on genotype 259 weighed 1.8 times more than those from caterpillars reared on genotype 259. Masses of male flies, however, did not differ between genotypes.

Relationship of parasitoid performance to aspen phytochemicals and host mass

Larval survivorship of *C. concinnata* was correlated relatively weakly, but significantly, with phenolic glycoside and condensed tannin concentrations, and with host mass (Table 5). Development times of both sexes, and adult mass of male parasitoids, were not significantly related to aspen quality or host mass. For females, however, 62% of the variance in adult mass could be explained by a combination of aspen secondary metabolites and host mass.

Discussion

Foliar chemistry

Elevated CO_2 had little effect on both primary and secondary metabolites of aspen. Nitrogen concentrations declined slightly, but the magnitude of change was small relative to decreases observed in former studies of aspen under enriched CO_2 (Lindroth et al. 1993a, 1997, 2001a, 2001b; Roth and Lindroth 1995; Zak et al. 2000). Starch concentrations were unaltered by CO_2, a result different from those of previous studies, in which starch concentrations increased (Lindroth 1996b). However, other studies of quaking aspen at the FACE site have reported similar, minimal effects of CO_2 on nitrogen and starch levels (Lindroth et al. 2002b; Kopper and Lindroth 2003a, 2003b). Carbon-based secondary metabolites also showed weak responses, in contrast to the general trend of increased concentrations of phenolics under elevated CO_2 (Koricheva et al. 1998). Other studies of aspen at the FACE facility have documented small to moderate changes in phenolic glycoside and condensed tannin concentrations under enriched CO_2 (Lindroth et al. 2001a, 2002b; Kopper and Lindroth 2003a, 2003b).

Several explanations exist for the minimal effects of elevated CO_2 observed in this study. First, the level of CO_2 enrichment was considerably lower than that of previous, related studies (560 vs 650–700 μl l^{-1}). Given that Herms et al. (1995) found much more pronounced effects of CO_2 on forest tent caterpillar performance using the same aspen clones, but 750 μl l^{-1} concentrations of CO_2 in growth chambers, this explanation seems likely. Second, high nutrient availability, such as occurs at the FACE site (Dickson et al. 2000), can dampen CO_2-mediated changes in foliar chemistry (Lavola and Julkunen-Tiitto 1984; Kinney et al. 1997; Lindroth et al. 2001a).

Elevated O_3 altered foliar composition to a greater extent than did CO_2. Concentrations of nitrogen decreased marginally, while those of starch increased. Previous work has shown that responses of these two metabolites to elevated O_3 are quite variable, but levels tend to decrease (Koricheva et al. 1998). Recent studies of aspen genotype 216 at the FACE facility found an O_3-mediated decline in nitrogen, and no change or an increase in starch, concentrations (Lindroth et al. 2001b; Kopper and Lindroth 2003a, 2003b). For secondary metabolites, enriched O_3 decreased levels of phenolic glycosides while increasing those of condensed tannins. Exposure to O_3 often results in increased activity of defense-related plant enzymes, including those regulating the shikimic acid pathway from which phenolics are derived (Kangasjärvi et al. 1994). Thus, production of phenolic compounds may be expected to increase, as was observed in condensed tannins. The converse reduction in phenolic glycosides was likely a result of competition between branches of the shikimic acid pathway for substrates (Keinanen et al. 1999), as such competition can be influenced by abiotic factors such as air pollution (Loponen et al. 1998).

Foliar chemistry was minimally affected by interactions between pollutants or by interactions between pollutants and aspen genotype. As predicted, CO_2 and O_3 additively decreased nitrogen, and increased condensed tannin, concentrations. In general, the two aspen genotypes, irrespective of O_3 tolerance, responded similarly. The primary exception was phenolic glycoside concentrations. Enriched O_3 elicited a greater reduction in levels of salicortin and tremulacin in the O_3-tolerant genotype 216 than in the sensitive genotype 259. Thus, this study shows that, depending upon the foliar chemical, the independent effects of CO_2 and O_3 on plant chemistry can be amplified or dampened when the two pollutants occur together, and that plant responses may be generally similar between genotypes. Previous studies, however, with larger numbers of aspen genotypes, have documented greater differences in responses to elevated CO_2 among genotypes (Lindroth et al. 2001a, 2002b).

Herbivore performance

Independently, elevated CO_2 had few biologically significant effects on forest tent caterpillar performance. CO_2 marginally increased development time, and differentially so for insects on the two aspen genotypes, but had no effect on larval and pupal masses. Lack of CO_2-mediated alterations in forest tent caterpillar performance mirrors the minimal changes observed in foliar composition. Other research, however, has documented greater alterations of aspen foliar chemistry under elevated CO_2 and, in turn, more pronounced reductions in the growth and development of lepidopterans, including forest tent caterpillars (Lindroth et al. 1993a, 2002b; Roth and Lindroth 1995; Roth et al. 1998). Herms et al. (1995) did not evaluate foliar chemistry, but also found marked negative impacts of CO_2 on tent caterpillar performance.

Conversely, elevated O_3 significantly improved herbivore performance. Caterpillars (fourth instars) were larger, and females developed faster, under the $+O_3$ treatment. The O_3-mediated trend of increased mass was also apparent in pupae, but was not statistically significant, and thus may reflect a decreased sensitivity of late instar larvae to changes in food quality (Scriber and Slansky 1981). Increased herbivore performance corresponds with the decreased phenolic glycoside concentrations under elevated O_3. Previous studies with forest tent caterpillars have shown that performance declines as concentrations of phenolic glycosides increase (Lindroth and Bloomer 1991; Hemming and Lindroth 1995; Hwang and Lindroth 1997; Kopper and Lindroth 2003b). In a related "chamber" experiment, using the same aspen genotypes as in our work, Herms et al. (1995) also found that ozone fumigation led to improved tent caterpillar performance. Similarly, other studies of leaf-chewing insects have documented O_3-mediated declines in larval development times coupled with increased larval or pupal masses (Trumble et al. 1987; Chappelka et al. 1988; Jackson et al. 2000).

Overall, the consequences of interactions between CO_2 and O_3 for herbivore performance were generally few, and were themselves influenced by plant genotype ($CO_2 \times O_3 \times$ genotype interactions). This result is similar to those of Kopper and Lindroth (2003b) who conducted a related study with forest tent caterpillars at the Aspen FACE site.

Regression analyses showed that phenolic glycoside and nitrogen concentrations explained a small portion of the variation in larval development times and pupal masses. The detrimental effects (e.g., prolonged development time) of phenolic glycosides on forest tent caterpillars are amplified when protein levels are low (Lindroth and Bloomer 1991). Hence, in addition to the direct impact of reduced nutritional quality, decreases in nitrogen may have increased the influence of phenolic glycosides on larval performance. This interaction could explain why larvae reared on genotype 216, in which phenolic glycoside concentrations were higher relative to those in genotype 259, were more responsive to pollutant-mediated changes in foliar nitrogen than those on genotype 259. Accordingly, performance was lower for larvae on genotype 216 relative to that of larvae on genotype 259. These results suggest that, for leaf-chewing insects, the consequences of CO_2- and O_3-mediated changes in foliar chemical composition may depend upon the overall suitability of host plants as food sources.

Parasitoid performance

Elevated CO_2, alone, had no impact on *Compsilura concinnata*, consistent with its minimal effects at the first and second trophic levels. This result parallels those of an earlier study, in which elevated CO_2 was shown to have relatively weak effects on the fitness of a hymenopteran parasitoid (Roth and Lindroth 1995). However, other aspects of parasitoid fitness, not addressed in this study, may be altered by elevated CO_2. For example, many parasitoids use chemical cues to locate hosts (Vet and Dicke 1992). Air pollutants, such as CO_2, could interfere with reception of these cues, thereby potentially altering searching efficiencies. Conversely, parasitoids could benefit indirectly from increased levels of CO_2. Prolonged development time (as observed in males under the $+CO_2$ $+O_3$ treatments) and increased consumption are common CO_2-mediated responses of leaf-chewers and may increase exposure of herbivores to natural enemies. Indeed, Stiling et al. (1999, 2003) found that under elevated CO_2, leafminers produced larger mines and suffered increased rates of attack by natural enemies.

In contrast to CO_2, elevated O_3 significantly altered parasitoid fitness. In the $+O_3$ treatment, where tent caterpillar performance improved, *C. concinnata* associated with genotype 216 suffered marked declines in survivorship. Similarly, Karowe and Schoonhoven (1992) found that parasitoid survivorship declined in hosts that developed faster and grew larger. Additionally in the $+O_3$ treatment, adult female parasitoids associated with genotype 216 tended to be larger relative to those in the control treatment. This trend was likely a response to O_3-mediated changes in phenolic glycoside concentrations and host masses, as adult female mass was strongly and negatively correlated with phenolic glycoside concentration, and weakly but positively correlated with host mass. In fact, Roth et al. (1997) found that both a lepidopteran host (*Lymantria dispar*) and its parasitoid (*Cotesia melanoscela*) were larger when the host was reared on a diet low in phenolic glycoside concentration.

Aspen genotype strongly affected all parameters of parasitoid fitness. Larval survivorship of parasitoids associated with genotype 259, on which tent caterpillars grew faster and larger, was much lower than for those associated with genotype 216. This result suggests that improved host performance decreased parasitoid survivorship. Similarly, Cheng (1970) found that a tachinid endoparasitoid had lower larval survivorship in winter moth larvae feeding on oak, a preferred host plant, relative to those feeding on other plants, and suggested the difference in parasitoid survivorship was due in part to increased performance of the caterpillars feeding on oak. *Compsilura concinnata* (females only) also grew slower as larvae, but weighed more as adults, from hosts on genotype 259 relative to those from hosts on genotype 216. In addition to phenolic glycosides, condensed tannin concentrations explained a relatively large amount of variation in, and were negatively correlated with, adult masses of *C. concinnata*. Given the minimal influence of aspen condensed tannins on forest tent caterpillar performance (Hemming and Lindroth 1995), this finding suggests that the foliar chemicals limiting the parasitoid may be different from those limiting its host.

In combination, the O_3- and genotype-mediated changes in parasitoid performance present conflicting results of diet-mediated host suitability for the parasitoid. Trees on which herbivores grew faster and weighed more led to declines in parasitoid survivorship but increased adult masses of surviving female parasitoids. We offer several explanations for this apparent discrepancy. First, whether the influence of host plant quality on parasitoid performance is positive or negative may differ among parasitoid life stages. Second, our results may reflect size-dependent selection of parasitoid larvae. Smaller larvae may have been particularly susceptible to mortality in treatments (e.g., $+O_3$, genotype 259) in which overall survivorship was poor. Ellers and van Alphen (2002) found a similar trend of increased size of female parasitoids under environmental conditions that elicited higher larval mortality. In short, plant-mediated changes in host quality may have led to a shift in the size distribution of emerging female parasitoids. Increased size of adult females may have implications for the potential fecundity of *C. concinnata*, as size is positively correlated with number of ovarioles (Bourchier 1991).

In summary, atmospheric concentrations of CO_2 predicted for the future are likely to have minimal plant-mediated impact on performance of the forest tent caterpillar and its endoparasitoid *C. concinnata*. In contrast, elevated O_3 concentrations may lead to improved caterpillar performance (see also Kopper and Lindroth 2003b) but markedly reduced fitness (especially survivorship) of *C. concinnata*. Moreover, the independent and interactive effects of the pollutants may themselves be influenced by interactions with host plant quality.

Results from this and related research suggest that the impact of herbivory on biomass accrual may shift in relation to the relative concentrations of CO_2 and O_3 in forests of the future. Aspen growth is significantly increased under high CO_2, but decreased under high O_3, concentrations (Percy et al. 2002). That difference is likely to be exacerbated by the impacts of CO_2 and O_3 on herbivorous insects and parasitoids as documented in this study.

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