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Atmospheric change alters performance of an invasive forest insect

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Abstract

Atmospheric change and species invasions are arguably two of the most important factors affecting the long-term sustainability of natural ecosystems. We examined the independent and interactive effects of atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) on the foliar quality of two host species and performance of an invasive folivorous insect. Trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) were grown at the Aspen FACE research site in northern Wisconsin, USA, under all combinations of ambient and elevated CO_2 and O_3. We measured the effects of elevated CO_2 and O_3 on aspen and birch phytochemistry and on the survivorship, development time, growth, and fecundity of the gypsy moth (*Lymantria dispar*). Elevated CO_2 had little effect on, whereas elevated O_3 altered, the composite phytochemical profiles of aspen and birch. Nutritional quality in aspen and birch leaves was marginally affected by elevated CO_2 and reduced by elevated O_3. Both gases increased concentrations of phenolic and structural compounds in aspen and birch. Elevated CO_2 offset reduced foliar quality under elevated O_3, but only in aspen, and to a greater extent later than earlier in spring. Elevated CO_2 generally had beneficial effects on, while elevated O_3 detrimentally affected, gypsy moth performance. Elevated CO_2 ameliorated most of the reductions in gypsy moth performance under elevated O_3. Our findings suggest that atmospheric change can alter foliar quality in gypsy moth hosts sufficiently to influence gypsy moth performance, but that these responses will depend on interactions among CO_2, O_3, and tree species. Our findings also contrast with those of earlier studies at Aspen FACE, indicating that foliar quality responses to environmental change are likely influenced by tree stand age and longevity of exposure to pollutants to the extent that they affect plant-herbivore interactions differently over decadal time spans.

Keywords: Aspen FACE, atmospheric change, gypsy moth, phytochemistry, plant-insect interactions

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Introduction

Atmospheric change and species invasions are widely recognized as environmental stressors that can strongly impact the long-term sustainability of natural ecosystems (Ward & Masters, 2007). Although considerable scientific effort has focused on these problems in isolation, very little has been done to address their interactions, especially in forest ecosystems. Such work is essential for predicting the long-term health and sustainability of forest ecosystems. Anthropogenic activities are significantly increasing levels of greenhouse gases (Intergovernmental Panel on Climate Change (IPCC), 2007) that impact tree physiology, to the extent that major ecosystem processes are being altered (Norby *et al.*, 2005; Holmes *et al.*, 2006; Taneva *et al.*, 2006; Cole *et al.*, 2010). Enriched concentrations of atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) have received considerable attention because they exert significant, yet opposite, influences on plants. Elevated CO_2 generally stimulates plant growth by increasing photosynthesis (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007). Elevated O_3, however, is arguably the most damaging environmental phytotoxin, inhibiting plant growth by damaging foliage and reducing photosynthesis (Broadmeadow & Jackson, 2000; Oksanen, 2001; Karnosky, 2005; Karnosky *et al.*, 2005, 2007; Wittig *et al.*, 2009). The impacts of these gases on forest ecosystems are of great concern for many reasons, primary among them that forests have the potential to sequester anthropogenic carbon by storing it in slow-turnover carbon pools (DeLucia *et al.*, 1999; Wittig *et al.*, 2009).

Both CO_2 and O_3 can alter foliar chemical composition, which can significantly influence the performance of folivorous insect pests (Lindroth, 1996, 2010; Zvereva & Kozlov, 2006; Valkama *et al.*, 2007). Elevated CO_2 generally decreases concentrations of nitrogen, and increases concentrations of carbohydrate and phenolic

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compounds and carbon to nitrogen (C : N) ratios. The effects of O_3 on foliar nutritional composition are variable, but generally include increases in production of phenolic compounds, potentially as an antioxidant response (Valkama *et al*., 2007). Tree responses to elevated O_3 largely depend on tree sensitivity (Karnosky *et al*., 1999) and longevity of exposure, such that prolonged exposure compounds the negative effects of O_3 (Oksanen, 2003a,b).

Invasive species represent one of the greatest threats to forest health, as they can directly and indirectly influence multiple aspects of short- and long-term forest structure and function (Lovett *et al*., 2006). For example, outbreaks by the gypsy moth (*Lymantria dispar*) can remove nearly 100% of photosynthetic tissue in a forest canopy, significantly limiting primary production. Thus, outbreaks can reduce the potential for trees to sequester anthropogenic carbon in long-term carbon pools by shifting carbon allocation patterns away from woody growth to reflush of leaves (Mattson & Addy, 1975; Cook *et al*., 2008). Gypsy moth outbreaks can also alter nutrient cycling (Lovett *et al*., 2002), ecosystem nutrient retention (Townsend *et al*., 2004), and forest succession patterns (Fajvan & Wood, 1996; Lovett *et al*., 2006). Understanding how climate change will affect interactions between outbreak insect herbivores, such as the gypsy moth, and forest ecosystems is crucial to fostering forest health (Volney & Fleming, 2000; Logan *et al*., 2003).

Previous research has shown that gypsy moths generally experience decreased growth rates when feeding on CO_2-enriched trees in their native European range (Hättenschwiler & Schafellner, 2004). Responses by gypsy moths to North American trees grown under elevated CO_2 have been limited to young trees (Lindroth *et al*., 1993, 1997; Williams *et al*., 2000, 2003). Little research has addressed how elevated CO_2 and O_3, independently or interactively, will affect gypsy moth performance when feeding on mature North American trees. Moreover, no research has investigated the effects of environmental change on reproductive capacity of gypsy moths, an important component of their population dynamics. How the gypsy moth responds to future atmospheric conditions is critical, considering that climate change is predicted to increase the frequency and intensity of insect outbreaks (Stireman *et al*., 2005; Jepsen *et al*., 2008).

The purpose of this study was to examine how exposure to levels of CO_2 and O_3 predicted for the year 2050 alter foliar quality of pole-stage aspen and birch and the performance of the gypsy moth. Recent findings from this site have shown that aspen and birch exposed to elevated CO_2 and O_3 alter the short-term (i.e. single instar) performance of gypsy moths (Couture *et al*., 2012); in this study we examined effects of these gases on the long-term performance (survivorship, development time, pupal weight, and fecundity). Specifically, we predicted that: (1) elevated CO_2 and O_3 will alter the composite phytochemical profiles of aspen and birch, (2) elevated CO_2 and O_3 will decrease aspen and birch foliar quality through reductions in nitrogen concentrations and increases in concentrations of phenolic and structural compounds and C : N ratios, (3) responses to elevated CO_2 and O_3 will vary between tree species, and (4) reductions in foliar quality will decrease gypsy moth performance.

Materials and methods

Experimental design

This experiment was conducted in northern Wisconsin, USA (W 89.5°, N 45.7°) at the Aspen Free Air CO_2 and O_3 Enrichment (FACE) research facility, a 32 ha research facility located near Rhinelander, WI. The site contained twelve, 30 m diameter experimental rings, with three blocks of four treatments. The full-factorial design allowed for all possible treatment combinations of ambient and elevated (560 ppm) CO_2 and ambient and elevated (1.5 × ambient) O_3 levels. A mixed aspen-birch stand was planted with one-year-old aspen and birch seedlings in the southwestern quadrant of each ring in 1997 and fumigation treatments began in 1998. Within each ring we randomly selected three trees each of aspen genotype 216 and birch from the mixed aspen-birch quadrant for foliar collections and herbivore bioassays. At the time of the experiment (2008) the trees were 12 years old and the canopy had closed. Detailed information about the Aspen FACE research facility can be found in Dickson *et al.* (2000).

Phytochemical analyses

Leaves were collected twice (30 May and 20 June) for phytochemical analyses, approximately 2 weeks after the beginning and 2 weeks before the end of the insect bioassays. We used pole pruners to clip short-shoots from trees, excised 10–12 leaves from petioles, and stored them on ice while in the field. Leaves were subsequently flash frozen in liquid nitrogen, lyophilized, ground, and stored at −20° C until chemical analysis. Leaves were assayed for nitrogen, C : N, soluble sugars (i.e. hexoses and sucrose), condensed tannins, total phenolic glycosides [reported as the sum of salicortin and tremulacin, the dominant phenolic glycosides in aspen (Lindroth & Hwang, 1996)], fiber (reported as the total amount of cellulose and lignin), and lignin.

We quantified all of the above phytochemical constituents, except fiber and lignin, using near infrared reflectance spectroscopy (NIRS) following the methods of Rubert *et al.* (*in review*). NIRS is an economical, methodological tool for the rapid, nondestructive determination of the chemical structure of plant tissues (Foley *et al*., 1998). Spectra are based on the reflectance of light by the C-H, N-H, and O-H bonds of

organic molecules in plant tissue across the visible and infrared regions, revealing information about the chemical composition of the sample.

First, spectra are collected from all samples, and then a subset of the samples is used to build a calibration model. Second, spectra from each sample of the subset used to build the calibration model are paired with the corresponding known value (e.g. concentration), determined by an analytical method, of a particular phytochemical constituent. Third, the calibration model is built using multivariate statistics to relate variation in the spectra with the analytical data. Finally, the calibration model is validated by comparing relationships between observed and predicted values to determine the robustness of the model. This calibration model can then be used to predict phytochemical concentrations of the remaining unknown samples on the basis of their spectra.

Calibration models used for the prediction of the above phytochemicals were based on archived spectra corresponding to aspen (including aspen genotype 216) and birch green leaves collected across 3 years of sampling (2006–2008) at Aspen FACE. Spectra were produced by scanning dried, ground leaf material across visible and near infrared wavelength bands (400–2500 nm) at 2-nm increments using a FOSS-NIRSystems Model 6500 instrument controlled via NIRS3 software. We then developed calibration models using modified partial least squares regression (mPLSR) with cross validation to avoid over-fitting the model (Shenk & Westerhaus, 1991). Statistics evaluating the precision of the calibration models can be found in the Supporting Information (Table S1).

Standard analytical determination of carbon and nitrogen was performed using a Thermo Finnigan Flash 1112 elemental analyzer. Simple sugars (i.e. hexoses and sucrose) were measured spectrophotometrically using a modified dinitrosalicyclic acid assay (Lindroth *et al.*, 2002). Condensed tannins were determined spectrophotometrically using a butanol-HCl method (Porter *et al.* 1986), with condensed tannins purified from aspen and birch leaves used as standards. Tremulacin and salicortin were analyzed using high-performance liquid chromatography (HPLC) following a method similar to that of Lindroth *et al.* (1987). Briefly, phenolic glycosides were extracted in methanol with sonication for 15 min and analyses were performed on a Beckman-126 system, using a 1.5 mL gradient flow of 98 : 2 H_2O-tetrahydrofuran (mobile A) with increasing methanol (mobile B) content on a Beckman ODS, 5-μ column over the course of 35–50 min. Phenolic glycoside peaks were measured by UV absorbance at 274 nm using a Beckman-168 photodiode array detector and quantified via internal or external standardization using butylated hydroxyanisole or resorcinol, respectively. Fiber and lignin were determined gravimetrically using sequential extraction in a hot acid-detergent solution in an Ankom 200 Fiber Analyzer and incubation in 72% H_2SO_4.

Insect bioassays

Bioassays were conducted to determine the independent and interactive effects of CO_2, O_3, and tree species on gypsy moth performance variables, including early-instar development time and survivorship, development time from egg hatch to pupation, pupal weights, and fecundity (i.e. number of eggs produced, total weight of eggs, and average weight per egg). Low densities of gypsy moths near Aspen FACE precluded the use of insects from local populations. Thus, gypsy moth egg masses were obtained from USDA-APHIS (Otis Air National Guard Base, MA, USA), surface sterilized in a solution of 0.1% sodium hypochlorite and 1% Tween 80, then placed into a Percival® growth chamber, in 2.5 × 15 cm plastic dishes, under a 24 : 18° C and 15 : 9 h light : dark cycle. Many spring folivores are highly synchronized with host budburst and this synchronization influences larval development (Feeny, 1970; Hunter, 1992; Jones & Despland, 2006). Budburst at the Aspen FACE site generally occurred in early May and leaf expansion continued throughout May (McGrath *et al.*, 2010). We timed gypsy moth egg hatch (16 May) such that larvae were fed expanding leaves during early-instar development. Because we were primarily concerned with insect responses to CO_2 and O_3-mediated effects on plant quality, we used detached leaves, as opposed to *in situ* feeding on trees, for all insect bioassays. Using detached leaves allowed us to focus specifically on plant-mediated effects, independent of any potentially confounding direct effects of CO_2 and O_3 on insect performance.

To determine how elevated CO_2, O_3, and tree species influenced gypsy moth early-instar development time and survivorship, we recorded the number of days required by larvae to complete their second stadium and the proportion of larvae surviving at that time. We randomly selected 20 neonate larvae and placed them into 2.5 × 15 cm plastic dishes in a Percival® chamber as described above. Larvae were fed either aspen or birch foliage from one of the four Aspen FACE treatments. Foliage was kept hydrated by inserting the petioles into 6 ml florist water picks filled with water. Experimental leaves were field-collected in the same manner as leaves for chemical analysis. All rearing dishes were examined daily to ensure adequate foliage was present; additional leaves were provided if more than 50% of the foliage was consumed. Leaves were replaced every second day, regardless of consumption, to ensure that the quality of the leaves fed to gypsy moths reflected the quality of the leaves in the canopy (Hemming & Lindroth, 1999). Each dish was treated as an experimental unit, with a total of 72 dishes (12 rings × 2 tree species × 3 replicate trees/tree species × 1 dish/tree = 72) and 1440 larvae (20 larvae/dish) used in this study.

To determine how elevated CO_2, O_3, and tree species influenced long-term gypsy moth performance, we reared a subset of larvae from the early-instar trials through to pupation. We randomly selected 3–5 larvae and placed them into 20 × 15 × 10 (L : W : H) cm plastic containers. Containers were kept on a table in an open-air room. We reared larvae to pupation on foliage from the same tree species and fumigation treatment they had received during their early-instar development. Leaves were collected and larvae were fed in the same manner as described previously for the early-instar assays. Each container was treated as an experimental unit, with a total of 72 containers (12 rings × 2 tree species × 3 replicate trees/tree species × 1 container/tree = 72 containers) and 344

larvae (3–5 larvae/container) used in this study. Upon pupation, we recorded total larval development time, pupal sex, and pupal weight.

To evaluate how elevated CO_2, O_3, and tree species influenced gypsy moth fecundity, we mated males and females from within individual tree species and fumigation treatments, collected egg masses, and recorded the number and total weight of eggs from each egg mass. We pooled all pupae from either aspen or birch within a ring and placed paired male and female pupae into 27 × 20 × 10 (L : W : H) cm plastic containers lined with kraft paper. After eclosion and copulation, females deposited egg masses. We collected the egg masses and removed the hair deposits by running individual egg masses gently through a #20 sieve. The egg masses were then lyophilized, weighed, and the number of eggs in each egg mass counted. We excluded from analysis four egg masses produced from females whose paired male did not eclose. If the number of males needed for mate pairing from a specific FACE ring and tree species was less than four, we added a male from the same tree species and fumigation treatment, but from a different ring. The total number of egg masses collected was 116 (12 rings × 2 tree species × 4–6 egg masses/tree species/ring).

Statistical analyses

We determined the influence of CO_2 and O_3 on shifts in composite phytochemical profiles of aspen and birch separately, using permutational analysis of variance (PERMANOVA; Anderson, 2001). In these analyses, we used Bray-Curtis measurements of resemblance and 999 permutations. Composite phytochemical profiles were visualized with nonmetric multidimensional scaling (NMDS) using Bray-Curtis dissimilarities. If PERMANOVA identified a statistically significant shift in the composite phytochemical profile under elevated CO_2 or O_3, we further examined the contribution of each chemical constituent to the overall change using similarity percentage analysis (SIMPER). This test allowed us to discriminate between two groups of data by calculating the overall percent contribution that each individual chemical constituent adds to the average dissimilarity between the two sets of information.

We analyzed individual chemical constituents of aspen and birch separately by analysis of variance with a split-plot design, using the model $Y_{ijkl} = b_i + C_j + O_k + CO_{jk} + e_{ijk} + T_l + CT_{jl} + OT_{kl} + COT_{jkl} + \varepsilon_{ijkl}$. In this model b represents block i, C represents CO_2 level j, O represents O_3 level k, e_{ijk} represents the whole-plot error, T represents time l, and ε_{ijkl} represents the sub-plot error. Y_{ijkl} represents the average response of block i, CO_2 level j, O_3 level k, and time l. Early-instar survivorship and development time and moth fecundity were analyzed using a similar model as phytochemical responses, with the exception that the effect of time was replaced with tree species. We analyzed gypsy moth development time from egg hatch to pupation and pupal weights using a similar model as we used for analyzing early-instar responses, except we added an additional splitting factor to include gypsy moth sex. F-tests were conducted with degrees of freedom assigned using the Satterthwaite approximation. Means and pooled standard errors are reported for all combinations of CO_2, O_3, and tree species for phytochemical responses, early-instar survivorship, development time, and moth fecundity, and for those same factors plus gypsy moth sex for development time from egg hatch to pupation and pupal weights.

For evaluation of the influence of foliar quality variables on gypsy moth performance, we used partial least squares regression (PLSR) analysis (Wold *et al*., 1984, 2001) following Couture *et al*. (2012). Data used in PLSR were averaged across replicates to the ring level (n = 12) for each tree species to increase data precision. We validated our models by examining the relationship between the predicted and observed responses. We related early-instar larval survivorship and development time to foliar quality data from only the first foliar collection date. We related gypsy moth female pupal weight, number of eggs produced, and total weight of eggs to the averaged foliar quality data from both foliar collection dates. Because of the limited treatment effects of CO_2 and O_3 on male and female gypsy moth total development time and male pupal weights, we did not relate foliar quality to those performance responses.

Statistical analyses were performed by using PRIMER (PRIMER-E, 2008) and JMP v. 9.0 statistical software (SAS Incorporated, (2008), Cary, NC, USA). The low replication of the Aspen FACE design increases the potential for type II errors. To balance the potential for committing type I vs. type II errors, we report P values $0.05 < 0.10$ as 'marginally significant' and $P < 0.05$ as 'significant' (Filion *et al*., 2000). Exact P values provided by the statistical analyses are reported in Tables 2, 3, 5, and 6.

Results

NIRS predictions

Near infrared reflectance spectroscopy successfully predicted most chemical constituents of aspen and birch foliage (Table S1). Models predicting aspen and birch nitrogen, C : N ratios, and condensed tannins, as well as aspen phenolic glycosides, performed well. Models developed for aspen and birch sugar values performed only marginally well as predictors of absolute concentrations. Nonetheless, because we were interested in relative effects of the treatments, as well as in absolute concentrations, we report aspen and birch sugar concentrations. We stress that values for sugar concentrations should be interpreted with caution and include the caveat that qualitative interpretations (i.e. relative increases or decreases) are most appropriate. We excluded aspen and birch sugar concentrations from analyses relating foliar quality to herbivore performance.

Foliar quality

We found that CO_2 had little effect on the phytochemical profile of either tree species, while O_3 altered the

composite chemical response of leaves from both aspen and birch (Fig. 1). Subsequent SIMPER analyses revealed that increased fiber and sugar concentrations and C : N ratios explained close to 70% of the differences in composite chemical profiles between ambient and elevated O_3 in aspen (Table 1). Similarly, increased condensed tannin and sugar concentrations and C : N ratios explained over 75% of the differences in the chemical profile of birch under elevated, compared with ambient, O_3 (Table 1).

Aspen and birch nutritional qualities (i.e. nitrogen and sugar concentrations and C : N ratios) were influenced by elevated CO_2, O_3, time, and their interactions (Table 2; Figs. 2 and 3). Independently, elevated CO_2 nominally affected the nutritional composition of aspen or birch, and these responses were consistent from early to late spring. Conversely, elevated O_3 had a strong, negative effect on both aspen and birch nutritional quality. Elevated O_3 reduced aspen nitrogen concentrations by 7%, and this response was consistent from early to late spring. Elevated CO_2 offset reduced aspen nitrogen concentrations in late, but not early, spring. Elevated O_3 reduced birch nitrogen concentrations, but to a greater extent in early spring (14%) than late spring (8%). Elevated CO_2 did not offset reduced birch nitrogen concentrations in either early or late spring. Elevated O_3 increased aspen C : N ratios by 9%, and this response was consistent from early to late spring. Elevated CO_2 offset increased C : N ratios in aspen during late, but not early, spring. Elevated O_3 increased birch C : N ratios, but to a greater extent in early spring (17%) than late spring (7%). Elevated CO_2 did not ameliorate increased birch C : N levels in either early or late spring. Elevated CO_2 did not alter aspen or birch sugar levels. Elevated O_3 increased aspen sugar concentrations; the response was more pronounced in late spring (11%) compared with early spring (6%), but was only marginally significant. Elevated O_3 increased birch sugar concentrations by 14% in late spring, whereas the increase was negligible in early spring.

Elevated CO_2, O_3, time, and their interactions also influenced concentrations of individual phenolic and structural compounds for aspen and birch

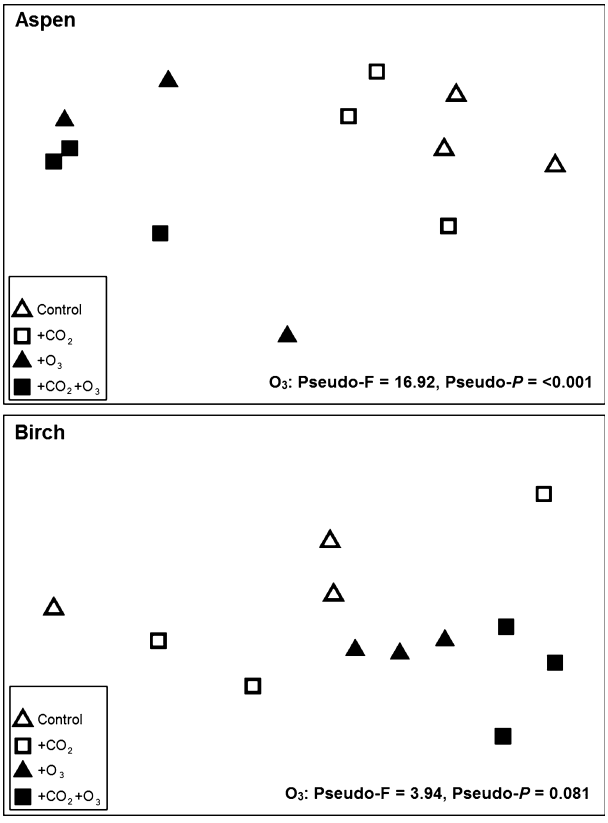

Fig. 1 Nonmetric multidimensional scaling (NMDS) ordination plots demonstrating differences between composite chemical profiles of aspen (upper panel) and birch (lower panel) leaves exposed to all possible combinations of elevated and ambient CO_2 and O_3. Statistical differences among treatments were calculated using PERMANOVA. Foliar quality data used to construct both plots were averaged across time.

Table 1 Mean concentrations (% dry weight ± SE) of individual chemical constituents in aspen and birch leaves exposed to ambient or elevated O_3. Data are averaged across both collection periods and ranked in order of largest contribution (%) to the overall dissimilarity between leaves from ambient compared with elevated O_3, as determined by SIMPER analyses. C : N, ratio of carbon to nitrogen

Tree species and chemical constituent	Ambient O_3 Concentration	Elevated O_3 Concentration	Contribution (%)
Aspen			
Fiber	23.4 ± 0.4	28.9 ± 0.4	40.9
Condensed tannins	12.5 ± 0.5	14.2 ± 0.5	15.2
Sugar	19.2 ± 0.5	20.8 ± 0.5	14.1
C:N	16.9 ± 0.3	18.5 ± 0.3	11.8
Lignin	12.9 ± 0.2	14.3 ± 0.2	11.1
Phenolic glycosides	5.4 ± 0.3	6.2 ± 0.3	5.1
Nitrogen	2.8 ± 0.1	2.6 ± 0.1	1.8
Birch			
Condensed tannins	13.1 ± 0.9	16.5 ± 0.9	38.8
C:N	16.7 ± 0.6	18.7 ± 0.9	24.2
Sugar	12.4 ± 0.6	13.1 ± 0.6	15.6
Lignin	14.0 ± 0.2	15.0 ± 0.2	10.6
Fiber	22.3 ± 0.3	22.3 ± 0.3	7.1
Nitrogen	2.7 ± 0.1	2.4 ± 0.1	3.6

Table 2 Summary of *P* values for the effects of CO_2, O_3, time, and their interactions on the foliar quality of aspen and birch. $P < 0.05$ values are in bold and *P* values $0.05 < P < 0.10$ are italicized

Tree species and treatments	df	*N*		C : N		Sugar		Condensed tannins		Phenolic glycosides		Fiber		Lignin	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Aspen															
CO_2	1,6	0.37	0.565	0.52	0.497	3.28	0.120	1.23	0.311	1.27	0.302	6.90	**0.039**	1.69	0.241
O_3	1,6	16.04	**0.007**	13.44	**0.010**	5.65	*0.055*	4.94	*0.068*	4.73	*0.072*	101.29	**<.001**	18.92	**0.005**
$CO_2 \times O_3$	1,6	0.11	0.756	0.02	0.902	1.63	0.248	0.65	0.449	0.24	0.644	0.01	0.950	2.58	0.159
Time	1,56	376.98	**<0.001**	332.88	**<0.001**	5.53	**0.022**	51.24	**<0.001**	100.16	**<0.001**	0.09	0.764	0.77	0.383
$CO_2 \times$ time	1,56	0.10	0.798	0.01	0.950	0.52	0.473	10.25	**0.002**	0.26	0.615	7.95	**0.007**	1.21	0.277
$O_3 \times$ time	1,56	0.73	0.398	0.63	0.432	3.49	*0.067*	0.10	0.749	0.93	0.339	11.98	**<0.001**	2.44	0.124
$CO_2 \times O_3 \times$ time	1,56	3.91	*0.053*	3.03	*0.088*	0.69	0.408	0.34	0.560	0.02	0.883	0.35	0.556	2.70	0.106
Birch															
CO_2	1,6	0.83	0.397	1.27	0.303	0.75	0.418	1.37	0.285	na	na	2.38	0.174	0.28	0.618
O_3	1,6	7.18	**0.037**	6.04	**0.049**	0.76	0.415	6.57	**0.043**	na	na	0.00	0.999	12.85	**0.012**
$CO_2 \times O_3$	1,6	0.14	0.717	0.12	0.740	0.06	0.803	1.29	0.299	na	na	0.62	0.461	0.09	0.780
Time	1,56	50.75	**<0.001**	40.73	**<0.001**	74.4	**<0.001**	1.42	0.238	na	na	3.96	*0.051*	7.95	**0.007**
$CO_2 \times$ time	1,56	0.02	0.902	0.06	0.804	1.49	0.227	0.04	0.849	na	na	3.22	*0.078*	0.87	0.355
$O_3 \times$ time	1,56	8.37	**0.005**	3.76	*0.058*	5.01	**0.029**	3.58	*0.064*	na	na	0.55	0.463	4.12	**0.047**
$CO_2 \times O_3 \times$ time	1,56	1.86	0.178	2.60	0.113	1.02	0.317	1.93	0.170	na	na	0.63	0.432	0.07	0.798

Numerator and denominator degrees of freedom (df: numerator, denominator) were calculated using the Satterthwaite approximation.

N, nitrogen; C : N, ratio of carbon to nitrogen; PG, total phenolic glycosides, reported as the sum of salicortin and tremulacin; na, not applicable.

(Table 2; Figs. 2 and 3). Elevated CO_2 increased aspen condensed tannins by 13% during early spring, but levels converged during late spring. Elevated CO_2 had no effect on birch condensed tannins. Elevated O_3 increased aspen condensed tannin concentrations by 13%. This marginally significant response was consistent from early to late spring. Elevated O_3 also increased birch condensed tannins, but the response magnitude was greater in early spring (33%) than that in late spring (18%). Elevated CO_2 had no effect on aspen phenolic glycoside concentrations, although elevated O_3 increased concentrations by 15%. The response was only marginally significant, but consistent from early to late spring (Fig. 2). Elevated CO_2 increased aspen fiber concentrations more in early spring (10%) compared with a negligible response in late spring, whereas elevated O_3 increased aspen fiber concentrations more in late spring (32%) compared with that in early spring (16%). Elevated CO_2 and O_3 had minimal effects on birch fiber concentrations. Elevated CO_2 had little effect on either aspen or birch lignin concentrations. Elevated O_3 increased aspen lignin concentrations by 11%, and the response was consistent in both early and late spring. Elevated O_3 also increased birch lignin concentrations by 11% in early spring, but the response was negligible in late spring.

Herbivore performance

Gypsy moth early-instar survivorship and development time were influenced by elevated CO_2, O_3, and their interactions. Survivorship increased by 8% and decreased by 16% when fed foliage from elevated, compared with ambient, CO_2 and O_3, respectively, and the response was consistent across both tree species (Fig. 4, Table 3). Elevated CO_2, however, ameliorated increased mortality under elevated O_3. Early-instar development time was nominally affected by foliage from elevated CO_2, but increased when larvae were fed foliage from elevated O_3, and the response was consistent across both tree species (Fig. 4; Table 3). Although statistically significant, the increase in development time when fed foliage from elevated O_3 was small (5%) and ameliorated by elevated CO_2.

Partial least squares regression models relating gypsy moth early-instar survivorship and development time to aspen and birch foliar quality produced significant relationships between predicted and observed responses (Fig. S1). When fed aspen foliage, early-instar

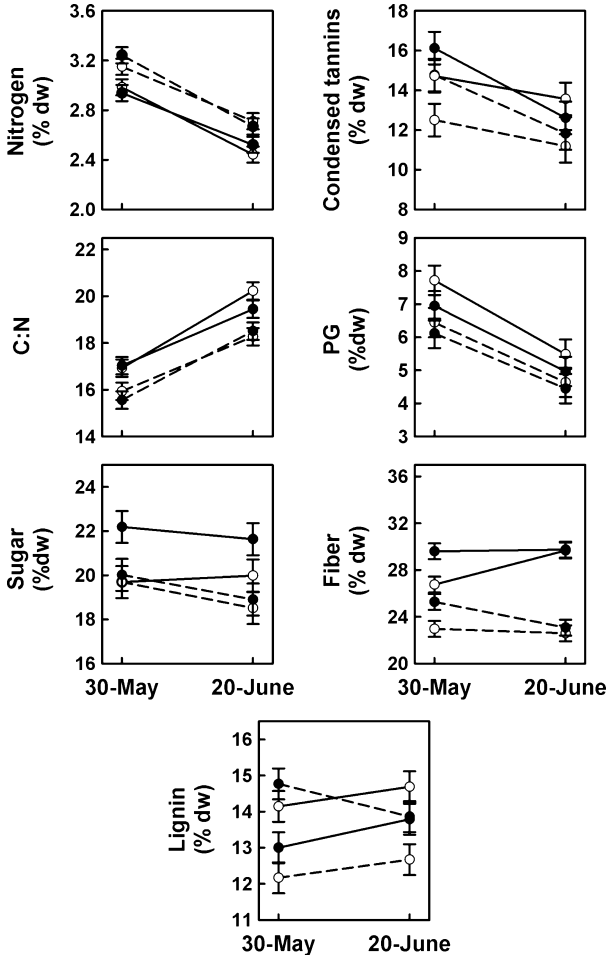

Fig. 2 Independent and interactive effects of elevated CO_2 and O_3 on aspen foliar quality. Open circles (○) represent ambient CO_2; closed circles (●) represent elevated CO_2. Dashed lines (– –) represent ambient O_3; solid lines (——) represent elevated O_3. C : N, ratio of carbon to nitrogen; PG, total phenolic glycosides, reported as the sum of salicortin and tremulacin. Error bars represent ± 1 SE.

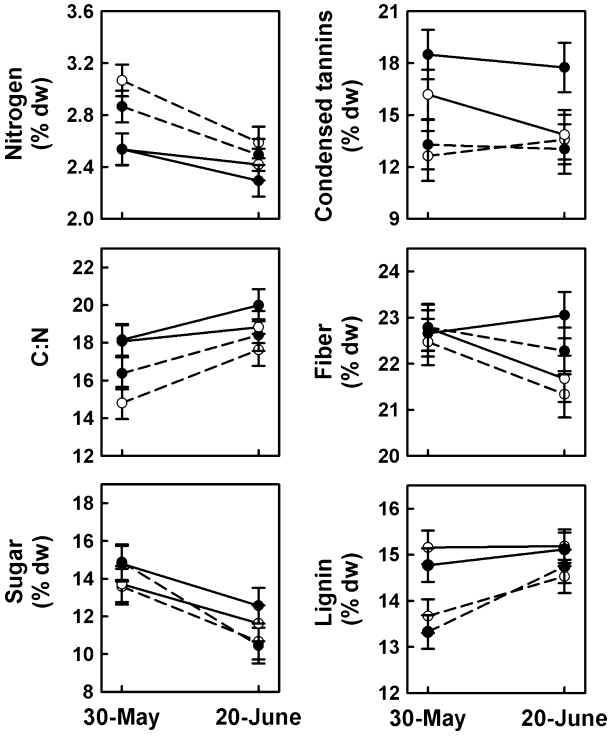

Fig. 3 Independent and interactive effects of elevated CO_2 and O_3 on birch foliar quality. Open circles (○) represent ambient CO_2; closed circles (●) represent elevated CO_2. Dashed lines (– –) represent ambient O_3; solid lines (——) represent elevated O_3. C : N, ratio of carbon to nitrogen. Error bars represent ± 1 SE.

survivorship was most positively and negatively related to concentrations of nitrogen and phenolic glycosides, respectively, and development time through the second stadium was most positively related to concentrations of phenolic glycosides (Table 4). When fed birch foliage, early-instar survivorship was most positively and negatively related to concentrations of nitrogen and C : N ratios, respectively, and development time through the second stadium was most positively related to concentrations of lignin and C : N ratios and negatively related to concentrations of nitrogen (Table 4).

Gypsy moth pupal weights were influenced by elevated CO_2, O_3, tree species, sex, and their interactions (Fig. 5; Table 5). On average, pupal weights were 12% greater on aspen, compared with birch (Table 5). Female pupal weights were 130% larger than male pupal weights (Fig. 5); this response, however, was influenced by ozone level and tree species. Female pupal weights decreased by 8% when insects were fed ozonated foliage, whereas the effect of ozone on male pupal weights was negligible and the size difference between female and male pupal weights was greater on aspen (140%) compared with that on birch (120%). Gypsy moth development time from egg hatch to pupation was only marginally affected by elevated CO_2 and O_3, and more influenced by tree species and sex (Fig. 5; Table 5). Overall, larvae fed aspen pupated a full day (3%) faster than those fed birch and male moths developed over 3 days (10%) faster than female moths (Fig. 5; Table 5). Elevated CO_2 reduced female gypsy moth development times more than that of males when fed aspen. The response, however, was small (3%) and offset by elevated O_3.

Gypsy moth fecundity was influenced by elevated CO_2, O_3, tree species, and their interactions (Fig. 6; Table 6). Overall, moth egg production increased when larvae were fed foliage from elevated, compared with ambient, CO_2 and the response was larger on aspen than that on birch. The response to elevated CO_2, however, was only marginally significant and predominantly

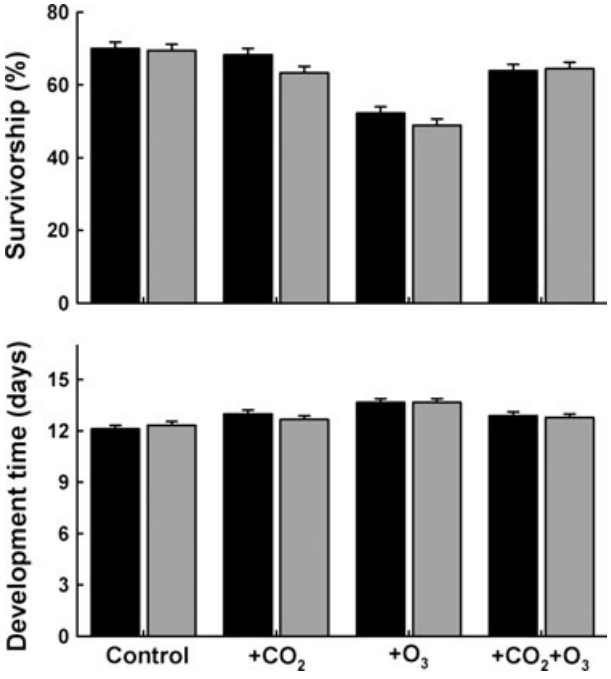

Fig. 4 Independent and interactive effects of elevated CO_2 and O_3 on gypsy moth early-instar survivorship and development time when fed aspen (dark bars) or birch (light bars). Error bars represent + 1 SE.

Table 3 Summary of *P* values for the effects of CO_2, O_3, tree species, and their interactions on early-instar gypsy moth survivorship and development time. $P < 0.05$ values are in bold

		Survivorship		Development time	
Treatments	df	F	*P*	F	*P*
CO_2	1,6	27.63	**0.002**	0.43	0.535
O_3	1,6	126.88	**<0.001**	18.27	**0.005**
$CO_2 \times O_3$	1,6	89.53	**<0.001**	18.27	**0.005**
Tree spp.	1,56	1.98	0.165	0.18	0.672
$CO_2 \times$ tree spp.	1,56	0.01	0.926	1.63	0.208
$O_3 \times$ tree spp.	1,56	0.22	0.641	0.00	1.000
$CO_2 \times O_3 \times$ tree spp.	1,56	1.98	0.165	0.72	0.399

Numerator and denominator degrees of freedom (df: numerator, denominator) were calculated using the Satterthwaite approximation.

driven by a reduction in egg production under elevated O_3 in the absence of elevated CO_2. When fed foliage from elevated, compared with ambient, O_3 moth egg production decreased by 28%, and this response was consistent whether larvae were fed aspen or birch. Elevated CO_2 ameliorated reduced egg production under elevated O_3, but the offset was larger on aspen than birch. The total weight of eggs followed a similar pattern as did number of eggs produced. When fed foliage from elevated, compared with ambient, CO_2, moth total egg weight increased by 11%. However, the response was only marginally significant and driven by a large reduction in total egg weight when larvae were fed foliage from elevated O_3 in the absence of elevated CO_2. Foliage from elevated, compared with ambient, O_3 caused moth egg production to decrease by 29% and this response was consistent across both aspen and birch (Fig. 6; Table 6). Elevated CO_2 ameliorated the reduction in total weight of eggs produced under elevated O_3, but the response was greater on aspen than that on birch. Elevated CO_2 and O_3 had little effect on the average weight per egg (Table 6). Number of eggs produced ($r = 0.503$, $P = <0.001$; data not shown) and total weights of eggs ($r = 0.491$, $P = <0.001$; data not shown) were positively correlated, but weight per eggs was not correlated ($r = 0.143$, $P = 0.124$; data not shown), with female pupal weight.

Partial least squares regression relating gypsy moth fecundity indices to aspen and birch foliar quality produced significant relationships between predicted and observed responses (Figure S2). When fed aspen foliage, female pupal weights were most positively and negatively related to condensed tannins and phenolic glycoside concentrations, respectively (Table 7). Number of eggs produced and total weight of eggs were most positively and negatively related to nitrogen concentrations and C : N ratios, respectively (Table 7). When fed birch foliage, female pupal weights, number of eggs produced, and total weight of eggs were most positively and negatively related to nitrogen concentrations and C : N ratios, respectively (Table 7).

Discussion

Foliar quality

Contrary to our first hypothesis, elevated CO_2 had little influence on the composite phytochemical profiles of aspen and birch. And contrary to our second hypothesis, elevated CO_2 had minimal effects on both aspen and birch nutritional composition. Decreased nitrogen concentrations and increased C : N ratios are generally reported for plants grown under elevated CO_2 (Zvereva & Kozlov, 2006; Stiling & Cornelissen, 2007; Lindroth, 2010; Robinson *et al*., 2012). However, recent reports from this study system have found nominal effects of elevated CO_2 on foliar nitrogen concentrations (Agrell *et al*., 2005; Vigue & Lindroth, 2010; Couture *et al*., 2012). In addition, these reports contrast with earlier findings of reduced foliar nitrogen levels under elevated CO_2 at this site (Lindroth *et al*., 2001, 2002; Holton *et al*., 2003; Kopper & Lindroth, 2003a,b),

Table 4 Standardized coefficients calculated using partial least squares regression relating aspen and birch foliar quality to gypsy moth early-instar survivorship and development time. Variables included in the final model were selected using variable importance for the projection (VIP) criteria from Wold *et al*. (1984, 2001). Negative or positive weighted coefficients indicate a negative or positive influence, respectively, of foliar quality on herbivore performance. Largest positive and negative weighted coefficients are in bold

	Aspen		Birch	
Foliar quality variable	Survivorship	Developmental time	Survivorship	Development time
Nitrogen	**0.248**	ns	**0.271**	**−0.220**
C:N	−0.299	ns	**−0.284**	0.212
Condensed tannins	ns	ns	ns	ns
PG	**−0.569**	**0.323**	na	na
Fiber	ns	0.270	ns	ns
Lignin	ns	0.245	ns	0.195

ns, not selected for final model; na, not applicable.
C : N, ratio of carbon to nitrogen; PG, total phenolic glycosides, reported as the sum of salicortin and tremulacin.

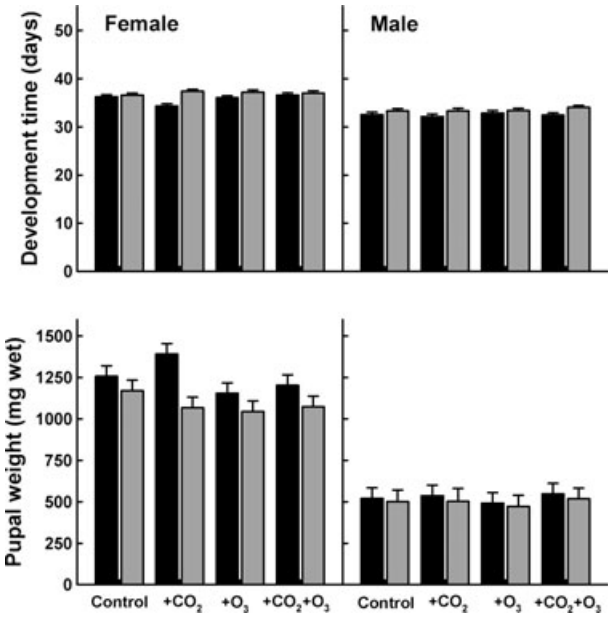

Fig. 5 Independent and interactive effects of elevated CO_2 and O_3 on gypsy moth development time from egg hatch to pupation and pupal weight when fed aspen (dark bars) or birch (light bars). Error bars represent + 1 SE.

Table 5 Summary of *P* values for the effects of CO_2, O_3, tree species, sex, and their interactions on gypsy moth development time from egg hatch to pupation and pupal weight. $P < 0.05$ values are in bold

	Development time			Pupal weight		
Treatments	df	F	*P*	df	F	*P*
CO_2	1,6.1	0.14	0.720	1,6.1	0.51	0.501
O_3	1,6.1	2.95	0.136	1,6.1	1.94	0.212
$CO_2 \times O_3$	1,6.1	1.02	0.351	1,6.1	0.16	0.700
Tree spp.	1,8.1	11.98	**0.008**	1,8.1	5.72	**0.043**
$CO_2 \times$ tree spp.	1,8.1	1.94	0.201	1,8.1	0.81	0.393
$O_3 \times$ tree spp.	1,8.1	0.44	0.524	1,8.1	0.32	0.589
$CO_2 \times O_3 \times$ tree spp.	1,8.1	1.13	0.318	1,8.1	0.51	0.494
Sex	1,111.2	324.01	**<0.001**	1,111.1	881.29	**<0.001**
$CO_2 \times$ sex	1,111.2	0.32	0.642	1,111.1	9.53	0.948
$O_3 \times$ sex	1,111.2	0.22	0.509	1,111.1	0.00	**0.035**
$CO_2 \times O_3 \times$ sex	1,111.2	0.02	0.557	1,111.1	1.72	0.834
Tree spp. × sex	1,111.2	0.44	0.573	1,111.1	4.55	**0.002**
$CO_2 \times$ tree spp. × sex	1,111.2	1.44	0.881	1,111.1	0.89	0.192
$O_3 \times$ tree spp. × sex	1,111.2	0.35	0.232	1,111.1	0.05	0.348
$CO_2 \times O_3 \times$ tree spp. sex	1,111.2	7.63	**0.006**	1,111.1	1.41	0.237

Numerator and denominator degrees of freedom (df: numerator, denominator) were calculated using the Satterthwaite approximation.

suggesting potentially complex interactions between tree developmental stage and atmospheric CO_2 levels (Körner, 2006).

Partially consistent with our second hypothesis and in agreement with our third, elevated CO_2 altered concentrations of foliar phenolic and structural compounds, but the response was detectable only in aspen and varied across time. Elevated CO_2 increased aspen condensed tannin and fiber concentrations, but the responses were greater earlier than later in spring. Elevated CO_2 had little effect on birch condensed tannin and fiber concentrations, aspen and birch lignin concentrations, and aspen phenolic glycoside concentrations.

The limited effect of elevated CO_2 on foliar quality is surprising and may potentially be due to the multiple

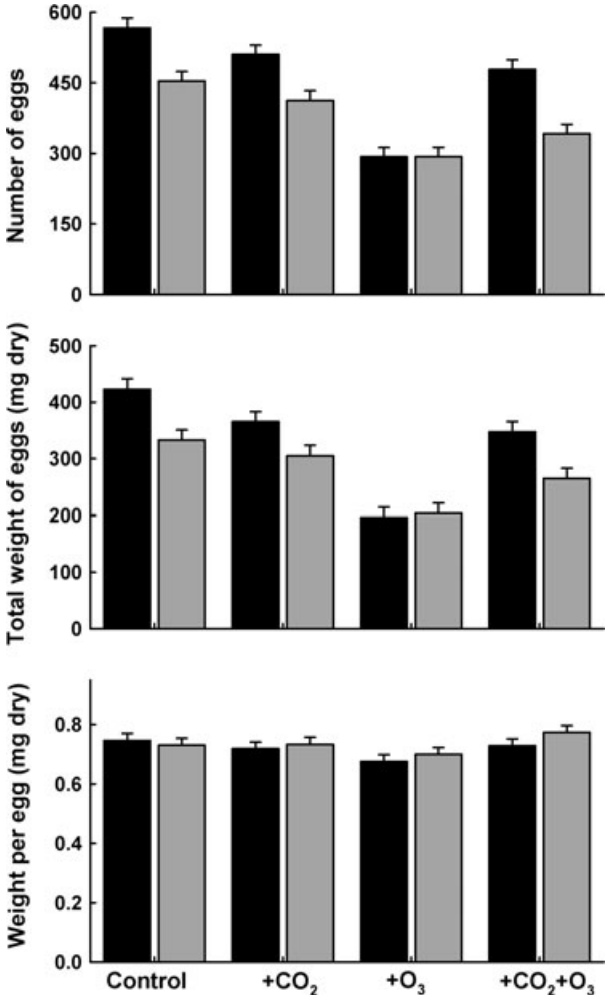

Fig. 6 Independent and interactive effects of elevated CO_2 and O_3 on gypsy moth fecundity when fed aspen (dark bars) or birch (light bars). Error bars represent + 1 SE.

ways CO_2 can affect forest ecosystems. Elevated CO_2 increases foraging for and uptake of nitrogen by plants, likely due to increased fine root production, soil organic matter decomposition, and allocation of carbon to mycorrhizal fungi (Finzi *et al*., 2007; Drake *et al*., 2011). Consistent with the notion that enhanced microbial association can facilitate increased nitrogen uptake, Andrew & Lilleskov (2009) found that ectomycorrhizal sporocarp production increased under elevated CO_2. If trees were able to uptake nitrogen proportionally with growth, then foliar nitrogen concentrations were less likely to be reduced whereas increased CO_2 levels stimulate tree productivity. Competitive interactions among trees, forest stand developmental stage, and nutrient availability are all suggested to influence responses by trees to elevated CO_2 (McDonald *et al*., 2002; Körner, 2006). Accumulating evidence from Aspen FACE, including recent chemical analyses of root sprouts from coppiced trees (RL Lindroth, unpublished data), also suggests that responses of some phytochemicals to enriched CO_2 are most pronounced in young trees.

Consistent with our first hypothesis, elevated O_3 altered the overall phytochemical profile of both tree species. In agreement with our second hypothesis and contrary to our third, elevated O_3 greatly reduced both aspen and birch foliar quality, reducing nitrogen levels and increasing C : N and sugar levels. Our findings contrast with a recent meta-analysis showing that elevated O_3 generally does not influence foliar nutrient and carbohydrate composition (Valkama *et al*., 2007). However, prolonged exposure to elevated O_3 increases sensitivity, potentially compounding the negative effects of O_3 on foliar quality (Oksanen, 2003a,b).

Consistent with our second and third hypotheses, elevated O_3 increased concentrations of phenolic and structural compounds, but the response varied by tree species. Elevated O_3 can upregulate phenolic production, via enhanced shikimic acid pathway activity, producing a number of compounds involved in stress

Table 6 Summary of P values for the effects of CO_2, O_3, tree species, and their interactions on gypsy moth fecundity. $P < 0.05$ values are in bold and P values $0.05 < P < 0.10$ are italicized

	Number of eggs			Total weight of eggs			Weight per egg		
Treatments	df	F	*P*	df	F	*P*	df	F	*P*
CO_2	1,6	4.79	*0.071*	1,5.8	4.66	*0.076*	1,5.8	2.00	0.209
O_3	1,6	73.83	**<0.001**	1,5.8	49.43	**<0.001**	1,5.8	0.49	0.509
$CO_2 \times O_3$	1,6	28.22	**0.002**	1,5.8	25.60	**0.003**	1,5.8	4.39	*0.083*
Tree spp.	1,100.2	47.24	**<0.001**	1,100.0	28.72	**<0.001**	1,100.0	1.42	0.236
$CO_2 \times$ tree spp.	1,100.3	5.91	**0.017**	1,100.0	2.10	0.151	1,100.0	0.78	0.379
$O_3 \times$ tree spp.	1,100.4	2.19	0.142	1,100.1	3.23	*0.075*	1,100.1	1.53	0.218
$CO_2 \times O_3 \times$ tree spp.	1,100.3	8.97	**0.003**	1,100.1	7.87	**0.006**	1,100.1	0.03	0.869

Numerator and denominator degrees of freedom (df: numerator, denominator) were calculated using the Satterthwaite approximation.

Table 7 Standardized coefficients calculated using partial least squares regression relating aspen and birch foliar quality to female gypsy moth pupal weight, number of eggs produced, and total egg weight. Variables included in the final model were selected using variable importance for the projection (VIP) criteria from Wold *et al*. (1984, 2001). Negative or positive weighted coefficients indicate a negative or positive influence, respectively, of foliar quality on herbivore performance. Largest positive and negative weighted coefficients are in bold

	Aspen			Birch		
Foliar quality variable	Pupal weight	Number of eggs	Total egg weight	Pupal weight	Number of eggs	Total egg weight
Nitrogen	ns	**0.252**	**0.249**	**0.246**	**0.286**	**0.297**
C:N	ns	**−0.264**	**−0.259**	**−0.259**	−0.219	−0.229
Condensed tannins	**0.396**	ns	ns	ns	ns	ns
PG	**−0.694**	ns	ns	na	na	na
Fiber	ns	ns	ns	ns	ns	ns
Lignin	−0.088	−0.230	−0.239	ns	**−0.653**	**−0.510**

ns, not selected for final model; na, not applicable.
C : N, ratio of carbon to nitrogen; PG, total phenolic glycosides, reported as the sum of salicortin and tremulacin.

responses (Close & McArthur, 2002; Cabané *et al*., 2004; Heath, 2008; Betz *et al*., 2009). We are not certain if the increase in phenolic glycosides in aspen was a response to oxidative stress caused by elevated O_3, but if so it could indirectly provide protection against unadapted herbivores (Lindroth & Hwang, 1996). Increased lignin concentrations may also be a stress response to elevated O_3, potentially providing a structural barrier and/or anti-oxidant protection from oxidative stress caused by elevated O_3 (Cabané *et al*., 2004).

Elevated CO_2 generally offset reductions in foliar nutrient composition. This finding again contrasts with the meta-analysis by Valkama *et al.* (2007) who reported reductions in foliar nutrient concentrations under the combination of elevated O_3 and CO_2. Moreover, these findings contrast with earlier findings of phytochemical responses at Aspen FACE. The reason for this contrast with earlier Aspen FACE studies is potentially a product of how longevity of exposure to elevated CO_2 and O_3 influences phytochemical responses. Over a decade of research examining phytochemical responses at Aspen FACE suggests that qualitative and quantitative responses by trees to elevated CO_2 and O_3 are indeed influenced by longevity of exposure to these gases (JJ Couture and RL Lindroth, unpublished data).

Spring folivores are highly synchronized with host phenology, in large part due to foliar quality (Feeny, 1970; Hunter, 1992; Jones & Despland, 2006). We found that elevated O_3 reduced foliar quality to a greater extent earlier in spring than later, and that CO_2 offset the reduction greater later in spring than earlier. These findings suggest that folivores feeding on ozonated foliage from early-successional, northern temperate forests will encounter reduced foliar quality during early-instar development, regardless of CO_2 level.

Herbivore performance

Elevated CO_2 generally improved gypsy moth performance. Our findings contrast with recent meta-analyses showing that herbivore performance is reduced when herbivores are fed foliage from elevated CO_2 (Zvereva & Kozlov, 2006; Stiling & Cornelissen, 2007). A number of studies, however, have shown that insect performance varies, and can even improve, under CO_2 and that responses are dependent on both tree and insect species studied (Lindroth *et al*., 1993; Roth & Lindroth, 1995; Kinney *et al*., 1997; Kopper *et al*., 2001; Holton *et al*., 2003; Hättenschwiler & Schafellner, 2004; Knepp *et al*., 2007; Peltonen *et al*., 2010). Our findings agree with the notion that environmentally mediated changes in herbivore performance often vary among herbivore and tree species (Tylianakis *et al*., 2008; Lindroth, 2010; Robinson *et al*., 2012).

Elevated O_3 greatly reduced gypsy moth early-instar performance. Consistent with our fourth hypothesis, reductions in performance were linked to decreased foliar quality. Levels of nitrogen, lignin, C : N ratios, and aspen phenolic glycosides all demonstrated strong relationships with early-instar survivorship and development time. Elevated O_3 altered all of these variables, suggesting that O_3-induced reductions in foliar quality negatively affected herbivore performance.

Reductions in foliar nitrogen generally induce compensatory feeding in herbivorous insects (Mattson, 1980; Scriber & Slansky, 1981); however, compensatory feeding can increase consumption of potentially toxic secondary compounds. Phenolic glycosides are known as effective defensive compounds in aspen against gypsy moths (Lindroth & Hwang, 1996), potentially causing oxidative stress and gut lesions (Palo, 1984; Lindroth *et al*. 1988). Reduced foliar nitrogen concentra-

tions in aspen under elevated O_3 likely induced compensatory feeding by gypsy moths, thereby increasing consumption of phenolic glycosides and reducing early-instar survivorship.

Although studies examining the short-term performance of insects fed foliage grown under elevated CO_2 and/or O_3 are abundant, few have examined the long-term performance of herbivores fed foliage from these environments (Lindroth, 2010; Robinson *et al.*, 2012). Reports vary across the few studies conducted (Lindroth *et al.*, 1997; O'Neill *et al.*, 2008; Hillstrom *et al.*, 2010). Gypsy moths fed aspen foliage from elevated CO_2 increased consumption and prolonged development, but experienced no change in female pupal weight and fecundity (Lindroth *et al.*, 1997). The weevil *Polydrusus sericeus* experienced reduced longevity and female fecundity when fed foliage from elevated CO_2, but was not affected by O_3, alone or in combination with CO_2 (Hillstrom *et al.*, 2010). The Japanese beetle *Popilla japonica* prolonged development and females increased fecundity when fed soybean from elevated CO_2, but beetles were not affected by O_3, alone or in combination with CO_2 (O'Neill *et al.*, 2008).

In the current study, we report that female gypsy moths nominally increased fecundity when fed foliage from elevated, compared with ambient, CO_2 (responses pooled across O_3 environments), but decreased fecundity when fed ozonated foliage from both tree species. These responses, however, were driven by a strong interaction between CO_2 and O_3, such that ozone reduced fecundity only in the absence of elevated CO_2. Interestingly, average weight per egg was only nominally affected by elevated CO_2 or O_3, suggesting a homeostatic control of allocation of biomass to individual eggs regardless of the number of eggs produced. We did not, however, measure additional components (e.g. vitellogenin) of egg quality that influence the performance of the resulting larvae and cannot state for certain that eggs of similar weight were of similar quality. However, egg weight is correlated with vitellogenin levels in gypsy moths (Rossiter *et al.*, 1993), suggesting that in the current study egg production was more negatively affected than egg quality when gypsy moths were fed ozonated foliage.

Partial least squares regression identified condensed tannin and phenolic glycoside concentrations as being positively and negatively related with female pupal weights, respectively, when larvae were fed aspen. Other studies have reported positive relationships between gypsy moth performance and condensed tannins (Rossiter *et al.*, 1988; Kleiner & Montgomery, 1994; Hemming & Lindroth, 1995). The positive relationship between increased female pupal weights and condensed tannins observed in this study is likely an artifact of the negative correlation ($r = -0.352$) between condensed tannins and phenolic glycosides, as has been previously suggested (Hemming & Lindroth, 1995). Nitrogen concentrations were positively related and lignin concentrations and C : N ratios were negatively related to female pupal weight when larvae were fed birch. Similarly, nitrogen concentrations were positively related and C : N ratios and lignin concentrations were negatively related to both the number of eggs produced and total egg weight when larvae were fed foliage from aspen or birch. Elevated O_3 reduced nitrogen concentrations and increased phenolic glycoside and lignin concentrations and C : N ratios, again suggesting that O_3-induced changes in foliar quality negatively affected gypsy moth performance. Our findings suggest that gypsy moth fecundity will be altered in early-successional, northern temperate forests exposed to elevated CO_2 and O_3, but highlight that responses are likely to vary among insect and tree species studied.

Elevated CO_2 ameliorated most of the negative responses by gypsy moths fed foliage from elevated O_3. Although surprising, these findings are supported by our measurements of foliar quality and have important implications for gypsy moth population dynamics and insect-mediated ecosystem processes. The independent and interactive effects of elevated CO_2 and O_3 on gypsy moth populations, via changes in foliar quality are important considering that concentrations of CO_2 are increasing at a global scale and concentrations of tropospheric O_3, while increasing, are likely to vary both spatially and temporally (Wittig *et al.*, 2009).

We cannot definitively state that altered fecundity under elevated CO_2 and O_3 will influence gypsy moth outbreak events, because of the effect of abiotic conditions, stochastic events, and natural enemies, and pathogens on gypsy moth population dynamics (Elkinton & Liebhold, 1990; Liebhold *et al.*, 1992; Sharov *et al.*, 1999). However, gypsy moth larval and egg mass densities are positively related to increased defoliation events (Liebhold *et al.*, 1998) and changes in background populations of gypsy moths under elevated CO_2 and O_3 may alter the occurrence of outbreak population levels. Our findings suggest that atmospheric change may potentially alter the population dynamics of a highly damaging, invasive forest pest. Uncoupled enemy-herbivore dynamics and climatic warming are suggested to increase the frequency and duration of insect outbreaks (Stireman *et al.*, 2005; Jepsen *et al.*, 2008) and we propose that changes in foliar quality under elevated levels of CO_2 and O_3 will also affect the incidence of insect outbreaks under future atmospheric conditions.

In summary, elevated CO_2 and O_3 independently and interactively altered the foliar quality of aspen and birch sufficiently to influence the performance of one of the most destructive, invasive folivores in North America. Our findings suggest that gypsy moths will likely be affected by phytochemical changes in host trees under future levels of CO_2 and O_3, but emphasize that responses will vary depending on interactions among CO_2, O_3, and tree species. Our findings contrast with earlier studies at this research site, suggesting that phytochemical responses by trees, and plant-herbivore interactions themselves are influenced by both tree age and longevity of exposure to elevated levels of CO_2 and O_3. These contrasts also highlight the need to continue long-term global change research in ecologically relevant environments. Longitudinal studies examining multitrophic interactions within forest ecosystems under elevated CO_2 and O_3 provide insight not only into the interactions themselves but also how they vary temporally, a key knowledge gap in experimental climate change research.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of calibration statistics for NIRS models predicting aspen and birch phytochemistry.
Figure S1. Observed versus predicted values from partial least squares regression (PLSR) models predicting gypsy moth early-instar survivorship and development time for insects when fed aspen or birch foliage.
Figure S2. Observed versus predicted values from partial least squares regression (PLSR) models predicting gypsy moth female pupal weight, number of eggs, and total egg weight for insects fed aspen or birch foliage.

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