

Elevated atmospheric CO₂: effects on phytochemistry, insect performance and insect-parasitoid interactions

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Abstract

This study was conducted to examine the effects of CO₂-mediated changes in tree chemistry on the performance of the gypsy moth (*Lymantria dispar* L.) and the parasitoid *Cotesia melanoscela* (Ratz.). We used carbon-nutrient balance theory to develop hypotheses regarding changes in tree chemistry and the performance of both insects under elevated CO₂. As predicted, levels of foliar nitrogen declined and concentrations of carbon-based compounds (e.g. starch and phenolics) increased under elevated CO₂. Gypsy moth performance (e.g. growth, development) was altered by CO₂-mediated changes in foliar chemistry, but the magnitude was small and varied across tree species. Larvae feeding on high CO₂ aspen exhibited the largest reduction in performance, relative to larvae feeding on birch, oak, or maple. Parasitism by *C. melanoscela* significantly prolonged gypsy moth development and reduced growth rates. Overall, the effect of parasitism on gypsy moth performance did not differ between CO₂ treatments. Altered gypsy moth performance on high CO₂ foliage in turn affected parasitoid performance, but the response was variable: parasitoid mortality increased and adult female size declined slightly under high CO₂, while development time and adult male size were unaffected. Our results suggest that CO₂-induced changes in plant chemistry were buffered to the extent that effects on third trophic level interactions were weak to non-existent for the system examined in this study.

Keywords: *Cotesia melanoscela*, elevated CO₂, global change, *Lymantria dispar*, tritrophic interactions

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Introduction

Recent studies have shown that elevated concentrations of atmospheric CO₂ modify tree physiology, growth, and phytochemistry (Kramer & Sionit 1987; Eamus & Jarvis 1989; Lindroth *et al.* 1993). Changes in chemical composition may in turn alter interactions between trees and associated phytophagous insects (Lindroth *et al.* 1993; Roth & Lindroth 1994b). Although changes in such interactions are likely to have consequences for higher trophic levels, few, if any, studies have investigated this possibility. The absence of multitrophic-level studies hinders our ability to predict responses of forest communities to enriched CO₂ environments.

Trees grown under elevated CO₂ conditions often exhibit decreases in foliar nitrogen, while levels of starch and/or carbon-based secondary metabolites tend to

increase (Lindroth *et al.* 1993; Roth & Lindroth 1994b). These changes in plant chemistry may affect herbivorous insects in a variety of ways (Lincoln *et al.* 1993; Lindroth 1995). Typical responses include prolonged development, increased food consumption, and reduced growth. Increased consumption may reflect compensatory feeding in response to low nitrogen content of high CO₂ foliage. Growth reductions occur in spite of increased consumption because of reduced food processing efficiencies. These changes in performance suggest that herbivore susceptibility to natural enemies may be altered as well. Extended development times may increase herbivore exposure to natural enemies, whereas nutrient deficiencies may reduce immune responses against endoparasitoids (Barbosa *et al.* 1982).

In addition to altering herbivore susceptibility to natural enemies, CO₂-mediated changes in tree chemistry may also affect the fitness of natural enemies. The size of adult endoparasitoids, for instance, may be influenced

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by the diet consumed by the herbivore host (Vinson & Barbosa 1987). Host plant allelochemicals may affect the parasitoid's ability to digest host tissues and obtain necessary nutrients (Greenblatt *et al.* 1982), thereby altering parasitoid success.

The purpose of this research was to evaluate the effects of CO₂-mediated changes in tree chemistry on herbivore-parasitoid interactions. Our specific objectives were to (1) examine the direct and interactive effects of CO₂-mediated changes in plant quality and of parasitism on performance of the gypsy moth (*Lymantria dispar* L.), and (2) assess the effects of changes in gypsy moth quality on performance of *Cotesia melanoscela* (Ratz.), a gypsy moth parasitoid.

Carbon-nutrient balance theory provides a basis for predicting trends in tree and herbivore responses to elevated CO₂. In short, this theory contends that resource availability influences patterns of allocation to growth, defence, and reproduction in plants (Bryant *et al.* 1983; Coley *et al.* 1985; Bazzaz *et al.* 1987; Tuomi *et al.* 1988). Thus, conditions that increase carbon, relative to nutrient, availability limit growth more than photosynthesis, resulting in accumulation of carbon-based compounds such as starch or phenolics. Moreover, species adapted to resource-rich environments respond more strongly to shifts in resource availability than do species adapted to resource-poor environments (Bryant *et al.* 1983, 1987). Accordingly, we formulated the following five hypotheses.

H1: Elevated CO₂ concentrations will cause levels of foliar nitrogen to decline and levels of starch and/or secondary compounds to increase. The magnitude of change will be greatest for fast-growing species and least for slow-growing species.

H2: Phytochemical changes (H1) will reduce growth and development rates, but increase consumption rates, of gypsy moth larvae.

H3: Parasitism by *Cotesia melanoscela* will alter gypsy moth performance by reducing growth and development rates.

H4: The combination of elevated CO₂ foliage and parasitism will reduce gypsy moth performance to a greater extent than will either factor alone.

H5: As gypsy moth performance declines on high CO₂ foliage, *Cotesia melanoscela* will exhibit reductions in growth and/or survival.

Materials and methods

Experimental system

We used four species of deciduous trees for this study: quaking aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.), red oak (*Quercus rubra* L.), and sugar maple (*Acer saccharum* Marsh.). These species were

selected for several reasons: (i) they are important components of forest ecosystems in the Great Lakes region; (ii) they span a range from fast-growing (aspen, birch) to intermediate- (oak) and slow-growing (maple); (iii) they differ in their responses to enriched CO₂ (Lindroth *et al.* 1993; Roth & Lindroth 1994b); and (iv) all are suitable gypsy moth hosts.

The gypsy moth (*Lymantria dispar* L.) is an outbreak species common throughout hardwood forests in northcentral and northeastern USA. Despite its polyphagous nature, larval performance varies across host species and in relation to CO₂-mediated changes in host quality (Lindroth *et al.* 1993; Roth & Lindroth 1994a, b).

The solitary endoparasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae) parasitizes young gypsy moth larvae by ovipositing directly into the body cavity (Burgess & Crossman 1929). After a 10–12 day larval period, third instar *C. melanoscela* bore through the host's integument, spin cocoons, and pupate. Development and survival of *C. melanoscela* are closely related to the diet on which the host larvae feed (Werren *et al.* 1992).

Experimental design and set-up

This experiment was conducted in controlled environment rooms at the University of Wisconsin Biotron. We used a split-plot design, with CO₂ levels (369 and 700 ppm) as whole plots. Three rooms for each CO₂ level were used to achieve true replication at the whole plot level. Subplots consisted of the four tree species.

Evergreen Nursery Co. (Sturgeon Bay, WI) provided one-year-old paper birch seedlings, and the Wisconsin Department of Natural Resources (Boscobel, WI) provided one-year-old red oak and two-year-old sugar maple seedlings. Quaking aspen were grown from seed during the year prior to the experiment in a greenhouse on the UW campus (seed source: University of Minnesota North Central Experiment Station, Grand Rapids, MN).

We planted dormant seedlings in 10 L pots containing a 2:2:1 mixture of peat, sand, and topsoil. Within each room, pots were arranged randomly, and CO₂ fumigation began immediately. We rotated pots twice during the experiment to minimize within-room variation in environmental conditions. Each room contained 14 trees of each of the 4 species. Trees were watered to saturation twice daily with half-strength Hoagland's solution (Hoagland & Arnon 1950); every third day pots were flushed with water to prevent excess salt accumulation. Rooms were maintained at 15:9 L:D, and incandescent lighting was used during the first and last hour of each light cycle to supplement red light. Photon flux density (photosynthetically active radiation, PAR) was 600 ± 36 (SD) μmol m⁻² s⁻¹. Light:dark temperatures and humidity were 25:18 °C and 60:60%, respectively.

Foliar chemistry

Midway through the insect feeding bioassays (see below), foliage (1.5–2 g per tree) was collected, flash frozen in liquid nitrogen, freeze-dried, ground, and stored at –20 °C until further analysis. Samples ($n = 240$) were collected from the same trees as used in the gypsy moth performance bioassay described below and were analysed for primary and secondary compounds known to influence gypsy moth performance. Detailed methods for each assay can be found in Roth & Lindroth (1994a, b). Foliar nitrogen was quantified with a modified Kjeldahl procedure (Parkinson & Allen 1975) followed by Nesslerization (Lang 1958). We used the Bradford protein assay (Bradford 1976; Jones *et al.* 1989), with BSA (bovine serum albumin) as the standard to measure soluble protein. Total nonstructural carbohydrates (starch, simple sugars) were analysed according to the method of Schoeneberger *et al.* (1995).

Phenolic compounds are the predominant secondary metabolites in the species used in this study. Condensed tannins were quantified in all species by the method of Porter *et al.* (1986), using purified aspen condensed tannin as a standard. Hydrolysable tannins were measured in oak and maple samples only, as aspen and birch do not contain measurable levels of these compounds (Lindroth *et al.* 1993; Roth & Lindroth 1994b). We used gallic acid and ellagic acid as standards to quantify gallotannins and ellagitannins by the methods of Inoue and Hagerman (1988) and Wilson and Hagerman (1990), respectively. Results for condensed tannins, gallotannins, and ellagitannins are presented in terms of aspen tannin, gallic acid, and ellagic acid equivalents, respectively. Finally, high-performance thin layer chromatography (HPTLC) was used to quantify the aspen phenolic glycosides salicortin and tremulacin (Lindroth *et al.* 1993).

Insect bioassays

First instar gypsy moths were obtained from Otis Air National Guard Base (Massachusetts, USA), the USDA-ARS Beneficial Insects Research Laboratory (Delaware, USA), and the USFS Northeastern Forest Experiment Station (Connecticut, USA). All larvae were reared on artificial diet (ODell *et al.* 1985) to avoid treatment effects prior to the bioassays. Hajek (1989) showed that switching between artificial diet and foliage did not negatively affect performance of gypsy moth larvae. Because the gypsy moth maintains quarantine status in Wisconsin, we were forced to conduct our bioassays on excised foliage in the laboratory. All larval rearing and bioassays were conducted in Percival environmental chambers at 24 °C, 15:9 L:D.

Cotesia melanoscela cocoons were obtained from the USDA-ARS Beneficial Insects Research Laboratory (Dela-

ware, USA). Cocoons were maintained at 12 °C for 5 days and then moved to 25 °C to initiate emergence. Females were mated and ready for oviposition after 24 h.

Twenty-two days after average tree flush (which spanned ≈ 3 days), two separate bioassays were begun. For each, larvae from different sources were distributed across treatments to minimize source effects.

Gypsy moth performance trial. This bioassay was designed to examine the direct and interactive effects of CO₂-mediated changes in foliage quality and of parasitism on gypsy moth performance. Larvae were reared through the second stadium on artificial diet; at the molt to the third stadium, half of the larvae were parasitized and half were reserved as unparasitized controls. Parasitism was accomplished by introducing a larva into a 7 mL glass vial containing a single, mated *C. melanoscela* female. Oviposition was usually rapid and was easily observed. Larvae were then placed singly into plastic petri dishes containing excised foliage in water piks. Procedures for standard, single-stadium feeding trials were followed for the duration of the third stadium (Roth & Lindroth 1994a,b). Nutritional indices (Waldbauer 1968) were calculated using standard formulae (Table 1). Two larvae (one control, one parasitized) were bioassayed for each of 10 trees per species, per room, for a total of 480 feeding trials.

Cotesia melanoscela performance trial. This bioassay was designed to determine whether *C. melanoscela* performance is affected by plant-mediated changes in gypsy moth quality under elevated CO₂. Gypsy moth larvae were reared through the second stadium on artificial diet; at the molt into the third stadium, cohorts of 6 parasitized larvae were placed in plastic petri dishes containing excised foliage in water piks. For each room × tree species combination, 4 dishes were set up and monitored until *C. melanoscela* cocoons formed. At that time, the duration of *C. melanoscela* larval development was recorded, and cocoons were removed, weighed and monitored at 25 °C until adults emerged. Metatibia lengths of adults were measured as an overall index of parasitoid fitness (Godfray 1994; M. Strand, personal communication).

Statistical methods

Foliar chemistry. We used analysis of variance (ANOVA; SAS Institute 1989; PROC GLM) to analyse phytochemical data. The split-plot model was:

$$Y_{ijk} = \mu + C_i + R_j(C_i) + S_k + (CS)_{ik} + \epsilon_{ijk}$$

where Y_{ijk} represents the average response over all trees in CO₂ level i , room j , and species k . Fixed effects included

Table 1 Larval nutritional indices (Waldbauer 1968)*

GR	Growth rate = biomass gained/day
CR	Consumption rate = food ingested/day
AD	Approximate digestibility = [(food ingested - faeces)/food ingested] × 100
ECD	Efficiency of conversion of digested food = [biomass gained/(food ingested - faeces)] × 100
ECI	Efficiency of conversion of ingested food = [biomass gained/food ingested] × 100

* all weights are expressed in mg dry weight

CO_2 level (C_i), species (S_k), and the $\text{CO}_2 \times$ species interaction [$(CS)_{ik}$]. Whole plot and subplot errors are represented by rooms nested in CO_2 [$R_j(C_i)$] and ε_{ijk} , respectively. F -tests were conducted for C_i with $R_j(C_i)$ as the error term ($F_{1,4}$), while F -tests for S_k and $(CS)_{ik}$ were computed with ε_{ijk} as the error term ($F_{3,12}$ for both). Our analysis for hydrolysable tannins was modified by omitting aspen and birch, which do not contain hydrolysable tannins. Because phenolic glycosides occur in aspen only, we used a one-way ANOVA (PROC GLM) with F tests for C_i calculated with $R_j(C_i)$ as the error term ($F_{1,4}$). We used an LSD procedure to make pairwise comparisons within a subplot (Milliken & Johnson 1992; pp. 300–304). Throughout this paper, we report results as marginally significant for comparisons with $0.05 \leq P \leq 0.10$ and as significant for comparisons with $P < 0.05$.

Gypsy moth performance trial. We performed two separate analyses for these data. The first was conducted to assess the direct and interactive effects of CO_2 and plant species, irrespective of parasitism treatment, on gypsy moth performance. To do so, we averaged the responses of the control and parasitized insects for each tree. These values were then averaged over all trees for each species within each room and analysed with the split-plot model described above. Our second analysis assessed the effects of parasitism within CO_2 levels and tree species. Due to chemical variation from tree to tree, comparisons of pooled responses (across trees) of control insects vs. pooled responses of parasitized insects were invalid. Therefore, we calculated the difference between the control and parasitized insect responses for each tree and averaged these differences over all trees for each species within each room. These data were then analysed with the split-plot model described above, using the same error terms to conduct F -tests. By combining the results of the two analyses, we were able to assess the main effects of CO_2 , species, and parasitism, as well as the interactive effects of these factors. We performed a modification of this analysis for the parameters growth rate, final weight, consumption rate, and total consumption. Because these parameters are often highly correlated with insect weight at the onset of feeding trials, we used a covariate analysis to account for the effect of initial insect weight on these parameters (see Roth & Lindroth 1994b for details).

To make pairwise comparisons across CO_2 levels within a species, we used the LSD procedure described for foliar chemistry. To make pairwise comparisons between control and parasitized insects within each $\text{CO}_2 \times$ species combination, we employed an LSD procedure (the standard error of the difference between control and parasitized insects within each $\text{CO}_2 \times$ species combination served as the error term; R. Nordheim, personal communication).

Cotesia melanoscela performance trial. Parasitoid performance was analysed by ANOVA (PROC GLM) using the model described for the phytochemical analyses. Main and interactive effects of CO_2 and tree species were determined, F -tests were conducted ($F_{1,4}$), and comparisons of means within each subplot were performed using an LSD procedure as described for foliar chemistry.

Results

Due to the complex nested design of this experiment, we present our results by describing each of the significant main effects as well as the interactive effects. Since the magnitude of responses varied among the four tree species examined, we will also identify the species for which specific trends are the greatest.

Foliar chemistry

Phytochemical analyses revealed that primary metabolite levels were significantly affected by CO_2 , species, and their interaction (Fig. 1). Foliar nitrogen declined under elevated CO_2 for all species except oak (i.e. $\text{CO}_2 \times$ species effect). Aspen contained on average twice as much nitrogen as did the other species. Analysis of soluble protein revealed a pattern similar to that for nitrogen, but the trends were less significant. Effects of elevated CO_2 on carbohydrate concentrations were variable. Levels of foliar hexose increased in high CO_2 aspen foliage only. Maple foliage contained only one-sixth as much hexose as the other species. Sucrose concentrations rose (15%) under elevated CO_2 for aspen only. Aspen and birch foliage contained 2–3 times more sucrose than oak or maple. Starch levels showed the most dramatic and significant response to elevated CO_2 , with concentrations increasing 100–200% in enriched CO_2 atmospheres. The magnitude of the CO_2 -mediated increase in starch levels

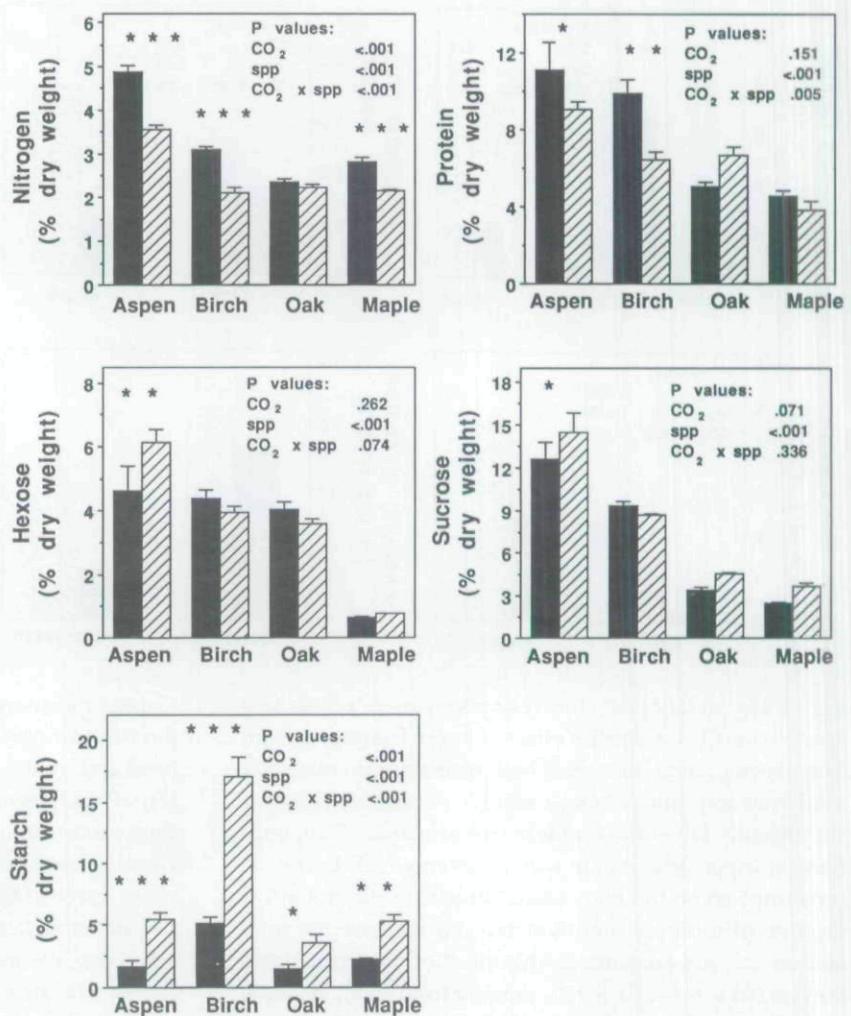


Fig. 1. Concentrations of foliar primary metabolites in quaking aspen, paper birch, red oak, and sugar maple trees grown under ambient (solid bars) or elevated (hatched bars) CO₂ conditions. Vertical lines indicate 1 SE. Asterisks represent significant pairwise comparisons within a species (*P < 0.10; **P < 0.05; ***P < 0.01).

was greatest in aspen and birch and least in oak and maple. Birch foliage contained 3–4 times as much starch as the other species.

Tree secondary chemistry was also altered in enriched CO₂ environments (Fig. 2). Levels of condensed tannins rose significantly under high CO₂ in birch and maple only (i.e. CO₂ × species effect). Concentrations of condensed tannins were 2–10 times higher in birch than in the other species. Hydrolysable tannin levels also increased under enriched CO₂ conditions, and responses were generally greater in maple than in oak. Maple contained 8 times more ellagitannins than did oak. Concentrations of the aspen phenolic glycosides salicortin and tremulacin also rose significantly under enriched CO₂ conditions.

Insect bioassays

Gypsy moth performance trial. Gypsy moth performance was influenced by CO₂-mediated changes in foliar quality, tree species, parasitism, and their interactions, although the magnitude of these effects varied among the

parameters measured (Fig. 3). Duration of the third stadium was unaffected by CO₂ level but differed across species, with larvae developing fastest on birch and slowest on oak. Parasitization significantly increased stadium duration, and the response varied across tree species, from 33% on aspen to 11% on oak (i.e. species × parasitism effect).

The response of gypsy moth growth rates and final weights to elevated CO₂ differed across tree species. Gypsy moths fed high CO₂ aspen exhibited reductions in growth rates and final weights, while larvae fed high CO₂ birch exhibited increases in final weights; growth rates and final weights of larvae fed oak or maple were unaffected by CO₂ treatment (i.e. CO₂ × species effect). Species differences were also prominent. Larvae fed aspen or birch grew 145% faster and gained 78% more weight than did larvae fed oak or maple. Parasitism reduced growth rates 26% and final weights by 8% across all species.

Increases in consumption rates and total amounts of food consumed under elevated CO₂ were marginally

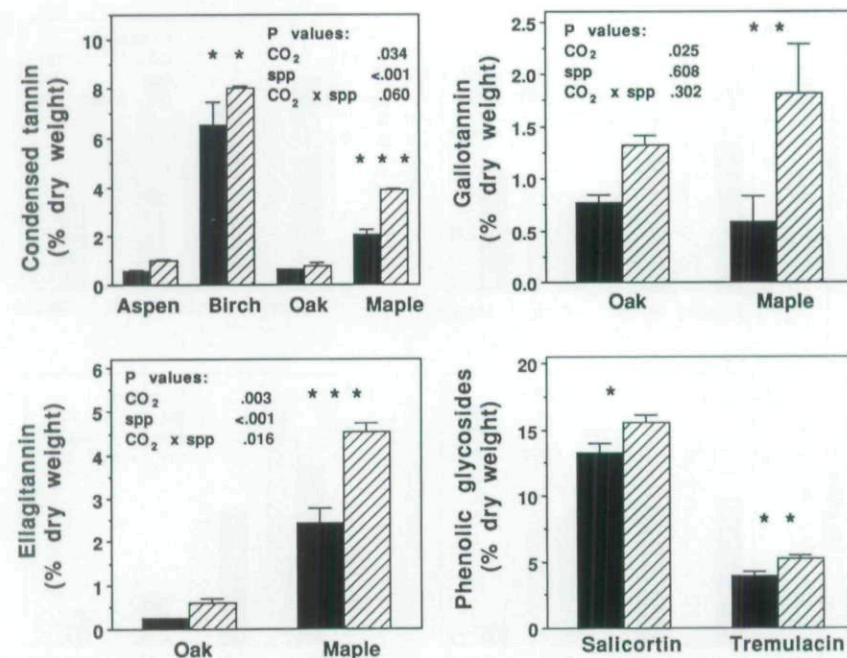


Fig. 2. Concentrations of foliar secondary metabolites in quaking aspen, paper birch, red oak, and sugar maple trees grown under ambient (solid bars) or elevated (hatched bars) CO_2 conditions. Phenolic glycoside data are for aspen only. Vertical lines indicate 1 SE. Significant pairwise comparisons are as in Fig. 1.

significant, and, again, the response varied across tree species (i.e. $\text{CO}_2 \times$ species effect). Larvae feeding on high CO_2 aspen or birch increased both consumption rates and total consumption, whereas neither parameter changed significantly when the diet was oak or maple. Consumption on aspen and maple was on average 1.5 times the consumption on birch and oak. Parasitism did not affect the rate of food consumption but did increase the total amount of food consumed. This effect on total consumption varied across CO_2 levels, ranging from an 8% increase in ambient CO_2 to a 25% increase in elevated CO_2 (i.e. $\text{CO}_2 \times$ parasitism effect).

With respect to food digestion, approximate digestibility (AD) varied most in relation to species, with aspen diets being the most digestible and birch diets the least. Parasitism resulted in an overall 10% improvement in AD. We observed no main effect of CO_2 on conversion efficiencies of digested food (ECD); ECDs declined under elevated CO_2 only for larvae on birch (i.e. $\text{CO}_2 \times$ species effect). Larvae feeding on birch were 3–7 times more efficient at converting digested food to biomass than were larvae on other diets, irrespective of CO_2 treatment. Parasitism significantly reduced ECDs only when larvae were fed aspen or birch (i.e. species \times parasitism effect). Conversion efficiencies of ingested food (ECI, the product of AD and ECD) declined under elevated CO_2 , but the change was significant only for larvae fed aspen or birch (i.e. $\text{CO}_2 \times$ species effect). Similar to the pattern observed for ECD, birch-fed larvae were most efficient at converting food to biomass, while oak-fed larvae were the least efficient. Conversion efficiencies of ingested food were reduced by parasitization, with aspen-fed larvae exhibiting the largest reduction.

Cotesia melanoscela performance trial. Performance of the parasitoid *Cotesia melanoscela* was affected both by CO_2 level and by the tree species on which the larval host fed (Fig. 4). CO_2 level had no effect on parasitoid development time except for a slight decline when hosts were fed oak. Development was prolonged 13% on oak relative to the other species. Fitness of adult parasitoids, as indicated by metatibia lengths, exhibited a significant but very small decline under elevated CO_2 for females but not for males. The strongest treatment effects on *C. melanoscela* performance occurred with respect to parasitoid mortality. Mortality included parasitoids that failed to form cocoons as well as those which formed cocoons but failed to emerge. Mortality increased when hosts were fed high CO_2 foliage, and the response was greatest on aspen. Mortality also varied among species and was highest on aspen-fed hosts.

Discussion

Foliar chemistry

Elevated CO_2 atmospheres altered foliar chemistry as predicted. We observed an overall 24% decline in nitrogen levels, a reduction similar in magnitude to those observed in previous studies (Lindroth *et al.* 1993; Roth & Lindroth 1994b). Total nonstructural carbohydrates increased under elevated CO_2 , with starch levels increasing 100–200%. In contrast, sugar levels were not strongly affected by CO_2 level. Similar trends were documented previously by Lindroth *et al.* (1993) and Roth and Lindroth (1994b) for the same species as used in this study.

Enriched CO_2 environments also affected the concentra-

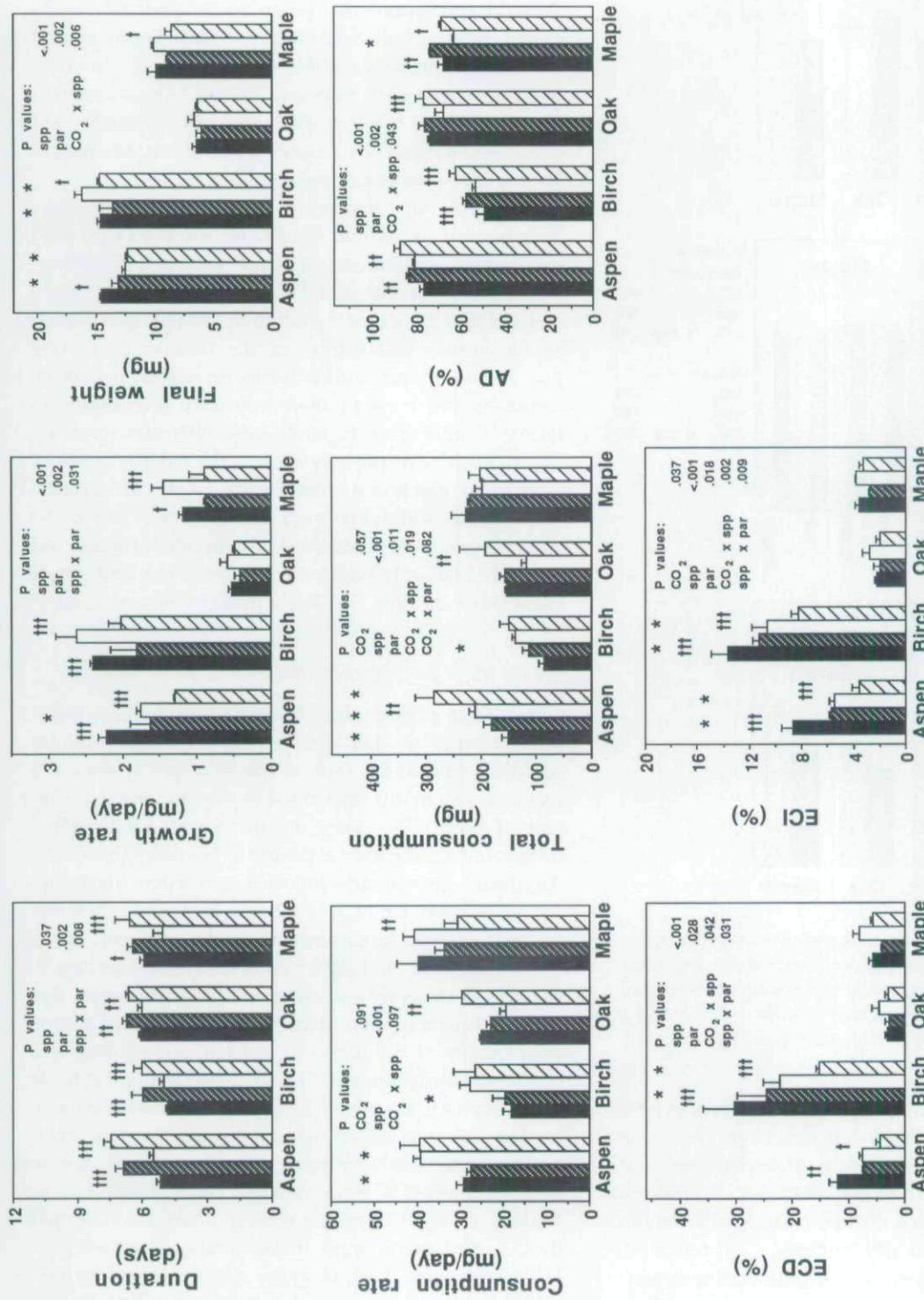


Fig. 3. Performance of control (solid bars) and parasitized (hatched bars) third instar gypsy moths from quaking aspen, paper birch, red oak, and sugar maple leaves grown in ambient (dark bars) or elevated (light bars) CO₂ atmospheres. For main and interactive effects, only significant ($P < 0.10$) P-values are shown; spp = species effect, par = parasitism effect. Vertical lines indicate 1 SE. Asterisks represent significant comparisons between CO₂ levels within each tree species, irrespective of parasitism (i.e. dark bars vs. light bars; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$). Crosses represent significant comparisons between control and parasitized insects (solid bars vs. hatched bars) within each CO₂ \times species combination († $P < 0.10$; †† $P < 0.05$; ††† $P < 0.01$). AD, approximate digestibility; ECD, conversion efficiency of digested food; ECI, conversion efficiency of ingested food.

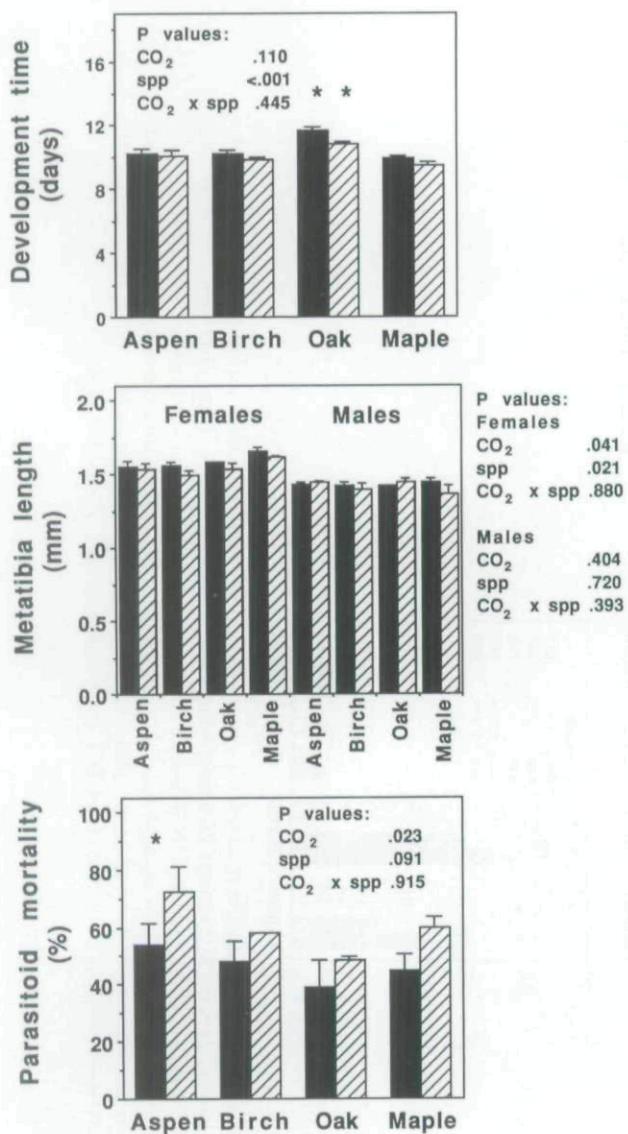


Fig. 4. Performance of *C. melanoscelsa* reared from gypsy moths fed foliage from quaking aspen, paper birch, red oak, and sugar maple trees grown in ambient (solid bars) or elevated (hatched bars) CO₂ atmospheres. Vertical lines indicate 1 SE. Within a species, pairwise comparisons were made as in Fig. 1.

tions of tree allelochemicals. Significant increases in both condensed and hydrolysable tannins were observed under high CO₂ conditions, results generally consistent with earlier studies (Lindroth *et al.* 1993; Roth & Lindroth 1994b), with the exception of oak hydrolysable tannin responses. In contrast to our findings, Lindroth *et al.* (1993) documented decreases in oak hydrolysable tannins under elevated CO₂. Genetic variation in tree stock or environmental variation such as light conditions (22% higher PAR in this study) may have influenced tree responses, particularly with respect to hydrolysable tannins. The increase in aspen phenolic glycoside levels

under high CO₂ is consistent with previous studies (Lindroth *et al.* 1993). Aspen foliage from both ambient and elevated CO₂ treatments contained phenolic glycosides in concentrations known to deleteriously affect gypsy moths (Roth & Lindroth 1994a; Hemming and Lindroth, in press). Condensed tannin levels were relatively low in aspen, reflecting the negative correlation that often exists between foliar phenolic glycosides and condensed tannins (Hemming and Lindroth, in press; S. Hwang, personal communication).

Phytochemical responses to elevated CO₂ were strongest for aspen and birch with respect to primary metabolites only. In contrast, maple exhibited the strongest response to elevated CO₂ in terms of secondary metabolite production. Lindroth *et al.* (1993) observed similar trends and attributed the differences to tree growth rates. Aspen and birch may preferentially allocate carbon to starch rather than secondary compounds to facilitate rapid growth, while inherently slow-growing species such as maple may allocate the relative excess of carbon to defensive compounds such as tannins. Although we did not measure tree growth in this study, we have previously noted that growth rates of aspen and birch are markedly higher than those of oak and maple (Lindroth *et al.* 1993; S.K. Roth, personal observation).

Gypsy moth performance trial

Gypsy moth performance was influenced by CO₂-mediated changes in diet quality, but the magnitude of response differed on each of the four tree species. On aspen, gypsy moths responded to the low nitrogen content of high CO₂ foliage by increasing consumption, thereby increasing their exposure to phenolic glycosides. As a result, growth rates and food conversion efficiencies (ECIs) declined. On high CO₂ birch, increased starch and lowered nitrogen levels also appeared to stimulate larval feeding, despite the high levels of condensed tannins in the foliage. In contrast to aspen-fed larvae, however, this increased consumption successfully compensated for the poor quality of the high CO₂ foliage, and growth was not deleteriously affected. CO₂-mediated changes in foliar chemistry of oak were slight, and gypsy moth performance on oak was accordingly unaffected. Overall, larval performance was very poor on oak, perhaps due to leaf toughness (S.K. Roth, personal observation). Larvae feeding on high CO₂ maple were in general not affected by CO₂-mediated changes in diet quality, despite higher levels of tannins. Lack of gypsy moth response to high tannin levels in elevated CO₂ foliage may reflect the fact that gypsy moths prefer to feed on tannin-rich species and thus are likely to be tannin-adapted.

In contrast to previous studies (Lindroth *et al.* 1993; Roth & Lindroth 1994b), we observed no CO₂ effect on

growth rates, except for larvae fed aspen. These differences might be due to the fact that we conducted our feeding trials with third instead of fourth instar gypsy moths. Duration of our feeding bioassays in this experiment averaged three days less than in previous studies (Lindroth *et al.* 1993; Roth & Lindroth 1994b); changes in diet quality therefore had less time to affect gypsy moth performance.

As predicted, parasitism by *C. melanoscela* reduced gypsy moth performance within the third stadium, prior to mortality. Responses to parasitism were stronger for insects on aspen and birch than for those on oak or maple. The prolonged stadium duration of parasitized larvae was likely due to the longer period of time required to gain sufficient biomass to molt successfully. Growth rates of parasitized larvae were significantly reduced relative to control larvae because of both the prolonged stadium and the lower final weights obtained. Parasitized larvae generally increased total food consumption, ostensibly to compensate for nutrients utilized by the parasitoid. Parasitism effects on AD, ECD, and ECI may reflect changes in gypsy moth consumption or manipulation of host metabolism by the parasitoid, irrespective of shifts in diet quality.

We found no support for our hypothesis that CO₂ level alters the response of gypsy moth larvae to parasitism. We observed no significant CO₂ × sting effects, except for a slight effect on total consumption. These results are not too surprising if we consider that gypsy moth performance was affected more strongly by parasitism than by CO₂-mediated changes in foliar quality. Changes in diet quality were apparently of insufficient magnitude to alter the effects of parasitism on gypsy moth performance.

Cotesia melanoscela performance trial

Our hypothesis that parasitoid performance should decline under elevated CO₂ was strongly supported only in terms of parasitoid mortality. Mortality represents the sum of mortality prior to emergence from the host (pre-emergence) and mortality after emergence but prior to eclosion (pre-eclosion). The effect of CO₂ treatment on mortality was not significant for each component individually, but was significant when the two were combined. Given that the survival of an endoparasitoid is closely linked to its host's diet, parasitoid mortality can reflect a nutrient imbalance in the host, a lack of utilizable nutrients, or a difficulty in digesting host tissues (Vinson & Barbosa 1987). Allelochemicals in the host's diet may be directly toxic to the parasitoid's tissues or may indirectly influence the parasitoid by altering the availability of nutrients in the gut (Vinson & Barbosa 1987). Parasitoid mortality was highest on aspen, which may reflect sensit-

ivity to phenolic glycosides in particular. Why diet-mediated changes in gypsy moth quality affected parasitoid mortality but not growth and development remains unclear. Allelochemicals and/or toxins in the diet could have caused mortality of parasitoid eggs (pre-emergence mortality) and may also have deleteriously affected metamorphosis (pre-eclosion mortality).

Implications and Conclusions

This study is in accordance with earlier studies that documented CO₂-mediated changes in tree chemistry, with subsequent effects on herbivorous insects (Lindroth *et al.* 1993; Roth & Lindroth 1994b). Insect performance is altered by increases and decreases in carbon and nitrogenous compounds, respectively, under enriched CO₂ conditions. Compensatory insect responses such as increased consumption may, however, modify the magnitude of such effects. Compensatory responses may buffer insects against declines in nutrient contents of foliar diets but may increase exposure to plant toxins in some instances (e.g. aspen-fed larvae). Failure of CO₂-mediated phytochemical changes to significantly impact gypsy moth performance may result in little or no effect on higher trophic levels, which appears to have been the case in our study. Systems in which herbivores respond more strongly to CO₂-mediated changes in foliar chemistry may exhibit more pronounced changes at higher trophic levels. In addition, CO₂-mediated changes in foliar quality may have stronger impacts in systems where parasitoids are intimately associated with the herbivore gut.

This study is, to our knowledge, one of the first to examine how plant-mediated changes in herbivore quality under elevated CO₂ translate into effects on higher trophic interactions. We found no evidence to suggest that CO₂-mediated changes in foliar quality enhanced the effect of parasitism on gypsy moth performance. In fact, parasitoid mortality was higher on hosts fed high CO₂ foliage. These results suggest that, at the population level, effects of parasitism on particular plant-insect associations may decrease under CO₂ environments of the future.

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