Oecologia (1994) 98:133–138

ORIGINAL PAPER

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Effects of CO_2-mediated changes in paper birch and white pine chemistry on gypsy moth performance

Received 7 October 1993 / Accepted: 9 February 1994

Abstract We examined the effects of CO_2-mediated changes in the foliar chemistry of paper birch (*Betula papyrifera*) and white pine (*Pinus strobus*) on performance of the gypsy moth (*Lymantria dispar*). Trees were grown under ambient or enriched CO_2 conditions, and foliage was subjected to plant chemical assays and insect bioassays. Enriched CO_2 atmospheres reduced foliar nitrogen levels and increased condensed tannin levels in birch but not in pine. Foliar carbohydrate concentrations were not markedly altered by CO_2 environment. Gypsy moth performance was significantly affected by CO_2 level, species, and the $CO_2 \times$ species interaction. Under elevated CO_2 conditions, growth was reduced for larvae fed birch, while development was prolonged for larvae fed pine. Although gypsy moths performed better overall on birch than pine, birch-fed larvae were influenced more by CO_2-mediated changes in host quality.

Key words *Betula papyrifera* · Carbon dioxide Feeding trials · *Lymantria dispar* · *Pinus strobus*

Introduction

The availability of carbon influences plant foliar chemistry, which in turn influences performance of phytophagous insects. Most research in this field has addressed the impacts of indirect modification of carbon availability, for example, by altering light environments (e.g., Larsson et al. 1986; Dustin and Cooper-Driver 1992; Iason and Hester 1993). More recently, and consistent with a growing awareness of the ecological consequences of global change, researchers have begun to address the direct effects of atmospheric CO_2 on plant chemistry and plant-insect interactions.

Despite the fact that forests are responsible for approximately two thirds of global photosynthesis (Kramer 1981), few studies have addressed the effects of elevated concentrations of atmospheric CO_2 on tree chemistry and tree-insect interactions. Those that have done so have shown that the impact of CO_2 is influenced by the particular plant and insect species involved (Lindroth et al. 1993). The ability of scientists to identify general patterns of response is hampered by a paucity of empirical studies.

The purpose of this research was to promote our understanding of CO_2 mediation of interactions between trees and the generalist defoliator, *Lymantria dispar*, by extending our studies to two additional host species, paper birch (*Betula papyrifera*) and white pine (*Pinus strobus*). Our specific aims were to assess (1) the effects of elevated CO_2 concentrations on the chemical composition (nutrient and allelochemical content) of a deciduous species, paper birch, and an evergreen species, white pine, and (2) how shifts in host chemistry influence the growth and food processing efficiencies of gypsy moth larvae.

Paper birch and white pine are commonly found in mixed stands throughout Canada and the northern United States (Elias 1980). In terms of secondary chemistry, both species produce phenolic compounds (e.g., condensed tannins), and white pine also produces volatile terpenoids (Mirov 1967). Paper birch is considered a preferred host of the gypsy moth, and although white pine is a less acceptable host, feeding by late-instar larvae is common (Barbosa 1978; Lechowicz and Mauffette 1986; Rossiter 1987).

Carbon-nutrient balance theory (Bryant et al. 1983; Coley et al. 1985; Bazzaz et al. 1987; Tuomi et al. 1988) provides a basis for predicting responses of trees and herbivores to elevated concentrations of CO_2. The theory contends that plant allocation to defense is influenced by carbon availability; carbon in excess of that required for growth is invested in carbon-based allelochemicals or storage compounds. Of particular interest here are phenolics, which derive from the shikimic acid pathway, and terpenoids, which derive from the mevalonate pathway.

Accordingly, we predicted that concentrations of nitrogen would be lower and concentrations of starch

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and/or carbon-based secondary compounds would be higher in foliage of trees grown under elevated CO_2. Moreover, because species adapted to resource-rich environments exhibit greater chemical responsiveness to changes in resource availability than do species adapted to less rich environments (Bryant et al. 1983, 1987), we anticipated the increase in allocation to carbon-based compounds to be greater in birch than in pine. Last, we predicted that performance of gypsy moths would decline for larvae reared on foliage grown under elevated CO_2, and that the magnitude of this effect would be greater for insects feeding on birch than on pine.

Materials and methods

Experimental design

We used a split-plot design for this study, with CO_2 levels (350 and 650 ppm) as whole plots and tree species (paper birch and white pine) as split plots. Trees were grown in controlled environment rooms at the University of Wisconsin Biotron; four separate rooms for each CO_2 treatment afforded true replication at the whole plot level.

Treatment of trees

We obtained 1-year old paper birch and white pine seedlings from Evergreen Nursery Co. (Sturgeon Bay, WI) and the Wisconsin Department of Natural Resources (Boscobel, WI), respectively. Trees were planted in 8 l plastic pots with a 2:2:1 mixture of peat:sand:forest soil and were arranged randomly among the eight rooms. Each room contained 8–10 paper birch and 3–5 white pine trees. Within each room, trees were rotated twice during the experiment to minimize effects of within-room environmental variation. An automatic drip irrigation system watered trees to saturation twice daily with half-strength Hoagland's solution. On every 3rd day trees received only water, in order to prevent salt buildup. Rooms were maintained at 15:9 L:D (photon flux density 501±83 (SE) μmol/m^2/s), with light:dark temperatures and humidity of 25°C:20°C and 70:85% respectively. Light and relative humidity measurements were made throughout the study to ensure constant conditions both within and among rooms.

Foliar chemistry

Fifty-three days after initiation of the experiment and midway through the insect feeding trials (see below), foliage samples (2–3 leaves per tree for birch, 4–5 fascicles per tree for white pine) were collected and flash-frozen in liquid nitrogen. All birch samples were freeze-dried, ground (Wiley Mill, no. 40 mesh), and stored at −20°C until further analysis. We divided each white pine sample into two subsamples. The first was freeze-dried, ground, and stored as described for birch samples; the second was not dried but stored at −80°C for monoterpene analysis.

We analyzed leaf samples for primary and secondary compounds likely to be affected by CO_2 concentrations. Concentrations of foliar water, nitrogen, protein, and nonstructural carbohydrates, including hexose, sucrose, and starch, were measured for each sample. We also quantified levels of condensed tannins in both species and monoterpenes in white pine. A survey of both species revealed no detectable hydrolyzable tannins.

Leaf water content was measured as the proportional difference between fresh and dry weight. To determine nitrogen content, samples were digested according to a modified Kjeldahl procedure (Parkinson and Allen 1975) and then analyzed by micro-Nesslerization (Lang 1958). Glycine *p*-toluene-sulfonic acid (5.665% N; Hach Co.) served as a standard.

Although nitrogen content is generally considered to be an index of protein concentrations, we performed the Bradford (1976) protein procedure as described by Jones et al. (1989) to quantify extractable proteins. Extracts [10–25 mg plant sample in 3 ml 0.1 *M* NaOH with 0.05% sodium dodecyl sulfate (SDS)] were assayed with 2.5 ml dilute Bio-Rad dye reagent (Bio-Rad Co.) to determine protein concentrations. SDS was used to improve solubility of the major plant enzyme, ribulose bisphosphate carboxylase, at the low pH required by the Bradford assay. Bovine serum albumin (BSA) served as a standard.

The method of M.M. Schoeneberger, K. Ludovici, and P. Faulkner (unpublished method) was employed to quantify nonstructural carbohydrates. This method sequentially converts fructose, sucrose, and starch to glucose, which is then quantified indirectly as the reduction of NADP to NADPH via coupled enzyme reactions. Thus we obtained measures of foliar hexose (glucose and fructose), sucrose, and starch.

To prepare extracts for condensed tannin analysis, plant material (50 mg) was extracted exhaustively in 70% acetone (with 0.1 *M* ascorbic acid) at 4°C. Condensed tannins were quantified according to the method of Porter et al. (1986), utilizing the hydrolytic conversion of proanthocyanidins to anthocyanidins. We constructed a standard curve using purified quebracho tannin.

Monoterpene analyses were conducted on the white pine samples. Needles (50 mg) were ground in liquid nitrogen, then extracted in 2 ml pentane + 200 μg *p*-cymene (Aldrich) at room temperature for 48 h. We used *p*-cymene as a standard because it is not present in detectable levels in white pine foliage and because it enables quantitative estimation of monoterpene concentrations via the internal standard method (Raffa and Steffeck 1988). Monoterpenes were analyzed by gas chromatography-mass spectrometry using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector. The column was a 30.0 m × 0.25 mm DB-1 bonded phase fused silica open tubular capillary column. After sample injection, the temperature was maintained at 60°C for 0.5 minutes, followed by an increase to 280°C at 10°C/min. Flow rate of the helium carrier gas was 1.20 ml/min. Monoterpenes were identified based on purity and fit comparisons between mass spectra of samples and those of a terpene library and the National Bureau of Standards (NBS). Using the internal standard technique, we then normalized samples to the standard, *p*-cymene and express our results as μg/g fresh weight.

Insect bioassays

Gypsy moth egg masses (New Jersey Standard Strain) were obtained from Otis Air National Guard Base (Massachusetts, USA) and surface sterilized in a 0.1% sodium hypochlorite solution. We reared insects through the third stadium on artificial diet (ODell et al. 1985) in a Percival environmental chamber at 25°C, 15:9 L:D. On day 46 of the study we began feeding trials with fourth instars. Newly-molted larvae (85–125 mg each) were placed singly into plastic petri dishes (25 × 1.5 cm) containing excised foliage in water piks. Larvae from individual egg masses were distributed across treatments to minimize genetic or maternal effects. For birch treatments, we selected leaf numbers 5 or 6 from the terminal end of each branch. For white pine, we collected one or two fascicles of new growth needles. Foliage was replaced every 1–3 days, as necessary, to maintain leaf quality. We bioassayed two insects per tree for a total of 172 feeding trials.

At the end of the fourth stadium, larvae were frozen, sexed, dried at 60°C for 1 week, and weighed. We estimated initial dry weights of larvae based on the proportional dry weight of a subsample of newly-molted fourth instars taken from the same egg masses as experimental larvae. Similarly, we obtained estimates of foliage initial dry weights from samples collected midway through the feeding trials. All frass and uneaten food were also collected, dried for 1 week at 60°C, and weighed.

Nutritional indices (Waldbauer 1968; Scriber 1977) were calculated using standard formulas for approximate digestibility

(AD) and efficiency of conversion of digested and ingested food (ECD and ECI). We used the following formulas for growth and consumption rates (GR and CR):

GR = biomass gained/day

CR = food consumed/day

Statistical methods

Analysis of variance (SAS Institute 1989; PROC GLM) was used to analyze data for tree chemistry and insect performance with respect to stadium duration, AD, ECD, and ECI. The model was as follows:

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

CO_2 level (C_i), species (S_k), and the $CO_2 \times$ species interaction $[(CS)_{ik}]$ represent fixed effects. Rooms nested in CO_2 $[R_j(C_i)]$ and ε_{ijk} are whole plot and subplot error terms, respectively. Within each subplot (species), pairwise comparisons of means were made using an LSD procedure (appropriate P values are given in the figure legends, $\alpha = 0.10$, 6 *df*).

Because the remaining insect performance parameters (growth rate, consumption rate, total consumption, and final larval weight) are typically highly correlated with insect weights at the onset of feeding trials, these values were analyzed in a manner that removes such effects. We first constructed separate regression equations relating each dependent variable (performance parameter) to initial weight for each of our subplots (= cells). Next, we calculated the grand mean initial weight over all insects used in this study; this value thus represented the "average" insect. Using this value as the independent variable, we then solved each regression equation to obtain a new data point for each cell. This data point represented the "average" insect's response to a given treatment and thus corrected for variation in initial insect weights. These data were then analyzed by ANOVA with CO_2, species, and $CO_2 \times$ species as fixed effects, as described previously.

Results

Foliar chemistry

Phytochemical analyses revealed significant main effects of CO_2 and species but no interactions of the factors (Fig. 1). Leaf water concentrations were not affected by CO_2 level for either species; pine contained slightly less water than did birch. Foliar nitrogen declined 20% in high CO_2 birch but did not change in pine. Birch leaves contained approximately twice the level of nitrogen as did pine needles. Patterns of response for soluble protein were similar to those for nitrogen.

Carbohydrate concentrations changed little in response to CO_2 treatment but differed substantially between tree species. Levels of hexose declined 18% in high CO_2 pine, and overall levels in pine were 2 times higher than in birch. In contrast, foliar concentrations of sucrose were 8–13 times greater in birch than in pine. An apparent increase in starch concentrations in both birch and pine did not prove to be statistically significant; levels were 5-fold greater in birch than in pine.

In terms of secondary chemistry, enriched CO_2 caused a marginally significant increase in condensed tannin concentrations across tree species, and the change was most pronounced (22%) for birch. Monoterpene analysis revealed six identifiable compounds present in all pine samples (Table 1). CO_2 level did not alter concentrations of any constituent.

Insect bioassays

We observed significant CO_2, species, and $CO_2 \times$ species effects on several insect performance parameters (Fig. 2). Duration of the fourth larval stadium increased an average of 15% under elevated CO_2, with the difference between CO_2 treatments greater for pine than for birch. The effect of host species on stadium duration was more pronounced than was that of host CO_2 environment; development was substantially delayed for larvae on pine diets compared to those on birch diets.

Enriched CO_2 atmospheres led to reduced growth rates and final weights for insects fed birch, but not for insects fed pine. Overall growth performance on pine was poor, however; growth rates of larvae fed pine were only 12% of those fed birch.

With respect to consumption parameters, the main effect of CO_2 (across tree species) was marginally significant only for total consumption. Larvae fed birch showed no changes in consumption, whereas those fed pine exhibited slightly elevated consumption rates and amounts for high CO_2 foliage. Significant species effects were evident; consumption rates were 2-times higher on birch than on pine, explaining in part the difference in growth for insects fed the two host species.

Variation in gypsy moth growth rates can be explained further by shifts in food digestion and conversion efficiencies. Overall digestibility (AD) and the efficiency with which digested food is converted into body mass (ECD) were not affected by CO_2 treatment, but were 1.7-times higher for insects fed birch than for those fed pine. Conversion efficiencies of ingested food (ECI, the mathematical product of AD and ECD) were reduced 20% and 23% for larvae feeding on high CO_2 birch and pine foliage, respectively.

Table 1 Effects of CO_2 level on white pine monoterpenes ($\bar{x} \pm 1$ SE); concentrations are μg/g fresh weight

	α-Pinene	Camphene	β-Pinene	Myrcene	β-Phellandrene	β-Caryophyllene
Ambient CO_2	502.1 (41.1)	60.0 (10.6)	274.6 (18.0)	67.5 (15.3)	78.8 (9.7)	92.9 (11.4)
Elevated CO_2	519.2 (81.3)	74.2 (13.1)	295.4 (59.1)	88.8 (15.6)	206.7 (113.4)	116.3 (20.6)
P values	0.858	0.434	0.747	0.369	0.304	0.359

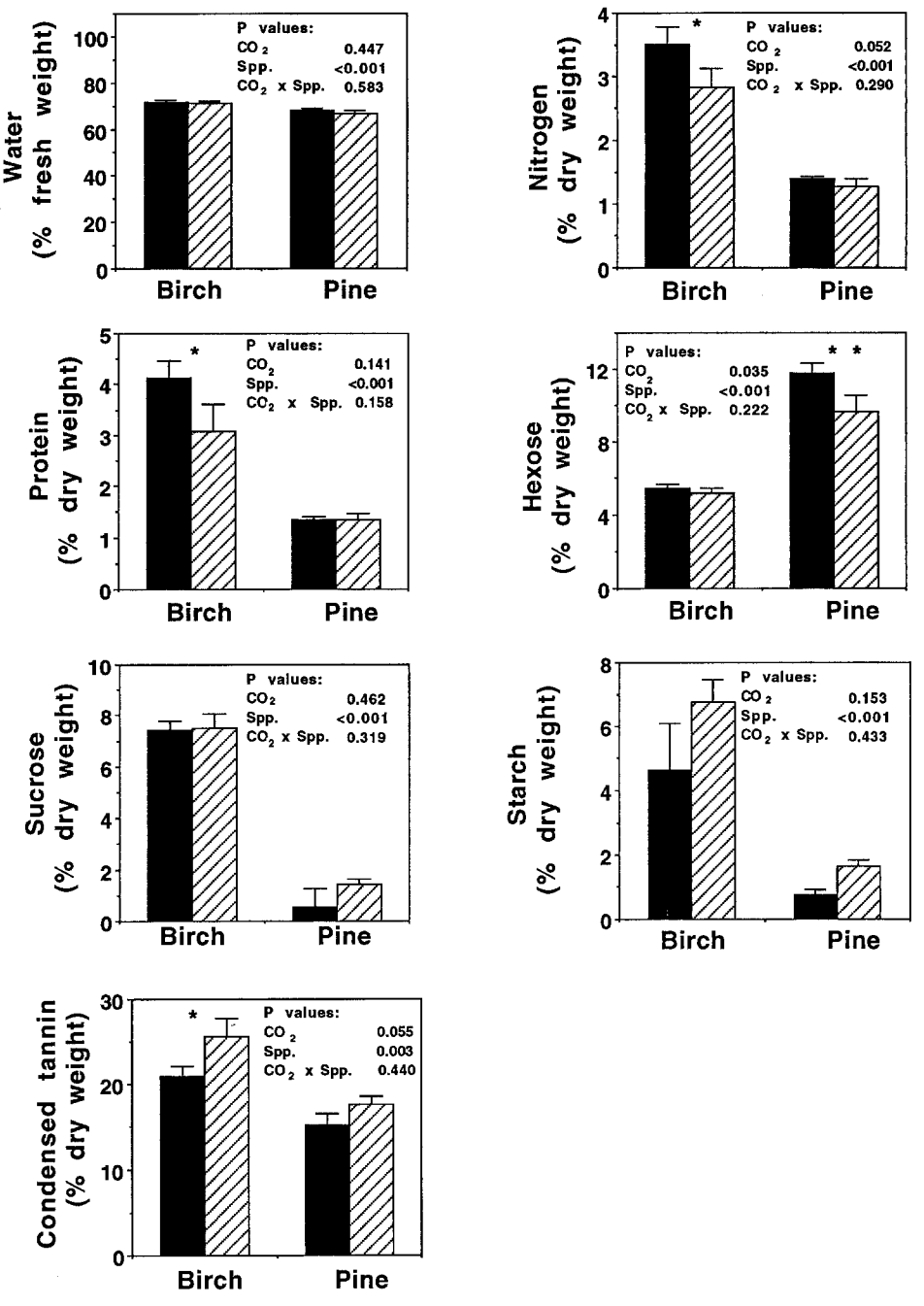

Fig. 1 Foliar nutrient and allelochemical concentrations of birch and pine trees grown in ambient (*solid*) or elevated (*hatched*) CO_2 atmospheres. *Asterisks* represent significant pairwise comparisons within a species (*$P<0.10$; **$P<0.05$; ***$P<0.01$). *Vertical lines* indicate 1 SE

Discussion

Enriched CO_2 atmospheres influenced nutrient profiles of birch and pine foliage, but the magnitude of effects was generally small. Nitrogen concentrations usually, but not invariably, decline in plants grown in high CO_2 environments (Ayres 1993; Lincoln et al. 1993). The decline of 20% observed for birch is comparable to reductions reported for quaking aspen and sugar maple (Lindroth et al. 1993). For simple carbohydrates, the only significant response observed was a reduction in hexose concentration in pine. These results are similar to those of Lindroth et al. (1993), who found no detectable shifts in simple carbohydrates in three deciduous species.

Contrary to our predictions and the results of other studies (Lincoln et al. 1993), CO_2 environment did not significantly affect starch concentrations. Many other factors, notably carbohydrate sink strength, influence starch accumulation. These factors, in combination with a lack of statistical power due to low replication, likely precluded detection of starch accumulation in birch and pine.

Only a few studies, notably with trees, have detected changes in plant allelochemical concentrations in response to enriched CO_2 atmospheres (Lindroth et al. 1993; K.K. Kinney, R.L. Lindroth, S.M Jung and E.V. Nordheim, submitted). We found that CO_2 treatment increased condensed tannin levels in birch, but did not alter tannin or monoterpene levels in pine. We caution that

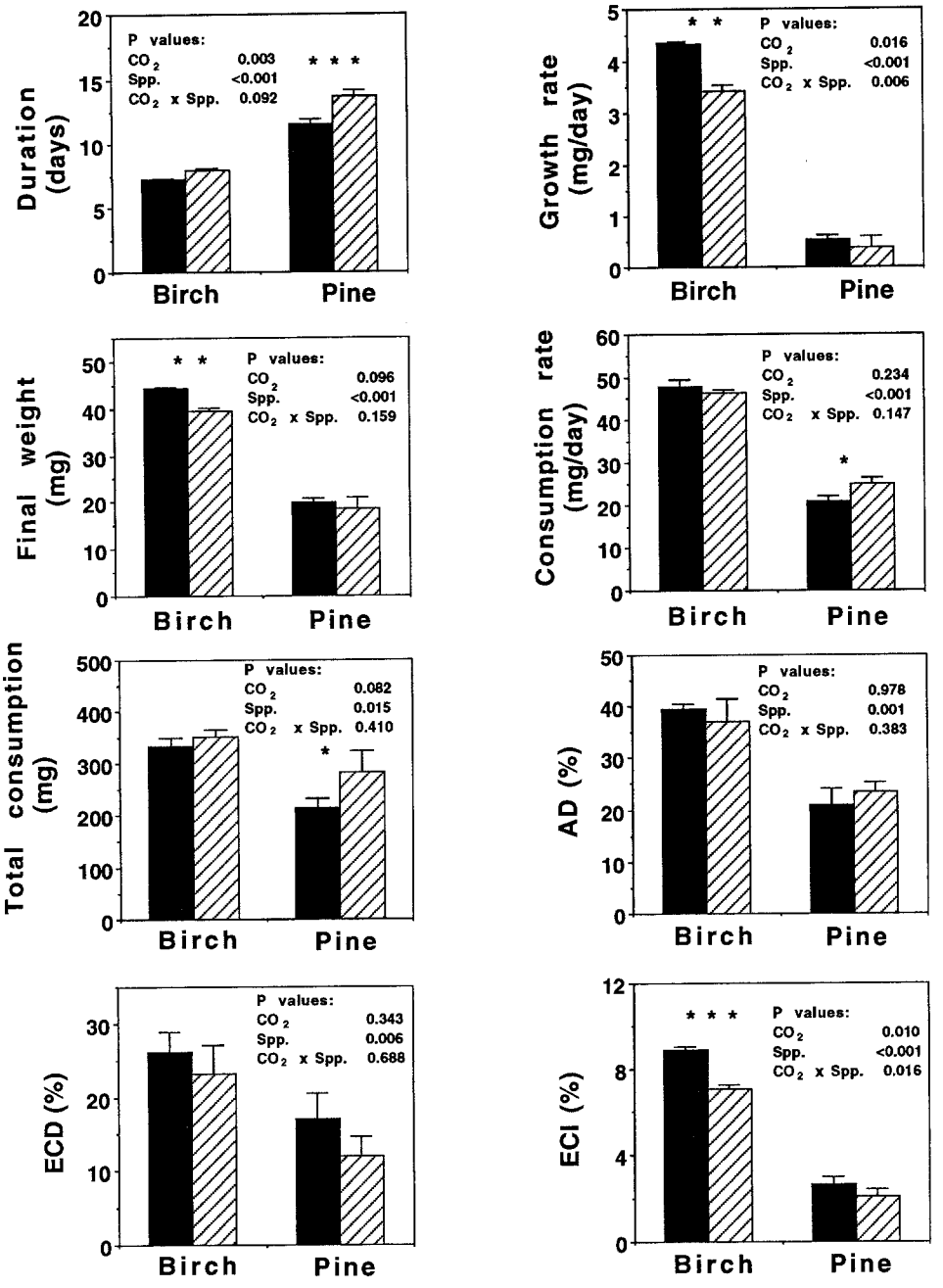

Fig. 2 Performance of fourth instar gypsy moths fed foliage from birch or pine trees grown in ambient (*solid*) or elevated (*hatched*) CO_2 atmospheres. *Asterisks* represent significant pairwise comparisons as in Fig. 1. *Vertical lines* indicate 1 SE

failure to detect an increased concentration of monoterpenes does not mean that biosynthesis of the compounds was unaltered. For allelochemicals such as monoterpenes that may experience fairly rapid turnover (but see Mihaliak et al. 1993), foliar concentrations represent the dynamic equilibrium between anabolic and catabolic processes (Reichardt et al. 1991; Herms and Mattson 1992).

Although the magnitude of CO_2-induced changes in foliar chemistry was generally small, patterns of plant responses were largely consistent with the predictions of carbon-nutrient balance theory. Levels of nitrogen declined while levels of condensed tannins (and possibly starch?) increased. Moreover, as predicted, responses were stronger in birch than in pine.

Altered foliar chemistry in turn effected changes in insect performance, reducing growth in insects fed birch and prolonging development in insects fed pine. For larvae fed birch, decreased levels of nitrogen and increased levels of condensed tannins reduced the efficiency with which ingested food was converted to body mass (ECI). Lindroth et al. (1993) documented similar reductions in growth rates and ECIs for gypsy moths fed high CO_2 aspen foliage. Consistent with our prediction, the magnitude of effects of CO_2-mediated changes in host quality was greater for insects fed birch than for insects fed pine.

Host species affected larval performance much more strongly than did host CO_2 environment. Larvae feeding

on pine ate less food, gained less weight, and required more time to develop to the fifth instar than did their counterparts on birch. Poor performance may have resulted from low nitrogen and starch levels, the presence of monoterpenes, or from leaf quality factors not measured, such as resin acids or toughness.

In summary, results from this study reveal once more that enriched CO_2 atmospheres can significantly alter the foliar chemical composition of trees and that these changes may elicit changes in the performance of phytophagous insects. As was reported previously for research with quaking aspen, red oak, and sugar maple (Lindroth et al. 1993), the directions and magnitudes of change vary among tree species. Such species-specific effects suggest that the community dynamics of interactions between the gypsy moth and its hosts will shift in forest environments under elevated concentrations of atmospheric CO_2.

Acknowledgements We thank T. Buss, S. Jung, and A. Nussbaum for technical assistance, T. Mayhew and J. Phillips for conducting the GC analyses, K. Kinney and R. Nordheim for statistical advice, and Evergreen Nursery Co. and the Wisconsin DNR for providing trees. Research funds were provided by NSF grant BSR-8918586 to RLL and an NSF Graduate Research Fellowship to SKR. This work contributes to the Core Research Programme of the Global Change in Terrestrial Environments (GCTE) Core Project of the International Geosphere-Biosphere Programme (IGBP).

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