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CONSEQUENCES OF ENRICHED ATMOSPHERIC CO_2 AND DEFOLIATION FOR FOLIAR CHEMISTRY AND GYPSY MOTH PERFORMANCE

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Abstract—Elevated concentrations of atmospheric CO_2 are likely to interact with other factors affecting plant physiology to alter plant chemical profiles and plant-herbivore interactions. We evaluated the independent and interactive effects of enriched CO_2 and artificial defoliation on foliar chemistry of quaking aspen (*Populus tremuloides*) and sugar maple (*Acer saccharum*), and the consequences of such changes for short-term performance of the gypsy moth (*Lymantria dispar*). We grew aspen and maple seedlings in ambient (~360 ppm) and enriched (650 ppm) CO_2 environments at the University of Wisconsin Biotron. Seven weeks after budbreak, trees in half of the rooms were subjected to 50% defoliation. Afterwards, foliage was collected for chemical analyses, and feeding trials were conducted with fourth-stadium gypsy moths. Enriched CO_2 altered foliar levels of water, nitrogen, carbohydrates, and phenolics, and responses generally differed between the two tree species. Defoliation induced chemical changes only in aspen. We found no significant interactions between CO_2 and defoliation for levels of carbon-based defenses (phenolic glycosides and tannins). CO_2 treatment altered the performance of larvae fed aspen, but not maple, whereas defoliation had little effect on performance of insects. In general, results from this experimental system do not support the hypothesis that induction of carbon-based chemical defenses, and attendant effects on insects, will be stronger in a CO_2-enriched world.

Key Words—*Acer saccharum*, carbohydrates, carbon dioxide, feeding trials, induced defenses, *Lymantria dispar*, phenolic glycosides, plant-insect interactions, *Populus tremuloides*, tannins.

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INTRODUCTION

Allocation of fixed carbon to growth, reproduction, and defense is closely linked to internal source-sink dynamics in plants. These, in turn, are shaped by plant responses to variation in resource availability over ecological and evolutionary time scales (Bryant et al., 1983; Herms and Mattson, 1992; Lerdau et al., 1994; Mole, 1994).

Availability of one critical plant resource, carbon dioxide, has been increasingly globally since the late 18th century, and atmospheric concentrations continue to rise at about 0.4% per year (Houghton et al., 1996). One biological effect of this change is a shift in the chemical composition of plants. In general, foliar C:N ratios increase because of decreases in nitrogen and increases in carbon-based compounds such as starch and phenolics (Lincoln et al., 1993; Watt et al., 1995; Lindroth, 1996). Such changes in plant chemical composition can alter the feeding and growth performance of phytophagous insects (Lincoln et al., 1993; Watt et al., 1995; Lindroth, 1996). The magnitudes of change in both foliar chemistry and insect performance differ, however, among plant and insect species and in relation to the availability of other required resources (Kinney et al., 1997; Roth et al., 1997).

Herbivory itself can alter plant chemical composition either through changes in source-sink dynamics or through elicitation of active defensive responses (Tallamy and Raupp, 1991; Baldwin, 1994; Lerdau et al., 1994; Karban and Baldwin, 1997). Moreover, the magnitude of induced responses, and effects on herbivores, can be influenced by resource availability (Bryant et al., 1993; Yates and Peckol, 1993; Hunter and Schultz, 1995).

Recognizing the importance of both enriched CO_2 and herbivory to community dynamics and ecosystem function, researchers have begun to address how those factors *interact* to influence plant–herbivore associations. The few studies conducted to date, however, have emphasized interactive effects on plant physiology and growth (e.g., Fajer et al., 1991; Pearson and Brooks, 1996; Wilsey et al., 1997; Kruger et al., 1998). Exceedingly few have addressed the consequences of such interactions for plant chemical composition and herbivore performance. Such studies are vitally important for improving our understanding of trophic dynamics in a CO_2-enriched world.

Here we assess the independent and interactive effects of enriched atmospheric CO_2 and defoliation on the foliar chemistry of deciduous trees and the performance of a leaf-feeding insect. We predicted that growth under enriched CO_2 would enhance production of induced, carbon-based defenses in quaking aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh.). Furthermore, we predicted that enhanced defensive capacity would reduce the growth and feeding performance of gypsy moth (*Lymantria dispar* L.) larvae.

Several factors influenced selection of this experimental system. Firstly,

both aspen and maple produce carbon-based secondary metabolites, concentrations of which are responsive to atmospheric CO_2 levels (Lindroth et al., 1993; Roth and Lindroth, 1995; Kinney et al., 1997). Secondly, CO_2-mediated changes in foliage can alter performance of gypsy moth larvae (Lindroth et al., 1993; Roth and Lindroth, 1995; Kinney et al., 1997). Thirdly, these tree species are known to respond to defoliation by increasing foliar concentrations of phenolics, including phenolic glycosides in aspen (Clausen et al., 1989) and condensed and hydrolyzable tannins in maple (Baldwin and Schultz, 1983). Finally, aspen and maple are important constituents of forest ecosystems in the Great Lakes region, where gypsy moths are now becoming established.

METHODS AND MATERIALS

Experimental Design. This study was conducted in eight environmental control rooms at the University of Wisconsin Biotron. We employed a split-plot design, with two crossed factors (CO_2 and defoliation) as whole plots and tree species (aspen and maple) as the subplot. CO_2 treatments were designated as ambient ($\sim$360 ppm) and enriched (650 ppm). Defoliation treatments consisted of control (0% defoliation) and defoliated (50% defoliation) rooms. Two separate rooms for each CO_2 $\times$ defoliation combination (7–10 trees/species/room) afforded true, although little, replication at the whole-plot level. An alternative design, with defoliation treatments nested within CO_2 treatments, would have afforded greater statistical power to detect defoliation and CO_2 $\times$ defoliation interactions. We did not use such a design because of concerns about elicitation of defensive responses in undamaged trees due to "communication" with neighboring defoliated trees (Farmer and Ryan, 1990).

Controlled Growth Conditions. One-year-old aspen and 2-year-old maple seedlings were used in this study. Aspen were grown from seed the previous year and stored, bare-rooted, in a cold room over winter. [Aspen seeds (full sibs) were obtained from the University of Minnesota North Central Experiment Station (Grand Rapids, Minnesota)]. Maple seedlings were provided by the Wisconsin Department of Natural Resources (Wilson Nursery, Boscobel, Wisconsin). Trees were randomly assigned to CO_2 and defoliation treatments. Another set of trees, identically treated, was used for assessments of treatment effects on gas exchange and growth. Results from that parallel study are reported by Kruger et al. (1998).

Dormant seedlings were planted in 11-liter pots with a 1:2:2 mixture of forest topsoil, peat, and sand. An automatic drip irrigation system provided nutrients and/or water twice a day. The watering regime consisted of two days with nutrient solution followed by one day of water only to prevent accumulation of salts. The nutrient solution (half-strength Hoagland's solution) provided nitrate

at a concentration of 7.5 mM, sufficient for excellent tree growth (Kinney and Lindroth, 1997). We used a 15L:9D photocycle as representative of early summer in southern Wisconsin. At maximum light intensity, photon flux density (photosynthetically active radiation) averaged 501 ± 83 (SE) μmol/m^2/sec; intensity was reduced to 40% of maximum during the first and last 30 min of each photocycle. Light–dark temperatures and humidities were maintained at 25:20°C and 70:85%, respectively. Pots were rotated within rooms twice during the experiment.

Defoliation Treatment. The defoliation treatment was conducted seven weeks after average budbreak. Half of each leaf on each defoliated tree was removed by cutting, with hair texturizing scissors, along one side of the midrib. This technique produced a ragged leaf edge, mimicking feeding by large caterpillars.

Foliar Chemistry. Chemical analyses were conducted on leaf tissues to determine concentrations of nutritional factors (water, nitrogen, protein, and carbohydrates) and secondary metabolites (various phenolics). Sampling of each tree was accomplished by clipping several leaves cleanly at the petiole, then flash-freezing tissue in liquid nitrogen. Subsequently, samples were freeze-dried, ground (No. 40 mesh), and stored at −20°C. We sampled leaves from 7–10 trees per species per room on two occasions. The first sample was taken one day prior to the defoliation treatment; this collection allowed us to ascertain whether any chemical differences existed between control and "defoliated" trees before the treatment was applied. The second was six days following defoliation, during the period of insect feeding bioassays.

Leaf water content was determined gravimetrically. Tissue nitrogen concentrations were measured by conducting Kjeldahl digestions (25–50 mg tissue) (Parkinson and Allen, 1975), followed by nitrogen determinations using the micro-Nesslerization technique of Lang (1958). Glycine *p*-toluene-sulfonic acid was used as the standard.

Soluble protein was quantified by the procedure of Jones et al. (1989). Samples of 10–25 mg were extracted in 0.1 N sodium hydroxide containing 0.05% sodium dodecyl sulfate (SDS). SDS was added to prevent precipitation of protein upon addition of the acidic Bio-Rad dye reagent. Bovine serum albumin served as the standard. Values for protein concentrations obtained by this method are typically less than those derived from the common practice of multiplying total nitrogen concentrations by 6.25 (Jones et al., 1989; Lindroth, unpublished data), indicating that a portion of plant protein may not be soluble in this assay and/or that other foliar components contribute significantly to total nitrogen concentrations.

The carbohydrate procedure of Schoeneberger, Ludovici, and Faulkner (unpublished) was used to analyze starch and soluble sugars [hexose (fructose, glucose) and sucrose]. Leaf tissue (25 mg) was extracted in 80% ethanol. Sugars

and starch were then enzymatically converted to glucose and quantified indirectly via coupled enzyme reactions that reduce NADP to NADPH in amounts proportional to the glucose content in each sample.

The major secondary metabolites of aspen and maple are phenolics, including phenolic glycosides (salicylates) in aspen, hydrolyzable tannins (gallotannins and ellagitannins) in maple, and condensed tannins in both species. We measured levels of salicortin and tremulacin, the most abundant salicylates in aspen, via high performance thin-layer chromatography (HPTLC) (Lindroth et al., 1993). Leaf samples (50 mg) were extracted in methanol and duplicate aliquots (1 μl) were developed on HPTLC plates (silica gel 60, 10 $\times$ 20 cm). The plates were scanned at 274 nm using a Camag Scanner II (Camag Scientific, Inc., Wrightsville Beach, North Carolina), and chromatograms were analyzed using Camag TLC software (CATS 3.11). Salicortin and tremulacin purified by sequential flash chromatography and thin-layer chromatography served as standards.

Concentrations of gallotannins were measured by the procedure of Inoue and Hagerman (1988) as modified by Lindroth et al. (1993). Maple leaf tissue (50 mg) was extracted in 70% acetone containing 10 mM ascorbic acid. Extracts were then hydrolyzed in acid (1 M H_2SO_4) and the hydrolysate was assayed for total gallic acid. A similar analysis was completed on nonhydrolyzed extract to measure levels of free gallic acid. Gallotannin concentrations were then calculated as the difference between total and free gallic acid, and values are presented as gallic acid equivalents. Ellagitannins were quantified using the method of Wilson and Hagerman (1990) as modified by Lindroth et al. (1993). Tissue samples (15 mg) were hydrolyzed in acid (1 M H_2SO_4) and ellagic acid was measured according to the standard method. Ellagitannin concentrations are given in terms of ellagic acid equivalents.

For condensed tannin determinations, leaf tissue was extracted as described previously for gallotannins. Tannin concentrations were measured via the hydrolytic conversion of proanthocyanidins to anthocyanidins according to the method of Porter et al. (1986). Results are expressed in terms of quebracho tannin equivalents. Because tannins from different species react differently in the assay, interspecific differences should be interpreted cautiously.

Gypsy Moth Bioassays. Gypsy moth egg masses (New Jersey Standard Strain) were obtained from the Otis Methods Development Center (Otis Air National Guard Base, Massachusetts). Larvae were reared in an environmental chamber (Percival, Boone, Iowa) in the Department of Entomology's Gypsy Moth Quarantine Facility. Insects were maintained at 25°C with a photoperiod of 15L:9D. During stadia 1–3, larvae were fed artificial diet (ODell et al., 1985).

Bioassays were conducted with fourth (penultimate) instars to assess effects of CO_2 and defoliation treatments on insect consumption, growth, and food

processing efficiencies. We conducted assays with one to two larvae for each of 7–10 trees per species per room (total of 20–26 insects per CO_2 × defoliation × tree species combination). The trials commenced one day following defoliation of the trees. Newly molted caterpillars were placed individually into 15- × 1.5-cm plastic Petri dishes containing a single damaged or undamaged leaf. Leaves were excised from trees at the base of the petiole, and petioles were inserted into water picks to maintain leaf hydration. Leaves were replaced, as necessary, at least every two to three days. Upon molting into the fifth stadium, larvae were frozen. Larvae, frass, and uneaten leaf material were dried (65°C) and weighed. To estimate the initial dry weights of larvae used in the bioassays, we determined the proportional dry weight of 25 larvae from the same pool of insects as used in the bioassays. Calculated initial weights of larvae were not statistically different among experimental treatments. To estimate initial dry weights of leaves used in bioassays, we regressed fresh weight on dry weight for leaves used in the chemical analyses. The resulting regression equations were then used to estimate initial dry weights of bioassay leaves from their measured fresh weights. We calculated the following insect performance parameters: growth rate, final weight, consumption rate, total consumption, approximate digestibility {AD: [(food ingested − frass)/food ingested] × 100}, and efficiency of conversion of digested food {ECD: [biomass gained/(food ingested − frass)] × 100}.

Statistical Analyses. We used analysis of variance (ANOVA) (PROC GLM, SAS Institute, Inc., 1989) for statistical analyses of plant chemical and insect performance data. The general split-plot model with two factors at the whole plot level and one at the subplot level was:

$$Y_{ijkl} = \mu + C_i + D_j + (CD)_{ij} + E_{ijk} + S_l + (CS)_{il} + (DS)_{jl} + (CDS)_{ijl} + e_{ijkl}$$

where Y_{ijkl} represents the average response (phytochemical or insect) in CO_2 level i, defoliation treatment j, room k, and tree species l. Fixed effects consisted of CO_2 level (C_i), defoliation (D_j), CO_2 × defoliation interaction $(CD)_{ij}$, species (S_l), CO_2 × species interaction $(CS)_{il}$, defoliation × species interaction $(DS)_{jl}$, and CO_2 × defoliation × species interaction $(CDS)_{ijl}$. Random effects included whole-plot error (E_{ijk}) and subplot error (e_{ijkl}). F tests for C_i, D_j, and $(CD)_{ij}$ were computed using E_{ijk} as the error term ($F_{1,4}$); F tests for S_l, $(CS)_{il}$, $(DS)_{jl}$, and $(CDS)_{ijl}$ were computed using e_{ijkl} as the error term ($F_{1,4}$). In situations where not all the terms were relevant to the basic model (e.g., for secondary metabolites occurring in only one tree species), the model parameters were adjusted accordingly. Cell means for CO_2 × defoliation × species × room were computed using the SAS MEANS procedure prior to analysis of variance. The standard errors we report are for the treatment means, $\bar{Y}_{ijl}$, computed by the SAS MEANS procedure ($N = 2$ rooms).

Because of the low level of replication of CO_2 and defoliation treatments in this study, the probability of type II statistical errors was high. We therefore refer to P values in the range 0.05–0.10 as "marginally significant."

Growth and feeding of insect larvae are correlated with size. We used the regression procedure described by Kinney et al. (1997) to remove the effect of initial size on growth and consumption variables. Adjusted values for growth rate, final larval weight, consumption rate, and total consumption were then analyzed with the general linear model previously described.

RESULTS

Foliar Chemistry. We found no statistically significant differences in foliar chemical composition between control and defoliated trees one day prior to the defoliation event (data not shown). Thus, the following responses attributed to defoliation can be construed as true treatment effects rather than to preexisting variation between nondefoliated and defoliated trees.

Enriched CO_2 and defoliation decreased and increased, respectively, foliar water content (Figure 1). Overall changes were slight, however, and tended to be stronger in aspen than in maple, although species interaction effects were not significant. Nitrogen concentrations declined 12 and 24%, respectively, in aspen and maple under high CO_2 concentrations, but did not change in relation to defoliation (Figure 1). Overall nitrogen concentrations were higher in aspen than in maple. Responses of soluble protein differed from those of nitrogen. Protein levels declined under enriched CO_2, but the response was much greater in aspen than in maple (Figure 1). Defoliation led to a 24% decline in protein concentration of aspen under ambient CO_2, a 53% increase under enriched CO_2, and no significant change in maple (significant three-way interaction).

CO_2 concentration and defoliation also altered leaf carbohydrates, affecting levels in aspen more than those in maple. With respect to hexose concentrations, responses were dominated by the three-way interaction among CO_2, defoliation, and species (Figure 2). That is, enriched CO_2 led to a much greater increase of hexose in aspen than in maple, but this response was ameliorated in defoliated trees. In contrast, sucrose levels were unaffected by both CO_2 and defoliation treatments (Figure 2). Responses of foliar starch mostly paralleled those of hexose (Figure 2); levels were 2.7-fold higher in enriched-CO_2, nondefoliated aspen but only 1.6-fold higher in enriched-CO_2, defoliated aspen.

Foliar phenolic levels responded to the direct, but not interactive, effects of CO_2 and defoliation treatments. In aspen, levels of the phenolic glycoside tremulacin tended to increase (24–27%) in high-CO_2 foliage, whereas levels of both tremulacin and salicortin tended to increase (31–75%) in damaged foliage (Figure 3). In maple, concentrations of gallotannins and ellagitannins increased

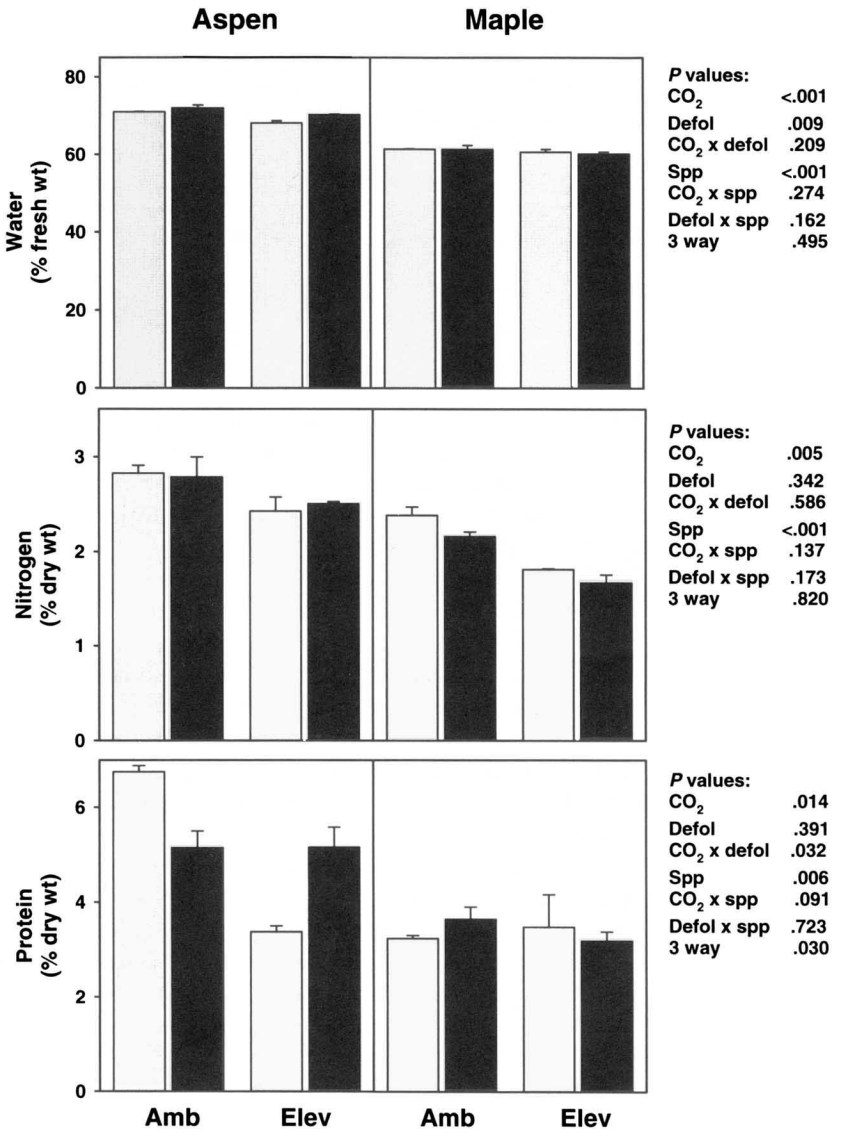

FIG. 1. Concentrations of water, nitrogen, and soluble protein in foliage of nondefoliated (light bars) and partially defoliated (dark bars) trees grown in ambient (Amb) or elevated (Elev) concentrations of CO_2. Vertical lines represent 1 SE. Defol = defoliation effect; Spp = species effect; 3-way = CO_2 × defoliation × species interaction.

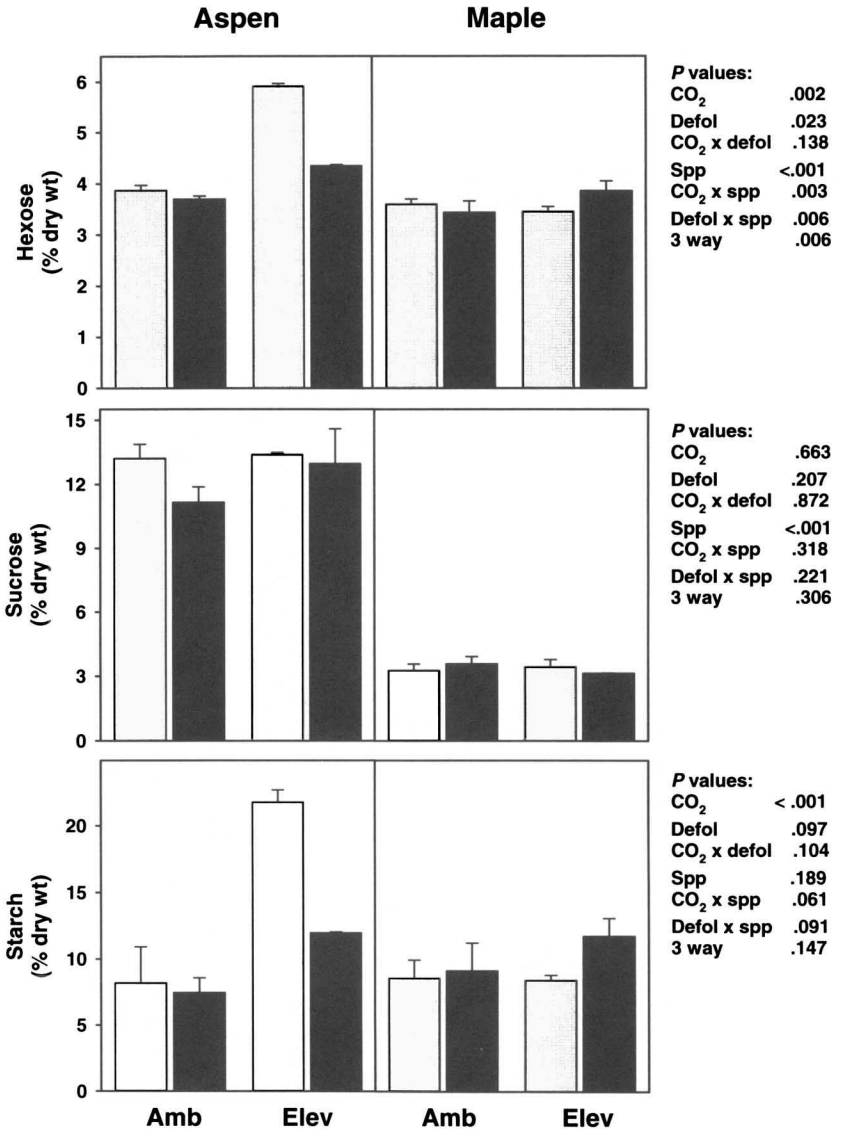

FIG. 2. Concentrations of carbohydrates in foliage of nondefoliated (light bars) and partially defoliated (dark bars) trees grown in ambient (Amb) or elevated (Elev) concentrations of CO_2. Format as described for Figure 1.

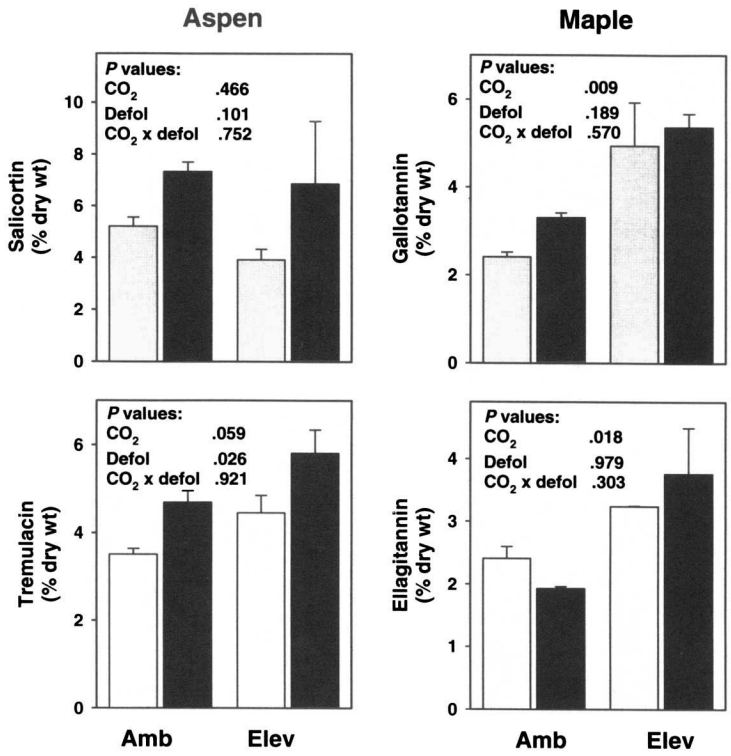

FIG. 3. Concentrations of phenolic glycosides (salicortin and tremulacin) in aspen foliage and hydrolyzable tannins (gallotannins and ellagitannins) in maple foliage of nondefoliated (light bars) and partially defoliated (dark bars) trees grown in ambient (Amb) or elevated (Elev) concentrations of CO_2. Format as described for Figure 1.

80 and 61%, respectively, under enriched CO_2, but were unaffected by defoliation (Figure 3). Similarly, levels of condensed tannins increased under enriched CO_2 in aspen (44%) and maple (59%), but were not affected by defoliation (Figure 4).

C:N ratios were calculated as the concentration of carbohydrates + phenolics relative to the concentration of nitrogen. C:N ratios increased substantially in response to high CO_2 in both aspen and maple, but were not significantly affected by defoliation in either species (Figure 4).

Insect Performance. In general, both CO_2 and defoliation treatments had little to moderate impact on insect performance. Duration of the fourth larval stadium was prolonged in insects reared on high-CO_2 foliage, especially in

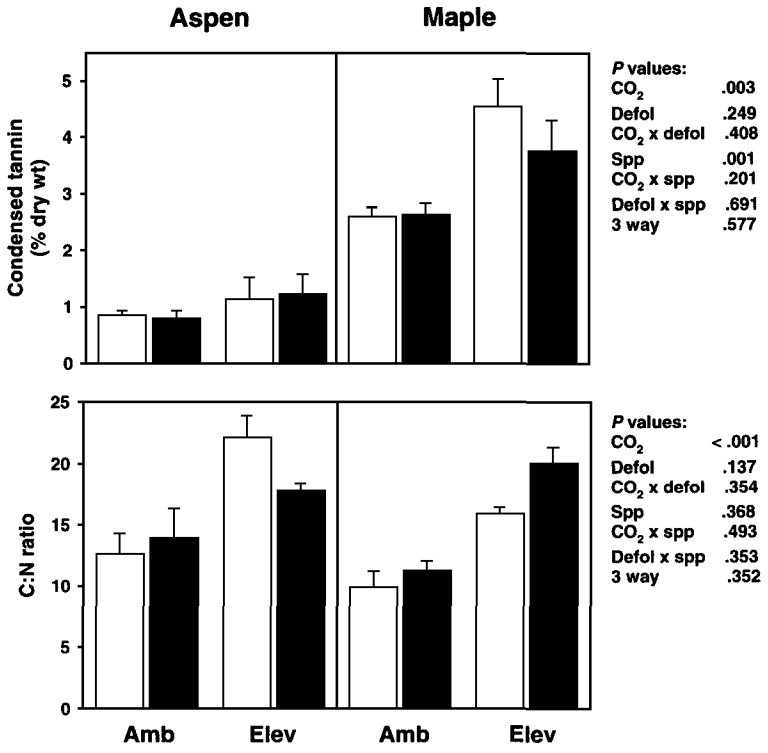

FIG. 4. Concentrations of condensed tannins and C:N ratios in foliage of nondefoliated (light bars) and partially defoliated (dark bars) trees grown in ambient (Amb) or elevated (Elev) concentrations of CO_2. Format as described for Figure 1.

larvae fed aspen (Figure 5). The changes, however, were small in magnitude. Defoliation did not significantly alter developmental rates overall, although the marginally significant three-way interaction suggests that the effects of defoliation may vary for different CO_2 levels and tree species. Enriched CO_2 reduced growth rates of insects fed aspen by 30%, but did not alter growth of insects fed maple (Figure 5). Reduced growth rates were offset by prolonged development times such that final larval weights of larvae fed ambient- and high-CO_2 aspen differed by only 15% (Figure 5). Leaf damage had no effect on growth rates or final larval weights. Prior defoliation did, however, tend to reduce consumption rates of larvae (Figure 6). Total leaf consumption was marginally increased and decreased, respectively, by CO_2 and defoliation treatments (Figure 6). Approximate digestibility declined slightly for insects fed damaged foliage,

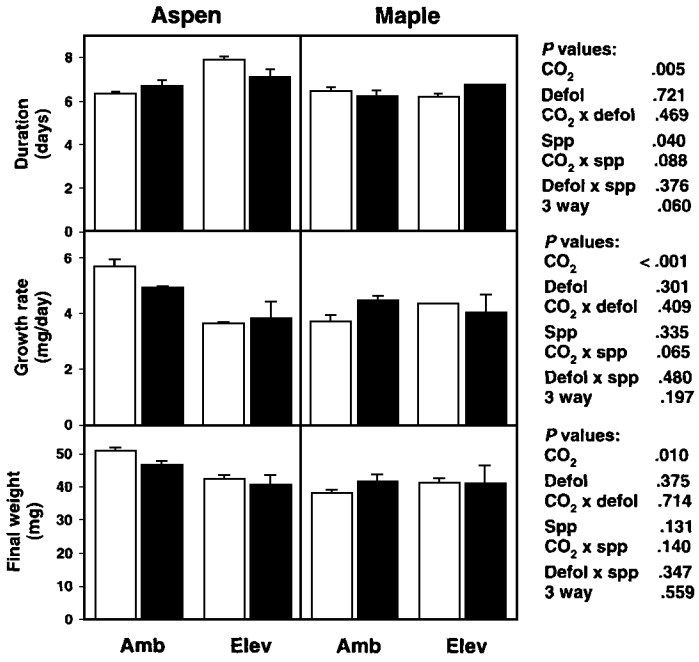

FIG. 5. Developmental time and growth performance of fourth-instar gypsy moths fed foliage from nondefoliated (light bars) and partially defoliated (dark bars) trees grown in ambient (Amb) or elevated (Elev) concentrations of CO_2. Format as described for Figure 1.

and values were much higher for insects fed aspen than for insects fed maple (Figure 6). The efficiency with which larvae converted digested food into biomass (ECD) was reduced in the high-CO_2 treatment for insects fed aspen, but not maple (Figure 6). Efficiency values tended to be higher for insects fed previously damaged foliage and were substantially higher for insects fed maple than for insects fed aspen.

DISCUSSION

Trees grown under enriched atmospheric CO_2 typically exhibit accelerated rates of photosynthesis, although negative acclimation (downregulation) occurs for some species and certain growing conditions after prolonged CO_2 exposure (Ceulemans and Mousseau, 1994; Curtis, 1996). Moreover, defoliation by leaf-chewing insects generally increases photosynthetic rates (Welter, 1989). Rea-

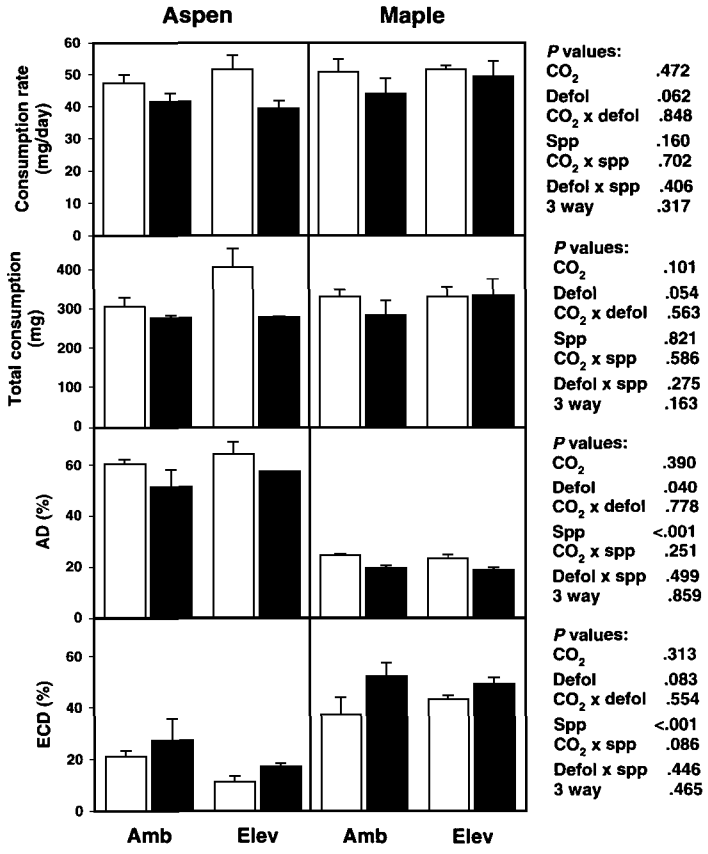

FIG. 6. Consumption and food processing parameters of fourth-instar gypsy moths fed foliage from nondefoliated (light bars) and partially defoliated (dark bars) trees grown in ambient (Amb) or elevated (Elev) concentrations of CO_2. Format as described for Figure 1.

sons for the latter response are multiple and varied, and include factors such as increased light penetration through the canopy and reduced feedback inhibition of photosynthesis. [Defoliation increases relative sink strength by increasing assimilate demand per unit of remaining leaf tissue (Welter, 1989)].

Whether enriched CO_2 and defoliation have additive effects on photosynthesis rates in trees is largely unknown. In a companion study to that described here, Kruger et al. (1998) assessed the effects of enriched CO_2 and defoliation on photosynthesis and growth in aspen and maple. They found that in aspen,

photosynthesis rates increased in high-CO_2 plants but were unaffected by defoliation. In maple, however, photosynthesis was not influenced by CO_2 level or defoliation. These results differ somewhat from those of Pearson and Brooks (1996), who showed that enriched CO_2 increased photosynthesis in *Rumex obtusifolius* and that herbivory by a chrysomelid beetle (*Gastrophysa viridula*) increased photosynthesis rates of plants in ambient, but not enriched CO_2. Kruger et al. (1998) also found that defoliation increased relative growth rates of maple trees in enriched CO_2 environments, and of aspen trees in both ambient and enriched CO_2 environments.

Foliar Chemistry. Here, we first discuss our results with respect to the direct effects of CO_2 and defoliation. Then we describe how those factors *interact* to affect foliar chemistry and compare our results with a similar open-top chamber study.

Phytochemical responses to CO_2 differed among compounds and plant species. Nitrogen (aspen and maple) and protein (aspen) levels declined in high-CO_2 plants, responses consistent with numerous other studies (McGuire et al., 1995; Watt et al., 1995; Lindroth, 1996; Roth and Lindroth, 1995). Enriched CO_2 led to increased carbohydrate (hexose and starch) accumulation in aspen, but not maple, responses linked to the differential effect of CO_2 on photosynthesis in the two species (Kruger et al., 1998).

Considering carbon-based seconary compounds, we found that levels of tremulacin, but not salicortin, increased in high-CO_2 aspen foliage. In six other studies (e.g., Lindroth et al., 1993; Roth and Lindroth 1995) of the effects of enriched CO_2 on aspen chemistry, our research group has usually found similar, marginal increases. Relatively weak responses by this group of compounds may be due to the fact that they are dynamic metabolites rather than stable end-products. Concentrations of the latter, such as tannins, may better reflect shifts in resource availability (Reichardt et al., 1991). Levels of condensed tannins in aspen, however, also showed small increases in response to CO_2. In contrast, gallotannin, ellagitannin, and condensed tannin concentrations all increased appreciably in high-CO_2 maple, results parallel to those of our other studies (e.g., Roth and Lindroth, 1995; Roth et al., 1997). The average 5% increase in leaf weight allocated to tannins in maple is particularly interesting given the fact that enriched CO_2 did not significantly accelerate photosynthesis in that species (Kruger et al., 1998). Slightly (although not significantly) elevated photosynthesis rates, sustained over many days, could account for some of the increase, as could a change in carbon metabolic processes (e.g., dark respiration) not measured by Kruger et al. (1998).

Phytochemical responses to defoliation also differed among compounds and tree species. Nitrogen concentrations were not affected by defoliation. Soluble protein levels in aspen, however, declined with defoliation under ambient CO_2, but increased with defoliation under enriched CO_2 (see discussion of interaction

below). Defoliation had little if any effect on carbohydrate levels in maple. Leaf damage reduced hexose and starch concentrations in aspen, and the magnitudes of change were affected by CO_2 treatment (interaction discussed below).

Carbon-based secondary metabolites exhibited varying responses to defoliation. In short, levels of dynamic metabolites (phenolic glycosides) increased, whereas those of static end-products (gallotannins, ellagitannins, condensed tannins) did not.

Overall, we observed few interactive effects of CO_2 and defoliation on foliar chemistry, and these were largely restricted to aspen. The strongest interactive effect occurred for soluble protein levels. The most abundant soluble protein in plant tissue is the carboxylating enzyme ribulose-1,5-bisphosphate (rubisco) (Jones et al., 1989). Reductions in rubisco concentrations in enriched-CO_2 aspen, especially in nondefoliated trees, is likely linked to carbohydrate metabolism and allocation. In high-CO_2, nondefoliated trees, levels of rubisco and other Calvin cycle enzymes may decline due to dilution from accumulating starch. In addition, declines may result from reallocation of nitrogen to other proteins (e.g., those required for electron transport or starch and sucrose synthesis) in photosynthetic tissue or to nonphotosynthetic tissue (Stitt, 1991; Ceulemans and Mousseau, 1994). (We did not, however, observe corresponding changes in foliar nitrogen levels.) In high-CO_2, defoliated aspen, protein levels did not decline. Because of a decrease in the carbohydrate source-sink ratio, the dilution effect did not occur and the demand for carboxylation activity remained high.

In research conducted following this study, Roth et al. (1998) evaluated the effects of enriched CO_2 and defoliation (by forest tent caterpillars, *Malacosoma disstria*) on aspen and maple in open-top chambers. Phytochemical results from that study and this one are nearly identical, with the exception that Roth et al. found that condensed tannin levels in aspen increased in response to defoliation as well as to CO_2, whereas ellagitannin levels in maple were unresponsive to CO_2.

A significant and similar finding in both studies was the lack of an interaction between CO_2 and defoliation with respect to allelochemical concentrations. Thus, at least for these species, CO_2 concentrations appear to affect levels of *constitutive*, but not *induced*, chemical defenses. We recognize that our studies have addressed only short-term induction responses (active defense mechanisms). CO_2 concentrations could conceivably play more important roles in mediating long-term induction responses in trees, as such responses may be more strongly affected by resource availability (Bryant et al., 1991). Finally, CO_2 is more likely to influence phytochemical induction in plants that respond more strongly to damage than do aspen and maple.

Insect Performance. Performance of gypsy moth larvae was affected by CO_2-mediated shifts in foliar chemical composition, although the magnitudes

of responses were generally small and differed between host species. Overall, CO_2 treatment did not significantly affect growth and consumption parameters of insects fed maple. These results are consistent with those of previous studies on CO_2, maple, and gypsy moths (Lindroth et al., 1993, Roth and Lindroth, 1995; Kinney et al., 1997), but differ from those of studies using forest tent caterpillars (*Malacosoma disstria*) (Lindroth et al. 1993; Roth et al. 1997) and whitemarked tussock moths (*Orgyia leucostigma*) (Agrell and Lindroth, unpublished data).

Gypsy moths reared on high-CO_2, nondefoliated aspen grew more slowly, even though eating more rapidly, due to marked declines in food conversion efficiencies. This is a common pattern of response for gypsy moths fed aspen (Lindroth et al., 1993, Roth and Lindroth, 1995) and can be explained on the basis of changes in foliar protein and phenolic glycoside levels. Gypsy moths increase consumption when fed diets low in protein (Sheppard and Friedman, 1992; Lindroth et al. 1997a). Moreover, phenolic glycosides are toxic to gypsy moths at moderate to high concentrations (Hemming and Lindroth, 1995; Hwang and Lindroth, 1997). Thus, growth performance of insects fed high-CO_2, undamaged trees deteriorated because compensatory consumption increased their toxic load. In contrast, growth of insects fed high-CO_2, defoliated trees declined not because of changes in consumption, but directly because of high levels of phenolic glycosides in those plants. Prolonged stadium duration did not entirely offset the reductions in growth rates of larvae, resulting in slightly smaller insects at the conclusion of the short-term feeding trial. Given that such effects can be magnified over long-term feeding regimes, it is possible that the consequences of enriched CO_2 atmospheres for fitness of gypsy moths reared on aspen may be greater than indicated in this short-term study. Of central importance in studies of this kind, however, is assessment of how CO_2 affects concentrations of those phytochemical constituents that most strongly shape insect performance (Lindroth et al., 1997b).

Defoliation had relatively minor effects on insect performance. Both consumption rates and approximate digestibilities tended to decline for insects fed damaged foliage, but these changes were offset by improved conversion efficiencies. As a result, growth was not altered. These results differ somewhat from those of Roth et al. (1998), who evaluated the effects of CO_2 and defoliation (of aspen and maple) on performance of forest tent caterpillars. They found that damage to aspen did not alter insect performance. Defoliation of maple, however, reduced larval growth via decreases in consumption and food processing efficiencies.

We originally predicted that growth under enriched CO_2 atmospheres would enhance defoliation-induced accumulation of carbon-based defensive compounds, resulting in an additive, detrimental effect on the performance of gypsy moth larvae. Given that we found no such effect on foliar chemistry, it is not

surprising that we found little evidence of a CO_2 × defoliation effect on larval performance. This finding is consistent with those of Roth et al. (1998), who observed direct, but not interactive, effects of CO_2 and defoliation on forest tent caterpillars.

A growing body of literature suggests that the impact of enriched CO_2 on plant–insect interactions will be modulated by other abiotic factors, such as nutrient, water, and light availability (Johnson and Lincoln, 1991; Lawler et al., 1997; Kinney et al., 1997; Roth et al., 1998; Agrell and Lindroth, unpublished data). Relatively few studies have addressed how atmospheric CO_2 may interact with biotic factors to affect plant–insect interactions. To date, however, whether those biotic factors have been prior defoliation (this study; Roth et al., 1998), insect pathogens (Lindroth et al., 1997b), or parasitoids (Roth and Lindroth, 1995), true interactive effects have been mostly negligible. Ideally, future research will be expanded to include additional species, both short- and long-term induction responses, and more natural growing conditions. Emerging FACE (free air CO_2 enrichment) (Hendrey, 1993) technology at research sites located in many of the world's most important biotic communities may afford such opportunities.

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