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Article *in* Ecology · April 1993

DOI: 10.2307/1940804 · Source: OAI

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RESPONSES OF DECIDUOUS TREES TO ELEVATED ATMOSPHERIC CO_2: PRODUCTIVITY, PHYTOCHEMISTRY, AND INSECT PERFORMANCE[1]

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Abstract. Although rising levels of atmospheric carbon dioxide are expected to directly affect forest ecosystems, little is known of how specific ecological interactions will be modified. This research evaluated the effects of enriched CO_2 on the productivity and phytochemistry of forest trees and performance of associated insects. Our experimental system consisted of three tree species (quaking aspen [*Populus tremuloides*], red oak [*Quercus rubra*], sugar maple [*Acer saccharum*]) that span a range from fast to slow growing, and two species of leaf-feeding insects (gypsy moth [*Lymantria dispar*] and forest tent caterpillar [*Malacosoma disstria*]). Carbon–nutrient balance theory provided a framework for tests of three hypotheses; in response to enriched CO_2: (1) relative increases in tree growth rates will be greatest for aspen and least for maple, (2) relative decreases in protein and increases in carbon-based compounds will be greatest for aspen and least for maple, and (3) relative reductions in performance will be greatest for insects fed aspen and least for insects fed maple. We grew 1-yr-old seedlings for 60 d under ambient (385 ± 5 μL/L) or elevated (642 ± 2 μL/L) CO_2 regimes at the University of Wisconsin Biotron. After 50 d, we conducted feeding trials with penultimate-instar gypsy moth and forest tent caterpillars. After 60 d, a second set of trees was harvested and partitioned into root, stem, and leaf tissues. We subsequently analyzed leaf material for a variety of compounds known to affect performance of insect herbivores.

In terms of actual dry-matter production, aspen responded the most to enriched CO_2 atmospheres whereas maple responded the least. Proportional growth increases (relative to ambient plants), however, were highest for oak and least for maple. Effects of elevated CO_2 on biomass allocation patterns differed among the three species; root-to-shoot ratios increased in aspen, decreased in oak, and did not change in maple. Enriched CO_2 altered concentrations of primary and secondary metabolites in leaves, but the magnitude and direction of effects were species-specific. Aspen showed the largest change in storage carbon compounds (starch), whereas maple experienced the largest change in defensive carbon compounds (condensed and hydrolyzable tannins). Consumption rates of insects fed high-CO_2 aspen increased dramatically, but growth rates declined. The two species of insects differed in response to oak and maple grown under enriched CO_2. Gypsy moths grew better on high-CO_2 oak, whereas forest tent caterpillars were unaffected; tent caterpillars tended to grow less on high-CO_2 maple, whereas gypsy moths were unaffected. Changes in insect performance parameters were related to changes in foliar chemistry. Responses of plants and insects agreed with some, but not all, of the predictions of carbon–nutrient balance theory.

This study illustrates that tree productivity and chemistry, and the performance of associated insects, will change under CO_2 atmospheres predicted for the next century. Changes in higher level ecological processes, such as community structure and nutrient cycling, are also implicated.

Key words: carbon dioxide; carbon–nutrient balance theory; feeding trials; global change; Lymantria dispar; Malacosoma disstria; *nutritional indices; plant–insect interactions; primary production; productivity and phytochemistry vs. increased CO_2; secondary compounds; tannins.*

INTRODUCTION

Increasing concentrations of atmospheric carbon dioxide and attendant climate change are anticipated to exert significant impacts on forest ecosystems (Eamus and Jarvis 1989, Graham et al. 1990, Melillo et al. 1990). Although numerous studies have addressed the direct effects of CO_2 on tree physiology and growth, few have considered the consequences of elevated CO_2 for ecological interactions among trees and other organisms. Such research is needed for valid assessments of the consequences of elevated CO_2 for forest dynamics. Moreover, given that forests cover one third of the Earth's land surface and conduct about two thirds of

[1] Manuscript received 19 September 1991; revised 10 April 1992; accepted 29 May 1992.

the world's photosynthesis (Kramer 1981), such studies are important for predicting perturbations in the global carbon cycle.

Of the many interactions likely to be influenced by elevated CO_2, that of trees and associated insects is one of the most important. Phytophagous insects are the major primary consumers in forests, and can directly and indirectly alter energy flow and nutrient cycling dynamics (Schowalter et al. 1986, Choudhury 1988). The quality of plant tissue for insect herbivores is determined largely by its composition of primary and secondary metabolites, and concentrations of these are closely linked to plant carbon metabolism. Thus atmospheric CO_2 levels are likely to influence interactions between forest trees and insects, as has been shown to occur between herbaceous plants and insects (Lincoln et al. 1984, 1986, Fajer et al. 1989).

Carbon–nutrient balance theory (Bryant et al. 1983, Bazzaz et al. 1987, Tuomi et al. 1988, 1991) provides a framework for predicting tree responses to elevated atmospheric CO_2. The central principle of this theory is that the availability of resources (e.g., carbon, nutrients, light) determines resource allocation patterns in plants. Tuomi et al. (1988) consider plant carbon gain to be allocated to two functional pools: carbon used for growth (e.g., cellulose and primary metabolic processes) and carbon used for defense and storage (e.g., phenolics and starch). They, and Bazzaz et al. (1987), argue that carbon is preferentially allocated to growth, and that carbon accumulated in excess of growth requirements is allocated to carbon-based allelochemicals or carbohydrate storage. Accordingly, environmental conditions that increase availability of carbon relative to nutrients will lead to the accumulation of carbon-based compounds. Furthermore, plant species adapted to high nutrient availability typically exhibit high potential growth rates, low investment in defensive compounds, and considerable plasticity in chemical response to changes in resource availability (Chapin 1980, Bryant et al. 1983, 1987*a*, Coley et al. 1985, Bazzaz et al. 1987, Tuomi et al. 1991). In contrast, plant species evolutionarily adapted to low nutrient availability generally exhibit low potential growth rates, high investment in defensive compounds, and little plasticity in chemical response to changes in resource availability. Thus evolutionary adaptations of plants to resource availability are likely to influence their responses to atmospheric CO_2.

The purpose of this research was to investigate the effects of elevated atmospheric CO_2 on the productivity and phytochemistry of deciduous trees and the performance of tree-feeding insects. Although the study was not designed to be a definitive assessment of the effects of elevated CO_2 on plant productivity, those results are provided as a context for interpreting outcomes for plant chemistry and insect performance. Our experimental system consisted of three temperate forest tree species, quaking aspen (*Populus tremuloides* Mich.), red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* Marsh.), and two "outbreak" insect species, gypsy moth (*Lymantria dispar* L.) and forest tent caterpillar (*Malacosoma disstria* Hbn.). The tree species are important components of several North American forest types, and represent a range of inherent growth rates and successional states. Aspen is a fast-growing, early-successional species, oak is a slower-growing, early-to mid-successional species, and maple is a slow-growing, late-successional species (Curtis 1959, Loehle 1988). Both insect species are major forest pests in the northeastern and northcentral United States.

We tested three specific hypotheses concerning the effects of elevated atmospheric CO_2 on trees and insects:

Hypothesis 1—Trees grown under elevated CO_2 conditions will exhibit enhanced growth rates; relative increases will be greatest for aspen and least for maple. Because aspen is an inherently fast-growing species, it should respond more strongly to resource (CO_2) augmentation than do oak and maple.

Hypothesis 2—Trees grown under elevated CO_2 will contain proportionately less protein but more starch and phenolic allelochemicals; relative changes will be greatest for aspen and least for maple. As the balance of carbon and nutrients available to trees changes in favor of carbon, foliar concentrations of nitrogen-based compounds should decrease whereas concentrations of carbon-based compounds should increase. Because fast-growing species show greater chemical flexibility in response to shifts in resource availability, the magnitude of accumulation of carbon-based compounds should be greatest in aspen and least in maple.

Hypothesis 3—Insects fed foliage from trees grown under elevated CO_2 will exhibit reduced performance; relative changes in performance will be greatest for larvae fed aspen and least for larvae fed maple. As foliar concentrations of protein decrease and phenolics increase, growth rates of gypsy moth and forest tent caterpillars should decline. The magnitude of change in performance should reflect the magnitude of change in plant chemistry as described for Hypothesis 2.

Methods

Experimental design

We used a split-plot experimental design for our studies. Whole plots consisted of environmental control rooms, with three rooms each for ambient ($\approx$350 μL/L) and elevated ($\approx$650 μL/L) levels of CO_2. Subplots consisted of tree species (aspen, maple, and oak). Within each room we grew one set of trees for productivity measurements and chemical analyses and a second set for use in insect bioassays.

Tree growth

We used 1-yr-old seedlings in the studies. Sugar maple and red oak were obtained from a Wisconsin Department of Natural Resources nursery (Boscobel, Wisconsin). Aspen had been grown the previous year from

seed obtained from the University of Minnesota North Central Experiment Station (Grand Rapids, Minnesota); seedlings were full-sibs. For each dormant seedling we measured total tree mass, stem length, root volume, and root collar diameter. In addition, we determined root, stem, and total dry mass for 10–25 individuals of each species. Trees were planted in 6-L pots in a 1:1:2 mixture of forest topsoil (containing native mycorrhizal inoculum), peat, and sand.

We grew the trees in environmental control rooms at the University of Wisconsin Biotron. Each room contained ≈16 aspen, 20 maple, and 20 oak trees, which were watered to saturation twice daily with an automatic drip irrigation system. To prevent excessive salt build-up, the watering rotation consisted of 2 d with half-strength Hoagland's solution followed by 1 d with water. We used a 15:9 light : dark photocycle as representative of early summer conditions at this latitude (43° N). Light : dark temperatures and humidities were maintained at 25:20°C and 70:85%, respectively. Photon flux density (photosynthetically active radiation, PAR) 70 cm above the pots was 490 ± 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($\bar{X}$ ± 1 SE) and did not differ between ambient and elevated CO_2 rooms. We initiated CO_2 control when approximately half of the trees began to break bud (day 0); all trees completed budbreak within several days. CO_2 concentrations were monitored continuously (every 2 min) throughout the study. Analysis of mean daily CO_2 concentrations indicated actual levels of 385 ± 5 and 642 ± 2 μL/L in ambient and elevated rooms, respectively, during the study. Trees were randomly rearranged within rooms twice during the study to reduce effects of within-room environmental variation. Seedlings grew for 60 d under the two CO_2 treatments.

To evaluate the effects of CO_2 on tree productivity, we destructively harvested 5–8 trees of each species from each room at the conclusion of the growth study. We separated stems, leaves, and roots, and recorded root collar diameter and stem height (collar to apical bud). Stems and roots were dried at 60°C for 2 wk and then weighed. Maple and oak exhibited multiple leaf flushes, each of which was processed separately. We recorded fresh leaf mass and measured total leaf area per plant with a LI-COR leaf area meter. We quantified leaf toughness with a McCormick dynamometer; separate multiple measurements were made for each leaf flush in maple and oak, whereas for aspen (an indeterminate grower) toughness was measured with leaf number 18. (Leaf number 1 was the most terminal leaf with length >1 cm.) We then flash-froze leaf tissue in liquid nitrogen and stored it at −75°C until freeze-dried. After drying, leaves were weighed, ground (425-μm mesh) and stored at −20°C for later chemical analysis.

Foliar chemistry

We analyzed foliar tissue for a variety of compounds likely to be altered by atmospheric CO_2 and known to influence insect performance. Separate analyses were performed on tissue from each leaf flush of each tree processed for growth assessment. Results of these analyses provided a test of predictions of Hypothesis 2.

Foliar nutritional analyses included determinations of water, total nitrogen (an index of protein), hexose, sucrose, and starch. Water content was estimated as the proportional difference between fresh and dry leaf mass. For Kjeldahl nitrogen determinations, we modified the procedure of Parkinson and Allen (1975) for digestion of 20–50 mg quantities of leaf tissue. The nitrogen content of digests was quantified in duplicate samples by a micro-Nesslerization technique (Lang 1958), using glycine *p*-toluene-sulfonic acid (5.665% N) as a standard. We used the procedure of M. M. Schoeneberger, K. Ludovici, and P. Faulkner (*unpublished manuscript*) for determination of total nonstructural carbohydrates. This method allows for separate quantification of hexoses (glucose and fructose), sucrose, and starch in 25-mg samples. Fructose, sucrose, and starch are enzymatically converted to glucose, which is then quantified indirectly via coupled enzyme reactions that simultaneously reduce NADP to NADPH.

The major secondary metabolites produced by aspen, oak, and maple are all phenolic compounds, and include phenolic glycosides and condensed tannins in aspen (Palo 1984, Bryant et al. 1987*b*, Lindroth et al. 1987*a*) and hydrolyzable and condensed tannins in oak (Rossiter et al. 1988) and maple (Baldwin et al. 1987). Quaking aspen produces a suite of four phenolic glycosides, of which salicortin and tremulacin constitute >85% by mass (Lindroth et al. 1987*b*) and are the most biologically active against Lepidoptera (Lindroth et al. 1988, Lindroth and Peterson 1988). Earlier work in our laboratory (Lindroth and Pajutee 1987) had shown that phenolic glycoside analyses are best done on fresh tissue; more recently we found that flash-freezing followed by freeze-drying works equally well (R. L. Lindroth, *unpublished data*). We measured concentrations of salicortin and tremulacin by high-performance thin-layer chromatography (HPTLC). We extracted 100 mg of tissue in 4 mL of methanol for 15 min, with sonication. We then centrifuged the extracts (to remove plant material) and evaporated 2-mL aliquots to dryness on a Savant Speed-Vac. Phenolic glycosides were redissolved in 0.25 mL of methanol, from which we applied duplicate 1-μL aliquots to high-performance thin-layer chromatography plates (Silica gel 60, 10 × 20 cm). Purified salicortin and tremulacin standards were applied similarly. We developed the plates in 6:1:1 methylene chloride : methanol : tetrahydrofuran. Plates were then scanned (274 nm) in a Camag TLC Scanner II (Camag Scientific, Inc., Wrightsville Beach, North Carolina, USA), and chromatograms were analyzed using Camag TLC evaluation software (CATS 3.11).

The hydrolyzable tannins are comprised primarily of ellagitannins and gallotannins, hexahydroxydiphenic acid and gallic acid esters, respectively, of carbohydrates or polyols. Procedures now exist for selective

TABLE 1. Effects of elevated atmospheric carbon dioxide on gypsy moth performance ($\bar{X} \pm 1$ SE).‡,§

Treatment	Duration (d)	RGR ($mg \cdot mg^{-1} \cdot d^{-1}$)	Final mass (mg)	RCR ($mg \cdot mg^{-1} \cdot d^{-1}$)
Ambient CO_2 (385 μL/L)				
Aspen	6.7 ± 0.2**	0.13 ± 0.00**	29.6 ± 0.8*	2.62 ± 0.26***
Oak	6.6 ± 0.2	0.11 ± 0.00†	27.4 ± 0.4*	1.54 ± 0.04
Maple	5.4 ± 0.2	0.16 ± 0.01	32.0 ± 0.7	1.57 ± 0.02
Elevated CO_2 (642 μL/L)				
Aspen	8.4 ± 0.5**	0.08 ± 0.01**	25.9 ± 1.2*	4.94 ± 0.30***
Oak	6.2 ± 0.2	0.13 ± 0.01†	30.3 ± 0.6*	1.74 ± 0.04
Maple	5.7 ± 0.2	0.15 ± 0.01	30.7 ± 0.8	1.48 ± 0.06
P values				
CO_2	0.017	0.079	0.431	0.003
Species	<0.001	<0.001	0.002	<0.001
CO_2 × Spp.	0.034	0.006	0.003	<0.001

‡ Symbols following values indicate significant pairwise comparisons within species; † $P < .10$, * $P < .05$, ** $P < .01$, *** $P < .001$.

§ All calculations based on dry mass measurements: RGR = relative growth rate = biomass gained·(mean larval biomass)$^{-1}$·day^{-1}; RCR = relative consumption rate = food ingested·(mean larval biomass)$^{-1}$·day^{-1}; TC = total consumption = food ingested; AD = approximate digestibility = (food ingested − feces excreted)·(food ingested)$^{-1}$·100; ECD = efficiency of conversion of digested food = biomass gained·(food ingested − feces excreted)$^{-1}$·100; ECI = efficiency of conversion of ingested food = biomass gained·(food ingested)$^{-1}$·100.

quantification of ellagitannins and gallotannins, based on acid hydrolysis and measurement of liberated ellagic acid or gallic acid. Our preliminary analyses confirmed the observation of Bryant et al. (1987*b*) that aspen does not contain hydrolyzable tannins, so subsequent analyses were restricted to oak and maple.

We adapted the method of Wilson and Hagerman (1990) for quantifying levels of ellagitannin. We measured samples (10–15 mg) into 10 × 75 mm test tubes, added 1 mL of 1 mol/L H_2SO_4, and loosely capped the tubes. The tubes were placed into a stainless steel vacuum/pressure chamber that was then purged several times with nitrogen and placed into a 100°C oven for 16 h. Sample residues were washed twice with 7:3:1 acetone : water : hydrochloric acid, once with acetone, dried under a stream of nitrogen, and dissolved in pyridine. We measured ellagic acid concentrations of duplicate pyridine samples according to the standard method.

We used a single extract preparation from each tissue sample for the remaining three tannin assays. Leaf material (100 mg) was exhaustively (4×) extracted with 2 mL of 70% acetone, each for 30 min (with sonication) at 4°C. We modified the procedure of Inoue and Hagerman (1988) for quantifying concentrations of free and esterified (gallotannin) gallic acid. We added 1 mL of acetone extract to a 10 × 75 mm test tube, removed the acetone under a stream of nitrogen, and added 1 mL of 1 mol/L H_2SO_4. Samples were hydrolyzed as described for ellagitannins, but for 26 h at 100°C. We diluted the hydrolysates to 10 mL with water and assayed duplicate samples for total gallic acid. We performed similar analyses on nonhydrolyzed extracts to measure concentrations of free gallic acid. Gallotannin concentrations were calculated as the difference between total and free gallic acid. Note that following standard protocols, our data for ellagitannin and gallotannin concentrations are provided in terms of ellagic acid and gallic acid equivalents, and thus underrepresent actual concentrations of intact tannin esters.

We measured condensed tannin concentrations by a modification of the method of Porter et al. (1986), which is based on the hydrolytic conversion of proanthocyanidins to anthocyanidins. In the hydrolysis step, duplicate samples were immersed in boiling water for 50 min. Quebracho tannin (provided by A. E. Hagerman, Miami University, Oxford, Ohio) was purified by absorption chromatography (with Sephadex LH-20) and used to construct a standard curve. Thus condensed tannin data are presented as quebracho equivalents.

Protein-binding capacity of our tannin extracts was determined by radial diffusion in agarose gels containing 0.1% bovine serum albumin (Hagerman 1987). We evaporated acetone from 2-3 mL aliquots under a stream of nitrogen and freeze-dried remaining aqueous portions. Samples were redissolved in 75 μL of 70% acetone and applied in duplicate 16-μL volumes to agarose gels. Plates were incubated at 30°C for 96 h. We measured the diameter of rings, and express our results as diameter squared per milligram of dry leaf tissue.

Insect bioassays

To test the predictions of Hypothesis 3, we conducted insect performance trials with gypsy moth and forest tent caterpillars. Gypsy moth egg masses (New Jersey Standard strain) was obtained from the Otis Methods Development Center (Otis Air National Guard Base, Massachusetts). Forest tent caterpillar egg bands were collected in Menominee County, Wisconsin. Larvae of each species were reared on standard artificial

TABLE 1. Continued.

TC (mg)	AD (%)	ECD (%)	ECI (%)
373 ± 43***	79.1 ± 1.8*	6.6 ± 1.2	5.0 ± 0.8**
207 ± 9	16.5 ± 3.6*	55.8 ± 7.7*	7.3 ± 0.1
191 ± 5	29.2 ± 1.9†	38.7 ± 3.6*	10.3 ± 0.6
775 ± 21***	89.9 ± 1.1*	2.0 ± 0.3	1.8 ± 0.3**
232 ± 7	24.9 ± 1.6*	34.8 ± 4.8*	7.7 ± 0.3
172 ± 16	21.3 ± 2.3†	55.9 ± 6.9*	10.2 ± 0.5
<0.001	0.173	0.441	0.020
<0.001	<0.001	<0.001	<0.001
<0.001	0.002	0.021	0.019

diets (Odell et al. [1985] for gypsy moth, Grisdale [1985] for forest tent caterpillar) from egg hatch to the penultimate larval stadium (fourth instar for gypsy moth, fifth instar for forest test caterpillar).

Feeding trials commenced on day 50 of the study. For each plant growth room we paired each of 12 gypsy moth and 10 forest tent caterpillars with individual aspen, oak, and maple trees. Thus the total number of insect bioassays conducted was 216 for gypsy moth and 180 for forest tent caterpillar. Larvae from at least 12 mothers of each species were used; siblings were distributed across treatments to minimize potential maternal or genetic effects.

For each bioassay a single newly molted larva (75–95 mg for gypsy moth, 35–95 mg for forest tent caterpillar) was placed into a 25 × 1.5 cm plastic petri dish containing a single leaf. Use of single larvae in the bioassays was consistent with the solitary feeding behavior of older instars of both species in the field. We removed leaves from trees using a razor blade or dissecting scissors, weighed them, and then immediately inserted petioles into 1.5-mL plastic microfuge tubes containing water. We used first-flush leaves in all bioassays for oak and maple. For aspen we removed leaves from the middle third of the tree, representing an intermediate age class. We replaced leaves, as necessary, every 1–3 d. Gypsy moth larvae were maintained in the Department of Entomology's Gypsy Moth Quarantine Facility. All larvae were reared for the duration of the penultimate stadium in Percival environmental chambers at 25°C, with a 15:9 L:D photocycle. Larvae were frozen at the end of each trial, and gypsy moth larvae were sexed. Afterwards, all larvae, uneaten leaves, and frass were dried (60°C, 1 wk) and weighed. We calculated nutritional indices on the basis of dry mass, using standard formulas (Waldbauer 1968, Scriber 1977, Table 1) for relative growth rate (RGR), relative consumption rate (RCR), total consumption (TC), approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI). To estimate the initial dry mass of larvae at the onset of the trials we calculated proportional dry mass of 20 newly molted larvae from the same egg masses as experimental larvae. The initial dry mass of leaves used in the bioassays was estimated based on dry mass : wet mass regressions of leaves used for chemical analyses.

Statistical analyses

Data for plant growth, plant chemistry, and gypsy moth performance were analyzed using the SAS GLM procedure (SAS Institute 1985). The overall model for the analyses was a split-plot, with CO_2 level (rooms) and species (within rooms) as whole-plot and sub-plot factors, respectively:

$$Y_{ijk} = \mu + C_i + R_k(C_i) + S_j + C \times S_{ij} + \epsilon_{ijk}.$$

In this model CO_2 level (C_i), species (S_j), and CO_2 × species interaction ($C \times S_{ij}$) are fixed effects, and whole-plot error [$R_k(C_i)$] and sub-plot error (ϵ_{ijk}) are random effects. F tests for C_i were computed with $R_k(C_i)$ as the error term ($F_{1,4}$), and F tests for S_j and $C \times S_{ij}$ were calculated with ϵ_{ijk} as the error term ($F_{2,8}$ for each). Cell means for CO_2 × species × room combinations were computed using the SAS MEANS procedure (SAS Institute 1985) prior to analysis of variance. The standard errors we report are for the treatment means, $\bar{Y}_{ij}$, computed by the SAS MEANS procedure ($n = 3$ rooms).

We used the SAS GLM/LSMEANS procedure (SAS Institute 1985) to construct whole-plot and sub-plot means. This procedure, however, incorrectly calculates the standard error of the difference between means for certain means comparisons in split-plot designs. In SAS GLM/LSMEANS, comparisons of the difference between whole-plot means at the same sub-plot level are computed using only the sub-plot error. The correct standard error of the difference between means for this comparison includes both the whole-plot error and the sub-plot error. Therefore, we hand-calculated LSD (least significant difference) values to compare whole-plot means at the same sub-plot level (Milliken and Johnson 1984:297–306). For nearly every parameter we

measured, the mean square for whole-plot error was not significantly larger than the mean square for sub-plot error, indicating that the variation between rooms was not statistically significant.

Our general approach was modified for several sets of data. First, because hydrolyzable tannins do not occur in aspen, that species was omitted from the analysis. F tests for C_i were computed as previously described but F tests for S_j and $C \times S_{ij}$ were calculated with ϵ_{ijk} as the error term and $F_{1,4}$. Second, because the phenolic glycosides tremulacin and salicortin occur only in aspen, these data were analyzed using a one-way ANOVA in SAS GLM (SAS Institute 1985). F tests for C_i were computed with $R_k(C_i)$ as the error term ($F_{1,4}$). Mortality of forest tent caterpillars in the feeding trials resulted in unequal numbers of sub-plot experimental units. Consequently, we used SAS CONTRAST and ESTIMATE procedures to construct the appropriate analysis for data with missing cells (Milliken and Johnson 1984:384–407).

Growth rates of fourth-instar gypsy moths fed high quality artificial diet differ between males and females (R. L. Lindroth, *unpublished data*). We examined growth data from this study for sexually dimorphic responses but found none. Thus data from males and females were pooled for analysis of gypsy moth performance.

Results

Tree growth and allocation

Trees responded to elevated atmospheric CO_2 with respect to every growth parameter measured, although responses varied among species (Fig. 1). Dry matter production (total plant growth) was 48, 121, and 44% greater under elevated than ambient CO_2 in aspen, oak, and maple, respectively, although the increase in maple was not statistically significant. Actual amounts (rather than proportions) of biomass produced in response to elevated CO_2 were greatest for aspen, intermediate for oak, and least for maple (33.2, 9.2, and 2.5 g of dry mass per tree, respectively). Ratios of elevated-to-ambient relative growth rates were 1.11 for aspen, 1.93 for oak, and 1.27 maple. Aspen grew much faster than oak or maple under both CO_2 concentrations. Oak grew faster than maple in terms of absolute growth rate, but the reverse was true for relative growth rate because of the large initial size of oak seedlings. Both total leaf mass and leaf mass per unit area increased in aspen and oak under elevated CO_2, but neither parameter was significantly altered in maple. Patterns of leaf toughness (Flush 1) paralleled those of leaf mass per unit area, with increases in aspen and oak but no change in maple. In contrast, toughness of Flush 2 oak and maple leaves did not differ according to either CO_2 level ($P = .679$) or species ($P = .335$) (data not shown). The increase in root collar diameter was greater under enriched CO_2 for aspen and oak, but did not differ between CO_2 treatments for maple.

Effects of elevated CO_2 on biomass allocation patterns also differed among tree species (Fig. 1). Under elevated CO_2, leaf mass ratios (leaf mass divided by total mass) decreased 16% in aspen, increased 35% in oak, and remained constant in maple. Correspondingly, root-to-shoot ratios increased markedly in aspen, decreased in oak, and did not change in maple.

Foliar chemistry

Figs. 2 and 3 present results from our chemical analyses of aspen leaves, and Flush 1 oak and maple leaves. If results of analyses of Flush 2 leaves differ markedly from those of Flush 1 leaves, they are described in the text.

We found significant CO_2, species, and interaction effects on concentrations of water and primary metabolites (Fig. 2). Growth under elevated CO_2 slightly reduced leaf water content in aspen and oak, but did not affect water content in maple. Flush 2 leaves of oak and maple contained 63 and 70% water, respectively, and were not affected by CO_2 treatment. Leaf nitrogen concentrations in high-CO_2 trees declined 24 and 17% in aspen and maple, respectively, but did not change in oak. Nitrogen concentrations were higher in maple than in aspen and oak, regardless of CO_2 treatment. Both hexose and sucrose concentrations were unaffected by CO_2 treatment and were much lower in maple than in aspen and oak. The accumulation of starch was affected much more strongly by elevated CO_2 than was that of sugar. Starch levels increased three-fold in aspen and over two-fold in oak, but did not change in maple. Thus under ambient CO_2 conditions starch levels in the three species varied by a factor of 1.4, but under elevated CO_2 conditions they varied by a factor of 3.6. For oak, starch levels in Flush 2 leaves were substantially higher than in Flush 1 leaves, but did not respond significantly to CO_2 treatment (6.4 $\pm$ 1.3 and 8.1 $\pm$ 0.7% dry mass [$\bar{X} \pm 1$ SE] for ambient and elevated trees, respectively). Patterns in concentrations of total nonstructural carbohydrates (TNC) largely reflected those of starch; aspen and oak showed significant increases under enriched CO_2 whereas maple did not.

Elevated atmospheric CO_2 also affected secondary metabolite profiles of our trees (Fig. 3). In aspen, concentrations of the phenolic glycoside salicortin increased 55%. The trend toward higher tremulacin levels was not statistically significant. Ellagitannin concentrations in oak and maple responded differently to elevated CO_2; levels declined 31% in oak but increased 83% in maple. Responses of Flush 2 leaves differed, with concentrations increasing 68% in oak and 36% in maple. Levels of free gallic acid in high-CO_2 foliage did not change for oak, but increased 10% in maple. Gallotannin concentrations were unaffected by CO_2 treatment and tended to be lower in maple than in oak. Condensed tannin concentrations averaged higher in all foliage grown under elevated CO_2, as indicated by the significant CO_2 main effect. The sub-

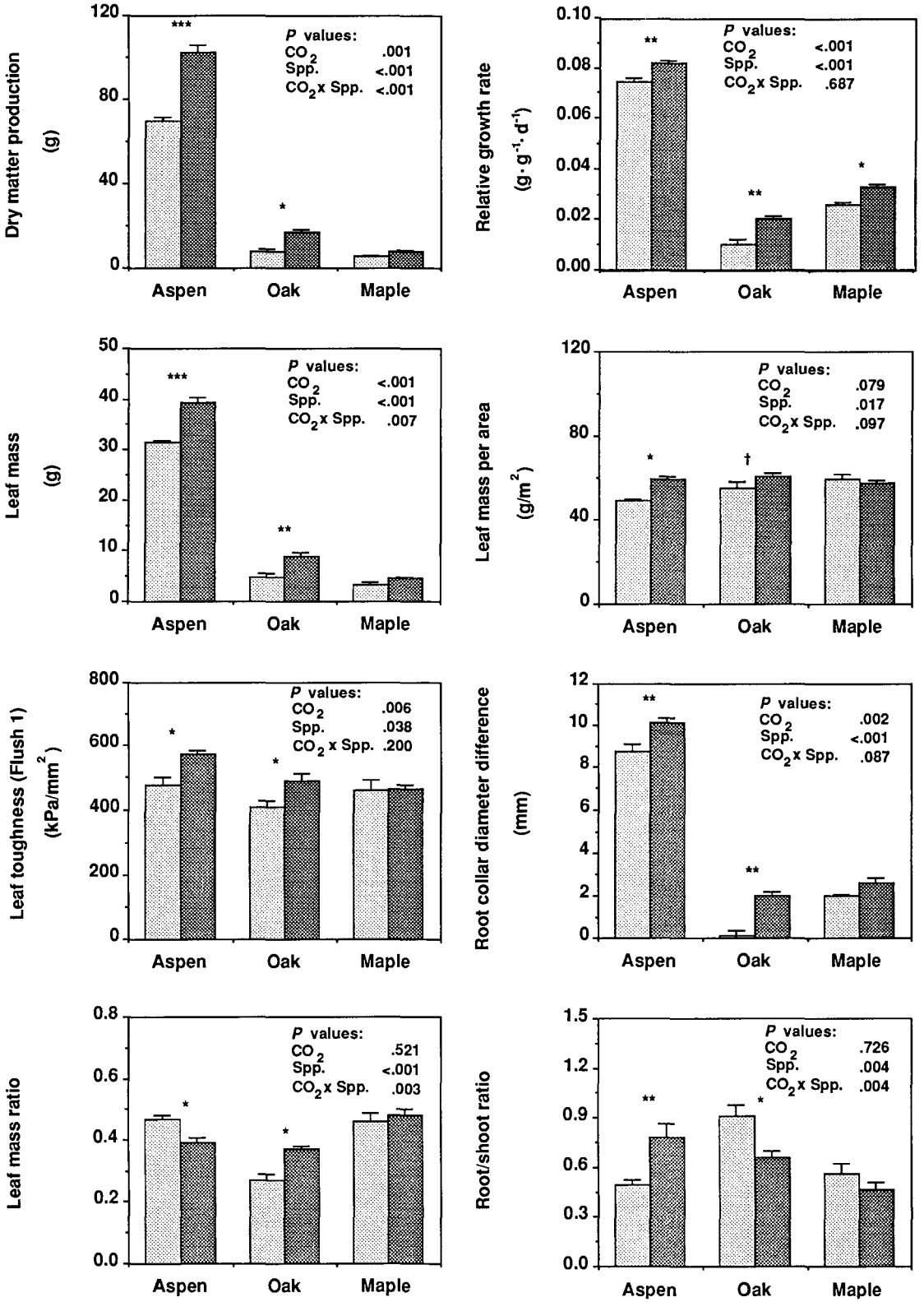

FIG. 1. Growth/allocation parameters and leaf toughness of quaking aspen, red oak, and sugar maple grown for 60 d under atmospheres with ambient (385 μL/L, light bars) and elevated (642 μL/L, dark bars) CO_2. Vertical lines indicate 1 SE above means. Within a species, statistical significance of pairwise comparisons is indicated above bars (†$P < .10$; *$P < .05$; **$P < .01$, ***$P < .001$).

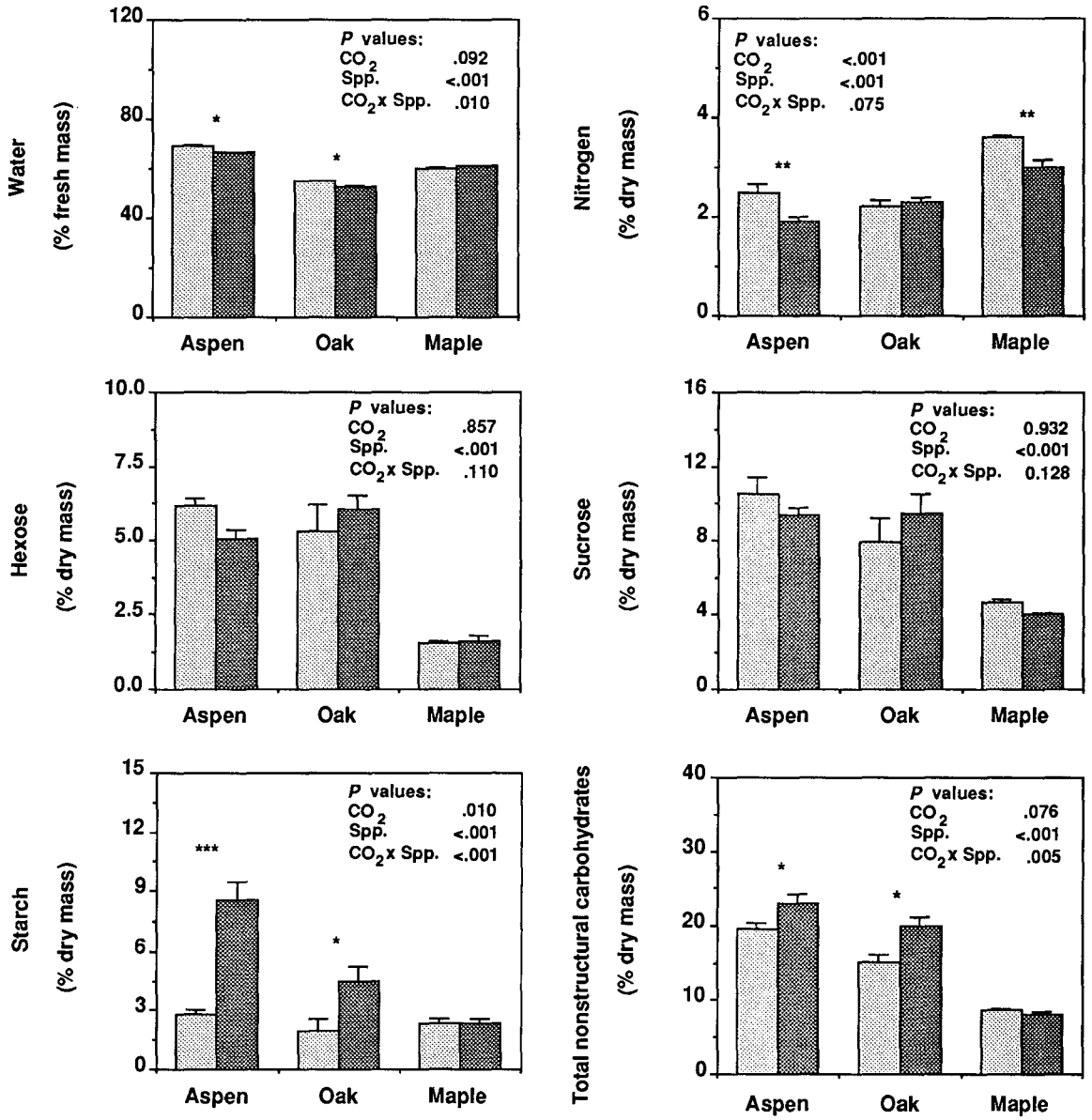

FIG. 2. Concentrations of foliar water and primary metabolites in quaking aspen, red oak, and sugar maple grown under atmospheres with ambient (385 μL/L, light bars) and elevated (642 μL/L, dark bars) CO_2. Vertical lines indicate 1 SE above means. Significant pairwise comparisons shown as in Fig. 1.

stantial variance associated with each mean, however, obscured significant differences (by paired *t* tests) for aspen and oak. Condensed tannin concentrations nearly doubled in high-CO_2 maple. Concentrations in Flush 2 foliage did not differ between oak and maple or according to CO_2 treatment. The protein precipitation capacity of leaf tissue was not affected by CO_2, and was much lower in aspen than in oak and maple. For Flush 2 maple leaves, however, elevated CO_2 increased protein precipitation capacity by 52%.

Insect performance

The main effects of CO_2 and species, and their interaction, influenced most indices of gypsy moth performance (Table 1). Larvae survived very well (≥97%) on all the treatments. Development time increased 25% in larvae fed high-CO_2 aspen, but did not change in larvae fed high-CO_2 oak or maple. The impact of CO_2 on relative growth rates (RGRs) of insects was strongly influenced by tree species, decreasing 35% in larvae fed aspen, increasing 20% in larvae fed oak, and not changing in larvae fed maple. Final larval masses reflected trends in growth rates; for the enriched-CO_2 treatments, values decreased for larvae fed aspen, increased for larvae fed oak, and did not change for larvae fed maple.

Food consumption rates varied in response to CO_2 treatment, tree species, and CO_2 × species interaction.

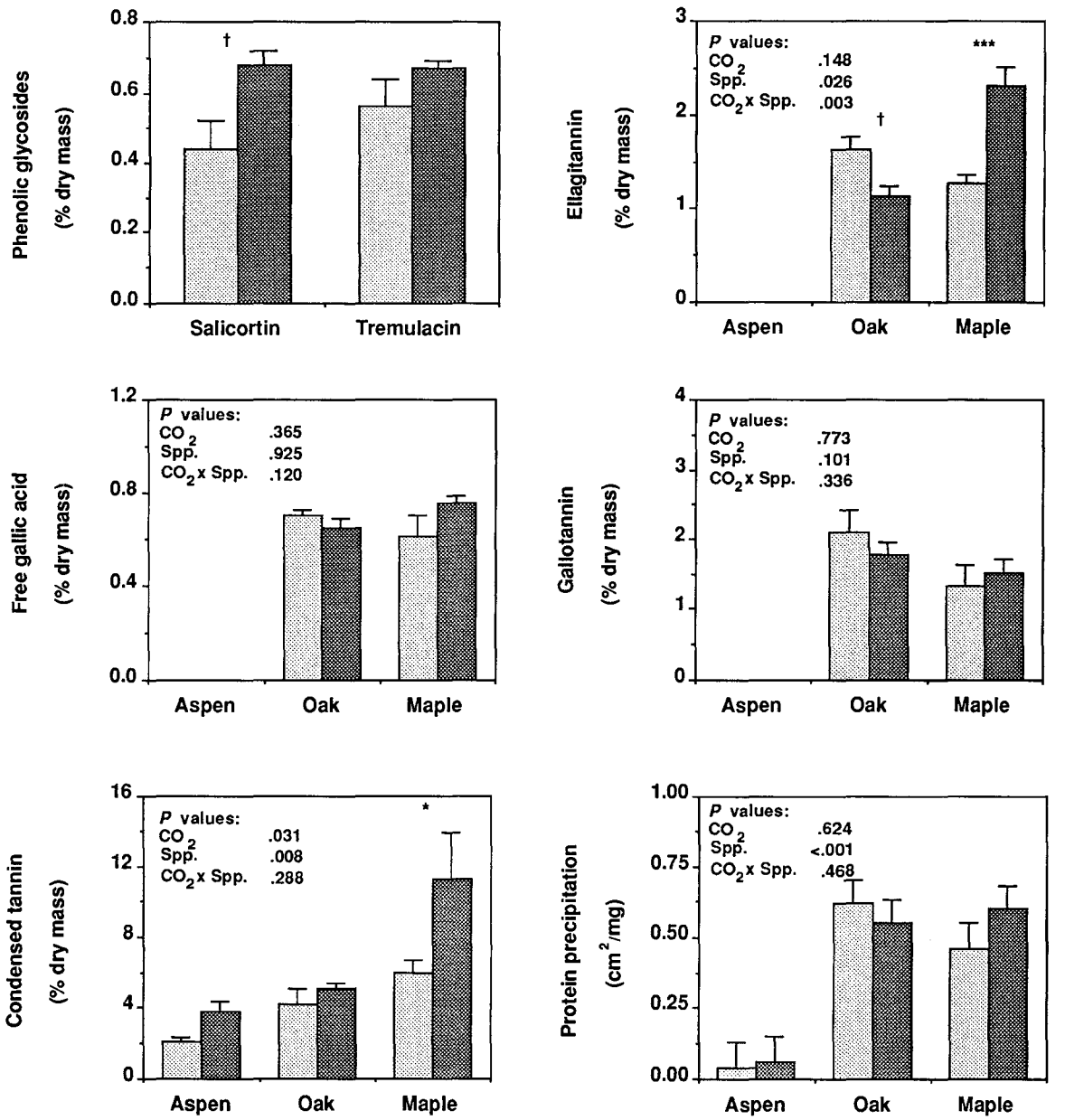

FIG. 3. Concentrations of foliar secondary metabolites in quaking aspen, red oak, and sugar maple grown under atmospheres with ambient (385 μL/L, light bars) and elevated (642 μL/L, dark bars) CO_2. Data for phenolic glycosides are for aspen only. Vertical lines indicate 1 SE above means. Significant pairwise comparisons shown as in Fig. 1.

Both relative consumption rates (RCRs) and total consumption doubled in gypsy moths fed high-CO_2 aspen, whereas consumption of oak and maple was independent of CO_2 treatment. Larvae consumed two- to fivefold more aspen than oak or maple, and similar amounts of oak and maple.

Changes in gypsy moth growth rates can be attributed to variation in food-processing efficiencies. High-CO_2 foliage was 14 and 51% more digestible (AD) than ambient CO_2 foliage in aspen and oak, respectively, but 27% less digestible in maple. The proportion of foliage digested was 3–4 times higher for larvae fed aspen than for larvae fed oak or maple. Although efficiency of conversion of digested food (ECD) appeared to decrease markedly in larvae fed high-CO_2 aspen, substantial variation obscured potentially significant effects. ECD values decreased and increased moderately for larvae fed elevated-CO_2 oak and maple, respectively. Larvae were much less efficient in converting digested aspen into body mass than they were in converting digested oak or maple into body mass. Finally, efficiencies of conversion of ingested food (ECIs) for larvae on high-CO_2 foliage decreased substantially for insects fed aspen, but were unaffected for insects fed oak or maple.

CO_2, species, and CO_2 × species interaction also

TABLE 2. Effects of elevated atmospheric carbon dioxide on forest tent caterpillar performance ($\bar{X} \pm 1$ SE).‡

Treatment	Duration (d)	RGR ($mg \cdot mg^{-1} \cdot d^{-1}$)	Final mass (mg)	RCR ($mg \cdot mg^{-1} \cdot d^{-1}$)
Ambient CO_2 (385 μL/L)				
Aspen	7.0 ± 0.1	0.15 ± 0.01†	24.4 ± 0.1	3.40 ± 0.18*
Oak	10.0 ± 0.1	0.06 ± 0.00	12.4 ± 0.2	0.83 ± 0.08
Maple	8.5 ± 0.1	0.09 ± 0.00	22.8 ± 0.9*	2.99 ± 0.09
Elevated CO_2 (642 μL/L)				
Aspen	7.8 ± 0.5	0.12 ± 0.01†	22.8 ± 1.3	5.96 ± 0.49*
Oak	10.2 ± 0.5	0.05 ± 0.00	14.1 ± 0.6	1.13 ± 0.01
Maple	9.3 ± 0.5	0.09 ± 0.01	20.6 ± 0.5*	2.48 ± 0.40
P values				
CO_2	0.153	0.067	0.344	0.067
Species	0.003	<0.001	<0.001	<0.001
CO_2 × Spp.	0.846	0.217	0.099	<0.001

‡ Abbreviations and significance symbols as in Table 1.

influenced performance of forest tent caterpillars (Table 2). Larvae survived well (≥83%) on all species but oak, for which survival rates were ≈50%. Survival rates were not affected by CO_2 or CO_2 × species interaction. Fifth-instar development time was significantly affected only by host species, with larvae developing fastest on aspen and slowest on oak. The elevated-CO_2 treatment lowered growth rates of larvae fed aspen by 20%, but did not affect growth of larvae fed oak or maple. Growth rates of insects on oak were less than half those of insects on aspen. Final larval mass was lower in larvae reared on high-CO_2 maple than in larvae reared on low-CO_2 maple, but was not affected by CO_2 treatment in larvae fed other hosts.

As for gypsy moths, food consumption by tent caterpillars was strongly influenced by CO_2 level, host species, and their interaction. RCRs increased 75% and total consumption doubled for larvae fed high-CO_2 aspen. CO_2 treatment did not alter RCRs of larvae fed oak or maple, but did affect total consumption. Total food consumed increased 145% for larvae fed high-CO_2 oak but decreased 25% for larvae fed high-CO_2 maple.

Foliage digestibility (AD) was unaffected by CO_2 treatment, but differed among species, with highest values for aspen and lowest for oak. ECDs were not significantly affected by CO_2 or species, but ECIs were significantly altered by both. ECIs declined for larvae fed high-CO_2 aspen or oak.

DISCUSSION

Tree growth and allocation

A comparison of relative growth rates of trees, irrespective of CO_2 treatment, reveals a pattern of aspen ≫ maple > oak. We expected this response in aspen, as it is an early-successional, rapidly growing species. That maple grew faster than oak is contrary to our expectation, however, as the reverse is typically true (Loehle 1988). This result may be due to the environmental conditions (e.g., suboptimal light) specific to our experiment.

Enriched atmospheric CO_2 promoted growth in each of the tree species studied, as has been shown in earlier studies for quaking aspen (Brown and Higginbotham 1986, Brown 1991), red oak (Williams et al. 1986) and sugar maple (Bazzaz et al. 1990). These results are consistent with responses of temperate tree seedlings in general (Eamus and Jarvis 1989). Moreover, the effects of elevated CO_2 on tree growth varied among species, but not in the manner predicted by Hypothesis 1. Exceptional plasticity of fast-growing species to resource availability should afford the capacity for marked responses to elevated atmospheric CO_2 concentrations. Proportional increases in both dry matter production and relative growth rate, however, were much higher in oak than in aspen and maple under conditions of enriched CO_2.

That aspen did not respond as strongly as expected to increased CO_2 is likely due to the lighting conditions used in our study. Photon flux density in the growth rooms was only about 50 and 60% of that required for maximum photosynthesis in aspen and oak, respectively, but exceeded that required for maximum photosynthesis in maple (Bazzaz and Carlson 1982, Monson and Fall 1989). Our results agree with those of Bazzaz et al. (1990), who concluded that under conditions of limited light and adequate nutrient availability, elevated CO_2 concentrations may enhance the growth of shade-tolerant trees more than that of shade-intolerant trees. As has been noted by many researchers (e.g., Sionit et al. 1985, Williams et al. 1986, Bazzaz et al. 1990, Ziska et al. 1991) such interspecific variation in response to elevated atmospheric CO_2 may change the composition of plant communities.

Within-plant allocation of carbohydrates is thought to be determined by the balance between carbon supply to leaves and nutrient supply to roots (reviewed by Eamus and Jarvis 1989). Accordingly, under conditions of nutrient limitation, increased availability of

TABLE 2. Continued.

TC (mg)	AD (%)	ECD (%)	ECI (%)
345 ± 5***	81.8 ± 0.7	7.9 ± 1.3	5.7 ± 0.7†
66 ± 9†	43.3 ± 12.5	11.4 ± 3.3	7.5 ± 0.9*
404 ± 20*	67.1 ± 1.6	5.5 ± 0.5	3.4 ± 0.1
691 ± 15***	90.1 ± 1.4	2.9 ± 0.4	2.4 ± 0.2†
162 ± 38†	47.0 ± 8.1	12.5 ± 7.8	3.5 ± 2.1*
302 ± 34*	58.6 ± 4.5	7.2 ± 0.7	3.8 ± 0.1
0.017	0.846	0.837	0.032
<0.001	0.003	0.289	<0.001
<0.001	0.479	0.635	0.217

carbon facilitates root growth, leading to proportional increases and decreases in root and leaf mass, respectively. In contrast, when nutrients are not limiting the root-to-shoot ratios typically decline or remain unchanged.

We found that with elevated CO_2 and a rich nutrient supply, proportional biomass allocation to roots increased in aspen, decreased in oak, and did not change in maple. Our results for aspen differ from those of Brown and Higginbotham (1986), who found no significant effect of elevated CO_2 on root mass ratios at any of three levels of nitrate supply. Reasons for the significant shift toward root production are unclear. Biomass partitioning of oak, however, followed expected patterns for trees grown with high CO_2 and nutrient availability. White oak seedlings grown under enriched CO_2 but low nutrient availability partition biomass differently, exhibiting significant increases in root-to-shoot ratios (Norby et al. 1986, O'Neill et al. 1987).

We caution that extrapolation of our plant growth results to responses of forest trees should be made with care. Growth and allocation were measured over only 60 d in seedlings preconditioned to ambient CO_2 concentrations. Our single harvest was not made in relation to the growth cycle of the trees, which complicates interpretation of the responses of episodically growing species such as oak and maple. Finally, many other factors (e.g., soil nutrient availability) are likely to alter tree growth responses to elevated atmospheric CO_2 in the field.

Foliar chemistry

Concentrations of primary metabolites most strongly affected by CO_2 treatment were those of nitrogen and starch. Nitrogen concentrations almost invariably decline in response to elevated CO_2 (Lincoln et al. 1984, Williams et al. 1986, Fajer 1989, Lincoln and Couvet 1989, Johnson and Lincoln 1990). The magnitude of decline in aspen and maple was in the upper end of the range (5–25%) typically observed. Foliar starch concentrations oftentimes increase in plants grown under enriched CO_2 (e.g., Johnson and Lincoln 1991), although accumulation rates are influenced by carbohydrate sinks such as root growth. We found striking increases in starch concentrations in aspen and no change in maple, a pattern consistent with the trend predicted (Hypothesis 2). Accelerated starch accumulation was probably caused by increased photosynthesis rates and/or decreased photorespiration rates, responses characteristic of plants under enriched CO_2 (Bazzaz 1990). Rates of photosynthesis were slightly higher in elevated-CO_2 aspen than in ambient-CO_2 aspen in weeks 4 and 6, but not week 8, of our study (P. Reich and R. L. Lindroth, *unpublished data*).

Other foliar constituents that serve as nutrients to insects were minimally affected or unaffected by tree CO_2 environment. Decreases in water concentration in high-CO_2 aspen and oak were statistically significant, but the change was trivial. We found no effect of CO_2 on foliar sugar concentrations. These results differ from those of Norby et al. (1986) and Johnson and Lincoln (1991), who documented increased sugar levels in white oak and sagebrush, respectively.

The major secondary compounds of trees in our study are products of the shikimic acid pathway. Precursors (e.g., erythrose-4-phosphate, phosphoenol pyruvic acid) for this biosynthetic pathway are derived from plant carbohydrate metabolism. Thus changes in carbon availability are likely to affect phenolic components of foliage.

In our study, effects of enriched CO_2 on tree allelochemistry varied among types of phenolics and among tree species. Phenolic glycoside concentrations increased in aspen, ellagitannin concentrations increased in maple (but decreased in oak), and condensed tannin concentrations tended to increase in all species, but predominantly in maple.

Concentrations of the various phenolic constituents in ambient foliage were similar to values reported elsewhere for field-grown trees, indicating that the phytochemical changes we observed, and resulting changes

in insect performance, are ecologically meaningful. Levels of phenolic glycosides in aspen registered at the low end of the very broad range exhibited naturally in aspen (Lindroth et al. 1987*b*; J. Hemming and R. L. Lindroth, *unpublished data*). Low concentrations probably resulted from genetic rather than experimental factors, however, because in a more recent CO_2 study, aspen of a different genetic stock exhibited substantially higher phenolic glycoside concentrations (K. K. Kinney and R. L. Lindroth, *unpublished data*). Levels of hydrolyzable tannins in our plants were in the general range of those measured in several oak species and sugar maple (Inoue and Hagerman 1988, Wilson and Hagerman 1990; S. Roth and R. L. Lindroth, *unpublished data*). Likewise, concentrations of condensed tannins in aspen, oak, and maple were within the range of values found in other studies (Bryant et al. 1987*b*, Inoue and Hagerman 1988; S. Roth and R. L. Lindroth, *unpublished data*).

The protein-precipitation capacity of tannins has been of ecological interest because it purportedly serves as an index of potential biological activity. Although little evidence exists to support the view that tannins disrupt protein digestibility in insects, protein-binding capacity may alter insect susceptibility to viral pathogens (Keating et al. 1988). We observed no effect of enriched CO_2 on the protein-binding capacity of tree foliage. The lack of response is surprising for maple, which accumulated large quantities of ellagitannins and condensed tannins under elevated CO_2. Aspen had low protein-precipitation capacity in comparison to oak and maple, a consequence of the absence of hydrolyzable tannins in this species.

This study provides the strongest evidence to date of significant changes in foliar allelochemistry in response to elevated atmospheric CO_2. Although several studies have considered the influence of CO_2 on concentrations of carbon-based secondary compounds (including coumarins, flavonoids, iridoid glycosides, sesquiterpene lactones, and other terpenoids), few significant responses have been observed (Fajer et al. 1989, Lincoln and Couvet 1989, Johnson and Lincoln 1990, 1991). Our study differs from the preceding ones in that it addressed CO_2 effects on trees rather than on herbaceous plants or shrubs, and the species we studied allocate considerable amounts of resources to production of carbon-based allelochemicals. We are aware of only one other study that addressed the impact of enriched CO_2 on tree allelochemicals; Norby et al. (1986) reported significant increases in tannin concentrations of white oak grown under elevated CO_2. Consequences of changes in global CO_2 levels may have greater impact on the chemical composition of forest vegetation than heretofore anticipated based on studies of herbaceous plants and woody shrubs.

Carbon–nutrient balance theory was only partly successful in predicting the phytochemical responses of trees to elevated atmospheric CO_2 (Hypothesis 2). In general, C/N ratios (as indicated by levels of carbohydrates, phenolics and N) increased in the foliage of elevated CO_2 plants, as expected. Moreover, storage carbohydrate (starch) increased most in aspen and least in maple, as expected. Accumulation of carbon-based defensive compounds, however, did not follow the predicted pattern. Proportional increases of phenolic compounds were substantially higher in maple than in aspen and oak. Our results suggest that allocation of carbon to storage vs. defense forms may be related to rates of plant photosynthesis (highest in aspen, lowest in maple; P. Reich and R. L. Lindroth, *unpublished data*) and growth. Starch is readily mobilized and transported within plants, whereas tannins are considered immobile due to their inability to cross cell membranes. Thus, accumulation of starch by aspen may facilitate rapid production of new tissues. Maple, however, is inherently slow-growing; fitness of these individuals may be enhanced by preferential allocation of carbon to defensive compounds. An alternative explanation for high starch concentrations in aspen foliage is that accumulation may have occurred as carbon metabolism became sink limited due to root restriction. Starch may accumulate, however, in leaves of plants grown under elevated CO_2 even when pot size is not limiting, as shown by Thomas and Strain (1991) for cotton.

Insect performance

Our study clearly demonstrates that the impact of elevated atmospheric CO_2 on plant–insect interactions will be influenced by the particular plant and insect species involved. Moreover, altered interactions will be mediated to a large extent by changes in plant chemistry.

Reduced performance of gypsy moth and forest tent caterpillars on high-CO_2 aspen probably resulted from combined changes in foliar nitrogen (protein) and phenolic glycoside concentrations. Although markedly increased consumption by larvae could ostensibly have more than compensated for the 24% decline in aspen nitrogen concentration, insects may have been hampered by reduced efficiency of protein digestion. In spite of high overall digestibility (AD), the digestibility of protein itself may have declined as food moved rapidly through the gut. Improved overall digestibility for insects fed high-CO_2 foliage was probably due to accumulation of readily digested starch. A similar response was noted by Johnson and Lincoln (1991) for grasshoppers fed high-CO_2 sagebrush foliage. Phenolic glycosides reduce the growth of both gypsy moths (Lindroth and Hemming 1990) and forest tent caterpillars (Lindroth and Bloomer 1991). Although concentrations of salicortin and tremulacin in high-CO_2 aspen were relatively low, compensatory feeding by larvae on this foliage greatly increased their exposure to phenolic glycosides. Moreover, toxicity of phenolic glycosides is potentiated at low to moderate concentra-

tions of dietary protein (Lindroth and Bloomer 1991), as existed in aspen leaves. That larvae fed high-CO_2 aspen may have suffered from phenolic glycoside toxicity is consistent with the low food conversion efficiencies of those insects. Increased concentrations of condensed tannins probably did not contribute to the decline in caterpillar growth rates, as other research has found no negative impact of aspen tannins on performance of these insect species (J. Hemming and R. L. Lindroth, *unpublished data*).

In contrast to results with aspen, gypsy moths grew better when fed oak grown under elevated CO_2. Improved performance probably resulted from increased concentrations of starch and decreased concentrations of hydrolyzable tannins. Other researchers have shown that gypsy moth performance is positively correlated with total free sugars (Valentine et al. 1983) and negatively correlated with hydrolyzable tannins (Rossiter et al. 1988) in oak. Forest tent caterpillars survived and grew poorly on oak, independent of CO_2 treatment. Reasons for the poor performance are unclear, as oak is a favored host of forest tent caterpillars in the field (Batzer and Morris 1971).

Despite the fact that CO_2 environment substantially altered maple tannin concentrations, changes in larval performance were less pronounced for this species than for aspen and oak. Growth of gypsy moths was unaffected, although food digestibility decreased and conversion efficiency (ECD) increased for larvae fed high-CO_2 foliage. The final mass of forest tent caterpillars was lower in larvae fed elevated-CO_2 maple, due to reduced food consumption. Effects of high tannin concentrations may have been counteracted by high protein concentrations in maple.

Results from this study only partially agree with Hypothesis 3, that insect performance would change most for individuals on aspen and least for individuals on maple, due to corresponding changes in plant chemistry. Insects responded strongly to CO_2-induced phytochemical changes in aspen, as predicted. For high-CO_2 oak, however, gypsy moth performance actually improved and forest tent caterpillar performance was only marginally affected. Both species of insects showed little change in performance on elevated-CO_2 maple, in spite of substantial increases in tannin concentrations. These results illustrate that phytophagous insects will not exhibit generalized responses to CO_2-mediated changes in host chemistry; responses will be influenced by the degree of adaptation of insects to host allelochemicals.

The question arises as to whether the fertilization effect of elevated CO_2 on plant growth may be offset by increased insect feeding rates. Preliminary evidence from our study suggests that such may be the case for particular tree species. For example, leaf mass increased 25% in aspen under elevated CO_2, but leaf consumption by gypsy moths and forest tent caterpillars doubled. In contrast, growth of maple improved while consumption remained constant (gypsy moth) or declined (forest tent caterpillar). Assessments of the mutual impacts of CO_2 and insect herbivory on tree productivity are difficult to make, however, for several reasons. First, carbon storage in perennial tissue will influence leaf production in successive growing seasons, a factor for which our study did not account. Second, we evaluated larval performance over a single stadium; CO_2-mediated changes in insect performance may differ for different developmental stages. Third, our study did not address population-level effects likely to occur as a consequence of changes in individual growth rates. Fecundity of female gypsy moths and forest tent caterpillars is correlated with final larval mass. Thus, we would predict that population densities of gypsy moths and tent caterpillars may decrease in aspen forests, but that densities of gypsy moths may increase in oak forests, with corresponding changes in defoliation rates. Fourth, tree responses to defoliation, such as compensatory growth or allelochemical induction, may be altered under enriched-CO_2 atmospheres. Additional research on these and related topics is required to better assess the impact of elevated CO_2 on plant–insect interactions in forest ecosystems.

Results from this study provide insight into potential changes in forest community and ecosystem dynamics under CO_2 atmospheres predicted for the next century. As mentioned previously, differential growth responses of trees to elevated CO_2 may alter competitive regimes. Outcomes of competitive interactions, however, will likely be influenced by changes in tree susceptibility to herbivory. Moreover, altered chemical composition of leaf litter (green or senescent) can be expected to change decomposition rates and nutrient cycling processes. Tannins and tannin complexes are refractory to decomposition and thought to protract breakdown and mineralization of detritus in many ecosystems (Horner et al. 1988). Tannin concentrations of aspen, oak, and maple changed in response to elevated CO_2, but not in a consistent manner. Accordingly, our data indicate that changes in decomposition rates may be greater in forests dominated by maple than in forests dominated by oak. We caution, however, that these large-scale consequences are only suggested by our study; a variety of other biotic and abiotic factors are likely to influence the impact of elevated atmospheric CO_2 on forest dynamics.

In conclusion, this research illustrates that enriched-CO_2 atmospheres can markedly alter growth, biomass allocation, and chemical composition of forest trees, and the performance of associated insects. The direction and magnitude of change for both trees and insects, however, are species-specific. Moreover, our results suggest that the impact of elevated CO_2 on plants and herbivores will not only modify the dynamics of those interactions per se, but may have ramifications for larger scales of ecosystem structure and function, such as community composition and nutrient cycling. Addi-

tional research is required, however, to identify general patterns of responses and to determine how responses at the individual level may be altered at the community and ecosystem levels.

ACKNOWLEDGMENTS

This research could not have been completed without untiring technical support from Steve Jung, Rob Thiboldeaux, Erik Hemming, Tony Weiss, and Anita Feuker. Peter Reich and Mark Tjoelker provided advice on tree growth analysis and Ann Hagerman gave advice on tannin assays. We thank Gary Wyckoff for supplying aspen seeds and the Wisconsin Department of Natural Resources for providing oak and maple seedlings. The Biotron staff provided assistance in set-up and monitoring of environmental control rooms. We thank Erik Nordheim for excellent and thorough assistance with statistical analyses. Finally, we appreciate the helpful comments of John Bryant and two anonymous reviewers on the first version of this manuscript. This research was supported by NSF grant BSR-8918586, USDA grant 87-CRCR-2581, and by the College of Agricultural and Life Sciences, University of Wisconsin, Madison (Hatch Project 3211).

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