OIKOS 88: 259–272. Copenhagen 2000

Effects of CO_2 and light on tree phytochemistry and insect performance

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Agrell, J., McDonald, E. P. and Lindroth, R. L. 2000. Effects of CO_2 and light on tree phytochemistry and insect performance. – Oikos 88: 259–272.

Direct and interactive effects of CO_2 and light on tree phytochemistry and insect fitness parameters were examined through experimental manipulations of plant growth conditions and performance of insect bioassays. Three species of deciduous trees (quaking aspen, *Populus tremuloides*; paper birch, *Betula papyrifera*; sugar maple, *Acer saccharum*) were grown under ambient (387 ± 8 μL/L) and elevated (696 ± 2 μL/L) levels of atmospheric CO_2, with low and high light availability (375 and 855 μmol × m^{-2} × s^{-1} at solar noon). Effects on the population and individual performance of a generalist phytophagous insect, the white-marked tussock moth (*Orgyia leucostigma*) were evaluated. Caterpillars were reared on experimental trees for the duration of the larval stage, and complementary short-term (fourth instar) feeding trials were conducted with insects fed detached leaves.

Phytochemical analyses demonstrated strong effects of both CO_2 and light on all foliar nutritional variables (water, starch and nitrogen). For all species, enriched CO_2 decreased water content and increased starch content, especially under high light conditions. High CO_2 availability reduced levels of foliar nitrogen, but effects were species specific and most pronounced for high light aspen and birch. Analyses of secondary plant compounds revealed that levels of phenolic glycosides (salicortin and tremulacin) in aspen and condensed tannins in birch and maple were positively influenced by levels of both CO_2 and light. In contrast, levels of condensed tannins in aspen were primarily affected by light, whereas levels of ellagitannins and gallotannins in maple responded to light and CO_2, respectively.

The long-term bioassays showed strong treatment effects on survival, development time, and pupal mass. In general, CO_2 effects were pronounced in high light and decreased along the gradient aspen > birch > maple. For larvae reared on high light aspen, enriched CO_2 resulted in 62% fewer survivors, with increased development time, and reduced pupal mass. For maple-fed insects, elevated CO_2 levels had negative effects on survival and pupal mass in low light. For birch, the only negative CO_2 effects were observed in high light, where female larvae showed prolonged development. Fourth instar feeding trials demonstrated that low food conversion efficiency reduced insect performance. Elevated levels of CO_2 significantly reduced total consumption, especially by insects on high light aspen and low light maple.

This research demonstrates that effects of CO_2 on phytochemistry and insect performance can be strongly light-dependent, and that plant responses to these two environmental variables differ among species. Overall, increased CO_2 availability appeared to increase the defensive capacity of early-successional species primarily under high light conditions, and of late-successional species under low light conditions. Due to the interactive effects of tree species, light, CO_2, and herbivory, community composition of forests may change in the future.

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Accepted 31 May 1999

ISSN 0030-1299

Rising levels of atmospheric carbon dioxide represent a global change in plant resource availability. Although tree species vary tremendously with respect to carbon allocation to growth, reproduction and chemical defense, research during the last decade has yielded some general conclusions regarding effects of enriched CO_2. First, trees respond to increased CO_2 concentrations by increasing photosynthetic and growth rates, at least over the short term (Ceulemans and Mousseau 1994, Curtis 1996). Second, the chemical composition of plants grown under enriched CO_2 changes, typically reflecting an increase in C:N ratios due to accumulated carbohydrate or phenolic constituents (Lincoln et al. 1993, Lindroth 1996a, Poorter et al. 1997, Peñuelas and Estiarte 1998). And third, patterns of allocation of photosynthate to growth versus defense in plants grown under elevated CO_2 are modulated by the availability of other critical resources, such as nutrients and water (Kinney et al. 1997, Lawler et al. 1997, Pritchard et al. 1997, Roth et al. 1997). Results of these and related studies suggest that rising levels of atmospheric CO_2 may be accompanied by changes in ecosystem structure and function, through changes in trophic interactions and nutrient cycling dynamics.

Two frameworks with a mechanistic basis for predicting growth and phytochemical responses are carbon-nutrient balance (CNB) theory (Bryant et al. 1983, Bazzaz et al. 1987, Tuomi et al. 1988) and growth-differentiation balance (GDB) theory (Herms and Mattson 1992). Both CNB and GDB theories are functionally based on source-sink relationships and make similar predictions for phytochemical responses to changes in factors directly affecting carbon assimilation (Tuomi 1992, Peñuelas and Estiarte 1998). In general, any environmental factor that affects the carbon supply from assimilating source tissues (e.g. CO_2 and light availability) and/or the demand by growing sink tissues (e.g. nutrient and water availability) will influence the amount of carbon that is available for storage or secondary metabolism. In general, any environmental factor (e.g., CO_2 and light availability) that affects the carbon supply from assimilating source tissues and/or the carbon demand by growing sink tissues will influence the amount of carbon that is available for storage or secondary metabolism. Both CO_2 and light are essential resources strongly affecting tree growth and chemical composition, but for which little is known with respect to interactive effects. Moreover, although availability of both resources influences accumulation of photosynthates, they are not identical in terms of effects on growth and chemical composition (McDonald et al. 1999). Thus, given the importance of variation in light availability for community and ecosystem processes, evaluation of potential interactions between light and CO_2 is important for anticipating ecological impacts of a carbon-enriched atmosphere.

Responses of trees to enriched CO_2 also vary in relation to interspecific differences in growth strategies (Loehle 1995). Plant species evolutionarily adapted to resource rich environments typically exhibit high growth rates, low investment in defensive compounds, and considerable plasticity in chemical response to changes in resource availability, whereas the reverse is true for plant species adapted to resource poor environments (Chapin 1980, Bryant et al. 1983, 1987, Coley et al. 1985, Bazzaz et al. 1987). Consequently, researchers have predicted that elevated CO_2 levels should increase growth rate and/or concentrations of carbon based defensive compounds more for fast-growing, early-successional species, than for slow-growing, late-successional species. Accumulated data to date, however, are not entirely consistent with the predicted patterns (Bazzaz et al. 1990, Lindroth et al. 1993, Kinney et al. 1997). Such inconsistencies have partly been explained by the modulating influence that light and nutrient availability may have on the effects of CO_2 on physiology and growth of early- and late-successional species (Bazzaz and Miao 1993, Kubiske and Pregitzer 1996).

Interacting effects of CO_2 and light on foliar chemical composition of trees are likely to affect the performance of associated herbivores. The independent effects of these factors are becoming well established; enriched CO_2 and high light environments increase the C:N ratio of foliage such that insects often exhibit prolonged development, increased (compensatory) feeding, decreased growth and reduced feeding efficiencies (Larsson et al. 1986, Lindroth 1996b, Lawler et al. 1997). How CO_2 and light availability may interact to affect herbivore performance, however, is poorly understood.

This study evaluated the direct and interactive effects of CO_2 and light on tree phytochemistry, and consequences for performance of a folivorous insect. Our experimental system consisted of three species of deciduous trees (quaking aspen, *Populus tremuloides*; paper birch, *Betula papyrifera*; sugar maple, *Acer saccharum*) and a generalist herbivore, the white-marked tussock moth (*Orgyia leucostigma*). The tree species chosen for this study vary with respect to intrinsic growth rate and shade tolerance (Loehle 1987). Aspen and birch are both early-successional species, although the former has a higher growth rate and lower shade tolerance than the latter. In contrast, maple is a slow-growing, shade tolerant, late-successional species.

We anticipated that interspecific differences in chemical plasticity with respect to resource availability would influence the phytochemical responses of these species to CO_2 and light levels, and subsequently alter performance of tussock moths. Specifically, we predicted that high levels of both CO_2 and light, through increases in carbon assimilation and carbohydrate concentrations (i.e., an increase in source strength), would increase levels of starch and phenolic compounds and decrease

levels of foliar nitrogen. Phytochemical responses should be strongest in aspen, somewhat lower in birch and weakest in maple. How the interactive effects of CO_2 and light might vary among tree species was less clear. Based on previous research on tree growth responses to enriched CO_2 (Bazzaz et al. 1990, Bazzaz and Miao 1993) we expected that the effects of CO_2 would be stronger for aspen and birch in high light, and for maple in low light. We also predicted that insect performance would mirror changes in concentrations of plant nutrients and defensive substances. Insects reared on trees grown under high levels of CO_2 and/or light would experience diets with comparably low levels of nitrogen and high levels of phenolic compounds, and consequently exhibit prolonged development time and reduced survival, pupal mass, and food conversion efficiency. Overall, negative effects of CO_2 on insect performance would likely be most pronounced on high light aspen and birch, and on low light maple.

Methods

Experimental design

This study was performed at the Univ. of Wisconsin Biotron from April to July 1996. We used eight greenhouse rooms (4×5 m) in which CO_2 and light levels were manipulated. We employed a split-split-plot experimental design, in which the whole-plot was CO_2 level (ambient and elevated), each replicated four times (rooms). The subplot was light level (low and high), with each room divided into a low and high light treatment. The sub-subplot was tree species (aspen, birch, and maple).

In all greenhouse rooms, thermal regimes were set on a 15:9 h schedule of 26 and 18°C for day and night, respectively. Four of the rooms operated at ambient CO_2 levels (387 ± 8 μL/L, mean ± SE), and four rooms had elevated CO_2 levels (696 ± 2 μL/L). All rooms were naturally illuminated and divided into a high light and a low light treatment. The high light treatment was left unshaded. In the low light treatment light intensity was reduced to 44% of available light by a double layer of optically neutral shadecloth attached to the sides and top of a square metal frame 2.5 m above the floor. Every third week throughout the experiment we measured light intensity at solar noon, at ten fixed positions (plant height) in each room. In high light treatments, the average solar noon light level during the experiment was 855 $\mu mol \times m^{-2} \times s^{-1}$ (range: 615–1232). Thus, the high light treatment could be considered as moderate availability relative to full sun levels. The corresponding value in the low light treatment was 375 $\mu mol \times m^{-2} \times s^{-1}$ (range: 295–490). We grew equal numbers of aspen, birch and maple, which were randomly located within each room and light treatment. This study lasted from 27 April to 2 July (66 d).

Plant material

Trees used in the study were one-year-old quaking aspen (*Populus tremuloides*), and two-year-old paper birch (*Betula papyrifera*) and sugar maple (*Acer saccharum*). We grew quaking aspen the previous year with seed collected by the Univ. of Minnesota North Central Experiment Station (Grand Rapids, Minnesota) from a single tree in Oneida County, Wisconsin. Birch seedlings were obtained from Wallace-Woodstock Co. (Neillsville, Wisconsin), and sugar maples were supplied by the Wisconsin Dept of Natural Resources (Boscobel, Wisconsin). The trees were planted in plastic pots containing 14 L of a 3:1 soil mixture of torpedo sand and shredded silt-loam topsoil. Each tree received 45 g of a complete slow release fertilizer (3 4 month release, 17-6-12 NPK with micronutrients, Sierra, Milpine, California). This provides a moderately high level of nutrients for fast-growing trees such as aspen (Hemming and Lindroth 1999). Trees were watered to saturation every other day, or as necessary. We planted four individuals of each species in each light treatment of each greenhouse room. Two of the trees were used for phytochemical assays and insect bioassays, and the remaining two were used either to replace individuals that did not flush, or to rear insects for the fourth instar feeding trials (see below). Plants were observed daily to determine the date of bud break.

Foliar chemistry

Leaves for foliar chemistry were collected half way through the experiment to coincide with the fourth instar feeding trials (see below). First flush leaves were cut at the base of the petiole, weighed, and flash frozen in liquid nitrogen. After freeze-drying the leaf material was reweighed, ground in a dental amalgamator (IDE-1A, IDE-Interstate, Amityville, New York), and stored at −20°C. Nutritional analyses conducted for all three tree species included water, nitrogen and starch. Previous studies have shown that CO_2 levels have limited influence on hexose and sucrose concentrations in these species (Lindroth 1996b), so we did not assay those sugars. All major secondary metabolites in aspen, birch and maple are phenolic compounds (Palo 1984, Baldwin et al. 1987, Lindroth et al. 1987). We analyzed for levels of condensed tannins in all species, for the phenolic glycosides salicortin and tremulacin in aspen, and for hydrolyzable tannins (ellagitannins and gallotannins) in maple.

Analytical methods for leaf chemistry are described extensively by Lindroth et al. (1993). In short, water

content was calculated gravimetrically, as the difference between fresh and dry foliage. Nitrogen analyses were performed with a micro-Kjeldahl method (modified from Parkinson and Allen 1975). Samples of leaf material were digested in a H_2SO_4-peroxide solution, and nitrogen content was determined using a micro-Nesslerization technique (Lang 1958). Glycine *p*-toulenesulfonate was used as a standard. To determine levels of foliar starch, we employed the method of M. M. Schoeneberger, K. Ludovici, and P. Faulkner (unpubl.), in which starch is enzymatically converted to glucose, which is then indirectly quantified by measuring the enzyme-mediated reduction of NADP to NADPH. Concentrations of condensed tannins were analyzed using a modification of the butanol-HCl method of Porter et al. (1986). We determined the hydrolytic conversion of proanthocyanidins to anthocyanidins in acetone (70%) extracts of leaf samples. Condensed tannin purified from aspen via absorption chromatography served as the standard. Because condensed tannins from different plant species respond differently in the acid butanol assay, interspecific comparisons in this study should be interpreted cautiously.

We measured concentrations of salicortin and tremulacin, the most abundant phenolic glycosides in aspen (Lindroth et al. 1987), using high performance thin layer chromatography (HPTLC). Leaf tissue was extracted in methanol, and 1 μL aliquots were applied in duplicate to HPTLC plates. Plates were developed, then scanned at 274 nm (Camag Scanner II, Camag Scientific, Incorporated, Wrightswille Beach, North Carolina). Chromatograms were analyzed with Camag TLC software (Cats 3.11). Purified salicortin and tremulacin were used as standards.

To selectively determine levels of ellagitannins and gallotannins in maple leaves, we employed procedures relying on acid hydrolysis and subsequent measurements of gallic or ellagic acid. For ellagitannin assays we used the method described by Wilson and Hagerman (1990), and modified by Lindroth et al. (1993). Leaf tissue samples were hydrolyzed in H_2SO_4 in a pressure chamber purged with nitrogen. Sample residues were concentrated, dissolved in pyridine, and levels of ellagitannins were quantified as ellagic acid equivalents. For gallotannins we followed the method of Inoue and Hagerman (1988), with the modifications described by Lindroth et al. (1993). In this assay, the acetone extracts previously prepared for condensed tannin analyses were used. After a hydrolysis procedure similar to that described for ellagitannins, hydrolysates were diluted and assayed for total gallic acid. Levels of free gallic acid in the samples were obtained by performing the same analyses on nonhydrolyzed extracts, and gallotannin concentrations (as gallic acid equivalents) were calculated as the difference between total and free gallic acid.

Bioassays

Bioassays were performed with the white-marked tussock moth (*Orgyia leucostigma*), a lymantriid species known to exhibit occasional, localized outbreaks on deciduous trees (Rose and Lindquist 1982). Larvae have five and six stadia for males and females, respectively, and pupal masses exhibit pronounced sexual dimorphism. We obtained tussock moth egg masses from the Forest Pest Management Inst., Canadian Forest Service (Sault Ste. Marie, Ontario, Canada). With few exceptions, previous studies on effects of atmospheric CO_2 on plant-insect interactions have emphasized short-term bioassays, usually the duration of one larval stadium. Such bioassays have been useful in highlighting treatment effects on growth and consumption rates, and food processing efficiencies, of insects. They are of less value, however, in elucidating long-term population-level effects (e.g. survival rates) or in detecting marginal changes in performance, the impact of which may increase over time. We conducted parallel long- and short-term bioassays.

To obtain long-term data on survival, development and growth rates, we reared cohorts of insects on foliage of treated trees for the duration of the larval stage. Egg masses were hatched 18 d after budbreak (day 24 of the experiment). For each of the 96 experimental trees (two per species, per light treatment, per glasshouse) ten larvae were randomly selected from 14 egg masses hatching simultaneously. Due to their very small size (< 0.2 mg), neonates could not be reared in mesh bags directly on the trees. We therefore reared insects on detached leaves in Petri dishes for the duration of the first stadium. Petri dishes (15×1.5 cm) were kept in a Percival growth chamber (Percival Manufacturing Company, Boone, Iowa) under conditions simulating those in the glass houses, i.e. a photoperiod of 15:9 L:D, with L:D temperatures of 26 and 18°C, respectively. Each set of larvae (experimental cohort) was fed leaves from its assigned tree. Leaves were excised from the trees with a razor blade and petioles were immediately inserted into a 1.5-mL plastic microfuge tube containing water. We replaced the leaf tissue every third day, or at shorter intervals if more than half of the leaf had been consumed. Seven days after hatching, when the majority of the larvae had molted into the second stadium, we recorded the number of surviving larvae in each Petri dish, the instar of each individual, and the aggregate mass of the individuals. Five of the surviving individuals were then transferred from the Petri dish to a fine-mesh nylon bag (20×14 cm), which enclosed one leaf (maple) or 2–3 leaves (aspen and birch) on the assigned tree. Bags were moved, usually every 2–3 d, to ensure that sufficient foliage was available. At 7-d intervals we recorded larval survival, development stage, and mass. When a majority of the individuals within a bag had molted

into the third instar, they were transferred to a larger fine-mesh branch bag (35 × 20 cm). These bags covered whole branches and were securely attached with twist ties around the base of the branch. We recorded the time when each larva initiated pupation; 2 d later the larva was removed from its cocoon, sexed, and weighed. The experiment continued until all larvae in all bags had either died or pupated.

From the long-term bioassay data we calculated survival, male and female pupal mass, and male and female development time. Survival in the cohorts was calculated for each 7-d period throughout the experiment. For the statistical analysis we then used total survival, i.e. the percentage of individuals surviving until pupation. Because numbers were reduced to five individuals per tree after one week, total mortality over the larval period was calculated as the proportional mortality during the first week added to the proportional mortality after that time. Survival until pupation was then calculated for each bag as 1 − total mortality. Data on average insect body mass per rearing bag were obtained at 7-d intervals by dividing the aggregate mass by the number of individuals in the bag. Finally, development time was calculated as the number of days elapsed from hatching until onset of pupation.

In this bioassay, insects were subjected to the same CO_2 and light regimes as were the plants. Thus, the *direct* effects of CO_2 and light on insect performance were confounded with the *plant-mediated* effects of CO_2 and light. We expected the former to be minimal to nonexistent. As confirmation, we evaluated the direct effects of CO_2 and light levels on larval performance by rearing larvae on artificial diet in each of the CO_2 × light environments. Coincident with onset of the long-term study, eight sets of ten newly hatched larvae were placed into well-ventilated Petri dishes (15 × 3 cm), which were evenly distributed among the rooms and treatments (two for each CO_2 × light combination). The larvae were fed gypsy moth artificial diet (ODell et al. 1985), and survival, developmental stage, and mass were recorded weekly until pupation.

To measure food consumption rates and conversion efficiencies, we performed separate short-term feeding trials with fourth instar tussock moths. In order to obtain individuals similar to those in the long-term bioassays, the larvae used in the fourth instar trials were reared on separate "rearing" trees grown parallel to the experimental trees. For each CO_2 × light combination, four trees of each species were individually enclosed in fine mesh nylon bags (50 × 70 cm) and 30 newly hatched larvae were placed in each bag. These larvae showed the same development rate as those from corresponding treatments reared in small bags. When the first larva in the long-term study molted into the fourth instar, all individuals on rearing trees were removed and placed in Petri dishes (15 × 3 cm) in the Percival growth chamber. They were then fed foliage from their respective feeding tree, and checked twice daily for individuals molting into the fourth instar. Molting individuals were placed individually into Petri dishes (15 × 1.5 cm) without access to foliage, and monitored every 4 h. Newly molted individuals were weighed, and then provided with a single leaf from a corresponding experimental tree. Leaves were excised, weighed, and petioles immediately inserted into a microfuge tube containing water. For each experimental tree we performed two separate short-term bioassays, for a total of 192 (16 bioassays per tree species, per CO_2 and light treatment). On average, these feeding assays were initiated 20 d after egg hatch.

The short-term feeding trials continued for the duration of the fourth larval stadium. Leaves were replaced, as necessary, every 1–3 d. Foliage was removed from dishes when individuals started to molt into the fifth instar. After molting, each larva was frozen and sexed. The larvae, uneaten leaf material and frass were dried for one week (60°C), and then weighed. Following Scriber (1977), we calculated the relative growth rate (RGR) and the average total consumption (TC) for each larva. Data on total consumption reflect the impact that individuals in insect populations would have on their respective host plants. In order to determine if individuals adjusted feeding *rates* to foliage quality (i.e. the extent of compensatory feeding) we also calculated absolute (ACR) and relative (RCR, adjusted for body mass) consumption rates. Finally, we calculated approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI). To obtain estimates of the initial dry mass of larvae, a sample of ten newly molted fourth instars from each tree species and CO_2 × light treatment was weighed, frozen, dried and reweighed. Initial dry mass of leaves fed to larvae was similarly determined by performing regressions between fresh and dry mass of leaves used for the phytochemical analyses, using separate regressions for each treatment combination.

Statistical analyses

To analyze data on tree chemistry and insect performance we used a split-split-plot design with a mixed model analysis of variance (ANOVA). Statistical computations were performed with the SAS GLM procedure (SAS Institute 1989). In the basic model CO_2 level was the whole-plot factor, light level was the sub-plot factor, and tree species was the sub-sub-plot factor:

$$Y_{ijkl} = \mu + C_i + E_{ij} + L_k + (CL)_{ik} + e_{ijk} + S_l + (CS)_{il} + (LS)_{kl} + (CSL)_{ikl} + e_{ijkl}.$$

Y_{ijkl} represents the average tree response or insect response to CO_2 level i, room j, light level k, and tree species l. CO_2 level (C_i), light level (L_k), CO_2 × light interaction $(CL)_{ik}$, species (S_l), CO_2 × species interaction $(CS)_{il}$, light × species interaction $(LS)_{kl}$ and CO_2 × light × species interaction $(CLS)_{ikl}$ were fixed effects. Random effects consisted of the whole-plot error (E_{ij}), the subplot error (e_{ijk}), and the sub-subplot error (ϵ_{ijkl}). F tests ($F_{1,6}$) were computed for C_i using E_{ij} as the error term and for L_k and $(CL)_{ik}$ using ϵ_{ijkl} as the error term. F tests ($F_{2,24}$) were calculated for S_l, $(CS)_{il}$, $(LS)_{kl}$ and $(CLS)_{ikl}$ using ϵ_{ijkl} as the error term. Before data were analyzed by ANOVA, cell (room) means for each CO_2, light and species combination were calculated using the SAS MEANS procedure. SAS MEANS was also used for computing standard errors for the treatment means ($n = 4$ rooms). To correct for heterogeneity of variances, data computed as proportions were transformed (arcsine-square root) before statistical analyses. To facilitate data presentation, only main and interactive effects with P values less than 0.10 are provided in the figures. Mean comparisons between CO_2 levels for each light level and between light levels for each CO_2 level were calculated separately for each species using the SAS MIXED/LSMEANS procedure (see Appendices 1–4).

The ANOVA model was modified for some specific analyses. Because phenolic glycosides occur only in aspen and hydrolyzable tannins occur only in maple, statistical analyses of concentrations of these compounds were performed with a simple split-plot model. F tests ($F_{1,6}$) were computed for C_i using the error term E_{ij}, and for L_k and $(CL)_{ik}$ using the error term e_{ijk}. Also, to determine if results from the short-term feeding trials were influenced by differences between male and female larvae, we performed an extensive analysis on the effect of sex. We expanded the basic model into a split-split-split-plot design by adding the main effect of sex, as well as all interactions involving sex. Analyses of RGR, TC, ACR, RCR, AD, ECD, and ECI revealed no significant effect of sex ($P > 0.3$). We thus conclude that our data were not influenced by intersexual differences in larval performance, and data from both sexes were pooled in subsequent analyses.

Results

Foliar chemistry

Both CO_2 and light influenced levels of nutritional constituents of foliage (i.e., water, starch and nitrogen). Water content showed a small but consistent decrease in response to elevated CO_2 across all species (Fig. 1). The species responded differently to light treatments, such that reductions in water content in high light were somewhat more pronounced for aspen and birch than for maple. Foliar starch concentrations varied greatly among treatments (Fig. 1), with concentrations elevated by both high CO_2 and high light environments. Moreover, effects of enriched CO_2 were strongest in high light, increasing starch content an average of 79%, compared with an average increase of 66% in low light treatments (CO_2 × light interaction). Starch levels increased along the species gradient from aspen to birch to maple. Foliar nitrogen concentrations declined in high CO_2 and high light environments, and the magnitude of change differed among tree species (Fig. 1). The decline was most pronounced in aspen and maple under high light conditions, and in birch foliage under low light conditions (CO_2 × light × species interaction). Nitrogen content was also more light sensitive in birch than in aspen and maple (light × species interaction).

CO_2 and light availability also strongly affected levels of carbon-based secondary compounds. Concentrations of condensed tannins increased in trees grown under enriched CO_2 or high light, but responses differed among species (Fig. 2). Aspen re-

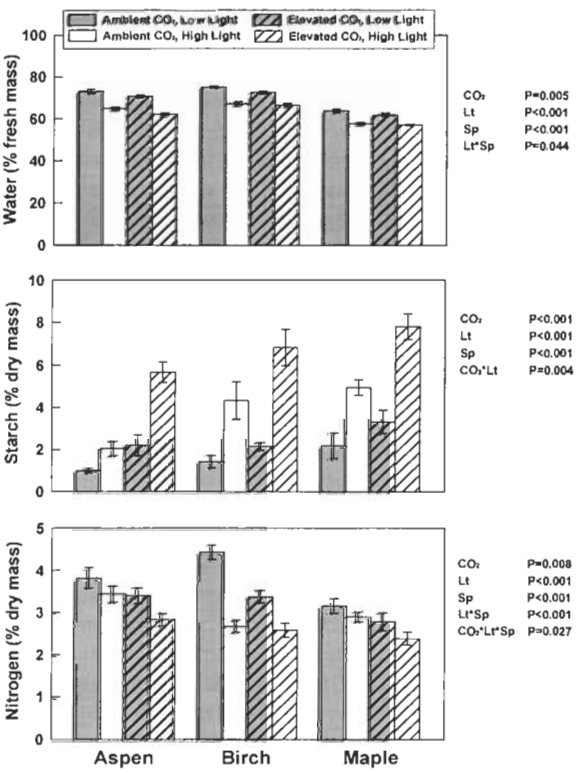

Fig. 1. Concentrations of foliar water, starch and nitrogen for quaking aspen, paper birch and sugar maple in response to CO_2 and light availability. Vertical lines represent ± 1 SE. Only P values < 0.100 are shown. Mean comparisons within whole plots and subplots are provided in Appendix 1.

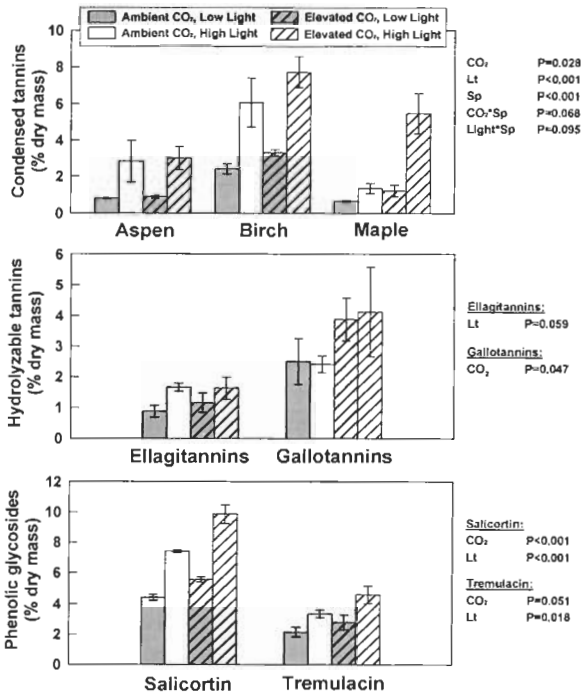

Fig. 2. Concentrations of foliar condensed tannins (all species), hydrolyzable tannins (maple) and phenolic glycosides (aspen) in response to CO_2 and light availability. Vertical lines represent ± 1 SE. Only P values < 0.100 are shown. Mean comparisons within whole plots and subplots are provided in Appendix 2.

sponded to light but not to CO_2, whereas birch and maple responded to both light and CO_2. CO_2 and light differentially affected levels of hydrolyzable tannins in maple (Fig. 2). Concentrations of ellagitannins showed a marginally significant increase in response to high light but were unaffected by CO_2 conditions. In contrast, concentrations of gallotannins averaged 63% higher in enriched CO_2, but did not vary between light treatments. Finally, levels of phenolic glycosides in aspen were strongly affected by both CO_2 and light conditions (Fig. 2). Salicortin concentrations increased 30% in high CO_2 and 72% in high light treatments. The general pattern was similar for tremulacin, although effects were less pronounced.

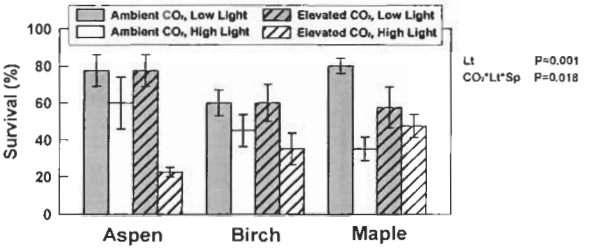

Fig. 3. Survival of white-marked tussock moth caterpillars reared on leaves from aspen, birch and maple grown under ambient or elevated levels of CO_2, and low or high light intensity. Data recorded from hatching to pupation. Vertical lines represent ± 1 SE. Mean comparisons within whole plots and subplots are provided in Appendix 3.

Bioassays

CO_2 and light had no direct effect on the performance of white-marked tussock moths reared on a standard artificial diet (Table 1). Thus, the effects of CO_2 and light treatments reported below are likely mediated by changes in plant quality. The long-term bioassays revealed strong treatment effects on tussock moth performance. The effect of CO_2 on larval survival rates varied markedly among light treatments and tree species ($P = 0.018$ for $CO_2 \times$ light $\times$ species interaction; Fig. 3). Under high light conditions, enriched CO_2 resulted in 62% fewer survivors for insects reared on aspen, whereas no corresponding effect occurred for insects on low light aspen. For larvae fed birch, trends were similar but not statistically significant. In contrast, for insects reared on maple, the effects of enriched CO_2 were stronger under low light conditions than under high light conditions.

Final pupal mass was influenced primarily by the interactive effects of CO_2 and light, and female pupae exhibited more variation (and overall larger size) than did male pupae (Fig. 4). Male pupal mass was significantly reduced only on high CO_2–high light aspen. For females reared on aspen, enriched CO_2 had a limited impact on pupal mass under low light conditions, but decreased pupal mass under high light conditions. For females reared on birch, the primary effect of elevated CO_2 was to increase pupal mass in low light. On maple, enriched CO_2 decreased female pupal mass

Table 1. Performance of white-marked tussock moth caterpillars ($\bar{X} \pm 1$ SE) reared on artificial diet in experimental CO_2 and light environments.

CO_2	Light	Male pupal mass (mg)	Female pupal mass (mg)	Male development time (d)	Female development time (d)	Survival (%)
Ambient	Low	132.5 ± 25.0	375.8 ± 49.0	27.6 ± 2.7	33.2 ± 3.1	0.95 ± 0.05
Ambient	High	153.3 ± 30.9	427.0 ± 21.1	29.0 ± 0.7	31.3 ± 0.9	0.85 ± 0.05
Elevated	Low	148.2 ± 32.2	391.2 ± 9.5	29.8 ± 0.9	30.0 ± 0.4	0.90 ± 0.10
Elevated	High	125.6 ± 8.5	409.2 ± 68.2	27.0 ± 3.2	34.0 ± 2.8	0.95 ± 0.05
P values:						
CO_2		0.73	0.97	0.98	0.42	0.73
Light		0.96	0.37	0.76	0.99	0.73
$CO_2 \times$ Light		0.35	0.65	0.36	0.25	0.32

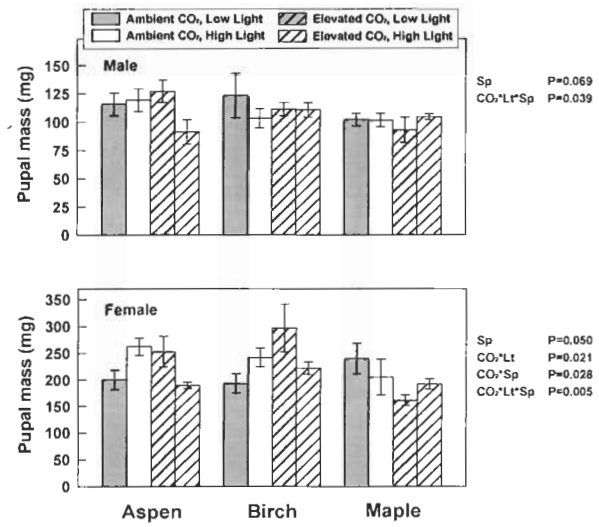

Fig. 4. Pupal mass of male and female white-marked tussock moth caterpillars reared on leaves from aspen, birch and maple grown under ambient or elevated levels of CO_2, and low or high light intensity. Vertical lines represent ± 1 SE. Only P values < 0.100 are shown. Mean comparisons within whole plots and subplots are provided in Appendix 3.

under low light conditions, but not under high light conditions.

Development times (egg hatch to pupation) showed that males and females responded similarly to experimental treatments (Fig. 5). In high light, increased CO_2 had a strong negative effect on development of insects

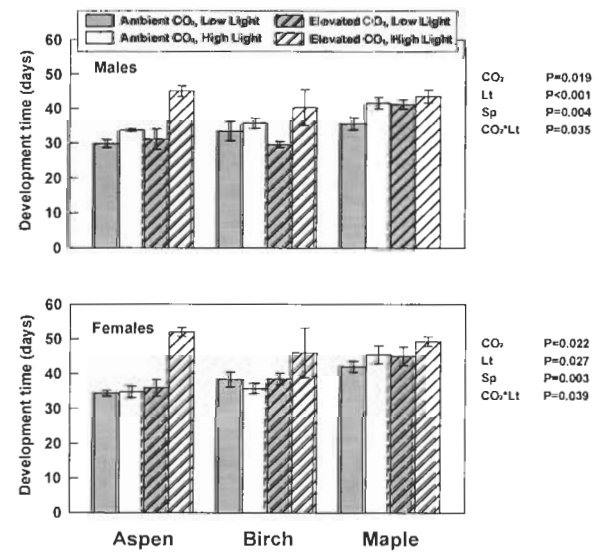

Fig. 5. Development time (from egg hatch to pupation) of white-marked tussock moth caterpillars reared on leaves from aspen, birch and maple grown under ambient or elevated levels of CO_2, and low or high light intensity. Vertical lines represent ± 1 SE. Only P values < 0.100 are shown. Mean comparisons within whole plots and subplots are provided in Appendix 3.

fed aspen, a moderate effect on insects fed birch, and a negligible effect on insects fed maple. Under low light conditions, however, CO_2 level increased development time only for insects on maple. Development times increased slightly along the gradient from aspen to birch to maple.

As in the long-term cohort study, the performance of larvae during the short-term (fourth instar) feeding trials was strongly affected by experimental treatments. Relative growth rates (RGR) of larvae were reduced by high light on aspen and birch, especially so in the high CO_2 treatments (Table 2). In contrast, RGRs of insects on maple tended to be highest in the high CO_2–high light treatment. Total consumption (TC) and absolute consumption rates (ACR) of larvae exhibited similar trends, generally declining in high CO_2 and increasing in high light treatments (Table 2). The significant light × species interaction for TC reflects that high light increased consumption more for insects on birch than for insects on aspen or maple. Overall, TC and ACR were lowest for larvae reared on aspen, intermediate for larvae on maple, and highest for larvae on birch. Relative consumption rates (RCR), which adjust consumption parameters for variation in the initial sizes of larvae, showed a CO_2 × light interaction: enriched CO_2 did not alter consumption by insects fed low light foliage, but markedly increased consumption by insects fed high light foliage (Table 2). RCR showed somewhat different interspecific variation than did TC and ACR, and increased along the gradient aspen < birch < maple.

Food digestibilities and conversion efficiencies were strongly affected by CO_2, light, host species, and their interactions. Overall, approximate digestibility (AD) values declined in response to high CO_2 in low light conditions, but increased in response to high CO_2 in high light conditions (CO_2 × light interaction; Table 2). The high light treatment generally improved digestibility of aspen and maple foliage, but reduced digestibility of birch foliage (light × species interaction). Conversion efficiencies (ECD, ECI) were strongly responsive to treatment conditions in larvae fed aspen, moderately responsive in larvae fed birch, and nonresponsive in larvae fed maple (Table 2). Larvae fed aspen foliage grown under high light exhibited reduced conversion efficiencies, especially for high CO_2 foliage (CO_2 × light interaction). A similar though less pronounced pattern was evident in insects fed birch.

Discussion

CO_2, light and foliar chemistry

Overall, increased levels of CO_2 and light strongly affected plant phytochemistry. Foliar water content was reduced with increased levels of both CO_2 and light, but

the magnitude of change was probably too small to be of significance to tree-feeding insects. With respect to starch and nitrogen, the observed changes agreed well with the predicted main effects of CO_2 and light treatments. Starch levels increased and nitrogen levels decreased in response to enhanced CO_2 and light availability. Moreover, CO_2 and light availability interacted to influence starch concentrations in all three species, and nitrogen concentrations in birch. Our results for these primary metabolites agree with previous research on CO_2 and/or light (Larsson et al. 1986, Lincoln et al. 1993, Julkunen-Tiitto et al. 1993, Lindroth 1996b, Wilkens et al. 1996, Lawler et al. 1997).

With respect to specific secondary compounds, we found that levels of condensed tannins in aspen responded to light, whereas in birch and maple they responded to both CO_2 and light, with evidence of an interactive effect in maple. In a parallel study on tree resource allocation, conducted at the same time and with the same tree species as this study, McDonald et al. (1999) found that CO_2 and light interacted strongly (more than additively) to affect condensed tannin concentrations. The reason for the difference in results is unclear, except that McDonald et al. sampled leaves several weeks earlier than was done in this study, suggesting that leaf phenology may influence $CO_2 \times$ light interactions. In the only other related study published to date, Lawler et al. (1997) found that condensed tannin levels in *Eucalyptus* are independently increased by both enriched CO_2 and high light. CO_2 and light conditions differentially affected levels of hydrolyzable tannins. Concentrations of ellagitannins increased in high light but were unresponsive to CO_2, whereas concentrations of gallotannins increased in high CO_2 but were unresponsive to light. Responses of hydrolyzable tannins in maple to enriched CO_2 are curiously unpredictable. Previous studies with elevated CO_2 detected increases in ellagitannins but no change in gallotannins (Lindroth et al. 1993, Kinney et al. 1997), increases in gallotannins but no change in ellagitannins (Roth et al. 1998), and increases in both ellagitannins and gallotannins (Roth and Lindroth 1995, Roth et al. 1997). Unknown genetic, phenological or environmental factors must influence the response of hydrolyzable tannins in maple to enriched CO_2. Finally, levels of phenolic glycosides in aspen increased in response to both high CO_2 and high light conditions. The responses to CO_2 and light were statistically independent, although concentrations of both phenolic glycosides tended to respond more strongly to CO_2 under high light.

The overall pattern of higher starch and secondary metabolite levels with increasing CO_2 and/or light availability is in general agreement with CNB and GDB theories when viewed as a response to an increase in source/sink ratio. Interpretation of the present study benefits from comparison with results from the parallel study of McDonald et al. (1999) showing that both phytochemical levels and tree relative growth rates were positively related to CO_2 and light levels. Furthermore, the relative investment in growth (vs secondary metabolism) was low when both CO_2 and light availability were low. Such patterns suggest that although the present study found secondary compound levels to be lowest in low CO_2–low light treatments, that result may reflect a comparably high *relative* investment in secondary metabolism. The positive relationship observed between resource levels and both growth and secondary metabolite levels agrees with GDB predictions given that our experimental conditions overall represented low to intermediate resource levels, i.e., moderately high nutrient levels and low to moderate light levels (cf. Herms and Mattson 1992). At resource levels exceeding moderate requirements GDB theory further predicts a negative relationship between growth and secondary metabolism. As we tested only two levels of CO_2 and light, and a single level of nutrients, our study does not offer support for the full array of GDB predictions.

Because plant species adapted to high resource environments typically exhibit considerable chemical plasticity in response to changes in resource availability (Chapin 1980, Bryant et al. 1983, Bazzaz et al. 1987), we expected that CO_2 effects on primary and secondary metabolites would be strongest for the early-successional aspen and birch. We also predicted that effects on these two species would be more pronounced under high light conditions, whereas the late-successional maple should respond less overall and primarily under low light conditions (Bazzaz and Miao 1993, Kubiske and Pregitzer 1996). In general, our results do not support those predictions. Starch levels in maple responded to resource availability in a manner similar to those in aspen and birch, and nitrogen levels in maple responded similarly to those in aspen. Also, low light maple did not respond more strongly to CO_2 enrichment than did high light maple. Similarly, Kinney et al. (1997) found no evidence of a clear "successional trend" in responsiveness of foliar carbohydrates and nitrogen to CO_2 and soil nitrate availability. Regarding the secondary metabolites, levels of various tannins in maple changed as markedly as did those of tannins and phenolic glycosides in aspen and birch. In short, maple exhibited no less chemical plasticity to changes in resource availability than did aspen and birch. Clearly, to identify trends of phytochemical responsiveness across life history or successional gradients will require evaluation of many more species than used in this study.

Effects on insect performance

Results from the bioassays showed that levels of CO_2 and light had a pronounced impact on insect perfor-

Table 2. Effects of CO_2 and light treatments on performance of white-marked tussock moth caterpillars ($\bar{X} \pm 1$ SE) reared on quaking aspen, paper birch and sugar maple foliage. Mean comparisons within whole plots and subplots are provided in Appendix 4.

Species	CO_2	Light	Insect performance parameter*						
			RGR (mg × mg $^{-1}$ × d $^{-1}$)	TC (mg)	ACR (mg × d $^{-1}$)	RCR (mg × mg $^{-1}$ × d $^{-1}$)	AD (%)	ECD (%)	ECI (%)
Aspen	Ambient	Low	0.487 ± 0.049	37.3 ± 6.3	7.41 ± 1.04	3.41 ± 0.48	37.6 ± 2.7	44.1 ± 4.4	15.34 ± 0.92
	Ambient	High	0.472 ± 0.089	45.3 ± 2.0	8.62 ± 0.33	4.28 ± 0.14	40.4 ± 3.5	30.1 ± 7.0	11.00 ± 1.64
	Elevated	Low	0.463 ± 0.014	36.3 ± 2.3	7.05 ± 0.26	3.47 ± 0.06	31.9 ± 1.9	45.0 ± 5.0	13.30 ± 0.67
	Elevated	High	0.361 ± 0.049	29.8 ± 2.0	5.84 ± 0.36	4.94 ± 0.14	50.3 ± 2.5	14.3 ± 2.4	6.64 ± 0.88
Birch	Ambient	Low	0.571 ± 0.050	44.7 ± 3.7	8.58 ± 0.55	5.17 ± 0.23	38.5 ± 2.0	31.5 ± 3.9	11.22 ± 0.61
	Ambient	High	0.488 ± 0.080	69.5 ± 6.6	12.29 ± 0.91	5.68 ± 0.40	27.5 ± 2.7	33.1 ± 7.2	8.36 ± 1.21
	Elevated	Low	0.549 ± 0.040	52.3 ± 3.6	10.52 ± 0.59	4.98 ± 0.30	34.6 ± 3.0	37.6 ± 6.2	11.48 ± 1.18
	Elevated	High	0.402 ± 0.041	65.1 ± 7.3	11.83 ± 1.52	6.51 ± 0.58	30.5 ± 2.7	21.6 ± 1.6	6.07 ± 0.09
Maple	Ambient	Low	0.309 ± 0.025	61.3 ± 4.8	9.74 ± 0.76	6.34 ± 0.33	37.0 ± 2.1	14.6 ± 2.3	4.97 ± 0.64
	Ambient	High	0.283 ± 0.024	59.7 ± 2.0	11.31 ± 0.26	6.49 ± 0.24	36.2 ± 2.6	14.9 ± 2.3	4.44 ± 0.32
	Elevated	Low	0.250 ± 0.026	43.9 ± 5.2	8.17 ± 0.62	6.01 ± 0.40	35.0 ± 1.7	12.7 ± 2.0	4.24 ± 0.60
	Elevated	High	0.361 ± 0.045	54.2 ± 9.2	10.54 ± 1.56	8.28 ± 0.63	41.5 ± 2.2	13.5 ± 2.4	4.49 ± 0.47
P values:									
CO_2			0.202	0.019	0.087	0.001	0.512	0.150	0.039
Light			0.021	0.071	0.048	0.006	0.046	<0.001	<0.001
Species			<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
CO_2 × Light			0.643	0.526	0.308	0.063	0.001	0.004	0.034
CO_2 × Species			0.486	0.194	0.145	0.701	0.772	0.669	0.092
Light × Species			0.080	0.050	0.114	0.932	<0.001	0.008	0.001
CO_2 × Light × Species			0.174	0.161	0.324	0.353	0.448	0.334	0.375

* RGR, relative growth rate; TC, total consumption; ACR, absolute consumption rate; RCR, relative consumption rate; AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food.

mance. Especially striking were CO_2 effects on the survival of larvae reared on high light aspen, where elevated levels of CO_2 reduced final cohort size to about one third of those reared under ambient conditions. A corresponding, although less pronounced, negative effect of CO_2 was also found for insects reared on maple, but under low light conditions. Females tended to be affected more strongly than males by CO_2 and light treatments, as determined from data on pupal mass and development time. The performance of female larvae on aspen and birch was significantly reduced by the combination of high CO_2 and high light. For insects on maple, negative CO_2 effects were detectable only in low light (reduced pupal mass).

Data from the fourth-instar feeding trials on relative growth rates (RGR) of larvae largely mirrored the patterns observed for pupal mass, although some discrepancies existed. For insects on aspen and birch, significant RGR reductions were observed in elevated CO_2–high light, but not in ambient CO_2–low light, treatments (Table 2). Female pupal mass was surprisingly low on aspen and birch in ambient CO_2–low light, given that fourth instars had high RGRs and foliage had the highest nitrogen and lowest secondary metabolite levels. This counterintuitive finding may be related to conditions larvae experienced during their final (5 and 6) developmental stadia, when growth rates apparently declined. Induced defenses are an unlikely explanation, as results from a simultaneous study in the same experimental system revealed no differential induction effects among treatments (Agrell et al. 1999). A more likely explanation is that, late in the experiment, young leaves represented a greater proportion of available foliage in ambient CO_2–low light, and younger leaves may contain higher levels of secondary compounds than do older leaves (cf. Palo 1984). An additional possibility is that the largest larvae (presumably females) experienced disproportionally high mortality during the last stadia, thus reducing (female) pupal mass in these treatments.

The bioassays demonstrated somewhat stronger interactive effects of CO_2 and light on insect performance than would be expected from the phytochemical results alone. Given the interactive effects of CO_2 and light on phytochemical levels observed in the parallel study (McDonald et al. 1999), insects in our bioassays may have exhibited the effects of that earlier phytochemical variation among the treatments, which was reduced by the time foliage was collected for the present study. Analyses of temporal variations in phytochemistry are obviously necessary if detailed explanations for differences in insect performance are to be obtained.

Observed treatment effects on survival, development and pupal mass were related to altered food conversion efficiency of the larvae. Where elevated CO_2 or light levels had a strong negative impact on insect performance in the long-term study (primarily for insects on

aspen and birch), the efficiencies of conversion of digested (ECD) and ingested (ECI) food were reduced. Although pronounced, effects on ECD and ECI may have been underestimated if caterpillars in stressful treatments (high CO_2 and/or high light) had a relative performance advantage because of their smaller size: small individuals are likely to be more efficient in converting food to biomass due to a larger surface area to volume ratio (Reavey 1993). In contrast to ECD and ECI, approximate digestibility (AD) seemed to have limited influence on insect performance, since AD was actually increased in the treatments where insects performed the poorest.

CO_2 effects on performance of leaf-feeding Lepidoptera have rarely been examined over more than one instar. Fajer et al. (1989) found that CO_2-enriched foliage reduced survival of the buckeye butterfly (*Junonia coenia*). No corresponding effects were detected either for gypsy moths (Traw et al. 1996, Lindroth et al. 1997) or forest tent caterpillars (*Malacosoma disstria*, Roth 1996). However, most long-term studies, including this one, agree with respect to increased development time and/or reduced pupal mass in insects fed CO_2-enriched foliage. To date, our study is unique in demonstrating that light can have a pronounced influence on how CO_2 affects insect demography. In the only previous tree–insect study where CO_2 and light were simultaneously manipulated, the experimental effects on insect performance were limited (Lawler et al. 1997).

Interestingly, for all five performance variables measured during the long-term bioassays, the strongest negative effects of elevated CO_2 levels were found for insects fed high light aspen. Furthermore, among the high light treatments, the impact of elevated CO_2 levels almost consistently declined along the gradient aspen > birch > maple. Our data on long-term insect performance therefore support the hypothesis that the influence of CO_2 is light dependent, and that this interactive effect has a stronger influence on shade intolerant than on shade tolerant tree species. Moreover, responses to elevated CO_2 levels tended to be stronger for insects fed low light maple than for those fed high light maple. These findings suggest that enriched CO_2 affects insect performance most strongly under the environmental (light) conditions to which the host is evolutionarily adapted.

Consumption was determined from the fourth-instar feeding trials. Overall total consumption (TC) and absolute consumption rate (ACR) were reduced by high CO_2, with effects being most pronounced for insects on aspen in high light and maple in low light. In contrast, relative consumption rates (RCRs) were increased by elevated CO_2 levels in high light treatments, indicating compensatory feeding in response to reduced food quality. Despite compensatory feeding, elevated CO_2 levels reduced total defoliation for aspen grown under high light and for maple under low light. These data suggest a net benefit of increased CO_2 levels for trees: reductions in insect performance (growth/mass) more than compensates for accompanying increases in relative consumption. Additional effects in CO_2-enriched environments, such as lowered insect survival (see below), are likely to further reduce the net impact of defoliating insects.

CO_2 effects on tree–insect and tree–tree interactions

Long-term bioassay results indicate that specific combinations of CO_2, light and host species have a strong potential to reduce insect population growth. However, these effects are likely to be modified, and possibly often enhanced, under natural conditions. We found that elevated CO_2 levels affected the insects negatively, especially on high light aspen and low light maple, where both survival and female pupal mass decreased. The latter has a strong direct effect on reproductive output, since fecundity of female moths is highly correlated with pupal mass (e.g., Hough and Pimentel 1978, Haukioja 1993). In addition, the growth rate of insect populations would be strongly reduced by the prolonged development time (e.g., the CO_2-induced increase from 26 to 43 d observed for insects on high light aspen). Increasing development time also has important indirect effects, including increased exposure to predation and/or reduced food quality (Slansky 1993), and, perhaps more crucial for facultatively bivoltine species such as the white-marked tussock moth, reduced potential for a second generation within the current growing season.

From an ecosystem perspective it is of interest that CO_2 and light effects on both long- and short-term performance of folivorous insects varied among host species. Such differential CO_2 effects on tree–insect interactions could potentially alter the successional dynamics of temperate communities. This could result if enriched CO_2 increases the relative competitive advantage of early-successional tree species (e.g., aspen and birch) during early successional stages with high light availability, while promoting late-successional tree species (e.g., maple) during late successional stages with low light availability (cf. Bazzaz and Miao 1993, Kubiske and Pregitzer 1996, Roden et al. 1997).

To conclude, effects of elevated CO_2 levels on plant chemistry and insect performance depend on light conditions, as well as on the plant species examined. The impact of CO_2 availability seems to be especially strong for fast-growing species under high light, and for slow-growing species under low light. Plant phytochemistry was influenced mainly by the independent effects of CO_2 and light levels, whereas insect performance was influenced by both independent and interactive effects.

Overall, results from this study demonstrate the importance of experimental light levels when examining effects of elevated CO_2 on plant phytochemistry and plant-herbivore interactions. Finally, this study suggests that CO_2, light availability and herbivory may interact to influence successional processes and species composition of deciduous forests.

Acknowledgements – We thank J. Hemming, R. Herringa, S.-Y. Hwang, J. Keen, P. Koss, A. Lekies, S. Miranpuri, T. Osier and S. Roth for excellent assistance during this project. Thanks also to R. McCron and M. Abou-Zaid at the Canadian Forest Service for providing tussock moth eggs and rearing advice. K. Albrecht, W. Goodman, and K. Kmiecik kindly allowed use of laboratory equipment. During this research JA was supported by the Foundation for Strategic Environmental Research, Sweden. The work was funded by NSF grant DEB-9306981 to RLL.

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Appendix 1. Comparison of means within whole-plots (CO_2) and subplots (light) for leaf nutrient data (Fig. 1).

Species	Comparison	Water	Starch	Nitrogen
Within whole plot				
Aspen	AC-LL vs EC-LL	*	NS	NS
	AC-HL vs EC-HL	*	***	*
Birch	AC-LL vs EC-LL	*	NS	***
	AC-HL vs EC-HL	NS	**	NS
Maple	AC-LL vs EC-LL	†	NS	NS
	AC-HL vs EC-HL	NS	***	*
Within subplot				
Aspen	AC-LL vs AC-HL	***	NS	NS
	EC-LL vs EC-HL	***	***	*
Birch	AC-LL vs AC-HL	***	***	***
	EC-LL vs EC-HL	***	***	**
Maple	AC-LL vs AC-HL	***	***	NS
	EC-LL vs EC-HL	***	***	NS

Note: AC = ambient CO_2; EC = elevated CO_2; LL = low light; HL = high light. Mean comparisons within whole plots and subplots computed with a least significant difference (LSD) test with pooled variance (NS: $p > 0.100$; †: $p < 0.100$; *: $p < 0.050$; **: $p < 0.010$; ***: $p < 0.001$).

Appendix 2. Comparison of means within whole-plots (CO_2) and subplots (light) for phenolic compounds (Fig. 2).

Species	Comparison	Condensed tannin	Salicortin	Tremulacin	Ellagitannin	Gallotannin
Within whole plot						
Aspen	AC-LL vs EC-LL	NS	*	NS	–	–
	AC-HL vs EC-HL	NS	***	†	–	–
Birch	AC-LL vs EC-LL	NS	–	–	–	–
	AC-HL vs EC-HL	†	–	–	–	
Maple	AC-LL vs EC-LL	NS	–	–	NS	NS
	AC-HL vs EC-HL	***	–	–	NS	NS

Appendix 2. (Continued)

Species	Comparison	Condensed tannin	Salicortin	Tremulacin	Ellagitannin	Gallotannin
Within subplot						
Aspen	AC-LL vs AC-HL	*	***	†	–	–
	EC-LL vs EC-HL	*	***	*	–	–
Birch	AC-LL vs AC-HL	***	–	–	–	–
	EC-LL vs EC-HL	***	–	–	–	–
Maple	AC-LL vs AC-HL	NS	–	–	†	NS
	EC-LL vs EC-HL	***	–	–	NS	NS

Note: AC = ambient CO_2; EC = elevated CO_2; LL = low light; HL = high light. Mean comparisons within whole plots and subplots computed with a least significant difference (LSD) test with pooled variance (NS: $p > 0.100$; †: $p < 0.100$; *: $p < 0.050$; ***: $p < 0.001$). – denotes no data.

Appendix 3. Comparison of means within whole-plots (CO_2) and subplots (light) for insect performance during long term bioassays (Figs 3, 4 and 5).

Species	Comparison	Survival	Male pupal mass	Female pupal mass	Male development time	Female development time
Within whole plot						
Aspen	AC-LL vs EC-LL	NS	NS	NS	NS	NS
	AC-HL vs EC-HL	**	†	*	**	***
Birch	AC-LL vs EC-LL	NS	NS	**	NS	NS
	AC-HL vs EC-HL	NS	NS	NS	NS	***
Maple	AC-LL vs EC-LL	†	NS	*	†	NS
	AC-HL vs EC-HL	NS	NS	NS	NS	NS
Within subplot						
Aspen	AC-LL vs AC-HL	NS	NS	*	NS	NS
	EC-LL vs EC-HL	***	*	*	***	***
Birch	AC-LL vs AC-HL	NS	NS	NS	NS	NS
	EC-LL vs EC-HL	*	NS	*	NS	***
Maple	AC-LL vs AC-HL	***	NS	NS	†	NS
	EC-LL vs EC-HL	NS	NS	NS	NS	NS

Note: AC = ambient CO_2; EC = elevated CO_2; LL = low light; HL = high light. Mean comparisons within whole plots and subplots computed with a least significant difference (LSD) test with pooled variance (NS: $p > 0.100$; †: $p < 0.100$; *: $p < 0.050$; **: $p < 0.010$; ***: $p < 0.001$).

Appendix 4. Comparison of means within whole-plots (CO_2) and subplots (light) for insect performance during short term bioassays (Table 2).

Species	Comparison	RGR	TC	CR	RCR	AD	ECD	ECI
Within whole plot								
Aspen	AC-LL vs EC-LL	NS	NS	NS	NS	NS	NS	NS
	AC-HL vs EC-HL	†	*	*	NS	**	*	***
Birch	AC-LL vs EC-LL	NS	NS	NS	NS	NS	NS	NS
	AC-HL vs EC-HL	NS	NS	NS	NS	NS	†	†
Maple	AC-LL vs EC-LL	NS	*	NS	NS	NS	NS	NS
	AC-HL vs EC-HL	NS	NS	NS	**	NS	NS	NS
Within subplot								
Aspen	AC-LL vs AC-HL	NS	NS	NS	NS	NS	*	**
	EC-LL vs EC-HL	**	NS	NS	**	***	***	***
Birch	AC-LL vs AC-HL	NS	**	**	NS	**	NS	*
	EC-LL vs EC-HL	*	†	NS	**	NS	*	***
Maple	AC-LL vs AC-HL	NS	NS	NS	NS	NS	NS	NS
	EC-LL vs EC-HL	†	NS	†	***	†	NS	NS

Note: AC = ambient CO_2; EC = elevated CO_2; LL = low light; HL = high light. Mean comparisons within whole plots and subplots computed with a least significant difference (LSD) test with pooled variance (NS: $p > 0.100$; †: $p < 0.100$; *: $p < 0.050$; **: $p < 0.010$; ***: $p < 0.001$).