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Effects of elevated CO_2 and temperature-grown red and sugar maple on gypsy moth performance

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Abstract

Few studies have investigated how tree species grown under elevated CO_2 and elevated temperature alter the performance of leaf-feeding insects. The indirect effects of an elevated CO_2 concentration and temperature on leaf phytochemistry, along with potential direct effects on insect growth and consumption, may independently or interactively affect insects. To investigate this, we bagged larvae of the gypsy moth on leaves of red and sugar maple growing in open-top chambers in four CO_2/temperature treatment combinations: (i) ambient temperature, ambient CO_2; (ii) ambient temperature, elevated CO_2 (+300 μL L^{-1} CO_2); (iii) elevated temperature (+3.5°C), ambient CO_2; and (iv) elevated temperature, elevated CO_2. For both tree species, leaves grown at elevated CO_2 concentration were significantly reduced in leaf nitrogen concentration and increased in C:N ratio, while neither temperature nor its interaction with CO_2 concentration had any effect. Depending on the tree species, leaf water content declined (red maple) and carbon-based phenolics increased (sugar maple) on plants grown in an enriched CO_2 atmosphere. The only observed effect of elevated temperature on leaf phytochemistry was a reduction in leaf water content of sugar maple leaves. Gypsy moth larval responses were dependent on tree species. Larvae feeding on elevated CO_2-grown red maple leaves had reduced growth, while temperature had no effect on the growth or consumption of larvae. No significant effects of either temperature or CO_2 concentration were observed for larvae feeding on sugar maple leaves. Our data demonstrate strong effects of CO_2 enrichment on leaf phytochemical constituents important to folivorous insects, while an elevated temperature largely has little effect. We conclude that alterations in leaf chemistry due to an elevated CO_2 atmosphere are more important in this plant–folivorous insect system than either the direct short-term effects of temperature on insect performance or its indirect effects on leaf chemistry.

Keywords: elevated CO_2 and temperature, global climate change, insect performance, leaf nitrogen, *Lymantria dispar*, phenolics

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Introduction

Terrestrial ecosystems constitute a large pool of mobile carbon. Forest ecosystems contain upwards of 80% of the carbon in the above-ground biosphere and contribute significantly to the flux of carbon between the biosphere and atmosphere (Dixon *et al*. 1994). The anticipated doubling of atmospheric CO_2 concentration in the next century (Houghton *et al*. 1996) will potentially alter carbon sequestration and release due to the effects CO_2 enrichment has on plant productivity (Bazzaz 1990). Studies using both hardwood and conifer tree species have demonstrated the substantial effects of an elevated CO_2 atmosphere on tree biomass production (Norby *et al*. 1995; Tissue *et al*. 1997; Curtis & Wang 1998; Norby *et al*. 1999). When expressed as a canopy productivity index (i.e. annual above-ground growth per unit leaf area), numerous studies using broadleaf tree species demonstrate an overall 29% increase in plants grown under CO_2 enrichment (Norby 1996). Herbivorous insects act as regulators of primary productivity (Mattson & Addy

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1975) and play a pivotal role in carbon sequestration and nutrient cycling in forest ecosystems (Schowalter *et al*. 1986). Changes in phytochemical constituents (e.g. nitrogen) important to folivorous insects due to increasing CO_2 concentration can potentially alter the nature of important plant–insect interactions (Lincoln *et al*. 1993; Watt *et al*. 1995). Of equal importance to increasing CO_2 concentration is the projected accompanying rise in global mean temperature of 1.5–4.5 °C (Houghton *et al*. 1996). To understand how terrestrial ecosystems may change in the future, an examination of the independent or possibly interactive effects of increasing atmospheric CO_2 and temperature is needed (Rawson 1992; Thornley & Cannell 1996).

Leaf nitrogen is known to be an important element for insect success (Mattson 1980) and numerous studies have shown decreases in foliar nitrogen in plants grown in an enriched CO_2 atmosphere (Cotrufo *et al*. 1998). Specifically, those examining both conifer (Williams *et al*. 1994, 1997) and hardwood (Lindroth *et al*. 1993; Traw *et al*. 1996; Kinney *et al*. 1997; Williams *et al*. 1998) tree species have demonstrated that concentrations of this important nutrient decline in leaf tissue grown at elevated CO_2, primarily because of increased leaf dry matter content (Norby *et al*. 1999). When coupled with increased levels of nonstructural carbohydrates (Lindroth *et al*. 1993; Roth & Lindroth 1994; Williams *et al*. 1998), carbon-based allelochemicals (Kinney *et al*. 1997; Lindroth *et al*. 1997; Roth *et al*. 1998), and C:N ratio (Williams *et al*. 1998), it is clear that tree leaves grown under an elevated CO_2 atmosphere are a comparably poorer food source for insects compared to ambient CO_2-grown leaves. Insects feeding on CO_2 enriched tree foliage exhibit reduced larval growth (Roth & Lindroth 1994, 1995), increased leaf consumption (Williams *et al*. 1994), prolonged larval development (Lindroth *et al*. 1997) and reduced female fecundity (Traw *et al*. 1996).

Although a substantial body of research has focused on elevated CO_2 effects on plant–insect interactions, few studies have examined the combined effects of CO_2 concentration and temperature. Strong interactions between elevated CO_2 concentration and temperature on carbon gain have been shown (Long 1991), where positive carbon gain in plants is partially offset by higher growth temperatures (Hogan *et al*. 1991; Baker *et al*. 1992; Callaway *et al*. 1994). In some cases the increase in CO_2 concentration and temperature combine to alter plant phenology (Murray *et al*. 1994; Koike 1995), while in others CO_2 concentration independent of temperature is responsible for plant growth responses (Tesky 1997). For folivorous insects, the independent or interactive effects of elevated CO_2 and temperature acting directly, or possibly indirectly via effects on leaf phytochemistry, have important implications for insect success. Direct effects on insect performance may be particularly important with regard to temperature. An extensive body of literature demonstrates that insects are temperature sensitive (see Taylor 1981). Rising global temperature is predicted to alter the range distribution, diversity, and abundance of agricultural insect pests in the UK (Cannon 1998). In herbaceous plants an elevated temperature increased leaf nitrogen concentration (CO_2 concentration had no effect), and the abundance of aphids increased on both elevated CO_2 and temperature-grown plants (Bezemer *et al*. 1998). Due to the experimental design it was not possible to ascertain any combined effects of CO_2 and temperature on plant chemistry or insect performance. In the only study to date using tree species, CO_2 and temperature had no interactive effects on *Quercus robur* L. leaf chemistry (Dury *et al*. 1998). Temperature alone resulted in decreased leaf nitrogen concentration and increased condensed tannin concentration, while CO_2 treatment increased total carbon-based phenolics, suggesting a larger primary role of temperature than CO_2 concentration in the phytochemical responses in this tree species (Dury *et al*. 1998).

In the study reported here, we examined the effects of elevated CO_2 and temperature-grown red maple (*Acer rubrum* L.) and sugar maple (*Acer saccharum* Margh.) leaves on the growth and consumption of an important folivorous insect, *Lymantria dispar* L. The gypsy moth has established itself as a serious defoliator of many hardwood tree species since its accidental introduction into the United States in the late 19th century. The design of this experiment allowed us to assess increased CO_2 and temperature effects, either independently or interactively, on leaf phytochemical constituents important to gypsy moth performance. In addition, the exposure of the feeding larvae to the same treatment conditions as the plants provided a way to ascertain any direct effects of increased CO_2 concentration or temperature on insect performance. We predicted that: (i) an enriched CO_2 atmosphere would result in a decline in leaf nutritional quality in both maple species, including an increase in carbon-based phenolics; (ii) an elevated temperature would ameliorate some of the CO_2 effects on leaf chemistry, particularly by reducing accumulations of nonstructural carbohydrates and thus reducing carbohydrate:nitrogen ratios; (iii) the combined effects of elevated CO_2 and temperature on leaf phytochemistry would result in alterations in the performance of the gypsy moth larvae; and (iv) insect performance would be affected directly by an elevated temperature but not an elevated CO_2 atmosphere.

Materials and methods

Plant growth conditions

This study was conducted at the Global Change Research site in the National Environmental Research Park at Oak Ridge National Laboratory, Tennessee. In the spring of 1994, 10 each of one-year-old bare-rooted saplings of red (*Acer rubrum*) and sugar (*A. saccharum*) maple were planted in the ground in 3 m diameter by 2.4 m tall open-top chambers. Saplings that did not survive the first year were replaced in the winter of 1995. A randomized complete block design used 3 replicate chambers for each of 4 treatments: (i) ambient temperature, ambient CO_2, ATAC; (ii) ambient temperature, elevated CO_2 (+300 μL L^{-1} CO_2), ATEC; (iii) elevated temperature (+3.5°C), ambient CO_2, ETAC; and (iv) elevated temperature, elevated CO_2, ETEC. The CO_2 and temperature treatments were maintained 24 h per day during the growing season, while the temperature treatment continued all year. Temperatures were regulated by passing the airflow through PID-controlled evaporative coolers and resistance heaters. Levels of CO_2 were regulated with rotameters and monitoring with an infrared gas analyser. For a more detailed description of the chamber design and experimental set-up see Norby *et al*. (1997) and Edwards & Norby (1999). Shade cloth was draped over the chambers throughout the plant growing season to reduce ambient light to 27%. At the time of this experiment the plants used for insect feeding were beginning their third growing season within the chambers (1996) and averaged 2.0 m in height. The trees used for this feeding study were grown as part of an overall larger study on the effects of elevated CO_2 and temperature on above and below-ground plant processes. Therefore, in order to minimize impacts due to insect damage, only two trees of each maple species were available per chamber.

Insect rearing

Gypsy moth egg masses were obtained from Otis Methods Development Center, USDA-APHIS. The gypsy moth is not endemic to the United States, having become established after an accidental release in the late 19th century. Because this insect is subject to federal quarantine regulations, and because the state of Tennessee is outside of its accepted infestation range, reproductive populations of the gypsy moth are not allowed in Tennessee. The insects used in this experiment were produced as part of the sterile male release programme for gypsy moth control (for a more detailed description of this programme see Schwalbe *et al*. 1991 and Mastro 1993). Previous studies have found these insects to be competitive with wild populations (Hansen 1988), and to be a valuable resource for studying gypsy moth out of its established range (Strom *et al*. 1996; Williams *et al*. 1998).

Twenty egg masses were placed on standard artificial diet (provided by Otis Methods Development Center) and reared until hatch. Newly eclosed larvae were separated among egg masses in rearing cups to 'mix' the population and avoid maternal effects. Larvae used for both the red and sugar maple feeding experiments were reared through the first instar on artificial diet, then transferred to foliage from trees adjacent to the study area for the respective tree species within the chambers (see below). Larvae were reared through the second instar (red maple) and third instar (sugar maple) in 17 cm × 12 cm × 6.5 cm rearing containers.

Red maple feeding experiment

In order to use plants of similar developmental stage in the experiment, within each chamber leaf phenology was matched for each plant; leaves were very near full expansion but not fully sclerified (approximately 10–12 days postbudbreak). There was variation among plants and chambers for budbreak, such that at the beginning of the experiment not all plants were available. Plants growing at an elevated temperature emerged on average 3 days earlier than those grown at ambient temperature (unpubl. data). However, due to large variation between chambers we were able to mix the insect set-up across all treatments over a period of 4–5 days to prevent any treatment bias. Depending on the plant size, 8–10 newly moulted third instar larvae were weighed and placed on a single branch per maple sapling in a 1 mm × 1 mm mesh-diameter nylon bag. The bags were approximately 35.5 cm wide × 35.5 cm long. The end of the bag was tied snugly and attached to the tree on which it was placed to support it. This design allowed for free movement within the bag for the larvae. Each branch contained 4–6 leaves. At no time during the larval feeding period was foliage limited. After 6 days insects were removed and weighed. Also at this time the number of dead larvae was recorded.

Insect performance was measured in two ways. (i) average weight per larva was calculated as the total weight of all larvae in a bag divided by the number of larvae present. This allowed us to account for larval mortality from the beginning to end of insect feeding. The average weight gain per larva (mg) was determined by measuring the difference from the beginning and ending weights. A dry weight conversion was determined from the mean proportional dry weight of 12 larvae. (ii) In order to quantify leaf biomass consumption, the length of each leaf within the bag was measured prior to insect feeding. The area of each leaf was

determined from the equation $A = 9.76(L) - 35.85$ ($P < 0.001$, $r^2 = 0.88$), where A is area (cm^2) and L is length (cm). The relationship between leaf length and area was calculated from leaves sampled for the phytochemical analyses (see below). Initial leaf area was converted to initial biomass (dry weight) using the mass: area ratio from sampled leaves. After larvae were removed from the bags, leaves fed upon were removed, oven-dried, and weighed to obtain the biomass of leaves remaining after the insects fed. Shredded pieces of leaves were collected from the bags and dried. This quantity was subtracted from the total biomass at the end of feeding to adjust for leaf material not consumed by the larvae. Consumption was calculated as the initial leaf biomass – final leaf biomass divided by average larval weight ($mg\,mg^{-1}$).

Leaf samples were collected for the determination of leaf nitrogen, non-structural carbohydrates, water, carbon-based phenolic concentrations, and for leaf mass-to-area ratio calculations. As insect bags were set up, two leaves were removed from the branch opposite that which the insect bag was placed (i.e. one branch per plant). In addition, two attached leaves on the same branch had the length recorded to track leaf expansion. Leaves were found to increase their length less than 1% from the starting length (data not shown). The length of each sampled leaf was measured and the area determined using a LiCor LI-3100 leaf area meter. These data were used to determine the relationship between leaf length and area (see above). Each leaf was then cut down the midvein into two pieces. One piece was weighed, the area taken and then dried at 40°C; this portion of the leaf was used for nitrogen, non-structural carbohydrate, water concentration, and leaf mass-to area relationship. The other leaf portion was placed immediately on ice and transported to a –80°C freezer for storage and subsequent phenolic quantification. This leaf sampling procedure was repeated at the end of the insect feeding period. Therefore, there were four leaves sampled per plant, two at the beginning and two at the end of the feeding experiment. Leaf water, nitrogen, nonstructural carbohydrates concentrations and leaf mass-to-area relationships were determined by combining the results from individual leaves collected. This represents a more accurate representation of what the larvae experienced over the several days feeding experiment.

Sugar maple feeding experiment

The methodology was similar to the red maple with a few exceptions. Newly moulted fourth instar larvae were used for the sugar maple feeding. The older larvae were used primarily because the sugar maples had more foliage available to support the higher consumption levels of later instar gypsy moth larvae. Even though on average saplings grown at an elevated temperature emerged 9 days earlier than those growing at ambient temperature, large variation among plants and chambers allowed us to choose plants of similar phenology across treatments so that all insect bags were set up over a 3-day period. Six larvae were placed in each bag and allowed 36 h to feed. This represented a reasonable time period to allow for significant consumption of foliage on a branch (i.e. 50–75% area removed). Methodologies for the calculation of insect growth and consumption and for phytochemistry sampling followed that used for the red maple described above. On these plants the leaf length to area relationship was explained by $A = 12.42(L) - 54.21$ ($P < 0.001$, $r^2 = 0.89$).

Chemical and statistical analysis

Leaf nitrogen concentration was analysed using a Carlo-Erba NA-1500 C: N analyser. Non-structural carbohydrates were analysed following the acid hydrolysis procedure of Tissue & Wright (1995). Soluble sugars and starch were extracted from ground leaf material using a methanol: chloroform: water solution and quantified spectrally after acid hydrolysis with concentrated sulphuric acid. The total non-structural carbohydrates are the sum of starch and soluble sugars. Total phenolics were analysed using the Folin–Ciocalteu's reagent technique of Singleton & Rossi (1965). Tannic acid (Sigma Chemical Company) was used in the development of the standard curve. The authors recognize that the total phenolics assay is subject to interference with other chemically reactive compounds in the leaf (see Mole & Waterman 1987). In spite of their limitations, these assays are useful for making relative comparisons between treatments, especially when the same plant species is used. Total phenolics are expressed as percent tannic acid equivalents.

Data were analysed using a General Linear Model Procedure (SAS for Power PC 1996). Effects of CO_2, temperature, and their interaction on leaf phytochemical measures were determined by comparing replicate chambers using an *a priori* CONTRAST statement. In our statistical model replication is at the level of the chamber, with bags within chambers combined in the overall larger model. The *F*-statistic for these comparisons was calculated using the Block Treatment interaction as the Type III mean-square error term. For all measures d.f. = 1, 6. The determination of insect performance used the same overall model, including the use of the initial starting insect weight as a covariate (Ancova). By utilizing the initial insect weight in our calculations we have avoided some of the problems associated with gravimetric quantities (Raubenheimer & Simpson 1992). Reported treatment means and standard errors were

calculated among chambers within CO_2 and temperature treatment. Because our limited replication made the demonstration of significant treatment effects difficult, results where $0.10 > P > 0.05$ are reported as marginally significant.

Results

Red maple

The effects of CO_2, temperature, and their interaction on leaf chemical constituents are presented in Table 1 and Fig. 1. Leaf water concentration was significantly lower in CO_2 enriched grown leaves (ATAC = 696 mg g^{-1} ± 11 (SE), ATEC = 660 ± 8, ETAC = 667 ± 13, ETEC = 631 ± 17), but was unaffected by temperature. Averaged among temperature treatments, leaf nitrogen concentration declined 16% and 13% in elevated CO_2-grown leaves at ambient and elevated temperature, respectively (Fig. 1). Of the non-structural carbohydrates, only soluble sugars were significantly affected by CO_2 treatment, increasing approximately 16% over ambient CO_2-grown leaves. Both the soluble sugar: nitrogen ratio and

Table 1 Significance values for effects of CO_2, temperature, and their interaction ($P <$) on red maple leaf constituents and third instar gypsy moth performance (Proc GLM with Contrast). See the Materials and methods for a detailed description of the statistical model. For all measures d.f. = 1, 6, $N = 3$. TNC, total nonstructural carbohydrates.

Leaf constituents	CO_2	Temp.	$CO_2 \times$ Temp.
Water concentration	0.060	0.121	0.984
Nitrogen concentration	0.020	0.325	0.570
Sugar concentration	0.039	0.803	0.946
Starch concentration	0.547	0.284	0.452
TNC	0.184	0.419	0.519
Sugar: nitrogen	0.050	0.899	0.633
Starch: nitrogen	0.092	0.176	0.281
TNC: nitrogen	0.017	0.254	0.302
Tannic acid equivalents	0.196	0.385	0.932
Insect performance			
Av. wt. gain/larva	0.026	0.324	0.097
Consumption	0.522	0.209	0.105

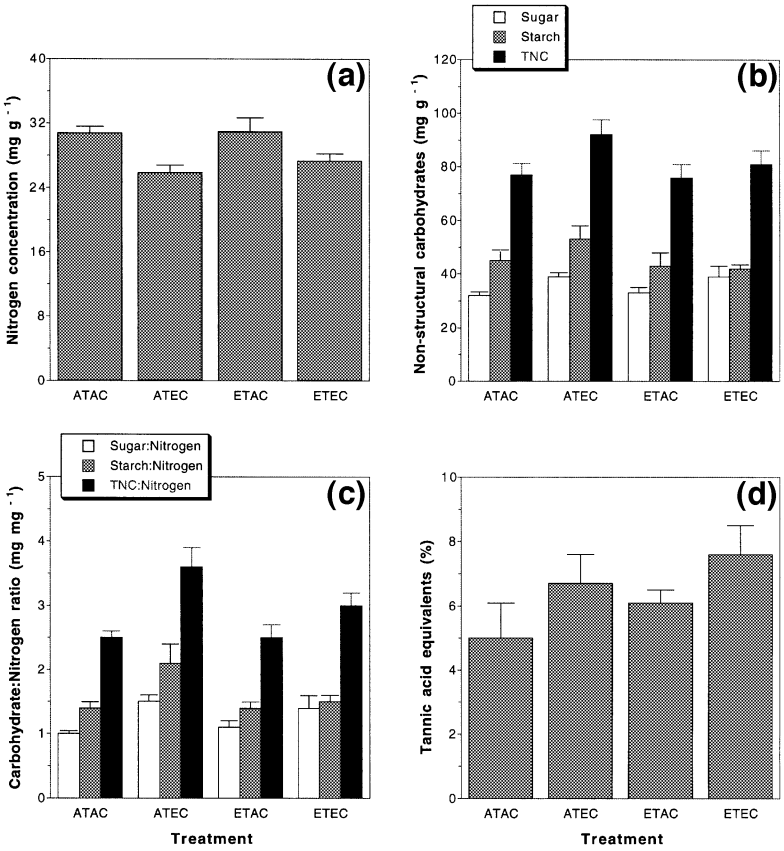

Fig. 1 Effects of CO_2 and temperature treatments on leaf phytochemical constituents in red maple leaves: (a) leaf nitrogen concentration; (b) non-structural carbohydrate concentration; (c) carbohydrate:nitrogen ratio, and (d) tannin acid equivalents. ATAC, ambient temperature, ambient CO_2; ATEC, ambient temperature, elevated CO_2; ETAC, elevated temperature, ambient CO_2; ETEC, elevated temperature, elevated CO_2. See Materials and methods for phytochemical assay descriptions and replicate numbers per treatment. Bars represent Standard Error of the mean.

TNC: nitrogen ratio (TNC = soluble sugars + starch) were significantly increased by CO_2 concentration (Table 1, Fig. 1). An enriched CO_2 atmosphere resulted in a 31% increase at ambient and 17% increase at elevated temperature relative to ambient CO_2 -grown leaves in TNC: nitrogen (Fig. 1). There were no significant effects on carbon-based phenolics in red maple.

The performance of gypsy moth larvae was generally more responsive to CO_2 concentration than to temperature (Table 1). The average weight gain per larva declined 39% in larvae feeding on CO_2 enriched leaves at an ambient temperature and 7% at an elevated temperature (Fig. 2). A marginally significant interaction between CO_2 concentration and temperature was observed on growth (Table 1). Consumption by larvae was, on average, 16% higher at the higher temperature but was not significantly related to either CO_2 concentration or temperature (Table 1, Fig. 2). There was minimal mortality in this feeding study, ranging from 0 to 8% across treatments. Mortality was not related to CO_2, temperature, or their interaction (data not shown).

Sugar maple

The effects of CO_2, temperature, and their interaction on leaf phytochemical constituents are presented in Table 2 and Fig. 3. There were no significant interactions between CO_2 and temperature treatment for any measure. Leaf water concentration declined in plants grown at the higher temperature (ATAC = 662 mg g^{-1} ± 3; ATEC = 630 ± 16; ETAC = 622 ± 12; ETEC = 617 ± 12). An elevated CO_2 atmosphere resulted in 21% and 15% declines (respectively) in leaf nitrogen concentration on ambient and elevated temperature-grown trees (Fig. 3). Responses of non-structural carbohydrates were inconsistent, with a significant effect of CO_2 concentration found only in the total non-structural carbohydrates (TNC) (Table 2). Averaged across temperature treatments TNC increased 17% in elevated compared to ambient CO_2-grown leaves. The ratios of sugar, starch, and TNC: nitrogen were all significantly increased in elevated CO_2-grown leaves (Fig. 3). Leaves grown in an enriched CO_2 atmosphere were 37% and 28% higher at ambient and elevated temperature (respectively) for TNC: nitrogen ratio. The amount of carbon-based phenolics relative to the tannic acid standard (i.e.%TAE) was significantly increased by CO_2 concentration and unaffected by temperature (Table 2, Fig. 3).

The average weight gain per larva was similar among the treatments except for a 35% increase for larvae in the elevated temperature/elevated CO_2 treatment (Fig. 4). There were no significant effects of CO_2, temperature, or their interaction on larval growth (Table 1). Consumption by larvae, averaged across CO_2 treatments, increased 27% at the higher temperature (Fig. 4), although no significant temperature effect was demonstrated (Table 2). During the feeding period only two larvae died, from two separate treatments and chambers (data not shown).

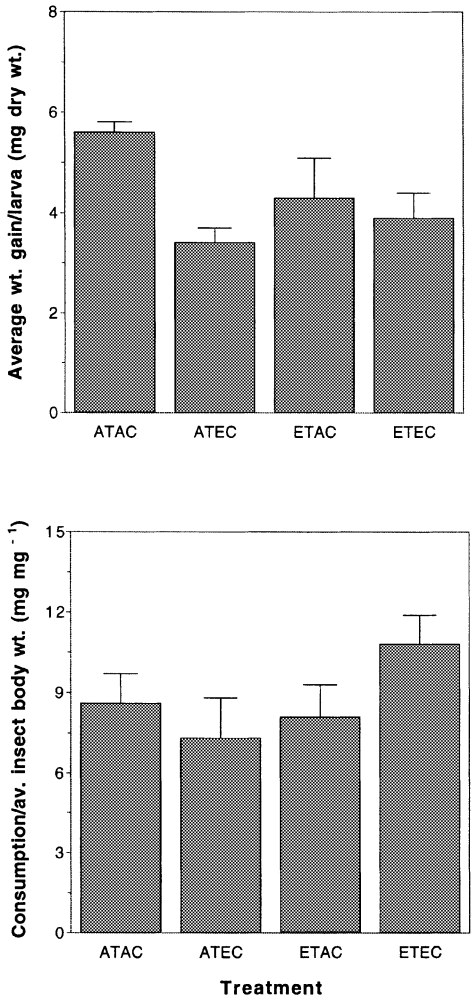

Fig. 2 Effects of red maple leaves grown under different CO_2 and temperature treatments on gypsy moth average weight gain per larva (Top) and consumption per average insect body weight (Bottom). See Materials and methods for insect performance calculations and replicate numbers per treatment and Fig. 1 legend for a description of the treatments. Duration of feeding = 6 days. Bars represent standard error of the mean.

Discussion

A considerable body of evidence exists demonstrating that the effects of elevated CO_2 on plants can influence insect herbivores. Few investigations, however, have incorporated CO_2 concentration with temperature treatment such that the independent or interactive effects of these conditions on plants and insects can be evaluated.

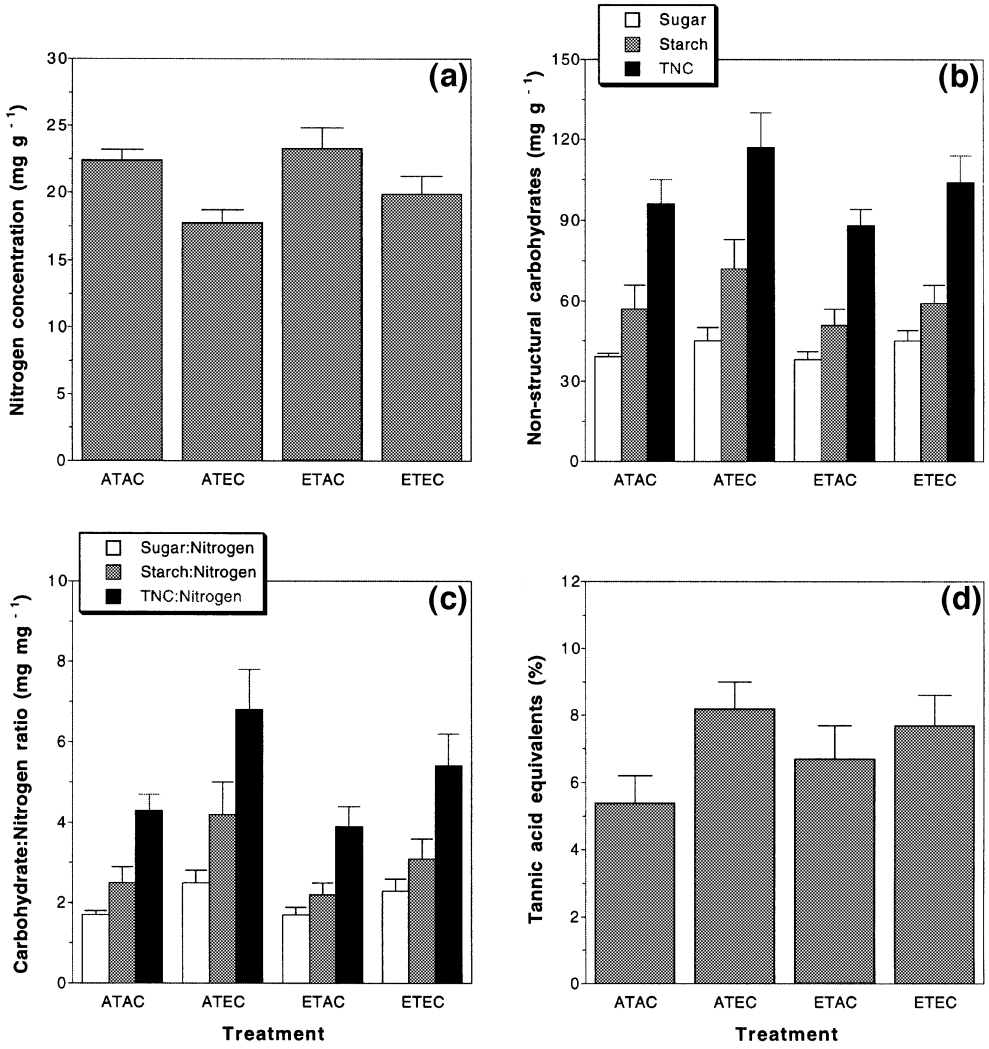

Fig. 3 Effects of CO_2 and temperature treatments on leaf phytochemical constituents in sugar maple leaves: (a) leaf nitrogen concentration; (b) non-structural carbohydrate concentration; (c) carbohydrate: nitrogen ratio; and (d) tannic acid equivalents. See Materials and methods for phytochemical assay descriptions and replicate numbers per treatment and Fig. 1 legend for a description of the treatments. Bars represent standard error of the mean.

In this study, we found that maple leaf phytochemistry was more sensitive to CO_2 concentration than to temperature and that the responses of the gypsy moth were generally more dependent on CO_2 concentration than temperature (Figs 2, 4). Our data suggest that the effects on leaf phytochemistry caused by CO_2 enrichment are more profound than those caused by elevated temperature and that the responses of this insect to short-term increases in temperature are minimal.

Reductions in leaf nutritional constituents (especially nitrogen) important for insect success in elevated CO_2-grown plants are well documented (Lincoln *et al*. 1993; Cotrufo *et al*. 1998). In our study, both red and sugar maple leaves had a significant decline in leaf nitrogen concentration when grown under CO_2 enrichment (Tables 1, 2, Figs 1, 3), partially supporting one of our original predictions (i.e. a decline in leaf nutritional quality with increasing CO_2 concentration). This observed reduction is in general agreement with other studies with hardwood tree species exposed to an elevated CO_2 atmosphere (Lindroth *et al*. 1997; Williams *et al*. 1998; Norby *et al*. 1999), including sugar maple (Lindroth *et al*. 1993; Roth *et al*. 1998). Temperature had no effect on nitrogen concentration of leaves for either species (Tables 1, 2) and there was no interaction between temperature and CO_2 concentration. Our results differ somewhat from studies with *Q. robur*, where an increased temperature resulted in a decline in foliar nitrogen, whereas CO_2 concentration had no effect (Dury *et al*. 1998). Because the exposure times, plant growth

Table 2 Significance values for effects of CO_2, temperature, and their interaction ($P <$) on sugar maple leaf constituents and fourth instar gypsy moth performance (Proc GLM with Contrast). See the Materials and methods for a detailed description of the statistical model. For all measures d.f. = 1, 6, $N = 3$

Leaf constituents	CO_2	Temp.	$CO_2 \times$ Temp.
Water concentration	0.121	0.040	0.234
Nitrogen concentration	0.021	0.285	0.689
Sugar concentration	0.217	0.911	0.903
Starch concentration	0.106	0.178	0.633
TNC	0.020	0.138	0.684
Sugar:nitrogen	0.050	0.634	0.797
Starch:nitrogen	0.029	0.173	0.396
TNC:nitrogen	0.013	0.192	0.427
Tannic acid equivalents	0.012	0.521	0.144
Insect performance			
Av. wt. gain/larva	0.850	0.253	0.284
Consumption	0.562	0.235	0.767

condition (i.e. pots vs. trees planted in the ground in our study), and stage of leaf development where responses were observed differed between our studies, direct comparisons are difficult. An elevated temperature did result in reduced leaf water concentration of sugar maple leaves in our study (Table 2), while leaf water responded more to CO_2 concentration in red maple leaves, exhibiting a marginally significant decline (Table 1). Leaf water concentration has been examined in other CO_2 enrichment studies providing contradictory results (Lincoln *et al*. 1993). Certainly the role of leaf water should not be underestimated, due to its effects on insect performance (Scriber & Slansky 1981). Non-structural carbohydrates (i.e. starch and soluble sugars and their sum, TNC) generally increased in red and sugar maple grown in enriched CO_2, although the extent of the responses varied substantially between the species (Figs 1, 3). Previous work with both hardwood (Williams *et al*. 1998) and conifer (Williams *et al*. 1997) tree species demonstrates that leaf age can be an important determining factor in leaf phytochemical alterations between ambient and elevated CO_2-grown plants. Because red maple leaves were younger than the sugar maple leaves used in our feeding studies, such age-dependent responses could have been responsible for the differences observed between tree species. In trees, CO_2-induced increases in carbohydrates are known from several studies, including those with maple (Roth & Lindroth 1994; Roth *et al*. 1998). In both the red and sugar maple in our study, none of the carbohydrates were related to plant growth temperature or its interaction with CO_2 concentration (Tables 1, 2), contradicting our prediction that carbon accumulation may be affected by plant growth temperature.

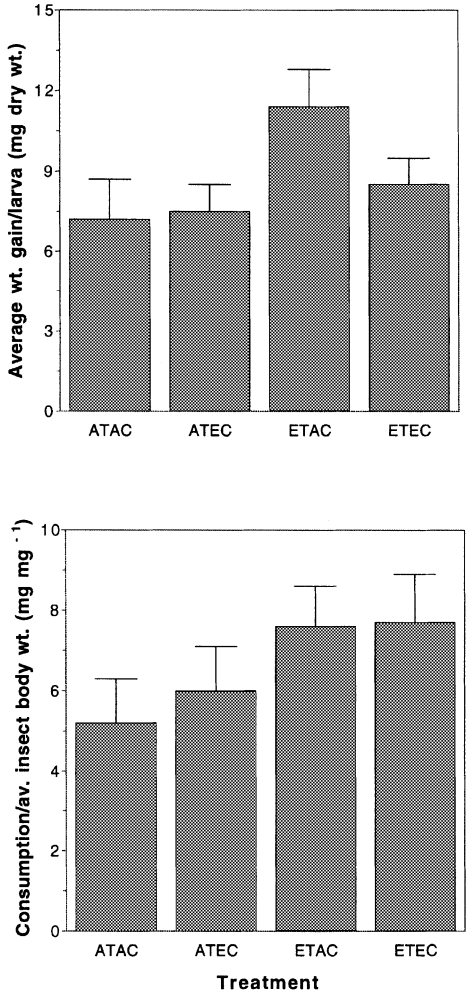

Fig. 4 Effects of sugar maple leaves grown under different CO_2 and temperature treatments on gypsy moth average weight gain per larva (Top) and consumption per average insect body weight (Bottom). See Materials and methods for insect performance calculations and replicate numbers per treatment and Fig. 1 legend for a description of the treatments. Duration of feeding = 1.5 days. Bars represent standard error of the mean.

Perhaps just as relevant to leaf-chewing insects as nitrogen and carbohydrates is the ratio of carbohydrate: nitrogen. Increasing carbohydrate: nitrogen ratio in plants grown at an elevated CO_2 atmosphere is thought to be important to observed insect responses (Williams *et al*. 1997, 1998). Both maple species in our study had significant increases in the ratio of non-structural carbohydrates to nitrogen, clearly demonstrating a decline in leaf nutritional quality in elevated CO_2-grown foliage (Figs 1, 3). Again, temperature and its interaction with CO_2 concentration had no effect on this measure. It appears that the ameliorating effect of temperature on CO_2 concentration observed in studies of plant growth responses (Baker *et al*. 1992; Callaway *et al*. 1994) is not expressed at the level of most of the leaf nutritional

constituents we measured in the maple species used in our study. Carbon-based phenolic responses to CO_2 concentration were different in the two species (Tables 1, 2). Leaves of sugar maple had significantly higher total phenolics when grown in an enriched CO_2 atmosphere (Fig. 3) while no significant treatment effects were observed in red maple (Fig. 1). This increase in sugar maple leaf secondary compounds is in general agreement with other studies examining related phenolic compounds found in hardwood tree species (Traw *et al*. 1996; Lindroth *et al*. 1997), although studies with maple have reported both significant (Lindroth *et al*. 1993; Roth & Lindroth 1994) and nonsignificant (Roth *et al*. 1998) results. As with all of the nutritional measures except leaf water content in sugar maple leaves, there were no effects of temperature or its interaction with CO_2 concentration on phenolics in red and sugar maple leaves in our study, further supporting the notion that CO_2 concentration, not temperature, had a principal effect on leaf constituents.

Although in both maple species studied we found substantial effects of an elevated CO_2 atmosphere on leaf phytochemical constituents (especially leaf nitrogen and its ratio with carbohydrates), insect responses were somewhat dependent on plant species (Figs 2, 4). The only significant effect on gypsy moth performance was a decline in the average weight gain in larvae feeding on red maple leaves grown in an elevated CO_2 atmosphere (Table 1, Fig. 2), partially supporting our prediction that alterations in leaf constituents would affect insect performance. Similarly, the marginally significant interaction between CO_2 concentration and temperature on insect growth for larvae feeding on red maple ($P = 0.097$) partially supports this notion. Temperature had no effect on growth or consumption of gypsy moth larvae in our study, despite a substantial increase in consumption of leaf tissue for larvae feeding on sugar maple leaves at the elevated temperature (Fig. 4). The lack of any direct effects of temperature on insect performance may be explained partially by the relatively short duration of exposure of insects to an elevated temperature. In addition, our previous study with this insect found larval age to be an important component of tree–gypsy moth interactions (Williams *et al*. 1998). Larval age could have been a factor in the current study: we observed growth declines in earlier instars feeding on red maple, while older instars feeding on sugar maple were unaffected. Even though both of the tree species used in this study are fed upon by the gypsy moth, previous work suggests that this insect much prefers sugar maple to red maple. Therefore, insect responses based on host plant susceptibility cannot be ruled out entirely. In spite of this, the observed growth reduction of larvae feeding on red maple leaves is presumably related to reductions in leaf nitrogen, water, and increases in the ratio of carbohydrate: nitrogen and total phenolics in CO_2-grown foliage. We conclude that because temperature had little or no effect on these leaf measures, any expected effects of elevated temperature on leaf chemistry, as well as direct effects on insect performance, are not supported by this study.

In conclusion, this study demonstrates changes due to CO_2 enrichment in leaf constituents important to folivorous insects. For example, declines in leaf nitrogen and increases in carbohydrate: nitrogen ratios were observed. Contrary to our original prediction was the lack of independent or interactive effects of an elevated temperature. With the exception of leaf water content of sugar maple leaves, plant growth temperature had no effect on leaf phytochemistry. Gypsy moth responses to treatments were minimal and plant species dependent. Direct comparisons of insect responses on red vs. sugar maple must, however, consider the potential effects of leaf and larval age and the duration of the feeding study. We were limited by our ability to demonstrate significant differences between treatments, but nonetheless conclude that CO_2-induced changes in leaf phytochemistry can reduce gypsy moth larval growth, while neither the direct nor indirect effects of an elevated temperature had any effect in this short-term feeding study. Our data provide little evidence to suggest that larvae in an elevated CO_2 atmosphere are negatively affected, in agreement with previous expectations (Nicolas & Sillans 1989). Our experimental design did not allow us to access what long-term effects an increased temperature and CO_2 concentration would have on this plant–insect association. Certainly longer-term experiments, examining multiple generations of non-laboratory strains of insects, are needed to make predictions on how future increases in atmospheric CO_2 concentration and global mean temperature could alter important plant–insect interactions.

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