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Development of gypsy moth larvae feeding on red maple saplings at elevated CO_2 and temperature

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Abstract Predicted increases in atmospheric CO_2 and global mean temperature may alter important plant-insect associations due to the direct effects of temperature on insect development and the indirect effects of elevated temperature and CO_2 enrichment on phytochemicals important for insect success. We investigated the effects of CO_2 and temperature on the interaction between gypsy moth (*Lymantria dispar* L.) larvae and red maple (*Acer rubrum* L.) saplings by bagging first instar larvae within open-top chambers at four CO_2/temperature treatments: (1) ambient temperature, ambient CO_2, (2) ambient temperature, elevated CO_2(+300 μl $l^{-1}CO_2$), (3) elevated temperature (+3.5°C), ambient CO_2, and (4) elevated temperature, elevated CO_2. Larvae were reared to pupation and leaf samples taken biweekly to determine levels of total N, water, non-structural carbohydrates, and an estimate of defensive phenolic compounds in three age classes of foliage: (1) immature, (2) mid-mature and (3) mature. Elevated growth temperature marginally reduced (P <0.1) leaf N and significantly reduced (P <0.05) leaf water across CO_2 treatments in mature leaves, whereas leaves grown at elevated CO_2 concentration had a significant decrease in leaf N and a significant increase in the ratio of starch:N and total non-structural carbohydrates:N. Leaf N and water decreased and starch:N and total non-structural carbohydrates:N ratios increased as leaves aged. Phenolics were unaffected by CO_2 or temperature treatment. There were no interactive effects of CO_2 and temperature on any phytochemical measure. Gypsy moth larvae reached pupation earlier at the elevated temperature (female =8 days, P <0.07; male =7.5 days, P <0.03), whereas mortality and pupal fresh weight of insects were unrelated to either CO_2, temperature or their interaction. Our data show that CO_2 or temperature-induced alterations in leaf constituents had no effect on insect performance; instead, the long-term exposure to a 3.5°C increase in temperature shortened insect development but had no effect on pupal weight. It appears that in some tree-herbivorous insect systems the direct effects of an increased global mean temperature may have greater consequences for altering plant-insect interactions than the indirect effects of an increased temperature or CO_2 concentration on leaf constituents.

Keywords Elevated temperature · Pupal weight · *Lymantria dispar* · *Acer rubrum* · Global climate change

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Introduction

The anticipated doubling of atmospheric CO_2 concentration and the resulting rise in global mean temperature of 1.5–4.5°C in this century (Houghton et al. 1996) could potentially alter large-scale ecosystems, including forests. Previous studies examining the effects of CO_2 enrichment on both hardwood and conifer tree species have demonstrated substantial impacts on tree biomass production (Tissue et al. 1997; Curtis and Wang 1998; Norby et al. 1999; Kerstiens 2001) and changes in leaf phytochemical constituents important to herbivorous insects (Lindroth et al. 1993; Williams et al. 1998, 2000). Because plant-feeding insects act as regulators of primary productivity (Mattson and Addy 1975) and play a pivotal role in carbon sequestration and nutrient cycling in forest ecosystems (Schowalter et al. 1986), CO_2-induced alterations in phytochemical constituents important to insects can potentially alter the nature of plant-insect interactions (Lincoln et al. 1993; Watt et al. 1995; Coviella and

Trumble 1999). Given the expected increases in both atmospheric CO_2 concentration and temperature, an examination of the interactive effects of these climatic factors on ecosystems is needed (Thornley and Cannell 1996; Morison and Lawlor 1999).

A number of previous studies have shown decreases in foliar N concentration in plants grown in an enriched CO_2 atmosphere (Cotrufo et al. 1998), including conifer (Williams et al. 1994) and hardwood (Kinney et al. 1997; Williams et al. 2000) tree species. Increased levels of non-structural carbohydrates (Roth and Lindroth 1994; Williams et al. 1998), carbon-based allelochemicals (Lindroth et al. 1997b; Booker and Maier 2001), and carbon:nitrogen ratio (Williams et al. 1998) in foliage demonstrate that tree leaves grown under an elevated CO_2 atmosphere are a comparably poorer food source for insects compared to ambient CO_2-grown plants. Because many essential nutrients found in plants affect insect growth and development (especially N; Mattson 1980), observed changes in nutritional components due to CO_2enrichment produce an array of effects on leaf-feeding insects. These effects include reduced larval growth (Roth and Lindroth 1994, 1995), increased leaf consumption (Williams et al. 1994), prolonged larval development (Lindroth et al. 1997b) and reduced female fecundity (Traw et al. 1996).

For insects that feed on leaves, the independent or interactive effects of elevated CO_2 and temperature on phytochemistry (indirect effect) or the exposure of feeding insects to these factors (direct effect) have important implications for the insects' success. An extensive body of literature demonstrates that insects are temperature sensitive (see Taylor 1981) and thus potentially subject to the direct effects of an increased temperature. Indeed, the direct effects of temperature on insects may be more important than any other factor associated with global climate change (i.e. elevated CO_2 concentration, alterations in precipitation, etc.; Bale et al. 2002). In a previous study where herbaceous plants were grown at either elevated CO_2 concentration or elevated temperature, leaf N concentration was increased at an elevated temperature, while the elevated CO_2 concentration had no effect (Bezemer et al. 1998). The abundance of aphids increased on both elevated $CO_2$and elevated temperature-grown plants. Few studies to date have examined the potential interactive effects of CO_2 and temperature on leaf chemistry and insect performance. In *Quercus robur*, temperature alone resulted in decreased leaf N concentration and increased condensed tannin concentration, while elevated CO_2 increased total carbon-based phenolics (Dury et al. 1998). These responses were somewhat dependent on days post budbreak, illustrating the importance of considering changing phytochemical profiles as foliage ages (Raupp and Denno 1983). A lepidopteran feeding on detached leaves from these plants grown at an elevated temperature had reduced fecundity (i.e. number of eggs produced), while leaves grown at elevated CO_2 did not have a similar effect on the insect (Buse et al. 1998). Interestingly, the fecundity of insects feeding directly on plants at elevated temperature and CO_2 was unaffected. In our previous work where red and sugar maple saplings were grown under CO_2 enrichment and elevated temperature, we found changes in plant phytochemistry due to increased CO_2 concentration but not temperature (Williams et al. 2000). We observed little effect on insect performance during a 3- to 6-day feeding period. Although the aforementioned studies on whole leaf-feeding insects demonstrate no interactive effects of CO_2 and temperature, a study with a leaf mining insect feeding on a herbaceous plant species demonstrated that CO_2 enrichment and elevated temperature can interactively negatively impact larval survivorship and adult weight (Johns and Hughes 2002).

In the study reported here, we examined the effects elevated CO_2 and elevated temperature-grown *Acer rubrum*leaves had on the development of an important folivorous insect, *Lymantria dispar*, by rearing larvae from first instar to pupation directly on plants within chambers. This insect is a widespread exotic defoliator of many hardwood tree species, and has caused extensive damage to eastern forests since its accidental introduction into the United States in the late 19th century. Our experiment allowed us to determine if increased CO_2 concentration and temperature either independently or interactively affected phytochemical constituents important to gypsy moth development. These indirect effects were considered in association with the direct effects of increased CO_2 concentration and temperature on larval feeding. By carrying out the experiment through the larval life cycle, a better understanding of long-term effects of CO_2 concentration and temperature on insect performance could be gained. We predicted that: (1) an enriched CO_2 atmosphere would result in a decline of leaf nutritional quality (especially N), and an increase in defensive phenolics; (2) an elevated temperature would reduce accumulations of non-structural carbohydrates and thus reduce carbohydrate:N ratios; (3) the combined effects of elevated CO_2 and temperature on phytochemistry would result in alterations in the development of the gypsy moth larvae (i.e. days to pupation and pupal weight); and (4) insect development would be directly affected by an elevated temperature but not an elevated CO_2 atmosphere.

Materials and methods

Plant growth conditions

In spring 1994, ten 1-year-old bare rooted seedlings of *A. saccharum* and *A. rubrum* were planted in the ground in 3-m diameter by 3.6-m tall open-top chambers at the Global Change Research site in the National Environmental Research Park at Oak Ridge National Laboratory, Tennessee. At the time of the experiment reported here (1997), the plants were beginning their fourth growing season within the chambers and averaged over 2.5 m in height. There were three replicate chambers, arranged in a randomized complete block design, for each of four treatments: (1) ambient temperature ambient CO_2, ATAC; (2) ambient temperature elevated CO_2 (+300 μl l^{-1} CO_2), ATEC; (3) elevated temperature (+3.5°C) ambient CO_2, ETAC; and (4) elevated temperature

elevated CO_2, ETEC. Carbon dioxide treatments were maintained 24 h per day during the growing season, while temperature treatments continued all year. Temperatures were regulated by passing the airflow through PID-controlled evaporative coolers and resistance heaters. Levels of CO_2 were controlled by regulating CO_2 additions with rotameters and monitoring with an infrared gas analyzer. For a more detailed description of the chamber design and experimental set-up see Norby et al. (1997) and Edwards and Norby (1999). Shade cloth draped over the chambers throughout the plant growing season reduced ambient light to 27%. The *A. rubrum* saplings used for insect feeding in this experiment were part of an overall larger experiment examining the effects of elevated CO_2 and temperature on maple tree physiological processes (Norby et al. 2000). In order to minimize negative impacts due to insect herbivory on this larger experiment, only two saplings per chamber were available for insect feeding.

Insect rearing

Gypsy moth egg masses were obtained from Otis Methods Development Center, United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). Because the gypsy moth is not indigenous to the United States, it is subject to federal quarantine regulations. The state of Tennessee is outside of its range of infestation and, therefore, reproductive populations of the gypsy moth are not permitted in Tennessee. The insects used in this experiment were produced as part of the sterile male release program for gypsy moth control (for a more detailed description of this program see Schwalbe et al. 1991 and Mastro 1993). Briefly, male pupae are irradiated and subsequent sterile adult males mated with normal females, producing sterile offspring. Previous studies with F_1-sterile insects have found them 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, 2000).

Twenty-two egg masses were placed on standard artificial diet (provided by Otis Methods Development Center). Newly eclosed larvae were separated among egg masses in rearing cups to "mix" the population and minimize potential maternal effects across treatments. Larvae used for this red maple feeding experiment were maintained on diet until 5 days post eclosion, then randomly chosen from the rearing cups for transfer to plants. In order to assure that larval age was initially the same among all four treatments, larvae chosen for the experiment were closely matched for date of egg hatch. Egg hatch was spread over several days between the 22 egg masses such that larvae of equal age were available over several days for set-up on the plants within chambers (see below).

Insect rearing on A. rubrum within chambers

We recognize that red maple is not an especially preferred tree species for the gypsy moth. Due to the overall larger collaborative nature of this study, our experimental design allowed us access to only red and sugar maple. A variety of factors made the red maple a more practical choice for this experiment, including the more desirable timing of budbreak to insect life stage compared to the sugar maple (see below). Insects were reared on plants within chambers so they would be exposed to both CO_2 and temperature treatments throughout the greater portion of the larval life cycle. The date larvae hatched from eggs was closely synchronized with red maple leaf emergence. For each chamber, leaf phenology was matched with insect age for each of the two plants used. Leaves were actively expanding but not curled (approximately 5–7 days post budbreak). There was considerable variation among plants and chambers for budbreak, such that not all plants within each chamber were available for insect feeding on the same date. Leaves of plants growing at an elevated temperature emerged, on average, 10 days earlier than those growing at ambient temperature (R. Norby, unpublished data). However, due to the large variation in leaf emergence dates between plants in all chambers, we were able to alternate insect set-up across all temperature and CO_2treatments over a period of 4 days. By only using plants with very similar leaf phenology, we prevented any potential treatment bias that would compromise our ability to distinguish treatment effects from leaf phenology or environmental effects. Ten- to 15 5-day-old larvae were weighed and placed on a single branch per red maple sapling in a 1×1 mm nylon mesh bag. The starting weight of larvae within bags was the same for all treatments (P =0.488, data not shown) thus preventing any potential treatment bias in initial weights. Nylon bags were approximately 35.5 cm wide by 35.5 cm long, with the end of the bag tied snugly and attached to the tree on which it was placed. This design allowed for free movement of larvae within the bag and provided support for bags when they became wet due to rain. Over the course of the feeding period, the bags and insects were moved to new branches on the same plant as the insects' consumption within bags became considerable. At no time was foliage availability limited and overall tree-wide defoliation never exceeded 5% (visual estimate). The number of dead insects within bags was recorded throughout the experiment and carcasses were removed at times when bags were being moved between branches.

Insect performance was measured in three ways: (1) percent of larval mortality, (2) days from set-up on plants to pupation, and (3) pupal fresh weight. Pupae were sexed at the end of the experiment using the differences between males and females in antennal sheath and genital markings.

Phytochemistry sampling and analyses

Leaf samples were collected to determine the concentration of leaf N, non-structural carbohydrates, water, and an estimate of defensive phenolic compounds. As insect bags were set up, two leaves were removed from the branch opposite that on which the insect bag was placed. Each leaf was then cut down the midvein into two pieces, with one piece being weighed, then dried at 40°C. This portion of the leaf was analyzed for N, non-structural carbohydrates, and water concentration. The other leaf portion was stored in a −80°C freezer until phenolics quantification. This leaf-sampling procedure was repeated approximately every 2 weeks until larvae began to pupate within the bags. When bags were moved on plants, leaf collection continued on the branch opposite the insect bag. Over the length of the experiment (i.e. approximately 6 weeks) a total of eight leaves (two for each of the four sampling dates) were collected per plant for phytochemistry analyses. Leaf samples were combined within chambers at each sample date and leaf chemistry analyzed and reported for three leaf age classes: (1) immature, (2) mid-mature, and (3) mature. Because there was insufficient tissue available to conduct the phytochemical assays on the young, expanding leaves (i.e. first sample date), the immature age class contains analyses from the first two sampling dates. On two sample dates, some phytochemical measures were not calculated in one chamber of two treatments due to problems in the processing of leaf samples for chemistry. This included one date for immature leaves for leaf nitrogen (ATEC) and one date for mid-mature leaves for carbohydrates (ETEC).

The concentration of N in leaves was determined using a Carlo-Erba NA-1500 carbon-nitrogen analyzer. Non-structural carbohydrates were analyzed following the acid hydrolysis procedure of Tissue and Wright (1995) in which soluble sugars and starch are extracted from ground leaf material using a methanol:chloroform:water solution and quantified spectrally after acid hydrolysis with concentrated sulfuric acid. The total non-structural carbohydrates (TNC) are the sum of starch and soluble sugars. Defensive phenolic compounds were analyzed using the Folin-Ciocalteau's reagent technique of Singleton and Rossi (1965). Tannic acid (Sigma Chemical Company) was used in the development of the standard curve. We recognize that this assay is subject to interference with other chemically reactive compounds in the leaf (see Mole and Waterman 1987) and that this redox-based assay does not allow for quantification of foliar phenolics (Appel et al. 2001). Therefore, we report an estimate of defensive phenolic

compounds expressed as percent tannic acid equivalents in the red maple leaf.

Statistical analysis

We used a General Linear Model Procedure (Proc GLM, SAS for Power PC; SAS Institute 1996) to analyze the effects of CO_2, temperature, and their interaction on insect performance 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. Reported treatment means and standard errors were calculated among chambers within CO_2 and temperature treatment. Data for percent mortality were arcsine transformed to normalize the data.

Phytochemical data were analyzed using a Repeated Measures ANOVA (Proc GLM, SAS) to accommodate the multiple measurements of each plant across sampling dates (representing leaf age). In this analysis, measures for each leaf age class are entered into the model and analyzed for main effects of leaf age, CO_2, temperature and the CO_2 × temperature interaction. Measures significantly affected by leaf age (P <0.05) were further analyzed by comparing the effects of CO_2, temperature and CO_2 × temperature interaction between the immature leaves (i.e. first two sample dates) and mid-mature and mature leaves using CONTRAST. Reported treatment means and standard errors for phytochemical measures were calculated among chambers within CO_2 and temperature treatment for each sample date. Prior to analysis, all data were checked for normality (Proc UNIVARIATE, SAS). Data not normally distributed were log transformed to normalize the data. 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

Although CO_2 enrichment altered some phytochemical constituents, there were no significant main effects of temperature or its interaction with CO_2 concentration (Table 1). Leaf N and water both significantly declined as leaves aged (significant effect of leaf age), while CO_2 concentration had a significant effect on leaf N and a marginally significant effect on leaf water (Table 1, Fig. 1A, B). Averaged across temperature treatments, compared to leaves grown at ambient CO_2, leaves in elevated CO_2 were 21% lower in leaf N in immature and 11% lower in leaf N in mature leaves (Fig. 1A). There was a marginally significant effect of temperature in

Table 1 Significance values (P) and df[a] for main effects of leaf age, CO_2, temperature and their interaction on *Acer rubrum* leaf constituents using a repeated measures ANOVA (Proc GLM). See the Materials and methods for a detailed description of the statistical model. *TNC* Total non-structural carbohydrates. Values of$P$ <0.1 appear in *bold type*

Leaf constituents	Leaf age	CO_2	Temp	CO_2 × Temp
Water concentration	**0.001**	**0.085**	0.141	0.492
N concentration	**0.001**	**0.001**	0.772	0.227
Sugar concentration	0.178	0.776	0.507	0.617
Starch concentration	0.310	0.213	0.550	0.967
TNC	0.628	0.288	0.892	0.684
Sugar:N ratio	0.443	0.134	0.805	0.609
Starch:N ratio	**0.004**	**0.038**	0.246	0.813
TNC:N ratio	**0.028**	**0.041**	0.388	0.682
Tannic acid equivalents	0.548	0.192	0.474	0.146

[a] Nitrogen, sugar, starch and TNC concentration: df=2,14 for leaf age, 1,7 for CO_2, temperature and CO_2 × temperature. Sugar:N, starch:N and TNC:N ratio: df=2,12 for leaf age, 1,6 for CO_2, temperature and CO_2 × temperature. Water and tannic acid equivalents: df=2,16 for leaf age, 1,8 for CO_2, temperature and CO_2 × temperature

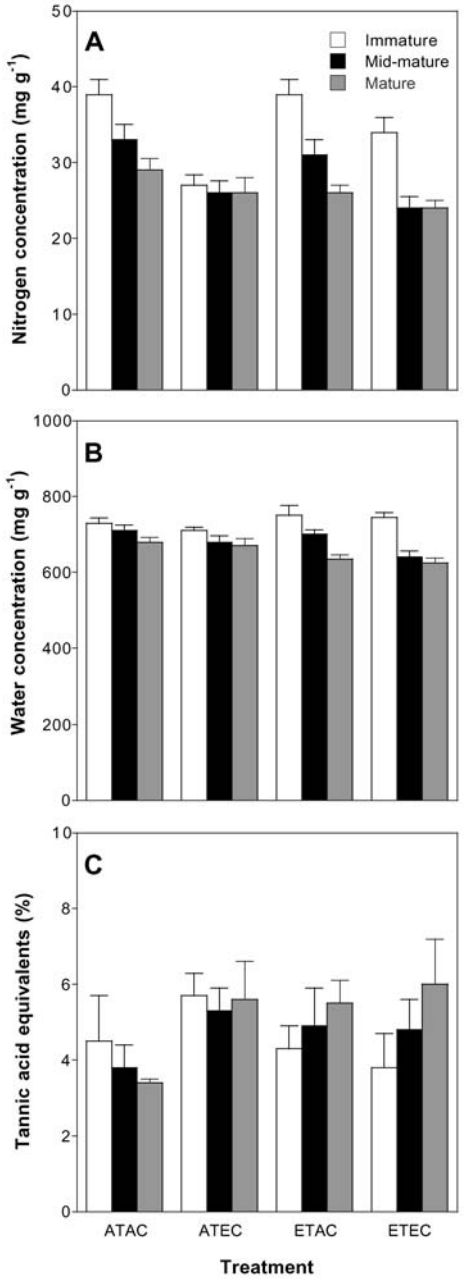

Fig. 1 Effects of CO_2 and temperature treatments on phytochemical constituents in three age classes of red maple leaves: **A** N concentration, **B** water concentration, and **C** tannic acid equivalents. *ATAC* Ambient temperature, ambient CO_2; *ATEC* ambient temperature, elevated CO_2; *ETAC* elevated temperature, ambient CO_2; *ETEC* elevated temperature, elevated CO_2. *Bars* represent standard error of the mean

Table 2 Significance values (P) and df[a] for CO_2, temperature and their interaction on immature versus mid-mature and mature leaves using repeated measures ANOVA (Proc GLM) with CONTRAST. See the Materials and methods for a detailed description of the statistical model. Values of P <0.1 appear in *bold type*

Leaf constituents	Mid-mature			Mature		
	CO_2	Temp	CO_2 × temp	CO_2	Temp	CO_2 × Temp
Nitrogen concentration	0.881	0.201	0.332	0.274	**0.072**	0.264
Water concentration	0.306	0.138	0.275	0.933	**0.013**	0.393
Starch:N ratio	0.674	**0.086**	0.266	0.721	0.816	0.773
TNC:N ratio	0.639	0.132	0.303	0.657	0.707	0.636

[a] df =1,7 for N, 1,8 for water and 1,6 for starch:N and TNC:N ratio

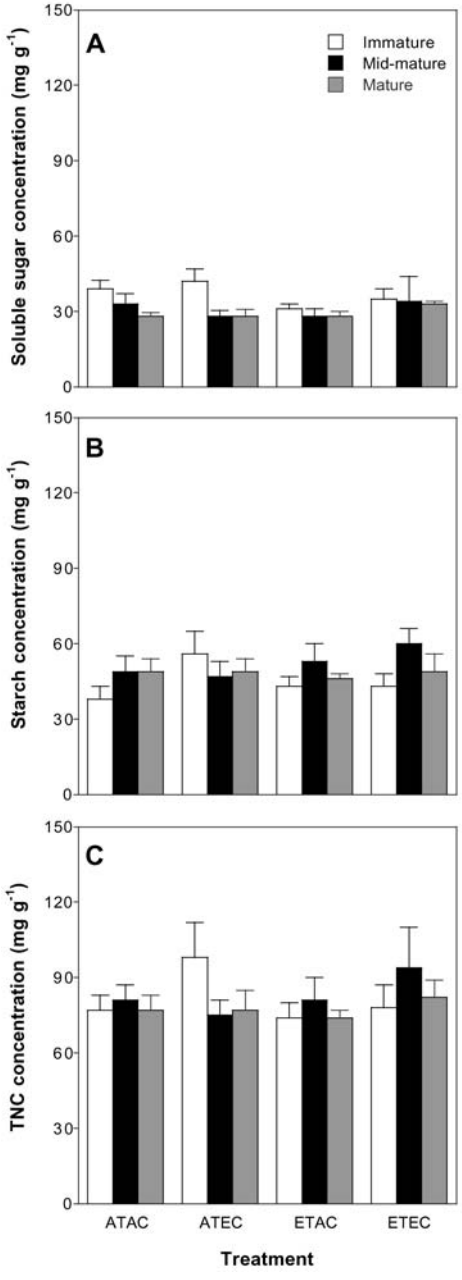

Fig. 2 Effects of CO_2 and temperature treatments on phytochemical constituents in three age classes of red maple leaves: **A** soluble sugar concentration, **B** starch concentration, and **C** total non-structural carbohydrates concentration. See Fig. 1 legend for a description of the treatments. *Bars* represent standard error of the mean

Table 3 Significance values (P) and df[a] for CO_2, temperature and their interaction on *Lymantria dispar*performance (Proc GLM with a priori CONTRAST). See the Materials and methods for a detailed description of the statistical model. Values of P <0.1 appear in *bold type*

Insect performance	CO_2	Temp	CO_2 × Temp
Mortality (%)	0.376	0.216	0.716
Pupal fresh weight (g)			
Female	0.367	0.166	0.887
Male	0.919	0.214	0.103
Days to pupation			
Female	0.848	**0.065**	0.958
Male	0.540	**0.032**	0.966

[a] For all measures df =1,6

mature compared to immature leaves for leaf N (Table 2). Averaged across CO_2 treatments, mature leaves grown at the elevated temperature were 9% lower in N compared to those grown at the ambient temperature (Fig. 1A). Similarly, temperature had a significant effect on leaf water concentration in mature leaves (Table 2), with leaves grown at the elevated temperature 6% lower in water compared to ambient temperature-grown leaves (Fig. 1B). Although percent tannic acid equivalents (%Tae) were unrelated to leaf age, CO_2, or temperature, there was a general tendency for %Tae to increase as leaves aged at the elevated temperature (Fig. 1C).

While none of the measured carbohydrates were affected by CO_2 concentration or leaf age (Table 1, Fig. 2A–C), the ratio of both starch:N and total non-structural carbohydrates:N (TNC:N) were significantly increased by CO_2treatment and leaf age (Table 1, Fig. 3B, C). Averaged across temperature treatments and leaf age classes, leaves grown at elevated CO_2 were 19% higher in TNC:N ratio than leaves grown at ambient CO_2(Fig. 3C). Temperature had a marginally significant effect on starch:N in leaves at mid-maturity, although this effect was not evident in mature leaves (Table 2, Fig. 3B).

The percentage of larval mortality was not significantly related to any treatment: [ATAC =16±4% (SE), ATEC =11±4, ETAC =28±10, ETEC =19±3; Table 3). The pupal fresh weight of males and females was unaffected by CO_2, temperature or their interaction (Table 3, Fig. 4A). The time it took the insects to complete the larval stages (i.e. reach pupation) was significantly reduced for both males and females

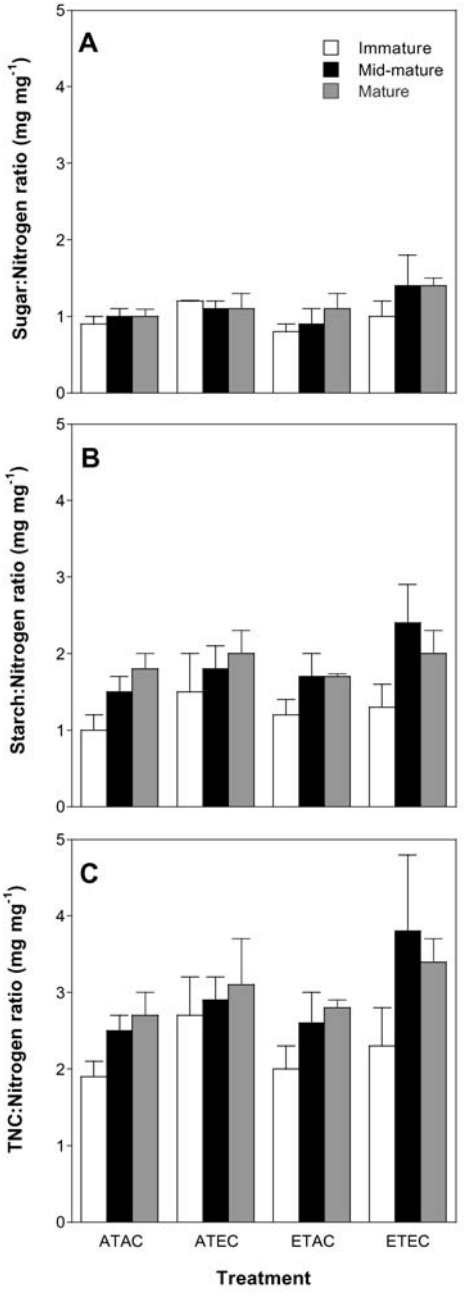

Fig. 3 Effects of CO_2 and temperature treatments on the ratio of carbohydrate: N in three age classes of red maple leaves: **A** soluble sugar:N ratio, **B** starch:N ratio, and **C** total non-structural carbohydrates:N ratio. See Fig. 1 legend for a description of the treatments.*Bars* represent standard error of the mean

(marginally significant) at the elevated temperature (Table 3). Averaged among treatments, males pupated 7.5 days and females 8 days earlier than larvae exposed to an ambient temperature (Fig. 4B).

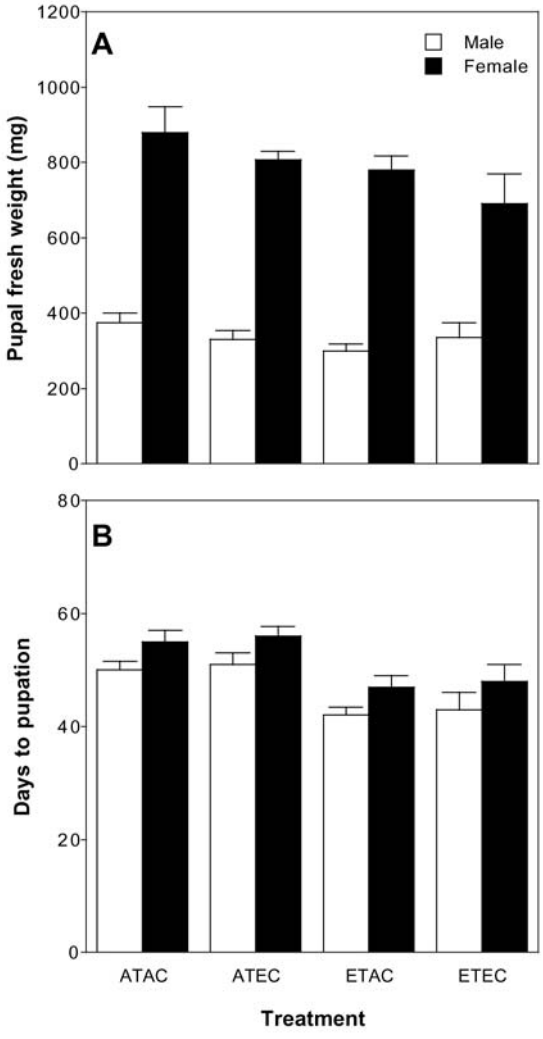

Fig. 4 Effects of CO_2 and temperature treatments on gypsy moth performance: **A** pupal fresh weight, and **B** days to pupation. See Fig. 1 legend for a description of the treatments. *Bars* represent standard error of the mean

Discussion

Phytophagous insect development is mediated by a variety of factors present in their host plants, including both nutritional and defensive allelochemical constituents that collectively reflect food quality (Scriber and Slanksy 1981; Stamp 1990). Acting concurrently with diet quality are abiotic factors such as temperature, which can have a profound effect on ectothermic organisms like insects (Taylor 1981; Casey 1993). Although the independent effects of nutritional quality and temperature on insect performance are known for a number of species, it is much less well understood how these factors may simultaneously affect insect development (Stamp and Yang 1996). A previous artificial diet study examining the long-term sensitivity of the gypsy moth to alterations in temperature and dietary N found these factors acted independently on insect development (Lindroth et al.

1997a). Whether diet quality and temperature independently or interactively affect the performance of plant-feeding insects has particular relevance within the context of predicted changes in global mean temperature (Houghton et al. 1996) and reduced foliar quality (Lincoln et al. 1993) as atmospheric CO_2 concentration increases due to anthropogenic activities. Much of our current understanding about how elevated CO_2-induced changes in host plants alter important plant-insect interactions comes from studies where elevated CO_2 is the only global climate change variable investigated. Many such studies demonstrate that an elevated CO_2concentration can alter key constituents important for insect performance, resulting in effects on growth, consumption and development (recently reviewed in Coviella and Trumble 1999). Less well understood are what effects concurrent increases in global mean temperature may have on folivorous insects and their host plants. Our study reared insects directly on a hardwood tree species at two CO_2 concentrations and temperatures to examine the independent and/or interactive effects of CO_2 and temperature-induced changes in leaf chemistry and insect development. This study represents one of the few to date that incorporates simultaneous treatment conditions, allowing for the assessment of both direct and indirect effects on an important folivorous hardwood pest.

We set forth a number of predictions at the outset to address the independent or interactive effects of an elevated CO_2 atmosphere and temperature on plant phytochemistry and insect performance. First, we expected a CO_2-enriched atmosphere to result in reductions in foliar N and increases in defensive phenolics in *A. rubrum* leaves grown at an elevated CO_2 concentration. We found that maple leaves grown at +300 $\mu l\ l^{-1}$ elevated CO_2 were significantly lower in N, and that as leaves aged an elevated plant growth temperature caused a slight reduction in leaf N (marginally significant; Tables 1, 2, Fig. 1A). Additionally, our data show that a reduction in leaf N due to CO_2enrichment occurs in both immature and mature leaves, a result similar to a previous study with *Quercus alba* L. (Williams et al. 1998). Therefore, in the study reported here both early and late instar larvae experienced a CO_2-induced reduction in foliage quality measured as leaf N. Our observed decline in leaf N under elevated CO_2 growth conditions agrees with our previous work with *A. rubrum* in this experimental system (Williams et al. 2000) and demonstrates that in this tree species there is no interactive effect of CO_2 concentration and temperature on foliar N. Because reduced foliar N (in particular) can affect the performance of insects feeding on CO_2-enriched plants (Lincoln et al. 1993; Lindroth et al. 1993), observed N reductions in the *A. rubrum*leaves in our study constitute a potential indirect effect on gypsy moth performance. Our observation that effects of temperature on leaf N occurred in older versus young leaves is in contrast to a previous work where *Q. rober* L. was grown under elevated CO_2 and temperature (Dury et al. 1998). In addition, that study found phenolics increase at elevated CO_2 concentration as leaves aged. These defensive compounds are thought to be important in the responses of insects to elevated CO_2-grown trees in other studies (Lindroth et al. 1993; Traw et al. 1996). Our estimate of phenolics (measured as tannic acid equivalents) was unrelated to either CO_2 or temperature treatment in our study. Some caution should be exercised in directly comparing results from our study to Dury et al. (1998), as differences in plant growth conditions and exposure times were substantial.

In addition to N, carbohydrates and water contribute to leaf nutritional quality. Previous studies with sugar maple (*A. saccharum* L.) demonstrate that leaves grown under CO_2 enrichment increase in leaf carbohydrates (Roth and Lindroth 1994; Roth et al. 1998), while leaf water is unchanged (Lindroth et al. 1993). We measured the levels of non-structural carbohydrates (i.e. sugar and starch) as leaves aged throughout the several-week larval feeding period and found that although the concentration of carbohydrates was unrelated to either CO_2or temperature, the ratio of starch:N and TNC:N was significantly increased at elevated CO_2but not elevated temperature (Table 1, Fig. 3B, C). Temporal changes in leaves were important, as both the starch:N and TNC:N ratio significantly increased as leaves matured (Table 1), although the effect of CO_2 concentration was evident in the youngest leaves (Fig. 3B, C). Observed levels of carbohydrates and carbohydrate:N ratio run contrary to one of our predictions, namely that an elevated temperature would reduce carbohydrates and their ratio with leaf N. The increased carbohydrate:N ratio under elevated CO_2demonstrates that larvae feeding on CO_2-enriched foliage ate a comparably poorer food source than those feeding on ambient CO_2-grown plants, irrespective of temperature (Fig. 3). The importance of leaf water in an insect's diet (see Slansky 1993) makes changes in this leaf constituent due to CO_2enrichment and elevated temperature relevant. We found a marginally significant reduction in leaf water due to CO_2 enrichment (Table 1) and a significant effect of temperature as leaves reached maturity (Table 2). Caterpillars feeding on the oldest leaves, irrespective of plant growth CO_2 concentration, had less water available in their diet (Fig. 1B). Because CO_2-induced reductions in foliage quality (e.g. N and water) were unrelated to insect mortality, development rate and pupal weight (Table 3), we conclude that phytochemical changes resulted in no negative effects on gypsy moth performance. This contradicted our prediction that alterations in leaf phytochemistry would affect gypsy moth development.

The only significant effect on *L. dispar* performance in our study was a reduction in the development time of male and female (marginally significant) larvae at the elevated temperature (Table 3). Irrespective of CO_2 concentration, on average, male larvae pupated 7.5 days earlier and female larvae 8 days earlier at elevated temperature (Fig. 4A). This result agrees somewhat with Buse et al. (1998), where caged winter moth caterpillars developed faster at higher temperature on oak leaves. Our observed response of *L. dispar* to an increased temper-

ature is not surprising from what we know of the phenology of this insect (Casagrande 1981) and its response to varying temperature in previous studies (Lindroth et al. 1997a). Because an insect's development rate may strongly influence survival and reproductive success (Taylor 1981), the direct effect of an increased temperature on reducing development time observed in our study has potentially important implications. For example, factors such as reduced host plant quality may in some cases prolong the development of insects, potentially exposing them to greater predation and parasitism risk (Clancy and Price 1987). A shortened development time due to a higher insect growth temperature could, therefore, serve to mitigate the effects of reduced host plant quality for some tree feeding insects. However, insect species that feed on other portions of leaves to whole leaf feeders (e.g. miners) may well be affected by elevated CO_2-induced reductions in foliage quality and an elevated growth temperature in other ways. In an herbaceous plant system, declines in leaf quality at elevated CO_2 dampened the effects of more rapid development of larvae at an elevated temperature (Johns and Hughes 2002). Our observed reduction in development time supports one of our original predictions, i.e. the performance of *L. dispar* would be directly affected by an elevated temperature but not CO_2concentration. Our observed lack of treatment effects on pupal weight generally agrees with Buse et al. (1998), where third instar caterpillars placed on leaves and reared in situ at elevated CO_2 and temperature had similar pupal weights among temperature and CO_2treatments. Although the insects in our experiment were not deleteriously affected by reduced host plant quality brought by an elevated CO_2 concentration (and in limited cases temperature), our finding of a temperature-induced reduction in development time supports the notion that the development of some insect species can be expected to change in a future warmer world.

In conclusion, by exposing *L. dispar* for the greater part of its larval life-cycle to simultaneous increases in both CO_2concentration and temperature, we were able to more closely mimic a future climate change scenario than in other studies. Our previous work in this maple system (Williams et al. 2000) was too short in duration to fully explore the potential direct effects of temperature on larval development. The study reported here demonstrates clear responses of *A. rubrum* leaves to an enriched CO_2 atmosphere, as well as to a lesser degree elevated temperature, when leaf age is considered. The fact that no interaction between CO_2 and temperature on leaf nutritional and defensive constituents important to folivorous insects was observed suggests that in some tree species climate change factors may act independently. Because the phytochemical constituents we measured in our study were primarily affected by CO_2 enrichment but not temperature, we conclude that potential effects on herbivorous insects may well depend upon their ability to deal with the reductions in leaf quality brought by CO_2 enrichment (indirect effects), while being exposed to higher temperatures that affect larval phenology (direct effects). Direct effects on insect development in a warmer world may be more important than chemical changes in host plants in some plant-insect systems as atmospheric CO_2 concentration increases. More studies using preferred host plant species at plant and insect population levels are needed if we are to fully understand how a suite of climate change factors may alter important tree-herbivorous insect associations.

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