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Decline in gypsy moth (*Lymantria dispar*) performance in an elevated CO_2 atmosphere depends upon host plant species

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Abstract Plant species differ broadly in their responses to an elevated CO_2 atmosphere, particularly in the extent of nitrogen dilution of leaf tissue. Insect herbivores are often limited by the availability of nutrients, such as nitrogen, in their host plant tissue and may therefore respond differentially on different plant species grown in CO_2-enriched environments. We reared gyspy moth larvae (*Lymantria dispar*) in situ on seedlings of yellow birch (*Betula allegheniensis*) and gray birch (*B. populifolia*) grown in an ambient (350 ppm) or elevated (700 ppm) CO_2 atmosphere to test whether larval responses in the elevated CO_2 atmosphere were species-dependent. We report that female gypsy moths (*Lymantria dispar*) reared on gray birch (*Betula populifolia*) achieved similar pupal masses on plants grown at an ambient or an elevated CO_2 concentration. However, on yellow birch (*B. allegheniensis*), female pupal mass was 38% smaller on plants in the elevated-CO_2 atmosphere. Larval mortality was significantly higher on yellow birch than gray birch, but did not differ between the CO_2 treatments. Relative growth rate declined more in the elevated CO_2 atmosphere for larvae on yellow birch than for those on gray birch. In preference tests, larvae preferred ambient over elevated CO_2-grown leaves of yellow birch, but showed no preference between gray birch leaves from the two CO_2 atmospheres. This differential response of gypsy moths to their host species corresponded to a greater decline in leaf nutritional quality in the elevated CO_2 atmosphere in yellow birch than in gray birch. Leaf nitrogen content of yellow birch dropped from 2.68% to 1.99% while that of gray birch leaves only declined from 3.23% to 2.63%. Meanwhile, leaf condensed tannin concentration increased from 8.92% to 11.45% in yellow birch leaves while gray birch leaves only increased from 10.72% to 12.34%. Thus the declines in larval performance in a future atmosphere may be substantial and host-species-specific.

Key words *Lymantria dispar* · Betulaceae · Elevation CO_2 · Tannin · Nitrogen

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Introduction

Atmospheric CO_2 levels are expected to double by the end of the 21st century (Houghton 1990). Within temperate forests, an increase of this magnitude has been shown to directly impact plants through changes in physiological processes (Eamus and Jarvis 1989; Bazzaz 1990; Bazzaz and Fajer 1992). Plants grown in an elevated CO_2 atmosphere commonly exhibit increased photosynthetic rates and carbohydrate storage, leading to higher ratios of carbon to nitrogen in leaves (Fig. 1), the "nitrogen dilution effect" (Wong 1979; Lincoln et al. 1993). Tree species differ in their leaf nitrogen dilution in an elevated CO_2 atmosphere (Oberbauer et al. 1986; Rochefort and Bazzaz 1990; Lindroth et al. 1993) with observed declines ranging from 4.8% in *Ledum palustre* (Oberbauer et al. 1986) to 46.2% in *Alnus glutinosa* (Norby 1987).

Insect herbivores, which are often limited by dietary nitrogen (Mattson 1980), have experienced lower growth rates (Lincoln et al. 1986; Fajer 1989; Johnson and Lincoln 1990; Lindroth et al. 1993; Roth and Lindroth 1994) and higher larval consumption rates (Lincoln et al. 1986; Lincoln and Couvet 1989; Johnson and Lincoln 1990, 1991; Lindroth et al. 1993; Lindroth 1996) on plants grown in an elevated CO_2 atmosphere. The observed correspondence between declines in insect performance and

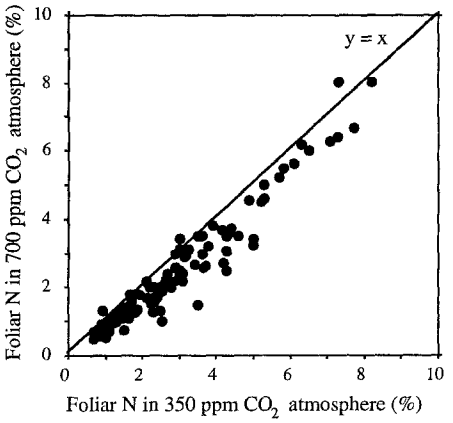

Fig. 1 Leaf nitrogen dilution of plant species grown in enriched CO_2 atmospheres. Leaf nitrogen content at an elevated (600–750 ppm) CO_2 atmosphere is presented as a function of leaf nitrogen content at an ambient (300–450 ppm) CO_2 atmosphere. The *line* $y = x$ represents no effect of an enriched CO_2 atmosphere on leaf nitrogen content. All *points* falling below the line indicate leaf nitrogen dilution. Studies with CO_2 treatments outside the specified ranges were excluded. Each point represents the mean value of the replicates in a treatment. Methods of selecting and analyzing leaves for leaf nitrogen varied among the studies. Values were compiled from the following references (number of points abstracted, number of plant species included): Brown 1991 (7,1); Coleman et al. 1991 (6,3); Coleman and Bazzaz 1992 (4,2); Curtis et al. 1989 (10, 2); Fajer et al. 1989 (1,1); Fajer et al. 1991 (2,1);Garbutt et al. 1990 (5,5); Johnson and Lincoln 1990 (1,1); Johnson and Lincoln 1991 (2,1); Kuehny et al. 1991 (6,1); Larigauderie et al. 1988 (8,1); Lincoln et al. 1984 (1,1); Lincoln et al. 1986 (1,1); Lincoln and Couvet 1989 (2,1); Lindroth et al. 1993 (6,3); Norby 1987 (3,3); Norby and O'Neill 1991 (4,1); Norby et al. 1986 (2,1); Oberbauer et al. 1986 (6,6); Rochefort and Bazzaz 1990 (4,4); Roth and Lindroth 1994 (2,2); Thomas et al. 1991 (4,1); Williams et al. 1988 (6,6); Wong 1979 (8,2)

leaf nitrogen dilution in the host plants has led to widespread speculation that leaf nitrogen dilution has an important role in the response of insects to an elevated CO_2 atmosphere (Lincoln et al. 1986; Fajer 1989; Johnson and Lincoln 1990; Lindroth et al. 1993; Roth and Lindroth 1994).

When generalist herbivores have been reared on several host plant species grown in an ambient or elevated CO_2 atmosphere, the effect of the CO_2 treatment on insect performance has depended upon the host plant species (Lindroth et al. 1993; Roth and Lindroth 1994). Insect herbivores commonly exhibit feeding preferences for plant tissues that provide the highest level of performance (Kimmerer and Potter 1987; Minkenberg and Ottenheim 1990; Basset 1991; but see Reavey 1991). Differential plant response to an elevated CO_2 atmosphere may therefore affect insect herbivore behavior. In an elevated CO_2 atmosphere, relative host plant preferences of a generalist herbivore may shift in favor of those host species that experience the least dilution of leaf nitrogen.

In studies of insect performance on plants in enriched CO_2 atmospheres, researchers have primarily considered one insect species on a single host plant species and few studies have reported information on insect behavior. Insects are also rarely reared directly on the host plants, nor are they measured over their entire developmental period.

This study addressed the in situ performance and host plant preferences of gypsy moths reared from egg to pupa on yellow and gray birch in an ambient or elevated CO_2 environment. Specifically, we asked the following questions:

1. Does the performance of gypsy moth larvae decrease when the larvae are reared on plants grown in an enriched CO_2 atmosphere?
2. Is there a correspondence between the larval response on a given species and that species' leaf nitrogen dilution in an elevated CO_2 environment?
3. Does the order of larval preference for gray and yellow birch shift when the plants are grown in an enriched CO_2 atmosphere?

Materials and methods

Study species

Gray birch (*Betula populifolia* Marsh.) and yellow birch (*B. allegheniensis* Britt.) are ecologically important temperate tree species and host plants of the gypsy moth (*Lymantria dispar* Linnaeus). These sympatric tree species are abundant in temperate forests throughout New England and Canada, especially in treefall gaps and along forest margins. Gray birch is a pioneer tree that colonizes recently disturbed forest, requires open sunlight, and grows well on nutritionally poor soil. Yellow birch is a mid-successional species, surviving in the understory, living longer, and requiring higher soil fertility than gray birch (Burns and Honkala 1990). Both species produce new leaves throughout the growing season.

In the field, gypsy moth larvae have been observed to prefer gray birch to yellow birch (Mauffette et al. 1983). However, the difference in observed preference was not large (yellow birch was ranked 15 and gray birch 13 out of 29 species). The gypsy moth is an abundant, introduced pest of New England's temperate forests. Gypsy moths are univoltine and overwinter as eggs, which hatch in early spring. Larvae feed from April until they pupate in June. In 1992, gypsy moth larvae defoliated over 15 million hectares in the United States alone, an area the size of Maine, Massachusetts, New Hampshire, and Vermont combined (Liebhold et al. 1993). Female moths are flightless and lay eggs next to the pupal case. Host plant selection is performed primarily by larvae (Barbosa et al. 1979).

Plant growth

In late May 1992 gray and yellow birch seeds from the Harvard Forest in Petersham, Massachusetts, were germinated in one thousand 5.7-cm peat pots containing a mixture of sand, unsterilized soil, turface, and peat in a 2:2:2:1 ratio. Seedlings were thinned to one per pot, watered daily, and fertilized with 10 ml of quarter-strength Peter's 20:20:20 NPK fertilizer per pot once every 2.5 weeks for 8 weeks. This fertilization rate resulted in very slow growth of the seedlings and was adjusted during the 8th week to 10 ml of half-strength Peter's 20:20:20 NPK fertilizer per pot applied twice per week. During the 10th week, we transplanted seedlings and the soil around their roots into 10.2-cm pots. After the transplant, plants were fertilized with 135 ml of quarter-strength Peter's 20:20:20 NPK fertilizer per pot once every 9 days on average through the remainder of the experiment. Plants continuously produced new leaves throughout the duration of the experiment.

Seedlings were grown in temperature and CO_2-controlled glass chambers (1×1×3m) at Harvard University. Temperature was a constant 25°C (day) and 20°C (night) (± 2°C), for a 16-h day, 8-h night, with a 2-h linear step up and step down. Natural light was supplemented by halogen bulbs when ambient light dropped below 600 μmol m^{-2} s^{-1}. Atmospheric CO_2 concentration was monitored by a Horiba infra-red gas analyzer and maintained at either 350 ppm (± 50 ppm) or 700 ppm (± 50 ppm). Chambers were blocked by adjacent pairs and plants were rotated between chambers within blocks once every 3 weeks.

Initially, eight glass chambers were used. During the 10th week of growth, four more chambers (two more blocks) were added. Because obtaining enough plant foliage for the larvae was a concern, a non-random method was used to expand into the additional chambers. Tall plants were left in the original eight chambers and spaced at half the previous density. Short plants were moved into the additional four chambers at the same density as previously. Two weeks later, when plants in the additional blocks began competing for light, half of the plants in each chamber were randomly removed and placed in a pair of CO_2-controlled glasshouse units for use in preference tests.

Larval rearing

Larvae were reared in cages in the same chambers and conditions as the plants and accordingly were blocked in the same way. Cages were constructed from nylon mesh sleeves internally supported by two PVC rings (20.3 cm diameter) 40 cm apart. One end of the sleeve was tied shut with fishing twine and hung from a suction cup on the chamber's glass ceiling. The other end of the sleeve was slipped over the top of one plant and attached securely to the 10.2 cm pot by a rubber band. A 13 cm gap was left along the seam of the nylon sleeve to permit watering and access to the larvae. Eight cages were suspended inside each of the 12 chambers, four cages containing yellow birch and four with gray birch.

Gypsy moth eggs in diapause were obtained from the USDA Animal and Plant Methods Center at Otis Air Force Base, Massachusetts, and were the 38th generation of captive stock reared on larval medium (Bell et al. 1981). Larvae emerged roughly simultaneously from eggs that had been removed from a refrigerator and left overnight at room temperature. On 29 September, we placed one newly emerged larvae into each of 96 cages. On six occasions during the next 13 days, a survey was made of larval presence or absence. Missing or dead larvae were replaced by two more larvae, which had been maintained in petri dishes with detached leaves of the same experimental plants of that species. If both larvae then survived, one was removed. After 5 days, 69 cages had established larvae. By the 13th day, 90 of the cages had established larvae and no more larvae were added to the cages. The total number of replacements inserted per treatment were as follows: gray birch, ambient (14), enriched (23); yellow birch, ambient (24), enriched (41). The total number of cages per treatment where establishment problems continued past the third day were as follows: gray birch, ambient (2), enriched (5); yellow birch, ambient (9), enriched (11). When larvae had consumed over 50% (a visual estimate) of the leaf area of a plant, it was replaced and not reused. We replaced plants so that larvae would always have plenty to eat and a range of leaf ages to choose from.

Data collection

Once every 6–10 days, larvae were removed from their host plants by means of a paint brush, put into containers, weighed, and returned to their plants. Larval mortality was recorded from day 13 (when the larval replacements were stopped) until the last larva pupated on day 60. As soon as a larva was observed to have pupated, it was removed from the cage, weighed, and stored. Adults were sexed after eclosion.

A sub-sample of plants was harvested on 10 October, the 13th day of larval rearing. Only plants from the eight chambers in the original four blocks were harvested. Three plants of each species were harvested from each chamber. We sequentially removed from the apex all main stem leaves greater than 1 cm in length, weighed them, and determined leaf area. Branch leaves were collected and measured together. Leaves were dried at 80°C for 4 days, then reweighed to obtain dry mass. Leaves were then ground in a Cyclotec Sample Mill (Tecator, Höganäs, Sweden). We measured the concentration of leaf nitrogen per dry weight using a Kjeldahl Autoanalyzer (Technicon, Tarrytown, New York), which we calibrated against standards of known nitrogen concentration. Three leaves were selected from the top, middle, and bottom of each plant so as to cover the range of possible values within a plant. A second subsample of plants was selected on 10 December for analysis of leaf sucrose, hexose, starch, and condensed tannin concentration. These plants were reared identically to those fed to the larvae and consisted of seven yellow birch from each of the two CO_2 levels, five gray birch from 350 ppm CO_2 , and four gray birch from 700 ppm CO_2. On each plant we removed the third, sixth, and tenth leaf down from the plant apex, flash-froze them in liquid nitrogen, and then freeze-dried the plant tissue. For carbohydrate analysis, we used the method of M.M. Schoeneberger, K. Ludovici, and P. Faulkner (unpublished work). This enzymatic assay allowed for separate determinations of hexoses (in glucose equivalents), sucrose, and starch. For condensed tannin analysis, we exhaustively extracted leaf tissue in 70% acetone (with 0.1 *M* ascorbic acid as antioxidant). Condensed tannin content of extracts was then determined by the acid butanol method of Porter et al. (1986), using condensed tannin purified from aspen leaves as a reference standard. Because tannins of different plant species may react differently in the acid butanol assay, we caution that between-species differences must be interpreted carefully.

Preference tests

Preference tests were conducted simultaneously with the larval rearing. Third-instar larvae, previously reared on artificial medium (Bell et al. 1981), were offered the choice between two leaves in a petri dish (15 cm diameter) lined with wet filter paper. The preference tests were conducted using plants from the two CO_2 controlled zones that were grown as described in the plant growth section. Only the fourth and fifth mainstem leaves from the plant apex were used. The four preference test treatments were as follows: (1) yellow birch (Yb) vs. gray birch (Gb) at 350 ppm; (2) Yb vs. Gb at 700 ppm; (3) Yb 350 ppm vs. 700 ppm; (4) Gb 350 ppm vs. 700 ppm. Each treatment contained between 38 and 50 replicates. To control for leaf shrinkage, we put leaves, but no larvae, into ten of the petri dishes in each treatment. Leaves were paired by size and measured for initial leaf area using a Licor 1700 leaf area meter. Leaves were then stapled to the moistened filter paper and a lid was placed on the petri dish. When all of the petri dishes were prepared, one larva was placed in each dish. After 24 h, the filter paper of each petri dish was re-moistened with water. After 45 h, larvae were removed from the petri dishes and leaf areas remeasured.

Statistical analysis

Mortality data were analyzed by the chi-square test. Growth rate was estimated as the pupal mass divided by the larval stage duration. Relative growth rate was calculated as the slope of the function of log(larval mass) versus larval age. We used a split-plot ANOVA to test for the effects of block, birch species, CO_2 treatment, and larval sex on four measures of larval performance. Our whole plot factor was CO_2 level and the whole plot error was the CO_2 × Block interaction. Our split-plot factors, plant species and larval sex, were completely randomized within CO_2 level. The split-plot error was the residual (Table 1). We also used the same ANOVA design to test for the effect of block, birch species, and CO_2 treatment on leaf nitrogen and water content. Statistical analyses of leaf tannin, hexose, sucrose, and starch content were performed differently because plants were sampled completely randomly within CO_2

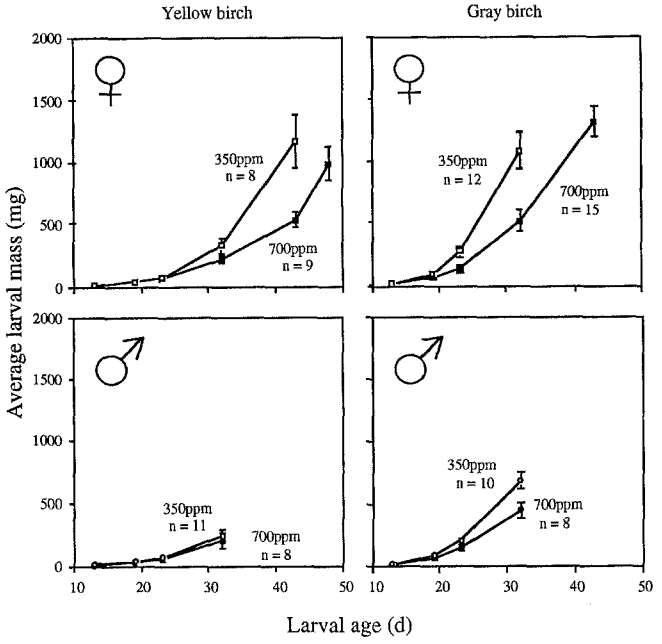

Fig. 2 Average mass of gypsy moth larvae on yellow and gray birch as a function of larval age. Results are shown separately for male and female larvae. Larvae reared in the elevated CO_2 atmosphere are indicated by the *filled points*. Larval masses are repeated measurements on the same larva, recorded six times over the course of the experiment. In order to not bias treatment averages toward slowly developing larvae, data are not shown for treatments after two or more larvae had pupated

treatment. The blocks from which the sampled plants came were not recorded. We used a two-way ANOVA to test the effect of CO_2 treatment and birch species on these leaf traits.

In preference tests, larvae ate an amount (A_1, A_2) of the two leaves, which each had an initial surface area (S_1, S_2). Larval preference for leaf 1 (P_1) was calculated as $A_1/(A_1+A_2) - S_1/(S_1+S_2)$. In words, preference for leaf 1 was calculated as the proportion of herbivory on leaf 1 that was in excess of leaf 1's proportion of available surface area. One preference value was calculated for each replicate dish. These values were normally distributed and were analyzed by a *t*-test (Snedecor and Cochran 1989) to assess whether the population mean differed significantly from zero with a type I error level of 5%.

Results

The performance of gypsy moth larvae reared *in situ* on yellow and gray birch was generally negatively affected by CO_2 treatment. Larvae grew more slowly on plants of both species in the 700 ppm than in the 350 ppm CO_2 treatment (Fig. 2). As a result, larvae achieved both a lower final larval mass and a lower pupal mass when reared on plants growing in an enriched CO_2 atmosphere (Fig. 3; Table 1) despite having fed for 3 days longer on average.

Female larvae were affected more than male larvae by growth on plants in an elevated CO_2 atmosphere (Fig. 3). Female gypsy moths on yellow birch experienced a greater decline in pupal mass in the elevated CO_2 treatment than did males, as indicated by the significant (P<0.0361) CO_2 treatment by species by larval sex interaction term (Table 1).

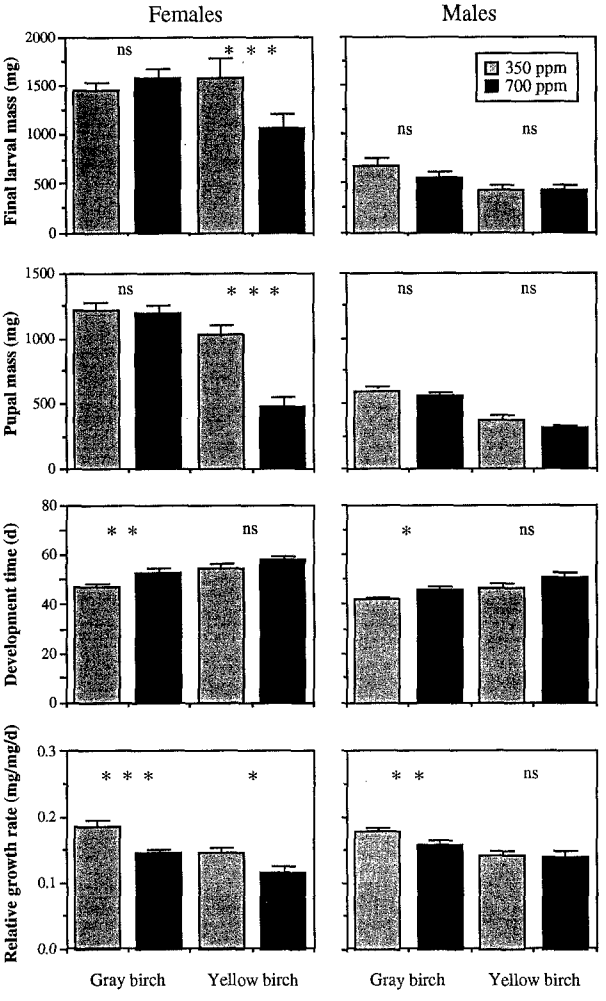

Fig. 3 Final larval mass, pupal mass, larval development time, and relative growth rate of larvae grown on gray or yellow birch grown in an ambient or elevated CO_2 atmosphere. Values are shown for each treatment ($\bar{x}$ ± 1SE). * P<0.05, ** P<0.01, *** P<0.001

Larval responses to the elevated CO_2 treatment were dependent upon the host plant species (Fig. 3). On yellow birch, female pupae were 38% smaller in the elevated CO_2 treatment (mean = 1024.6 mg, SE = 76.13, n = 7) than in the ambient CO_2 treatment (629.9, 57.27, 5). Pupae on gray birch experienced no decrease in pupal mass on plants in the enriched CO_2 atmosphere. This interaction between CO_2 treatment and host plant species was significant (Table 1; P<0.0198). Larval mortality on yellow birch was higher in the elevated CO_2 atmosphere (7/24) than in the ambient CO_2 atmosphere (5/24). On gray birch, larval mortality was higher in the ambient CO_2 atmosphere (2/24) than in the elevated CO_2 atmosphere (0/24). The greater larval mortality on yellow birch than gray birch was significant (P<0.01). Larval mortality was not affected by CO_2 atmosphere, nor by the CO_2 × species interaction.

Yellow and gray birch leaves differed in their response to an elevated CO_2 atmosphere (Fig. 4). Foliar nitrogen content of both yellow birch and gray birch was

Table 1 Summary of split-plot ANOVA results for gypsy moth final larval mass, pupal mass, developmental time, and relative growth rate (RGR). Male and female larvae were reared on yellow or gray birch grown in an ambient or elevated CO_2 atmosphere. The mean squares (MS) and P values from each ANOVA are shown below. P values greater than α=0.05 are listed as nonsignificant (ns)

Source	*df*	Final larval mass		Pupal mass		Development time		RGR	
		MS	*P*	MS	*P*	MS	*P*	MS	*P*
Block	5	71833	ns	23794	ns	16.1	ns	0.002	0.0002
CO_2	1	311335	0.0343	346924	0.0099	273	0.0012	0.01	0.0416
Block×CO_2	5	37346	ns	21267	ns	6.36	ns	0.0012	0.0047
Species	1	777898	0.0009	1613012	0.0001	530	0.0001	0.017	0.0001
CO_2×Species	1	272914	0.0421	141955	0.0198	10.0	ns	0.001	ns
Sex	1	12152936	0.0001	4312206	0.0001	670	0.0001	0.00005	ns
CO_2×Sex	1	7668	ns	52604	ns	2.50	ns	0.001	ns
Species×Sex	1	259	ns	71335	ns	27.2	ns	0.001	ns
CO_2×Species×Sex	1	502549	0.0065	113897	0.0361	0.61	ns	0.00028	ns
Residual	60	63402		24785		15.7		0.00034	

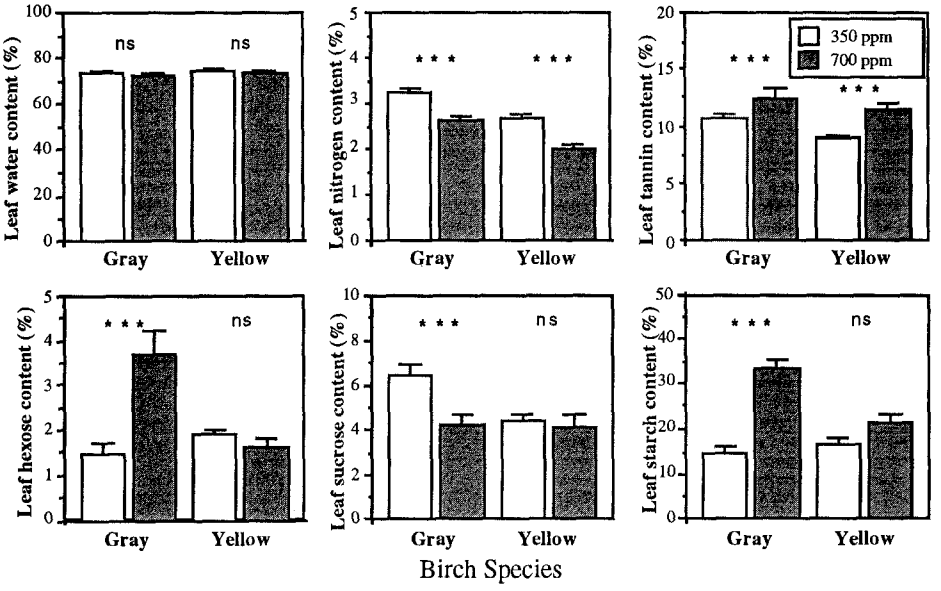

Fig. 4 Changes in leaf percent water, nitrogen, condensed tannin, hexose, sucrose, and starch for yellow and gray birch grown in an ambient or elevated CO_2 atmosphere. The CO_2 treatment had a significant effect on leaf concentration of nitrogen, condensed tannins, hexose and starch. There was a significant CO_2 × plant species interaction for hexose, sucrose, and starch. Values are shown for each treatment ($\bar{x}$ ± 1SE). * P<0.05, ** P<0.01, *** P<0.001

significantly lower in plants grown at 700 ppm CO_2 than at 350 ppm CO_2 (P<0.025). The nitrogen dilution of yellow birch in the elevated CO_2 atmosphere (from 2.68% to 1.99%) represented a decrease of 26% of the levels under ambient CO_2 while the gray birch dilution (from 3.23% to 2.63%) represented a decrease of 18% of the ambient CO_2-grown foliage. Foliar water content was not affected by CO_2 treatment in either yellow or gray birch. The concentration of condensed tannins was higher in the elevated CO_2-grown plants of both yellow birch and gray birch. The magnitude of increase of condensed tannins was higher in yellow birch (28%) than in gray birch (15%). However, the interaction between CO_2 treatment and plant species was not significant for either leaf nitrogen or condensed tannin content. In gray birch, leaf concentration of hexose was higher (P<0.001) in the elevated CO_2 treatment, while concentration of sucrose was lower (P<0.01). However, in yellow birch leaves the concentration of these sugars was not affected by CO_2 treatment. Leaf starch concentration was higher in the elevated CO_2 atmosphere in both gray and yellow birch (P<0.001), however the magnitude of increase in the 700 ppm CO_2 treatment was much greater in gray birch (127%) than in yellow birch (28%).

When pooled across experimental treatments, larval growth rate was positively correlated with leaf nitrogen content (Fig. 5). Larval relative growth rate and pupal mass were also positively correlated with leaf nitrogen content for both females (P<0.0007, P<0.0084) and males (P<0.0034, P<0.0001) respectively. Larval development time was negatively correlated with leaf nitrogen content for both females (P<0.0202) and males (P<0.0001). Leaf water content was not correlated with any of the larval performance parameters measured.

In preference tests, larvae preferred ambient CO_2-grown leaves of yellow birch to those grown in elevated CO_2 (P<0.05). The larvae in this preference test consumed an average of 10.97% more area of the ambient CO_2-grown leaves than would be expected if larval feeding was random within each petri dish (mean preference = 0.1097, SE = 0.05, n = 33). When offered the choice of gray birch leaves from the two CO_2 levels, lar-

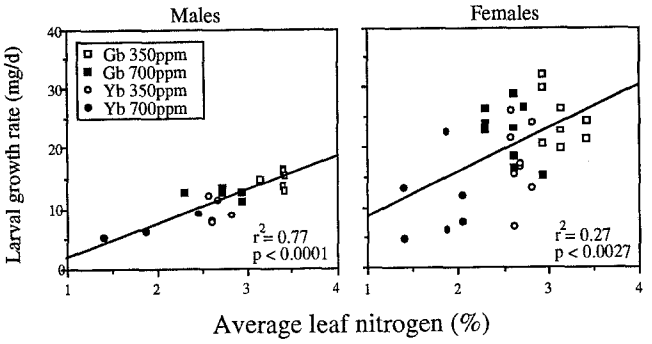

Fig. 5 Larval growth rate as a function of average leaf nitrogen content for a subset of the larvae. Each data *point* represents the growth rate of a single larva and the average leaf nitrogen content of host plants in its growth chamber. The chamber leaf nitrogen values are averages of three leaves (one young, medium and old leaf) from three plants harvested on 10 October (Gb gray birch, Yb yellow birch)

vae exhibited no significant preference for the ambient CO_2-grown leaves (mean preference = -0.0311, SE = 0.108, n = 35). Larvae had an insignificant preference for yellow birch leaves over gray birch leaves at both CO_2 levels.

Discussion

In an enriched CO_2 atmosphere, declines in gypsy moth larval performance were substantial and depended strongly upon the identity of the host species. On yellow birch, female larvae had a 50% decline in pupal mass when reared on plants in a twice ambient (700 ppm) CO_2 atmosphere. On gray birch, female gypsy moths achieved similar pupal masses on plants grown in ambient and elevated CO_2 atmospheres. As female pupal mass is highly correlated with fecundity in gypsy moths (Hough and Pimentel 1978; Miller et al. 1991), fecundity of gypsy moth larvae on yellow birch may also decline in an enriched CO_2 atmosphere, while gypsy moth fecundity on gray birch may not change.

On gray birch, larval mortality was actually higher on plants grown in the ambient CO_2 atmosphere (8%) than on plants grown in the elevated CO_2 atmosphere (0%). On yellow birch, larval mortality was higher in the elevated (29%) than in the ambient CO_2 atmosphere (21%). These data on female fecundity and survivorship both suggest that gypsy moth populations will decline in an enriched CO_2 atmosphere on yellow birch, but not gray birch.

Foliar concentration of nitrogen and condensed tannin were the most likely of the leaf traits measured to be responsible for the differential response of larvae on the two host plant species to growth in the elevated CO_2 atmosphere. Yellow birch exhibited a 24% decline in leaf nitrogen content in the elevated CO_2 atmosphere as compared to only an 18% decline in gray birch leaves. Similarly, condensed tannin concentrations of yellow birch leaves in the elevated CO_2 atmosphere were 28% higher than yellow birch in the ambient CO_2 atmosphere, while for gray birch there was only a 15% difference between CO_2 treatments. When we plotted the growth rate of larvae against the leaf nitrogen content of their diet, regardless of the host plant species or CO_2 treatment, the relationship was positive and highly significant. Fajer (1989) found a similar positive correlation between leaf nitrogen content and larval performance of the butterfly, *Junonia coenia*, fed leaves of its host plant, *Plantago lanceolata*, from ambient and elevated CO_2 atmospheres. Interestingly, Lindroth et al. (1993) found that although both aspen (*Populus tremuloides*) and sugar maple (*Acer sacharrum*) exhibit nitrogen dilution in an elevated CO_2 atmosphere, gypsy moth performance only declined on aspen but not sugar maple. In the only other experiment with gypsy moths, Roth and Lindroth (1994) recorded a leaf nitrogen dilution of 20% in white birch (*Betula papyrifera*) but not white pine (*Pinus strobus*). Gypsy moth larvae experienced a greater effect of the elevated CO_2 atmosphere on white birch than on white pine. Leaf nitrogen content is widely recognized as a limiting resource for many insect herbivores (reviewed by Mattson 1980; Scriber and Slansky 1981) and has been previously implicated in larval declines on plants grown in an elevated CO_2 atmosphere (Lincoln et al. 1984, 1986; Osbrink et al. 1987; Lincoln and Couvet 1989; Fajer 1989; Fajer et al. 1989, 1991; Johnson and Lincoln 1990, 1991; Lindroth et al. 1993, 1995; Roth and Lindroth 1994). Whether condensed tannins have an effect on gypsy moth performance is unclear. When larvae were reared on hydrolyzable tannin diets, there was a positive impact on early instar gypsy moth larvae followed by a negative impact on late instar larvae, especially for females (Bourchier and Nealis 1993). Although Roth and Lindroth (1994) found that gypsy moth growth declined on paper birch in elevated CO_2 atmosphere while condensed tannins increased, other studies have reported mixed results. Lindroth et al. (1993) found that although sugar maple increased foliar condensed tannin concentration when grown in an enriched CO_2 atmosphere, gypsy moth larval growth rate, consumption, and final mass were not significantly different between CO_2 treatments. Similarly, Hemming and Lindroth (1995) reported no correlation between leaf condensed tannin content of aspen and gypsy moth performance. Thus, in our experiment, it is unclear whether an increase in condensed tannins in yellow birch reduced larval performance. There may well be other plant traits that we did not measure (e.g., toughness, other secondary compounds) that could actually be responsible for the greater decline in gypsy moth performance on yellow birch than gray birch in the elevated CO_2 atmosphere. Leaf water content, which is also often correlated with insect herbivore performance (Scriber and Slansky 1981), did not change in leaves of either species in the enriched CO_2 atmosphere and was not correlated with gypsy moth performance in this study.

In preference tests, larvae choosing between ambient and elevated CO_2-grown leaves of the same species made choices that would result in the highest pupal mass. Lar-

vae on yellow birch preferred ambient CO_2-grown over elevated CO_2-grown leaves, while larvae on gray birch showed no preference. The preference test result suggests that in elevated CO_2 the foliage quality of yellow birch declined while that of gray birch did not. In preference tests where larvae were offered the choice between yellow and gray birch leaves, it was expected that larvae would prefer gray birch, however, no preference was found. This result suggests that field observations of larval preference for gray birch over yellow birch (Mauffette et al. 1983) may be due to factors other than foliage quality, such as trunk texture (Smitley et al. 1993). It is also possible that the artificiality of our preference bioassay and morphological differences between gray and yellow birch leaves may have prevented us from truly testing differences in leaf nutritional quality. Arnone et al. (1995) also found no effect of an elevated CO_2 atmosphere on insect foraging behavior. However, in that experiment there was also no CO_2 effect on the leaf nutritional quality of any of the seven plant species tested, likely because these plants were grown at low nutrient levels (Arnone et al. 1995).

An enriched CO_2 atmosphere had a greater effect on the performance of female larvae than male larvae. In the elevated CO_2 atmosphere, female larvae experienced declines in growth rate and pupal mass whereas male larvae did not. Even in measures of performance such as larval development time and relative growth rate, where male larvae exhibited differences in the elevated CO_2 atmosphere, the relationships were less strong than the same effects on female larvae. Interestingly, Fajer et al. (1989) found the reverse to be true for the buckeye butterfly, *Junonia coenia*. Male larvae exhibited reduced growth and longer developmental time in the elevated CO_2 atmosphere while female larvae did not. Male gypsy moths contain lower concentrations of nitrogen and require a lower intake of this resource than do females (Montgomery 1982). Therefore, plant species with high nitrogen dilution in an enriched CO_2 atmosphere would be expected to be relatively worse for gypsy moth females than for males, as was found in this study. It is certainly possible that the respective nutritional requirements of male and female buckeye butterfly larvae differ from the gypsy moth.

Insect herbivores may have an important influence on the competitive interactions among plant species (Strong et al. 1984). An interesting further step in this research would be to test whether the effects of an insect herbivore on the competitive interactions between two plant species changes in an elevated CO_2 atmosphere. Any such indirect effects of an elevated CO_2 atmosphere on plant-plant interactions would be of interest because these processes are important regulators of productivity and diversity in forest ecosystems (Eamus and Jarvis 1989; Bazzaz 1990; Bazzaz and McConnaughay 1992).

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