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Gypsy moth feeding in the canopy of a CO_2-enriched mature forest

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Abstract

Rising atmospheric carbon dioxide (CO_2) concentration is expected to change plant tissue quality with important implications for plant–insect interactions. Taking advantage of canopy access by a crane and long-term CO_2 enrichment (530 μ mol mol^{-1}) of a natural old-growth forest (web-free air carbon dioxide enrichment), we studied the responses of a generalist insect herbivore feeding in the canopy of tall trees. We found that relative growth rates (RGR) of gypsy moth (*Lymantria dispar*) were reduced by 30% in larvae fed on high CO_2-exposed *Quercus petraea*, but increased by 29% when fed on high CO_2-grown *Carpinus betulus* compared with control trees at ambient CO_2 (370 μ mol mol^{-1}). In *Fagus sylvatica*, there was a nonsignificant trend for reduced RGR under elevated CO_2. Tree species-specific changes in starch to nitrogen ratio, water, and the concentrations of proteins, condensed and hydrolyzable tannins in response to elevated CO_2 were identified to correlate with altered RGR of gypsy moth larvae. Our data suggest that rising atmospheric CO_2 will have strong species-specific effects on leaf chemical composition of canopy trees in natural forests leading to contrasting responses of herbivores such as those reported here. A future change in host tree preference seems likely with far-ranging consequences for forest community dynamics.

Keywords: elevated CO_2, forest ecosystems, global change, herbivory, leaf chemistry, *Lymantria dispar*, plant–herbivore interactions, Swiss Canopy Crane

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Introduction

Forest canopies are structurally and functionally among the most complex and species-rich terrestrial habitats (Ozanne *et al*., 2003). More than 25% of all terrestrial herbivorous invertebrates are supposedly unique to forest canopies (Basset *et al*., 2003). Human activities highly threaten these habitats, most obviously through massive deforestation and more subtly through changes in atmospheric composition. The rising carbon dioxide (CO_2) concentration in the atmosphere for example is expected to change forest canopy composition because of altered regeneration success of tree species (Hättenschwiler & Körner, 2000) and accelerated successional dynamics as a result of strong liana growth stimulation (Granados & Körner, 2002). CO_2-induced shifts in forest community structure have major implications for insect herbivores; specialists among them may lose their food source and generalists may change their host preferences (Lindroth, 1996).

In addition to changes in host tree abundance, herbivores will be confronted with substantially altered chemical composition of their diet with important consequences for insect performance, growth, and development (see reviews by Watt *et al*., 1995; Lindroth, 1996; Bezemer & Jones, 1998; Coviella & Trumble, 1998; Whittaker, 1999; Hunter, 2001). Atmospheric CO_2 enrichment is regularly observed to cause increases in nonstructural carbohydrates (NSCs) (Körner & Arnone, 1992; Körner & Miglietta, 1994; Poorter *et al*., 1997), decreases in nitrogen (N) (Poorter *et al*., 1997; Cotrufo *et al*., 1998), and less consistently increases in secondary metabolites (Lavola & Julkunen-Tiitto, 1994; Kinney *et al*., 1997; Peñuelas & Estiarte, 1998). Leaf-chewing insects often respond with slower growth and larval

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development to these changes in leaf chemistry (Lincoln *et al.*, 1986; Roth & Lindroth, 1995; Hättenschwiler & Schafellner, 1999; Veteli *et al.*, 2002), but may compensate for the lower food quality with increasing feeding rates (Lindroth *et al.*, 1993; Williams *et al.*, 1994; Docherty *et al.*, 1996).

Former experiments including more than one tree species revealed a wide variation in CO_2-mediated responses in leaf chemistry among species and of generalist herbivores feeding leaves from those trees (Lindroth *et al.*, 1993; Traw *et al.*, 1996; Kinney *et al.*, 1997; Agrell *et al.*, 2000). These observations suggest shifts in host tree preferences by generalist herbivores with rising atmospheric CO_2. Altered insect growth, food consumption, and food preference are all expected to change plant–herbivore interactions fundamentally in forest canopies. However, it is highly uncertain whether the results obtained with young tree seedlings often planted under artificial growth chamber conditions can be extrapolated to the natural situation of a forest canopy composed of mature trees (Hunter, 2001). Recently, Goverde *et al.* (2002) showed that the clear CO_2 effects on larval development of a satyrid butterfly obtained in a greenhouse study disappeared completely under field conditions. The difficulty of extrapolation of the current experimental evidence to natural conditions is seriously limiting predictions of plant–herbivore interactions in a changing environment.

The Swiss Canopy Crane (SCC) project and the new CO_2 enrichment technology web-free air carbon dioxide enrichment (FACE) (Pepin & Körner, 2002) made it possible for the first time to study insect herbivore performance in the canopy of a 35 m tall old-growth forest in the second year of elevated-CO_2 exposure. Larvae of the generalist folivore gypsy moth (*Lymantria dispar* L.) as one of the most serious insect pests of temperate forests were allowed to feed on top-canopy branches of three deciduous tree species (oak, beech, hornbeam) for 2 weeks. Gypsy moth mass outbreaks have strong ecological and economical impacts on hardwood forests of both its European and Asian native range, and cause serious problems as an exotic pest species in North America.

Materials and methods

Study site and experimental design

In 1999, the SCC was installed within a species-rich mixed forest near Hofstetten, about 15 km SW of Basel (47°28′N, 7°30′E) at an elevation of 550 m a.s.l. (see Körner, 2000 for a detailed description of the SCC site). The site is characterized by a typical humid temperate zone climate with a long-term mean annual precipitation of 885 mm and daily mean temperatures of −2.0 °C in January and +18 °C in July, respectively. The growing season length of deciduous trees is approximately 165 days, starting at the end of April and ending in early October.

The forest is about 120 years old, with tree heights between 30 and 36 m, a tree density of 415 individuals ha^{-1} (tree diameter ≥ 0.1 m), a stem basal area of 46 $m^2 ha^{-1}$, and a leaf area index of the tree canopy of 5 $m^2 m^{-2}$. The forest area accessible by the crane (2830 m^2) is dominated by *Fagus sylvatica* L. and *Quercus petraea* L., and the co-occurring species *Carpinus betulus* L., *Q. robur* L., *Tilia platyphyllos* Scop., *Acer campestre* L., and *Prunus avium* L.

CO_2 enrichment of canopy trees by web-FACE started in autumn 2000 (Pepin & Körner, 2002). Basically, pure CO_2 is released under pressure from thin plastic tubes (0.5 cm diameter) with fine laser-punched holes (Drip Store Inc., Escondido, CA, USA). Overall, a network of approximately 10 km of tubing was woven into the crowns of 14 broad-leaved trees in one part of the crane area, leaving most of the remaining 50 trees within the crane radius as non-enriched controls. CO_2 concentrations in the CO_2-enriched canopy were around 530 μmol mol^{-1} (see Pepin & Körner, 2002).

Plant and animal material

We selected two individual canopy trees per CO_2 treatment (ambient, elevated) from each of the three hardwood tree species *Q. petraea* (sessile oak), *F. sylvatica* (European beech), and *C. betulus* (hornbeam) (12 trees in total). Within the crown of each tree, one large branch at each cardinal direction was determined for larval feeding (48 branches in total). Five, third instar *L. dispar* larvae were placed on each branch and enclosed with a 0.6 m long and 0.3 m wide nylon mesh bag (containing approximately 80–150 leaves per branch) on 18 May 2002 and stayed there for 15 days. Three additional bags without insect larvae were used to assess potential bag effects on microclimatic conditions. During windy days, there were no measurable differences in temperature or humidity within and outside bags. However, on the rare days with some calm periods, air temperature and humidity could be somewhat higher (maximum differences of 0.6 °C and 3% RH) within bags than outside bags during periods without any wind. Since there was typically some wind at our experimental site, we consider the bag effect on microclimate of minor importance for larval development.

Gypsy moth egg masses were collected in the field from a natural mixed oak forest in Burgenland

(Austria) in April 2002 and brought to the lab of the Institute of Forest Entomology, BOKU (Vienna). After hatching, larvae from five egg masses were reared at 20 °C and a 16L : 8D photoperiod and fed with a high wheat germ diet (Bell *et al.*, 1981). Approximately, 1500 second instar larvae were then transferred to Basel. The experiment started when the bulk of larvae molted into the third instar. A total number of 240 third instar larvae were selected and kept without food for 9 h to empty their guts before weighing. Larvae were weighed individually, ranging from 15.6 to 26.5 mg of fresh mass (mean, 20.4 mg) and randomly assigned into groups of five to one of the 48 branches. From the pool of unused larvae, 20 individuals were taken to determine the fresh to dry mass ratio.

Data collection and analysis

Larvae were allowed to feed in the canopy for 2 weeks before they were removed on 2 June 2002 as early fourth instars. Larvae were again kept without food for 9 h and then weighed. Immediately after weighing, they were flash-frozen in liquid air, freeze–dried, and reweighed to determine larval dry mass. Relative growth rates (RGR) were calculated following Waldbauer (1968). The mean across the five individuals per branch was used as the sample unit ($n = 48$).

Leaf material was collected at start (22 May) and at the end of the experiment (2 June) from branches immediately adjacent to the bagged branches larvae fed on. Six individual leaves without any signs of herbivory or diseases were taken from each branch between 17 and 19 h, stored on ice and brought to the lab. In the lab, leaf area was determined with an area meter (LI-3050A, Licor Inc., Lincoln, NB, USA) to calculate specific leaf area (SLA). The fresh leaves were weighed, flash-frozen in liquid air, and freeze-dried. After determining leaf dry mass, leaves were ground and stored at −30 °C for chemical analyses. Leaf material was analyzed for starch, sugars (sucrose, glucose and fructose), N, carbon, total phenolics, condensed tannins, hydrolyzable tannins, fiber, and protein ($n = 48$ branches). Starch and sugar were analyzed using an enzymatic starch digestion and a spectrophotometric glucose test after invertase and isomerase addition (Körner & Miglietta, 1994). Total N and carbon were measured using a CHN-analyzer (Model 900, LECO Instruments, St Joseph, MI, USA). Leaf extracts with boiling 50% aqueous methanol were the basis of total phenolic and condensed tannin analyses. The Folin–Denis technique was used to assay for total phenolics (Swain & Hillis, 1959; Waterman & Mole, 1994), with tannic acid (Sigma-Aldrich, St. Louis, MO, USA) used as a standard. Total phenolics are expressed as percent dry weight tannic acid equivalents. Condensed tannins (proanthocyanidins) were measured following the procedure of Porter *et al.* (1986). Purified quebracho tannin served as a standard. Results are given in percent quebracho tannin equivalents. Hydrolyzable tannins (gallotannins) were determined by the Rhodanine assay according to Inoue & Hagerman (1988). Commercially available gallic acid (Sigma) was used to construct a calibration curve. Values are expressed as % gallic acid. Leaf protein content was determined by the Lowry *et al.* (1951) method modified according to Peterson (1977) with bovine serum albumin (BSA Fraction V, Biotrade, Vienna, Austria) as a standard. Proteins were determined by comparison with a standard curve of BSA. Values are given in % protein of total leaf dry mass. For determining the fiber content of the plant material, 250 mg of leaf powder was extracted with cold acetone, followed by hot acid and alkaline digestion (Tecator Fibertec M, 1020 Hot Extractor, 1021 Cold Extractor, 1022 Hot Plate, Foss, Silver Spring, MD, USA). The residue was dried, weighed, and ashed. After cooling, samples were reweighed and the difference before and after ashing was addressed as crude fiber.

Data were analyzed for each tree species separately according to a split-plot design with individual trees as a random factor (plot), CO_2 treatment as a fixed factor between plots, and exposition (cardinal direction) of the four branches within a tree as a fixed factor within plots. Accordingly, the CO_2 effect was tested against the mean square (MS) of 'trees within CO_2' as the error term, and the effect of exposition, as well as the interaction between CO_2 and exposition were tested against the MS of 'exposition by trees within CO_2' as the error term. However, 'exposition' and the interaction between CO_2 and exposition remained insignificant in all of the species (i.e. there were no significant differences among cardinal directions). Moreover, variances among branches within trees were much greater than the variances between trees of a given species and CO_2 treatment, wherefore individual branches were treated as independent sample units for further analyses for comparisons among tree species (model I analysis of variance with the factors CO_2, tree species, and their interaction). Differences between individual tree species pairs were tested using Fisher's LSD *post hoc* tests. Correlations between larval growth of gypsy moth and leaf quality traits were tested with simple linear regression analyses. All data expressed in percent of leaf dry mass were arc sin square root transformed to homogenize variances and to meet the requirement of normal distribution. SYSTAT, version 5.2.1 (Systat Inc., Evanston, IL, USA) was used for statistical analyses.

Results

Growth of gypsy moth larvae

Larval mortality was very low. Out of the 240 individual caterpillars, we lost only nine during the 2-week experiment, which is less than 4%. At ambient CO_2, two larvae died on hornbeam, one on beech, and none on oak. At elevated CO_2, four larvae died on beech, two on oak, and none on hornbeam. Larval biomass increased on average by a factor of 3.8 over the entire feeding period, but there were clear differences among tree species and CO_2 treatments. Not surprisingly, at ambient CO_2 we observed the highest RGR on oak as the preferred host species of gypsy moth and the lowest on hornbeam (Fig. 1). At elevated CO_2, larval growth was affected distinctly depending on tree species ($P < 0.01$ for the species $\times$ CO_2 interaction). For oak, RGR was significantly reduced by 30% in larvae feeding on high CO_2-exposed trees compared with those feeding on ambient-CO_2 trees (Fig. 1). Similarly, larvae feeding on beech showed a nonsignificant trend of reduced growth at elevated CO_2. Unexpectedly, however, larval RGR increased by 29% on high CO_2-exposed hornbeam trees compared with those grown at ambient CO_2 (Fig. 1).

Leaf quality

Leaf quality differed greatly among tree species at both sampling dates and there was a highly significant species effect on all of the measured leaf traits (Table 1). Leaf chemical data for May, at the start of larval feeding, are summarized in Table 2. Briefly, oak leaves had the highest water and fiber content followed by beech and hornbeam, while hornbeam leaves had the highest SLA, N, sugar, and NSC concentrations. Interspecific differences in protein concentration did not fully match those measured for total N, with the highest protein concentration in oak, the lowest in hornbeam, and intermediate in beech (at ambient CO_2 in May, Table 2). The carbon concentration was similar in oak and beech, but significantly lower in hornbeam. Carbon-based secondary metabolites were analyzed with a common standard for all three species, and should be compared cautiously among species. Total phenolics and hydrolyzable tannins were highest in hornbeam, whereas condensed tannins were highest in beech. The absolute values, however, depend strongly on the standard chosen for the analysis and should only be compared in relative terms within species.

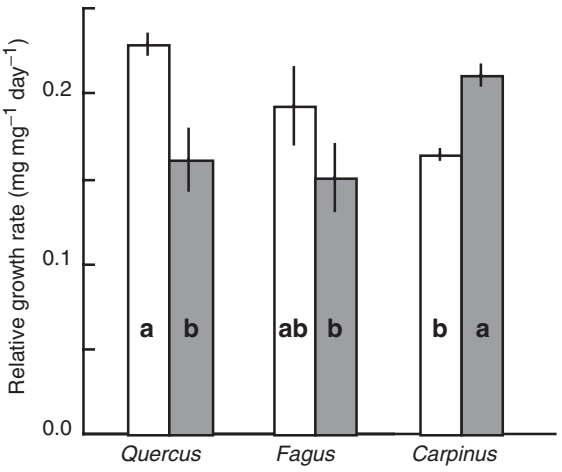

Fig. 1 Relative growth rate of *Lymantria dispar* larvae feeding on three different tree species within the canopy of an old-growth forest for 15 days. Open bars represent trees grown at ambient-CO_2 and dark bars represent trees exposed to an elevated-CO_2 atmosphere (mean $\pm$ SE of $n = 8$ branches). Different letters indicate significant differences between CO_2 treatments across species at $P < 0.05$ based on two-factorial ANOVA. Alternatively, *P*-values from tests of the CO_2 effect based on a split-plot design were $P = 0.074$ for *Quercus*, $P = 0.14$ for *Fagus*, and $P = 0.020$ for *Carpinus*.

Significant main effects of canopy CO_2 concentration were found on SLA, the concentrations of protein, sugars, and NSC in leaves collected in May (Tables 1 and 2). However, most CO_2 effects differed significantly among species. In oak, leaf water and SLA were reduced at elevated CO_2, whereas water content was higher and SLA not significantly different in beech. In hornbeam, water content and SLA did not significantly change at elevated CO_2. The total amount of carbon remained unchanged by elevated CO_2 in oak and hornbeam, and decreased slightly yet significantly in beech. Starch responded strongly, but in a species-specific way to CO_2 (Table 1). In oak and beech leaves, starch concentrations increased by 42% and 21%, respectively, but decreased in hornbeam (−32%) at elevated CO_2. While there was an increase in NSC in the order oak < beech < hornbeam in leaves from control trees, the species-specific CO_2 responses in starch equalized these differences, resulting in similar leaf–NSC concentrations among species in the CO_2-enriched atmosphere (Table 2, Fig. 2). Similarly, the relative differences in N concentration among species changed as a consequence of the species-specific responses to elevated CO_2 (Table 2, Fig. 2). For example, at elevated CO_2, N concentration tended to decrease in oak and to increase in hornbeam. This effect amplified the relative difference between oak and hornbeam from + 5% at ambient CO_2 to + 21% at elevated CO_2 in May (Table 2) and from −5% at ambient CO_2 to + 10% at elevated CO_2 in June (Fig. 2).

Elevated CO_2 distinctly affected polyphenol concentration among species. Condensed tannins showed a trend to increase in oak, remained unchanged in hornbeam, and decreased in beech (Table 2). In

Table 1 Effects of species, CO_2 treatment and their interaction on leaf quality traits measured on 22 May and on 2 June 2003

	May						June					
	Species		CO_2		Species × CO_2		Species		CO_2		Species × CO_2	
Leaf trait	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Water	77.5	146***	0.9	1.8	8.3	15.7***	55.0	36.5***	1.1	0.7	1.5	1.0
SLA	5384	40.4***	792.3	5.9*	802.7	6.0**	6712	34.6***	1027	5.3*	490.7	2.5
Fiber	76.0	99.8***	1.6	2.1	1.4	1.9	43.5	174***	0.4	1.4	1.5	6.0*
Carbon	5.1	22.1***	0.4	1.8	1.7	7.1**	3.5	11.0***	1.0	3.0	0.4	1.3
Nitrogen	3.5	14.6***	0.01	0.02	0.5	2.4	1.2	6.6**	0.06	0.4	0.4	2.5
Proteins	16.8	20.3***	69.2	83.9***	59.3	71.9***	9.7	5.7**	0.4	0.2	2.9	1.7
Sugars	87.6	62.5***	7.7	5.5*	2.4	1.7	43.9	33.9***	12.2	9.4**	1.3	1.0
Starch	72.8	19.4***	4.0	1.1	19.6	5.2**	38.8	6.6**	60.6	10.3**	20.8	3.5*
NSC	25.3	8.1**	15.6	5.0*	5.6	1.8	29.4	7.9**	67.3	18.1***	2.4	2.4
Phenolics	194.8	182***	0.9	0.9	0.4	0.3	161.4	109***	3.4	2.3	7.6	5.0**
CT	6687	436***	27.8	1.8	84.0	5.5**	5474	270***	5.8	0.3	74.4	3.7*
HT	1205	886***	2.7	2.0	11.5	8.4***	1029	387***	0.2	0.08	8.4	3.2*

*Significance level $P<0.05$, following the *F* ratio.
**Significance level $P<0.01$, following the *F* ratio.
***Significance level $P<0.001$ following the *F* ratio.
SLA, specific leaf area; NSC, nonstructural carbohydrates; phenolics, total phenolics; CT, condensed tannins; HT, hydrolyzable tannins; MS, mean square.

Table 2 Leaf quality of the studied tree species grown at ambient and elevated CO_2 on 22 May 2003 (mean ± SE)

	Quercus		*Fagus*		*Carpinus*	
Leaf trait	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Water content	65.2 ± 0.5	62.1 ± 0.3*	56.4 ± 0.3	58.2 ± 0.5*	57.0 ± 0.4	56.9 ± 0.6
SLA	149 ± 5	128 ± 3*	124 ± 4	131 ± 6	168 ± 2	158 ± 5
Fiber	22.1 ± 0.2	19.8 ± 0.1*	20.7 ± 0.5	19.0 ± 0.2	10.6 ± 0.5	11.3 ± 0.5
Carbon	44.7 ± 0.2	44.6 ± 0.3	45.4 ± 0.2	43.9 ± 0.4*	42.6 ± 0.3	43.3 ± 0.3
Nitrogen	2.33 ± 0.1	2.12 ± 0.1	1.98 ± 0.1	2.06 ± 0.1	2.44 ± 0.1	2.56 ± 0.1
Proteins	11.6 ± 0.5	5.1 ± 0.2*	10.0 ± 0.3	10.2 ± 0.3	9.3 ± 0.2	8.7 ± 0.3
Sugars	9.6 ± 0.6	11.0 ± 0.7	9.8 ± 0.1	9.7 ± 0.2	13.9 ± 0.3	15.3 ± 0.4*
Starch	3.8 ± 0.5	5.4 ± 0.6	5.8 ± 0.4	7.0 ± 0.4	4.0 ± 0.6	2.7 ± 0.3
NSC	13.4 ± 0.9	16.4 ± 1.3	15.6 ± 0.4	16.7 ± 0.5	17.9 ± 0.7	18.0 ± 0.5
Total Phenolics	10.2 ± 0.3	10.1 ± 0.4	11.6 ± 0.7	10.9 ± 0.2	18.0 ± 0.3	17.9 ± 0.5
Condensed tannins	1.2 ± 0.3	1.8 ± 0.2	53.4 ± 5.0	41.8 ± 0.2*	3.0 ± 0.4	3.1 ± 0.2
Hydrolyzable tannins	2.4 ± 0.3	2.8 ± 0.6	0.5 ± 0.1	0.6 ± 0.1	14.4 ± 0.5	11.6 ± 0.4*

Water content is given as % of total leaf fresh weight, SLA as $cm^2 g^{-1}$ leaf dry mass, and all other variables as % of total leaf dry mass.
*Significant differences at $P<0.05$ between ambient and elevated CO_2 within tree species.
SLA, specific leaf area; NSC, nonstructural carbohydrates.

contrast, hydrolyzable tannins were not significantly altered in oak and beech, but decreased in hornbeam. There were no significant CO_2 and no species × CO_2 effect on the concentration of total phenolics in May (Table 1). However, at the end of the larval feeding period in June, total phenolics were lower in beech and showed a nonsignificant trend to increase in oak and to decrease in hornbeam under elevated CO_2 (Table 1, Fig. 2).

Leaf chemistry changed over the 2-week observation period according to what is expected for maturing leaves. Water content, SLA, N, protein, and total

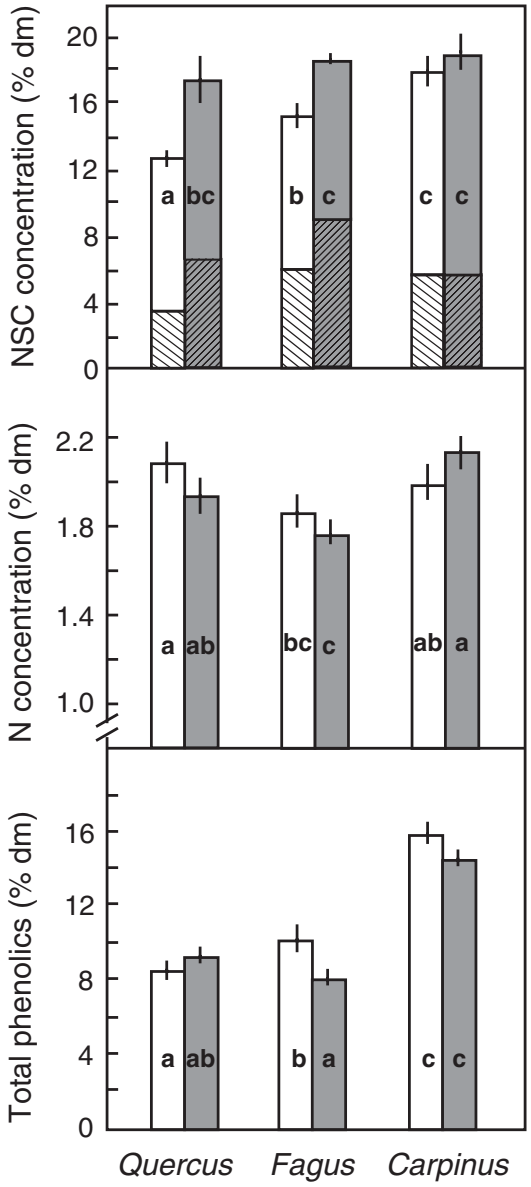

Fig. 2 Concentrations of nonstructural carbohydrates (hatched for starch with the upper nonhatched part for sugars), nitrogen, and total phenolics in top-canopy leaves from three different tree species collected on 2 June 2003. Open bars indicate trees at ambient-CO_2 and dark bars indicate those exposed to an elevated-CO_2 atmosphere (mean ± SE of $n = 8$ branches). Different letters indicate significant differences between CO_2 treatments across species at $P < 0.05$ based on two-factorial ANOVA.

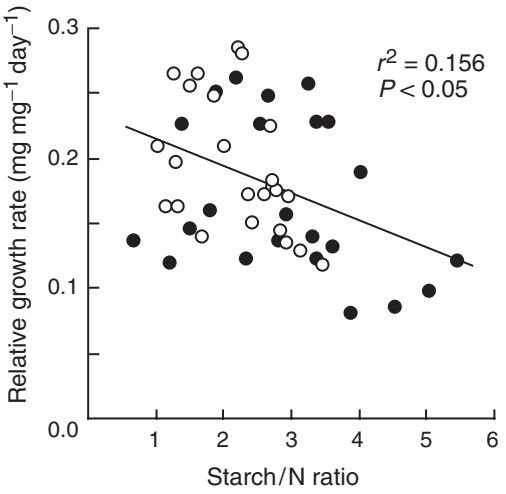

Fig. 3 Relative growth rate of *Lymantria dispar* larvae as a function of the starch/nitrogen (N) ratio of their food (2 June 2003) across all tree species and CO_2 treatments ($n = 48$ branches). Open circles represent trees grown at ambient CO_2 and black symbols represent trees grown at elevated CO_2. The line and corresponding r^2- and P-values are shown for simple linear regression.

phenolics generally decreased, while the concentrations of total carbon, fiber, starch, and condensed tannins increased from May to June. The concentrations of sugars and hydrolyzable tannins remained essentially the same or decreased slightly. The relative differences among species were largely unaffected by these changes in leaf quality over time. Also, the CO_2-induced changes in leaf chemistry remained roughly the same, except for protein in May (the effect disappeared in June) and starch in June (there was no effect in May) (Table 1). The species-specific differences in the CO_2 response increased for fiber and total phenolics, leading to a significant species × CO_2 interaction in June while a similar effect did not occur in May. In contrast, the initially significant species × CO_2 interactions on water, carbon, and protein concentrations were not significant in June (Table 1).

Relationship between leaf quality and larval growth

Across all tree species and both CO_2 treatments, the RGR of gypsy moth larvae showed a significant negative correlation with the starch to N ratio (Fig. 3). Leaf starch or N concentration alone were not significantly correlated with RGR ($r^2 < 0.06$). None of the other leaf quality traits were correlated with larval growth across tree species and CO_2 treatments.

Regressions tested for each species separately gave distinct results depending on the species. The only relatively consistent relationship between larval RGR and leaf quality traits among all three tree species was found for the starch to N ratio that correlated negatively with RGR in beech ($r^2 = 0.55$, $P < 0.01$) and hornbeam ($r^2 = 0.25$, $P < 0.05$) and as a nonsignificant trend also in oak ($r^2 = 0.19$, $P = 0.15$). Apart from this common

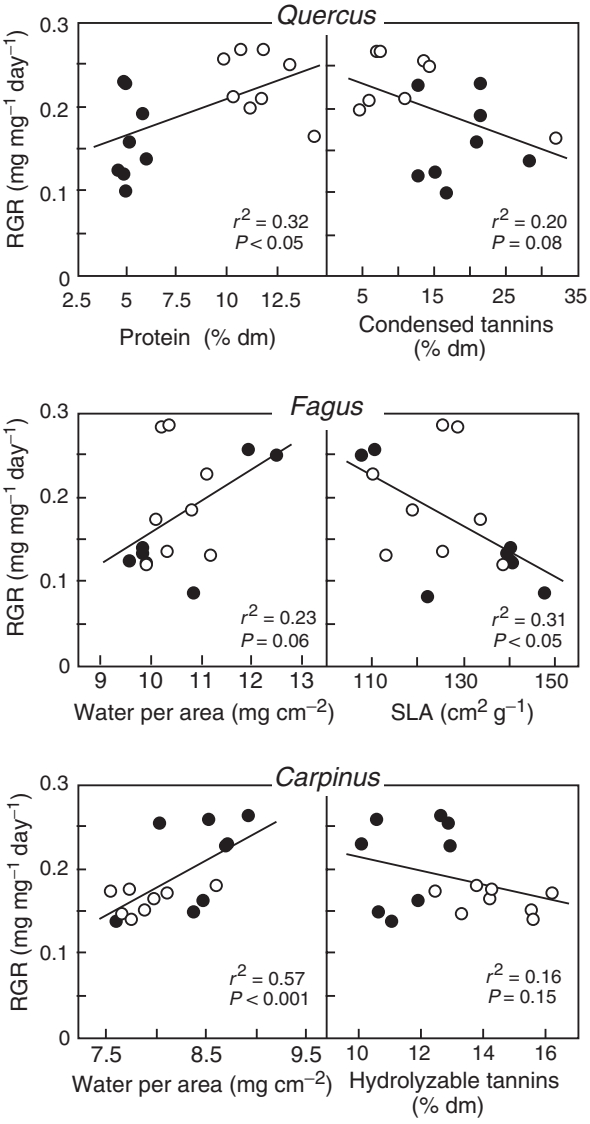

Fig. 4 Relative growth rate (RGR) of *Lymantria dispar* larvae as a function of different leaf traits within each of three tree species ($n = 16$ branches). Open circles represent trees grown at ambient CO_2 and black symbols represent trees grown at elevated CO_2. Lines and corresponding r^2- and P-values are shown for simple linear regression.

pattern among species, protein concentration showed a trend to correlate positively and condensed tannins tended to correlate negatively with RGR in oak (Fig. 4). In beech, the SLA showed a positive correlation and the amount of water per unit leaf area showed a negative correlation with RGR (Fig. 4). In hornbeam, the strongest correlation occurred between RGR and the amount of water per unit leaf area (Fig. 4), a similar response as seen in beech. Correspondingly, RGR on hornbeam also decreased with increasing SLA ($r^2 = 0.49$, $P < 0.01$). Although statistically not significant, the relationship between high concentrations of hydrolyzable tannins and low larval RGR in hornbeam (Fig. 4) might contribute to the understanding of the tree species-specific responses in larval growth.

Discussion

Here we show how an elevated atmospheric CO_2 concentration expected to occur within the next 30–50 years will possibly affect the growth of a generalist herbivore feeding in the canopy of a mature, species-rich temperate deciduous forest. Our data suggest that elevated CO_2 has a strong influence on larval growth of gypsy moth through changes in leaf chemistry of its host trees. The CO_2 effects on gypsy moth larvae differed substantially among tree species, ranging from a clearly negative effect when larvae fed on oak to a pronounced positive effect when they fed on hornbeam. Because of its pest status and great economic importance, gypsy moth is one of the most frequently studied herbivorous insects with respect to the impact of rising atmospheric CO_2 (Lindroth *et al.*, 1993; Traw *et al.*, 1996; Kinney *et al.*, 1997; Henn & Schopf, 2001; Williams *et al.*, 2003). However, so far it remains speculative as to whether previous findings from feeding trials in petri dishes or feeding experiments on artificially planted tree saplings, and the use of laboratory gypsy moth populations can be scaled up to natural conditions in a mature, mixed-species forests and gypsy moth larvae from natural populations. Our results agree with previous reports of significant CO_2 effects on gypsy moth performance (Lindroth *et al.*, 1993; Traw *et al.*, 1996) and support the conclusion from multispecies comparisons that the CO_2 responses depend on plant species (Roth & Lindroth, 1995; Traw *et al.*, 1996; Kinney *et al.*, 1997). It thus appears that the CO_2 effects on herbivores observed in studies with planted tree saplings are maintained in mature trees within a natural forest community. Whether this is fully true for a particular tree species, however, is still difficult to judge because most former studies with gypsy moth employed North American tree species. *Q. petraea* as the main native host of gypsy moth, and *C. betulus* have not been studied previously. In a recent experiment with gypsy moth feeding on high CO_2-exposed beech seedlings in the greenhouse, no significant changes in RGR were observed (Henn & Schopf, 2001). Although this result is in accordance to the nonsignificant (though trend-wise negative) CO_2 effect we found in beech, the data by Henn & Schopf (2001) are difficult to interpret because they did not report if and how elevated CO_2 altered leaf chemistry.

Opposite CO_2 effects on gypsy moth feeding on oak and hornbeam are particularly important results.

Obviously, because of the CO_2-induced changes in leaf quality of hornbeam, gypsy moth larvae attained similar RGR on this commonly less favored host tree species as did larvae that fed on the preferred oak grown under ambient CO_2. Apart from a marginally significant positive CO_2 effect on gypsy moth larvae feeding detached leaves of *Q. rubra* (Lindroth *et al*., 1993), which could not be confirmed in a later experiment (Roth & Lindroth, 1995), there exists no other report on significantly increased growth rates under elevated CO_2 in any other leaf-chewing insect. However, depending on host species variable, negative or no CO_2 effects are common findings in experiments with *L. dispar* (Traw *et al*., 1996; Kinney *et al*., 1997), other lepidopteran species (Roth *et al*., 1997; Agrell *et al*., 2000; Goverde & Erhardt, 2003), or grasshoppers (Asshoff & Hättenschwiler, 2004). Hence, for generalist herbivores, it seems reasonable to assume changing host plant preferences with the continuing rise of atmospheric CO_2 concentration. In its native range of European temperate forests, gypsy moth might increasingly abandon its former preferred host species and switch to hornbeam, which is a common and very abundant tree species in mixed oak forests, or possibly other, not yet studied tree species. Since insect herbivores have a significant long-term impact on tree growth (Trotter *et al*., 2002), such a shift in host tree preference likely leads to altered competition among tree species, and consequently to changes in forest community composition.

Although CO_2-mediated changes in leaf chemistry affected larval growth, elevated CO_2 may have only little impact on population dynamics of gypsy moth, because it apparently maintains similar growth rates on alternative host species as under current ambient-CO_2 concentrations. Development time and final pupal mass, thus, may not change significantly in a CO_2-enriched environment as long as herbivores can choose their food plant freely as it was shown for a satyrid butterfly in CO_2-exposed grassland (Goverde *et al*., 2002), and if different host species are sufficiently abundant. Additionally, the growth responses might change in later instars (Fajer 1989; Asshoff & Hättenschwiler, 2004), which are usually less sensitive to differences in food quality than early instars, and the CO_2 effects reported here might become smaller over the entire larval development.

Even if generalist herbivores are confined to a single food plant, they may compensate less nutritive leaf material to some extent by increasing relative consumption rates, or nitrogen utilization efficiency (Williams *et al*., 1994, 1998; Hättenschwiler & Schafellner, 1999). Such compensatory responses may lead to no net changes in development time or adult mass under elevated CO_2 (Docherty *et al*., 1996; Buse *et al*., 1998; Williams *et al*., 2003), or may still be insufficient for coping with reduced food quality (Traw *et al*., 1996; Agrell *et al*., 2000). N availability is widely recognized as the major growth-limiting factor for insect herbivores (Mattson, 1980; Scriber & Slansky, 1981), and accordingly, growth reductions in insects feeding high CO_2-grown leaves are frequently associated with lower N concentrations (Watt *et al*., 1995; Bezemer & Jones, 1998). Other changes in food composition such as low water content and high concentrations of secondary metabolites and NSCs have also been invoked to explain changes in insect growth under elevated CO_2 (e.g. Lindroth *et al*., 1993; Roth *et al*., 1997). Here, we found a weak and only marginally significant CO_2 effect on leaf N concentration during the 2-week feeding period, in contrast to what is commonly reported for tree seedlings exposed to elevated CO_2 (Cotrufo *et al*., 1998; Norby *et al*., 1999). The negative correlation between RGR of larvae and leaf starch to N ratio documented in our study suggests a greater importance of the relative amounts of N and carbon-rich starch for larval performance than of N alone. The species-specific changes in starch and N concentrations in response to elevated CO_2 also help to explain the opposite CO_2 effects on larval RGR on oak and hornbeam with higher (oak) and lower (hornbeam) starch to N ratios at elevated compared with ambient CO_2. Additionally, lower protein concentrations and higher concentrations of condensed tannins in high CO_2-grown oak could have contributed to decreased RGR compared with larvae feeding on ambient-CO_2-grown trees. In contrast, higher water intake and somewhat lower concentrations of hydrolyzable tannins in hornbeam at elevated CO_2 may be linked to higher larval RGR compared with ambient CO_2. On the basis of the present study and on previous work, it seems evident that a number of different leaf chemical parameters influence insect performance concomitantly, but leaf quality is distinctly affected by elevated CO_2 varying with host plant species and general plant growth conditions. It is therefore of foremost importance to study the impacts of rising atmospheric CO_2 on plant–herbivore interactions in natural ecosystems within their natural range to be able to make reasonable predictions on future changes in herbivore population dynamics and forest community composition.

In conclusion, we have demonstrated that elevated CO_2 significantly and distinctly alters the performance of gypsy moth larvae feeding in the canopy of three co-occurring hardwood tree species in a mature mixed European temperate forest. Tree species-specific changes in starch to N ratio, water, proteins, and condensed and hydrolyzable tannins were related to

reduced (oak), stimulated (hornbeam), and not significantly changed (beech) growth rates of gypsy moth larvae. We suggest that gypsy moth might change its host tree preference with rising atmospheric CO_2 concentration in the long term, but that population dynamics of this insect pest might not necessarily be influenced negatively. The resulting altered herbivore pressure on future forests will likely change competitive interactions among tree species with consequences for forest biodiversity, community composition, and ecosystem properties.

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