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The greenhouse diet: gypsy moth performance in a CO_2-enriched world

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Abstract: Nahrung aus dem Treibhaus: die Entwicklung des Schwammspinners in einer CO_2-angereicherten Atmosphäre. Steigende Konzentrationen an atmosphärischem CO_2 beeinflussen den Chemismus von Pflanzen, vornehmlich durch eine Ankurbelung der Fotosynthese, wodurch sich auch die Nahrungsqualität für herbivore Insekten ändert. Die vorliegende Studie dokumentiert erste Ergebnisse aus einem Freilandversuch mit Raupen des Schwammspinners *Lymantria dispar* (Lep., Lymnatriidae) und seinem natürlichen Gegenspieler, der parasitischen Schlupfwespe *Glyptapanteles liparidis* (Hym., Braconidae), die sich auf einer web-FACE (free air CO_2 enrichment) Versuchsfläche in einer 540 ppm CO_2-Atmosphäre entwickelten. Die Ergebnisse verknüpfen Daten der Blattchemie von Eiche, Buche und Hainbuche mit jenen des Wachstums und der Entwicklung von Raupen und Parasitoiden. Unter erhöhten CO_2 Konzentrationen produzierten die Blätter generell mehr Kohlenhydrate. Blieb in den Bäumen die Stickstoff (N)-Aufnahme hinter dem CO_2-bedingten Anstieg an Kohlenstoff zurück (z.B. Eiche), konsumierten die Raupen über 50% mehr Blattmasse. Dennoch waren die Wachstumsraten dieser Tiere um ca. 15% niedriger und sie entwickelten sich langsamer als Raupen in normaler CO_2 Atmosphäre (ca. 378 ppm). Bei einem gleichzeitigen Anstieg der N-Gehalte in den Blättern (z.B. Hainbuche) erreichten die Raupen trotz kürzerer Entwicklungsdauer eine deutlich höhere Biomasse. Die Puppen der Tiere, die sich in erhöhter CO_2 Atmosphäre entwickelt hatten, waren bei allen 3 Baumarten schwerer als die der Kontrolltiere. Überraschenderweise zeigten sich die in der Leibeshöhle der Schwammspinnerraupen heranwachsenden Parasitoidenlarven von der veränderten Nahrungsqualität ihrer Wirte nicht beeinträchtigt. Ein etwaiges geringeres Nährstoffangebot glichen sie durch Verlängerung der endoparasitischen Entwicklungsphase aus. Die adulten Wespen von beiden CO_2 Varianten unterschieden sich nicht voneinander.

Key words: CO_2 enrichment, insect herbivores, trophic interactions, *Lymantria dispar*, *Glyptapanteles liparidis*

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Rising atmospheric CO_2 is regarded as the main driver of global warming (CROWLEY, 2000). While temperature changes directly affect plants and animals (ROOT et al., 2003; PARMESAN, 2006), the effects of CO_2 on herbivores are mediated through changes in nutrient quality. Elevated concentrations of atmospheric CO_2 are likely to increase photosynthetic activity and thus provide more C-based compounds which may alter plant chemical profiles and plant–herbivore–natural enemy interactions. There are several scenarios how insects will react when confronted with a different food quality. A nutrient poor diet, induced by nitrogen dilution, may result in compensatory feeding with either no adverse effects on insect performance or with negative effects on insect growth due to low digestibility of plant structural compounds (e.g. lignin) or toxic effects of secondary metabolites (e.g. tannins).

Here we present data from on-tree feeding trials with larvae of the generalist herbivore *Lymantria dispar* and one of its natural enemies, the hymenopteran endoparasitoid *Glyptapanteles liparidis*, studied in 2005. The experiments were conducted at the Swiss free-air CO_2 enrichment (FACE) site near Basel, in an approximately 80-100-yr-old, mixed-species forest. The data link changes in foliar chemistry of three tree species (*Quercus petraea, Fagus sylvatica, Carpinus betulus*) exposed to 540 ppm CO_2 with herbivore and parasitoid performance.

Material and Methods

Study site and trees. The web-FACE experimental facilities are located in a mature deciduous forest near Basel, Switzerland (PEPIN & KÖRNER, 2002). Since spring 2001 trees are exposed to a mean CO_2 concentration of 540±20ppm during daytime hours from bud break in spring to leaf fall in autumn (about 260 days). A crane supplied with a gondola gives access to 3,000 m^2 of the tree canopy (62 trees). The feeding experiments were conducted on oak (*Quercus petraea*), beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*) in May and June 2005, corresponding to the fifth year of CO_2-enrichment. For each tree species, two individuals were selected from the CO_2-enriched site and two individuals from the CO_2-ambient (control) site (12 trees in total).

Insects. For the on-tree feeding trials, we used larvae of the generalist herbivore *Lymantria dispar* (Lep., Lymantriidae) from our laboratory culture. Until start of experiments, the larvae were reared on meridic diet (wheat germ) at 20±1°C, ~70% R.H. and a photoperiod of LD 16:8h. The natural enemy *Glyptapanteles liparidis* (Hym., Braconidae), a gregarious endoparasitic braconid wasp, was used for the parasitization experiment. Under the laboratory conditions described above, parasitoid larvae emerge as newly molted third instars from a developmentally arrested host larva approximately 17 days post parasitization.

Feeding trials. In mid-May 2005, two weeks after bud break, eight groups of 10 third instar *L. dispar* larvae from a laboratory culture were bagged on randomly chosen branches from the upper crown of each tree (960 larvae in total). Larvae were allowed to feed for two, four, and six weeks, respectively. After removal, the biomass of the larvae and faeces were determined, freeze-dried and reweighed to measure fresh and dry biomass. One group of larvae was allowed to feed on the trees until pupation (six weeks). Pupae were carefully removed, sexed, and their biomass determined. Additionally, we exposed one group of 20 third instar *L. dispar* larvae per tree that were previously parasitized by *G. liparidis* wasps. The parasitized larvae were kept on the trees for 12 days and then recollected to record parasitoid developmental parameters.

Gypsy moth and parasitoid performance. Growth and consumption rates, efficiency of converting ingested and digested food into biomass (ECI, ECD), and approximate digestibility (AD) were calculated following WALDBAUER (1968). An estimation of leaf consumption was achieved by determining leaf area and leaf fresh and dry mass, and by counting the number of leaves before and after larval feeding. Life history parameters recorded for *G. liparidis* included total and endoparasitic development time, cocoon weight as well as mortality, duration of pupation, and sex of adult wasps.

Leaf chemistry. For nutritional analyses, leaves from *Q. petraea*, *F. sylvatica*, and *C. betulus* were collected in bi-weekly intervals throughout the on-tree feeding trial with *L. dispar* larvae, from mid-May until the end of June. The leaf material was flash-frozen in liquid nitrogen at the study site, and then freeze-dried, ground, and analyzed for nutritional parameters in the laboratory. Starch was determined after enzymatic digestion as glucose with HPLC, carbohydrates (sugars, cyclitols) with GC, protein content with the Lowry method, total phenolics as tannic acid equivalents with the Folin-Denis method, and fiber after the Weender protocol. Total N and carbon were quantified using a CHN analyzer.

Results and Discussion.

Leaf quality parameters are summarized in Table 1. The trees produced significantly more carbohydrates (sugar, starch, NSC) under elevated-CO_2 conditions ($P<0.05$, ANOVA). A reduction in tissue water, nitrogen, protein, and fiber, and an increase in total phenolic content were observed in elevated-CO_2 *Quercus*. Little variations or changes opposite to those found in oak occurred in elevated-CO_2 *Fagus* and *Carpinus*.

On all elevated-CO_2 tree species, *L. dispar* larvae consumed up to 50% more leaf matter than larvae on ambient-CO_2 trees (Table 2). Although they ingested more N and carbohydrates, only larvae on elevated-CO_2 *Fagus* and *Carpinus* increased their biomasses significantly compared to control larvae ($P<0.05$, ANOVA). Larvae on elevated-CO_2 *Quercus* lagged behind (see ECI, ECD) and it took them longer to develop (Table 2). Yet, when larvae finally ecdysed to pupae, they showed a higher biomass than those from the controls at ambient-CO_2 trees.

A significant positive correlation was found between larval biomass and the amount of N ingested (Fig. 1). The dilution of N in foliage, which is often observed in an elevated-CO_2 atmosphere, is likely to be mitigated by increasing atmospheric N deposition. In a previous paper we already demonstrated this effect with *L. monacha* (Lep., Lymantriidae) larvae feeding on N-fertilized spruce trees (Hättenschwiler & Schafellner, 1999).

Table 1. Leaf quality parameters of the tree species exposed to ambient and elevated CO_2.

	Quercus		Fagus		Carpinus	
Treatment	ambient	elevated	ambient	elevated	ambient	elevated
SLA	107.5±5.82	97.1±4.97	116.9±6.56	121.1±6.66	151.5±13.18	144.1±4.68
Water	62.15±1.82	58.53±0.48	54.09±1.02	54.85±0.91	54.94±3.03	55.04±0.70
Nitrogen	2.55±0.07	2.08±0.08	1.85±0.09	1.97±0.06	1.92±0.08	2.11±0.09
C/N	19.39±0.48	23.66±0.84	26.91±1.06	25.29±0.54	25.06±1.04	22.86±0.80
Protein	8.71±0.55	5.71±0.26	8.80±0.49	8.42±0.56	8.33±0.35	8.00±0.40
Sugar	9.07±0.33	10.70±0.71	9.15±0.48	9.52±0.15	12.06±0.36	13.33±0.32
Starch	3.53±0.39	6.67±0.89	6.04±0.31	9.01±1.00	5.79±0.61	5.62±0.77
NSC	12.60±0.36	17.37±0.80	15.19±0.39	18.53±0.57	17.85±0.48	18.96±0.55
Fiber	22.27±0.41	20.00±0.14	20.27±0.81	20.07±0.31	12.16±0.73	12.28±0.79
Phenolics	93.8±4.85	97.2±3.68	108.8±6.03	95.5±3.70	167.9±5.73	163.8±4.58

Data (means±SE) refer to eight groups of 10 sample leaves from two trees per species and CO_2 treatment. Intact leaves were collected from bags with feeding larvae in two-week intervals. SLA, specific leaf area; NSC, non-structural carbohydrates. SLA is given as cm^2 g^{-1} dry mass, water as % of total leaf fresh mass, all other parameters as % of total leaf dry mass.

Table 2. Growth and development of *L. dispar* larvae on ambient and elevated CO_2 trees.

	Quercus		Fagus		Carpinus	
Treatment	ambient	elevated	ambient	elevated	ambient	elevated
Mass gain	147.7±18.07	122.9±14.72	78.5±9.97	122.3±16.52	72.4±3.66	106.3±11.18
Consumption	2527±383	3966±937	1035±122	1423±167	960±22	1338±191
N uptake	65.02±9.63	83.40±20.48	19.12±2.72	28.11±2.96	19.08±0.62	28.19±4.07
ECI	6.10±0.89	3.40±0.44	7.78±1.10	8.58±0.43	7.55±0.38	8.58±1.72
ECD	4.06±0.85	2.58±0.41	5.02±1.03	6.14±1.35	6.74±0.79	6.59±1.73
AD	79.52±3.43	83.59±0.83	76.05±3.72	71.07±5.43	67.69±2.70	72.96±3.38
Development m	38.00±0.00	38.08±0.08	38.63±0.30	38.10±0.10	38.90±0.38	38.31±0.14
Development f	38.91±0.34	40.36±0.36	41.70±0.39	39.45±0.33	43.53±0.35	40.27±0.40
Pupal mass m	424.7±9.08	394.4±14.8	375.2±27.9	334.6±9.8	310.8±10.4	336.4±11.8
Pupal mass f	971.6±38.5	1114.8±47.8	855.1±45.6	928.9±35.0	751.2±45.7	950.1±60.6

Data (means±SE) refer to eight groups of 10 larvae kept on two trees per species and CO_2 treatment and values were calculated after a four-week on-tree feeding period (except development time and pupal biomass). Biomass gain, consumption, and N-uptake are given as mg dry mass, ECI, ECD, and AD as %, larval development as days, pupal mass as mg fresh mass. m, male; f, female.

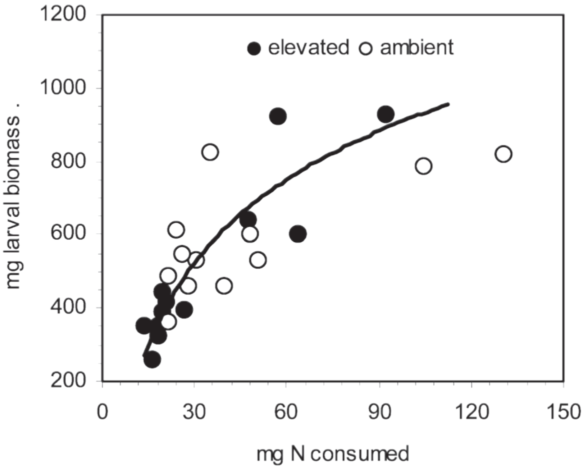

Figure 1. Relationship between larval biomass and amount of nitrogen (N) consumed by *L. dispar* larvae from ambient-CO_2 (open circles) and elevated-CO_2 (closed circles) foliage. Biomass and N consumption were determined after a four-week on-tree feeding period (May – June 2005). Each circle represents the mean of 10 larvae. Log regression Rsq=0.69, df=22, F=49.8, $P<0.0001$, N=24).

The developing parasitoids were not adversely affected by CO_2-mediated changes in food quality across the trophic levels (Table 3). Interestingly, endoparasitic development accelerated significantly in hosts from elevated-CO_2 *Carpinus,* indicating an increase in the nutritional quality of *Carpinus* foliage mediated by the host larvae.

Table 3. *G. liparidis* parasitoid performance on *L. dispar* larvae feeding on foliage from ambient and elevated CO_2 trees (May 2005).

	Quercus		Fagus		Carpinus	
Treatment	ambient	elevated	ambient	elevated	ambient	elevated
Host final mass	141.3±16.66	197.2±9.95	124.3±6.44	138.6±6.34	134.3±6.59	157.7±7.06
Host RGR	0.21±0.025	0.14±0.019	0.05±0.018	0.07±0.002	0.07±0.003	0.10±0.011
Par development	17.86±0.22	18.16±0.26	18.58±0.64	18.50±0.31	20.03±0.49	18.15±0.30
Cocoon mass	3.06±0.08	2.87±0.12	2.85±0.12	3.03±0.26	3.09±0.12	3.33±0.31
Par pupation	6.05±0.10	6.07±0.07	6.32±0.11	6.20±0.12	6.33±0.10	6.26±0.13

Data (means±SE) refer to 30 larvae kept on two tree individuals per species and CO_2 treatment. Host final biomass, and cocoon biomass are given as mg fresh biomass, host RGR as mg fresh biomass per mg and day, par development and par pupation as days. Par, parasitoid.

In summary, we showed that *L. dispar* larvae responded differently to CO_2-induced changes in food quality. Adverse effects occurring in *Quercus* (e.g. less N) were compensated until pupation by increased and longer feeding. Favourable changes observed in *Fagus* and *Carpinus* (e.g. more nitrogen and carbohydrates) resulted in higher body mass and shorter development time. Responses of generalist herbivores to a changing diet, therefore, seem highly host-plant specific. Previous short-term feeding studies providing data for only third and fourth instars (Hättenschwiler & Schafellner, 2004) have to be modified in that the performance of *L. dispar* is unlikely to decline in a CO_2-enriched atmosphere, even though the food quality might deteriorate. In contrast, host plant quality of some species may increase as CO_2 concentrations rise, suggesting a possible shift of *L. dispar* host plant preferences with important consequences during mass outbreaks of this insect pest.

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