Oecologia (2004) 139: 383–391
DOI 10.1007/s00442-004-1516-4

PLANT ANIMAL INTERACTIONS

Marcel Goverde · Andreas Erhardt · Jürg Stöcklin

Genotype-specific response of a lycaenid herbivore to elevated carbon dioxide and phosphorus availability in calcareous grassland

Received: 16 March 2003 / Accepted: 21 January 2004 / Published online: 24 February 2004

Abstract Effects of elevated CO_2 and P availability on plant growth of the legume *Lotus corniculatus* and consequences for the butterfly larvae of *Polyommatus icarus* feeding on *L. corniculatus* were investigated in screen-aided CO_2 control chambers under natural conditions on a calcareous grassland in the Swiss Jura mountains. Elevated CO_2 conditions and P fertilisation increased the biomass production of *L. corniculatus* plants and affected the plant chemical composition. CO_2 enrichment increased the C/N ratio and sugar concentration and decreased the N and P concentrations. C- and N-based allelochemicals (cyanoglycosides, total polyphenols and condensed tannins) were only marginally affected by CO_2 enrichment. P fertilisation increased the specific leaf area and concentrations of water, N, sugar and P, while the C/N ratio and the concentration of total polyphenols decreased. Furthermore, P availability marginally enhanced the effect of elevated CO_2 on the total dry mass and sugar concentration while the opposite occurred for the total polyphenol concentration. The changes in food-plant chemistry as a result of P fertilisation positively affected larval mass gain and accelerated the development time of *P. icarus*. Only a marginal negative effect on larval mass gain was found for CO_2 enrichment. However, we found genotype-specific responses in the development time of *P. icarus* to elevated CO_2 conditions. Larvae originating from different mothers developed better either under elevated CO_2 or under ambient CO_2 but some did not react to CO_2 elevation. As far as we know this is the first finding of a genotype-specific response of an insect herbivore to elevated CO_2 which suggests genetic shifts in insect life history traits in response to elevated CO_2.

Keywords Elevated carbon dioxide · *Lotus corniculatus* · Nutrient availability · Plant-insect interaction · *Polyommatus icarus*

M. Goverde (✉) · A. Erhardt
Department of Integrative Biology, Section of Conservation Biology, University of Basel,
St Johanns-Vorstadt 10,
4056 Basel, Switzerland
e-mail: marcel.goverde@bluemail.ch
Tel.: +41-61-4220355
Fax: +41-61-4220357

J. Stöcklin
Department of Integrative Biology, Institute of Botany, University of Basel,
Schönbeinstrasse 6,
4056 Basel, Switzerland

Present address:
M. Goverde
Melchtalstrasse 21,
4102 Binningen, Switzerland
e-mail: marcel.goverde@bluemail.ch

Introduction

The current anthropogenic increase in atmospheric CO_2 is likely to affect plant-insect interactions (e.g. Watt et al. 1995; Coviella and Trumble 1999). Changes in leaf chemistry under elevated CO_2, especially reduced leaf protein concentration which is most limiting for phytophagous insect development (Schoonhoven et al. 1998), can lead to decreased insect herbivore growth, increased larval development time, or compensatory feeding (e.g. Fajer et al. 1989; Ayres 1993; Lincoln et al. 1993; Bezemer and Jones 1998).

The impact of CO_2 enrichment on herbivores has been investigated in about 60 studies (see e.g. Coviella and Trumble 1999; Goverde et al. 2002b). Most of these studies have been conducted under standardised experimental conditions with a relatively high power to detect even small effects of experimental treatments. However, the extrapolation of greenhouse and growth-chamber data to natural conditions is difficult and of limited value since the conditions of these experiments may neglect processes of ecological relevance and lead to the detection of effects not occurring in the field (Peters 1993; Körner 2000). For example, the effect of elevated CO_2 on the development of a satyrid butterfly found in a greenhouse study (Goverde and Erhardt 2003) could not be found when using a

screen-aided CO_2 control (SACC) system under natural conditions on a calcareous grassland (Goverde et al. 2002b). The lack of agreement between these two experiments might be a result of natural climatic conditions, food-plant choice and long-term effects in the field experiment which mask the effect of elevated CO_2 found in the greenhouse (Körner 1995; Goverde et al. 2002b).

The present study investigates effects of elevated CO_2 on biomass production and leaf chemistry of the legume *Lotus corniculatus* L. (Fabaceae), and consequences for the butterfly larvae of *Polyommatus icarus* Rottemburg (Lepidoptera, Lycaenidae) feeding on *L. corniculatus* in a SACC system under field conditions [for further details on the SACC see Leadley et al. (1999)]. The same plant-insect system has been investigated in a growth-chamber and greenhouse study (Goverde et al. 1999; Bazin et al. 2002) and, therefore, comparisons of results can be made. Under elevated CO_2 conditions *L. corniculatus* plants grown either in growth-chambers or in the greenhouse, increased concentrations of starch, condensed tannins and total polyphenols, and decreased cyanoglycoside concentrations (Goverde et al. 1999; Bazin et al. 2002). In both experiments, *P. icarus* developed faster under elevated CO_2 than under ambient CO_2 conditions. Since *L. corniculatus* plants were grown in a nutrient-rich soil, they were not N limited and, hence CO_2 enrichment had no effect on the protein concentration of *L. corniculatus*. The faster development of the larvae on plants grown under elevated CO_2 conditions was probably a result of the higher starch concentration of these plants compared to plants grown under ambient CO_2 conditions (Goverde et al. 1999).

As shown by Stöcklin and Körner (1999) the biomass increase of legumes as a result of elevated CO_2 conditions is much larger when plants are fertilised with P. This indicates that legumes' responsiveness is largely controlled by P availability. Therefore, in the present study, we added a P-fertiliser treatment to investigate its direct and interactive effect with CO_2 enrichment on the growth of *L. corniculatus* and its consequence for insect performance. Positive effects of fertilisation on insect growth were found in many other studies (e.g. Kinney et al. 1997; Kerslake et al. 1998; Hättenschwiler and Schafellner 1999). Larvae of the satyrid butterfly *Coenonympha pamphilus* reared on fertilised plants showed a lower development time and higher weights than larvae feeding on unfertilised plants (Goverde and Erhardt 2003). Furthermore, adult butterflies showed lower preferences for amino acid-rich nectar mimics when reared on fertilised plants, which indicates that their fertilised larval food plants were more nutritious than unfertilised control plants (Mevi-Schütz et al. 2003). However, in these studies interactive effects of elevated CO_2 and fertilisation were found neither for larval development nor for adult nectar preference. Increased effects of elevated CO_2 as a result of fertilisation were found for plant characters such as dry mass, leaf N and starch concentrations (e.g. Conroy et al. 1992; Entry et al. 1998; Goverde et al. 2002a), but so far not for insect growth (e.g. Williams et al. 1997; Hättenschwiler and Schafellner 1999; Goverde and Erhardt 2003 but see Kerslake et al. 1998).

Finally, the effect of elevated CO_2 on insect performance has been shown to be species-specific or to depend on the insects' feeding guild (Bezemer and Jones 1998). Genotype-specific responses to elevated CO_2 have been shown for plants (e.g. Leadley and Stöcklin 1996; Goverde et al. 1999; Mansfield et al. 1999; Roumet et al. 1999; Lindroth et al. 2001). However, so far few studies have investigated the effect of elevated CO_2 on different genotypes of herbivores. Larvae of *P. icarus* originating from different mothers show differences in larval, pupal and adult mass, in development time and in conversion efficiency (Goverde et al. 1999; Bazin et al. 2002). Thus the question arises if there are interactive effects of larval genotype and elevated CO_2 conditions, which could result in an evolutionary advantage for a specific genotype within a population.

In the present study, we tested the following hypotheses:

1. Elevated CO_2 conditions increase starch and C-based allelochemical concentrations of *L. corniculatus* plants.
2. Growth of *L. corniculatus* plants increases with P fertilisation, and the latter amplifies the effect of CO_2 enrichment for plant growth.
3. The changes in leaf chemistry of *L. corniculatus* plants as a result of elevated CO_2 and fertilisation positively affect the performance of *P. icarus*.
4. Larvae of *P. icarus* originating from different mothers differ in their responses to elevated CO_2.

Materials and methods

Study site and CO_2 enrichment

The present study was carried out in a semi-natural, undisturbed calcareous grassland near the village of Nenzlingen (47°27′N, 7°34′ E) in the Swiss Jura mountains. The site is a moderately steep (ca. 20°), south-west-facing slope at an altitude of 520 m a.s.l.. The Rendzina soil consists of a 10- to 15-cm silty clay loam top horizon underlain by calcareous debris (Leadley et al. 1999). Originally covered by beech forest, these grasslands have been grazed by cattle for many centuries, leading to the characteristic Teucrio-Mesobrometum vegetation (Schläpfer et al. 1998). Snow usually covers the area for <1 month per year.

Beginning with the onset of the growing season in late March 1994, elevated CO_2 was applied using SACC (for details see Leadley et al. 1997, 1999). Twenty-four experimental plots were arranged in a randomised block design. Each block contained six hexagonal SACC chambers reaching a height of 50 cm and enclosing 1.27 m^2. In three plots per block, the atmospheric CO_2 concentration was raised to 600 $\mu l\ l^{-1}$, whereas the other three plots were exposed to present-day CO_2 concentrations (368 $\mu l\ l^{-1}$). In the outer part of the 1.27-m^2 area of each plot several tubes (diameter 9.5 cm, depth approximately 30 cm) were dug in. For the present study *L. corniculatus* plants were planted in six tubes per plot (three tubes were situated in the upper left part of the plot and three in the lower right part).

Plant and insect material

Lotus corniculatus L. (Fabaceae) seeds were obtained from a local distributor (Wildstaudengärtnerei Willi-Durrer, Eschenbach), and were germinated in compost and transplanted after 12 days into small pots. On 12 March 1998, 27 days after sowing, two *L. corniculatus* plants per pot (10 cm diameter) were planted. The soil in these pots originated from the study site at Nenzlingen described above. To ensure nodulation of all plants, pots were watered with a solution containing rhizobial bacteria. Nodules of roots of *L. corniculatus* plants collected at Nenzlingen were crushed with a mortar and pestle, and left for 24 h in 100 mmol mannitol in tapwater (J. Müller, personal communication). This procedure was repeated 1 week later to ensure nodulation. Plants were grown in a greenhouse at the Botanical Institute Basel for 6 weeks. On 2 April 1998, *L. corniculatus* plants were randomly assigned to the tubes described above and planted in the field. To ensure growth, plants were watered on 25 April, and 4 8 11 and 13 May.

Polyommatus icarus Rottemburg (Lepidoptera, Lycaenidae) females were captured at different sites in the Swiss Jura mountains. Butterflies were kept in cages in the greenhouse to oviposit [for further detail see Goverde et al. (1999)]. Eggs from six different females were obtained and these were kept, separated by female, on moist filter paper in petri dishes covered by a nylon mesh. After hatching, batches of eight to ten larvae per female were placed in petri dishes containing shoots of *L. corniculatus*. Since the mortality of young larvae is high, *P. icarus* caterpillars were reared in the greenhouse until reaching their third larval instar (I_3).

Experimental design

The six tubes per plot were randomly assigned to two groups. One group acted as control while the other group received granulated P fertiliser on 3 April and on 4 May. The total amount of P fertiliser per tube was 15 kg P ha^{-1}.

On 8 June, 67 days after planting, shoots and leaves of all *L. corniculatus* plants were counted. Additionally, the cages for larval rearing were installed in the field. Each tube was covered by a fine nylon mesh of 30 cm height which was attached to a stick placed in the centre of the tube. Between 10 and 13 June freshly emerged I_3 *P. icarus* larvae were weighed and released into the cages. Four of the six cages (two control and two with P fertiliser) per plot received one I_3 caterpillar originating from one specific female. The two remaining tubes were used as control without herbivory. Per female genotype, two ambient and two elevated CO_2 plots were used, resulting in four plots per female genotype and a total of six female genotypes.

Twelve days after they had been released in the cages, larvae were collected, weighed and returned to the original plants from which they were obtained. Between 7 and 9 July all larvae had reached their pupal stage. Pupae were collected and weighed, and singly placed in one of 18 compartments of a plastic box (21 cm×12 cm) with a nylon webbing floor. Boxes were placed in a greenhouse 5 cm above a pan with water to maintain high humidity. Boxes were daily checked for emerging adults. After they emptied their gut, emerged butterflies were weighed.

After removing the pupae, plant characteristics were measured. First, the number of leaves and shoots of the remaining *L. corniculatus* plants were counted. Then, to analyse cyanide concentration, six to seven leaves from each plant were removed, frozen in liquid nitrogen and stored at −80°C until analysis. To determine the specific leaf area (cm^2 g^{-1} dry mass), a subsample of leaves was harvested and their leaf area was measured using a planimeter (LI-3100 area meter; LI-COR, Lincoln, Neb.). Finally, all remaining plants (above-ground and 20 cm of the root system) were harvested, weighed and dried at 60°C for 48 h. Leaf water concentration was measured as the proportional difference between fresh and dry leaf mass. Total shoot and root dry mass were determined. N and C concentrations were determined from dried leaves using a CHN analyser (model 932; LECO Instruments, St. Joseph, Mich.), which uses a combustion procedure. Non-structural carbohydrates (NSC) were determined using a series of enzymatic digestions (Wong 1990; Körner and Miglietta 1994). With this method, soluble-neutral sugar and NSC concentrations are measured whereas the starch concentration was calculated as the difference between NSC and the soluble-neutral sugar concentration. The P concentration was determined by using the molybdate blue ascorbic acid method (Watanabe and Olsen 1965). Cyanide samples were ground with liquid N using a mortar and pestle, and the HCN released was fixed in 0.5 ml of 1 N NaOH in a 37°C oven for 12 h. The HCN concentration was estimated by the method of Lambert et al. (1975) and expressed in equivalent mg CN g^{-1} dry mass. A standard absorption curve at 725 nm was established with commercial KCN (Prolabo, Briare, France). For total polyphenol and condensed tannin analysis, approximately 70-mg dried leaf samples were extracted in 10 ml of 70% acetone overnight (15 h) at 4°C in the dark. After filtration, the condensed tannin concentration [estimated at 550 nm and expressed by the extinction coefficient ($E‰_{550}$)=150 in units of mg g^{-1}; condensed tannins measured as anthocyanidin equivalents] was assessed using the acid butanol assay (Porter et al. 1986). For total polyphenols, the Prussian blue assay (Price and Butler 1977) was used with a standard curve of

Table 1 Skeleton ANOVA for measured plant characters and insect characters. Split-plot ANOVA, sum of squares type I, n =144 for plant characters; n =96 for weight of third instar larvae; n =81 for weight of larvae after 12 days; n =68 for pupal weight, adult weight and development time

Plant characters				Insect characters			
Source of variation	*df*	Mean square (MS)	Variance-ratio (*F*)	Source of variation	*df*	Mean square	Variance-ratio (*F*)
Block (B)	3	MSB	MSB/MSP	CO_2	1	$MSCO_2$	$MSCO_2$/MSP
CO_2	1	$MSCO_2$	$MSCO_2$/MSP	Female genotype (FG)	5	MSFG	MSFG/MSP
Plot (P)	19	MSP	MSP/MSR	CO_2×FG	5	$MSCO_2$×FG	$MSCO_2$×FG/MSP
Fertilisation (F)	1	MSF	MSF/MSR	Covariate sex (S)	1	MSS	MSS/MSP
Herbivory (H)	1	MSH	MSH/MSR	Plot (P)	11	MSP	MSP/MSR
CO_2×F	1	$MSCO_2$×F	$MSCO_2$×F/MSR	Fertilisation (F)	1	MSF	MSF/MSR
CO_2×H	1	$MSCO_2$×H	$MSCO_2$×H/MSR	CO_2×F	1	$MSCO_2$×F	$MSCO_2$×F/MSR
F×H	1	MSF×H	MSF×H/MSR	FG×F	5	MSFG×F	MSFG×F/MSR
CO_2×F×H (all)	1	MSall	MSall/MSR	CO_2×FG×F (all)	5	MSall	MSall/MSR
Error (residuals; R)	114	MSR		S	1	MSS	MSS/MSR
				Error (residuals; R)	59 [a]	MSR	

[a]Model *df*, i.e. since there were some larvae or pupae lost, the effective error *df* is <59

Table 2 Summary of plant parameters (means±SEM) under ambient and elevated CO_2 conditions without (*Control*) and with P fertiliser. The proportional effect of elevated CO_2 and P fertilisation (*P*) is indicated. Significant CO_2 or P main effects are shown (for interactive and marginal effects see the text). *n* No. of samples per treatment, *SLA* specific leaf area, *d.m.* dry mass

	n	Ambient CO_2		Elevated CO_2		CO_2 effect			P effect		
		Control	P fertiliser	Control	P fertiliser	Control	P fertiliser		Ambient CO_2	Elevated CO_2	
Number of shoots (before herbivory)	12	20.06±0.56	23.28±0.98	20.25±0.53	24.00±0.99	1.0%	3.1%		16.1%	18.5%	$F_{1,114}$ =17.64,*P* <0.001
No. of leaves (before herbivory)	12	129.50±4.11	163.31±8.39	131.22±5.91	161.61±6.17	1.3%	−1.0%		26.1%	23.2%	$F_{1,114}$ =27.57,*P* <0.001
No. of shoots (after herbivory)	12	25.19±0.96	28.78±1.27	26.06±0.86	29.44±1.66	3.4%	2.3%		14.2%	13.0%	$F_{1,114}$ =8.40,*P* =0.005
No. of leaves (after herbivory)	12	159.83±8.82	225.28±13.42	160.89±5.79	220.53±14.02	0.7%	−2.1%		40.9%	37.1%	$F_{1,114}$ =40.87,*P* <0.001
Total dry mass (g)	12	2.99±0.20	3.96±0.16	3.63±0.21	5.40±0.38	21.5%	36.3%	$F_{1,19}$ =11.48,*P* =0.003	32.6%	48.8%	$F_{1,114}$ =46.06,*P* <0.001
Leaf water (%)	12	67.5±0.6	69.1±0.8	66.6±0.7	68.2±0.8	−1.4%	−1.2%		2.4%	2.5%	$F_{1,114}$ =11.92,*P* <0.001
SLA (cm^2g^{-1}d.m.)	4	167.94±7.93	180.19±11.54	147.07±17.73	187.80±20.76	−12.4%	4.2%		7.3%	27.7%	$F_{1,9}$ =9.84,*P* =0.012
N (%)	12	2.82±0.08	3.13±0.06	2.54±0.05	2.78±0.05	−10.0%	−11.2%	$F_{1,19}$ =22.98,*P* <0.001	10.9%	9.4%	$F_{1,114}$ =32.95,*P* <0.001
C/N ratio	12	14.99±0.44	13.72±0.30	16.74±0.34	15.44±0.31	11.7%	12.5%	$F_{1,19}$ =21.04,*P* <0.001	−8.5%	−7.8%	$F_{1,114}$ =24.34,*P* <0.001
Sugar (%)	12	3.74±0.20	4.16±0.36	4.33±0.38	5.47±0.23	15.7%	31.5%	$F_{1,19}$ =6.90,*P* =0.017	11.1%	26.3%	$F_{1,114}$ =9.59,*P* =0.002
Starch (%)	12	9.74±1.05	8.64±1.09	10.89±1.11	10.46±1.42	11.9%	21.1%		−11.2%	−4.0%	
P (%)	12	4.49±0.31	6.59±0.23	3.54±0.22	5.34±0.21	−21.1%	−19.1%	$F_{1,19}$ =15.50,*P* <0.001	46.9%	50.7%	$F_{1,114}$ =102.41,*P* <0.001
Cyanide (mg g^{-1})	12	0.97±0.08	1.03±0.10	0.87±0.09	0.85±0.05	−10.6%	−17.7%		6.2%	−2.2%	
Total polyphenols (mg g^{-1})	12	2.98±0.12	2.93±0.13	3.48±0.15	3.04±0.17	16.8%	3.8%		−1.9%	−12.7%	$F_{1,114}$ =4.78,*P* =0.031
Condensed tannins (mg g^{-1})	12	96.31±4.59	97.11±6.39	119.17±7.01	101.36±7.49	23.7%	4.4%		0.8%	−14.9%	

absorption at 725 nm established with commercial tannic acid (catechin; Fluka Chemie, Switzerland).

Statistical analysis

The data were analysed using the skeleton ANOVA presented in Table 1 which corresponds to a hierarchical split-plot design. For plant characters, first the variation of the factor *block* was fitted. Then, the main effect of elevated CO_2 was fitted and tested against the variation among plots. All remaining factors and interactions were then tested against the residuals (Table 1). For insect characters the factor *block* could not be tested since the factor *female genotype* was not orthogonal to the factor *block*. The main factors *CO_2* and *female genotype* were tested against the variation in plots. The factor *fertilisation* and all other interactions were then tested against the residuals (Table 1). To eliminate the variation of differences between males and females, the covariate *sex* was fitted for both strata (plot-stratum and plots-by-units stratum; Table 1). Two statistical models were used for analysing larval mass after 12 days of growth in the field. First, larval mass was analysed using the model described above. Second, larval mass was analysed using the larval mass at the beginning of the experiment in the field facility as covariate. Finally, to meet the assumptions for ANOVA, some data were transformed. Square root transformation was applied for shoot/root ratio and total polyphenol concentration, log-transformation was used for sugar concentration and weight of third instar larvae, and inverse sine transformation was applied for leaf water concentration (Zar 1999). All statistical analyses were performed using Genstat 5 release 3.2 (Payne 1993). Values are given as mean ± 1 SEM.

Results

Effects of elevated CO_2 and P fertilisation on plant characters

The effects of elevated CO_2 and P fertilisation on plant parameters measured are given in Table 2. Elevated CO_2 increased the total dry mass, C/N ratio and sugar concentration of *L. corniculatus*, and decreased the N and P concentrations (Table 2). Marginal effects were found for all allelochemicals measured. The cyanide concentration marginally decreased ($F_{1,19}$ =3.13, P =0.09) while the total polyphenol and condensed tannin concentrations of *L. corniculatus* plants marginally increased under elevated CO_2 conditions ($F_{1,19}$ =3.19, P =0.09 and $F_{1,19}$=3.13, P =0.09, respectively).

Most plant parameters were positively affected by P fertilisation (Table 2). The strongest effects of P fertilisation were found for the number of shoots and leaves, for total dry mass and for P concentration of *L. corniculatus* plants (Table 2). A negative effect of P fertilisation was only found for the C/N ratio and the total polyphenol concentration (Table 2).

Additionally, one significant and some marginally significant interactive effects of elevated CO_2 and P fertilisation were found. The effect of elevated CO_2 on root dry mass was stronger when plants were fertilised ($F_{1,114}$ =4.80, P =0.031). For total dry mass as well as for sugar concentration the effect of elevated CO_2 tended to be more pronounced in fertilised plants (Table 2; $F_{1,114}$=3.88, P =0.051 and $F_{1,114}$=2.91, P =0.091, respectively). The opposite effect appeared for concentrations of total polyphenols and condensed tannins, where P fertilisation tended to decrease the effect of elevated CO_2 (Table 2, $F_{1,114}$=3.39, P =0.068 and $F_{1,114}$=3.72, P =0.056, respectively).

Effects of herbivory on plant characters

The feeding of *P. icarus* caterpillars negatively affected the number of *L. corniculatus* leaves ($F_{1,114}$ =5.03, P =0.027). Plants that experienced herbivory had 11.2% fewer leaves than control plants without caterpillars. The number of shoots and the total dry mass, however, were unaffected ($F_{1,114}$ =0.67, P =0.42 and $F_{1,114}$=0.10, P =0.76, respectively). Feeding of *P. icarus* caterpillars increased the concentration of N by 4.2% ($F_{1,114}$ =5.31, P =0.023). Corresponding to this, the C/N ratio was significantly reduced by herbivory ($F_{1,114}$ =5.95, P =0.016). The strongest effect of herbivory was found for starch concentration, where plants with herbivore damage had 26.2% less starch than control plants without caterpillars ($F_{1,114}$ =26.43, P <0.001). Additionally, this effect depended on P fertilisation ($F_{1,114}$ =5.16, P =0.025). The effect of herbivory on starch concentration was stronger in unfertilised (34.0%) than in fertilised (16.3%) *L. corniculatus* plants. Finally, herbivory marginally increased condensed tannin concentrations by 10.4% in plants with herbivore damage compared to control plants ($F_{1,114}$ =3.66, P =0.058).

Effects on insect development and insect genotype-specific responses

Larval mass after 12 days of growth in the field was marginally reduced under elevated CO_2 conditions (Fig. 1, Table 3). Larval mass was increased by P fertilisation (Fig. 1), and additionally this increase depended on the female genotype (fertilisation-by-female genotype interaction; Table 3). Some larvae originating from a specific mother reacted more strongly to P fertilisation than others. However, when using larval mass at the beginning of the experiment as a covariate only the main effect of fertilisation remains significant ($F_{1,44}$ =23.57, P <0.001). For pupal and adult fresh mass no significant main effect of elevated CO_2 was found (Table 3). However, a three-way interaction of CO_2, female genotype and fertilisation appeared, i.e. the effect of elevated CO_2 on pupal and adult mass depended on the origin of the larvae as well as on P fertilisation. Strong differences in pupal and adult fresh mass among larvae from different females were found (Table 3; female genotype effect). Additionally, as for larval mass, female genotype affected the outcome of the P fertilisation effect on pupal and adult fresh mass, while only a marginal main effect of P fertilisation on pupal fresh mass was found (Table 3). Finally, the effect of elevated CO_2 on development time depended on female genotype, i.e. some larvae originating from a specific mother developed better under elevated CO_2, some under

ambient CO_2 and some did not react to CO_2 elevation (Fig. 2). As for larval mass, development time was affected by fertilisation (Table 3). Larvae feeding on fertilised *L. corniculatus* plants developed within 32.4 ±0.3 days, while they took 34.0±0.6 days to fully develop on unfertilised plants.

Discussion

The novelty of the present study is to investigate the interactive effects of maternal butterfly-specific, i.e. genotype-specific, responses to elevated CO_2 and P fertilisation under natural conditions in the field, thus larvae from a specific mother might react differently to elevated CO_2 and/or P fertilisation than other larvae. Many studies have demonstrated that the effect of elevated CO_2 on plants can influence insect herbivores. However, most studies investigating this effect were conducted under controlled conditions in greenhouse or growth-chamber experiments and, so far, genotype-specific responses of an insect herbivore have not been considered.

Although there are some studies investigating the effect of elevated CO_2 on insect herbivores under more natural conditions (e.g. Bezemer et al. 1998; Jones et al. 1998; Stiling et al. 2003) there is still a lack of studies which connect findings from greenhouse studies with findings from experiments conducted under more natural conditions (but see Bezemer et al. 1999; Goverde et al. 2002b). The plant-insect system used here had already been investigated in a growth-chamber and a greenhouse experiment (Goverde et al. 1999; Bazin et al. 2002). In these studies elevated CO_2 conditions caused *L. corniculatus* to increase its biomass, to accumulate starch and C-based allelochemical and to reduce N-based allelochemicals. However, the N concentration did not change. Larvae of the butterfly *P. icarus* feeding on plants grown under elevated CO_2 conditions showed higher rates of consumption, conversion efficiency and consequently accelerated growth.

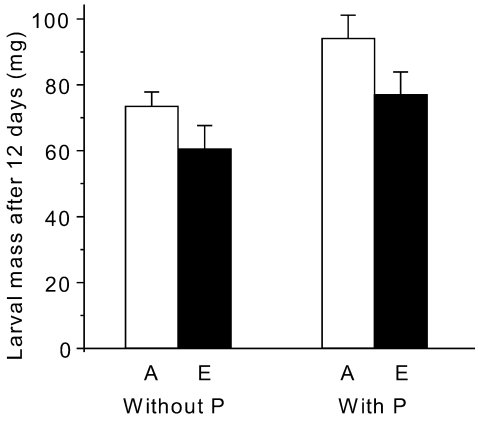

Fig. 1 Mass of *Polyommatus icarus* larvae after feeding for 12 days on *Lotus corniculatus* plants exposed to ambient (*A*) and elevated (*E*) CO_2 conditions, without or with P fertilisation. Means +SEM, n =48

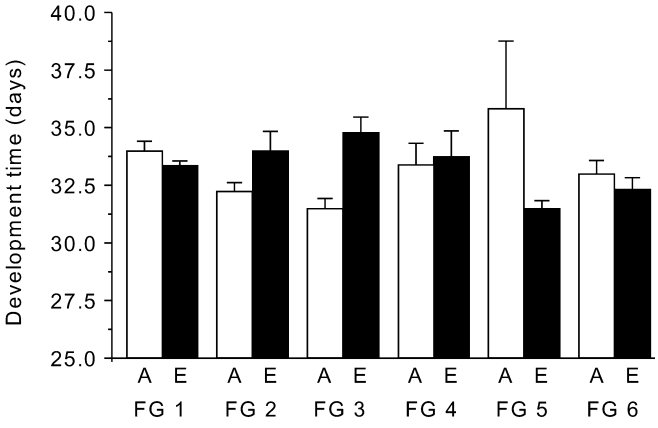

Fig. 2 Development time from third larval instar to adult eclosion of *P. icarus* under ambient (*open bars*) and elevated (*black bars*) CO_2 conditions. Means+SEM for the six different female genotypes (*FG*) are presented, n =8 in each case. For other abbreviations, see Fig. 1

In the present study, an increase in atmospheric CO_2 affected *L. corniculatus* plant growth and foliar chemistry. *L. corniculatus* plants grown under elevated CO_2 conditions showed a decrease in N concentration. A lower N concentration under elevated CO_2 conditions has been found in many plants (Cotrufo et al. 1998), but was not expected in the present study due to earlier findings from a greenhouse experiment (Goverde et al. 1999). The difference might be an effect of soil type. In the greenhouse study a nutrient-rich soil (compost) was used while in the present study plants were grown in their original soil in a nutrient-poor calcareous grassland. Results, thus, confirm that the choice of soil type and/or the environmental conditions of the experiments with elevated CO_2 can affect the outcome. It is, therefore, difficult to extrapolate results gained from greenhouse experiments to natural conditions (see also Körner 2000). The surplus C in the elevated CO_2 treatment was mainly stored as sugar and not as starch as predicted. However, since sugar and starch are both easily digested carbohydrates for insect herbivores, the surplus of carbohydrates might positively affect insect growth (Chapman 1998). Finally, the predicted increase in C-based allelochemicals under elevated CO_2 conditions was only marginally significant.

Amplification of CO_2 effects by P fertilisation

N-fixing plants obtain a large amount of their N requirement from the atmosphere and, therefore, N-fixing plants might rather be P-limited than N-limited and interactive responses of P availability and elevated atmospheric CO_2 are likely (Stöcklin et al. 1998; Stöcklin and Körner 1999). The predicted interactive effect of P fertilisation and CO_2 enrichment on the growth of *L. corniculatus* was significant for root dry mass and some marginal effects (for details see Results). As suggested by Stöcklin and Körner (1999), nutrient availability has an

Table 3 ANOVA for larval, pupal and adult fresh mass and development time of *Polyommatus icarus* reared on *Lotus corniculatus* plants grown under ambient or elevated CO_2 conditions with or without P fertilisation

Source of variation	Larval mass after 12 days				Pupal fresh mass				Adult fresh mass				Development time			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
CO_2	1	3,270.1	4.55	0.056	1	190.7	2.33	0.155	1	25.5	1.65	0.225	1	3.7	1.31	0.277
Female genotype (FG)	5	70.5	0.10	0.990	5	847.6	10.36	<0.001	5	192.0	12.42	<0.001	5	2.6	0.91	0.507
CO_2×FG	5	1,099.8	1.53	0.258	5	43.1	0.53	0.752	5	32.7	2.12	0.139	5	16.2	5.71	0.008
Covariate sex (S)	1	8,794.2	12.25	0.005	1	517.3	6.32	0.029	1	62.3	4.03	0.070	1	20.7	7.32	0.020
Plot (P)	11	718.1	2.34	0.035	11	81.8	1.52	0.176	11	15.5	0.91	0.546	11	2.8	0.39	0.950
Fertilisation (F)	1	7,028.0	22.88	<0.001	1	207.3	3.84	0.059	1	21.2	1.24	0.274	1	50.6	7.02	0.013
CO_2×F	1	391.2	1.27	0.269	1	32.8	0.61	0.441	1	53.6	3.14	0.086	1	0.5	0.07	0.792
FG×F	5	792.6	2.58	0.049	5	372.2	6.90	<0.001	5	69.2	4.05	0.006	5	10.8	1.49	0.220
CO_2×FG×F	5	291.4	0.95	0.466	5	205.3	3.80	0.008	5	51.8	3.03	0.024	5	8.6	1.20	0.333
S	1	1,278.1	4.16	0.051	1	442.0	8.19	0.007	1	1.9	0.11	0.738	1	0.8	0.11	0.738
Error (residual)	27	307.2	1.11		31	54.0			31	17.1			31	7.2		

over-riding effect on CO_2 responsiveness, and species-specific responses in biomass to elevated CO_2 and fertilisation will affect community structure and biodiversity by suppressing the less responsive species. Thus, N-fixing plants may play a very important role in a future CO_2-enriched environment (Cotrufo et al. 1998). Furthermore, as predicted in our second hypothesis, the growth of *L. corniculatus* plants was enhanced by P fertilisation. Fertilised plants had more shoots, leaves and a higher dry mass. Also, most other plant characteristics were increased (Table 2). Probably a combination of all these changes did affect insect growth positively. *P. icarus* larvae feeding on fertilised plants had higher weights and developed faster. However, the difference in larval weight disappeared in the adult stage, indicating compensatory feeding (Bezemer and Jones 1998).

The effect of herbivory on *L. corniculatus*

The feeding of *P. icarus* larvae on *L. corniculatus* plants had several, probably connected effects. Plants with herbivore damage had less leaves but their dry mass was the same. This indicates compensatory growth after herbivory which has already been found in this plant species (Bazin et al. 2002). Since young or fresh leaves have higher protein concentrations (e.g. Gleadow and Woodrow 2000; Alonso and Herrera 2002; Riipi et al. 2002), compensatory growth might also explain the higher N concentration in *L. corniculatus* leaves with herbivore damage. The lower starch concentration in *L. corniculatus* plants with herbivore damage indicates the preference for digestible C by *P. icarus* larvae. Herbivores feeding on N-fixing legumes might rather be limited by the accessibility of digestible carbohydrates than by protein (Goverde et al. 1999), and plants with higher starch concentrations are more digestible for herbivores (Slansky and Rodriguez 1987; Lincoln et al. 1993). Finally, the marginal increase in condensed tannin concentrations in *L. corniculatus* plants with herbivore damage may indicate a potential of the plant to show an induced response (Schoonhoven et al. 1998).

Effects on insect performance

Especially the higher C/N ratio of plants grown under elevated CO_2 conditions reduces the tissue quality for herbivorous insects. This reduction in food-plant quality is likely to reduce herbivore fitness (Bezemer and Jones 1998; Stacey and Fellowes 2002). We predicted a positive effect of elevated CO_2 on insect performance, since such an effect had been found in greenhouse studies for the same insect-plant system (Goverde et al. 1999; Bazin et al. 2002). However, the present study does not support this prediction. Elevated CO_2 had no effect on pupal and adult mass, but a marginally negative effect on larval mass after 12 days—which, however, disappears when using larval mass at the beginning as a covariate. These results differ clearly from previous ones from a growth-chamber study where mass gain of *P. icarus* larvae was increased under elevated CO_2 conditions (Goverde et al. 1999). As mentioned above, the choice of a low-fertility soil and the environmental conditions did affect the outcome of the experiment.

Larval genotype matters

In the present study we found insect genotype-specific responses to elevated CO_2 and to P fertilisation. The effect of elevated CO_2 on the development time of *P. icarus* depended on the origin of the larvae. Larvae of two mothers prolonged their development time while larvae of three mothers did not react, and the larvae from one mother even accelerated their development time under elevated CO_2 conditions. A similar effect was found in the growth chamber and greenhouse study for the same plant-insect system (Goverde et al. 1999; Bazin et al. 2002). CO_2 concentration can thus be considered as a potential

selective factor for some genotypes of this butterfly species if rapid development is advantageous, i.e. a change in selective pressure might affect the genetic diversity of single species. Thus, we find not only species-specific but also genotype-specific responses to elevated CO_2 in insect herbivores (Bezemer and Jones 1998). In addition, larvae from a specific mother reacted more strongly to P fertilisation-induced changes in larval food quality than other larvae (significant interaction between P fertilisation and female genotype). As in the response of development time to elevated CO_2 this finding suggests genetic variation in insect life history traits in response to P fertilisation. Finally, the effect of elevated CO_2 on pupal and adult mass depended not only on the origin of larvae but also on P fertilisation (three-way interaction). A further three-way interaction among CO_2 enrichment, insect genotype and plant genotype does appear for several insect life history traits of *P. icarus* feeding on different *L. corniculatus* genotypes under ambient and elevated CO_2 (Goverde et al. 1999). These results show the complexity of ecological systems and the challenge to investigate real effects of the ongoing increase in atmospheric CO_2 on plant-insect dynamics.

Conclusion

In conclusion, we showed that CO_2 enrichment in SACC chambers affected the growth and chemical composition of *L. corniculatus* plants. These changes in food-plant quality partially affected the development of the butterfly *P. icarus*. Remarkably, the interactive effect of CO_2 enrichment and origin of larvae showed that there is genetic variability in the insect population leading them to react differently to changes in the quality of larval food-plants caused by increased atmospheric CO_2 conditions and P fertilisation. Thus, the effect of environmental changes on insect-plant interactions can depend on genotype-specific responses in both plants and insects. So far, genotype-specific responses in insect-plant interactions have been poorly investigated. However, both are of high relevance for evolutionary processes, i.e. a change in selective pressure will affect the genetic composition within populations of a species and hence the genetic diversity of single species. Our study suggests that rising levels of CO_2 might be such a selective factor, affecting both plant and herbivore populations and their interaction. Therefore, genotype-specific responses must be considered because this will affect the outcome of elevated CO_2 for plant-herbivore interactions.

Acknowledgements We are especially grateful to Kathrin Schweizer, Daniel Bretscher, Monika Wohlfender, Olivier Bignucolo, Susanna Pelaez-Riedl for technical support, to Bernhard Schmid and Christian Körner for providing us with the facility in Nenzlingen. This research was supported by grants from the Swiss Priority Program Environment of the Swiss National Science Foundation to A. Erhardt (no. 5001–044622/1) and to C. Körner (no. 5001–035214).

References

Alonso C, Herrera CM (2002) Seasonal variation in leaf characteristics and food selection by larval noctuids on an evergreen Mediterranean shrub. Acta Oecol 21:257–265

Ayres MP (1993) Plant defense, herbivory, and climate change. In: Kareiva PM, Kingsolver JG, Huey RB (eds) Biotic interactions and global change. Sinauer, Mass., pp 75–94

Bazin A, Goverde M, Erhardt A, Shykoff JA (2002) Influence of atmospheric CO_2 enrichment on induced defence and growth compensation after herbivore damage in *Lotus corniculatus*. Ecol Entomol 27:271–278

Bezemer TM, Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. Oikos 82:212–222

Bezemer TM, Jones TH, Knight KJ (1998) Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. Oecologia 116:128–135

Bezemer TM, Knight KJ, Newington JE, Jones TH (1999) How general are aphid responses to elevated atmospheric CO_2? Ann Entomol Soc Am 92:724–730

Chapman RF (1998) The insects. Structure and function. Cambridge University Press, Cambridge

Conroy JP, Milham PJ, Barlow WR (1992) Effect of nitrogen and phosphor availability on the growth response of *Eucalyptus grandis* to high CO_2. Plant Cell Environ 15:843–847

Cotrufo MF, Ineson P, Scott A (1998) Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biol 4:43–54

Coviella CE, Trumble JT (1999) Effects of elevated atmospheric carbon dioxide on insect-plant interactions. Conserv Biol 13:700–712

Entry JA, Runion GB, Prior SA, Mitchell RJ, Rogers HH (1998) Influence of CO_2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings. Plant Soil 200:3–11

Fajer ED, Bowers MD, Bazzaz FA (1989) The effects of enriched carbon dioxide atmospheres on plant-insect herbivore interactions. Science 243:1198–1200

Gleadow RM, Woodrow IE (2000) Temporal and spatial variation in cyanogenic glycosides in *Eucalyptus cladocalyx*. Tree Physiol 20:591–598

Goverde M, Erhardt A (2003) Effects of elevated CO_2 on development and larval food-plant preference in *Coenonympha pamphilus* (Satyridae). Global Change Biol 9:74–83

Goverde M, Bazin A, Shykoff JA, Erhardt A (1999) Influence of leaf chemistry of *Lotus corniculatus* (Fabaceae) on larval development of *Polyommatus icarus* (Lepidoptera, Lycaenidae): effects of elevated CO_2 and plant genotype. Funct Ecol 13:801–810

Goverde M, Arnone JA, Erhardt A (2002a) Species-specific reactions to elevated CO_2 and nutrient availability in four grass species. Basic Appl Ecol 3:221–227

Goverde M, Erhardt A, Niklaus PA (2002b) In situ development of a satyrid butterfly on calcareous grassland exposed to elevated carbon dioxide. Ecology 83:1399–1411

Hättenschwiler S, Schafellner C (1999) Opposing effects of elevated CO_2 and N deposition on *Lymantria monacha* larvae feeding on spruce trees. Oecologia 118:210–217

Jones TH, Thompson LJ, Lawton JH, Bezemer TM, Bargett RD, Blackburn TM, Bruce KD, Cannon PF, Hall GS, Hartley SE, Howson G, Jones CG, Kampichler C, Kandeler E, Ritchie DA (1998) Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. Science 280:441–443

Kerslake JE, Woodin SJ, Hartley SE (1998) Effects of carbon dioxide and nitrogen enrichment on a plant-insect interaction: the quality of *Calluna vulgaris* as a host for *Operophtera brumata*. New Phytol 140:43–53

Kinney KK, Lindroth RL, Jung SM, Nordheim EV (1997) Effects of CO_2 and NO_3^- availability on deciduous trees: phytochemistry and insect performance. Ecology 78:215–230

Körner C (1995) Biodiversity and CO_2: global change is under way. Gaia 4:234–243
Körner C (2000) Biosphere responses to CO_2-enrichment. Ecol Appl 10:1590–1619
Körner C, Miglietta F (1994) Long-term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. Oecologia 99:343–351
Lambert JL, Ramasamy J, Paukstelis JV (1975) Stable reagents for the colorimetric determination of cyanide by the modified Konig reactions. Anal Chem 47:916–918
Leadley PW, Stöcklin J (1996) Effects of elevated CO_2 on model calcareous grasslands: community, species, and genotype level responses. Global Change Biol 2:389–397
Leadley PW, Niklaus P, Stocker R, Körner C (1997) Screen-aided CO_2 control (SACC): a middle ground between FACE and open-top chambers. Acta Oecol 18:207–219
Leadley PW, Niklaus PA, Stocker R, Körner C (1999) A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. Oecologia 118:39–49
Lincoln DE, Fajer ED, Johnson HJ (1993) Plant-insect herbivore interactions in elevated CO_2 environments. Trends Ecol Evol 8:64–68
Lindroth RL, Roth S, Nordheim EV (2001) Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO_2 enrichment. Oecologia 126:371–379
Mansfield JL, Curtis PS, Zak DR, Pregitzer KS (1999) Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO_2 and in high- and low-fertility soil. Am J Bot 86:1154–1159
Mevi-Schütz J, Goverde M, Erhardt A (2003) Effects of fertilisation and elevated CO_2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus*. 54:36–43
Payne RW (1993) Genstat 5, Release 3. Reference Manual. Clarendon Press, Oxford
Peters RH (1993) A critique for ecology. Cambridge University Press, Cambridge
Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223–230
Price ML, Butler LG (1977) Rapid visual estimations and spectrophotometric determination of tannin content of sorghum grain. J Agric Food Chem 25:1269–1273
Riipi M, Ossipov V, Lempa K, Haukioja E, Koricheva J, Ossipova S, Pihlaja K (2002) Seasonal changes in birch leaf chemistry: are there trade-offs between leaf growth and accumulation of phenolics? Oecologia 130:380–390
Roumet C, Laurent G, Roy J (1999) Leaf structure and chemical composition as affected by elevated CO_2: genotypic responses of two perennial grasses. New Phytol 143:73–81
Schläpfer M, Zoller H, Körner C (1998) Influences of mowing and grazing on plant species composition in calcareous grassland. Bot Helv 108:57–67
Schoonhoven LM, Jermy T, van Loon JJH (1998) Insect-plant biology. Chapman & Hall, London
Slansky F Jr, Rodriguez JG (1987) Nutritional ecology of insects, mites, spiders, and related invertebrates. Wiley, New York
Stacey DA, Fellowes DE (2002) Influence of elevated CO_2 on interspecific interactions at higher trophic levels. Global Change Biol 8:668–678
Stiling P, Moon DC, Hunter MD, Colson J, Rossi AM, Hymus GJ, Drake BG (2003) Elevated CO_2 lowers relative and absolute herbivore density across all species of a scrub-oak forest. Oecologia 134:82–87
Stöcklin J, Körner C (1999) Interactive effects of elevated CO_2, P availability and legume presence on calcareous grassland: results of a glasshouse experiment. Funct Ecol 13:200–209
Stöcklin J, Schweizer K, Körner C (1998) Effects of elevated CO_2 and phosphorus addition on productivity and community composition of intact monoliths from calcareous grassland. Oecologia 116:50–56
Watanabe FS, Olsen SR (1965) Test of an ascorbic acid method for determing phosphorus water and NAH-CO_3 extracts from soil. Soil Sci Soc Am Proc 29:677–678
Watt AD, Whittaker JB, Docherty M, Brooks G, Lindsay E, Salt DT (1995) The impact of elevated atmospheric CO_2 on insect herbivores. In: Harrington R, Stork NE (eds) Insects in a changing environment. Academic Press, London, pp 197–217
Williams RS, Thomas RB, Strain BR, Lincoln DE (1997) Effects of elevated CO_2, soil nutrient levels, and foliage age on the performance of two generations of *Neodiprion lecontei* (Hymenoptera: Diprionidae) feeding on loblolly pine. Environ Entomol 26:1312–1322
Wong SC (1990) Elevated atmospheric partial pressure of CO_2 and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. Photosynth Res 23:171–180
Zar JH (1999) Biostatistical analysis. Prentice Hall, N.J.