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Effects of elevated CO_2 on flowering phenology and nectar production of nectar plants important for butterflies of calcareous grasslands

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Abstract Effects of elevated CO_2 on flowering phenology and nectar production were investigated in *Trifolium pratense*, *Lotus corniculatus*, *Scabiosa columbaria*, *Centaurea jacea* and *Betonica officinalis*, which are all important nectar plants for butterflies. In glasshouse experiments, juvenile plants were exposed to ambient (350 $\mu l\ l^{-1}$) and elevated (660 $\mu l\ l^{-1}$) CO_2 concentrations for 60–80 days. Elevated CO_2 significantly enhanced the development of flower buds in *C. jacea*. *B. officinalis* flowered earlier and *L. corniculatus* produced more flowers under elevated CO_2. In contrast, the number of flowers decreased in *T. pratense*. The amount of nectar per flower was not affected by elevated CO_2 in the tested legumes (*T. pratense* and *L. corniculatus*), but was significantly reduced (!) in the other forbs. Elevated CO_2 did not significantly affect nectar sugar concentration and composition. However, *S. columbaria* and *C. jacea* produced significantly less total sugar under elevated CO_2. The nectar amino acid concentration remained unaffected in all investigated plant species, whereas the total of amino acids produced per flower was reduced in all non-legumes. In addition, the amino acid composition changed significantly in all investigated species except for *C. jacea*. The observed effects are unexpected and are a potential threat to flower visitors such as most butterflies which have no alternative food resources to nectar. Changes in nectar production due to elevated CO_2 could also have generally detrimental effects on the interactions of flowers and their pollinators.

Key words Elevated CO_2 · Phenology · Nectar · Sugar · Amino acids

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Introduction

The atmospheric CO_2 concentration is expected to increase from the present ambient level of 345 $\mu l\ l^{-1}$ to 650 $\mu l\ l^{-1}$ within the next 50–75 years (Hanson et al. 1981). Several recent studies have shown that plants grown under enriched CO_2 conditions generally contain less nitrogen, a limiting nutrient for insect larval development (Mattson 1980), than those grown under ambient CO_2 (Lincoln et al. 1986; Osbrink et al. 1987; Fajer 1989; Johnson and Lincoln 1990; Lindroth et al. 1995). Consequently, Lepidoptera larvae reared on plants grown under elevated CO_2 for their entire larval period suffer from reductions in fitness-related parameters such as growth rates and pupal weight, increased mortality and longer development times (Osbrink et al. 1987; Akey and Kimball 1989; Fajer et al. 1991; Lindroth et al. 1995). However, elevated CO_2 will not only affect interactions between larvae and their host plants (Lincoln et al. 1986; Osbrink et al. 1987; Fajer et al. 1989; Johnson and Lincoln 1990; Lindroth et al. 1995), but also interactions of adult butterflies with their nectar plants.

Most butterflies are frequently seen feeding on flowers and for many butterfly species nectar is the most important food in the adult stage. Although nectar contains a wide variety of chemical constituents, three sugars – glucose, fructose and sucrose – dominate the solutes (Baker and Baker 1975, 1983). Free amino acids are also regularly found in floral nectar and can play a significant role for pollinators (Baker and Baker 1982, 1986). In addition, Baker and Baker (1975, 1982, 1990) found the composition of nectar to be remarkably constant within species. The same authors also detected great variation in nectar composition between plant species, in particular in the proportions of sugars and in amino acid concentrations. These findings suggested that different pollinator classes select for different nectar types according to their specific preferences and physiological requirements (Baker and Baker 1975, 1983, 1990).

The expected increase in atmospheric CO_2 concentration could cause changes in the phenology and nectar quantity and quality of nectar-producing plants. Such changes could affect the fecundity and longevity of nectar-feeding butterflies and could even further reduce the fitness of butterflies which may already suffer in their larval stage from losses in quality of their host plants due to elevated CO_2 (Osbrink et al. 1987; Akey and Kimball 1989; Fajer et al. 1991; Lindroth et al. 1995), since several studies have shown that nectar feeding in butterflies contributes to somatic maintenance and increases longevity and reproduction (Gilbert 1972; Dunlap-Pianka et al. 1977; Boggs 1986, 1988; Karlsson 1987; Hill 1989; Hill and Pierce 1989; Lederhouse et al. 1990). However, except for one study (Rathcke 1992), no investigation has examined the response of nectar production to elevated CO_2.

The objective of the present study was therefore to determine effects of elevated CO_2 on flowering phenology and nectar production in nectar plants important for butterflies, in order to assess possible effects of elevated CO_2 on the interactions between butterflies and their nectar plants. Extended field work in the Jura mountains has shown that five plant species, i.e. red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.), knapweed (*Centaurea jacea* L.), small scabious (*Scabiosa columbaria* L.) and betony (*Betonica officinalis* L.) are all important nectar plants for butterflies and can even be called keystone nectar plants for butterflies in calcareous grasslands (H.P. Rusterholz and A. Erhardt, unpublished data). Since the flowers of these plant species are also visited and pollinated by a variety of other insects (Müller 1873; Knuth 1898–1905), the findings of the present study are also generally relevant for interactions between flowers and their pollinators. While only nectar volume and sugar concentration were measured in the study of Rathcke (1992), nectar sugar composition as well as amino acid concentration and composition were also determined in the present study.

Materials and methods

Plant material

Plants of *T. pratense* and *L. corniculatus* as representatives of legumes and of *C. jacea*, *S. columbaria* and *B. officinalis* as representatives for non-leguminous nectar plants were grown from seeds collected from the field site Vicques, situated in the northern part of the Swiss Jura mountains. The seeds were germinated in typical calcareous substratum (sieved humus/marly soil/sand 5:1:1). After 2 weeks, seedlings were transplanted to pots (10 cm diameter) with the same substratum and were grown for 1 month in a cold-glasshouse, before being used for the experiments.

Experimental procedure

In total, 40 plants of each species of the same age and size were transferred to daylight growth cabinets for a period of 60–80 days until they flowered. Plants were grown under controlled environmental conditions of 24°C 16 h light/16°C 8 h darkness, at daytime CO_2 concentrations of 350 μl l^{-1} CO_2 and 660 μl l^{-1} CO_2. A photon flux density of 200–250 μmol m^{-2} s^{-1} at plant height was maintained by additional light from two 1000 W mercury vapour lamps outside each chamber. To avoid chamber effects, plants and treatments were exchanged between the two growth cabinets at 10-day intervals. Furthermore, the plants were fertilised once a week with 25 ml 0.5 Hoagland solution per pot.

Data collection

Phenological observations were recorded each day and included the presence of flower buds, the time of the first open flower and the course of anthesis of each individual flower. To standardise nectar sampling, nectar samples from different plants were taken at the same time of day (11.30 a.m.) from flowers of the same age, 4 h after they had been watered (25 ml water/pot). Nectar samples were taken from one selected flower per plant with carefully drawn out glass micropipettes flamed at the tip to avoid scratching the floral tissue. Samples were spotted on filter paper (Whatman no. 1). Nectar volumes were calculated from the spot area of the nectar samples on the filter paper (Baker 1979). Nectar sugar concentration and composition were determined with the aid of high-performance anion exchange chromatography with pulsed amperometric detection (Martens and Frankenberger 1990). Calculations of the concentrations of individual nectar sugars were based on internal standardisation with trehalose. Amino acids were analysed as their AccQ derivatives according to Cohen and Micheaud (1993), and were separated by reverse-phase HPLC chromatography. Sample injection was 20 μl, run time 50 min at 37°C. Amino acids were identified by comparing retention time in the sample against standards, and quantified by comparing the areas of the individual peaks. This procedure allowed identification and quantification of the following 24 amino acids: alanine, γ-amino-butyric acid, arginine, asparagine, aspartic acid, citruline, cysteine, glutamine, glutamic acid, glycine, histidine, hydoxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. However, several components such as unknown amino acids or proteins appearing in the chromatograms of the nectar samples could not be identified. After nectar sampling was finished, the plants were harvested and separated into leaves, roots and flowers for biomass measurements.

Data analysis

Only means ± SE per species and treatment are presented in the figures and tables. Flowering probability for each species was calculated by dividing the number of individuals which bloomed during the experimental period by the total number of individuals in the experiment. The influence of elevated CO_2 on the flowering probabilities was tested with contingency analysis. The effect of elevated CO_2 on the time to produce the first open flower was analysed with the Wilcoxon rank sum test.

Differences between species and the species-CO_2 interaction were tested using analyses of variance. The factor CO_2 was tested against the residual error term (df 1,150), the species effect was tested against the species-CO_2 interaction (df 4,4), and the species-CO_2 interaction was tested against the residual error term (df 4,150). The single plant species were also analysed separately. Since these data were not normally distributed, the Wilcoxon rank sum test was used to determine the effects between the CO_2 treatments. To reduce the overall probability of type I error caused by multiple testing, sequential Bonferroni tests (Rice 1989; Zolman 1993) with a significant level $\alpha = 0.05$ were conducted on both nectar sugar and amino acid data sets. The statistical analyses were conducted with the software packages JMP version 3.1 (SAS 1994) and Genstat Release 3 (McCullagh and Nelder 1989; Payne and Lane 1993; Payne et al. 1993).

Results

Biomass development and flowering phenology

Elevated CO_2 tended to increase the vegetative biomass of the investigated plant species. However, neither the overall CO_2 effect, nor the differences between the species, nor the species by CO_2 interaction were significant with regard to the vegetative biomass at final harvest (data not shown). Except for *C. jacea*, no significant differences were found in the proportion of plants in bloom under the different CO_2 treatments (Table 1). Furthermore, elevated CO_2 caused *B. officinalis* to flower 1 week earlier, and *L. corniculatus* to produce more flowers. In contrast, the number of flowers was significantly reduced in *T. pratense* under enriched CO_2 conditions (Table 1).

Nectar secretion and nectar composition

Nectar sugar

All non-leguminous plant species showed a significant reduction in nectar volume per flower under elevated CO_2 (Table 1), whereas nectar volumes did not change significantly in the tested legumes (Table 1). Total nectar sugar concentration (Table 1) as well as the concentration of the single nectar sugars, glucose, fructose and sucrose – no other nectar sugars could be detected in the investigated plant species – tended to increase under elevated CO_2 (Table 2). However, these trends were not significant in any of the tested plant species. Thus, the nectar sugar composition of the investigated plant species was not significantly affected by elevated CO_2 (Table 2). In contrast, total sugar production was significantly changed under elevated CO_2. Analysis of variance indicated an overall significant CO_2 effect ($P < 0.05$), a significant difference between species ($P < 0.05$) and a significant species by CO_2 interaction in the amount of secreted glucose, fructose and sucrose per flower ($P < 0.05$). The responses in the nectar sugar production of the individual species to elevated CO_2 are shown in Fig. 1. While increases in nectar sugars in the two legumes (*L. corniculatus*, *T. pratense*) and in *B. officinalis* were not significant, sugar production was significantly reduced by 40–50% under enriched CO_2 conditions in *S. columbaria* and *C. jacea* (Fig. 1).

Nectar amino acids

The total amino acid concentration in floral nectar of the tested plant species remained constant under enriched CO_2 conditions (Table 3). However, the total amount of amino acids was significantly affected by elevated CO_2. Analysis of variance showed an overall significant CO_2 effect ($P < 0.05$) and a highly significant

Table 1 Flowering probability, time to produce the first flower (*Time to flower*), number of flowers per plant, amount of floral nectar per flower and total nectar sugar concentration (weight to total weight) of *Lotus corniculatus*, *Trifolium pratense*, *Betonica officinalis*, *Scabiosa columbaria* and *Centaurea jacea* under *ambient* (350 µl l^{-1}) and under *elevated* (650 µl l^{-1}) atmospheric CO_2 concentration. Values are the mean ± SE

	Flowering probability		Time to flower (days)		Number of flowers per plant		Nectar volume (µl)		Sugar concentration (%)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Lotus corniculatus (ambient *n* = 15; elevated *n* = 16)	0.75	0.80	61.0 ± 5.0	61.2 ± 3.2	24.3 ± 3.0	38.9 ± 5.2**	0.44 ± 0.05	0.42 ± 0.04	49.2 ± 2.9	55.3 ± 1.7
Trifolium pratense (ambient *n* = 19; elevated *n* = 16)	0.95	0.80	49.7 ± 2.3	51.2 ± 2.5	7.2 ± 0.7	4.8 ± 0.8**	0.57 ± 0.04	0.52 ± 0.06	28.4 ± 2.1	32.2 ± 1.4
Betonica officinalis (ambient *n* = 15; elevated *n* = 16)	0.75	0.80	45.2 ± 2.1	37.4 ± 0.9**	6.7 ± 0.6	7.8 ± 0.7	1.59 ± 0.13	1.15 ± 0.10*	54.0 ± 1.4	57.6 ± 0.6
Scabiosa columbaria (ambient *n* = 14; elevated *n* = 17)	0.70	0.85	56.8 ± 1.7	60.0 ± 1.6	4.5 ± 0.6	3.9 ± 0.5	0.22 ± 0.02	0.11 ± 0.11***	56.7 ± 0.9	59.9 ± 0.6
Centaurea jacea (ambient n = 13; elevated n = 20)	0.65	1.0***	70.7 ± 1.9	71.3 ± 0.9	2.8 ± 0.2	2.8 ± 0.1	0.32 ± 0.03	0.20 ± 0.02**	48.4 ± 1.9	52.4 ± 1.5

* $P < 0.1$; ** $P < 0.01$; *** $P < 0.001$

Table 2 Nectar sugar composition of *L. corniculatus*, *T. pratense*, *B. officinalis*, *S. columbaria* and *C. jacea* under *ambient* (350 μl l^{-1}) and under *elevated* (650 μl l^{-1}) atmospheric CO_2 concentration. Values are the mean ± SE

	μg glucose/μl nectar		μg fructose/μl nectar		μg sucrose/μl nectar		Glucose/fructose		Sucrose/(glucose + fructose)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
L. corniculatus (ambient *n* = 15; elevated *n* = 16)	219.1 ± 35.7	258.9 ± 27.7	225.3 ± 39.5	259.1 ± 27.7	1442.5 ± 187.0	2039.1 ± 295.8	1.0 ± 0.02:1	1.0 ± 0.01:1	3.7 ± 0.3:1	3.8 ± 0.2:1
T. pratense (ambient *n* = 19; elevated *n* = 16)	41.6 ± 4.1	61.0 ± 11.5	67.4 ± 6.7	97.5 ± 19.0	557.1 ± 78.6	613.2 ± 98.2	0.7 ± 0.02:1	0.7 ± 0.02:1	5.2 ± 0.5:1	5.2 ± 0.8:1
B. officinalis (ambient *n* = 15; elevated *n* = 16)	55.4 ± 6.5	98.3 ± 16.5*	177.5 ± 24.5	268.5 ± 33.0*	1288.5 ± 174.0	1918.0 ± 200.7*	0.3 ± 0.02:1	0.4 ± 0.02:1	5.7 ± 0.4:1	5.6 ± 0.5:1
S. columbaria (ambient *n* = 14; elevated *n* = 17)	458.8 ± 57.3	477.4 ± 57.5	381.0 ± 56.7	393.0 ± 49.6	758.3 ± 88.2	910.5 ± 94.6	1.2 ± 0.01:1	1.3 ± 0.03:1	1.0 ± 0.2:1	1.3 ± 0.1:1
C. jacea (ambient *n* = 13; elevated *n* = 20)	92.2 ± 19.3	93.5 ± 11.7	86.6 ± 19.9	84.9 ± 11.5	1056.2 ± 91.3	1265.0 ± 123.6	1.1 ± 0.03:1	1.1 ± 0.05:1	7.7 ± 0.8:1	7.7 ± 0.6:1

*$P < 0.1$

difference between species ($P < 0.001$). In contrast to the nectar sugars, the species by CO_2 interaction was not significant ($P = 0.23$).

Elevated CO_2 caused significant reductions in total amino acids per flower in all tested non-legumes (*B. officinalis*, *S. columbaria* and *C. jacea*; Fig. 2), whereas in both legumes (*L. corniculatus* and *T. pratense*), the total amino acids slightly increased (Fig. 2). Furthermore, elevated CO_2 caused significant shifts in the amino acid composition in the tested plant species except for *C. jacea* (Table 3). In each species, a limited number of the amino acids (asparagine, aspartic and glutamic acid, glutamine, glycine, hydoxyproline, phenylalanine, proline, threonine and tyrosine) accounted for most of the total amino acid concentration. The enriched CO_2 conditions mainly caused changes in these dominant amino acids (Table 3). In *L. corniculatus*, the fraction of cysteine, lysine, methionine and proline decreased under elevated CO_2, whereas asparagine, aspartic acid, hydoxyproline and tyrosine increased. In nectar of *T. pratense*, elevated CO_2 caused a significant reduction in aspartic acid, asparagine, glutamic acid, glycine, isoleucine, leucine, phenylalanine, tyrosine and valine, whereas the main component, proline, increased. The two dominant amino acids in the nectar of *B. officinalis* – hydoxyproline and proline – decreased under elevated CO_2, but the fractions of asparagine and isoleucine doubled and phenylalanine even tripled. In *S. columbaria* nectar, enriched CO_2 caused a significant increase in alanine and glutamine and a reduction in glutamic acid. In contrast to the other species, the amino acid composition in the nectar of *Centaurea jacea* was not affected by elevated CO_2.

Discussion

The observed effects of elevated CO_2 on flowering phenology were relatively small in the present experiment. Elevated CO_2 did not significantly affect the vegetative and/or reproductive biomass of any of the investigated plant species. This finding differs from several other studies in which most plant species responded with higher biomass production under enriched CO_2 conditions (Sionit et al. 1985; Nijs et al. 1988; Arnone and Gordon 1990). However, Tolley and Strain (1984) reported no significant increase in biomass of *Pinus taeda* seedlings under elevated CO_2. Furthermore, Leadly and Stöcklin (1996) showed that calcareous grassland plant species respond differentially in their biomass production to elevated CO_2, including neutral, negative and positive responses. These findings suggest that plant species generally differ in their biomass production under elevated CO_2. The missing increase in biomass production under elevated CO_2 in the plants investigated in the present study could also have been caused by the relatively low light intensity (200–250 PPFD) during the experiment. Since biomass was not significantly affected by elevated CO_2, the higher flowering

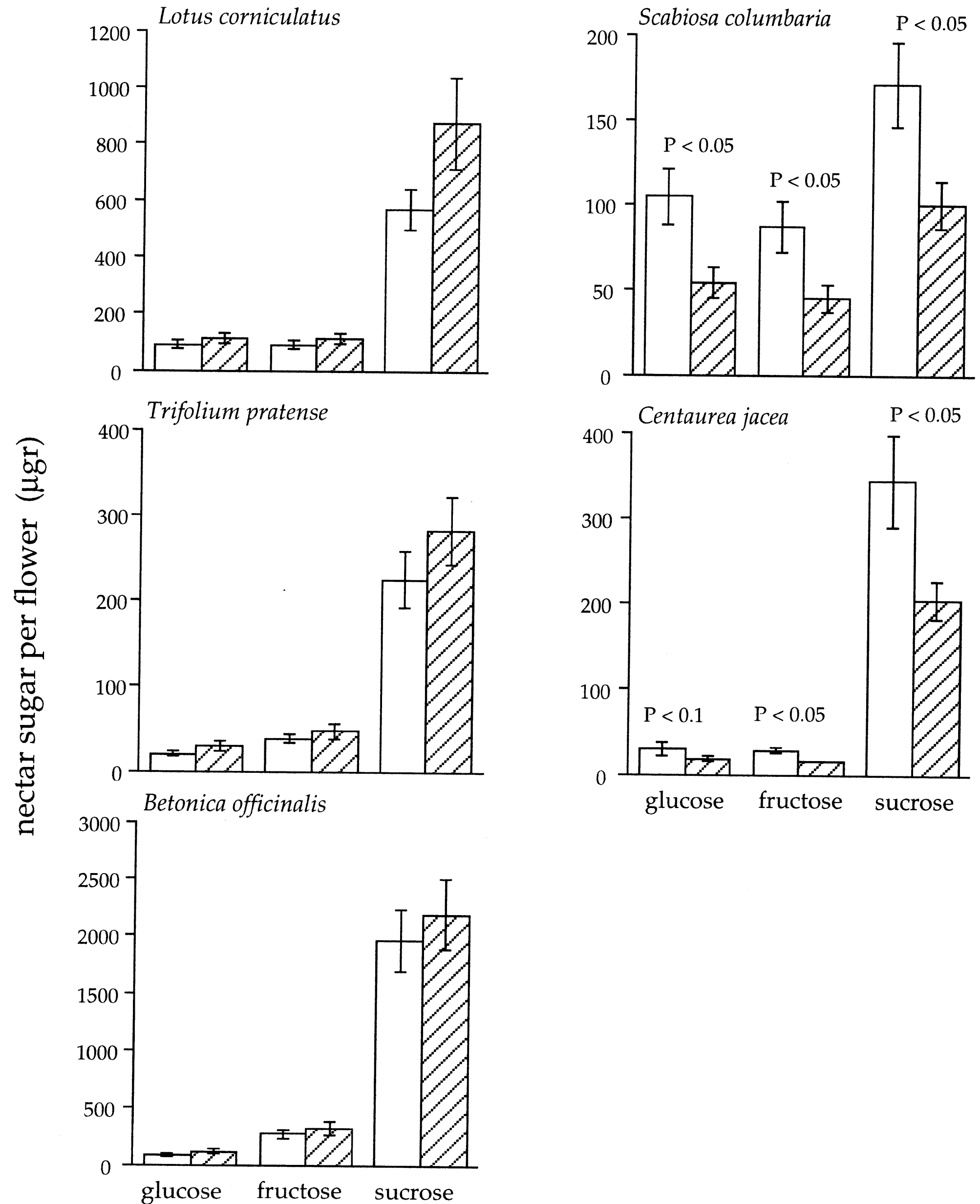

Fig. 1 Amount of glucose, fructose and sucrose (μg) per flower in floral nectar of *Lotus corniculatus*, *Trifolium pratense*, *Betonica officinalis*, *Scabiosa columbaria* and *Centaurea jacea* under ambient (350 μl l^{-1}, *open bars*) and elevated CO_2 (660 μl · l^{-1}, *hatched bars*). Mean values ± SE are shown (n = 15/16 *L. corniculatus*, 19/16 *T. pratense*, 15/16 *B. officinalis*, 14/17 *S. columbaria* and 13/20 *C. jacea*)

probabiliy in *C. jacea*, the increased number of flowers in *L. corniculatus* and the opposite reaction in *T. pratense* must have other causes. However, the present results parallel the findings of an earlier investigation on four annual plant species (Reekie and Bazzaz 1991). On the other hand, they are in disagreement with a later study of Reekie et al. (1994), in which earlier flowering and a higher reproductive biomass production of four long-day plant species were caused by higher vegetative biomass production under elevated CO_2. The shorter period of time taken by *B. officinalis* to attain anthesis in our experiment corresponds to the earlier flower bud development in perennial long-day plants under elevated CO_2 (Reekie et al. 1994). However, time to reach anthesis remained unchanged in the plants investigated by Reekie et al. (1994).

In contrast to effects on flowering phenology, effects of elevated CO_2 on nectar sugar and amino acid production were more pronounced. The reduction in nectar volume, total sugar and total amino acids per flower in the three investigated non-leguminous forbs under elevated CO_2 was unexpected, as was the absence of any effects of elevated CO_2 in these nectar parameters in the two investigated legumes. These results contrast with the findings of Rathcke (1992) in *Ipomoea purpurea*, which produced more nectar per flower under elevated CO_2. Nectar sugar concentration remained unchanged in *I. purpurea* (Rathcke 1992). However, effects of elevated CO_2 on amino acid concentration and composition were not studied in this species. Furthermore, *I. purpurea* is annual whereas the plant species investigated in the present study are all perennial.

Table 3 Total amino acid concentration (pmol/μl nectar) and amino acid composition (%) in floral nectar of *L. corniculatus*, *T. pratense*, *B. officinalis*, *S. columbaria* and *C. jacea* under ambient (350 μl l^{-1}) and elevated (650 μl l^{-1}) atmospheric CO_2 concentration. Values are the mean ± SE

	L. corniculatus		*T. pratense*		*B. officinalis*		*S. columbaria*		*C. jacea*	
	Ambient (n = 12)	Elevated (n = 12)	Ambient (n = 17)	Elevated (n = 17)	Ambient (n = 17)	Elevated (n = 17)	Ambient (n = 7)	Elevated (n = 11)	Ambient (n = 12)	Elevated (n = 12)
Amino acid concentration (pmol/μl)	1059.9 ± 122.4	1259.9 ± 201.4	727.1 ± 67.5	780.9 ± 81.9	1315.7 ± 145.2	1464.1 ± 154.8	488.7 ± 51.5	483.9 ± 52.7	818.2 ± 119.1	788.2 ± 100.2
Amino acid composition (%)										
Alanine	8.3 ± 0.87	7.0 ± 0.16	3.7 ± 0.32	3.5 ± 0.30	3.5 ± 0.32	4.7 ± 0.82	27.7 ± 4.1	35.6 ± 0.8**	8.0 ± 1.1	9.9 ± 1.0
Arginine	1.2 ± 0.16	1.1 ± 0.14	2.5 ± 0.59	1.7 ± 0.07	1.3 ± 0.06	1.5 ± 0.11	2.8 ± 1.2	1.0 ± 0.05	2.7 ± 0.56	1.9 ± 0.15
Asparagine	0.3 ± 0.16	1.6 ± 0.26***	4.4 ± 1.70	0.8 ± 0.30*	0.2 ± 0.11	0.4 ± 0.09*	–	–	0.9 ± 0.14	0.9 ± 0.11
Aspartic acid	0.5 ± 0.11	1.2 ± 0.17*	1.2 ± 0.26	0.3 ± 0.10*	0.2 ± 0.04	0.3 ± 0.06	7.2 ± 1.7	7.3 ± 0.9	1.3 ± 0.20	1.5 ± 0.21
Cysteine	0.02 ± 0.01	0.01 ± 0.005*	5.7 ± 0.91	4.5 ± 0.70	1.9 ± 0.65	0.8 ± 0.22	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Glutamine	2.6 ± 0.31	2.5 ± 0.39	1.3 ± 0.19	0.9 ± 0.12	0.7 ± 0.06	0.6 ± 0.07	6.5 ± 0.60	9.0 ± 0.51**	6.6 ± 0.68	7.5 ± 1.13
Glutamic acid	0.9 ± 0.83	1.1 ± 0.22	0.8 ± 0.30	0.2 ± 0.08*	0.1 ± 0.03	0.1 ± 0.3	7.3 ± 1.9	2.1 ± 0.42**	1.0 ± 0.66	0.5 ± 0.14
Glycine	1.0 ± 0.18	0.6 ± 0.10	1.4 ± 0.34	0.5 ± 0.22*	0.2 ± 0.10	0.3 ± 0.08	1.4 ± 0.33	1.5 ± 0.13	1.1 ± 0.29	1.1 ± 0.19
Hydroxyproline	3.0 ± 0.10	6.1 ± 0.15**	20.0 ± 1.77	23.1 ± 0.81	29.5 ± 1.0	25.7 ± 1.0*	10.2 ± 4.7	2.7 ± 0.60	4.9 ± 1.3	3.7 ± 0.48
Isoleucine	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.05	0.1 ± 0.03*	0.03 ± 0.01	0.05 ± 0.01*	0.4 ± 0.06	0.3 ± 0.02	0.3 ± 0.05	0.3 ± 0.04
Leucine	0.2 ± 0.05	0.2 ± 0.03	0.4 ± 0.05	0.2 ± 0.04*	0.1 ± 0.02	0.14 ± 0.02	0.3 ± 0.04	0.3 ± 0.03	0.3 ± 0.05	0.3 ± 0.7
Lysine	0.4 ± 0.06	0.2 ± 0.05*	0.3 ± 0.04	0.2 ± 0.03	0.13 ± 0.03	0.17 ± 0.02	0.5 ± 0.05	0.4 ± 0.01	0.3 ± 0.04	0.4 ± 0.04
Methionine	0.4 ± 0.08	0.2 ± 0.01*	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.003	0.6 ± 0.07	0.5 ± 0.01	0.3 ± 0.05	0.3 ± 0.04
Ornithine	1.7 ± 0.9	0.3 ± 0.47	0.2 ± 0.09	0.1 ± 0.07	0.1 ± 0.09	0.08 ± 0.02	8.6 ± 3.3	4.2 ± 0.57	–	–
Phenylalanine	20.5 ± 2.17	20.8 ± 3.42	0.3 ± 0.08	0.1 ± 0.05*	3.1 ± 1.5	10.9 ± 3.2*	0.3 ± 0.08	0.2 ± 0.01	0.2 ± 0.03	0.2 ± 0.06
Proline	23.9 ± 2.8	16.3 ± 1.28*	48.7 ± 2.65	58.8 ± 1.4**	54.9 ± 1.4	50.2 ± 2.2*	24.8 ± 3.9	29.7 ± 1.3	55.8 ± 2.1	51.4 ± 2.8
Threonine	6.3 ± 0.88	5.8 ± 1.55	4.3 ± 0.73	2.3 ± 0.18	1.8 ± 0.03	1.8 ± 0.11	8.7 ± 2.9	4.9 ± 0.33	16.0 ± 1.5	20.3 ± 3.8
Tyrosine	27.0 ± 3.77	40.3 ± 3.70*	0.5 ± 0.12	0.2 ± 0.05*	0.9 ± 0.06	1.05 ± 0.29	0.5 ± 0.07	0.5 ± 0.01	0.4 ± 0.04	0.4 ± 0.04
Valine	0.6 ± 0.08	0.6 ± 0.10	0.5 ± 0.11	0.2 ± 0.04*	0.05 ± 0.02	0.1 ± 0.02	0.6 ± 0.07	0.5 ± 0.05	0.5 ± 0.11	0.5 ± 0.13

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ [Wilcoxon rank sum test, after correction for multiple tests with sequential Bonferroni method ($\alpha = 0.05$, $k = 19$)]

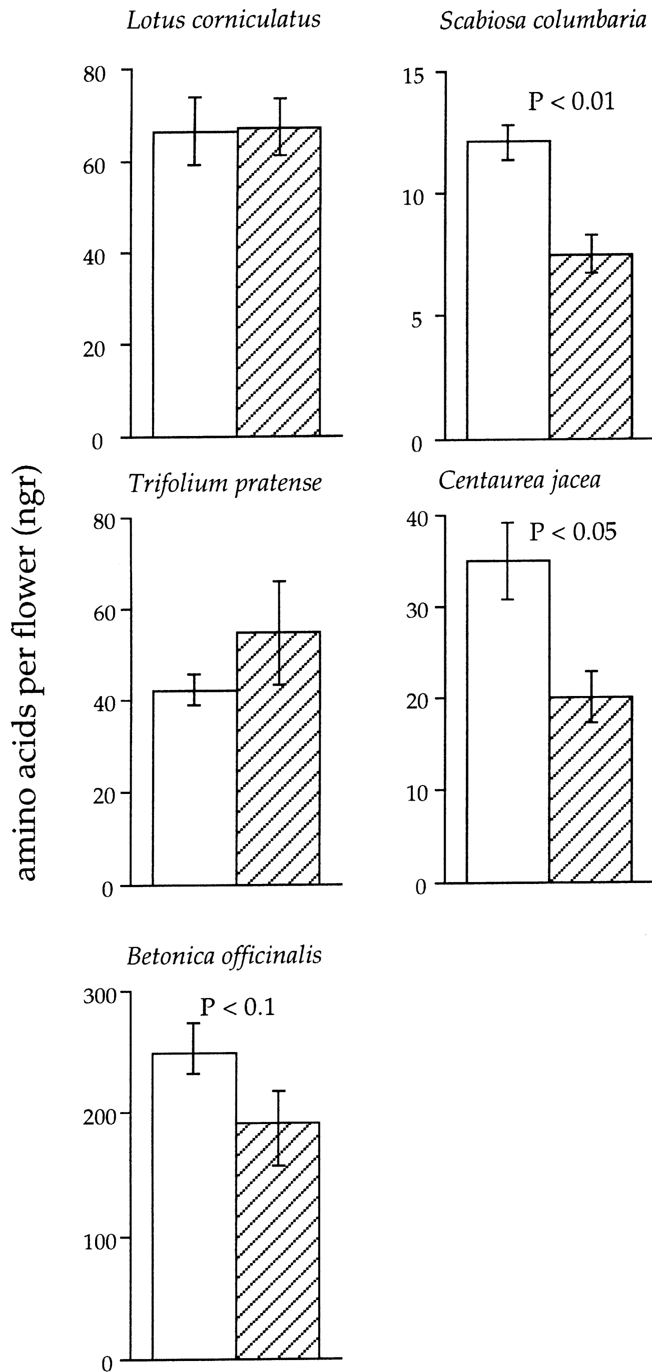

Fig. 2 Amount of nectar amino acids (ng) per flower of *L. corniculatus*, *T. pratense*, *B. officinalis*, *S. columbaria* and *C. jacea* under ambient (350 μl l^{-1}, *open bars*) and elevated (660 μl l^{-1}, *hatched bars*) CO_2. Mean values ± SE are shown (n = 12/12 *L. corniculatus*, 17/17 *T. pratense*, 17/17 *B. officinalis*, 7/11 *S. columbaria* and 12/12 *C. jacea*)

The causes for the observed changes are not clear and must remain speculative at this point. Opler (1983) found a strong positive correlation between flower biomass, nectar volume and sugar quantity produced per flower. However, in the present study, flower size and flower biomass did not change significantly under the different CO_2 regimes (H.P. Rusterholz and A. Erhardt, unpublished data), ruling out floral biomass as a factor for the observed reductions in nectar production. Since vegetative biomass was also not significantly affected by elevated CO_2, it too must be excluded as a potential factor for reduced nectar production in the non-leguminous forbs. Finally, the root/shoot ratio, which is an important factor for water balance and carbon allocation in plants, and which can be changed under elevated CO_2 (Tyree and Alexander 1993) was again not affected by elevated CO_2 in the plants in the present experiments and also fails, therefore, to explain the observed reduced nectar production.

The questions arise, whether the detected changes in nectar production under elevated CO_2 are relevant for nectar-feeding insects, in particular for butterflies which have no alternative food resources to nectar, and what the potential consequences could be. It has repeatedly been shown that restrictions in the adult diet of butterflies can directly reduce their reproductive output (Pivnick and McNeil 1985; Boggs and Ross 1993). For instance, *Colias* butterflies realise only 5% of their maximal reproductive output if they are not fed in the adult stage (Watt et al. 1974). Appropriate sugar resources in the adult diet can have an important effect on longevity and fecundity of females (Hill 1989; Hill and Pierce 1989). Amino acids can also strongly increase longevity and egg production as shown for the famous *Heliconius* butterflies (Gilbert 1972; Dunlap-Pianka et al. 1977). Since females of *Pieris rapae* and *Inachis io* prefer nectar mimics containing amino acids over corresponding plain sugar solutions (Alm et al. 1990; H.P. Rusterholz and A. Erhardt, in preparation), and since females of the Adonis blue, *Lysandra bellargus*, favour flowers with high levels of nectar amino acids (H.P. Rusterholz and A. Erhardt, in preparation), amino acids in the adult food may also generally play an important role for egg maturation in butterflies. Furthermore, males of *Papilio glaucus* increased their reproductive success when they were fed with amino acids (Lederhouse et al. 1990).

Adult feeding can also play an essential role for the energy requirements of butterflies. Species such as *P. rapae* cover over 50% of their energy demand from adult feeding (Gilbert and Singer 1975 and reference therein). Butterflies use up to 20% of their activity time for foraging (Wiklund and Ahrberg 1978; Dennis 1982, 1983). Furthermore, butterflies have distinct flower preferences which seem mainly to be related to nectar quality and quantity (H.P. Rusterholz and A. Erhardt, in preparation). If nectar production is reduced by 30–50% under elevated CO_2 as in *S. columbaria* and *C. jacea*, which are both essential nectar plants for butterflies (H.P. Rusterholz and A. Erhardt, unpublished data), this would cause significant increases in foraging time for butterflies, reducing time for other essential activities such as oviposition, courting and mating, and could therefore indirectly lead to decreases in longevity and reproductive output. This would pose further risks to butterflies in addition to those they are

already exposed to by habitat destruction, habitat fragmentation, habitat deterioration (Erhardt 1995) and deterioration in larval food quality by elevated CO_2 (Lincoln et al. 1986; Osbrink et al. 1987; Fajer 1989, Johnson and Lincoln 1990; Lindroth et al. 1995).

As outlined for butterflies, the observed changes in nectar production by elevated CO_2 could also affect other pollinators, and could further lead to a decreased attraction of flowers, to interspecific shifts in the attraction of flowers for pollinators and consequently to reductions and/or interspecific shifts in seed set.

Although little is known about specific preferences of pollinators for single amino acids (e.g. Potter and Bertin 1988), the observed changes in the nectar amino acid composition could cause changes in the taste of nectar, further disturbing potentially balanced relationships between plants and pollinators. Finally, elevated CO_2 could also affect the scent production of flowers which could have additional detrimental effects on the attraction of plants to their specific pollinators.

In conclusion, the observed changes in nectar production due to elevated CO_2 are not only a potential threat to pollinators such as butterflies, but could also critically disturb coevolved interactions between plants and their pollinators.

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