

Abdelmalek Boutaleb Joutei · Jacques Roy  
Georges Van Impe · Philippe Lebrun

## Effect of elevated CO<sub>2</sub> on the demography of a leaf-sucking mite feeding on bean

Received: 8 May 1999 / Accepted: 4 November 1999

**Abstract** The effect of elevated CO<sub>2</sub> on the demography of the arachnid species *Tetranychus urticae* feeding on *Phaseolus vulgaris* plants was analysed. This class of herbivores (Arachnida) and its feeding guild (cell content feeders) are under-represented in studies of the combined effects of herbivory and CO<sub>2</sub>. The growth of bean was strongly stimulated by elevated CO<sub>2</sub>. The number of leaves on lateral stems and of flowers increased but pod weight decreased. Leaf nitrogen content was 25% lower at elevated CO<sub>2</sub> due to an increase in non-structural sugar concentration. Leaf water content was lower at elevated CO<sub>2</sub> while leaf-specific mass and epidermis thickness were higher. Females of the mite raised at ambient or elevated CO<sub>2</sub>, but all fed with leaves grown at ambient CO<sub>2</sub>, had similar progenies. When females were raised on plants grown at elevated CO<sub>2</sub>, the numbers of their progeny were reduced by 34% and 49% in the first and second generation respectively. Later stages of development were more reduced in elevated CO<sub>2</sub>, suggesting that both fecundity and rate of development were affected. This study suggests that the abundance of *T. urticae*, and consequently the damage to the many crops it infests, might decrease in a future elevated-CO<sub>2</sub> environment.

**Key words** Carbon dioxide enrichment · Demography · Herbivory · *Phaseolus vulgaris* · *Tetranychus urticae*

### Introduction

An increase in CO<sub>2</sub> concentration directly causes an increase in photosynthesis and a decrease in stomatal opening (Drake et al. 1997). Indirectly it can lead to an increase in plant biomass (Bazzaz 1990; Field et al. 1992; Wolfe et al. 1998; Wand et al. 1999) although this increase can be marginal in natural ecosystems with generally low fertility (Körner 1996). A more general indirect effect of elevated CO<sub>2</sub>, found in laboratory experiments (Poorter et al. 1997) as well as in natural vegetation (Körner and Miglietta 1994), is a change in the chemical composition of the plant material that strongly affects plant-herbivore interactions (Lincoln et al. 1993; Watt et al. 1995; Bezemer and Jones 1998).

CO<sub>2</sub>-stimulated carbon fixation results in an accumulation of total non-structural carbohydrates (TNC) in the leaves that “dilutes” the concentration of the other leaf constituents, in particular nitrogen and minerals (Lincoln et al. 1993; Poorter et al. 1997). Nitrogen and mineral concentrations can also be lowered by elevated CO<sub>2</sub> independently of this dilution effect (Poorter et al. 1997). These changes increase the C/N ratio and strongly reduce the dietary quality of plants (Mattson 1980; Scriber and Slansky 1981; Ayres 1993). In many cases, insects fed with plants grown at elevated CO<sub>2</sub> increase their consumption rate, but this compensatory mechanism is often not enough to prevent a reduction in the insects’ growth rate (Lincoln 1993; Lindroth 1996a). Typically, reduced food-processing efficiency is observed with insects fed on plants grown at high CO<sub>2</sub> (Lindroth 1996a; Roth et al. 1998) although adjustments in digestive physiology can occur (Williams et al. 1994, 1998; Hättenschwiler and Schafellner 1999).

Reduction in growth efficiency of ingested food grown at high CO<sub>2</sub> may result from larger amounts of allelochemicals in the diet, due either to increased consumption or to a higher concentration of allelochemicals in plant tissues. The carbon/nutrient balance hypothesis (Bryant et al. 1983) predicts that if the availability of carbon relative to nutrients increases, carbohydrates in

A. Boutaleb Joutei · G. Van Impe · P. Lebrun  
Université catholique de Louvain,  
Unité d’Ecologie et de Biogéographie,  
Place Croix du Sud, 5, B-1348 Louvain-La-Neuve, Belgique

J. Roy (✉)  
Centre d’Ecologie Fonctionnelle et Evolutive,  
Centre National de la Recherche Scientifique,  
F-34293 Montpellier cedex 5, France  
e-mail: roy@cefe.cnrs-mop.fr  
Fax: +33-4-67412138

excess may be diverted to the production of carbon-based secondary metabolites often involved in the deterrence of herbivores (Lambers 1993; Ayres 1993). However, plants grown at high CO<sub>2</sub> do not consistently show an increase in secondary metabolites (Lincoln 1993; Lincoln et al. 1993; Lavola and Julkunen-Tiitto 1994; Lindroth 1996b; Penuelas et al. 1996; Poorter et al. 1997; Penuelas and Estiarte 1998; Roth et al. 1998).

Herbivores preferentially feed on leaves with a high water content (Scriber and Slansky 1981), but reduced stomatal conductance and increased water-use efficiency under elevated CO<sub>2</sub> (Bazzaz 1990) can be associated with either improved or deteriorated tissue water status (Lincoln et al. 1993). Elevated CO<sub>2</sub> can also increase leaf toughness and palatability, partially through an increase in leaf-specific weight and thickness, but this has been rarely studied (Lindroth et al. 1993). Leaf temperature can also be increased at elevated CO<sub>2</sub> and may affect insect development time (Brooks and Whittaker 1998).

Insect herbivores fed on plants grown at elevated CO<sub>2</sub> generally show a reduction in fitness (Lincoln et al. 1993). The longer development times may reduce survival by extending the exposure of individuals to predators, parasitoids and climatic adversity. Larval mortality increase in lepidopteran species with food grown at elevated CO<sub>2</sub> (Fajer et al. 1989; Traw et al. 1996; Lindroth 1996a). A reduction in pupal and/or adult weight, as found with food grown at elevated CO<sub>2</sub> (Lincoln et al. 1993; Traw et al. 1996; Lindroth 1996a), is positively correlated with male spermatophore size and adult female fecundity (Boggs 1981). However, few studies have measured the consequences for population size: the results from short-term studies examining a subset of life-history stages or from laboratory feeding trials with excised leaves are difficult to extrapolate to the population level. However, intergeneration effects (Brooks and Whittaker 1998) and trophic web interactions (Stiling et al. 1999) have been shown.

These general trends in the effect of elevated atmospheric CO<sub>2</sub> on herbivory are derived from studies with a very restrictive taxonomic representation. Among the 61 plant-herbivore interactions reviewed by Bezemer and Jones (1998), 60 focus on insects, mainly from the orders Lepidoptera and Hemiptera (34 and 17 interactions respectively). There is also a restrictive feeding guild representation since most focus on foliage-chewers (38) and phloem-feeders (11) (Bezemer and Jones 1998). Phloem-feeders do not all follow the trend typical of foliage-feeders: some species show a decrease in development time and an increase in population size under elevated CO<sub>2</sub> (Awmack et al. 1997; Bezemer and Jones 1998). There is insufficient data to draw conclusions for other guilds (leaf-miners, xylem-feeders, root feeders, seed eaters).

Here we investigate the effect of elevated CO<sub>2</sub> on the demography of an arachnid species (*Tetranychus urticae* Koch) raised on *Phaseolus vulgaris* L. (cultivar Con tender) plants. This mite can be found on more than 150

crop species (Jeppson et al. 1975) and causes large yield reductions (Sances et al. 1981, 1982). It feeds on the content of parenchyma cells (André and Remacle 1984; Tomczyk and Kropczynska 1985). Since sugars accumulate in the cytoplasm and sometimes in the vacuole (Matil 1987; Huber et al. 1992), we predict that the accumulation of TNC generally observed at elevated CO<sub>2</sub> will negatively affect the performance of this cell-feeding arachnid as it does for the foliage-chewers. Additionally, changes in the leaf anatomy due to elevated CO<sub>2</sub> can also contribute to changes in the feeding behaviour of leaf-sucking insects. We also tested the direct effect on the mite of doubling the current CO<sub>2</sub> concentration. It is generally assumed that there is no direct effect (Lincoln et al. 1993), but two tests (Fajer et al. 1991; Caulfield and Bunce 1994) have given contradictory results.

## Materials and methods

*P. vulgaris* plants were grown in 1.5-l pots filled with a mixture of horticultural compost (2/3) and sand (1/3). Thirty pots were placed in each of four growth chambers (Fabreguettes et al. 1992): two operated at a CO<sub>2</sub> concentration of 350 µmol mol<sup>-1</sup> and two at 700 µmol mol<sup>-1</sup>, with 600 µmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation (PAR) for 16 h per day at 24°C and 70–75% relative humidity. Three seeds per pot were sown on 18 March 1996 (day 1). The number of plants per pot was reduced to one 12 days after sowing. Soil humidity was maintained around 80% field capacity by adding daily the amount of water that had been evapotranspired during the previous 24 h. This amount, determined by weighing two control pots per growth chamber, did not differ significantly between the two CO<sub>2</sub> treatments (data not shown). On days 36 and 39, 200 ml of complete nutrient solution (500 µmol l<sup>-1</sup> nitrogen; described in Koch et al. 1987) was added to each pot.

Thirty plants per growth chamber were infested with *T. urticae* on day 15. The strain "White Eye", easily identifiable with its unpigmented eyes, was used because of our knowledge of its biology (Van Impe 1985, 1991; Lebrun et al. 1991; Nihoul et al. 1991). A stock of teleochrysalids was gathered the evening before the infestation. Males surround the females that emerge from the chrysalids and mating occurs immediately. These females, mated and less than 12 h old, were used to infest the plants. A single female, whose health was checked by observing its turgidity, movements and feeding activity, was deposited with a light brush on the upper side of one of the two primary leaves of each plant. Its health after deposition was also checked using a magnifying lens. Additionally, to test for the direct effect of elevated CO<sub>2</sub> on *T. urticae*, 40 leaves of *P. vulgaris* grown in a greenhouse at ambient CO<sub>2</sub> only were detached and placed in Petri dishes on a layer of cotton imbibed with deionized water. After one female had been placed on each leaf, the Petri dishes, without cover, were randomly distributed among the growth chambers at 350 and 700 µmol CO<sub>2</sub> mol<sup>-1</sup>.

Demographic data were collected 12 days after the infestation, since 12 days is the period after which the first females of the next generation hatch, at 24°C (Van Impe 1985). On each leaf where a founding female had been placed, the colony was located and the corresponding portion of the leaf was cut out, deposited in a Petri dish and frozen at -60°C. The remaining part of the plant was checked for the occurrence of secondary colonies. Immediately after removal from the freezer, leaf samples were examined under a magnifying lens (5, 10 and 25×) and eggs, larvae, nymphs, adult males and adult females were counted.

Before the colonies were deep-frozen, one female from each of 15 randomly chosen colonies in each growth chamber was collected and deposited on a mature trifoliate leaf. After 12 days (day

38), the second-generation colonies were located, harvested and analysed as described above.

Non-infested cotyledonous leaves were sampled on days 15, 24 and 27. Trifoliate leaves were sampled on days 27 and 38. One leaf from each of five plants per growth chamber (eight plants on day 15) was sampled randomly each time. They were weighed after being oven-dried at 55°C for 48 h. The cotyledonous leaves from day 24 were used for water content determination and leaf area measurement using a scanner (Delta-T Devices Ltd, Cambridge, UK). Total C and total N concentrations were determined on each leaf with a Carbon-Hydrogen-Nitrogen analyser (Carlo Erba instruments, model EA 1108, Milano, Italy). Total non-structural carbohydrate (TNC) analysis was carried out on the five cotyledonous leaves sampled on day 27 following the method of Farrar (1993). Samples were extracted in 95% (v/v) ethanol at 80°C to obtain ethanol-soluble sugars. Starch and fructans (water-soluble sugars) contained in the residue were extracted at 100°C for 1 h in water; starch was then hydrolysed using amyloglucosidase in an acetate:acetic acid buffer with a pH of 4.5. Total carbohydrates in each fraction were determined using the phenol-sulphuric acid method of Dubois et al. (1956).

Eight non-infested primary leaves per growth chamber were randomly sampled on day 27 and stored in a 70% ethyl alcohol solution. After soaking the leaf in deionized water, two 50-mm<sup>2</sup> blade samples were taken at half length of the leaf, one adjacent to the main rib and one adjacent to a secondary rib. Transverse blade sections were made using a microtome (Reichert-Jung, Austria). The sections were mounted with glycerine and analysed using a microscope (Reichert-Jung, model Polyvar, Austria) connected to a video camera. Epidermis thickness was measured.

At the end of the experiment (day 38), all plants were harvested. Internodes length was measured and the number of leaves, flowers and pods (longer than 1 cm) was counted. After oven-drying at 55°C for 48 h, the various above-ground parts were weighed.

Data were analysed using nested ANOVA (Statgraphics plus 4.0, Manugistic Inc). The linear model  $X_{ijk} = \mu + A_i + B_{j(i)} + e_{ijk}$  was used to describe the data (Underwood 1997, p. 250) where  $X_{ijk}$  is any measurement ( $k$ ) in any replicated growth chamber ( $j$ ) in a given CO<sub>2</sub> treatment ( $i$ ). Degrees of freedom for CO<sub>2</sub> treatments were  $a-1=1$ , for growth chambers within each CO<sub>2</sub> treatment  $a(b-1)=1$ , and for the error term  $ab(n-1)$  where  $a$  is the number of CO<sub>2</sub> treatments (2),  $b$  is the number of replicated growth chambers (2) and  $n$  is the number of measurements in each growth chamber.  $F$ -tests were conducted for  $A_i$  and  $B_{j(i)}$  with  $B_{j(i)}$  and  $e_{ijk}$  as the error terms, respectively. Variation among growth chambers was never significant ( $P>0.05$ ); however, according to Winer et al. (1991), nested components were only pooled (leading to a single-factor ANOVA with  $bn$  replicates in each treatment) when  $P>0.25$ . (In the results, a superscript a following  $F$ -values indicates that pooling was not possible). Normality tests indicated that no data transformation was necessary. Relationships between the mite performance and plant parameters were analysed using Pearson product-moment correlation coefficients.

## Results

### The plant response to elevated CO<sub>2</sub>

Neither internode length nor number of leaves on the main stem were affected by CO<sub>2</sub>, but plants grown at elevated CO<sub>2</sub> had a significantly larger number of leaves on lateral stems and a tendency to have more flowers (Table 1). Above-ground total plant weight was 65% greater at elevated CO<sub>2</sub>, but the weight (as well as the number) of pods was significantly lower at elevated CO<sub>2</sub> (Table 1). The nitrogen concentration of the leaves was significantly lower at elevated CO<sub>2</sub> throughout the growing period, resulting in a higher C/N ratio (Table 2). This decrease in nitrogen concentration was due to the higher non-structural sugar concentration at elevated CO<sub>2</sub> (Table 3). Leaf-specific mass was higher at elevated CO<sub>2</sub> and leaf water content was lower. Epidermal thickness was always greater at elevated CO<sub>2</sub> but it was significant only on the lower side near the secondary ribs (Table 3).

### Effects of elevated CO<sub>2</sub> on *T. urticae* demographic parameters

#### Direct effects

All females founded a colony at both CO<sub>2</sub> levels. The number and class structure of the offspring that developed on the detached leaves grown at ambient CO<sub>2</sub> were not significantly different in the growth chambers at 350 and 700 μmol CO<sub>2</sub> mol<sup>-1</sup> (Table 4). This suggests that elevated CO<sub>2</sub> does not directly influence the performance of this species.

#### Indirect effects on the first generation:

All the 120 females placed on intact plants in the growth chambers founded a colony on the leaf where they were originally placed. Though females were placed on the adaxial leaf surface, all colonies but one were established on the abaxial surface near a rib, as in nature. Colonies were not fragmented, suggesting that females immediately found a favourable site. This and the fact that most colonies had adults after 12 days indi-

**Table 1** Structure and weight of bean plants grown at ambient or elevated CO<sub>2</sub>

CO <sub>2</sub> (μmol mol <sup>-1</sup> )	Internode length (cm)			Number of leaves		Number of open flowers	Number of pods >1 cm	Aboveground dry weight (g)		
	1st	2nd	3rd	On main stem	On lateral stems			Vegetative biomass	Pods	Total
350	2.4	3.9	11.1	3.9	04.5	05.0	12.9	4.46	0.51	4.97
700	2.3	3.9	12.0	3.9	07.3	10.1	10.8	7.90	0.30	8.20
<i>F</i>	0.26 <sup>a</sup>	0.03 <sup>a</sup>	0.23 <sup>a</sup>	0.00 <sup>a</sup>	30.65	11.16 <sup>a</sup>	04.24	187.02	8.52	162.87
<i>P</i>	0.66	0.88	0.68	1.00	<0.001	0.079	0.046	<0.001	0.006	<0.001

<sup>a</sup> Growth chamber effect  $P<0.25$  so nested components were not pooled, see Materials and methods

**Table 2** Nitrogen and carbon concentrations of bean leaves grown at ambient or elevated CO<sub>2</sub>

	CO <sub>2</sub> ( $\mu\text{mol mol}^{-1}$ )	Cotyledonous leaves						Trifoliolate leaves			
		Day 15		Day 24		Day 27		Day 27		Day 38	
Nitrogen (mg g <sup>-1</sup> )	350	71.6	<i>F</i> =116.9 <sup>a</sup>	48.4	<i>F</i> =63.3	42.1	<i>F</i> =18.4	54.5	<i>F</i> =30.3	36.2	<i>F</i> =64.8 <sup>a</sup>
	700	57.7	<b><i>P</i>=0.008</b>	37.0	<b><i>P</i>&lt;0.001</b>	34.9	<b><i>P</i>&lt;0.001</b>	44.3	<b><i>P</i>&lt;0.001</b>	19.8	<b><i>P</i>=0.015</b>
Carbon (mg g <sup>-1</sup> )	350	419	<i>F</i> =4.35	397	<i>F</i> =1.93	397	<i>F</i> =0.30	421	<i>F</i> =1.04	397	<i>F</i> =5.64
	700	425	<i>P</i> =0.051	406	<i>P</i> =0.181	399	<i>P</i> =0.591	427	<i>P</i> =0.32	391	<b><i>P</i>=0.028</b>
C/N	350	5.8	<i>F</i> =52.5 <sup>a</sup>	8.3	<i>F</i> =135.7	9.5	<i>F</i> =13.3	7.7	<i>F</i> =29.54	11.1	<i>F</i> =124.6
	700	7.6	<b><i>P</i>=0.018</b>	10.9	<b><i>P</i>&lt;0.001</b>	11.6	<b><i>P</i>=0.001</b>	9.7	<b><i>P</i>&lt;0.001</b>	19.9	<b><i>P</i>&lt;0.001</b>

<sup>a</sup> See legend of Table 1

**Table 3** Characteristics of bean leaves grown at ambient or elevated CO<sub>2</sub>

CO <sub>2</sub> ( $\mu\text{mol mol}^{-1}$ )	Non structural sugars (mg g <sup>-1</sup> )		N (mg g <sup>-1</sup> structural biomass)	Specific mass (mg cm <sup>-2</sup> )	Water content (%)	Epidermis thickness ( $\mu\text{m}$ )			
	Ethanol soluble	Water soluble				Near main rib		Near secondary rib	
						Upper side	Lower side	Upper side	Lower side
350	61.5	53.1	47.5	4.87	83	1.97	1.21	1.96	1.34
700	87.9	144.8	45.6	6.54	77	2.49	1.28	2.58	1.74
<i>F</i>	8.96	14.6	1.8	32.8	34.4	4.32 <sup>a</sup>	0.23 <sup>a</sup>	6.48 <sup>a</sup>	5.45
<i>P</i>	<b>0.008</b>	<b>0.001</b>	0.197	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.173	0.679	0.125	<b>0.026</b>

<sup>a</sup> See legend of Table 1

**Table 4** Number of eggs and offspring per female raised at either ambient or elevated CO<sub>2</sub> and then placed on detached leaves all grown at ambient CO<sub>2</sub> (mean $\pm$ SE), and statistics for Fig. 1a,b

	CO <sub>2</sub> ( $\mu\text{mol mol}^{-1}$ )		Statistics		Statistics Fig. 1a		Statistics Fig. 1b	
	350	700	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Eggs	10.6 $\pm$ 1.0	8.4 $\pm$ 0.7	3.1	0.08	05.9 <sup>a</sup>	0.134	10.4	<b>0.003</b>
Larvae	6.6 $\pm$ 0.7	5.6 $\pm$ 0.7	1.0	0.31	10.9	<b>0.001</b>	6.6	<b>0.018</b>
Nymphs	16.9 $\pm$ 1.4	16.6 $\pm$ 1.5	0.0	0.91	34.1	<b>&lt;0.001</b>	10.0	<b>0.004</b>
Males	3.0 $\pm$ 0.4	3.9 $\pm$ 0.5	1.7	0.19	13.6 <sup>a</sup>	0.066	4.5	<b>0.043</b>
Females	2.1 $\pm$ 0.3	3.3 $\pm$ 0.7	2.2	0.14	17.1	<b>&lt;0.001</b>	2.8	0.107
Total	39.0 $\pm$ 2.6	37.7 $\pm$ 2.9	0.1	0.70	45.1	<b>&lt;0.001</b>	13.7	<b>0.001</b>

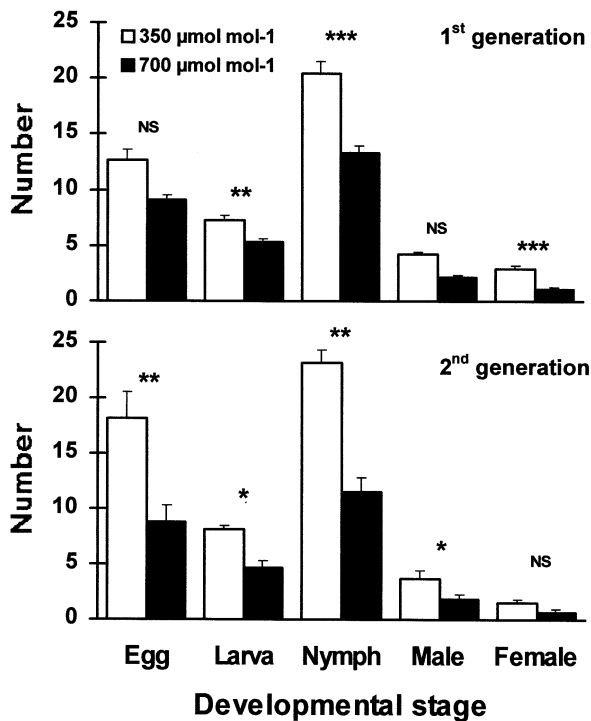
<sup>a</sup> See legend of Table 1

**Table 5** Correlation coefficients (*r*) between the number of eggs and offspring per female (1st generation) and plant parameters (*n*=20)

	Plant parameters day 24				Plant parameters day 27			
	Nitrogen %	Carbon %	Water %	Specific mass	Nitrogen %	Carbon %	Ethanol-soluble sugars	Water-soluble sugars
<i>r</i>	0.80	-0.25	0.76	-0.75	0.64	-0.14	-0.61	-0.45
<i>P</i>	<b>&lt;0.001</b>	0.29	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.002</b>	0.55	<b>0.004</b>	<b>0.046</b>

cate that the transfer and culture conditions were satisfactory. The total number of eggs and offspring per female was 34% lower at elevated CO<sub>2</sub> (31.2 vs. 47.5 at ambient CO<sub>2</sub>, statistics in Table 4). Figure 1a presents the effect on each developmental stage (*T. urticae* is a protandrous species: at 24°C, male development is about 12 h shorter than female; Van Impe 1985). Later stages of development were more affected than earlier ones (-24% for larvae, -62% for females), suggesting

that both fecundity and rate of development are affected. The Pearson correlation coefficients between the total number of eggs and offspring and the plant parameters are given in Table 5. Most plant parameters significantly correlated with female egg and offspring production (plant nitrogen content, water content, specific mass and ethanol-soluble sugar) had similar correlation coefficients.



**Fig. 1a, b** Impact of  $\text{CO}_2$  on the number of eggs and offspring in different developmental stages per female ( $\pm\text{SE}$ ; \*\*\* $P < 0.005$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; NS  $P > 0.05$ )

#### Indirect effects on the second generation:

The establishment of the second-generation colonies was less successful than that of the first generation, especially at elevated  $\text{CO}_2$ : 20 out of 30 females founded a colony at ambient  $\text{CO}_2$  and 15 out of 30 at elevated  $\text{CO}_2$ . Elevated  $\text{CO}_2$  reduced the total number of eggs and offspring per female that successfully established a colony (–49%, 27.5 vs. 54.7, statistics in Table 4). All stages, except the females, were significantly lower in numbers (Fig. 1b). A two-way ANOVA with  $\text{CO}_2$  and generation as factors showed no difference between generations except for the females which were less numerous at the second generation ( $F=3.96$ ,  $P=0.048$ ). There was no  $\text{CO}_2 \times$  generation interaction except for the number of eggs which were more affected by  $\text{CO}_2$  in the second generation ( $F=4.84$ ,  $P=0.029$ ).

## Discussion

The strong stimulation of the growth (+65%) of *P. vulgaris* at elevated  $\text{CO}_2$  was accompanied by a higher number of leaves and internodes on lateral stems and by a higher number of flowers (Table 1), suggesting reduced apical dominance. Such a developmental effect has been observed in other species but is not the rule. The mechanisms underlying this variability in response are not understood (Pritchard et al. 1999). Surprisingly, pod development (estimated by the number of pods

>1 cm) was not enhanced by elevated  $\text{CO}_2$  and the weight of pods was significantly reduced (Table 1). A lack of relationship between vegetative and reproductive mass responses to  $\text{CO}_2$  has been shown using published data from a variety of species (Ackerly and Bazzaz 1995), but is also true within genotypes of a single species (Jablonsky 1997) or of a single population (Potvin and Tousignant 1996). Such a variability might exist in *P. vulgaris*, since Jolliffe and Ehret (1985), working with the cultivar “Pure Gold Wax”, found vegetative and pod dry weights to be similarly stimulated by  $\text{CO}_2$ .

The observed  $\text{CO}_2$ -induced changes in leaf chemical composition (lower N concentration, as a result of an increase in TNC) and specific mass (Table 3) are in agreement with the results obtained on most species. In a literature review (Drake et al. 1997), ratios of parameters for plants grown at elevated  $\text{CO}_2$  to those of plants grown at normal ambient concentration were 0.83 for leaf nitrogen concentration, 2.62 for starch and 1.60 for sucrose. In our study these ratios were on average 0.75 for leaf nitrogen concentration and 2.02 for TNC. Radoglou and Jarvis (1992), on the same species, found a lower effect of  $\text{CO}_2$  on carbohydrates. We also observed a significant increase in the thickness of the lower epidermis (Table 3). Radoglou and Jarvis (1992) found an increase in the thickness of bean leaves at elevated  $\text{CO}_2$ , but only the parenchyma tissues were affected.

Although small variations in atmospheric  $\text{CO}_2$  concentration can be detected by insects and can play a role in host finding (Nicolas and Sillans 1989; Stange 1997), it is generally assumed that a general increase in atmospheric  $\text{CO}_2$  concentration has no direct effects on herbivores (Lincoln et al. 1993). Indeed, we found no direct effect of  $\text{CO}_2$  on the establishment and reproduction of *T. urticae*. Similarly, Fajer et al. (1991) found no effect of  $\text{CO}_2$  on size and survival of *Junonia coenia* butterfly larvae. However, the results of Caulfield and Bunce (1994) suggest a positive effect of elevated  $\text{CO}_2$  on the survival of *Spodoptera exigua* larvae, but only when fed with plants grown at elevated  $\text{CO}_2$ .

We report a considerable decrease in population size with elevated  $\text{CO}_2$ : the mean progeny per female was reduced by 34% in the first and 49% in the second generation, and fewer second-generation females founded a colony at elevated  $\text{CO}_2$ . The observed changes in both the leaf chemistry (C/N, water content) and anatomy (epidermis thickness) could be responsible for this decrease (Table 5). Wilson (1994) indeed showed a strong relationship between the fecundity of *T. urticae* and the nitrogen content of cotton. While there have been many studies on the effect of elevated  $\text{CO}_2$  on the performance of individuals, few studies have actually documented the consequences for population size and structure. Among the 61 plant-herbivore interactions reviewed by Bezemer and Jones (1998), 17 analyse the impact on population density. The phloem (8 studies) and whole-cell feeders (5 studies) on average respond positively to elevated  $\text{CO}_2$ , but there is a large variability and for 70% of the studies, the impact of  $\text{CO}_2$  is not significant (Bezemer and Jones

1998). One of the studies showing a significant increase in population size with elevated CO<sub>2</sub>, a result opposite to ours, was conducted with *T. urticae* (Heagle et al. 1994). However this experiment should be interpreted with caution since the white clover plants were partially defoliated before infestation with the mites and CO<sub>2</sub> was applied only about 7 days before the infestation. CO<sub>2</sub> stimulated plant growth, but no data on plant chemistry are given to further evaluate the effect of these treatments.

While most studies on the performance of individuals show a negative impact of CO<sub>2</sub> (Watt et al. 1995), studies at the population level give much more variable results. Three points can explain this paradox: (1) the proportion of studies on aphids (short-lived, phloem feeders, often not or positively affected by CO<sub>2</sub>) is high at the population level and low at the individual level; (2) in many studies at the population level, the insects were free to move on the plant and possibly could choose to consume the less altered plant parts (Williams et al. 1997), while in most studies at the individual level the insects were fed with a pre-determined plant part; and (3) several population studies were done with open-top chambers without confinement of the insect (Butler 1985; Butler et al. 1986). Here differences in population sizes could result from an effect of attraction or deterrence (Docherty et al. 1997).

More integrated studies are needed on a larger spectrum of herbivore types before reliable predictions of the impact of elevated CO<sub>2</sub> on plant-herbivore interaction can be made. Interactions with leaf age (Williams et al. 1998) and trophic web structure (Stiling et al. 1999) emphasise the need for more realistic experimental design, and the behavioural, demographic and genetic responses of the herbivores need a more thorough assessment.

**Acknowledgements** We would like to thank C. Collin, G. Laurent, M. Roche and L. Sonié for their help during cultivation and measurements of the beans; F. Jardon, J. Fabreguettes and J.L. Salager for maintaining the growth chambers, O. Gobin and C. Roumet for analysing the sugars, P. Berthet for statistical advice, and the referees for their suggestions.

## References

- Ackerly DD, Bazzaz FA (1995) Plant growth and reproduction along CO<sub>2</sub> gradients: non-linear responses and implications for community change. *Global Change Biol* 1:199–207
- André HM, Remacle C (1984) Comparative and functional morphology of the gnathosoma of *Tetranychus urticae* (Acari: Tetranychidae). *Acarologia* 25:179–190
- Awmack CS, Harrington R, Leather SR (1997) Host plant effects on the performance of the aphid *Aulacorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO<sub>2</sub>. *Global Change Biol* 3:545–549
- Ayres MP (1993) Plant defence, herbivory, and climate change. In: Kareiva PM, Kingsolver JG, Huey RB (eds) *Biotic interactions and global change*. Sinauer, Sunderland, pp 75–94
- Bazzaz FA (1990) The response of natural ecosystems to the rising global CO<sub>2</sub> levels. *Annu Rev Ecol Syst* 51:167–196
- Bezemer TM, Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO<sub>2</sub>: quantitative analyses and guild effects. *Oikos* 82:212–222
- Boggs CL (1981) Nutritional and life-history determinant of resource allocation in holometabolous insects. *Am Nat* 117:692–709
- Brooks GL, Whittaker JB (1998) Responses of multiple generations of *Gastrophysa viridula*, feeding on *Rumex obtusifolius*, to elevated CO<sub>2</sub>. *Global Change Biol* 4:63–75
- Bryant JP, Chapin FS III, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368
- Butler GD (1985) Populations of several insects on cotton in open-top carbon dioxide enrichment chambers. *Southwest Entomol* 10:264–267
- Butler GD, Kimball BA, Mauney JR (1986) Populations of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) on cotton grown in open-top field chambers enriched with CO<sub>2</sub>. *Environ Entomol* 15:61–63
- Caulfield F, Bunce JA (1994) Elevated atmospheric carbon dioxide concentration affects interactions between *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae and two host plant species outdoors. *Physiol Chem Ecol* 23:999–1005
- Docherty M, Wade FA, Hurst DK, Whittaker JB, Lea PJ (1997) Responses of tree sap-feeding herbivores to elevated CO<sub>2</sub>. *Global Change Biol* 3:51–59
- Drake BG, Gonzalez Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO<sub>2</sub>? *Annu Rev Plant Physiol Plant Mol Biol* 48:609–639
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
- Fabreguettes J, Salager JL, Roy J (1992) An automated CO<sub>2</sub> control system for plant growth chambers and its use to estimate net plant CO<sub>2</sub> exchange. *J Agric Eng Res* 51:129–138
- Fajer ED, Bowers MD, Bazzaz FA (1989) The effects of enriched carbon dioxide atmospheres on plant-insect herbivore interactions. *Science* 243:1198–1200
- Fajer ED, Bowers MD, Bazzaz FA (1991) The effects of enriched carbon dioxide atmospheres on the buckeye butterfly, *Junonia coenia*. *Ecology* 72:751–754
- Farrar JF (1993) Carbon partitioning. In: Hall DO, Scurlock JMO, Bohlar-Nordenkampf HR, Leegood SC, Long SP (eds) *Photosynthesis and production in a changing environment. A field and laboratory manual*. Chapman Hall, London, pp 232–246
- Field CB, Chapin FS, Matson PA, Mooney HA (1992) Responses of terrestrial ecosystems to the changing atmosphere: a resource-based approach. *Annu Rev Ecol Syst* 23:201–235
- Hättenschwiler S, Schafellner C (1999) Opposing effects of elevated CO<sub>2</sub> and N deposition on *Lymantria monacha* larvae feeding on spruce trees. *Oecologia* 118:210–217
- Heagle AS, Brandenburg RL, Burns JC, Miller JE (1994) Ozone and carbon dioxide effects on spider mites in white clover and peanut. *J Environ Qual* 23:1168–1176
- Huber SC, Huber JLA, McMichael RW Jr (1992) The regulation of sucrose synthesis in leaves. In: Pollock CJ, Farrar JF, Gordon AJ (eds) *Carbon partitioning within and between organisms*. Bios, Oxford, pp 1–26
- Jablonski LM (1997) Responses of vegetative and reproductive traits to elevated CO<sub>2</sub> and nitrogen in *Raphanus* varieties. *Can J Bot* 75:533–545
- Jeppson LR, Keifer HH, Baker EW (1975) *Mites injurious to economic plants*. University of California Press, Berkeley
- Jolliffe PA, Ehret DL (1985) Growth of bean plants at elevated carbon dioxide concentration. *Can J Bot* 63:2021–2025
- Koch GH, Winner WE, Nardone A, Mooney HA (1987) A system for controlling the root and shoot environment for plant growth studies. *Environ Exp Bot* 27:365–377
- Körner C (1996) The response of complex multispecies systems to elevated CO<sub>2</sub>. In: Walker B, Steffen W (eds) *Global change and terrestrial ecosystems*. Cambridge University Press, Cambridge, pp 20–42
- Körner C, Miglietta F (1994) Long-term effects of naturally elevated CO<sub>2</sub> on mediterranean grassland and forest trees. *Oecologia* 99:343–351

- Lambers H (1993) Rising CO<sub>2</sub>: secondary plant metabolism plant-herbivore interactions and litter decomposition. *Vegetatio* 104/105:263–271
- Lavola A, Julkunen-Tiitto R (1994) The effect of elevated carbon dioxide and fertilization on primary and secondary metabolites in birch *Betula pendula* (Roth). *Oecologia* 99:315–321
- Lebrun P, Van Impe G, Saint-Georges-Grèdelet D de, Wauthy G, André HM (1991) The life strategies of mites. In: Schuster R, Murphy PW (eds) *The Acari: reproduction, development and life-history strategies*. Chapman Hall, London, pp 1–22
- Lincoln DE (1993) The influence of plant carbon dioxide and nutrient supply on susceptibility to insect herbivores. *Vegetatio* 104/105:273–280
- Lincoln DE, Fager ED, Johnson RH (1993) Plant insect herbivore interactions in elevated CO<sub>2</sub> environments. *Trends Ecol Evol* 8:64–68
- Lindroth RL (1996a) CO<sub>2</sub>-mediated changes in tree chemistry and tree-Lepidoptera interactions. In: Koch GW, Mooney HA (eds) *Carbon dioxide and terrestrial ecosystems*. Academic Press, San Diego, pp 105–120
- Lindroth RL (1996b) Consequences of elevated atmospheric CO<sub>2</sub> for forest insects. In: Körner C, Bazzaz FA (eds) *Carbon dioxide populations and communities*. Academic Press, San Diego, pp 347–361
- Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO<sub>2</sub>: productivity and insect performance. *Ecology* 74:763–777
- Matil P (1987) The sap of plant cells (Tansley review 7). *New Phytol* 105:1–26
- Mattson WJ (1980) Herbivory in relation to plant nitrogen content. *Annu Rev Ecol Syst* 11:119–161
- Nicolas G, Sillans D (1989) Immediate and latent effects of carbon dioxide on insects. *Annu Rev Entomol* 34:97–116
- Nihoul P, Van Impe G, Hance T (1991) Characterizing indices of damage to tomato by the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) to achieve biological control. *J Hortic Sci* 66:643–648
- Penuelas J, Estiarte M (1998) Can elevated CO<sub>2</sub> affect secondary metabolism and ecosystem function? *Trends Ecol Evol* 13:20–24
- Penuelas J, Estiarte M, Kimball BA, Idso SB, Pinter PJ, Wall GW, Garcia RL, Hansaker DJ, LaMorte RL, Hendrix DL (1996) Variety of responses of plant phenolic concentration to CO<sub>2</sub> enrichment. *J Exp Bot* 47:1463–1467
- Poorter H, Van Berkel Y, Baxter R, Den Hertog J, Dijkstra P, Gifford RM, Griffin KL, Roumet C, Roy J, Wong SC (1997) The effect of elevated CO<sub>2</sub> on the chemical composition and construction costs of leaves of 27 C-3 species. *Plant Cell Environ* 20:472–482
- Potvin C, Tousignant D (1996) Evolutionary consequences of simulated global change: genetic adaptation or adaptive phenotypic plasticity. *Oecologia* 108:683–693
- Pritchard SG, Rogers HH, Prior SA, Peterson CM (1999) Elevated CO<sub>2</sub> and plant structure: a review. *Global Change Biol* 5:807–837
- Radoglou KM, Jarvis PG (1992) The effects of CO<sub>2</sub> enrichment and nutrient supply on growth morphology and anatomy of *Phaseolus vulgaris* L seedlings. *Ann Bot* 70:245–256
- Roth S, Lindroth RL, Volin JC, Kruger EL (1998) Enriched atmospheric CO<sub>2</sub> and defoliation: effects on tree chemistry and insect performance. *Global Change Biol* 4:419–430
- Sances FV, Wyman JA, Ting IP, Steenwyk RA van, Oatman ER (1981) Spider mite interactions with photosynthesis, transpiration and productivity of strawberry. *Environ Entomol* 10:442–448
- Sances FV, Toscano NC, Oatman ER, Lapré LF, Johnson MF, Voth V (1982) Reduction in plant processes by *Tetranychus urticae* (Acari: Tetranychidae). *Int J Acarol* 4:47–56
- Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. *Annu Rev Entomol* 26:183–211
- Stange G (1997) Effects of changes in atmospheric carbon dioxide on the location of hosts by the moth *Cactoblastis cactorum*. *Oecologia* 110:539–545
- Stiling P, Rossi AM, Hungate B, Dijkstra P, Hinkle CR, Knott WM, Drake B (1999) Decreased leaf-miner abundance in elevated CO<sub>2</sub>: reduced leaf quality and increased parasitoid attack. *Ecol Appl* 9:240–244
- Tomczyk A, Kropczynska D (1984) Feeding effects of *Tetranychus urticae* Koch on the physiology of some plants. In: Griffiths DA, Bowman CE (eds) *Proceedings 6th International Congress of Acarology*. Ellis Harwood, Chichester, pp 740–746
- Traw MB, Lindroth RL, Bazzaz FA (1996) Decline in gypsy moth (*Lymantria dispar*) performance in an elevated CO<sub>2</sub> atmosphere depends upon host plant species. *Oecologia* 108:113–120
- Underwood AJ (1997) *Experiments in ecology. Their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge
- Van Impe G (1985) Contribution à la conception de stratégies de contrôle de l'acarien tisserand commun *Tetranychus urticae* Koch (Acari: Tetranychidae) Thèse de doctorat Sciences, Unité d'Ecologie et de Biogéographie, Université catholique de Louvain
- Van Impe G (1991) The effects of mating on the main biological traits of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Belg J Zool* 121:45–46
- Ward SJE, Midgley GF, Jones MH, Curtis PS (1999) Responses of wild C4 and C3 grass (Poaceae) species to elevated CO<sub>2</sub> concentration: a meta-analytic test of current theories and perceptions. *Global Change Biol* 5:723–741
- Watt AD, Whittaker JB, Docherty M, Brooks G, Lindsay E, Salt DT (1995) The impact of elevated atmospheric CO<sub>2</sub> on insect herbivores. In: Harrington R, Stork NE (eds) *Insects in a changing environment*. Academic Press, London, pp 197–217
- Williams RS, Lincoln DE, Thomas RB (1994) Loblolly pine grown under elevated CO<sub>2</sub> affects early instar pine sawfly performance. *Oecologia* 98:64–71
- Williams RS, Lincoln DE, Thomas RB (1997) Effect of elevated CO<sub>2</sub>-grown loblolly pine needles on the growth consumption development and pupal weight of red-headed pine sawfly larvae reared within open-topped chambers. *Global Change Biol* 3:501–511
- Williams RS, Lincoln DE, Norby RJ (1998) Leaf age effects of elevated CO<sub>2</sub>-grown white oak leaves on spring-feeding lepidopterans. *Global Change Biol* 4:235–246
- Wilson LJ (1994) Plant-quality effect on life-history parameters of the two-spotted spider mite (Acari: Tetranychidae) on cotton. *J Econ Entomol* 87:1665–1673
- Winer BJ, Brown DR, Michels KM (1991) *Statistical principals in experimental design*, 3rd edn. McGraw-Hill, New York
- Wolfe DW, Gifford RM, Hilbert D, Luo Y (1998) Integration of photosynthetic acclimation to CO<sub>2</sub> at the whole plant level. *Global Change Biol* 4:879–893