

# Performance of the legume-feeding herbivore, *Colias philodice* (Lepidoptera: Pieridae) is not Affected by Elevated CO<sub>2</sub>

David N. Karowe · Angela Migliaccio

Received: 21 November 2008 / Accepted: 6 December 2010  
© Springer Science+Business Media B.V. 2011

**Abstract** Presumably due to their association with nitrogen-fixing bacteria, the nutritional quality of legumes decreases less than that of non-legume C<sub>3</sub> plants when grown under elevated atmospheric CO<sub>2</sub>. Therefore, it seems likely that legume-feeding herbivores will be less adversely affected than herbivores of non-legume C<sub>3</sub> plants by anthropogenic increases in atmospheric CO<sub>2</sub>. When the legumes *Medicago sativa* (alfalfa), *Trifolium repens* (white clover), and *Lotus corniculatus* (birdsfoot trefoil) were grown under elevated (756 ppm) CO<sub>2</sub>, leaf nitrogen remained the same or increased, and C:N ratio did not change. Unlike most insects fed non-legume C<sub>3</sub> plants, *Colias philodice* (sulfur butterfly) larvae fed elevated-grown *M. sativa* and *T. repens* did not exhibit reduced relative growth rate (RGR), and larvae fed elevated-grown *L. corniculatus* exhibited a nearly significant 37% increase in RGR. Pupal weight was unaffected by growth of host plants under elevated CO<sub>2</sub>. Relative nitrogen growth rate (RGRN) did not change for larvae fed elevated-grown *M. sativa* or *T. repens*, but increased by 34% for larvae fed elevated-grown *L. corniculatus*. These results suggest that legume-feeding herbivores will be relatively buffered against the adverse effects of elevated CO<sub>2</sub> typically experienced by herbivores of non-legume C<sub>3</sub> plants.

**Keywords** Elevated CO<sub>2</sub> · Legumes · Plant-herbivore interactions · Nutritional indices · *Colias philodice* · Global change

## Introduction

By 2100 the concentration of carbon dioxide in Earth's atmosphere is expected to reach 550–900 ppm (Meehl et al. 2007), and could be substantially higher if positive carbon cycle feedbacks are even moderately strong (Zeng et al. 2004; Matthews 2006). When grown under elevated CO<sub>2</sub>, most C<sub>3</sub> plants exhibit increased photosynthetic rates and, consequently, decreased nitrogen content and increased carbon:nitrogen ratios of foliage (Karowe et al. 1997; Nowak et al. 2004; Körner 2006). Presumably in response to decreased nitrogen content of their host plants, insect herbivores nearly always increase consumption rates, but compensatory feeding often does not prevent reduced larval growth and/or survivorship (Fajer et al. 1989; Lindroth et al. 1993; Coviella and Trumble 1999; Johns and Hughes 2002; Zvereva and Kozlov 2006).

Relative to that of non-legume C<sub>3</sub> plants, the nutritional quality of legumes is less affected by growth under elevated CO<sub>2</sub>. On average, foliar nitrogen content of legumes decreased by only 7% under elevated CO<sub>2</sub>, while that of non-legume C<sub>3</sub> species decreased by 15% (Cotrufo et al. 1998; Stiling and Cornelissen 2007). Similarly, legume seed nitrogen was unaffected by elevated CO<sub>2</sub> while that of non-legume species decreased by 15% (Jablonski et al. 2002). The attenuated response of legumes is likely due to increased nitrogen fixation under elevated CO<sub>2</sub> (de Graaff et al. 2006; van Groenigen et al. 2006).

Given that plant nitrogen content is the most important determinant of larval performance for insect herbivores

Handling Editor: Gary Felton.

D. N. Karowe (✉)  
Department of Biological Sciences, Western Michigan  
University, Kalamazoo, MI 49008-5410, USA  
e-mail: david.karowe@wmich.edu

A. Migliaccio  
Department of Ecology and Evolutionary Biology, University of  
Michigan, Ann Arbor, MI 48109, USA

(Scriber and Feeny 1979; Mattson 1980; Scriber and Slansky 1981), it seems likely that the reduced sensitivity of legume nutritional quality to elevated CO<sub>2</sub> would buffer legume-feeding herbivores against the typical adverse effects of host plant growth under elevated CO<sub>2</sub>. Although many dozens of studies have addressed the effects of elevated CO<sub>2</sub> on herbivores of non-legumes (Stiling and Cornelissen 2007; Wang et al. 2008), we are aware of studies addressing only six legume-herbivore systems. Not surprisingly, soybean is the best studied in this regard. Lincoln et al. (1984, 1986) found no difference in relative growth rate for soybean loopers fed elevated-grown soybean, and Osbrink et al. (1987) did not observe any reduction in performance of the cabbage looper fed unfertilized elevated-grown lima beans. More recent work from the SoyFACE experiment indicated that Japanese beetles and Mexican bean beetles prefer soybean leaves grown at elevated CO<sub>2</sub> (Hamilton et al. 2005) and that fecundity was approximately doubled and lifespan extended by 8–25% for Japanese beetles fed elevated-grown soybean (O'Neill et al. 2008). Soybean fields exposed to elevated CO<sub>2</sub> also had larger herbivore populations and levels of leaf damage (Dermody et al. 2008).

Very few studies have addressed the effect of elevated CO<sub>2</sub> on herbivores of legumes other than soybean. Goverde et al. (1999) found increased performance of lycaenid caterpillars fed elevated-grown birdsfoot trefoil, though the response depended on larval genotype (Goverde et al. 2004). In a previous study of the sulfur butterfly fed red clover and white sweet clover, Karowe (2007) observed no change in growth rate, instar duration, or pupal weight. Growth of cotton leafworm larvae was greater on elevated-grown yellow lupine, but lower on elevated-grown blue lupine (Schädler et al. 2007) and alfalfa (Agrell et al. 2006). The paucity of information about legume-feeding herbivores explains why no narrative (e.g. Penuelas and Estiarte 1998; Coviella and Trumble 1999; Hunter 2001) or meta-analytical (e.g. Zvereva and Kozlov 2006; Valkama et al. 2007) summary quantitatively addresses the effects of elevated CO<sub>2</sub> on legume-feeding herbivores.

The common sulfur butterfly, *Colias philodice* Latreille (Lepidoptera: Pieridae), feeds throughout the continental United States and southern Canada on at least 15 genera of herbaceous legumes (Klots 1951; Scott 1986). In northern Michigan, *Medicago sativa* (alfalfa) and *Trifolium repens* (white clover) are primary host plants and *Lotus corniculatus* (birdsfoot trefoil) is a secondary host (Karowe 1988). Therefore, *C. philodice* and its host plants provide an opportunity to assess the susceptibility of legumes and their herbivores to the direct effects of elevated CO<sub>2</sub>. Accordingly, in this study we ask:

1. Does growth under elevated CO<sub>2</sub> affect foliar nutritional quality of the legumes *M. sativa*, *T. repens*, and/or *L. corniculatus*?
2. Is performance of the legume-feeder *C. philodice* adversely affected by growth of its host plants under elevated CO<sub>2</sub>?

## Materials and methods

### Legumes

In early May, *Medicago sativa* (alfalfa), *Trifolium repens* (white clover), and *Lotus corniculatus* (birdsfoot trefoil) were sown from seed in 6-inch pots filled with potting soil and allowed to germinate in the greenhouse at the University of Michigan Biological Station (UMBS) in Cheboygan County, Michigan. Four days after germination, plants were thinned to two per pot and pots were placed into one of 46 0.5 m<sup>3</sup> open-topped chambers (Barbehenn et al. 2004; Karowe 2007) in a field adjacent to the greenhouse. Due to low seed germination rates, only seven pots containing *L. corniculatus* were placed in chambers at each CO<sub>2</sub> level. At the same time, inoculum was prepared by placing soil gathered from a nearby field containing *M. sativa*, *T. repens*, and *L. corniculatus* into a plastic container with approximately 20 gallons of water. Each pot was inoculated with 500 ml of this water. Visual inspection of roots of all plants used in feeding trials revealed no apparent difference in nodulation levels between treatments.

CO<sub>2</sub> concentration inside the elevated chambers was controlled by dispensing 100% CO<sub>2</sub> into the inlet port of an input blower and monitored by continuous sampling of chamber air. Twenty-three chambers were maintained at 756 ± 6 ppm CO<sub>2</sub> (elevated treatment) and 23 chambers were maintained at 371 ± 7 ppm CO<sub>2</sub> (ambient treatment). Pumps delivered air from elevated and ambient chambers to an adjacent control house containing a microcomputer-controlled valve manifold that directed the gas stream past an infrared gas analyzer (IRGA). Output from the IRGA was displayed and logged by the computer. The dispensing rate of CO<sub>2</sub> was adjusted with manual flow-meters. Plants were watered twice daily, inspected periodically for insect herbivores and, when necessary, sprayed with Safer Soap, a short-lived organic insecticide.

### *Colias philodice*

Eggs were obtained from 21 gravid *C. philodice* females collected from fields in the vicinity of UMBS in late June. Females were placed in oviposition chambers (25 cm clay

pots containing plant cuttings, covered with clear plastic wrap, and placed 5 cm below a 40 watt incandescent bulb) and fed a solution of 10% clover honey in water twice daily. On average, females laid approximately 200 eggs over 7 days. Upon hatching, larvae from all females were randomly placed into 25 ml polystyrene cups (10 larvae per cup) containing Soybean Looper artificial diet (BioServ Inc, Frenchtown, N.J.); larvae were reared on diet to prevent feeding induction (Karowe 1989). Cups were placed in an environmental chamber under a 16:8 L: D cycle and a corresponding 25:20°C temperature cycle. Fresh diet was provided every other day. When they ceased feeding at the end of the 4th instar, larvae were transferred to individual 25 ml polystyrene cups to molt. Immediately after molting into the 5th (ultimate) instar, test larvae were weighed and placed individually into 5.5 cm Petri dishes containing a preweighed amount of leaf tissue. Twenty-three larvae were placed on *M. sativa* and *T. repens* at each CO<sub>2</sub> level, and seven larvae were placed on *L. corniculatus* at each CO<sub>2</sub> level (each larva was fed leaf tissue from a different pot). Initial weights of test larvae did not differ between treatments ( $F_{5,101} = 0.78$ ,  $P > 0.5$ ). An additional 10 freshly molted 5th instar larvae were weighed, dried to constant weight at 60°C, and reweighed to provide a wet/dry conversion factor for estimating the initial dry weight of test larvae. Fresh leaves from the same plant were added every two days or more often if necessary, and an equal aliquot of leaves was similarly treated to provide a wet/dry conversion factor used to estimate the dry weight and water content of leaves provided to test larvae. The weight of leaves provided was such that at least 75% was consumed, in order to reduce error in estimating the dry weight of food provided (Schmidt and Reese 1986; Bowers et al. 1991). Immediately upon pupation, each pupa was weighed, frozen, dried to constant weight at 60°C, and reweighed. All frass produced during the 5th instar and all uneaten food were also dried to constant weight at 60°C and weighed.

All dried pupae, frass, and larval and plant samples used to generate conversion factors were ground under liquid nitrogen, dried again to constant weight at 60°C, and analyzed for nitrogen and carbon contents using a Perkin Elmer CHN Elemental Analyzer. Two replicates were run for each sample; if replicates differed by >10%, a third replicate was run. For all test larvae, whole food and nitrogen nutritional indices were calculated on a dry weight basis as in (Waldbauer 1968):

Relative consumption rate (RCR) = (food ingested)/(average larval weight × days)

Approximate digestibility (AD) = (food ingested—frass)/(food ingested)

Efficiency of conversion of digested food (ECD) = (weight gained)/(food ingested—frass)

Relative growth rate (RGR) = (weight gained)/(average larval weight × days)

Note that  $RCR \times AD \times ECD = RGR$ .

Nitrogen nutritional indices were calculated by substituting nitrogen for weight, e.g.

Relative nitrogen growth rate (RGRN) = (nitrogen gained)/(average larval nitrogen × days)

All variables were analyzed with a split-plot, Type III ANOVA (PROC MIXED) with CO<sub>2</sub> level as whole plots, plant species as sub-plots, and block as a random variable. Homogeneity of variances was verified by  $F_{\max}$  tests. The normality of residuals was evaluated by Kolmogorov–Smirnov tests using PROC UNIVARIATE (SAS Institute 2000). One variable (5th instar duration) could not be normalized, so was analyzed by pairwise Kruskal–Wallis tests using PROC NPAR1WAY. For all other variables, pairwise differences between least-squares means for CO<sub>2</sub> levels and plant species were evaluated by Tukey tests (Wilkinson 2000); all pairwise comparisons were planned a priori.

## Results

When *Medicago sativa*, *Trifolium repens*, and *Lotus corniculatus* were grown under elevated CO<sub>2</sub>, leaf nitrogen content did not change for *M. sativa* and *L. corniculatus*, but increased significantly (by 8%) for *T. repens*, producing a significant CO<sub>2</sub>\*Species interaction (Table 1). Neither C:N ratio nor carbon content was affected by elevated CO<sub>2</sub>. In contrast, leaf water content decreased under elevated CO<sub>2</sub> for all three species, though the decrease was significant only for *M. sativa* and *T. repens*. Leaf nitrogen, carbon, and C:N ratio did not differ among species, but *L. corniculatus* contained more water than *T. repens*, which contained more water than *M. sativa*.

Relative to *Colias philodice* larvae fed legumes grown under ambient CO<sub>2</sub> (hereafter “ambient-grown”), larvae fed *M. sativa*, *T. repens*, and *L. corniculatus* grown under elevated CO<sub>2</sub> (hereafter “elevated-grown”), consumed their host plants at significantly increased rates (RCR was 17, 11, and 30% higher, respectively; Table 2). Larvae fed elevated-grown plants digested leaf tissue less efficiently (AD was reduced by 9, 6, and 13%, respectively), though the difference was significant only for larvae fed *M. sativa*. On all species, larvae fed elevated-grown plants converted digested leaf tissue into larval biomass (ECD) as efficiently as larvae fed ambient-grown plants. As a consequence, relative growth rate (RGR) did not differ overall between larvae fed ambient- and elevated-grown plants. However, a nearly significant CO<sub>2</sub>\*Species interaction for RGR arose because feeding on host plants under elevated CO<sub>2</sub> resulted in a 1%

**Table 1** Measures of leaf nutritional quality (least square mean  $\pm$  SE) for *M. sativa*, *T. repens*, and *L. corniculatus* grown at ambient (371 ppm) and elevated (756 ppm) CO<sub>2</sub>

Variable	<i>Medicago sativa</i>		<i>Trifolium repens</i>		<i>Lotus corniculatus</i>		F values		
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	CO <sub>2</sub> d.f. = 1,9	Species d.f. = 2,83	CO <sub>2</sub> $\times$ Species d.f. = 2,83
Nitrogen	4.13 $\pm$ 0.06	4.15 $\pm$ 0.06	4.10 $\pm$ 0.08	4.41 $\pm$ 0.08	4.24 $\pm$ 0.15	4.12 $\pm$ 0.15	0.70	1.25	3.17*
Carbon	42.5 $\pm$ 0.4	42.8 $\pm$ 0.3	42.2 $\pm$ 0.3	43.3 $\pm$ 0.3	42.1 $\pm$ 0.9	42.0 $\pm$ 0.7	1.23	1.13	0.96
C:N Ratio	10.4 $\pm$ 0.2	10.4 $\pm$ 0.2	10.4 $\pm$ 0.2	9.9 $\pm$ 0.2	10.0 $\pm$ 0.5	10.3 $\pm$ 0.5	0.13	0.56	1.07
Water (%)	76.6 $\pm$ 0.6	71.9 $\pm$ 0.8	82.2 $\pm$ 0.6	77.2 $\pm$ 0.6	82.9 $\pm$ 0.8	80.6 $\pm$ 1.1	47.11***	56.06***	1.74

Nitrogen and carbon values are percent of dry weight. Sample sizes are 23 at each CO<sub>2</sub> level for *M. sativa* and *T. repens*, and 7 for *L. corniculatus*. Significance levels are indicated by asterisks, where \*  $P < 0.05$  and \*\*\*  $P < 0.001$

**Table 2** Whole food nutritional indices (least square mean  $\pm$  SE) for *C. philodice* larvae fed *M. sativa*, *T. repens*, and *L. corniculatus* grown at ambient (371 ppm) and elevated (756 ppm) CO<sub>2</sub>

Variable	<i>Medicago sativa</i>		<i>Trifolium repens</i>		<i>Lotus corniculatus</i>		F values		
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	CO <sub>2</sub> d.f. = 1,9	Species d.f. = 2,83	CO <sub>2</sub> $\times$ Species d.f. = 2,83
RCR (g/g/d)	1.41 $\pm$ 0.06	1.65 $\pm$ 0.06	1.51 $\pm$ 0.05	1.67 $\pm$ 0.05	1.02 $\pm$ 0.13	1.33 $\pm$ 0.14	46.02***	14.43***	0.11
AD (%)	50.1 $\pm$ 1.4	45.5 $\pm$ 1.2	48.3 $\pm$ 1.3	45.2 $\pm$ 1.2	43.9 $\pm$ 3.1	38.2 $\pm$ 3.2	14.93**	6.48**	1.17
ECD (%)	34.9 $\pm$ 1.5	32.3 $\pm$ 1.3	44.0 $\pm$ 1.8	41.8 $\pm$ 1.3	34.2 $\pm$ 3.6	36.9 $\pm$ 3.3	3.00	10.54***	2.22
RGR (g/g/d)	0.244 $\pm$ 0.6	0.242 $\pm$ 0.8	0.310 $\pm$ 0.6	0.316 $\pm$ 0.6	0.136 $\pm$ 0.8	0.186 $\pm$ 1.1	1.23	73.50***	3.06 <sup>†</sup>
Pupal Weight (mg dry)	29.1 $\pm$ 1.8	27.7 $\pm$ 1.6	41.1 $\pm$ 1.6	41.4 $\pm$ 1.4	26.4 $\pm$ 2.0	29.9 $\pm$ 2.1	0.38	44.51***	1.29
Instar Duration (days) <sup>a</sup>	4.21 $\pm$ 0.09	4.31 $\pm$ 0.08	3.89 $\pm$ 0.07	3.92 $\pm$ 0.06	6.71 $\pm$ 0.90	5.57 $\pm$ 0.41	0.26	47.6***	N.D.

Sample sizes are 23 at each CO<sub>2</sub> level for *M. sativa* and *T. repens*, and 7 for *L. corniculatus*. Significance levels are indicated by symbols, where <sup>†</sup> 0.10  $< P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$

<sup>a</sup> Data could not be normalized so were analyzed by Kruskal–Wallis tests, which precluded direct testing of the CO<sub>2</sub>\*Species interaction. Chi-square values are reported in place of F values for tests of the CO<sub>2</sub> and Species effects

RCR relative consumption rate; AD approximate digestibility; ECD efficiency of conversion of digested food; RGR relative growth rate

decrease in RGR for larvae fed *M. sativa*, a 2% increase for larvae fed *T. repens*, and a 37% increase for larvae fed *L. corniculatus*. Neither pupal weight nor fifth instar duration was affected by elevated CO<sub>2</sub> on any host plant (Table 2).

Nitrogen nutritional indices revealed a pattern similar to that observed for whole food nutritional indices (Table 3). Larvae fed elevated-grown plants consumed nitrogen at a higher rate (RCRN increased by 21, 16, and 40% on *M. sativa*, *T. repens*, and *L. corniculatus*, respectively). Overall, larvae fed elevated-grown plants digested nitrogen less efficiently (ADN decreased by 8, 4, and 8% on *M. sativa*, *T. repens*, and *L. corniculatus*, respectively), though the difference was not significant for any individual species. Growth of legumes under elevated CO<sub>2</sub> did not affect the efficiency with which larvae converted digested

nitrogen into larval nitrogen (ECDN did not exhibit a significant CO<sub>2</sub> effect or CO<sub>2</sub>\*Species interaction). As a consequence, for *C. philodice* fed elevated-grown plants, relative nitrogen growth rate (RGRN) was virtually unaffected on *M. sativa*, increased by 5% on *T. repens*, and increased by 34% on *L. corniculatus*; this difference resulted in a significant CO<sub>2</sub>\*Species interaction (Table 3).

All whole food nutritional indices differed significantly among host plant species. When both CO<sub>2</sub> levels were considered together, larvae fed *M. sativa* and *T. repens* exhibited higher RCR and AD than larvae fed *L. corniculatus*. ECD was higher for larvae fed *T. repens* than for larvae fed *M. sativa*, and RGR and pupal weight were highest for larvae fed *T. repens* and higher for larvae fed *M. sativa* than for larvae fed *L. corniculatus*. Instar

**Table 3** Nitrogen nutritional indices (least square mean  $\pm$  SE) for *C. philodice* larvae fed *M. sativa*, *T. repens*, and *L. corniculatus* grown at ambient (371 ppm) and elevated (756 ppm) CO<sub>2</sub>

Variable	<i>Medicago sativa</i>		<i>Trifolium repens</i>		<i>Lotus corniculatus</i>		F values		
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	CO <sub>2</sub>	Species	
							d.f. = 1,9	d.f. = 2,83	
RCRN (g/g/d)	0.56 $\pm$ 0.03	0.68 $\pm$ 0.03	0.62 $\pm$ 0.02	0.72 $\pm$ 0.03	0.43 $\pm$ 0.06	0.60 $\pm$ 0.06	50.48***	16.45***	0.43
ADN (%)	65.6 $\pm$ 1.6	60.5 $\pm$ 1.9	66.3 $\pm$ 1.1	63.9 $\pm$ 1.7	61.0 $\pm$ 3.2	56.1 $\pm$ 3.6	6.28*	4.42*	0.90
ECDN (%)	60.2 $\pm$ 3.3	56.3 $\pm$ 2.7	70.5 $\pm$ 2.2	65.7 $\pm$ 2.8	49.5 $\pm$ 5.2	50.6 $\pm$ 5.4	3.11	6.11***	2.09
RGRN (g/g/d)	0.221 $\pm$ 0.011	0.218 $\pm$ 0.011	0.287 $\pm$ 0.009	0.302 $\pm$ 0.010	0.130 $\pm$ 0.0178	0.174 $\pm$ 0.023	0.74	58.70***	3.12*

Sample sizes are 23 at each CO<sub>2</sub> level for *M. sativa* and *T. repens*, and 7 for *L. corniculatus*. Significance levels are indicated by symbols, where \*  $P < 0.05$  and \*\*\*  $P < 0.001$ . RCRN relative consumption rate of nitrogen; ADN approximate digestibility of nitrogen; ECDN efficiency of conversion of digested nitrogen; RGRN relative nitrogen growth rate

duration was shortest for larvae fed *T. repens* and shorter for larvae fed *M. sativa* than for larvae fed *L. corniculatus* (Table 2). Therefore, at both CO<sub>2</sub> levels, *T. repens* was a better host plant than *M. sativa* which, in turn, was a better host than *L. corniculatus*.

Like whole food nutritional indices, all nitrogen nutritional indices differed significantly among host plant species. RCRN and RGRN were highest for larvae fed *T. repens* and higher for larvae fed *M. sativa* than for larvae fed *L. corniculatus*. ADN was higher for larvae fed *M. sativa* and *T. repens* than for larvae fed *L. corniculatus*, and ECDN was higher for larvae fed *T. repens* than for larvae fed *M. sativa* or *L. corniculatus* (Table 3).

## Discussion

Several meta-analyses have concluded that the nutritional quality of legumes decreases less than that of non-legume C<sub>3</sub> species when plants are grown under elevated CO<sub>2</sub>. Cotrufo et al. (1998) determined that the decrease in foliar nitrogen under elevated CO<sub>2</sub> was only half as large for legumes as for non-legume C<sub>3</sub> species. Jablonski et al. (2002) observed a similar pattern for other plant parts, including seeds, and Ainsworth et al. (2002) concluded from over 100 studies that soybean foliar nitrogen increased on average by 8% under elevated CO<sub>2</sub>. The results of our study are consistent with this general trend: foliar nitrogen content of two herbaceous legumes, *M. sativa* and *L. corniculatus*, did not change under elevated CO<sub>2</sub>, while nitrogen increased by 8% in *T. repens*.

In this study, relative growth rate (RGR) of *C. philodice* larvae was not affected by elevated CO<sub>2</sub> on any of three legume host plants. RGR actually increased by 37%, albeit non-significantly, for larvae fed elevated-grown *L. corniculatus*. Goverde et al. (1999) observed a similar increase in growth of common blue lycaenid caterpillars fed elevated-grown *L. corniculatus*, though this increase disappeared when larvae were grown under field conditions (Goverde et al. 2004). Pupal weight, which is proportional to lifetime fecundity of *C. philodice* females (Karowe 1990) also was unaffected by elevated CO<sub>2</sub> on all three legumes. In a previous study (Karowe 2007), RGR and pupal weight were also unaffected for *C. philodice* fed elevated-grown legumes *Melilotus alba* and *T. pratense*.

Surprisingly, despite no change in foliar nitrogen or C:N ratio, larvae on all three species increased consumption (RCR) by at least 11%. Similarly, RCR also increased for *P. icarus* larvae fed elevated-grown *L. corniculatus* without a concomitant decrease in foliar nitrogen or increase in C:N ratio (Goverde et al. 1999). It is not clear which CO<sub>2</sub>-induced changes in foliar quality were responsible for increased consumption observed in this study. It is possible

that the reduced ability of larvae to digest nitrogen from elevated-grown plants (ADN was reduced by 4–8%) resulted in lower haemolymph amino acid concentrations, thereby stimulating consumption. However, on each host plant, consumption increased more (by 11–30%) than ADN decreased, suggesting that other factors were involved. Hamilton et al. (2005) suggested that increased sugars in soybean stimulated consumption by Japanese beetles. It is possible that reduced foliar water content may have caused increased consumption and decreased digestibility of elevated-grown plants. However, this seems unlikely, since *Manduca sexta* larvae fed a low-water diet exhibited no change in consumption and increased digestive efficiency (Martin and Van't Hof 1988).

On all plant species, elevated CO<sub>2</sub> increased the rate at which larvae consumed nitrogen (RCRN). There was an overall negative effect of elevated CO<sub>2</sub> on the efficiency with which larvae digested nitrogen, suggesting that elevated CO<sub>2</sub> may have altered the quality, rather than the quantity, of foliar nitrogen. Nonetheless, increased RCRN was sufficient to stabilize the rate at which larvae accumulated nitrogen (RGRN) on *M. sativa* and *T. repens* and to increase RGRN by 34%, albeit non-significantly, on elevated-grown *L. corniculatus*.

It is not clear why plant growth under elevated CO<sub>2</sub> appeared to have a more positive effect for larvae fed *L. corniculatus* than for larvae fed *M. sativa* or *T. repens*. It is possible that *L. corniculatus* contains one or more chemical defenses that are less abundant in elevated-grown plants. However, this seems unlikely since the three genera are similar in containing non-protein amino acids, protease inhibitors, and isoflavonoids, and lacking pyrrolizidine, indolizidine, indole and *Erythrina* alkaloids (Wink and Waterman 1999; Wink and Mohamed 2003).

Although some components of performance of 5th instar *C. philodice* differed between ambient- and elevated-grown legumes, the overall effects of elevated CO<sub>2</sub> were smaller than for most insects fed non-legume host plants (Watt et al. 1995; Coviella and Trumble 1999; Zvereva and Kozlov 2006). For instance, RGR was reduced by 25–60% for gypsy moth caterpillars fed trembling aspen (Lindroth et al. 1993), red maple (Williams et al. 2000), and sessile oak (Hättenschwiler and Schafellner, 2004), forest tent caterpillars fed trembling aspen (Lindroth et al. 1993), cotton bollworms fed cotton (Chen et al. 2005), beetles fed willow (Veteli et al. 2002), and grasshoppers fed sagebrush (Johnson and Lincoln 1991) and bog blueberry (Asshoff and Hättenschwiler 2005). Similarly, pupal, larval and/or adult weights were reduced by 10–35% for gypsy moths fed aspen and red maple (Lindroth et al. 1993), forest tent caterpillars fed trembling aspen (Percy et al. 2002), nun moths fed spruce (Hättenschwiler and Schafellner 1999), beet armyworms fed cotton (Akey and Kimball 1989),

cotton bollworms fed cotton and wheat (Chen et al. 2005; Wu et al. 2006), leaf miners fed Paterson's Curse (Johns and Hughes 2002), and grasshoppers fed bog blueberry (Asshoff and Hättenschwiler 2005). Most dramatically, elevated CO<sub>2</sub> increased mortality of buckeye larvae fed plantain by 180% (Fajer et al. 1989) and of leaf miners fed Paterson's Curse by 135% (Johns and Hughes 2002).

The few studies of legume-feeding herbivores suggest that they are less adversely affected by growth of their host plants under elevated CO<sub>2</sub>. When fed elevated-grown *L. corniculatus*, common blue lycaenid caterpillars converted ingested leaves into larval tissue more efficiently, grew faster, and contained more lipid as adults (Goverde et al. 1999). Growth of soybean under elevated CO<sub>2</sub> did not affect RGR of the soybean looper because RCR increased sufficiently to offset decreased ECD (Lincoln et al. 1984, 1986), and actually increased adult longevity and approximately doubled fecundity of the Japanese beetle (O'Neill et al. 2008). In a previous study of *C. philodice*, elevated CO<sub>2</sub> did not affect RGR, instar duration, or pupal weight for larvae fed red clover or white sweet clover (Karowe 2007). Similarly, performance of the cabbage looper did not differ between unfertilized ambient- and elevated-grown lima beans, though elevated CO<sub>2</sub> reduced pupal weight when plants were fertilized (Osbrink et al. 1987). In contrast, pupal weight decreased and development time increased for cotton leafworm larvae fed elevated-grown alfalfa, (Agrell et al. 2006), possibly due to increased saponin levels (Agrell et al. 2004).

Though there are still too few studies to warrant generalization, it appears that the association of legumes with nitrogen-fixing bacteria at least partially buffers legume herbivores against many of the adverse effects typically experienced by herbivores of nonlegume C<sub>3</sub> species when their host plants are grown under elevated CO<sub>2</sub>. The attenuated response of legume nutritional quality to elevated CO<sub>2</sub> is most likely a consequence of symbiotic nitrogen fixation, which generally increases under elevated CO<sub>2</sub> (Hungate et al. 1999; Edwards et al. 2006). Given that legumes are expected to make an increasingly important contribution to ecosystem nitrogen availability (West et al. 2005; de Graaff et al. 2006) and, therefore, to global carbon storage (Hungate et al. 2003) as atmospheric CO<sub>2</sub> continues to rise, additional studies of the effects of elevated CO<sub>2</sub> on legume-feeding herbivores, including other guilds than leaf chewers, would be valuable. Moreover, the observation that mortality of silkworm larvae increased under elevated CO<sub>2</sub> on birch, oak, and maple but not on alder, which harbors the nitrogen-fixing actinomycete *Frankia* sp. (Koike et al. 2006), suggests that such environmental buffering may also be accomplished by non-legumes that are associated with a range of nitrogen-fixing symbionts.

**Acknowledgments** We thank Chris Vogel for technical assistance, Mike Grant for processing CHN samples, the University of Michigan Biological Station for logistical support, and two anonymous reviewers for helpful comments. This research was supported United States Department of Agriculture NRICGP grant #9706410 and National Science Foundation grant #DEB-9796250 to D. Karowe and by National Science Foundation grant #DEB-9731615 to J. Teeri and D. Karowe.

## References

- Agrell J, Anderson P, Oleszek W, Stochmal A, Agrell C (2004) Combined effects of elevated CO<sub>2</sub> and herbivore damage on alfalfa and cotton. *J Chem Ecol* 30:2309–2324
- Agrell J, Anderson P, Oleszek W, Stochmal A, Agrell C (2006) Elevated CO<sub>2</sub> levels and herbivore damage alter host plant preferences. *Oikos* 112:63–72
- Ainsworth E, Davey P, Bernacchi C, Dermody O, Heaton E, Moore D, Morgan P, Naidu S, Yoo Ra H, Zhu X, Curtis P, Long S (2002) A meta-analysis of elevated [CO<sub>2</sub>] effects on soybean (*Glycine max*) physiology, growth and yield. *Glob Change Biol* 8:695–709
- Akey D, Kimball B (1989) Growth and development of the beet armyworm on cotton grown in an enriched carbon dioxide atmosphere. *Southwest Entomol* 14:255–260
- Asshoff R, Hättenschwiler S (2005) Growth and reproduction of the alpine grasshopper *Miramella alpina* feeding on CO<sub>2</sub> enriched dwarf shrubs at treeline. *Oecologia* 142:191–201
- Barbehenn R, Karowe D, Spickard A (2004) Effects of elevated atmospheric CO<sub>2</sub> on the nutritional ecology of C<sub>3</sub> and C<sub>4</sub> grass-feeding caterpillars. *Oecologia* 140:86–95
- Bowers M, Stamp N, Fajer E (1991) Factors affecting calculation of nutritional indices for foliage-fed insects: an experimental approach. *Ent Exp et Appl* 61:101–116
- Chen F, Wu G, Feng G, Parajulee M, Shrestha R (2005) Effects of elevated CO<sub>2</sub> and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Ent Exp Appl* 115:341–350
- Cotrufo M, Ineson P, Scott A (1998) Elevated CO<sub>2</sub> reduces the nitrogen concentration of plant tissues. *Glob Change Biol* 4:43–54
- Coviella C, Trumble J (1999) Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Cons Biol* 13:700–712
- de Graaff M-A, van Groenigen K-J, Six J, Hungate B, van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO<sub>2</sub>: a meta-analysis. *Glob Change Biol* 12:2077–2091
- Dermody O, O'Neill B, Zangerl A, Berenbaum M, DeLucia E (2008) Effects of elevated CO<sub>2</sub> and O<sub>3</sub> on leaf damage and insect abundance in a soybean agroecosystem. *Arthropod-Plant Int* 2:125–135
- Edwards E, McCaffery S, Evans J (2006) Phosphorus availability and elevated CO<sub>2</sub> affect biological nitrogen fixation and nutrient fluxes in a clover-dominated sward. *New Phytol* 169:157–167
- Fajer E, Bowers M, Bazzaz F (1989) The effects of enriched carbon dioxide atmospheres on plant-herbivore interactions. *Science* 243:1198–1200
- Goverde M, Bazin A, Shykoff J, Erhardt A (1999) Influence of leaf chemistry of *Lotus corniculatus* (Fabaceae) on larval development of *Polyommatus icarus* (Lepidoptera, Lycaenidae): effects of elevated CO<sub>2</sub> and plant genotype. *Funct Ecol* 13:801–810
- Goverde M, Erhardt A, Stöcklin J (2004) Genotype-specific response of a lycaenid herbivore to elevated carbon dioxide and phosphorus availability in calcareous grassland. *Oecologia* 139:383–391
- Hamilton J, Dermody O, Aldea M, Zangerl A, Rogers A, Berenbaum M, DeLucia E (2005) Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environ Entomol* 43:479–485
- 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
- Hättenschwiler S, Schafellner C (2004) Gypsy moth feeding in the canopy of a CO<sub>2</sub>-enriched mature forest. *Glob Change Biol* 10:1899–1908
- Hungate B, Dijkstra P, Johnson D, Hinkle C, Drake B (1999) Elevated CO<sub>2</sub> increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Glob Change Biol* 5: 781–789
- Hungate B, Dukes J, Shaw M, Luo Y, Field C (2003) Nitrogen and climate change. *Science* 302:1512–1513
- Hunter M (2001) Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Agric Forest Entomol* 3:153–159
- Institute SAS (2000) The SAS system for Windows, Version 8e. SAS Institute, Cary
- Jablonski L, Wang X, Curtis P (2002) Plant reproduction under elevated CO<sub>2</sub> conditions: A meta-analysis of reports on 79 crop and wild species. *New Phytol* 156:9–26
- Johns C, Hughes L (2002) Interactive effects of elevated CO<sub>2</sub> and temperature on the leaf-miner *Dialectica scariella* Zeller (Lepidoptera: Gracillariidae) in Paterson's Curse, *Echium plantagineum* (Boraginaceae). *Glob Change Biol* 8:142–152
- Johnson R, Lincoln D (1991) Sagebrush carbon allocation patterns and grasshopper nutrition: the influence of CO<sub>2</sub> enrichment and soil mineral nutrition. *Oecologia* 87:127–134
- Karowe D (1988) Ecological and evolutionary determinants of larval host plant range in the sulfur butterflies, *Colias philodice* and *Colias interior*. PhD thesis, University of Michigan, Ann Arbor
- Karowe D (1989) Facultative monophagy as a consequence of feeding experience: behavioral and physiological specialization in *Colias philodice* larvae (Lepidoptera: Pieridae). *Oecologia* 78:106–111
- Karowe D (1990) Predicting host range evolution: colonization of *Coronilla varia* by *Colias philodice* (Lepidoptera: Pieridae). *Evolution* 44:1637–1647
- Karowe D (2007) Are legume-feeding herbivores buffered against direct effects of elevated CO<sub>2</sub> on host plants? A test with the sulfur butterfly, *Colias philodice*. *Glob Change Biol* 13:2045–2051
- Karowe D, Siemens D, Mitchell-Olds T (1997) Species-specific response of glucosinolate content to elevated atmospheric CO<sub>2</sub>. *J Chem Ecol* 23:2569–2582
- Klots AB (1951) Butterflies of North America. Houghton Mifflin, San Francisco
- Koike T, Tobita H, Shibata T, Matsuki H, Konno K, Kitao M, Yamashita N, Maruyama Y (2006) Defense characteristics of several deciduous broad-leaved tree seedlings grown under differing levels of CO<sub>2</sub> and nitrogen. *Pop Ecol* 48:23–29
- Körner C (2006) Plant CO<sub>2</sub> responses: an issue of definition, time and resource supply. *New Phytol* 172:393–411
- Lincoln M, Strain B (1984) Growth and feeding response of *Pseudoplusia includens* (Lepidoptera: Noctuidae) to host plants grown in controlled carbon dioxide atmospheres. *Environ Entomol* 13:1527–1530
- Lincoln D, Sionit M, Strain B (1986) Response of an insect herbivore to host plants grown in carbon dioxide enriched atmospheres. *Oecologia* 69:556–560
- Lindroth R, Kinney K, Platz C (1993) Responses of deciduous trees to elevated atmospheric CO<sub>2</sub>: productivity, phytochemistry, and insect performance. *Ecology* 74:763–777

- Martin M, van't Hof H (1988) The cause of reduced growth of *Manduca sexta* larvae on a low-water diet: Increased metabolic processing costs or nutrient limitation? *J Insect Physiol* 34:515–525
- Matthews H (2006) Emissions targets for CO<sub>2</sub> stabilization as modified by carbon cycle feedbacks. *Tellus B* 58:591–602
- Mattson W (1980) Herbivory in relation to plant nitrogen content. *Ann Rev Ecol Syst* 11:119–161
- Meehl G, Stocker T, Collins W et al. (2007) Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Avery KB, Tignor M, Miller HL (eds) *Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the intergovernmental panel on climate change*
- Nowak R, Ellsworth D, Smith S (2004) Functional responses of plants to elevated atmospheric CO<sub>2</sub>—do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol* 162:253–280
- O'Neill B, Zangerl A, DeLucia E, Berenbaum M (2008) Longevity and fecundity of Japanese Beetle (*Popillia japonica*) on foliage grown under elevated carbon dioxide. *Environ Entomol* 37:601–607
- Osbrink W, Trumble J, Wagner R (1987) Host suitability of *Phaseolus lunata* for *Trichoplusia ni* (Lepidoptera: Noctuidae) in controlled carbon dioxide atmospheres. *Environ Entomol* 16:639–644
- Penuelas J, Estiarte M (1998) Can elevated CO<sub>2</sub> affect secondary metabolism and ecosystem function? *Trends Ecol Evol* 13:20–24
- Percy K, Awmack C, Lindroth R, Kubiske M, Kopper B, Isebrands J, Pregitzer K, Hendrey G, Dickson R, Zak D, Oksanen E, Sober J, Harrington R, Karnosky D (2002) Altered performance of forest pests under atmospheres enriched by CO<sub>2</sub> and O<sub>3</sub>. *Nature* 420:403–407
- Schädler M, Roeder M, Brandl R (2007) Interacting effects of elevated CO<sub>2</sub>, nutrient availability and plant species on a generalist invertebrate herbivore. *Glob Change Biol* 13:1005–1015
- Schmidt D, Reese J (1986) Sources of error in nutritional index studies of insects on artificial diets. *J Insect Physiol* 32:193–198
- Scott JA (1986) *The Butterflies of North America*. Stanford University Press, Stanford
- Scriber JM, Feeny P (1979) Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants. *Ecology* 60:829–850
- Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. *Ann Rev Ecol Syst* 26:183–211
- Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO<sub>2</sub>) affect plant-herbivore interactions? A field experiment and meta-analysis of CO<sub>2</sub>-mediated changes on plant chemistry and herbivore performance. *Glob Change Biol* 13:1823–1842
- Valkama E, Koricheva J, Oksanen E (2007) Effects of elevated O<sub>3</sub>, alone and in combination with elevated CO<sub>2</sub>, on tree leaf chemistry and insect herbivore performance: a meta-analysis. *Glob Chang Biol* 13:184–201
- van Groenigen K, Six J, Hungate B, de Graaff M, van Breemen N, van Kessel C (2006) Element interactions limit soil carbon storage. *Proc Natl Acad Sci USA* 103:6571–6574
- Veteli T, Kuokkanen K, Julkunen-Tiitto R, Roininen H, Tahvanainen J (2002) Effects of elevated CO<sub>2</sub> and temperature on plant growth and herbivore defensive chemistry. *Glob Change Biol* 8:1240–1252
- Waldbauer G (1968) The consumption and utilization of food by insects. *Adv Insect Physiol* 5:229–289
- Wang X, Ji L, Wang G, Liu Y (2008) Potential effects of elevated carbon dioxide on leaf-feeding forest insects. *Front Biol China* 3:68–77
- Watt A, Whittaker M, Docherty M, Brooks G, Lindsay E, Salt D (1995) The impact of elevated atmospheric CO<sub>2</sub> on insect herbivores. In: Harrington R, Stork ME (eds) *Insects in changing environment*. Academic Press, San Diego, California, pp 197–217
- West J, HilleRisLambers J, Lee T, Hobbie S, Reich P (2005) Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric [CO<sub>2</sub>]. *New Phytol* 167:523–530
- Wilkinson L (2000) SYSTAT: the system for statistics. SYSTAT Inc, Evanston
- Williams R, Norby R, Lincoln D (2000) Effects of elevated CO<sub>2</sub> and temperature-grown red and sugar maple on gypsy moth performance. *Glob Change Biol* 6:685–695
- Wink M, Mohamed G (2003) Evolution of chemical defense traits in the Leguminosae: mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the *rbcl* gene. *Biochem Syst Ecol* 31:897–917
- Wink M, Waterman P (1999) Chemotaxonomy in relation to molecular phylogeny of plants. *Annl Plant Rev* 2:300–341
- Wu G, Chen F-J, Ge F (2006) Response of multiple generations of cotton bollworm *Helicoverpa armigera* Hübner, feeding on spring wheat, to elevated CO<sub>2</sub>. *J Appl Entomol* 130:2–9
- Zeng N, Qian H, Munoz E, Iacono R (2004) How strong is carbon cycle-climate feedback under global warming? *Geo Res Lett* 31:L20203. doi:10.1029/2004GL020904
- Zvereva E, Kozlov M (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: a metaanalysis. *Glob Change Biol* 12:27–41