

Are legume-feeding herbivores buffered against direct effects of elevated carbon dioxide on host plants? A test with the sulfur butterfly, *Colias philodice*

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Abstract

When grown under elevated atmospheric carbon dioxide (CO₂), leaf nitrogen content decreases less for legumes than for nonlegume C₃ plants. Given that elevated CO₂ adversely affects insect herbivores primarily through dilution of plant nitrogen, it is reasonable to expect that legume-feeding herbivores will be relatively buffered against CO₂-induced reduction in performance. However, despite their ecological and economic importance, very few studies have addressed the effects of elevated CO₂ on legume-feeding herbivores. Unlike the responses of the vast majority of nonlegume C₃ plants, when the legumes *Trifolium pratense* and *Melilotus alba* were grown under elevated (742 ppm) CO₂, leaf nitrogen and carbon contents and C:N ratios did not change. For *Colias philodice* larvae fed *T. pratense*, elevated CO₂ had little or no effect on consumption, digestion, or conversion of whole food or nitrogen and, consequently, no effect on growth rate, instar duration, or pupal weight. For larvae fed *M. alba*, elevated CO₂ had little or no effect on consumption of whole food or nitrogen, increased digestion but decreased conversion of both and, consequently, had no effect on growth rate, instar duration or pupal weight. These results suggest that, relative to herbivores of nonlegume C₃ plants, legume-feeding herbivores will be less affected as atmospheric CO₂ continues to rise.

Keywords: *Colias philodice*, elevated CO₂, herbivory, legumes, *Melilotus alba*, nutritional indices, *Trifolium pratense*

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Introduction

It is now widely expected that the concentration of carbon dioxide (CO₂) in Earth's atmosphere will rise from the current level of 380 to 500–750 ppm by the end of this century (Falkowski *et al.*, 2000; Alley *et al.*, 2007). Such a change in atmospheric composition would likely have important consequences for interactions between plants and their insect herbivores. Elevated CO₂ generally stimulates plant growth (Curtis & Wang, 1998; Poorter & Navas, 2003), but it also results in the synthesis of leaves that have lower nitrogen and water contents, and higher carbon:nitrogen ratios (Lincoln *et al.*, 1984; Karowe *et al.*, 1997; Barbehenn *et al.*, 2004b; Körner, 2006). However, the response to elevated CO₂ differs between plant functional groups. In particular,

because total nitrogen fixation by legumes tends to increase under elevated CO₂ (Vogel *et al.*, 1997; Hartwig *et al.*, 2000; Edwards *et al.*, 2006), foliar nitrogen content of legumes decreases on average by only 7%, while that of nonlegume C₃ species decreases by 15% (Cotrufo *et al.*, 1998).

It has long been known that plant nitrogen content profoundly influences insect herbivore performance (Scriber & Feeny, 1979; Mattson, 1980; Scriber & Slansky, 1981; Karowe & Martin, 1989). Presumably to compensate for decreased leaf nitrogen content of plants grown under elevated CO₂, insect herbivores generally increase consumption rates but, nevertheless, often display reduced survivorship and/or growth (Fajer *et al.*, 1989; Lincoln *et al.*, 1993; Lindroth *et al.*, 1993; Percy *et al.*, 2002; Goverde & Erhardt, 2003; Chen *et al.*, 2005; Zvereva & Kozlov, 2006; but see Bezemer & Jones, 1998).

Given that elevated CO₂ appears to adversely affect insect herbivores primarily through dilution of plant

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nitrogen, it is plausible that legume-feeding herbivores may be relatively buffered against CO₂-induced changes in larval performance. However, despite the ecological and economic importance of legumes, remarkably few studies have addressed the effects of elevated CO₂ on legume-feeding herbivores. Growth rate was not reduced for soybean loopers fed elevated CO₂ grown (hereafter elevated-grown) soybean (Lincoln *et al.*, 1984, 1986) or cabbage loopers fed unfertilized elevated-grown lima beans (Osbrink *et al.*, 1987). When lycaenid caterpillars were fed elevated-grown birdsfoot trefoil, performance generally increased (Goverde *et al.*, 1999), though the response depended on larval genotype (Goverde *et al.*, 2004). In contrast, growth of cotton leafworm was reduced on elevated-grown alfalfa (Agrell *et al.*, 2006).

The common sulfur butterfly, *Colias philodice* Latreille, is widely distributed throughout the continental United States and contiguous regions of southern Canada and feeds throughout its range on at least 15 genera of herbaceous legumes (Klots, 1951; Scott, 1986). In northern Michigan, primary host plants include *Trifolium pratense* (red clover) and *Melilotus alba* L. (white sweet clover) (Karowe, 1988). *Colias philodice* and its host plants, therefore, provide an excellent opportunity to test the effect of elevated CO₂ on legume-herbivore interactions. Accordingly, this study addresses two related hypotheses:

1. Growth under elevated CO₂ will not alter the nutritional quality of legume foliage.
2. Consequently, performance of the legume-feeding herbivore, *C. philodice*, will not differ between plants grown under ambient and elevated CO₂.

Materials and methods

Legumes

In early May 2002, *T. pratense* and *M. alba* were sown from seed in 6-in. pots filled with potting soil and allowed to germinate in the greenhouse at the University of Michigan Biological Station (UMBS) in Cheboygan County, Michigan. Three days after germination, plants were thinned to three per pot, and two pots of each species were placed into each of 20 0.5 m³ open-topped chambers (Karowe *et al.*, 1997; Barbehenn *et al.*, 2004a) located in a field adjacent to the greenhouse. Ten chambers were maintained at 742 ± 7 ppm CO₂ (elevated treatment) and 10 chambers were maintained at 374 ± 8 ppm CO₂ (ambient treatment). CO₂ concentration inside the elevated chambers was controlled by dispensing 100% CO₂ into the inlet port of an input blower and monitored by continuous sampling of

chamber air. 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 CO₂ was adjusted with manual flowmeters. Plants were watered twice daily, inspected periodically for insect herbivores and, when necessary, sprayed with Safer Soap, a short-lived organic insecticide.

C. philodice

Eggs were obtained from 15 gravid *C. philodice* females collected from fields that contained both *T. pratense* and *M. alba* near UMBS in early July. 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 W incandescent bulb) and maintained on a solution of 15% clover honey in water. On average, females laid approximately 150 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, NJ, USA); larvae were reared on diet to prevent feeding induction (Lincoln *et al.*, 1984; Karowe, 1989). Cups were placed in a Precision 818 environmental chamber under a 16:8 L:D cycle and a corresponding 25:20 °C temperature cycle. Fresh diet was provided every 2 days.

Feeding trials

Upon reaching the end of the fourth instar, larvae were transferred to empty Petri dishes. Immediately after molting into the fifth (ultimate) instar, test larvae were weighed and placed individually into 5.5 cm Petri dishes containing a preweighed amount of leaf tissue from one of the four pots per chamber. Fifth instar larvae were used because earlier instars do not consume enough to provide accurate measurements. An additional 20 freshly molted fifth instar larvae were weighed wet, 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. A total of 20 larvae (two from each chamber) were placed on each species at each CO₂ level. Initial larval weights did not differ between the four treatment combinations ($F_{3,74} = 2.34$, $P > 0.1$). To avoid placing a disproportionate number of fast growing larvae on any treatment or chamber, one test larva was placed on leaves of each species from each chamber within each CO₂ treatment before a second larva was placed on any. Each test larva was given fresh leaves from the same pot every 2 days, and an equal aliquot of

leaves from the same pot was used to provide a wet/dry conversion factor used to estimate the dry weight 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 & Reese, 1986; Bowers *et al.*, 1991). All test larvae pupated successfully, with the exception of two larvae feeding on elevated-grown *M. alba* that were inadvertently lost. Immediately upon pupation, each pupa was weighed, frozen, dried to constant weight at 60 °C, and reweighed. All frass produced during the fifth 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 content using a Perkin Elmer CHN Elemental Analyzer (Perkin Elmer, Waltham, MA, USA). Two replicates were run for each sample; if replicates differed by >10%, a third replicate was run. For all test larvae, standard gravimetric nutritional indices and nitrogen nutritional indices were calculated (Waldbauer, 1968) as

Relative consumption rate (RCR)

$$= (\text{food ingested}) / (\text{average larval weight} \times \text{days})$$

Approximate digestibility (AD)

$$= (\text{food ingested} - \text{frass}) / (\text{food ingested})$$

Efficiency of conversion of digested food (ECD)

$$= (\text{weight gained}) / (\text{food ingested} - \text{frass})$$

Relative growth rate (RGR)

$$= (\text{weight gained}) / (\text{average larval weight} \times \text{days})$$

$$\text{RGR} = \text{RCR} \times \text{AD} \times \text{ECD}$$

Nitrogen nutritional indices were calculated by substituting nitrogen for weight.

The experimental design was a split-plot design, with CO₂ as whole plots and plant species (*T. pratense* and *M. alba*) as subplots. Pots were rotated among blocks every 3 days to minimize block effects. All variables were

analyzed with a split-plot Type III ANOVA (SAS, 2000, The SAS System for Windows, Version 8e, SAS Institute, Cary, NC, USA) with CO₂ level and plant species as main effects and block as a random variable. The normality of residuals was verified with Kolmogorov–Smirnov tests. If necessary, data were normalized by log transformation before analysis. Homogeneity of variances was verified by F_{max} tests.

Results

Overall, nitrogen content of the two legume species was not affected by growth under elevated CO₂ (Table 1). Leaf nitrogen content of both *T. pratense* and *M. alba* decreased by only 2.7%, and in both cases remained high. Leaf C:N ratio also did not change significantly under elevated CO₂, increasing by <3% for each species. Similarly, carbon content was unaffected by elevated CO₂, decreasing by <2% for each species. No measure of nutritional quality exhibited a significant CO₂ × Species interaction, indicating that the two species were similarly unaffected by elevated CO₂. However, regardless of CO₂ level, *M. alba* leaves had significantly higher nitrogen content and lower C:N ratio than did leaves of *T. pratense*.

Overall, relative consumption rate (RCR) of *C. philodice* larvae was not affected by growth of host plants under elevated CO₂ (Table 2). RCR increased by <4% for larvae fed each species. Overall, digestive efficiency (AD) did not differ between CO₂ levels, but a significant CO₂ × Species interaction arose because, under elevated CO₂, AD decreased by 3% for larvae fed *T. pratense*, but increased by 12% for larvae fed *M. alba*. Host plant growth under elevated CO₂ resulted in an overall 14% decrease in the efficiency with which larvae converted digested tissue into larval biomass (ECD), but this effect was due entirely to a 24% decrease for larvae fed *M. alba*. ECD was actually 3% higher for larvae fed elevated-grown *T. pratense* than for those fed ambient-grown *T. pratense*, resulting in a significant CO₂ ×

Table 1 Measures of leaf nutritional quality (mean ± SE) for *Trifolium pratense* and *Melilotus alba* grown at ambient (374 ppm) and elevated (742 ppm) CO₂

Variable	<i>Trifolium pratense</i>		<i>Melilotus alba</i>		F values		
	Ambient	Elevated	Ambient	Elevated	CO ₂ df = 1.9	Species df = 1.56	CO ₂ × Species df = 1.56
Nitrogen	4.01 ± 0.25	3.90 ± 0.22	5.07 ± 0.19	4.93 ± 0.22	2.90	27.64***	0.22
Carbon	42.7 ± 0.5	42.0 ± 0.4	43.0 ± 0.3	42.9 ± 0.4	0.91	1.90	0.64
C:N ratio	10.9 ± 0.6	11.2 ± 0.5	8.8 ± 0.5	9.0 ± 0.5	3.05	33.98***	0.92

Nitrogen and carbon values are percent of dry weight. Sample sizes are 18–20 for each species × CO₂ combination. *** $P < 0.001$.

Table 2 Whole food nutritional indices (mean \pm SE) for *Colias philodice* larvae fed *Trifolium pratense* and *Melilotus alba* grown at ambient (374 ppm) and elevated (742 ppm) CO₂

Variable	<i>Trifolium pratense</i>		<i>Melilotus alba</i>		F values		
	Ambient	Elevated	Ambient	Elevated	CO ₂ df = 1.9	Species df = 1.56	CO ₂ \times Species df = 1.56
RCR (g g ⁻¹ day ⁻¹)	1.45 \pm 0.05	1.46 \pm 0.05	1.48 \pm 0.05	1.53 \pm 0.05	3.43	0.07	2.41
AD (%)	65.1 \pm 1.6	63.4 \pm 1.6	60.3 \pm 1.5	67.3 \pm 1.5	3.18	0.08	8.77**
ECD (%)	19.0 \pm 1.2	19.6 \pm 1.2	31.5 \pm 1.0	23.9 \pm 1.0	7.95*	78.33***	18.87***
RGR (g g ⁻¹ day ⁻¹)	0.179 \pm 0.01	0.179 \pm 0.01	0.256 \pm 0.01	0.245 \pm 0.01	0.31	47.74***	0.30
Pupal weight (g dry)	0.032 \pm 0.002	0.033 \pm 0.002	0.061 \pm 0.002	0.058 \pm 0.002	2.04	203.64***	3.50 [†]
Fifth Instar duration (h) [‡]	138.9 \pm 6.0	133.2 \pm 6.0	129.0 \pm 5.7	133.6 \pm 5.7	0.01	0.35	0.59
Food eaten (g dry)	0.18 \pm 0.01	0.17 \pm 0.01	0.26 \pm 0.01	0.28 \pm 0.01	0.69	121.94***	0.88

Sample sizes are 18–20 for each CO₂ \times Species combination.

[†]0.10 < *P* < 0.05, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

[‡]Data were normalized by log transformation before analysis.

RCR, relative consumption rate; RGR, relative growth rate.

Table 3 Nitrogen nutritional indices (mean \pm SE) for *Colias philodice* larvae fed *Trifolium pratense* and *Melilotus alba* grown at ambient (374 ppm) and elevated (742 ppm) CO₂

Variable	<i>Trifolium pratense</i>		<i>Melilotus alba</i>		F values		
	Ambient	Elevated	Ambient	Elevated	CO ₂ df = 1.9	Species df = 1.56	CO ₂ \times Species df = 1.56
RCRN (g g ⁻¹ day ⁻¹)	0.59 \pm 0.03	0.59 \pm 0.03	0.69 \pm 0.02	0.69 \pm 0.02	0.03	16.44***	0.02
ADN (%)	79.8 \pm 1.3	76.9 \pm 1.3	74.6 \pm 1.1	77.8 \pm 1.2	0.02	2.96 [†]	6.25*
ECDN (%)	35.8 \pm 2.1	36.0 \pm 2.1	47.5 \pm 1.8	42.5 \pm 2.0	1.39	21.41***	1.74
RGRN (g g ⁻¹ day ⁻¹)	0.167 \pm 0.010	0.161 \pm 0.010	0.239 \pm 0.009	0.228 \pm 0.010	0.92	50.92***	0.03
Pupal nitrogen (%)	10.20 \pm 0.15	9.69 \pm 0.15	9.42 \pm 0.14	9.47 \pm 0.15	2.28	13.04***	4.15*

Sample sizes are 18–20 for each CO₂ \times Species combination.

[†]0.10 < *P* < 0.05, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Species interaction. Overall, larvae fed elevated-grown plants did not grow any more slowly than larvae fed ambient-grown plants; RGR was unchanged for larvae fed elevated-grown *T. pratense*, and was only 4% lower for larvae fed elevated-grown *M. alba*. Overall, larvae fed ambient- and elevated-grown legumes produced pupae of equivalent weight, though a nearly significant CO₂ \times Species interaction arose because pupal weight increased by 3% for larvae fed elevated-grown *T. pratense* but decreased by 5% for larvae fed elevated-grown *M. alba*. Neither species-specific change was significant, however (*P* = 0.68 and 0.13, respectively). Overall, larvae fed ambient- and elevated-grown legumes did not differ in the amount of time required to complete the fifth instar or the amount of food eaten during the instar.

Nitrogen nutritional indices revealed a similar pattern. Overall, relative nitrogen consumption rate (RCRN) of *C. philodice* larvae was not affected by growth of legumes under elevated CO₂ (Table 3). Like

AD, nitrogen digestive efficiency (ADN) did not differ between CO₂ levels, but a significant CO₂ \times Species interaction arose because, under elevated CO₂, ADN decreased by 4% for larvae fed *T. pratense* but increased by 4% for larvae fed *M. alba*. Unlike ECD, nitrogen conversion efficiency (ECDN) was unaffected by elevated CO₂ and did not exhibit a CO₂ \times Species interaction. Like RGR, relative nitrogen growth rate (RGRN) was unaffected by elevated CO₂. Interestingly, pupal nitrogen decreased by 5% for larvae fed elevated-grown *T. pratense* but was essentially unchanged for larvae fed elevated-grown *M. alba*, producing a significant CO₂ \times Species interaction.

Many measures of larval performance differed between legume species. Relative to larvae fed *T. pratense*, larvae fed *M. alba* consumed more food during the fifth instar (though at an equivalent rate relative to body size), digested it with equal efficiency but converted digested food to larval tissue much more efficiently and, consequently, grew faster and produced much

heavier pupae (Tables 2 and 3). Relative to larvae fed *T. pratense*, larvae fed *M. alba* consumed nitrogen at a faster rate, digested it less efficiently but converted digested nitrogen into larval tissue more efficiently and, consequently, accumulated tissue nitrogen much more rapidly. Nonetheless, because pupae from larvae fed *T. pratense* were much smaller, they actually contained more nitrogen as a percent of dry weight.

Discussion

In this study, growth of two legumes under twice ambient CO₂ did not result in any change in leaf nitrogen or carbon contents or C:N ratio. This result is consistent with conclusion of Cotrufo *et al.* (1998) that the nutritional quality of legumes is less affected by elevated CO₂ than is that of nonlegume C₃ plants. In their analysis of 75 studies, leaf nitrogen content of legumes decreased on average by only 7% under elevated CO₂, which was less than half the decrease exhibited by nonlegume C₃ plants. Other tissues behave similarly: for instance, a meta-analysis of crop and wild species by Jablonski *et al.* (2002) indicated that seed nitrogen was unaffected by legume growth under elevated CO₂, but decreased on average by 15% for nonlegume C₃ plants. The diminished response of legume nutritional quality to elevated CO₂ is likely a consequence of enhanced nitrogen fixation (Edwards *et al.*, 2006; van Groenigen *et al.*, 2006).

Insect herbivores can be considered to be buffered from the adverse effects of elevated CO₂ if plant quality does not change, or if herbivores are able to compensate for changes in plant quality sufficiently to prevent decreased RGR and pupal weight and increased larval development time. The assumption underlying the focus on RGR, pupal weight and development time is that these measures arguably are the three that most directly impact fitness. Pupal weight is frequently strongly positively correlated with fecundity in Lepidoptera (e.g. Karowe, 1990, 1992). Though relevant data are scarce, development time is generally accepted to be negatively correlated with mortality from predators and parasitoids (Price *et al.*, 1980, 1986; but see Gotthard, 2000), and RGR is a function of both weight gain and development time. Using this definition, the results of this study suggest that herbivores feeding on legumes may be buffered against the typical adverse effects associated with nonlegume C₃ host plants grown under elevated CO₂. Relative to larvae fed *T. pratense*, larvae fed *M. alba* grew more rapidly but were slightly more influenced by host plant exposure to elevated CO₂. For larvae fed *T. pratense*, elevated CO₂ had little or no effect on consumption, digestion, or conversion of whole food or nitrogen and, consequently, no effect on growth rate,

instar duration, or pupal weight. For larvae fed *M. alba*, elevated CO₂ had little or no effect on consumption of whole food or nitrogen, increased digestion but decreased conversion of both and, consequently, had no effect on growth rate, instar duration, or pupal weight. Because pupal weight is positively correlated with fecundity for *C. philodice* (Karowe, 1990), it is likely that fecundity also will be unaffected by elevated CO₂.

Although digestive efficiency (AD) increased and conversion efficiency (ECD) decreased for larvae fed elevated-grown *M. alba*, the changes were smaller than those exhibited by most lepidopterans fed nonlegume host plants grown under elevated CO₂ (Coviella & Trumble, 1999; Zvereva & Kozlov, 2006). For instance, when fed trembling aspen grown at 650 ppm CO₂, RGR of gypsy moth larvae decreased by 63% and pupal weight decreased by 14%, while RGR of the forest tent caterpillar decreased by 25% (Lindroth *et al.*, 1993). Gypsy moth RGR was also 30% lower when fed elevated-grown sessile oak (Hättenschwiler & Schafellner, 2004). Other insect orders may also be adversely affected by host plant exposure to elevated CO₂. When fed sagebrush grown at 650 ppm CO₂, RGR of the grasshopper *Melanoplus differentialis* decreased by 40% (Johnson & Lincoln, 1991), and RGR, larval development rate, female adult weight, and egg weight were reduced for the grasshopper *Miramella alpina* fed the alpine shrub *Vaccinium uliginosum* grown at 550 ppm (Asshoff & Hättenschwiler, 2005). Even more dramatic reductions in larval performance have been observed on some herbaceous C₃ plants. For instance, mortality over the entire larval period increased by 180% for buckeye larvae fed plantain grown at 700 ppm (Fajer *et al.*, 1989) and by 135% for leaf miners fed Paterson's Curse (Johns & Hughes, 2002). Interestingly, silkworm larval survivorship decreased under elevated CO₂ on oak, maple, and birch, but not on alder, a nitrogen fixer (Koike *et al.*, 2006).

Although elevated CO₂ generally adversely affects performance of insect herbivores (Coviella & Trumble, 1999; Zvereva & Kozlov, 2006), not all insects perform poorly when fed nonlegume host plants grown under elevated CO₂ (Bezemer & Jones, 1998). For instance, gypsy moth pupal weight was not significantly reduced for larvae fed elevated-grown red oak (Lindroth *et al.*, 1993), and RGR did not decrease significantly on elevated-grown European beech and increased by 30% on elevated-grown European hornbeam (Hättenschwiler & Schafellner, 2004).

Given the ecological and economic importance of legumes, it is surprising so few studies have addressed the effect of elevated CO₂ on legume-feeding herbivores. When fed soybean grown at 650 ppm, neither

RGR nor instar duration of soybean looper larvae changed significantly because, as was observed for *C. philodice* feeding on *M. alba* in this study, decreased ECD was offset by increased RCR and AD (Lincoln *et al.*, 1984, 1986). Similarly, performance of the cabbage looper, *Trichoplusia ni*, was unaffected when fed unfertilized elevated-grown lima beans. However, pupal weight was lower when elevated-grown lima beans were fertilized (Osbrink *et al.*, 1987). When fed elevated-grown *Lotus corniculatus*, larvae of the lycaenid butterfly *Polyommatus icarus* converted ingested leaves into larval tissue more efficiently, grew faster, and contained more lipid as adults (Goverde *et al.*, 1999). In contrast, when larvae of the cotton leafworm were reared from hatching to pupation on elevated-grown alfalfa, pupal weight decreased and development time increased (Agrell *et al.*, 2006). Although herbivore growth was not measured, overall herbivore damage to the legume *Galactia elliottii* decreased under elevated CO₂, but a similar change was observed for three oak species (Hall *et al.*, 2005).

Taken together, the results of this and previous studies generally support the hypothesis that legume-feeding herbivores will be relatively buffered against the typical adverse effects of elevated CO₂ on herbivore performance. Clearly, however, additional studies are needed, particularly ones that compare performance of a single herbivore (or closely related herbivores) feeding on both legumes and nonlegumes.

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References

Agrell J, Anderson P, Oleszek W, Stochmal A, Agrell C (2006) Elevated CO₂ levels and herbivore damage alter host plant preferences. *Oikos*, **112**, 63–72.

Alley R, Berntsen T, Bindoff N *et al.* (2007) *Climate Change 2007: The Physical Science Basis, Summary for Policymakers*. Intergovernmental Panel on Climate Change, Paris, France.

Asshoff R, Hättenschwiler S (2005) Growth and reproduction of the alpine grasshopper *Miramella alpina* feeding on CO₂ enriched dwarf shrubs at treeline. *Oecologia*, **142**, 191–201.

Barbehenn RV, Karowe DN, Chen Z (2004a) Performance of a generalist grasshopper on a C₃ and C₄ grass: compensation for the effects of elevated CO₂ on plant nutritional quality. *Oecologia*, **140**, 96–103.

Barbehenn RV, Karowe DN, Spickard A (2004b) Effects of elevated atmospheric CO₂ on the nutritional ecology of C₃ and C₄ grass-feeding caterpillars. *Oecologia*, **140**, 86–95.

Bezemer TM, Jones TH (1998) Plant–insect herbivore interactions in elevated atmospheric CO₂: quantitative analyses and guild effects. *Oikos*, **82**, 212–222.

Bowers MD, Stamp NE, Fajer ED (1991) Factors affecting calculation of nutritional indices for foliage-fed insects: an experimental approach. *Entomologia Experimentalis et Applicata*, **61**, 101–116.

Chen F, Wu G, Feng G, Parajulee MN, Shrestha RB (2005) Effects of elevated CO₂ and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Entomologia Experimentalis et Applicata*, **115**, 341–350.

Cotrufo M, Ineson P, Scott A (1998) Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Global Change Biology*, **4**, 43–54.

Coviella CE, Trumble JT (1999) Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Conservation Biology*, **13**, 700–712.

Curtis P, Wang X (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia*, **113**, 299–313.

Edwards EJ, McCaffery S, Evans JR (2006) Phosphorus availability and elevated CO₂ affect biological nitrogen fixation and nutrient fluxes in a clover-dominated sward. *New Phytologist*, **169**, 157–167.

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

Falkowski P, Scholes RJ, Boyle E *et al.* (2000) The global carbon cycle: a test of our knowledge of earth as a system. *Science*, **290**, 291–296.

Gottthard K (2000) Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. *Journal of Animal Ecology*, **69**, 896–902.

Goverde M, Bazin A, Shykoff JA, Erhardt A (1999) Influence of leaf chemistry of *Lotus corniculatus* (Fabaceae) on larval development of *Polyommatus icarus* (Lepidoptera, Lycaenidae): effects of elevated CO₂ and plant genotype. *Functional Ecology*, **13**, 801–810.

Goverde M, Erhardt A (2003) Effects of elevated CO₂ on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyridae). *Global Change Biology*, **9**, 74–83.

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.

Hall MC, Stiling P, Moon D, Drake B, Hunter M (2005) Effects of elevated CO₂ on foliar quality and herbivore damage in a scrub oak ecosystem. *Journal of Chemical Ecology*, **31**, 267–286.

Hartwig U, Lüscher A, Daepf M, Blum H, Soussana J-F, Nösberger J (2000) Due to symbiotic N₂ fixation, five years of elevated atmospheric pCO₂ had no effect on the N concentration of plant litter in fertile, mixed grassland. *Plant and Soil*, **224**, 43–50.

- Hättenschwiler S, Schafellner C (2004) Gypsy moth feeding in the canopy of a CO₂-enriched mature forest. *Global Change Biology*, **10**, 1899–1908.
- Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist*, **156**, 9–26.
- Johns CV, Hughes L (2002) Interactive effects of elevated CO₂ and temperature on the leaf-miner *Dialectica scariella* Zeller (Lepidoptera: Gracillariidae) in Paterson's Curse, *Echium plantagineum* (Boraginaceae). *Global Change Biology*, **8**, 142–152.
- Johnson RH, Lincoln DE (1991) Sagebrush carbon allocation patterns and grasshopper nutrition: the influence of CO₂ enrichment and soil mineral nutrition. *Oecologia*, **87**, 127–134.
- Karowe DN (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, 227 pp.
- Karowe DN (1989) Facultative monophagy as a consequence of feeding experience: behavioral and physiological specialization in *Colias philodice* larvae (Lepidoptera: Pieridae). *Oecologia*, **78**, 106–111.
- Karowe DN (1990) Predicting host range evolution: colonization of *Coronilla varia* by *Colias philodice* (Lepidoptera: Pieridae). *Evolution*, **44**, 1637–1647.
- Karowe DN (1992) Plant secondary chemistry and the evolution of feeding specialization among insect herbivores: a different perspective. *Proceedings of the 8th International Symposium on Insect-Plant Interactions*, Wageningen, The Netherlands, pp. 207–208.
- Karowe DN, Martin MM (1989) Effects of the quantity and quality of dietary nitrogen on the growth, consumption rate, feeding efficiency, nitrogen budget, and respiration rate of *Spodoptera eridania* larvae. *Journal of Insect Physiology*, **35**, 699–708.
- Karowe DN, Siemsen DS, Mitchell-Olds T (1997) Species-specific response of glucosinolate content to elevated atmospheric CO₂. *Journal of Chemical Ecology*, **23**, 2569–2582.
- Klots AB (1951) *Butterflies of North America*. Houghton Mifflin, San Francisco.
- Koike T, Tobita H, Shibata T *et al.* (2006) Defense characteristics of seral deciduous broad-leaved tree seedlings grown under differing levels of CO₂ and nitrogen. *Population Ecology*, **48**, 23–29.
- Körner C (2006) Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytologist*, **172**, 393–411.
- Lincoln DE, Fajer ED, Johnson RH (1993) Plant–insect herbivore interactions in elevated CO₂ environments. *Trends in Ecology and Evolution*, **8**, 64–68.
- Lincoln DE, Sionit M, Strain B (1984) Growth and feeding response of *Pseudoplusia includens* (Lepidoptera: Noctuidae) to host plants grown in controlled carbon dioxide atmospheres. *Environmental Entomology*, **13**, 1527–1530.
- Lincoln DE, Sionit M, Strain B (1986) Response of an insect herbivore to host plants grown in carbon dioxide enriched atmospheres. *Oecologia*, **69**, 556–560.
- Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO₂: productivity, phytochemistry, and insect performance. *Ecology*, **74**, 763–777.
- Mattson WJ (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.
- Osbrink WA, Trumble JT, Wagner RE (1987) Host suitability of *Phaseolus lunata* for *Trichoplusia ni* (Lepidoptera: Noctuidae) in controlled carbon dioxide atmospheres. *Environmental Entomology*, **16**, 639–644.
- Percy KE, Awmack CS, Lindroth RL *et al.* (2002) Altered performance of forest pests under atmospheres enriched by CO₂ and O₃. *Nature*, **420**, 403–407.
- Poorter H, Navas M (2003) Plant growth and competition at elevated CO₂: on winners, losers, and functional groups. *New Phytologist*, **157**, 175–198.
- Price PW, Bouton C, Gross P, McPherson BA, Thompson JN, Weiss A (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.
- Price PW, Westoby M, Rice B, Alsatt PR, Fritz R, Thompson JN, Mobley K (1986) Parasite mediation in ecological interactions. *Annual Review of Ecology and Systematics*, **17**, 487–505.
- Schmidt DJ, Reese JC (1986) Sources of error in nutritional index studies of insects on artificial diets. *Journal of Insect Physiology*, **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. *Annual Review of Ecology and Systematics*, **26**, 183–211.
- van Groenigen KJ, Six J, Hungate BA, de Graaff MA, van Breemen N, van Kessel C (2006) Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences*, **103**, 6571–6574.
- Vogel CS, Curtis PS, Thomas RB (1997) Growth and nitrogen accretion of dinitrogen-fixing *Alnus glutinosa* (L.) Gaertn. under elevated carbon dioxide. *Plant Ecology*, **130**, 63–70.
- Waldbauer G (1968) The consumption and utilization of food by insects. *Advances in Insect Physiology*, **5**, 229–289.
- Zvereva EL, Kozlov MV (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis. *Global Change Biology*, **12**, 27–41.