

**Elevated Atmospheric CO₂ Affects the Chemical Quality of
Brassica Plants and the Growth Rate of the Specialist, *Plutella
 xylostella*, but Not the Generalist, *Spodoptera littoralis***

GADI V. P. REDDY,[†] PAULA TOSSAVAINEN, ANNE-MARJA NERG, AND
 JARMO K. HOLOPAINEN*

Department of Ecology and Environmental Sciences, University of Kuopio, P.O. Box 1627,
 FIN-70211 Kuopio, Finland

Cabbage, *Brassica oleracea* subsp. *capitata* (cv. Lennox and Rinda), and oilseed rape, *Brassica rapa* subsp. *oleifera* (cv. Valo and Tuli), plants were grown under ambient CO₂ (360 ppm) or elevated CO₂ (720 ppm) at 23/18 °C and under a photoperiod of 22/2 h light (250 μmol m⁻² s⁻¹)/dark regime for up to 5 weeks. Afterward, the performance of the crucifer specialist *Plutella xylostella* (Lepidoptera: Plutellidae) and the generalist *Spodoptera littoralis* (Lepidoptera: Noctuidae) on those plants was studied. The mean relative growth rate (RGR) of *P. xylostella* larvae, feeding on both cultivars of oilseed rape or on the Lennox cultivar of cabbage leaves grown at an elevated CO₂ concentration, was significantly reduced as compared to ambient CO₂. A negative larval growth rate at elevated CO₂ was observed for *P. xylostella* on both oilseed rape cultivars, but the growth rate was reduced but positive on cabbage. Conversely, the RGR of *S. littoralis* on either plant species was not affected by CO₂ treatment but was lower on cabbage cv. Rinda than on cv. Lennox. The mortality of the larvae was not affected by CO₂ treatment either. At the same time, elevated CO₂ significantly decreased the concentrations of leaf phytochemical constituents in oilseed rape, i.e., total phenolics and total nitrogen, but not in cabbage. The effect of elevated CO₂ on the leaf glucosinolate concentrations of both plant species was marginal. In addition, the observed significant changes in individual glucosinolate concentrations of oilseed rape leaves were not consistent among cultivars. However, our results demonstrate for the first time quite strong effects of CO₂ enrichment on the larval performance of *P. xylostella*, which is an important pest of *Brassica* plants around the world. Further studies are still required to increase our understanding of why elevated CO₂ differently affects the performance of specialist and generalist insect herbivores on *Brassica* plants.

KEYWORDS: Elevated CO₂; *Plutella xylostella*; *Spodoptera littoralis*; larval growth; leaf nitrogen; total phenolics; glucosinolates; *Brassica*

INTRODUCTION

It is well-established that the amount of carbon dioxide (CO₂) in the atmosphere is continuously rising (1). This change is one of the greatest threats to human beings because the concentration of atmospheric CO₂ has risen from a preindustrial value of 270 μL l⁻¹ to the present value of about 360 μL l⁻¹ and is anticipated to double by 2100 (2). Elevated CO₂ affects the physiology and growth of plants (3, 4) and also crop production (5). To evaluate the total impact of the elevated CO₂ concentration on plant growth and yield, simultaneous changes in the occurrence of pests and diseases have to be taken into account (6).

Under elevated CO₂ concentrations, the rate of plant growth increases, and leaves contain decreased levels of nitrogen and increased carbon/nitrogen ratios (7). The classical carbon–nutrient balance hypothesis (CNBH) (8) predicts that the increased carbon/nitrogen ratio will subsequently increase the level of C-based secondary compounds of plants grown under elevated CO₂, although further studies have indicated the conceptual limitations of this hypothesis (e.g., 9, 10). Karowe and co-workers (11) reported that elevated CO₂ possibly reduces the concentration of N-containing plant secondary compounds, such as glucosinolates in mustard. Glucosinolates are amino acid-derived secondary compounds characteristic to the Brassicaceae family. They are grouped into aliphatic, aromatic, and indolyl glucosinolates based on the amino acid from which they are derived (12, 13). The important role of glucosinolates and their hydrolysis products as feeding deterrents and toxic compounds to nonadapted, generalist herbivores (14–17) and

* To whom correspondence should be addressed. Tel: +358/17 163 230. Fax: +358/17 163 191. E-mail: Jarmo.Holopainen@uku.fi.

[†] Present address: Agricultural Experiment Station, College of Natural and Applied Sciences, University of Guam, Mangilao, Guam 96923.

feeding and oviposition stimulants for crucifer specialists (18–20) has been reported.

An increased atmospheric CO₂ concentration affects the growth and development of chewing insects, resulting in increased developmental times, reduced fecundity, and decreased larval and pupal weights [see reviews by Watt et al. (21) and Docherty et al. (22)]. Changes in phytochemical constituents due to increased CO₂ concentrations can potentially alter the nature of important insect–plant interactions (21, 23). The effects of increased atmospheric CO₂ on damage caused by insect pests will be dependent on changes in insect performance both at the individual and at the population level (24, 25). The performance of chewing insects feeding on CO₂-enriched plants has decreased, followed by an increase in their food consumption to get adequate dietary nitrogen (26, 27). Also, a decrease in the water content of leaves after CO₂ exposure, reported by Johnson and Lincoln (28), might be a reason for the decreased insect performance.

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a specialist herbivore and one of the most serious pests of cruciferous plants throughout the world (29). This species can also migrate during the early summer to the polar areas where it does not manage to overwinter (30). Outbreaks in the north are related to solar activity peaks (31). The cotton leafworm, *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae), is a generalist herbivore that attacks many plant species in several plant families, including Solanaceae, Brassicaceae, Poaceae, Fabaceae, and Malvaceae, in many parts of the world (32).

The aim of the present study was to compare the performance of two lepidopteran larvae, a *Brassica* specialist *P. xylostella* and a generalist *S. littoralis*, on two *Brassica* plants [*Brassica rapa* subsp. *oleifera* L. and *Brassica oleracea* subsp. *capitata* L. (Brassicaceae)] exposed to ambient and elevated CO₂ concentrations. The specific objectives were (i) to determine the mean relative growth rate (RGR) for the larvae of both insect species reared on *Brassica* plants grown in ambient and elevated CO₂, (ii) to determine the total phenolics and total nitrogen concentrations in the leaves of *Brassica* plants grown in ambient and elevated CO₂, and (iii) to compare glucosinolate concentrations in the leaves of *Brassica* plants grown in ambient and elevated CO₂. Shifts in ecological interactions, such as the movement of an insect species from one type of host plant to another, are potential sources of speciation (33). Specialists and generalists are at opposite ends of the ecological specialization spectrum (34), and a longstanding notion in evolutionary theory assumes that specialists are derived from generalists (33). We hypothesize that since the specialists have adapted to feed only on a specific host plant family, any physiological changes in host plants caused by CO₂ enrichment, such as changes in total phenolics, total nitrogen, and glucosinolate concentration in plant leaves, will affect the growth of the specialist to a much greater extent and even in an opposite direction (35) to effects on the growth of generalists. Generalists have already adapted to a wide range of plant defenses, and they might be less affected by the phytochemical changes.

MATERIALS AND METHODS

Plant Growth Conditions. Seeds of oilseed rape (*B. rapa* subsp. *oleifera* L., cv. Valo and cv. Tuli; Boreal Ltd., Jokioinen, Finland) and cabbage (cv. Lennox, Enkhuizen, The Netherlands, and cv. Rinda, Bejo Zaden BV, Warmenhuizen, The Netherlands) were individually sown in 1 L plastic pots filled with peat and sand (3:1). Thereafter, 40 sown pots of each plant species were transferred to grow in computer-controlled growth chambers (36) with either ambient or elevated CO₂

concentrations. One chamber received 360 μL L⁻¹ CO₂ (ambient) and another received 720 μL L⁻¹ CO₂ (elevated) at a thermoperiod of 23/18 °C and a photoperiod of 22/2 h light (250 μmol m⁻² s⁻¹)/dark regime. The CO₂ exposure was maintained 24 h per day for 5 weeks. The seedlings were watered daily with tap water and fertilized with 0.1% 9-Superex (19:5:20 N:P:K, Kekkilä, Finland) at the rate of 0.5–1.0 dL/pot, starting 2 weeks after sowing. The CO₂ treatments and seedlings were rotated between the two chambers weekly to randomize any systematic chamber effect across the seedlings. The growth chamber conditions were constantly monitored.

Insect Rearing. *P. xylostella* pupae were obtained from INRA, Entomologie et Lutte Biologique, Antibes, France. The rearing of immature stages was carried out in acrylic polyester gauze cages (60 cm × 33 cm × 33 cm, external dimensions) at 25 °C, 50% relative humidity, and 16/8 h light/dark photoperiod. Each cage contained 5–6 week old broccoli (*B. oleracea* subsp. *italica*) seedlings, two small cups (3 cm diameter) of a honey/water solution (10 wt %/vol) for adult feeding, and 50 pairs of adult *P. xylostella*. The plants were replaced daily. After oviposition, the seedlings were transferred to another cage for mass rearing of the larvae. Fresh plants were provided every 3 or 4 days for larval feeding. This procedure consistently produced a large number of various stages of *P. xylostella*. The pupae of the *S. littoralis* were obtained from the Department of Biological Organic Chemistry, Institute of Chemical and Environmental Research-CSIC, Barcelona, Spain. The larvae were reared in the laboratory at 26 ± 1 °C and 75 ± 10% relative humidity with a 16/8 h light/dark photoperiod on slightly modified artificial diets from those previously reported (37). The eggs were deposited by *S. littoralis* females on strips of filter paper in a plastic Ziploc bag (23 cm long × 18 cm width). Groups of early instars larvae were transferred into small plastic containers (3.0 cm high × 5.5 cm i.d.), while later instars were placed in plastic boxes (7.5 cm high × 17.5 cm i.d.), in both cases with small cubes of artificial diet. The lid of the boxes had a few small holes to avoid fungal development. When the larvae stopped feeding, sawdust was provided for pupation. The pupae were sexed, and the adults were separated daily by age and kept in other plastic containers (18 cm long × 14 cm wide × 9 cm high). The adults were fed with a 10% sucrose solution. Both insect cultures were maintained at our laboratory for several generations before being used in the current experiments.

Chemical Analysis. Three leaves were detached from individual plants for total phenolics and total nitrogen (N) analyses. The total phenolics were extracted from oven-dried (40 °C) and powdered leaf material with 80% (vol/vol) aqueous acetone and analyzed with Folin–Ciocalteu reagent using tannic acid as the reference standard (38). For total N analysis, the leaf material was oven-dried at 60 °C, powdered, and analyzed using the Kjeldahl technique (39).

Glucosinolate Analysis. The extraction of glucosinolates from both plant species was based on the method reported in the *Official Journal of the European Communities* (40) with slight modifications. Two leaves were detached from the middle part of each 5 week old plant, immediately frozen in liquid nitrogen and freeze-dried for 75 h. The dried material was placed in a desiccator and kept in a freezer (–20 °C) until analysis. Each sample was individually ground with a mortar and pestle using liquid nitrogen, and a 300 mg sample was weighed. Certified reference seed material [obtained from the Community Bureau of Reference (BCR program of the Commission of the European Communities)], having indicative values for individual glucosinolates, was used to verify the correct application of the method. Therefore, 200 mg of sample of reference seed material was also weighed simultaneously into a separate test tube. The tubes were boiled for 3 min in a water bath. Four milliliters of boiling water and 200 μL of sinigrin solution (2.5 mmol/l) as an internal standard were added into each test tube. The extracts were shaken with a Vortex mixer, heated again in a water bath for 5 min, and centrifuged at 2200 rpm for 5 min. The supernatant solution was taken into a new test tube. One milliliter of boiling water was added to the sediment of extracts, shaken with a Vortex mixer, and centrifuged for 5 min. The supernatants were combined and filtered with 0.45 μm syringe filters (Millipore, France). One milliliter of the supernatant was loaded into a balanced minicolumn prepared from DEAE Sephadex A-25 (Fluka Chemie, Buchs, Switzerland) resin. The columns were washed twice with 2 mL of 0.02 M

sodium acetate buffer (pH 4), and glucosinolates were desulfated overnight by adding 75 μ L of purified sulfatase (*Helix pomatia* type H1) into the columns. The desulfated glucosinolates were eluted from the column with 1.5 mL of water. The samples were placed in the freezer (-20 °C) until analysis.

Glucosinolates were identified by high-performance liquid chromatography (Hewlett-Packard series 1050, 1040 M Series II detection system) using a reversed phase capillary column (HP LiChrospher 100 RP-18, 5 μ m, 250 mm \times 4 mm) with a guard column (HP LiChrospher, 100 RP-18, 5 μ m, 4 mm \times 4 mm). A gradient elution from 0% acetonitrile to 25% acetonitrile during 45 min was utilized. The flow rate of the mobile phase was 0.8 mL/min, the column temperature was 30 °C, and the detection wavelength was 229 nm. The identification of individual glucosinolates was based on corresponding peaks in reference material and their relative retention times. The results were calculated by using the peak area of each compound and the peak area and concentration of internal standard and expressed as μ mol g⁻¹ dry weight.

Larval Growth Rate. The RGR for *P. xylostella* and *S. littoralis* larvae feeding on oilseed rape (cv. Valo and Tuli) and cabbage (cv. Lennox and Rinda) leaves was measured. Fully expanded leaves from both plant species were cut and inserted individually into Eppendorf tubes containing tap water. *P. xylostella* or *S. littoralis* second instar larvae were weighed (Sartorius Microbalance MP3, Sartorius AG, Göttingen, Germany) and introduced individually onto the leaf inside a plastic container with a lid. The larvae were kept at 25 °C, 50% relative humidity, and 16/8 h light/dark photoperiod. *P. xylostella* larvae were allowed to feed for 3 days on cabbage and 2 days on oilseed rape. *S. littoralis* larvae were allowed to feed for 3 days on cabbage and 4 days on oilseed rape. The feeding periods were different because the larval feeding rate of *P. xylostella* was higher on oilseed rape than on cabbage, whereas for *S. littoralis*, the larval feeding rate was higher on cabbage than on the oilseed rape. After feeding, the larvae were weighed again. The mean RGR of larvae was calculated using the following formula:

$$\text{RGR} = [\ln(\text{final weight}) - \ln(\text{initial weight})] \times \text{time}^{-1} [\text{mg}(\text{mg} \times \text{day})^{-1}]$$

From each cultivar, 15–20 replicate plants were used in both *P. xylostella* and *S. littoralis* tests.

Statistical Analysis. All of statistical analyses were performed with SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL). Treatment means and standard errors were calculated among varieties within elevated CO₂ and ambient CO₂ treatments. The main effects and interactions of treatment and cultivar were analyzed with two way analysis of variance (ANOVA) (GLM procedure) in the cases of phenolics and nitrogen. The data from larval RGR were analyzed using two way ANOVA or Student's *t*-test. The major results from the glucosinolate analyses were not normally distributed, and the difference between treatments was analyzed using the nonparametric Mann–Whitney *U*-test.

RESULTS

Total Phenolics. There was a significant reduction in the total phenolics extracted from the leaves of oilseed rape grown at the elevated CO₂ concentration ($P < 0.001$, **Table 1**). The difference between the total phenolics extracted from the leaves of cabbage grown at ambient and elevated CO₂ concentrations was not significant ($P = 0.065$, **Table 1**).

Nitrogen. There was a significant reduction in the nitrogen content of leaves of oilseed rape grown at the elevated CO₂ concentration ($P < 0.001$, **Table 1**). The difference between the nitrogen content of cabbage grown at ambient and elevated CO₂ concentrations was not significant ($P = 0.282$, **Table 1**).

Glucosinolate Analysis. In cabbage leaves, 10 individual glucosinolates were found as follows: six alkenyl glucosinolates (glucoiberin, progointrin, gluconapoleiferin, gluconapin, glucobrassicinapin, and glucoerucin), three indolyl glucosinolates (4-

Table 1. Mean Concentration (\pm Standard Error) of Total Phenolics and Percent Total Nitrogen in the Leaves of Cabbage (Lennox and Rinda Cultivars) and Oilseed Rape (Valo and Tuli Cultivars) under Ambient and Elevated CO₂^a

	Cabbage				
	ambient CO ₂	elevated CO ₂	main effects/ <i>P</i> values (ANOVA)		
			CO ₂	cultivar	CO ₂ \times cultivar
	total phenolics (mg/g dry wt)				
Lennox	41.9 \pm 0.71	42.2 \pm 0.65	0.065	0.168	0.333
Rinda	42.5 \pm 0.40	43.9 \pm 0.44			
	nitrogen (%)				
Lennox	0.60 \pm 0.04	0.65 \pm 0.06	0.282	0.212	0.928
Rinda	0.53 \pm 0.01	0.60 \pm 0.07			
	Oilseed Rape				
	ambient CO ₂	elevated CO ₂	main effects/ <i>P</i> values (ANOVA)		
			CO ₂	cultivar	CO ₂ \times cultivar
	total phenolics (mg/g dry wt)				
Valo	15.2 \pm 0.5	12.2 \pm 1.4	<0.001	0.085	0.089
Tuli	13.7 \pm 0.3	12.1 \pm 0.2			
	nitrogen (%)				
Valo	3.2 \pm 0.1	1.8 \pm 0.0	<0.001	0.729	0.459
Tuli	2.9 \pm 0.3	1.9 \pm 0.2			

^a Statistical significance of the main effects is given. $N = 6$ for cabbage, $N = 8$ for oilseed rape phenolics, and $N = 4$ for oilseed rape nitrogen.

OH-glucobrassicin, glucobrassicin, and 4-methoxyglucobrassicin), and one glucosinolate belonging to the aromatic glucosinolates (gluconasturtin). The major glucosinolates were 4-OH-glucobrassicin, glucoiberin, and glucobrassicin, constituting about 28, 26, and 21% of the total glucosinolates, respectively. The proportion of gluconasturtin was about 15% of the total glucosinolates, whereas the other compounds were less common (proportion of each <5%). In the leaves of both cabbage varieties, the concentration of the total glucosinolates (Mann–Whitney, $P = 0.529$) was at the same level in the ambient and elevated CO₂ (data not shown). CO₂ only marginally (Mann–Whitney, $P = 0.064$) increased the concentration of the only aromatic glucosinolate (gluconasturtin) in the leaves of the Lennox cultivar. Other individual glucosinolates were not affected by CO₂. When the glucosinolate concentrations were grouped into indolyl, alkenyl, and aromatic glucosinolates (**Figure 1**), no other significant changes other than a change in the concentration of aromatic gluconasturtin could be found.

In oilseed rape leaves, 15 individual glucosinolates were found as follows: nine alkenyl, four indolyl, and two aromatic glucosinolates (**Table 2**). The major glucosinolate was gluconasturtin, constituting about 40% of the total glucosinolates. Progointrin, glucoraphanin, glucobrassicinapin, and glucobrassicin constituted about 10% of each, and the other compounds were less common. The concentration of all indole glucosinolates showed a decrease under elevated CO₂ being only significant for 4-OH-glucobrassicin (**Table 2**).

On the other hand, in the leaves of cv. Tuli, there was a general increase in the concentration of indole glucosinolates specifically in neoglucobrassicin (Mann–Whitney, $P = 0.026$). Gluconasturtin (Mann–Whitney, $P = 0.046$) concentration were also increased at elevated CO₂ (**Table 2**). When glucosinolate concentrations were grouped into indolyl, alkenyl, and aromatic glucosinolates (**Figure 2**), elevated CO₂ significantly increased the concentration of aromatic glucosinolates (Mann–Whitney, $P = 0.046$). The oilseed rape leaves of Tuli cultivars grown in elevated CO₂ had significantly (Mann–Whitney, $P = 0.016$)

Table 2. Concentrations ($\mu\text{mol g}^{-1}$ Dry wt) of Individual Glucosinolates (Standard Errors in Parentheses) in the Leaves of Oilseed Rape (Valo and Tuli Cultivars)^a

glucosinolate	trivial name	ambient CO ₂		elevated CO ₂	
		Valo	Tuli	Valo	Tuli
alkenyl glucosinolates					
3-methylsulfanylpropyl	glucoiberin	0.011 (0.007)	0.000 (0.000)	0.024 (0.024)	0.052 (0.037)
<i>R</i> -2-hydroxy-3-butenyl	progoitrin	0.046 (0.013)	0.086 (0.043)	0.102 (0.034)	0.038 (0.028)
<i>S</i> -2-hydroxy-3-butenyl	epiprogoitrin	0.020 (0.020)	0.062 (0.006)	0.008 (0.008)	0.077 (0.045)
4-methylsulfanylbutyl	glucoraphanin	0.069 (0.007)	0.132 (0.053)	0.038 (0.015)	0.050 (0.024)
2-hydroxy-4-pentenyl	gluconapoliiferin	0.005 (0.004)	0.000 (0.000)	0.000 (0.000)	0.014 (0.014)
5-methylsulfanylpentyl	glucoalysin	0.040 (0.022)	0.000 (0.000)	0.099 (0.072)	0.000 (0.000)
3-butenyl	gluconapin	0.009 (0.004)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
4-pentenyl	glucobrassicinapin	0.065 (0.027)	0.055 (0.036)	0.157 (0.092)	0.068 (0.056)
4-methylthiobutyl	glucoerucin	0.071 (0.037)	0.000 (0.000)	0.000 (0.000)	0.063 (0.063)
indolyl glucosinolates					
4-hydroxy-3-indolylmethyl	OH-glucobrassicin	0.059 (0.014)	0.018 (0.007)	0.011 (0.004)**	0.055 (0.033)
3-indolylmethyl	glucobrassicin	0.070 (0.023)	0.040 (0.014)	0.025 (0.010)	0.095 (0.032)
4-methoxy-3-indolylmethyl	4-methoxyglucobrassicin	0.019 (0.005)	0.048 (0.024)	0.013 (0.005)	0.023 (0.009)
1-methoxy-3-indolylmethyl	neoglucobrassicin	0.019 (0.006)	0.002 (0.002)	0.000 (0.000)*	0.055 (0.019)*
aromatic glucosinolates					
benzyl	glucotropaeolin	0.000 (0.000)	0.000 (0.000)	0.011 (0.011)	0.000 (0.000)
2-phenylethyl	gluconasturtin	0.301 (0.068)	0.257 (0.088)	0.224 (0.111)	0.498 (0.052)*
total glucosinolates		0.800 (0.077)	0.644 (0.176)	0.712 (0.185)	1.087 (0.136) [†]

^a Statistical differences between treatments among each according to the Mann–Whitney test; ** $P < 0.01$; * $P < 0.05$; $N = 8$; [†]statistical significance between treatments in the Tuli cultivar according to the *t*-test; $P = 0.067$.

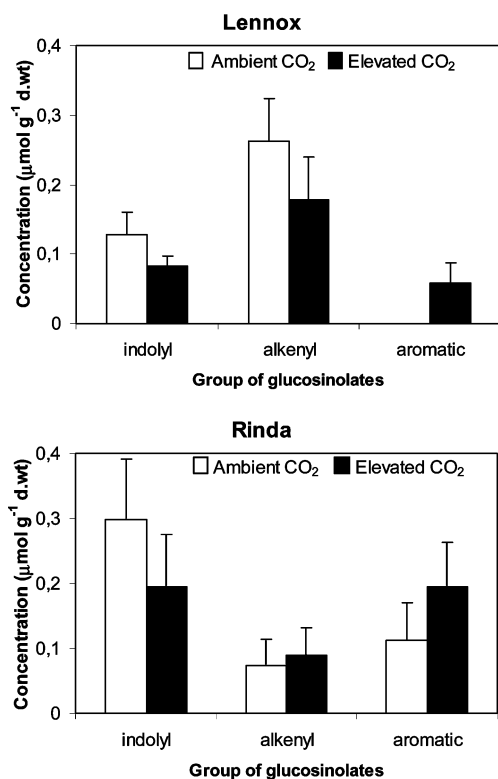


Figure 1. Concentrations of indolyl, alkenyl, and aromatic glucosinolates in cabbage leaves of Rinda and Lennox cultivars in ambient and elevated CO₂ treatment. Error bars are the standard errors; $N = 8$.

more aromatic glucosinolates than the corresponding leaves of the Valo cultivars (**Figure 2**).

Larval Growth. The mean RGR of *P. xylostella* larvae feeding on the CO₂-enriched Lennox cultivar of cabbage declined 60% as compared to ambient ($t_{11,9} = 2.191$, $P < 0.05$), while that of larvae feeding on the elevated CO₂-grown Rinda cultivar declined only 43% ($t_{11,10} = 1.363$, $P > 0.05$) (**Figure 3**). The RGR of the generalist *S. littoralis* showed a declining trend on CO₂-grown cabbage leaves as compared to ambient

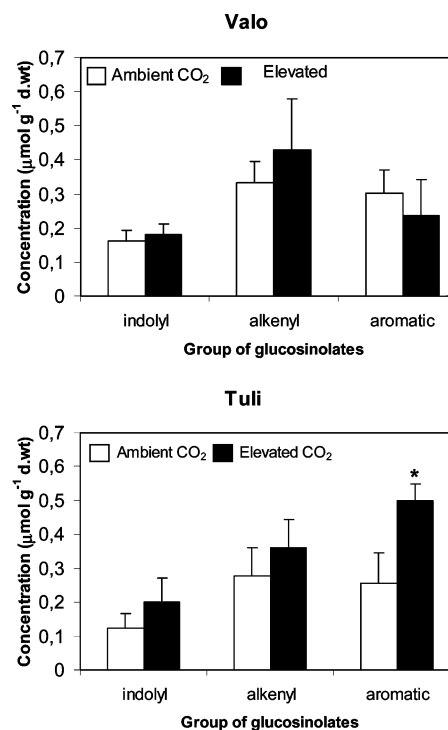


Figure 2. Concentrations of indolyl, alkenyl, and aromatic glucosinolates in oilseed rape leaves of Valo and Tuli cultivars in ambient and elevated CO₂ treatment. Error bars are the standard errors. Asterisks indicate significantly different levels according to the Mann–Whitney test ($P < 0.05$); $N = 8$.

(18% decrease with Lennox and 25% with Rinda), but these changes were not statistically significant (**Figure 3**).

P. xylostella feeding on oilseed rape leaves grown in the enhanced CO₂ environment lost weight during the experiment, producing negative RGR values, which were significantly lower than RGR values for larvae feeding on leaves grown in the ambient CO₂ environment (**Figure 4**; $t_{8,12} = 4.568$, $P < 0.001$ for the Valo cultivar; $t_{10,15} = 3.204$, $P = 0.004$ for the Tuli cultivar).

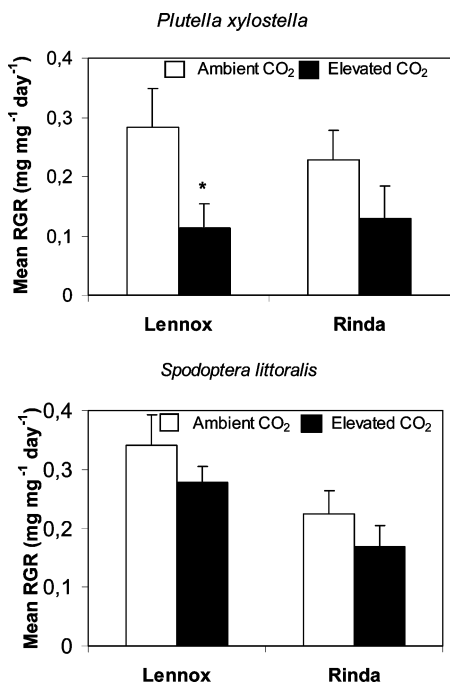


Figure 3. Mean RGR of *P. xylostella* and *S. littoralis* larvae during 3 days of feeding on cabbage leaves of Lennox and Rinda cultivars grown under ambient and elevated CO₂. Error bars are the standard errors. Asterisks indicate a significant difference at the $P < 0.05$ level according to the *t*-test. The sample size, $N = 20$, at the start of feeding experiment.

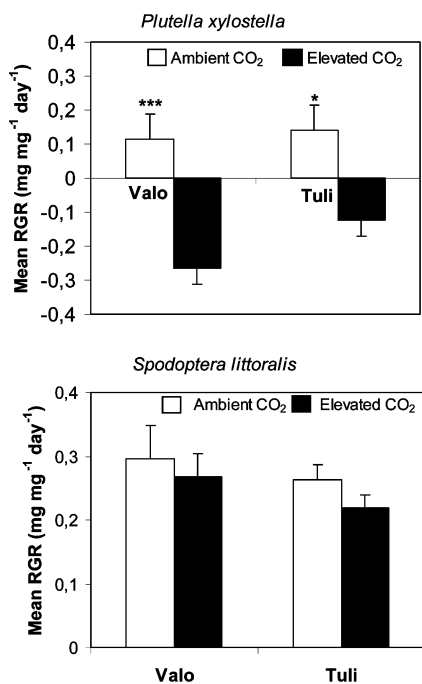


Figure 4. Mean RGR of *P. xylostella* larvae during 2 days of feeding and *S. littoralis* larvae during 4 days of feeding on oilseed rape leaves of Tuli and Valo cultivars grown under ambient and elevated CO₂. Error bars are the standard errors. Asterisks indicate a significant difference according to the *t*-test: *** $P < 0.001$; ** $P < 0.01$. The sample size, $N = 20$, at the start of feeding experiment.

RGR values for the generalist, *S. littoralis*, feeding on oilseed rape leaves grown in elevated CO₂ were only slightly lower than those measured for larvae feeding on leaves grown in ambient CO₂ levels: 9% lower in Valo and 17% lower in Tuli (Figure 4). These differences are not statistically significant.

The mortality was low in this feeding study, ranging from 0 to 10% across treatments.

DISCUSSION

We observed that the elevated CO₂ decreased the larval growth rate of the *Brassica* specialist (*P. xylostella*) feeding on both tested cultivars of cabbage and oilseed rape, while the generalist *S. littoralis* was less affected. The study by Stancy and Fellowes (41) provided the first empirical evidence that the cabbage aphid *Brevicoryne brassicae* (L.) (Homoptera: Aphididae) reared on Brussels sprout plants (*B. oleracea* subsp. *gemmifera*) grown under elevated CO₂ was larger and accumulated fat, while there was no change in the growth of the generalist green peach aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Some phloem-feeding insects have exhibited better performance when provided with plants grown under elevated CO₂ (7, 42). In contrast, in our study, the growth of the generalist (*S. littoralis*) larvae was not significantly affected by CO₂ on either of the studied plant species. However, the effect of elevated CO₂ on insect herbivores is dependent on both the plant and the insect species under study (43–45). Agrell et al. (46) reported that it would be possible to rank host plants grown under elevated CO₂ based upon their deleterious effects on insect performance.

In general, the glucosinolate concentrations in the leaves of Dutch cabbage cultivars and Finnish oilseed rape cultivars were rather low as compared to the studies conducted elsewhere (47–49). Oilseed rape leaves had about two times higher total glucosinolate concentrations than cabbage. At the time of harvest in separate experiments, all sampled plants were at the same developmental age, which is important when determining the differences between treatments and cultivars within experiments (50). CO₂ enrichment had only a marginal effect on the glucosinolate concentrations of cabbage leaves, while oilseed rape leaves were shown to be somewhat more responsive. However, the responses of different oilseed rape cultivars were not consistent with respect to the CO₂ enrichment. Accumulation of gluconasturtin in oilseed rape leaves, as detected in our study after elevated CO₂ in the Tuli cultivar, has earlier been reported to be correlated with disease resistance (51). Indolyl glucosinolates are known to increase after insect-feeding damage and chemical elicitor treatments (51–53), but in our study, elevated CO₂ decreased two indolyl glucosinolates in the leaves of the Valo cultivar. It has been suggested by Karowe et al. (11) that changes in nitrogen allocation with crucifers under elevated CO₂ are species specific. They found decreased foliar glucosinolate concentrations at elevated CO₂ only with mustard but not with radish or turnip. To our knowledge, there are no corresponding earlier studies with oilseed rape or cabbage.

Both nutrients and secondary metabolites in plant leaves are important in determining the leaf quality as food for herbivores (54). The leaf chemistry is known to be more sensitive to high CO₂ concentrations than to low CO₂ concentrations (55). Stancy and Fellowes (41) reported that *B. oleracea* plants reared under elevated CO₂ conditions (650 vs 350 ppm) were larger and had decreased water and nitrogen contents in the leaves. Reductions in gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), performance on aspen were associated with CO₂-induced increases in phenolic glycosides (56). Nutrients and the secondary chemistry of birch and maple were also affected by CO₂, but there were no parallel changes in gypsy moth performance. Although leaves of red maple, *Acer rubrum* L. (Aceraceae), had significantly higher total phenolics concentration when grown in enriched CO₂, no significant effect on the growth of *L. dispar*

larvae was observed (55). However, Coviella et al. (57) recently reported that cotton, *Gossypium hirsutum* L. (Malvaceae), plants had lower N concentrations and higher C:N ratios when grown in elevated CO₂, leading to adverse effects on growth and survival of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Although the results of Stancy and Fellowes (41) suggest to a certain extent that changes in host plant quality could affect the specialist sucking herbivore (*B. brassicae*) but not the generalist sucking herbivore (*M. persicae*), it still remains unclear from our study why the RGR of *P. xylostella* larvae was reduced on *Brassica* plants grown at elevated CO₂, while that of *S. littoralis* was not affected. However, this kind of study should be further expanded to investigate the impact of different plant surface structures, such as trichomes and trichome exudates, epicuticular waxes (58), and leaf toughness on insect herbivore performance to help us to understand why CO₂ enrichment affects the specialist and generalist performances differently.

In conclusion, our results suggest that larval growth of *P. xylostella* is negatively affected by elevated CO₂. However, long-term experiments under elevated CO₂ with multiple sequential insect generations are required to judge how elevated CO₂ concentration could affect the plant–herbivore interactions in the future, as already suggested by Hunter (25) and Williams et al. (55). In addition to these studies, the effects of elevated CO₂ on plant volatiles (59) and plant signaling to predators and parasitoids at the third trophic level should be encouraged and considered as a priority in the future research.

ACKNOWLEDGMENT

We thank Prof. A. Guerrero, IIQAB-CSIC, Barcelona, Spain, and Dr. E. Tabone, INRA, Antibes, France, for providing *S. littoralis* and *P. xylostella* cultures for the present study. We thank Dr. Aubrey Moore (University of Guam) for comments on a previous version of the manuscript.

LITERATURE CITED

- Keeling, C. D.; Whorf, T. P.; Wahlen, M.; Van Der Plicht, J. Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* **1995**, *375*, 660–670.
- Houghton, J. T.; Ding, Y.; Griggs, D. J.; Noguera, M.; Van Der Linden, P. J.; Xiaosu, D.; Maskell, K.; Johnson, C. A. *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*; Cambridge University Press: Cambridge, 2001; 892 pp.
- Bazzaz, F. A. The response of natural ecosystems to the rising global CO₂ levels. *Annu. Rev. Ecol. Syst.* **1990**, *21*, 167–196.
- Poorter, H.; Navas, M. L. Plant growth and competition at elevated CO₂: On winners, losers and functional groups. *New Phytol.* **2003**, *157*, 175–198.
- Parry, M. L. The potential effect of climate changes on agriculture and land use. *Adv. Ecol. Res.* **1992**, *22*, 63–91.
- Reilly, J. Agriculture in a changing climate: Impacts and adaptation, climate change 1995. In *Impacts, Adaptations and Mitigation of Climate Change: Scientific-Technical Analyses*; Watson, R. T., Zinyowera, M. C., Moss, R. H., Dokken, D. J., Eds.; Cambridge University Press: Cambridge, 1996; pp 429–467.
- Bezemer, T. M.; Jones, T. H. Plant-insect herbivore interactions in elevated atmospheric CO₂: Quantitative analyses and guild effects. *Oikos* **1998**, *82*, 212–222.
- Bryant, J. P.; Chapin, F. S.; Klein, D. R. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **1983**, *40*, 357–368.
- Koricheva, J. The carbon-nutrient balance hypothesis is dead; Long live the carbon-nutrient balance hypothesis? *Oikos* **2002**, *98*, 537–539.
- Sallas, L.; Luomala, E.-M.; Utriainen, J.; Kainulainen, P.; Holopainen, J. K. Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. *Tree Physiol.* **2003**, *23*, 97–108.
- Karowe, D. N.; Seimens, D. H.; Mitchell-Olds, T. Species-specific response of glucosinolate content to elevated atmospheric CO₂. *J. Chem. Ecol.* **1997**, *23*, 2569–2582.
- Louda, S.; Mole, S. Glucosinolates: chemistry and ecology. In *Herbivores: Their Interactions with Secondary Plant Metabolites*, 2nd ed.; Rosenthal, G. A., Berenbaum, M. R., Eds.; The Chemical Participants, Academic Press: New York, 1991; Vol. 1, pp 123–164.
- Halkier, B. A.; Du, L. The biosynthesis of glucosinolates. *Trends Plant Sci.* **1997**, *2*, 425–431.
- McCloskey, C.; Isman, M. B. Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the Bertha army worm, *Mamestra configurata* Walker. *J. Chem. Ecol.* **1993**, *19*, 249–266.
- Coleman, R. A.; Barker, A. M.; Fenner, M. Cabbage (*Brassica oleracea* var. *capitata*) fails to show wound-induced defense against a specialist and a generalist herbivore? *Oecologia* **1996**, *108*, 105–112.
- Hopkins, R. J.; Ekbom, B.; Henkow, L. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. *J. Chem. Ecol.* **1998**, *24*, 1203–1216.
- Li, Q.; Eigenbrode, S. D.; Stringham, G. R.; Thiagarajah, M. R. Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. *J. Chem. Ecol.* **2000**, *26*, 2401–2419.
- Nault, L. R.; Styer, W. E. Effects of sinigrin on host selection by aphids. *Entomol. Exp. Appl.* **1972**, *15*, 423–427.
- Hicks, K. P. Mustard oil glucosides: Feeding stimulants for adult cabbage flea beetles, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am.* **1974**, *67*, 261–264.
- Bartlett, E.; Parsons, D.; Williams, I. H.; Clark, S. J. The influence of glucosinolates and sugars on feeding by the cabbage stem flea beetle, (*Psylliodes chrysocephala*). *Entomol. Exp. Appl.* **1994**, *73*, 77–83.
- Watt, A. D.; Whittaker, J. B.; Docherty, M.; Brooks, G.; Lindsay, E.; Salt, D. T. The impact of elevated atmospheric CO₂ on insect herbivores. In *Insects in a Changing Environment*; Harrington, R., Stork, N. E., Eds.; Academic Press: London, 1995; pp 197–217.
- Docherty, M.; Salt, D. T.; Holopainen, J. K. The impacts of climate change and pollution on forest insect pests. In *Forests and Insects*; Watt, A. D., Stork, N. E., Hunter, M. D., Eds.; Hunter Chapman & Hall: London, 1997; pp 229–247.
- Lincoln, D. E.; Fajer, E. D.; Johnson, R. H. Plant-insect herbivore interactions in elevated CO₂ environments. *Trends Ecol. Evol.* **1993**, *8*, 64–68.
- Harrington, R.; Fleming, R. A.; Woiwod, I. P. Climate change impacts on insect management and conservation in temperate regions: can they be predicted. *Agric. For. Entomol.* **2001**, *3*, 233–240.
- Hunter, M. D. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Agric. For. Entomol.* **2001**, *3*, 153–159.
- Fajer, E. D.; Bowers, M. D.; Bazzaz, F. A. The effects of nutrients and enriched CO₂ environment on production of carbon-based allelochemicals in *Plantago*: a test of the carbon-nutrient balance hypothesis. *Am. Nat.* **1992**, *140*, 707–723.
- Docherty, M.; Hurst, D. K.; Holopainen, J. K.; Whittaker, J. B.; Lea, P. J.; Watt, A. D. Carbon dioxide-induced changes in beech foliage cause female beech weevil larvae to feed in a compensatory manner. *Global Change Biol.* **1996**, *2*, 335–341.

- (28) Johnson, R. H.; Lincoln, D. E. Sagebrush carbon allocation patterns and grasshoppers nutrition: the influence of CO₂ enrichment and soil mineral limitation. *Oecologia* **1991**, *87*, 127–134.
- (29) Talekar, N. S.; Shelton, A. M. Biology, ecology and management of diamondback moth. *Annu. Rev. Entomol.* **1993**, *38*, 275–301.
- (30) Smith, D. B.; Sears, M. K. Evidence for dispersal on diamondback moth, *Plutella xylostella* (Lepidoptera, Plutellidae) into southern Ontario. *Proc. Entomol. Soc. Ont.* **1982**, *113*, 21–27.
- (31) Kanervo, V. On the epidemiology of the diamond back moth (*Plutella maculipennis* Curt.). *Ann. Entomol. Fenn.* **1949**, *14* (Suppl.), 99–105.
- (32) Cabello, T.; Rodrigez, H.; Vargas, P. Development, longevity and fecundity of *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) reared on eight artificial diets. *Z. Angew. Entomol.* **1984**, *97*, 494–499.
- (33) Thompson, J. N. *The Coevolutionary Process*; The University of Chicago Press: Chicago, IL, 1994.
- (34) Futuyama, D. J.; Morena, G. The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* **1988**, *19*, 207–233.
- (35) Van Der Meijden, E. Plant defence, an evolutionary dilemma: Contrasting effects of (specialist and generalist) herbivores and natural enemies. *Entomol. Exp. Appl.* **1996**, *80*, 307–310.
- (36) Holopainen, T.; Kärenlampi, L. Injuries to lichen ultrastructure caused by sulphur dioxide fumigations. *New Phytol.* **1984**, *98*, 285–294.
- (37) Poitout, S.; Bues, R. Élevage de chenilles de vingt-huit espèces de lépidoptères Noctuidae et de deux espèces d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces. *Ann. Zool. Ecol. Anim.* **1974**, *6*, 431–441.
- (38) Julkunen-Tiitto, R. Phenolic constituents in leaves of northern willows: methods for the analysis of certain phenolics. *J. Agric. Food Chem.* **1985**, *33*, 213–217.
- (39) Allen, S. E. *Chemical Analysis of Ecological Materials*, 2nd ed.; Blackwell Science Publications: Oxford, 1989.
- (40) *Off. J. Eur. Commun.* **1990**, L170 (July 3), 33.
- (41) Stancy, D. A.; Fellows, M. D. E. Influence of elevated CO₂ on interspecific interactions at higher trophic levels. *Global Change Biol.* **2002**, *8*, 668–678.
- (42) Awmack, C. S.; Harrington, R.; Leather, S. R. Host effects on the performance of the aphid *Aulacorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO₂. *Global Change Biol.* **1997**, *3*, 545–549.
- (43) Lindroth, R. L.; Arteel, G. E.; Kinney, K. K. Responses of three saturniid species to paper birch grown under enriched CO₂ atmospheres. *Funct. Ecol.* **1995**, *9*, 306–311.
- (44) Traw, M. B.; Lindroth, R. L.; Bazzaz, F. A. Decline in gypsy moth (*Lymantria dispar*) performance in an elevated CO₂ atmosphere depends on host plant species. *Oecologia* **1996**, *108*, 113–120.
- (45) Coviella, C. E.; Trumble, J. T. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conserv. Biol.* **1999**, *18*, 700–712.
- (46) Agrell, J.; McDonald, E. P.; Lindroth, R. L. Effects of CO₂ and light on tree phytochemistry and insect performance. *Oikos* **2000**, *88*, 259–272.
- (47) Kirkegaard, J. A.; Sarwar, M. Biofumigation potential of brassicas. *Plant Soil* **1998**, *201*, 71–89.
- (48) Kirkegaard, J. A.; Sarwar, M. Glucosinolate profiles of Australian canola (*Brassica napus annua* L.) and Indian mustard (*Brassica juncea* L.) cultivars: implications for biofumigation. *Aust. J. Agric. Res.* **1999**, *50*, 315–324.
- (49) Kushad, M. M.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, B. P.; Wallig, M. A.; Jeffrey, E. H. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *J. Agric. Food Chem.* **1999**, *47*, 1541–1548.
- (50) Porter, A. J. R.; Morton, A. M.; Kiddle, G.; Doughty, K. J.; Wallsgrove, R. M. Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. I. Effect of leaf age and position. *Ann. Appl. Biol.* **1991**, *118*, 461–467.
- (51) Kiddle, G. A.; Doughty, K. J.; Wallsgrove, R. M. Salicylic acid-induced accumulation of glucosinolates in oilseed rape (*Brassica napus* L.) leaves. *J. Exp. Bot.* **1994**, *45*, 1343–1346.
- (52) Bodnaryk, R. P. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. *Phytochemistry* **1992**, *31*, 2671–2677.
- (53) Bartlett, E.; Kiddle, G.; Williams, I.; Wallsgrove, R. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. *Entomol. Exp. Appl.* **1999**, *91*, 163–167.
- (54) Scriber, J. M.; Slansky, F. The nutritional ecology of immature insects. *Annu. Rev. Entomol.* **1981**, *26*, 183–211.
- (55) Williams, R. S.; Norby, R. J.; Lincoln, D. E. Effects of elevated CO₂ and temperature-grown red and sugar maple on gypsy moth performance. *Global Change Biol.* **2000**, *6*, 685–695.
- (56) McDonald, E. P.; Agrell, J.; Lindroth, R. L. CO₂ and light effects on deciduous trees: growth, foliar chemistry, and insect performance. *Oecologia* **1999**, *119*, 389–399.
- (57) Coviella, C. E.; Stipanovic, R. D.; Trumble, J. T. Plant allocation to defensive compounds: interactions between elevated CO₂ and nitrogen in transgenic cotton plants. *J. Exp. Bot.* **2002**, *53*, 323–331.
- (58) Justus, K. A.; Mosdall, L. M.; Mitchell, B. K. Oviposition of *Plutella xylostella* (Lepidoptera: Plutellidae) and effects of phylloplane waxiness. *J. Econ. Entomol.* **2000**, *93*, 1152–1159.
- (59) Vuorinen, T.; Reddy, G. V. P.; Nerg, A.-M.; Holopainen, J. K. Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO₂ concentration. *Atmos. Environ.* **2004**, *38*, 675–682.

Received for review November 24, 2003. Resubmitted on April 21, 2004. Accepted April 27, 2004. This work was financially supported by the Research Council for Biosciences and Environment, the Academy of Finland (Project 48605), and the Jenny and Antti Wihuri Foundation (grant to A.-M.N.). Thanks are due to the Research Council for Biosciences and Environment, Academy of Finland, for providing a visiting scientist grant (Project 51656) to G.V.P.R.

JF049358V