

Effects of elevated CO₂ on an insect omnivore: A test for nutritional effects mediated by host plants and prey

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Received 1 March 2007; received in revised form 25 June 2007; accepted 26 June 2007

Available online 6 August 2007

Abstract

Elevated atmospheric CO₂ has generally been found to have negative impacts on the performance of insect herbivores via negative effects on plant quality. Yet, reduced food quality may lead to an increase in crop damage when pests compensate by consuming more plant tissue. The flow-on effects of elevated CO₂ level on higher trophic levels have rarely been investigated. We tested the effects of elevated CO₂ on the behavior and performance of the omnivorous bug *Oechalia schellenbergii* (Heteroptera: Pentatomidae) and its prey, a polyphagous chewing herbivorous pest (*Helicoverpa armigera*; Lepidoptera: Noctuidae), feeding on pea (*Pisum sativum*) foliage. We hypothesized that elevated CO₂ would impose negative nutritional effects on the omnivore by lowering the quality of plants, the prey, or both.

Plants grown at elevated CO₂ were significantly larger, with reduced N content, than plants grown at ambient CO₂. *H. armigera* larvae feeding on elevated CO₂-grown plants were significantly smaller than those grown on ambient-grown plants, but prey N content did not differ between CO₂ treatments. The omnivore required prey to complete its development, and performed best on a mixed plant-prey diet, regardless of CO₂ level. Bugs that failed to reach adulthood were slower to develop on the elevated CO₂-prey diet; those that successfully enclosed were not affected by CO₂ treatment in terms of development time, adult weight, adult longevity, hatch rate or pre-oviposition time. The bugs did not display compensatory feeding when offered prey of similar size from different CO₂ treatments. The bugs performed best when fed larvae from the elevated-CO₂ treatment apparently because these prey were smaller and thus easier to subdue. Taken together, results indicate that elevated CO₂ may benefit generalist predators through increased prey vulnerability, which would put pest species under higher risk of predation.

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Keywords: Climate change; *Helicoverpa armigera*; *Oechalia schellenbergii*; Omnivory; Tri-trophic interactions

1. Introduction

Atmospheric CO₂ has increased 35% since pre-industrial times, far exceeding the natural range over the past 65,000 years, and is expected to at least double by the end of the century (IPCC, 2007). Plants grown at elevated atmospheric CO₂ are nutritionally poorer than those grown at ambient levels, with flow-on effects to the performance of insect herbivores (e.g., Bezemer and Jones, 1998; Johns and

Hughes, 2002; Zvereva and Kozlov, 2006). Herbivores with chewing feeding habits that are reared on elevated-CO₂ foliage typically have longer developmental times, lower survivorship, reduced adult weight, and lower fecundity than conspecifics reared on plants under ambient CO₂ level (e.g., Stiling et al., 1999; Whittaker, 1999). In most cases, insects respond to variable host plant quality with increased food consumption (Bezemer and Jones, 1998; Coviella and Trumble, 1999). The decline in insect performance and increase in consumption have generally been attributed to decreased nitrogen concentration in plant tissues and an increase in the C:N ratio of foliage grown at elevated CO₂ levels (e.g., Cotrufo et al., 1998; Zvereva and Kozlov, 2006). In agricultural systems, such effects would act to reduce pest

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populations because of slow development and high mortality under elevated CO₂ conditions (Hunter, 2001). Yet, compensatory feeding by the pest may result in higher crop damage under these conditions (Lincoln et al., 1984).

While the effects of elevated CO₂ on interactions between individual herbivore species and their host plants are becoming better understood, little is known about how these effects may flow on to secondary consumers (predators and parasitoids) and thus affect whole food webs. Atmospheric CO₂ levels may affect the performance of natural enemies and/or susceptibility of prey via a variety of indirect “bottom-up” effects. Some of these impacts, which potentially make prey more susceptible to their enemies, include: (i) herbivores that feed on poor host plants under elevated CO₂ conditions often spend more time in the more vulnerable, early stages of development, and thus may suffer greater mortality from natural enemies (Stiling et al., 1999); (ii) herbivores may be physically weakened while feeding on poor hosts under elevated CO₂ conditions, and are thus less able to defend themselves against predators and parasitoids (Barbosa et al., 1982); and (iii) enriched CO₂ may alter enemy-avoidance behavior (Mondor et al., 2004); some aphids, for example, show reduced responses to alarm pheromones under elevated CO₂, potentially making them more susceptible to enemy attack (Awmack et al., 1997). Such effects would increase the susceptibility of herbivores to natural enemies, further reducing herbivore population size under elevated CO₂ conditions (Roth and Lindroth, 1995).

Elevated CO₂ may also have the opposite effect, making prey less susceptible and/or reducing enemy performance in the following ways: (i) plants growing under elevated CO₂ conditions tend to be larger, architecturally more complex, and have a larger leaf area (e.g., Pritchard et al., 1999; Stacey and Fellowes, 2002), all of which reduce the searching efficiency of natural enemies; (ii) prey species may be less suitable for their natural enemies, in terms of either size or composition, if they have developed on nutritionally poor host plants (Price et al., 1980; Barbosa et al., 1982; Vinson and Barbosa, 1987); and (iii) at the population level, elevated CO₂ conditions may disrupt the synchrony of natural enemies with their prey/host (Percy et al., 2002), allowing prey populations to escape regulation.

Other CO₂-mediated effects may be either to the advantage or disadvantage of natural enemies, depend on the circumstances. For example, elevated CO₂ has been shown to affect parasitoid detection of damage-induced plant volatiles which are important cues in prey-searching behavior (Vuorinen et al., 2004). Changes in prey distribution and apparency on the plant may also affect their vulnerability to natural enemies (e.g., Stiling et al., 1999, 2003; Stacey and Fellowes, 2002).

Elevated CO₂ may therefore make some herbivores more susceptible to their enemies, while making others less susceptible. It is thus not surprising that the few studies that have measured the impacts of CO₂ enrichment on interactions between insects and their enemies have reported

all possible outcomes. Predator and parasitoid performance and population levels have been found to increase (Stiling et al., 1999, 2002; Chen et al., 2005), decrease (Butler, 1985; Roth and Lindroth, 1995; Lindroth, 1996; Sanders et al., 2004) or be unaffected (Bezemer et al., 1998; Percy et al., 2002; Stacey and Fellowes, 2002; Holton et al., 2003; Hoover and Newman, 2004) at elevated levels of CO₂. In one study, natural enemies of aphids in a free-air CO₂ enrichment (FACE) experiment were present at higher densities in 1 year, but were unaffected in another (Percy et al., 2002).

As generalist natural enemies play a central role in shaping herbivore population dynamics and structuring ecological communities (Riechert and Bishop, 1990; Snyder and Wise, 1999; Riechert and Lawrence, 1997; Sunderland and Samu, 2000; Symondson et al., 2002), it is important to understand how such relationships will be affected by future changes in the atmosphere (Hoover and Newman, 2004). In this study, we extend the previous work on CO₂ impacts on predatory insects by investigating the effect of enriched CO₂ on the performance of an omnivorous insect. Omnivory is a widespread feeding strategy (Coll, 1998; Coll and Guershon, 2002). Elevated CO₂ levels may affect plant-feeding omnivores both directly, by altering the quality of their food plants, and indirectly, by changing the characteristics of their prey. In this study, we tested the effects of CO₂ enrichment on both plant quality and the nutritional and behavioral properties of an important pestiferous prey, and the subsequent impacts on an omnivorous consumer.

We hypothesized that elevated CO₂ conditions would impose a negative nutritional effect on the omnivore both directly, when it feeds on plants of inferior quality, and indirectly, when it feeds on poor quality prey. Specifically, we assessed the effects of elevated CO₂ levels on the omnivorous pentatomid bug (*Oechalia schellenbergii*; Heteroptera: Pentatomidae) and its prey, a chewing generalist herbivore (*Helicoverpa armigera*; Lepidoptera: Noctuidae), feeding on pea (*Pisum sativum*) foliage.

H. armigera is a highly polyphagous pest that feeds primarily on foliage and fruit of many crop plants. *H. armigera* suffers greater mortality, extended development time, and increased foliage consumption rate when developing on plants grown at elevated CO₂ (Stadler, 1999). *O. schellenbergii* is an important predator of *Helicoverpa* larvae as well as other herbivorous species (Awan, 1984). Like many other bugs, *O. schellenbergii* also derives moisture and nutrients directly from plants (Awan, 1986). In the present study, we addressed the following questions:

- (i) What are the direct (via the plant) and indirect (via the prey) effects of elevated CO₂ on the performance and behavior of the omnivorous bug?
- (ii) Does the bug increase its rate of feeding to compensate for low prey quality that may occur under elevated CO₂ conditions?

For the study, which focused on nutritional effects of players at three trophic levels, we employed laboratory and growth cabinet experiments that provided the needed controlled conditions. Furthermore, laboratory conditions were required to record the behavior of the omnivore and its prey, standardize their sizes, and retrieve prey remains to quantify omnivore consumption levels under different CO₂ treatments.

2. Materials and methods

2.1. Study species

Pea plants (*P. sativum*, cv. Greenfeast; YatesTM) were grown in potting soil in 15 cm pots. After germination, pots were randomly assigned to four growth cabinets (ThermolineTM) set at 25 °C and 14:10 L:D. Light was provided by eight 100 W incandescent lamps, two 400 W high-intensity discharge (HID) lamps, and three 1000 W HID lamps per cabinet. Two of the cabinets were maintained at ambient CO₂ concentration (360 µl l⁻¹) and the remaining two were supplied with approximately double the ambient concentration (700 µl l⁻¹). Plants were watered daily and fertilized (Thrive[®]) once a week, according to the manufacturer's instructions. At fertilization time, pots were randomly repositioned within the cabinet and CO₂ treatments were switched among cabinets.

H. armigera eggs were obtained from the Australian Cotton Collaborative Research Centre (Narrabri, NSW, Australia), and placed on experimental plants in cages (0.6 × 0.6 × 0.8 m *D* × *W* × *H*, with screened walls and ceiling) which were held in growth cabinets (ThermolineTM) at 25 °C, 14:10 L:D and ambient CO₂ conditions. Experimental plants were added as food for the caged *H. armigera* larvae every other day, and prey larvae were collected for experiments 13 days after egg hatch (as 3rd and 4th instars when reared on plants grown at elevated and ambient CO₂, respectively). This procedure was repeated three times at 3 d intervals before each experiment, to provide a continuous supply of prey.

A laboratory culture of *O. schellenbergii* was established from 51 adult females and 37 adult males collected in lucerne fields near Singleton, NSW, Australia. The bugs were allowed to feed *ad libitum* on *Drosophila* larvae and excised leaves held in water-filled vials. The bug culture and all experiments were held at room temperature (21 ± 2 °C).

2.2. Performance of *O. schellenbergii* feeding on plants and prey grown under different CO₂ conditions

To assess the nutritional effect of plant and prey food sources on bug performance, 90 5th instar nymphs,

moulted within the previous 12 h, were kept individually in containers (0.17 × 0.11 × 0.4 m *L* × *W* × *H* with mesh cover) and randomly assigned to one of the following six diet combinations (*n* = 15 per treatment): (i) leaves grown under elevated CO₂ (E-plants); (ii) leaves grown under ambient CO₂ (A-plants); (iii) 13-day old *H. armigera* larvae reared on elevated CO₂ plants (E-prey); (iv) 13-day old *H. armigera* larvae reared on ambient CO₂ plants (A-prey); (v) E-plants + E-prey; and (vi) A-plants + A-prey. Plant material (leaves with stems held in water-filled vials) was replaced twice weekly. To specifically test predator response to prey quality, rather than behavior, prey was offered as thawed larvae, which are readily consumed by the bugs (Awan, 1983). Each bug was offered five larvae daily, which is in excess of their daily consumption (Awan, 1990).

When the bugs moulted to adults, they were measured (maximum pronotal width), weighed, sexed and then paired with a bug of the opposite sex from the same treatment (*n* = 18 pairs). Adults were monitored daily for survival and oviposition until they died. All deposited eggs, mainly on sides of experimental containers, were counted and percent hatch determined. We measured bug performance in terms of time to adulthood, % survival to adulthood, adult pronotal width, adult fresh weight <1 day after eclosion, pre-oviposition time, oviposition per day, lifetime reproduction, and longevity.

To characterize pea properties, we collected the central leaflets of ten youngest but fully expanded leaves from each treatment, one leaflet per pea plant. The leaves were fresh weighed, and then stored at -20 °C until their nitrogen content was determined. To characterize prey, we measured the fresh weight of randomly selected, 13-day old larvae fed on plants grown under ambient and enriched CO₂ concentrations (*n* = 5 late 3rd and 5 early 4th instars, respectively). The larvae were then stored at -20 °C until their nitrogen content was determined. Leaf and prey samples were dried at 60 °C until reaching a constant mass, and then weighed. Dried materials were ground for total nitrogen analysis at Waite Analytical Services (University of Adelaide, SA, Australia), using Complete Combustion Gas Chromatography (Carlo Erba Instrument).

2.3. Compensatory feeding

To investigate whether *O. schellenbergii* displayed compensatory feeding in response to a particular diet, ten live third instar *H. armigera* larvae from each of the two CO₂ treatments were weighed and then offered individually to a total of 20 adult female bugs (i.e., one larva per female). Unconsumed prey and prey parts were weighed 24 h later to determine prey consumption by the bugs. Weight loss by unfed control prey larvae did not differ significantly between those that were fed on plants grown under ambient and elevated CO₂ conditions before the experiment

Table 1

Characteristics and results of ANOVA of pea host plants (1 S.E.) grown under ambient and elevated CO₂ conditions, and of *Helicoverpa armigera* larvae (1 S.E.) reared on them

Trait	Ambient CO ₂	Elevated CO ₂	d.f.	MS	F	P
Pea plants						
Leaflet N content ^a (% DW)	4.4 (0.23)	3.28 (0.28)	1,18	6.27	9.36	0.0067
Leaflet fresh weight ^a (g)	0.342 (0.033)	0.525 (0.037)	1,18	0.17	15.81	0.001
Leaflet dry weight ^a (g)	0.049 (0.006)	0.094 (0.005)	1,18	0.01	34.28	<0.001
<i>H. armigera</i> larvae						
N content ^b (% DW)	10.46 (1.50)	12.86 (2.44)	1,8	14.4	0.93	0.362
Fresh weight (g)	0.073 (0.008)	0.038 (0.004)	1,51	162.4	18.9	<0.001
Dry weight ^b (g)	0.008 (0.010)	0.006 (0.002)	1,18	0.71	4.44	0.049

Ambient and elevated CO₂ levels were 360 µl l⁻¹ and 700 µl l⁻¹, respectively.

^a Measurements were taken for the central leaflet of the youngest, fully expanded leaf of the plant.

^b Measured for a sub-set of the larvae used for fresh weight determination.

(0.011 ± 0.004 and 0.012 ± 0.006 g fresh weight ± S.D., respectively).

2.4. Impacts of CO₂ level on *O. schellenbergii* predation of live prey

2.4.1. *O. schellenbergii* behavior

To test the ability of *O. schellenbergii* to subdue prey from different CO₂ treatments, we offered pre-weighed, 13-d old *H. armigera* larvae to 10 individually held adult bugs that had been starved for 24 h. Each adult was offered a larva from either ambient ($n = 5$) or elevated CO₂ treatments ($n = 5$). Each predator was observed for approximately 6 h to record prey defensive and predator attack behaviors. Finally, we noted the success rate of predatory attempts on prey from elevated and ambient CO₂ treatments.

2.4.2. *O. schellenbergii* performance on live prey

Individually held, 12-h old, 4th instar bugs ($n = 36$) were each offered live pre-weighed 13-day old *H. armigera* larvae. Half of the bugs were offered larvae reared on plants grown under ambient CO₂ conditions, and the other half were given larvae reared on plants from elevated CO₂ (4th and 3rd instars, respectively). Each bug was offered 10 live larvae per day (i.e., excess prey; Awan, 1990), and the number consumed per day was recorded. Bugs were monitored daily until adulthood. Nymphal development time, survivorship, adult size (pronotal width), and fresh weight of the adults <1 day after eclosion were recorded.

2.5. Statistical analyses

Data were subjected to analyses of variance (ANOVA) and Fisher protected least significant difference (LSD) tests (SAS, 2001). Error variances were homogenized by data transformation when ANOVA of the residuals indicated a significant F statistic (Levene test, SAS, 2001). Unless otherwise noted, tests of significance used a probability level of 0.05.

3. Results

3.1. Effect of CO₂ level on plant and prey characteristics

Pea plants grown under elevated CO₂ had significantly higher biomass and lower nitrogen content than those grown under ambient conditions (Table 1). *H. armigera* larvae feeding on plants grown under elevated CO₂ conditions were significantly smaller than those fed ambient CO₂-grown plants of equivalent age (Table 1). Nitrogen content of the larvae, however, did not differ significantly between CO₂ levels (Table 1). Properties of larvae reared on ambient and elevated CO₂-grown plants were similar for live and thawed individuals.

3.2. Performance of *O. schellenbergii* feeding on plants and prey grown under different CO₂ conditions

Only 1 of the 30 *O. schellenbergii* nymphs fed on leaves alone survived to adulthood. This individual was a male feeding on ambient CO₂-grown plants. Longevity of nymphs that failed to reach adulthood was significantly affected by an interaction between CO₂ level and diet type (Table 2); nymph longevity was significantly lower on the elevated CO₂ prey diet (4.50 days ± 2.75) compared to the other treatment combinations (12.4 days ± 1.15), which did not differ significantly from each other (Fisher LSD tests at $p < 0.05$). Development time of 5th instar nymphs to adulthood was not affected by diet (prey or plant + prey), CO₂ level, or their interactive effect (Table 2). CO₂ level did not have a significant effect on nymph survival to adulthood, nor did it interact with the effect of diet treatment on survival. Yet, diet had a significant effect on nymph survival to adulthood; 77.8 ± 10.1, 82.3 ± 4.6, and 2.5 ± 1.5 percent survived when fed plant + prey, prey, and plant, respectively).

Weight of adult bugs was not affected significantly by diet, CO₂ level, or their interaction (Table 2). Nymphs feeding on plant + prey diets, irrespective of CO₂ treatments, moulted into larger adults than did those feeding on

Table 2

Results of ANOVAs for life history traits of *O. schellenbergii* feeding only on *H. armigera* larvae reared on either ambient- or elevated CO₂-grown plants, or on prey and respective host plants

Variable	Sex	CO ₂ level			Diet			CO ₂ level × diet		
		d.f.	F	p	d.f.	F	p	d.f.	F	p
Development time of 5th instar nymphs	Males	1,18	0.14	0.71	1,18	0.29	0.59	1,18	3.12	0.09
	Females	1,22	1.14	0.30	1,22	1.47	0.24	1,22	0.01	0.94
Nymph longevity ^a	M + F	1,33	3.64	0.06	2,33	4.43	0.20	2,33	4.13	0.02
Adult dry weight	Males	1,18	0.01	0.91	1,18	2.30	0.15	1,18	0.32	0.58
	Females	1,22	0.02	0.88	1,22	0.11	0.74	1,22	0.16	0.69
Adult shoulder width	Females	1,18	0.47	0.50	1,18	3.04	0.09	1,18	1.48	0.24
Pre-oviposition time	Females	1,13	1.38	0.26	1,13	0.68	0.42	1,13	0.19	0.67
Adult longevity	M + F	1,39	0.13	0.72	1,39	7.80	0.01	1,39	2.58	0.12
Lifetime reproduction (egg deposition)	Females	1,9	0.61	0.45	1,9	0.96	0.35	1,9	10.3	0.01
Total number of egg-clusters	M + F	1,9	0.81	0.39	1,9	0.98	0.35	1,9	4.28	0.07
Number of eggs per cluster	M + F	1,9	1.31	0.28	1,9	3.04	0.11	1,9	18.30	<0.01
Egg hatching success rate	M + F	1,9	1.06	0.33	1,9	1.10	0.32	1,9	0.58	0.46

Ambient and elevated CO₂ levels were 360 $\mu\text{l l}^{-1}$ and 700 $\mu\text{l l}^{-1}$, respectively. Analysis includes three diet treatments: prey, plant, and prey + plant. Data are analyzed separately for males and females whenever 3-way interactions (sex × CO₂ level × diet) were significant. Data were pooled for the sexes when all interaction terms for sex were not significant or when sexes could not be separated (e.g., number of eggs).

^a For nymphs that did not reach adulthood.

prey alone (pronotal “shoulder” width: females’: 6.11 ± 0.06 and 5.95 ± 0.05 mm, respectively; and males’: 5.03 ± 0.07 and 4.71 ± 0.06 mm, respectively). The difference in female size on the two diets was marginally non-significant ($p = 0.09$; Table 2). Adults reared on the prey and plant diet were not significantly different in size from individuals collected in the field (pronotal width: $F_{1,23} = 0.05$, $p = 0.82$ and $F_{1,31} = 0.26$, $p = 0.61$ for males and females, respectively; and dry weight: $F_{1,25} = 1.54$, $p = 0.23$ and $F_{1,35} = 1.81$, $p = 0.19$ for males and females, respectively).

Neither female pre-oviposition time nor adult longevity was affected significantly by CO₂ conditions or diet (Table 2). However, adults that were fed a plant + prey diet, were significantly longer-lived compared to those fed on prey alone (Table 2; Fig. 1). Two- or three-way interactive effects of CO₂, diet and sex on adult longevity were not statistically significant. There was a significant CO₂ × diet effect on lifetime reproduction (Table 2); it appears that while lifetime

reproductive output was significantly lower for females fed elevated- than ambient-CO₂ prey, the presence of plant material in the diet acted to negate, to some extent, the differential effect of the prey (Fig. 2c). Differential reproductive output appears to be the result of the effects of CO₂ level and diet on both the lifetime number of egg clusters produced per female (Table 2; Fig. 2a) and the number of eggs deposited per cluster (Table 2; Fig. 2b). Egg hatching rate did not differ significantly among CO₂ or diet treatments ($87.2\% \pm 6.2$; Table 2).

3.3. Compensatory feeding

We found no evidence for compensatory feeding in adult bugs; prey biomass consumption was not affected significantly by CO₂ treatment ($F_{1,9} = 0.06$, $p = 0.81$ and $F_{1,9} = 0.18$, $p = 0.68$ for consumed fresh and dry weights, respectively). Each bug consumed an average of 0.082 ± 0.03 and 0.062 ± 0.034 g fresh weight of prey when these were fed elevated and ambient CO₂ grown plants, respectively. Corresponding values for dry weight were 0.0060 ± 0.0033 and 0.0068 ± 0.0044 g.

3.4. Impacts of CO₂ level on *O. schellenbergii* predation of live prey

3.4.1. *O. schellenbergii* behavior

Prey larvae responded to predator attack with head waving and jerking, biting, rolling, regurgitation and abdomen wagging. The effectiveness of these defensive behaviors increased with larval size. Fourth instar larvae (i.e., those fed ambient CO₂ plants) were often successful in predator evasion: only 13.3% of predation attempts were successful. In comparison, 78.2% of predation attempts on 3rd instars (i.e., those fed elevated CO₂ plants) were

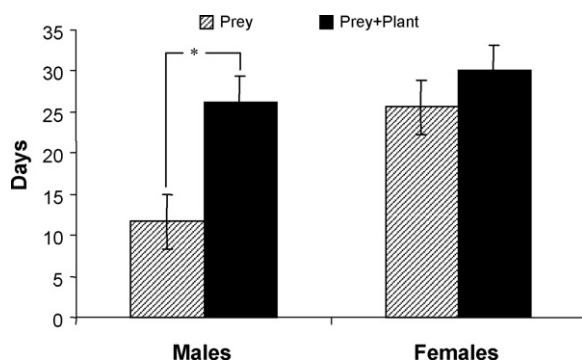


Fig. 1. Longevity of *Oechalia schellenbergii* adults (days ± 1 S.E.) when reared as nymphs on prey or plant + prey diets, pooled over CO₂ treatments, which did not differ significantly from each other. (*) Indicated bars are significantly different at $p < 0.05$ (protected Fisher LSD tests).

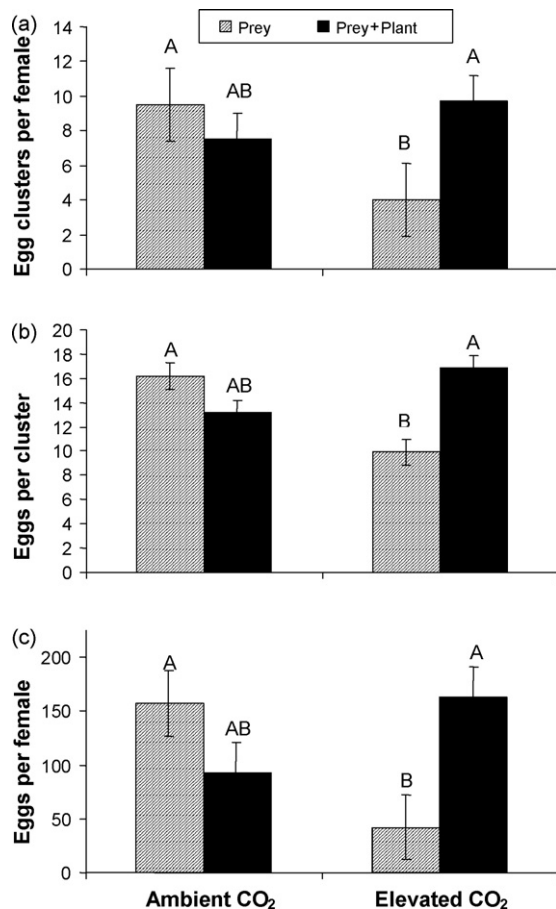


Fig. 2. Reproductive output of *Oechalia schellenbergii* when reared as nymphs on prey or prey + plant diets grown under ambient ($360 \mu\text{l l}^{-1}$) and elevated ($700 \mu\text{l l}^{-1}$) CO₂ levels: (a) lifetime egg cluster production per female (\pm S.E.); (b) number of eggs per cluster (\pm S.E.); and (c) lifetime egg deposition per female (\pm S.E.). Bars with same letter do not differ significantly (protected Fisher LSD tests, $p < 0.05$).

successful. Variation in prey size also affected the predators' attack strategy; they attacked any exposed part of the body when prey was small (3rd instars from elevated CO₂ treatment), while larger prey were selectively attacked behind the head.

3.4.2. *O. schellenbergii* performance on live prey

Development time of 5th instar female bugs was significantly shorter on elevated than ambient CO₂ prey

(Table 3). Further, 73% of nymphs feeding on elevated CO₂ prey reached the adult stage, compared to 67% of those feeding on ambient CO₂ prey. These findings for live prey, and the larger size of adult females in elevated than ambient CO₂ conditions, may be attributed to the significantly higher number of prey taken by nymphs feeding on elevated CO₂ prey (Table 3). The total amount of prey biomass subdued during the 5th stadium, however, was significantly higher for nymphs feeding on ambient than elevated CO₂ prey (Table 3), because ambient prey was significantly heavier than those from elevated CO₂ (Table 1).

4. Discussion

Elevated CO₂ had the expected effects on plant growth and herbivore performance. Plants grown at elevated CO₂ were larger, but with lower nitrogen content, consistent with most other studies (e.g., Cotrufo et al., 1998; Zvereva and Kozlov, 2006). *H. armigera* larvae were significantly smaller when grown on the nutritionally poorer hosts (reared under elevated CO₂), also consistent with many other studies on the performance of leaf chewing herbivores (e.g., Zvereva and Kozlov, 2006). The nitrogen content of the larvae, however, was not affected by the nitrogen content of the host plants, consistent with the notion that herbivorous insects actively maintain a relatively tight homeostasis in body elemental composition (Sterner and Elser, 2002; Fagan et al., 2002). The effect of CO₂ level on the composition of animal tissue has rarely been tested; while Chen et al. (2005) found that the soluble protein content of aphids was reduced when they were raised on elevated CO₂-grown plants, Stacey and Fellowes (2002) found that aphid quality as prey was not affected by CO₂ treatment.

Like other zoophytophagous omnivores (i.e., carnivores that also feed on plant material), the bugs in the present study required animal prey to successfully complete their development. Moreover, bugs' development, survival and longevity were superior on a mixed plant-prey diet, irrespective of CO₂ level. Bugs that failed to reach adulthood were slower to develop on the elevated CO₂-prey diet, but those that did successfully eclosed were not affected by CO₂ treatment in terms of their development time, adult weight, adult longevity, egg hatch rate or pre-oviposition time.

Table 3

Life history traits of, and prey intake by, *Oechalia schellenbergii* females (1 S.E.) when fed live *Helicoverpa armigera* larvae reared on plants grown at ambient or elevated CO₂ levels

Trait	Ambient CO ₂	Elevated CO ₂	d.f.	MS	F	p
Development time of 5th instar nymphs (days)	7.37 (0.46)	6.11 (0.26)	1,15	6.76	6.05	0.026
Female fresh weight (g)	0.201 (0.008)	0.221 (0.005)	1,15	0.002	5.28	0.036
Female dry weight (g)	0.009 (0.002)	0.013 (0.002)	1,14	0.000	1.50	0.24
Number of prey taken per 5th instar nymph	26.12 (1.88)	38.36 (2.18)	1,17	693.7	16.3	0.001
Prey biomass subdued per nymph during the 5th stadium (g)	0.192 (0.014)	0.147 (0.008)	1,17	9147	8.43	0.010

Ambient and elevated CO₂ levels were $360 \mu\text{l l}^{-1}$ and $700 \mu\text{l l}^{-1}$, respectively.

Lifetime reproductive output, however, was higher for the bugs reared on ambient-raised prey, indicating perhaps that some quality of the prey, other than nitrogen content, may be important. Further support for the potential importance of other, non-nitrogenous materials is provided by the interactive effects of CO₂ and diet on egg deposition; feeding on low nitrogen plants cancelled out the negative effect of prey under elevated CO₂ conditions.

Compensatory feeding is one way by which consumers may be able to mitigate some of the negative effects of reduced plant quality under elevated CO₂ conditions. Compensatory feeding is often exhibited by herbivores, especially mobile leaf chewers, in response to the reduction in host plant quality under elevated CO₂ levels. Yet, such compensatory feeding is often incomplete, because many species continue to exhibit a significant reduction in growth rate (Lindroth, 1996). We did not detect compensatory feeding by the omnivorous bug when it was offered prey of a similar size from the different CO₂ treatments, presumably because there was little or no difference in prey nitrogen content. It is possible, however, that we underestimated the effects of elevated CO₂ levels on the omnivore, because we did not measure impacts of different diets on the first three nymphal stages. Early instars of omnivorous insects tend to rely more heavily on plant material than do subsequent stages (Parker, 1981; Coll, 1996, 1998), and they may therefore experience a more substantial direct effect of low quality plants at elevated CO₂.

When bugs were fed live prey, the overall biomass of ambient CO₂-raised larvae that were subdued by the bugs was greater, due to the fact that larvae in this treatment were larger than larvae of equivalent age that were raised at elevated CO₂. However, the bugs performed best overall when fed larvae from the elevated-CO₂ treatment, consuming larger numbers of smaller larvae. The most likely explanation for this result is that the energetic costs of subduing the larger, more active prey outweighed the slight increase in biomass consumed, leading to a net overall benefit of the elevated-CO₂ treatment. These results indicate that generalist predators may benefit from, rather than be hampered by, increased CO₂ levels due to an increase in prey vulnerability. This is consistent with the slow development-high mortality hypothesis (Clancy and Price, 1987), and with similar effects of resistant crops on other omnivorous bugs (Bouton, 1984; Isenhour et al., 1989). Our results also suggest that behavioral as well as nutritional impacts need to be considered in the search for generality of CO₂ impacts on third-order trophic interactions. There currently have been too few studies on these types of interactions to draw general conclusions. Chen et al. (2005) found that lady beetles (*Leis axyridis*) had shorter duration of later instars and pupal stage, and higher overall mean relative growth rates when fed aphids from elevated CO₂, consuming approximately twice the number of prey individuals than at ambient levels, presumably as a compensatory response for the lower levels of soluble nitrogen in the prey. In contrast, the quality of aphids as prey did not appear to affect predation by

the ladybird *Hippodamia convergens* (Stacey and Fellowes, 2002).

A handful of studies have found that the impact of CO₂ level on enemy–prey interactions is either weak or non-existent. Roth and Lindroth (1995) reported that the effect of the parasitoid *Cotesia melanoscela* on the performance of the gypsy moth *Lymantria dispar* was not significantly affected by CO₂, and Salt et al. (1995) found that predators were not affected by high CO₂ in a free air CO₂ enrichment (FACE) experiment. Other experiments conducted either in open-topped chambers (Butler, 1985) or FACE (Sanders et al., 2004) have shown that populations of predators were reduced at elevated CO₂. Likewise, our results suggest that *O. schellenbergii* populations will be slower to increase when feeding on prey at elevated CO₂, because of lower reproductive output. Yet, the ability to feed on plant materials as well as prey, i.e., being an omnivore, negates this adverse effect of elevated CO₂ on prey nutritional quality. The results of this first study of the complex direct and indirect effects of CO₂ level on an omnivorous consumer suggest that feeding at two trophic levels acts to minimize the adverse effect of elevated CO₂ on omnivores through the reduction of prey nutritional value alone.

It has been proposed that the generally observed reduction in herbivore population size under elevated CO₂ levels is likely to be buffered, rather than reinforced, by natural enemy activity (e.g., Whittaker, 1999). This tentative conclusion, however, is based on only a few studies, most of which involved parasitoids. Such enemies are likely to be adversely affected by elevated CO₂ levels because their hosts are often smaller and show high mortality under these conditions. In contrast, our results suggest that generalist predators may exert greater pressure on their prey at elevated than at ambient CO₂ levels, because the smaller prey are more vulnerable to predation under the former conditions. Predators may thus reinforce the effect of elevated CO₂ through the plants, to further suppress population densities of herbivorous pests. Experiments that exclude the predators in the system may therefore underestimate effects of elevated CO₂ on insect pests. Nevertheless, ecological systems may still persist under elevated CO₂ conditions because, unlike pure carnivory, omnivorous feeding habits exhibited by many arthropods release herbivore populations from predation when plant-based resources are consumed.

5. Conclusion

The study greatly advances our mechanistic understanding of the effects of elevated carbon dioxide on insect populations, by assessing both bottom-up and top-down forces. Furthermore, this is the first study that measured the effect of global atmospheric change on an omnivorous consumer. Results indicate that (i) the omnivore enjoys higher lifetime fecundity when reared on plants and prey grown under ambient than elevated CO₂ levels; (ii) by

feeding at two trophic levels, it appears that the omnivore is able to mitigate most of the adverse effects of elevated CO₂ on its performance; and (iii) the bugs were better able to subdue and consume prey that were fed plants grown under elevated CO₂ levels. Earlier studies predicted that adverse effects of global increase in atmospheric CO₂ levels on pure carnivores, such as parasitoids, will act to increase pest-inflicted yield losses. Results of the present study, however, suggest the contrary; omnivorous natural enemies should provide higher level of pest suppression under elevated than ambient CO₂ conditions. Since omnivory is widespread in agroecosystems, we argue that yield loss to most pest species will be lower under elevated atmospheric CO₂ levels, compared to the current situation.

Acknowledgments

We thank Robert and Narelle Worth, and Graham Berry, for permission to collect *O. schellenbergii* on their properties; Colin Tan from the Australian Cotton Collaborative Research Centre for providing *H. armigera* eggs; and two reviewers for their thoughtful comments on the manuscript. MC's visit to Australia was partially funded by a Macquarie University Visiting Scholar Grant.

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