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RESEARCH ARTICLE

Effect of elevated CO₂ on tropical soda apple and its biological control agent *Gratiana boliviana* (Coleoptera: Chrysomelidae)

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Rising atmospheric CO₂ levels could have drastic effects on the performance of invasive weeds and their insect herbivores. Despite the importance of biological control as an effective management tool for environmental weeds, there have been few studies on the potential impact of climate change on the future efficacy of biological control. The objective of the present study was to evaluate the effect of elevated CO₂ on the performance of tropical soda apple *Solanum viarum* (Solanaceae) and its biological control agent *Gratiana boliviana* (Coleoptera: Chrysomelidae). We established three levels of CO₂ in environmental growth chambers: ambient (400 ppm), medium (580 ppm) and high (780 ppm). Plants growing at the high level had greater biomass compared to those growing at the ambient and medium levels of CO₂. Leaf water content and the amount of leaf nitrogen were reduced at high compared to ambient or medium CO₂ levels. *G. boliviana* immature survival and developmental time were negatively affected at high CO₂ but not at medium or ambient levels. Adults were lighter and smaller when reared at the high CO₂ level compared to ambient and medium treatments, while adult fecundity was higher at the medium CO₂ level. Leaf area consumed by fifth instars was lower when feeding on plants grown at the high CO₂ level either inside a Petri dish or on potted plants. These results suggest that beetle performance may be diminished under future climate. However, further studies should incorporate other factors such as temperature and precipitation as well as the evolutionary potential of herbivores and plants to adapt to a changing climate.

Keywords: climate change; insect–plant interactions; weed biological control; Solanaceae; *Solanum viarum*

1. Introduction

Atmospheric CO₂ levels have been steadily increasing since the mid-nineteenth century. The CO₂ level was approximately 280 ppm at the beginning of the industrial revolution, but by 2005 the level had increased to 379 ppm, which represents a 35% increase over a 200-year period (Intergovernmental Panel on Climate Change [IPCC] 2007). The impact of elevated CO₂ is particularly important for plants because of its central role in photosynthesis. Several changes in plant traits have been reported to occur under elevated CO₂ including increases in plant growth and biomass (Drake, Gonzalez-Meler, and Long 1997; Smith et al. 2000; Ainsworth and Long 2005), improvements in water- and light-use efficiencies (Drake et al. 1997;

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Kriticos, Sutherst, Brown, Adkins, and Maywald 2003) and changes in carbon allocation patterns (Pritchard, Rogers, Prior, and Peterson 1999; King et al. 2001). Because of their economic importance, studies on the impact of climate change have focused primarily on crops (e.g. Cure and Acock 1986; Rosenzweig and Tubiello 1997; Long, Ainsworth, Leakey, Nosberger, and Ort 2006); however, major interest is now being directed to weeds in agriculture and natural areas (Dukes and Mooney 1999; Ziska and Dukes 2011). According to Ziska (2003), the increase of atmospheric CO₂ during the twentieth century may have been a major factor leading to the invasiveness of six of the worst weeds in the US continent. Moreover, increasing CO₂ levels may promote plant invasions, particularly of fast-growing alien woody plants (Thuiller, Richardson, and Midgley 2007). For example, the invasive potential of *Acacia nilotica* L. in Australia may be enhanced by elevated CO₂ through improved water-use efficiency (Kriticos et al. 2003).

Climate change not only influences plant traits but may also affect insect herbivores as well as their natural enemies (Stireman et al. 2005; Thomson, Macfadyen, and Hoffmann 2010). In general, plant quality for herbivores is reduced under elevated CO₂ due to decreases in leaf nitrogen content (Mattson 1980; Cotrufo, Ineson, and Scott 1998; Reddy, Tossavainen, Nerg, and Holopainen 2004) and increases in carbon-based defensive compounds (Coviella, Stipanovic, and Trumble 2002; Bidart-Bouzat and Imeh-Nathaniel 2008). This reduction in the nutritional value of plants may be detrimental to insect herbivores resulting in higher mortality, longer developmental time and reduced adult size and fecundity (e.g. Lindroth, Kinney, and Platz 1993; Watt et al. 1995; Stiling et al. 1999; Knepp, Hamilton, Zangerl, Berenbaum, and DeLucia 2007). Food consumption by insect herbivores may increase in order to compensate for reduced leaf nitrogen concentration (Coviella and Trumble 1999; Kopper, Lindroth, and Nordheim 2001; DeLucia, Casteel, Nabity, and O'Neill 2008), but this is not always the case (Kopper and Lindroth 2003; Heisswolf, Obermaier, and Poethke 2005). Thus, the effect of climate change on insect–plant interactions seems to be dependent on the species of insects and plants involved in the interactions (Bidart-Bouzat and Imeh-Nathaniel 2008). Insects of different feeding guilds may respond differently; for example, leaf-mining insects were negatively affected by elevated CO₂ combined with higher temperature (Johns and Hughes 2002), whereas the survival and consumption rates of free-living beetles was affected to a lesser degree (Johns, Beaumont, and Hughes 2003).

Even though, many studies have now examined the effect of climate change on insect–plant interactions, fewer have specifically focused on potential impacts to weed biological control programmes. Under future climate conditions, some weeds may become more problematic due to increased plant growth, while the performance of biological control agents may be reduced. In the present study, we made initial steps towards understanding the impact of climate change on a successful biological control programme. Specifically, we explored the effect of elevated CO₂ levels on the performance of an invasive weed and the response of an established and effective biological control agent. Our model system included the South American weed, tropical soda apple, *Solanum viarum* Dunal (Solanaceae), and the leaf-feeding beetle *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), which was introduced and established in Florida for its control. We measured changes in plant characteristics at

different CO₂ levels over a two-month period and then measured several life history parameters of immature and adult beetles which had fed on those plants.

2. Materials and methods

2.1. Study system

Tropical soda apple is an herbaceous prickly shrub 1–2 m tall, native to South America, with C₃-type photosynthesis and is considered a major weed of pastures and natural areas in the south-eastern USA (Mullahey, Nee, Wunderlin, and Delaney 1993). Warm temperatures and high precipitation during summer months in Florida are conducive to rapid growth of tropical soda apple, but the cool, dry conditions experienced during the winter are detrimental (Overholt, Diaz, Markle, and Medal 2010). The spread of tropical soda apple is facilitated by cattle and small mammals that actively feed on the fruits and thereby transport seeds to new areas (Brown, Mullahey, and Akanda 1996). Infestations of tropical soda apple not only reduce cattle stocking rates (Mullahey, Shilling, Mislevy, and Akanda 1998), but also serve as a reservoir of several diseases of solanaceous crops (McGovern, Polston, and Mullahey 1994; Adkins, Kamenova, Roskopf, and Lewandowski 2007).

Gratiana boliviana is a host specific leaf-feeding beetle originally collected from northern Argentina and Paraguay and released in Florida in 2003 for biological control of tropical soda apple (Overholt et al. 2009; Diaz, Overholt, et al. 2011). Larvae and adults feed on the foliage forming distinctive shot-hole damage which often results in severe plant defoliation by the end of the growing season (Overholt et al. 2010; Diaz, Aguirre, et al. 2011). The beetle completes development from egg to adult in ≈ 30 days at 25°C, allowing it to complete several generations during the year (Diaz et al. 2008). A long-term study in central Florida demonstrated that density of tropical soda apple was reduced by 90% in three years following beetle release (Overholt et al. 2010). Beetle populations in central Florida increase during April to September, decrease during October to December and occur in very low numbers from January to March (Overholt et al. 2010). This reduction in numbers during winter is due in part to a reproductive diapause triggered by short photoperiods (Diaz, Overholt, et al. 2011). While tropical soda apple foliage is available throughout the year in central and South Florida, *G. boliviana* impact is noticeable from March to October (Overholt et al. 2010).

2.2. Rearing plants and insects

Experiments were conducted at the Biological Control Research and Containment Laboratory, University of Florida, Fort Pierce, FL, USA. Tropical soda apple plants were grown from seeds in seedling trays in germination soil (Fafard Superfine Germinating Mix, Agawam, MA, USA), and 10-day-old seedlings were transplanted individually to 3.8 L pots with potting soil (Fafard 3B potting soil, Agawam, MA, USA). All plants were kept inside a greenhouse, fertilised weekly with Miracle Gro (2 g per 3.79 L, 24N-8P-16K; Miracle Gro, Scotts, Marysville, OH, USA) and watered twice per week with 100 ml of water throughout the duration of the experiment. A colony of *G. boliviana* was maintained inside cages containing tropical

soda apple plants, and all cages were kept in a greenhouse. New adults were moved to clean plants, and plants were replaced as needed.

2.3. Effects of increased CO₂ on tropical soda apple performance

Experiments were conducted in three growth chambers (Conviron, Model: CMP4030 Winnipeg, Manitoba, Canada) set at three CO₂ levels: (1) ambient (400 μl^{-1} CO₂), (2) medium (580 μl^{-1} CO₂) and (3) high (780 μl^{-1} CO₂). The CO₂ levels were monitored daily with an external unit (SenseLife, Hana Engineering Co., Korea) and adjusted as needed. The projected concentrations of CO₂ in the year 2100 ranges from 540 to 970 ppm (IPCC 2007); thus, the CO₂ concentrations selected for this study fall into that range. All chambers were set at 25°C, 60–70% RH, 14:10 L:D photoperiod and had fluorescent and incandescent light bulbs which provided a range 6800–7000 lux at the chamber's floor. Data loggers were included in each chamber and temperature was checked twice per week.

Ten tropical soda apple seedlings (20-day-old, 4 cm tall) were placed in each growth chamber (30 total plants). The positions of plants were rotated twice per week and the level of CO₂ in the chambers was changed, along with the plants they contained, every 7 days to reduce incidental chamber effects (Bezemer, Thompson, and Jones 1998). Plant height was measured twice per week for a period of 65 days. At the end of the experiment (after 16 weeks), all plants were harvested, and several parameters were measured: (1) specific leaf area (SLA = leaf area/leaf dry weight) and per cent leaf water content [LWC = (fresh leaf weight – dried leaf weight) \times fresh leaf weight⁻¹] of second and third leaves (counting from top of stem), (2) C:N leaf ratios and (3) plant biomass (leaves, stems, roots). For plant biomass, 4 of the 10 plants (not exposed to herbivory) from each growth chamber were harvested, oven-dried for 10 days at 50°C, and dry biomass (roots, leaves and stems) was measured. Total C and N contents in plant samples (0.4 g per sample, oven dried basis) were determined using a CN-analyzer (Vario MAX CN Macro Elemental Analyzer; Elemental Analysensystem GmbH, Hanau, Germany).

2.4. Effect of increased CO₂ on *Gratiana boliviana* performance

Eight weeks after initiation of the experiment (day 56), six plants (\approx 40 cm tall) from each chamber were exposed to *G. boliviana* larvae, and four plants were left without herbivory. Similar to the study on the effects of CO₂ on tropical soda apple, the positions of plants in chambers were rotated twice per week and the level of CO₂ in the chambers was changed, along with the plants they contained, every 7 days. Six-second instar larvae were placed on each plant, and development and survival were recorded weekly. Pupae were counted and moved to clear plastic containers (15 cm height, 12 cm diameter) with ventilated lids and kept in the same growth chambers until adult emergence. The following insect variables were recorded: (1) time of development to adult, (2) per cent survival to adult and (3) size of newly emerged adults (length and fresh weight). For adult length, measurements were taken using ImageJ (Abramoff, Magalhaes, and Ram 2004) from scaled digital images.

Newly emerged adults reared at each CO₂ level were kept in clear plastic containers with tropical soda apple leaves from the same treatment and allowed to mature sexually for 1 week in each growth chamber. Two pairs of adults were placed

inside a mesh cage (70 cm height, 26 cm diameter) containing a 40-day tropical soda apple seedling grown under the same CO₂ treatments, and kept inside the same chamber (four to five replicates per treatment). After 30 days, all adults were counted and removed from plants, and total eggs laid were recorded (fecundity). The experiment was conducted a second time using 12 larvae per plant, and 5 plants per treatment. The same variables were recorded.

2.5. Effect of CO₂ and plant quality on leaf area consumption by fifth instars

Two-fifth instars from each plant (12 insects from each treatment) were used for a 48-h feeding trial during the first experiment. One larva was removed and placed inside a Petri dish (9 cm diameter) containing moist filter paper and a tropical soda apple leaf (coming from same CO₂ level). All Petri dishes were placed inside an environmental growth chamber at ambient CO₂ level, 25°C, 60% RH and 14:10 L:D photoperiod. The second larva was left on the plant but moved to an undamaged leaf which was labelled. Thus, larval consumption was measured either inside Petri dishes under the same conditions (insects exposed to plant quality only) or on potted plants at each growth chamber (insects exposed to both elevated CO₂ and plant quality). Leaf area consumed was measured after 48 h by scanning each leaf and measuring leaf area removed using the software ImageJ (Abramoff et al. 2004).

2.6. Statistical analyses

Repeated measures analyses were conducted to compare plant parameters (height) among CO₂ treatments over time. Insect parameters (time of development, per cent survival to adult and fecundity) and final plant parameters (SLA, LWC, C:N ratios and biomass) were compared among CO₂ treatments using one-way analysis of variance (ANOVA; SAS Institute 2009). The proportions of individuals surviving to adulthood were arcsine-square-root transformed prior to analysis (Zar 1999). Leaf area consumed by fifth instars was compared among CO₂ treatments and experimental setups (Petri dish vs. whole plants) using two-way ANOVAs (SAS Institute 2009). Adult size (weight and length) was compared among CO₂ treatments and genders using two-way ANOVAs (SAS Institute 2009). Means were separated using the *post hoc* Student–Newman–Keuls (SNK) test (SAS Institute 2009). A significance level of $\alpha = 0.05$ was used for all statistical analyses.

3. Results

3.1. Effects of increased CO₂ on tropical soda apple performance

Repeated measures analyses showed significant interactions of treatment \times time for plant height ($F = 13.5$; $df = 30, 397$; $P < 0.001$). Thus, single ANOVAs were conducted for each day for the last 20 days. There were no differences in plant height during the first 52 days of the experiment (Figure 1). Plants growing under the high CO₂ level were taller compared to those growing under ambient and medium treatments during days 56 ($F = 27.9$; $df = 2, 26$; $P < 0.0001$) and 65 ($F = 38.8$; $df = 2, 26$; $P < 0.001$; Figure 1).

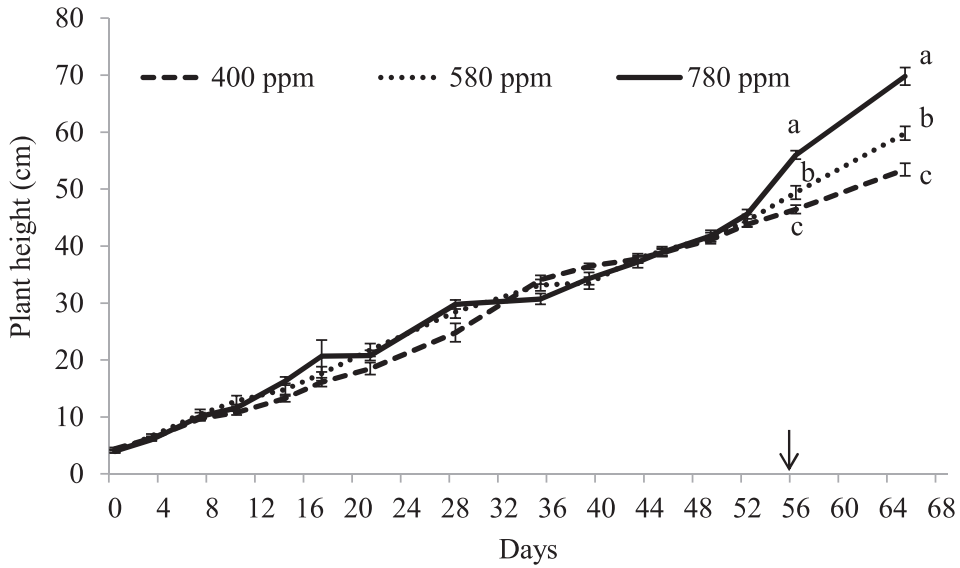


Figure 1. Plant height (cm) (mean \pm SE) of tropical soda apple growing under different levels of CO₂. Arrow indicates the date when *G. boliviana* (second instars) were placed on plants. Different letters indicate significant differences among CO₂ treatments ($P < 0.05$).

Specific leaf areas were 1.3-fold higher for plants grown at the medium CO₂ level compared to ambient and high treatments (Table 1). Plants exposed to the high CO₂ level had lower water contents (third leaf) and higher C:N ratios compared to ambient and medium treatments (Table 1). Final biomass of tropical soda apple exposed to the high CO₂ level was 1.5-fold higher compared to ambient and medium treatments (roots: $F = 6.9$; $df = 2, 9$; $P = 0.010$; leaves: $F = 9.8$; $df = 2, 9$; $P = 0.005$; stems: $F = 37.6$; $df = 2, 9$; $P < 0.001$; Figure 2).

3.2. Effect of increased CO₂ on *Gratiana boliviana* performance

No differences were detected in *G. boliviana* performance between experiments one (6 larvae per plant) and two (12 larvae per plant; survival to adulthood: $F = 0.21$,

Table 1. Specific leaf area, water content and C:N ratios (mean \pm SE) of tropical soda apple grown under different levels of CO₂.

	Specific leaf area 2	Specific leaf area 3	Percent water content 2	Percent water content 3	C:N ratio
400 ppm	0.28 \pm 0.01b	0.29 \pm 0.01b	77.3 \pm 0.3b	77.9 \pm 1.0b	14.2 \pm 0.4b
580 ppm	0.36 \pm 0.02a	0.38 \pm 0.02a	79.7 \pm 0.02a	80.9 \pm 0.6a	9.6 \pm 0.3c
780 ppm	0.24 \pm 0.01b	0.26 \pm 0.01b	75.0 \pm 0.6c	75.7 \pm 0.8b	16.5 \pm 0.9a
<i>F</i>	14.3	14.7	16.3	11.2	33.0
<i>df</i>	2, 26	2, 26	2, 26	2, 26	2, 26
<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001

Note: 2 = second leaf counting from top; 3 = third leaf counting from top. Measurements were conducted at the end of the experiment. Different letters indicate significant differences among CO₂ treatments ($P < 0.05$)

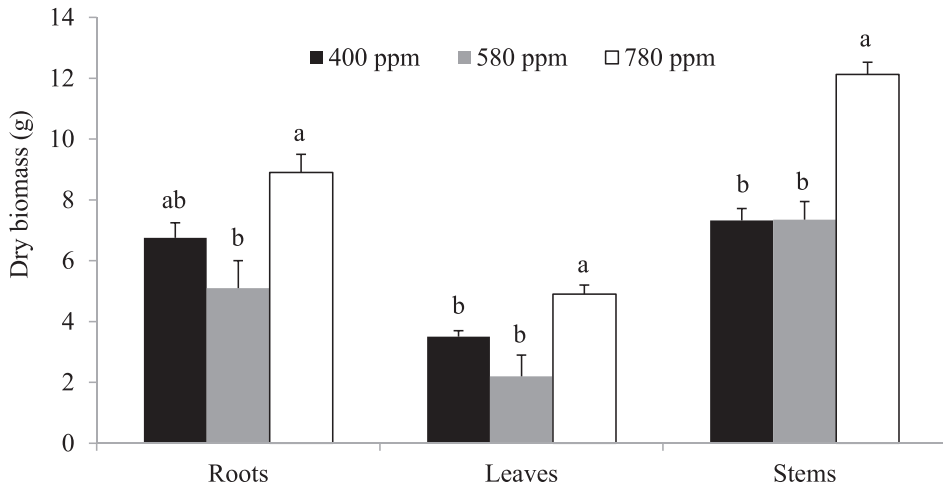


Figure 2. Final plant biomass of tropical soda apple grown under different CO₂ levels. Different letters indicate significant differences among CO₂ treatments ($P < 0.05$).

$df = 1, 27, P = 0.600$; time of development: $F = 0.65, df = 1, 176, P = 0.400$), and therefore, all data were combined for analyses. Survival to adulthood was 1.3-fold higher at ambient and medium CO₂ levels compared to the high CO₂ level (Table 2). Developmental time to adulthood occurred faster at ambient and medium CO₂ levels compared to the high CO₂ level (Table 2). Thus, insect performance was negatively affected when immatures were reared on plants at the highest CO₂ level (780 ppm).

Adult weights and lengths of *G. boliviana* reared at different levels of CO₂ are shown in Table 3. There were no interactions between treatment and gender for weight (treatment \times gender: $F = 1.6; df = 2, 162; P = 0.200$) or length (treatment \times gender: $F = 1.5; df = 2, 162; P = 0.200$). Adults were lighter and smaller when reared at the high CO₂ level compared to ambient and medium treatments (Table 3). Overall, females were larger and heavier than males (weight: $F = 109.6; df = 1, 162; P < 0.001$; length: $F = 66.6; df = 1, 162; P < 0.001$; Table 3). In addition, female fecundity (total eggs per female during a 30-day-period) was higher when reared at medium (97.3 ± 11.7 eggs) compared to ambient (62.3 ± 7.6 eggs) or high (61.5 ± 7.3 eggs) CO₂ levels ($F = 5.1; df = 2, 25; P = 0.010$).

Table 2. Survival and time of development (mean \pm SE) from second instar to adult of *G. boliviana* when reared on tropical soda apple exposed to different levels of CO₂.

CO ₂ levels	Survival (%)	Time of development (days)
400 ppm	65.9 \pm 3.6a	20.7 \pm 0.3b
580 ppm	70.4 \pm 4.1a	20.9 \pm 0.3b
780 ppm	52.3 \pm 13.6b	23.1 \pm 0.6a
<i>F</i>	6.3	9.4
<i>df</i>	2, 30	2, 179
<i>P</i>	0.005	<0.001

Note: different letters indicate significant differences among CO₂ treatments ($P < 0.05$).

Table 3. Adult size (mean \pm SE) of *G. boliviana* when reared on tropical soda apple exposed to different levels of CO₂.

Treatments	Weight (mg)	Length (mm)
Females 400 ppm	20.7 \pm 0.3a	6.57 \pm 0.04a
580 ppm	21.6 \pm 0.5a	6.65 \pm 0.03a
780 ppm	18.5 \pm 0.7b	6.36 \pm 0.09b
Males 400 ppm	17.6 \pm 0.3a	6.34 \pm 0.04a
580 ppm	17.4 \pm 0.3a	6.25 \pm 0.03a
780 ppm	15.6 \pm 0.4b	6.01 \pm 0.05b
<i>F</i> treatment	109.6	17.9
<i>df</i>	2, 162	2, 162
<i>P</i>	<0.0001	<0.0001

Note: different letters indicate significant differences among CO₂ treatments ($P < 0.05$).

3.3. Effect of CO₂ and plant quality on leaf area consumption by fifth instars

Leaf area consumed by fifth instars during a 48-h-period was lower when feeding on plants grown at the high CO₂ level either inside a Petri dish or on potted plants (experimental setups: $F = 17.5$; $df = 1, 29$; $P < 0.001$; treatments: $F = 4.8$; $df = 2, 29$; $P = 0.010$; setups \times treatments: $F = 1.1$; $df = 2, 29$; $P = 0.340$; Figure 3). However, the consumption rate of larvae was lower when feeding on potted plants (exposed to both elevated CO₂ and plant quality) compared to those placed inside Petri dishes held at ambient CO₂ (exposed to plant quality only; Figure 3). This suggests that a combination of host-plant quality and CO₂ levels affects larval consumption.

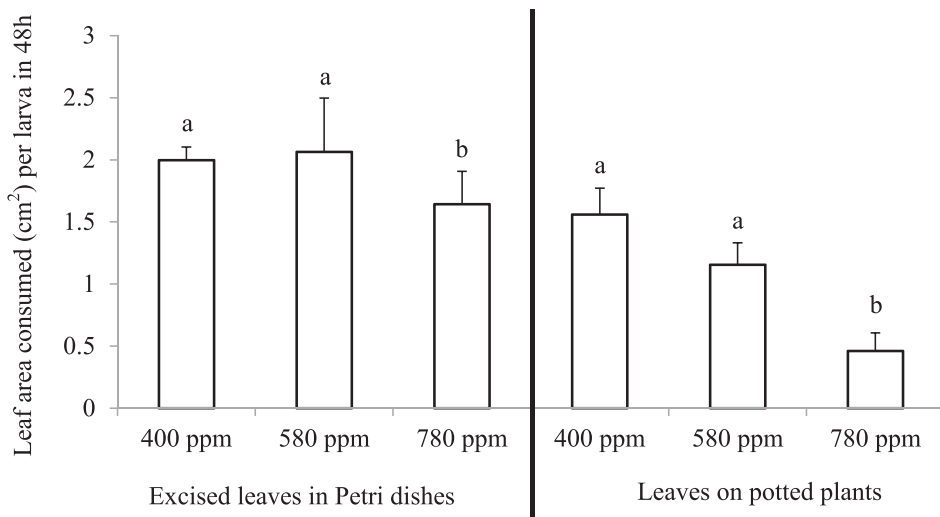


Figure 3. Leaf area consumed (mean \pm SE) (cm²) by fifth instars of *G. boliviana* when feeding on tropical soda apple leaves grown under different CO₂ levels. Different letters indicate significant differences among CO₂ treatments ($P < 0.05$).

4. Discussion

Atmospheric CO₂ concentration is expected to almost double from the current level by the end of this century (IPCC 2007). The increase of CO₂ will influence insect–plant interactions and possibly affect well established and successful weed biological control programmes. However, few studies have examined the effect of climate change on weed biological control (Johns and Hughes 2002; Johns et al. 2003). In our study, plants performed better at the medium (580 ppm) and high CO₂ levels (780 ppm) compared to those under ambient CO₂ (400 ppm). Differences in plant height were detected after 52 days, thus, there was a delay in the response in plant growth which is in agreement with other study with other herbaceous plants (Ziska, Namuco, Moya, and Quilang 1997; Morgan, Bollero, Nelson, Dohleman, and Long 2005). Moreover, plants grown at high CO₂ had greater above and below ground biomass at the end of the experiment (after a 2.5-month-period). These results are in accordance with other studies that have shown increased plant productivity under elevated CO₂ (Drake et al. 1997; Smith et al. 2000; Kriticos et al. 2003; Ainsworth and Long 2005). In particular, C₃ plants, such as tropical soda apple, are known to respond to rising CO₂ levels by increasing the rate of photosynthesis (Long, Baker, and Raines 1993; Griffin and Seemann 1996). These results suggest that tropical soda apple may become more aggressive with global climate change; however, other climatic factors such as rising temperatures and changes in precipitation patterns may also affect the outcome (Morison and Lawlor 1999; Williams et al. 2007; Bradley, Blumenthal, Wilcove, and Ziska 2010). In addition, this study was conducted in enclosed chambers which may modify the microclimate and amplify the effect of CO₂ on plant productivity (Long, Ainsworth, Rogers, and Ort 2004; Ainsworth and Long 2005).

In addition to plant growth, other characteristics of tropical soda apple were affected by elevated CO₂ levels. LWC and foliage nitrogen were reduced at high CO₂ (780 ppm) compared to ambient (400 ppm) and medium levels (580 ppm). The higher SLA, per cent water and N content at the medium level (580 ppm) found in this study are not consistent with the findings of other studies with C₃-photosynthesis plants (Cotrufo et al. 1998; Ainsworth and Long 2005). A possible explanation, at least for the lower C:N ratio at the medium CO₂, is that tropical soda apple actually became more efficient at accumulating N, as was observed in other *Solanum* spp. (Sage, Sharkey, and Seeman 1989). However, we are cautious about the results found at the medium level, and future studies should explore further the response of tropical soda apple to elevated levels of CO₂ including its interactions with higher temperatures and N supply. These changes in plant quality affected the performance of the biocontrol agent, *G. boliviana*. Beetles reared at high CO₂ had lower survival to adulthood and slower development than those reared at ambient or medium CO₂ levels. While immature survival was reduced at the medium level, the higher adult fecundity suggests that beetle performance may slightly increase when exposed to 580 ppm of CO₂. This result is consistent with plant quality changes because leaf nitrogen concentrations increased at 580 ppm of CO₂. However, adult size and fecundity were reduced at high CO₂ levels compared to ambient or medium CO₂ levels. Similarly, other studies have shown that insect herbivores are negatively affected when feeding on plants exposed to elevated CO₂ (Coviella and Trumble 1999; Knepp et al. 2007). For example, female longevity and

egg production of the invasive weevil *Polydrusus sericeus* Schaller were reduced under elevated CO₂ (Hillstrom, Vigue, Coyle, Raffa, and Lindroth 2010). Plant secondary defensive compounds may increase under high CO₂ levels (Coviella et al. 2002; Bidart-Bouzat and Imeh-Nathaniel 2008), and this may have been the case for tropical soda apple. However, the defensive compounds against herbivory in tropical soda apple are unknown and were not measured in this study.

A likely explanation for the inferior performance of beetles at high CO₂ was the drastic reduction in consumption as CO₂ increased; beetles consumed <50% as much leaf tissue at the high level of CO₂ compared to the ambient CO₂ treatment. In contrast to our findings, some defoliators are known to increase their consumption rates when exposed to high CO₂ in order to compensate for the reduced nitrogen contents in plant foliage (Bezemer and Jones 1998; DeLucia et al. 2008). Leaf consumption by fifth instars held at ambient CO₂ was reduced when feeding on leaf tissue grown at high CO₂, but much more reduced when feeding on whole plants in an elevated CO₂ environment. As far as we are aware, this is the first study that has explored direct effects of CO₂ on insect herbivores, and suggests that not only plant quality, but also the concentration of atmospheric CO₂, may affect insect performance. However, we are tentative in this conclusion because the nutritional quality and defensive chemistry of leaves may have changed after excision from the plant.

Under future CO₂ levels (~780 ppm), tropical soda apple may become more aggressive while the performance of *G. boliviana* may be reduced, resulting in a decline in the suppression of tropical soda apple by the beetle. Similarly, the performance of *Dialectica scalariella* (Zeller; Gracillariidae), a biological control agent of *Echium plantagineum* L. (Boraginaceae), was negatively affected at elevated CO₂ (Johns and Hughes 2002). However, climate models predict not only increases in atmospheric CO₂, but also changes in temperature and precipitation patterns (IPCC 2007; Ziska and Dukes 2011). According to Zvereva and Kozlov (2006), the predicted negative effects of CO₂ elevation on herbivores may be mitigated by temperature increases. However, this is not always the case (Johns and Hughes 2002; Johns et al. 2003), and, therefore, the influence of climate change on insect-plant interaction cannot be generalised. In order to improve predictions of how plants and herbivores respond to climate change, multi-factorial studies are needed. In addition, studies should attempt to incorporate the evolutionary potential of plant species and their insect herbivores to adapt to future climate (Whitney and Gabler 2008; Clements and DiTommaso 2011). For example, plant growth and insect performance could be measured under various climate change scenarios over several generations in order to determine which adaptive traits may rapidly evolve in response to climate change.

In summary, this study is one of only a few that has examined the effect of elevated CO₂ on weed growth and the performance of a biological control agent. Tropical soda apple plants grown under elevated CO₂ (780 ppm) had increased growth and biomass compared to those exposed to ambient CO₂ levels (400 ppm). Plant quality for insect herbivores was reduced under high CO₂ (lowered LWCs and foliar nitrogen), which was detrimental to *G. boliviana*. Survival to adulthood, adult size and fecundity of *G. boliviana* were all reduced when feeding on tropical soda apple plants under high CO₂ levels. These results suggest that beetle performance, and, therefore, efficacy of biological control, may be diminished under future climate.

However, further studies should incorporate other climatic factors expected to change, as well as the evolutionary potential of insect herbivores and their hosts to adapt to future conditions, in order to better predict the effects on presently successful biological control programmes.

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