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Effects of elevated CO_2 on feeding responses of biological control agents of *Pontederia crassipes*

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Abstract

Elevated carbon dioxide (eCO_2) and rising temperatures will have far-reaching effects on global plant-insect interactions, yet their implications for future biological control programs are not fully understood. Studies have shown that elevated CO_2 will affect insect feeding guilds differently and these responses can be predicted with some confidence. Water hyacinth, *Pontederia crassipes* Mart. (Pontederiaceae), is a native and representative species of the Del Plata wetlands (Argentina) that invades outside of its native environment. It is considered one of the world's worst aquatic weeds and a target for biological control. In this study, water hyacinth plants were grown under two CO_2 concentrations – current (400 p.p.m.) or elevated (800 p.p.m.) –, with and without two biocontrol agents representing different feeding guilds, the leaf-chewing *Cornops aquaticum* Brüner (Orthoptera: Acrididae) and the phloem-feeding *Megamelus scutellaris* Berg (Hemiptera: Delphacidae). Under eCO_2 concentration, photosynthetic rate, total dry weight, and relative growth rate of *P. crassipes* acclimated to eCO_2 conditions and plants showed very little CO_2 fertilization response in eutrophic water. Insect herbivory varied depending on feeding guilds at eCO_2; however, *P. crassipes* growth responses increased when exposed to insect herbivory. Chewing herbivory by *C. aquaticum* was consistent across CO_2 conditions, whereas the feeding by *M. scutellaris* increased substantially at eCO_2. These results indicate that successful biological control of *P. crassipes* under conditions of elevated CO_2 might rely on phloem-feeding insects, with chewers playing a lesser role.

KEYWORDS

Acrididae, biocontrol agents, *Cornops aquaticum*, Delphacidae, eCO_2, feeding guild, global change, Hemiptera, *Megamelus scutellaris*, Orthoptera, Pontederiaceae, water hyacinth, weed control

INTRODUCTION

The current rise in atmospheric carbon dioxide (CO_2) from post-industrial society is unprecedented (Ehleringer & Cerling, 2002) with a record rise in ambient CO_2 (aCO_2) at a rate of ca. 1 p.p.m. per year (Pachauri et al., 2014). The current global CO_2 concentration (415 p.p.m.) under the current 'no mitigation' scenario is expected to rise to between 600 and 1000 p.p.m. at the turn of the next century (Pachauri et al., 2014). Consequently, the elevated CO_2 (eCO_2) has resulted in a rise in average global temperatures, altered rainfall patterns, and affected global weather patterns (Pachauri et al., 2014). It is expected that predicted eCO_2 conditions will alter plant physiology by altering photosynthesis and affecting aspects of plant metabolism (Sage, 1994; Bowes, 1996; Ehleringer & Cerling, 2002).

Elevated CO_2 and climate change will not be selective nor simple in its impact on plant communities, including both valuable and undesirable species (Ziska & George, 2004). The effects of climate change and the increase in atmospheric CO_2 will have an effect at the individual level by altering plant photosynthetic and metabolic systems (Sage, 1994) as well as larger community effects, changing species ranges, interactions, and ecosystem structure and function (Walther et al., 2002; Root et al., 2003; Hellmann et al., 2008). It is widely thought that invasive plant species will experience a disproportionally positive response to this change (Ziska, 2003; Hellmann et al., 2008; Ziska & Dukes, 2011), suggesting biological invasions will become more prevalent (Reeves, 2017).

Pontederia (=*Eichhornia*) *crassipes* Mart. (Pontederiaceae), also known as water hyacinth, is a highly invasive aquatic weed that reduces water quality by decreasing dissolved oxygen content and light penetration (Villamagna & Murphy, 2010), altering ecosystem structure (Masifwa et al., 2001; Tofte et al., 2003; Coetzee et al., 2014) with adverse effects on human health and well-being (Gezie et al., 2018). The weed has invaded 55 countries across the globe, with a further threat of range expansion due to climate change (Hellmann et al., 2008; Hoveka et al., 2016). Due to the invasiveness of *P. crassipes* and the utilization of the C_3 photosynthetic mechanism, there is a strong possibility that it may show improved growth under eCO_2 (Spencer & Bowes, 1986; Liu et al., 2010).

Biological control plays an important role in the integrated management of *P. crassipes* and, to date, nine biocontrol agents have been released (mostly in South Africa) (Coetzee et al., 2009, 2011, 2021). The current suite of insect biocontrol agents consists of two weevils (*Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache), a moth (*Niphograpta albiguttalis* Hodges), two mirids (*Eccritotarsus catarinensis* Carvalho and *Eccritotarsus eichhorniae* Henry), a leaf-mining mite (*Orthogalumna terebrantis* Wallwork), the planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), and the grasshopper *Cornops aquaticum* Bruner (Orthoptera: Acrididae).

Megamelus scutellaris has been distributed across South Africa since its release in 2013 and has established across different climatic zones, from the subtropical Nseleni River, in KwaZulu Natal, to the temperate Kubusi River (Byrne et al., 2010; Coetzee et al., 2021). This planthopper is multivoltine and can complete its life cycle in 25 days, dependent on environmental temperatures (Sosa et al., 2005). When population densities reach sufficient levels, i.e., approximately 100 insects per plant (Fitzgerald & Tipping, 2013), the insect can cause extensive chlorosis of the leaves and an overall reduction in plant vigor (Miller et al., 2019). *Cornops aquaticum*, a semi-aquatic grasshopper that is oligophagous within the family Pontederiacae (Oberholzer & Hill, 2001), was a promising biocontrol agent in South Africa, effectively reducing plant growth and biomass accumulation in *P. crassipes* in laboratory experiments (Bownes et al., 2010). *Cornops aquaticum* thus provides a perfect model chewing biocontrol agent for this study, but it is yet to have established viable populations at any known release sites in South Africa (Coetzee et al., 2021); this has been attributed to the incompatibility of the tropical insect's thermal tolerance to South Africa's temperate environment (Venturi, 2020). It has also been suggested that the prolonged period in quarantine may have contributed to the poor climatic suitability of the insect in the field (Coetzee et al., 2021).

Insects may be affected by peaks in atmospheric CO_2 concentrations but unaffected by consistent eCO_2 conditions (Guerestein & Hildebrand, 2008), whereas plants typically experience increases in stored carbohydrates due to eCO_2, resulting in the dilution of nitrogen in leaves through either increased carbohydrate storage or the reallocation of resources affecting the C:N ratio of the plant (Stiling & Cornelissen, 2007; Robinson et al., 2012). For example, an increase in carbohydrates by an average of 35% and a decline in protein by 10% results in an average increase in C:N ratio of 19% (DeLucia et al., 2012; Robinson et al., 2012). Herbivorous insects may also experience an alteration in plant defenses, whereby eCO_2 stimulates the production of certain secondary defense compounds and inhibits others, altering the vulnerability of plants to attack (Zavala et al., 2008; Zhang et al., 2015). For example, in chewing species, alterations in plant palatability can result in 'compensatory feeding' or excess feeding, declines in fecundity and larval weight, and changes in population dynamics (Lavoie & Oberhauser, 2004), whereas in phloem-feeding insects, the changes are often more positive, as eCO_2 has been shown to stimulate feeding and population growth rates in various aphid species (Sun & Ge, 2011).

The biology and performance of weed biocontrol agents are thus likely to be affected by alterations in host plant physiology brought on by eCO_2 conditions (Thomson et al., 2010). For example, the efficacy of biocontrol agents that control four of the five worst aquatic plant invaders in South Africa was reduced as a result of eCO_2, with the exception of *Cyrtobagous salviniae* Calder & Sands for the control *Salvinia molesta* D.Mitch (Salviniaceae), which experienced an increase in insect damage (Baso et al., 2021). Although the literature is sparse, it is expected that the responses of insect biological control will be species-specific (Shabbir et al., 2014; Shabbir et al., 2019; Baso et al., 2021).

In this study, we tested the efficacy of two biocontrol agents, in different guilds, of the worst aquatic weed in South Africa under eCO_2 conditions (800 p.p.m.). We use the phloem-feeding *M. scutellaris* and the mobile leaf-chewing *C. aquarium* as model species to test the impact of eCO_2 on feeding guild responses. We hypothesized that under eCO_2, the compensatory growth of *P. crassipes* would be enhanced through increased CO_2 assimilation rates. We further hypothesized that the performance of the phloem-feeding *M. scutellaris* would improve under eCO_2 conditions, whereas *C. aquaticum* would experience 'compensatory feeding' responses indicative of mobile chewers under eCO_2 conditions.

METHODS AND MATERIALS

eCO_2 testing facility

This study was conducted at the Rhodes University Elevated CO_2 Facility, Eastern Cape, South Africa (33°18'41.0″S, 26°30'33.4″E). The experiment consisted of four decagonal open-top chambers (OTCs) of 3 m diameter and 2.8 m high. Each OTC was covered with F-Clean polyethene sheeting, allowing 94% of solar radiation at visible and near-visible wavelengths. The OTCs were ventilated with a 3-phase fan; airflow was distributed evenly via a 34-cm-diameter diffuser running the entire circumference of the chamber, positioned 1 m above the ground, perforated with 300 1-cm-diameter holes. The diffusers' airflow speed was regulated via a fan speed controlled by Senlan (Chengdu, China) SB150 variable speed drives that respond to variations in temperature and CO_2 concentration. To test the effects of eCO_2, two of the four OTC's atmospheric CO_2 concentrations were adjusted to the IPCC's RCP 8.5 'business as usual' atmospheric CO_2 projection for the year 2100, which equates to 800 p.p.m. (Pachauri et al., 2014), whereas the other two OTC's remained at ambient concentrations (400 p.p.m.). Atmospheric CO_2 was controlled and measured within all the OTCs by open-path CO_2 analyzers (GMP343, Visala, Finland), which alter the CO_2 injection into the chamber ventilation system. CO_2 is injected into the blower fan inlet, ensuring mixing and is controlled by a 2873 proportional valve (Bürkert Fuel Control Systems, Ingelfingen, Germany). Temperature was monitored via CS215 temperature/humidity sensors (Campbell Scientific, Logan, UT, USA), and data were processed via a CR6 datalogger and logger-net software (Campbell Scientific) (Raubenheimer et al., 2022). Data-logger and logger-net software use proportional-integral-derivative (PID) procedures to control fans speeds according to the temperature differentials.

Preparation of plant and insect cultures

The effect of elevated CO_2 on insect herbivory was measured by exposing test plants grown at eCO_2 conditions to either *C. aquaticum* or *M. scutellaris* herbivory. Mature *P. crassipes* plants were collected from the Centre for Biological Control (CBC) Mass Rearing Facility (Rhodes University, Makhanda, South Africa) on 15 December 2018. Mature *P. crassipes* plants (192) were grown in 20-L cylindrical mesocosms (30 cm diameter, 33 cm high) in sets of four. These plants were grown in the chambers for 62 days prior to the start of the herbivory impact study at 10 mg N L^{-1} Multisol 6.1.3(44) Foliage (Culterra, Nietgedacht, South Africa) to replicate the average nutrient conditions of invaded sites in South Africa (Coetzee & Hill, 2012), and 1.1 mg Fe L^{-1} 13% Fe chelate to prevent iron deficiency, allowing the plants to acclimate to the experimental eCO_2 conditions. Prior to the start of the insect herbivory study, all dead material and daughter plants were removed from each mesocosm, leaving four mature plants of approximately equal weight and size per mesocosm.

Cornops aquaticum nymphs used in this experiment were reared from adult *C. aquaticum* provided by the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu Natal (29°42'22.3″S, 31°02'43.1″E). *Megamelus scutellaris* nymphs were collected from the CBC Mass Rearing Facility's *P. crassipes* biological control program. The feeding impact study was conducted over 92 days from February to May 2019. Mesocosms within each chamber were divided into three groups of six containers; an insect-free control, a *C. aquaticum* herbivory treatment, and a *M. scutellaris* herbivory treatment, 72 mesocosms in total. Six first instars of *C. aquaticum* and 60 first instars of *M. scutellaris* were introduced to their respective treatment mesocosms to reflect field densities of biocontrol agents under strong control conditions of *P. crassipes*. Insect numbers of both agents were based on estimation from previous impact studies, namely Bownes et al. (2010) who used field observations from Silveira-Guido & Perkins (1975) for *C. aquaticum*, and Miller et al. (2019) who used estimations based on Sosa et al. (2007) for *M. scutellaris*. Due to the difficulty of identifying sex in nymphs of both species, nymphs were randomly selected, assuming a 1:1 sex ratio. A fine mesh screen was used to cover each mesocosm to prevent predation and the insects from escaping.

Experimental design and statistical analysis

In order to determine the combined effect of CO_2 and herbivory on *P. crassipes* growth, plant photosynthetic rates were recorded every 14 days, whereas various plant growth parameters were recorded at the beginning and at the end of the experiment. Mean insect population densities of *M. scutellaris* were measured at the end of the experimental period, for each experimental CO_2 treatment. The maturation rate of *C. aquaticum* did not allow for the development of F1 generations in the study, as the experiment was terminated at maturation but before oviposition as winter conditions would have increased *C. aquaticum* mortality, affecting the results of the study. Leaf gas exchange was measured for all treatments using a portable photosynthesis system with a blue-red LED light source (LI-6400; Li-Cor, Lincoln, NE, USA) on the adaxial surface of the second leaf of representative plants. The LI-6400 blue-red LED light source provided a standardized leaf area of 3 × 2 cm and leaf-to-air vapor deficit below 1.5 kPa, block temperature at a constant 30 °C, and photosynthetically active radiation (PAR) at 1500 µmol m^2 s^{-1}. Carbon assimilation rate (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (Ci) (µmol CO_2) from the same plants from all treatments were measured using the same photosynthesis system on weeks 3, 6, 10, and 13. Plant growth parameters were measured at the beginning of the experiment and every 2 weeks thereafter

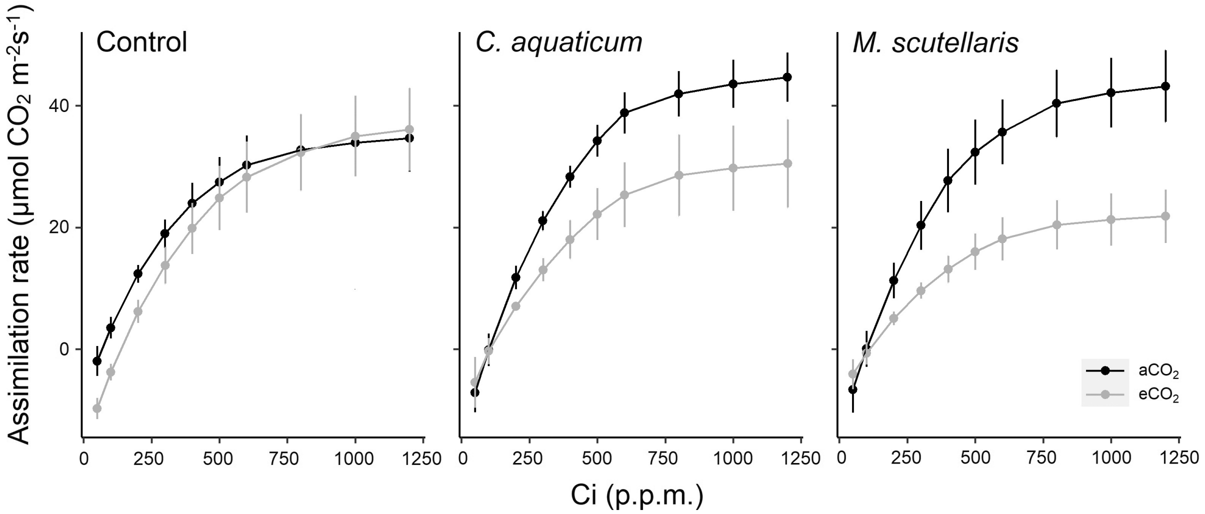

FIGURE 1 Mean (± SE) carbon assimilation rate (A, μmol CO$_2$ m^{-2} s^{-1}) by intercellular CO$_2$ concentration (Ci, p.p.m.) of *Pontederia crassipes* exposed to ambient or elevated CO$_2$ conditions (aCO$_2$, 400 p.p.m., or eCO$_2$, 800 p.p.m.) and insect herbivory (chewing herbivory by *Cornops aquaticum* or sap-feeding herbivory by *Megamelus scutellaris*).

for 13 weeks, on days with similar warm, sunny conditions between 25 and 30 °C and PAR of >1500 μmol m^2 s^{-1}.

After that term, plants were removed from the containers and material was separated into three components: above water biomass (petioles and leaves), below water biomass (roots and crown), and dead material; wet weight was measured for each, and total wet weight was calculated. The second and fourth leaf of each plant were removed and dried at 60 °C until a consistent dry weight was recorded. The samples were then prepared for C:N analysis and sent to the Stable Isotope Laboratory at the Mammal Research Institute (MRI) at the University of Pretoria for analysis. All remaining material was placed in the drying oven at the Department of Botany, Rhodes University, and dried at 60 °C until a consistent dry weight was recorded. Using linear regression to calculate the correlation between the plants' final wet and final dry weights, a proxy based on the linear equation y = mx + c was made. The approximate initial dry weight of *P. crassipes* prior to the start of the feeding trial was calculated using the initial wet weight of the plants and the linear regression proxy. The relative growth rate (RGR) was calculated using the formula:

$$\mathrm{RGR} = \frac{\left(\ln S_2 - \ln S_1\right)}{\left(t_2 - t_1\right)},$$

where S_1 and S_2 represent the dry biomass at times t_1 and t_2 (at the beginning and at the end of the experiment).

The differences in feeding guild damage caused by the chewing *C. aquaticum* and the phloem-feeding *M. scutellaris* at current and eCO$_2$ conditions were measured through image analysis and leaf gas exchange. The abaxial and adaxial surfaces of the second, third, and fourth leaf of a representative plant from each *C. aquaticum* treatment mesocosm were placed on a 1 × 1 mm laminated graph paper and photographed every day for 3 weeks, starting after 1 week of the study. Imaging software, ImageJ (Schneider et al., 2012), was used to quantify the feeding damage of *C. aquaticum* by calculating the area of feeding scars (mm^2) relative to that of the total area of the leaf. This was compared to control leaves to show the extent of damage by *C. aquaticum* to *P. crassipes*. The leaf area:damage ratio as impacted by *C. aquaticum* was calculated using the ratio between the predicted area of the leaf divided by the estimated damage of the leaf caused by *C. aquaticum*.

Statistical analyses were conducted in R v.3.5.3 (R Core Team, 2021). Normality and homogeneity of data were tested using Shapiro-Wilks and Bartlett tests. Photosynthetic CO$_2$ assimilation versus internal leaf CO$_2$ (AC$_i$) curves were fitted and analyzed using the CRAN *plantecophys* package (Duursma, 2015), using the models of Farquhar & Sharkey (1982) as described by Bernacchi et al. (2001). Impact of CO$_2$ and insect herbivory treatments on net assimilation rates, Vcmax, Jmax, relative growth rates, root weight-to-shoot weight ratio, and C:N ratio of *P. crassipes*, as well as the percentage feeding impact of *M. scutellaris* and *C. aquaticum* was analyzed using generalised linear regression model (glm). The statistical models tested the effect of eCO$_2$, insect herbivory, and their interaction on the selected dependent variables. The appropriateness of the model was tested using the function 'simulateResidual' from the DHARMa package [residual diagnostics for hierarchical (multi-level/mixed) regression models] (Hartig, 2022). In the case of poor diagnostics from the DHARMa package, data were log-transformed and re-run accordingly. To identify mechanistic relationships between eCO$_2$ and insect herbivory on the growth performance of *P. crassipes*, a structural equation model (SEM) was developed using *piecewiseSEM* (Lefcheck, 2016) and *MuMIn* (Barton, 2020) in RStudio.

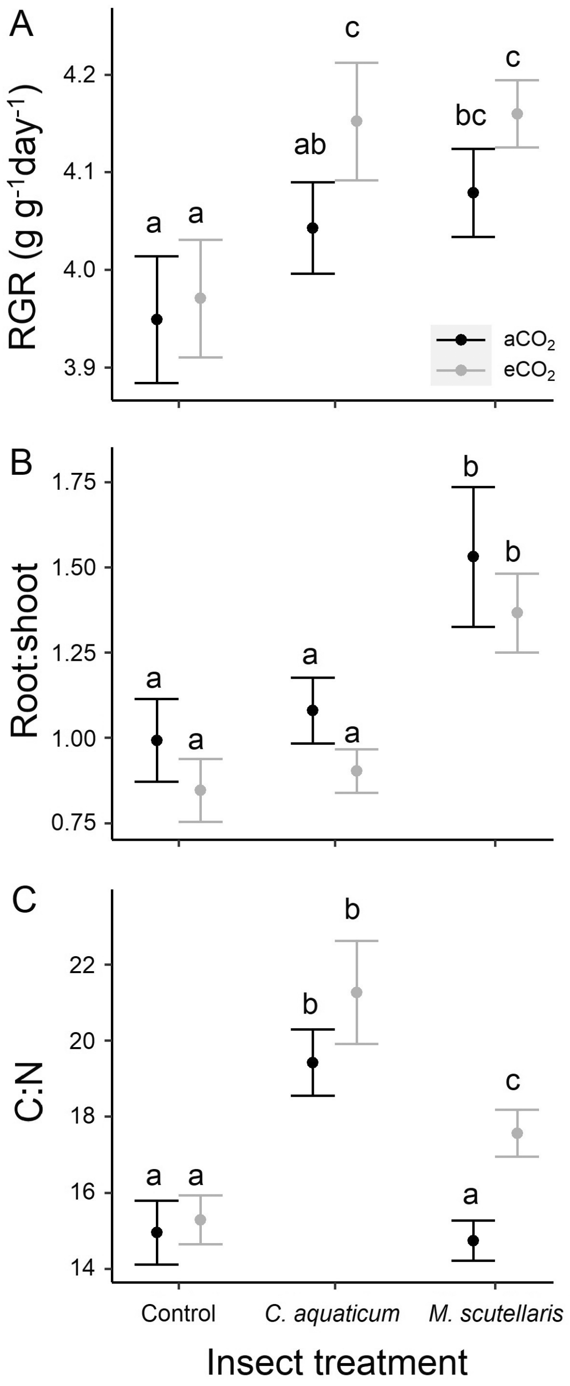

FIGURE 2 Mean (± SE) (A) relative growth rate (RGR, g g^{-1} day^{-1}), (B) root:shoot ratio, and (C) C:N ratio of *Pontederia crassipes* exposed to ambient or elevated CO_2 conditions (aCO_2, 400 p.p.m., or eCO_2, 800 p.p.m.) and insect herbivory (chewing herbivory by *Cornops aquaticum* or sap-feeding herbivory by *Megamelus scutellaris*). Means within a panel capped with different letters are significantly different (generalized linear model: $P < 0.05$).

RESULTS

Elevated CO_2 had no significant effect on carbon assimilation rates, Vcmax, Jmax, RGR, root weight-to-shoot weight ratio, or C:N ratio of *P. crassipes* when grown in the absence of insect herbivory (Figures 1 and 2, Tables 1 and S1). Both

TABLE 1 The effects of CO_2, insect herbivory type, and their interaction on net assimilation rate, maximum rate of Rubisco carboxylase activity (Vcmax), maximum rate of photosynthetic electron transport (Jmax), relative growth rate (RGR), root weight-to-shoot weight, and C:N ratio of *Pontederia crassipes* fed upon by *Megamelus scutellaris* or *Cornops aquaticum*.

Herbivore	Factor	d.f.	Net assimilation rate (μmol CO_2 m^{-2} s^{-1})		Vcmax (μmol CO_2 m^{-2} s^{-1})		Jmax (μmol CO_2 m^{-2} s^{-1})		RGR (g g^{-1} day^{-1})		Root:shoot ratio		C:N ratio	
			χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Megamelus scutellaris	CO_2	1	0.79	0.79	0.888	0.35	0.234	0.63	0.967	0.25	1.15	0.28	5.704	0.02
	Insect herbivory	1	0.049	0.05	0.689	0.41	0.319	0.57	8.918	0.003	13.291	0.0003	1.991	0.16
	CO_2*insect herbivory	1	0.009	0.01	4.491	0.03	5.645	0.02	0.317	0.57	0.004	0.95	3.395	0.06
Cornops aquaticum	CO_2	1	1.93	0.17	0.619	0.43	0.013	0.90	1.29	0.26	2.775	0.01	1.301	0.25
	Insect herbivory	1	0.058	0.81	0.033	0.86	0.001	0.97	5.303	0.02	0.567	0.45	28.579	<0.0001
	CO_2*insect herbivory	1	1.363	0.24	3.248	0.07	2.934	0.09	0.563	0.45	0.024	0.88	0.605	0.44

phloem-feeding and chewing insect herbivory had significant effects on plant performance, affecting net carbon assimilation rate, RGR, root weight-to-shoot weight, and C:N ratio (Figures 1 and 2, Table 1). Insect herbivory resulted in partial compensatory growth response and changes in leaf chemistry in *P. crassipes* with RGR and root:shoot ratio increasing in the presence of *M. scutellaris* and C:N ratio increasing significantly in the presence of *C. aquaticum* (Figure 2A). The significant interaction effects of eCO_2 and *M. scutellaris* herbivory on carbon assimilation rates, RGR, and root weight-to-shoot weight ratio show that the impact of phloem-feeding herbivory differed between CO_2 treatments. eCO_2 conditions in combination with *M. scutellaris* phloem-feeding herbivory significantly reduced carbon assimilation rates of *P. crassipes*, reducing Vcmax and Jmax, and significantly reduced net assimilation rates of *P. crassipes*, limiting the potential compensatory growth responses of the plant, whilst showing strong patterns towards increasing C:N ratio (by 19%) (Figures 1 and 2). The notable reduction in photosynthetic capacity and increasing C:N ratio reflects the apparent resource limitations implied by the increase in root allocation with exposure to *M. scutellaris* (Figure 2B).

The extent of insect impact on the plant is portrayed in Figure 3, with differing indices for each insect based on the feeding type: (A) the impact of *C. aquaticum* was determined by the proportional decline in leaf area (control leaf area – treatment/control leaf area; from 0 to 1), and (B) the impact of *M. scutellaris* was determined by the proportional decline in net assimilation rate (control – treatment/control; from 0 to 1). The proportion of impact on leaf area by *C. aquaticum* between aCO_2 and eCO_2 treatments did not differ significantly as the leaf area impacted by *C. aquaticum* was 19% lower than at aCO_2 conditions (Table 2, Figure 3A). The impact of *M. scutellaris* under eCO_2 conditions on net assimilation rate was on average 49% greater than the impact measured at aCO_2 conditions (Table 2, Figure 3B).

A structural equation model (SEM) path diagram displays the interactions between eCO_2 and insect herbivores on net assimilation rate (Figure 4, Table 3). The SEM indicated that in an environment experiencing changes in atmospheric CO_2 conditions, eCO_2 did not significantly stimulate net assimilation rates of *P. crassipes*, nor did it enhance the impact of the chewing herbivory by *C. aquaticum* (Figure 4, Table 3). Conversely, under eCO_2 conditions, the impact of phloem feeding by *M. scutellaris* was significantly improved as the net assimilation rate of *P. crassipes* was negatively impacted by the interaction of eCO_2 and *M. scutellaris* herbivory (Figure 4, Table 3).

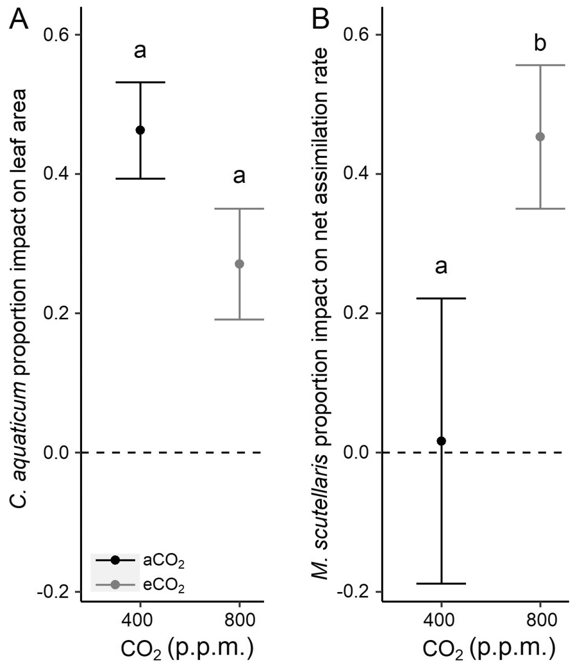

FIGURE 3 Mean (± SE) proportion of impact by (A) *Cornops aquaticum* on leaf area of *Pontederia crassipes*, and (B) *Megamelus scutellaris* on net assimilation rate, exposed to ambient or elevated CO_2 conditions (aCO_2, 400 p.p.m., or eCO_2, 800 p.p.m.). The dashed line indicates zero insect herbivory in the control treatments.

TABLE 2 Effects of CO_2, insect herbivory, and their interaction on impact (%) on leaf area of *Pontederia crassipes* by *Cornops aquaticum*, and on net assimilation rate by *Megamelus scutellaris*.

Response	Factor	χ^2 (d.f. = 2)	P
Impact (%) of *M. scutellaris* on net assimilation rate	CO_2	7.02	0.008
Impact (%) of *C. aquaticum* on leaf area	CO_2	3.313	0.07

DISCUSSION

Noxious alien weeds' invasive potential may likely benefit from the predicted increases in atmospheric CO_2 and climate change (Poff et al., 2002; Hellmann et al., 2008; Thuiller et al., 2008; Diez et al., 2012). In this study, there was no firm evidence supporting an increase in the invasive potential of *P. crassipes* under eCO_2 conditions alone, as expressed by plant growth. Environmental factors such as the thermal thresholds of *P. crassipes* and nutrient concentration of the water are critical in the growth of the plant (Gosset & Norris, 1971; Reddy et al., 1990; Heard & Winterton, 2000). Hoveka et al. (2016) modelled the impact of expected temperature changes as a result of climate change linked to eCO_2 on the invasive potential of *P. crassipes* in South Africa and showed that under expected rising temperature, the invasive range of *P. crassipes* is expected to expand into regions that are currently unfavorable to the plant. This coupled with highly eutrophic waters in South Africa (Villiers & Thiart, 2007; Oberholster & Ashton, 2008), suggests that the invasive potential of *P. crassipes* will be enhanced under future climates, potentially even more by the eCO_2 conditions.

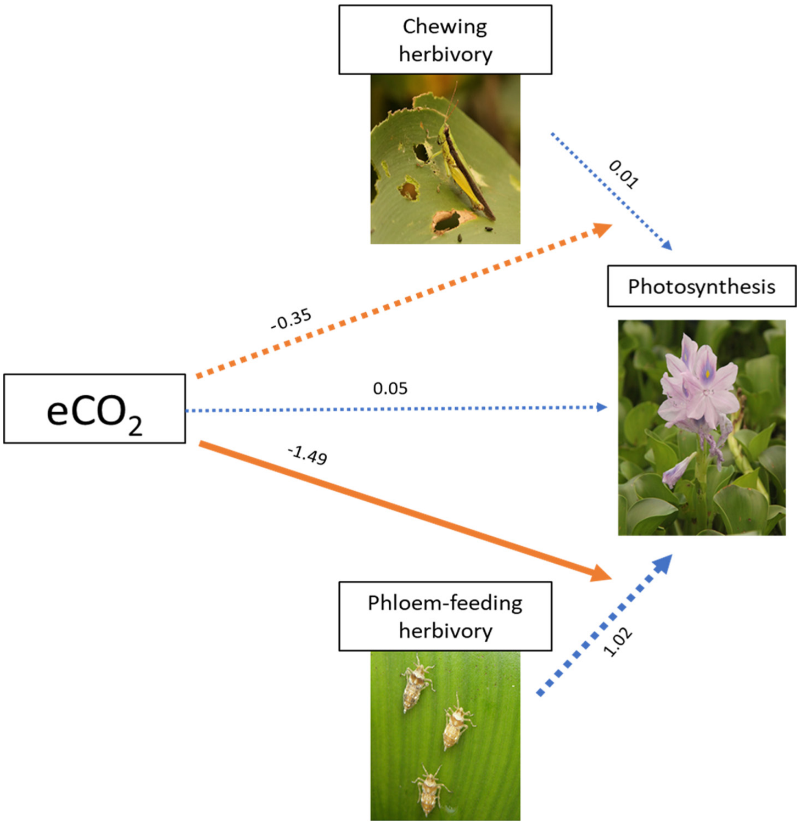

FIGURE 4 Structural equation model (SEM) path diagram showing the effect of eCO$_2$ conditions on the relationship between net assimilation rate of *Pontederia crassipes* and two host-specific biological control agents representing two distinct feeding guilds. The results are from a fitted SEM. The solid arrow indicates a significant effect, the dashed arrows indicate non-significant effects. Arrow width indicates the magnitude of the effect based on the standardized regression coefficient (next to the arrow). Orange arrows indicate a negative effect, blue arrows indicate a positive effect.

TABLE 3 Statistical outputs of the structural equation model (SEM) for the effects of elevated CO$_2$ (eCO$_2$), insect herbivory, and their interaction on net assimilation rate (μmol CO$_2$ m^{-2} s^{-1}) and relative growth rate (g g^{-1} day^{-1}) of *Pontederia crassipes* fed upon by *Megamelus scutellaris* or *Cornops aquaticum*.

Response	Factor	d.f.	Standardised regression coefficient	P
Net assimilation rate	eCO$_2$	2	0.49	0.18
	M. scutellaris	2	1.02	0.10
	eCO$_2$**M. scutellaris*	18	−1.49	0.03
	eCO$_2$	2	0.052	0.82
	C. aquaticum	2	0.014	0.98
	eCO$_2$**C. aquaticum*	39	0.35	0.46
Relative growth rate	eCO$_2$	2	0.08	0.80
	M. scutellaris	2	0.71	0.25
	eCO$_2$**M. scutellaris*	18	−0.2	0.76
	eCO$_2$	2	0.05	0.82
	C. aquaticum	2	0.01	0.97
	eCO$_2$**C. aquaticum*	39	0.36	0.46

In this study, *P. crassipes* responses to eCO$_2$ had mixed effects on insect feeding responses, depending on the insect feeding guild. For example, the impact of phloem-feeding insects was increased by eCO$_2$ conditions, and this can reduce the assimilation rates, represented by changes in maximum rate of Rubisco caboxylase activity (Vcmax) and the maximum rate of photosynthetic electron transport (Jmax) (Welter, 1989, 1993; Zvereva et al., 2010; White et al., 2016). Phloem feeding by *M. scutellaris* resulted in a substantial decline in photosynthetic capacity, affecting electron transport in *P. crassipes* to a greater extent than the leaf chewing by *C. aquaticum*, as well as marked declines in net assimilation rates and increases in C:N ratio at eCO$_2$. The mechanism whereby eCO$_2$ affects phloem-feeders is considered plant-mediated (Sun & Ge, 2011), and model studies on aphids have shown that when nitrogen is not limited within the system, phloem-feeders will gain considerable benefits from eCO$_2$ conditions (Newman et al., 2003). In this study, *M. scutellaris* followed similar enhanced feeding behavior of many phloem-feeders under eCO_2 conditions showing increased feeding damage and (non-significant) increases in population density by 57% (see Figure S1) (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001; Stiling & Cornelissen, 2007; Robinson et al., 2012). This improved feeding impact and population responses can act as greater nutrient sinks, removing metabolites to a greater extent from the phloem and xylem and interrupting source-to-sink transport flow of nutrients within the plant (Larson & Whitham, 1991, 1997; also see the meta-analysis by Zvereva et al., 2010). Root biomass is considered a carbon sink, and the enhanced interruption by *M. scutellaris* at eCO_2 resulted in enhanced compensatory growth of root biomass by 15%. Further increases in C:N ratio as a result of declining nitrogen content of the leaves, suggests that *P. crassipes* allocated resources to nutrient access in an attempt to overcome the potential nutrient limitation imposed by *M. scutellaris* herbivory.

eCO_2 conditions did not result in the hypothesized enhanced feeding response in *C. aquaticum* outlined by the compensatory feeding hypothesis, where leaf chewing insects experience an increase in feeding to compensate for a decline in foliar nitrogen as a result of increasing atmospheric CO_2 (Lincoln et al., 1986; Bezemer & Jones, 1998; Schädler et al., 2007; Robinson et al., 2012). Rather, feeding impact by *C. aquaticum* resulted in a proportional decrease in leaf area by 19% when compared to aCO_2 conditions. The increased chewing response of mobile folivores under eCO_2 is often associated with increases in leaf C:N ratio (Bezemer & Jones, 1998; Hunter, 2001; Robinson et al., 2012), but in this study, the C:N ratio of leaves remained similar, which would result in very little of the predicted compensatory feeding response of chewers under eCO_2 conditions. Although *P. crassipes* experienced a notable decline in photosynthetic capacity resulting from *C. aquaticum* feeding, it must be noted that eCO_2 conditions still allowed for the marginal increase in net assimilation rate at eCO_2. This, in conjunction with little change in C:N ratio due to high nutrient conditions, would reduce compensatory feeding and allow for *P. crassipes*' innate ability to tolerate chewing herbivory, as seen by the slight enhancement in RGR (4.5% when compared to control) and the decline in root:shoot ratio with the favoring of shoot renewal over root growth. Under current aCO_2 conditions, and when nutrients are not limited, *P. crassipes* can sufficiently tolerate foliar herbivory based on low-to-moderate biomass removal, experiencing improved growth and biomass renewal (Soti & Volin, 2010). Bownes et al. (2010) found similar compensatory growth responses of *P. crassipes* to *C. aquaticum* herbivory; however, the response was density-dependent and showed that with an increase in *C. aquaticum* density of 3–4 adults per plant (greater than used in this study), growth of *P. crassipes* was drastically reduced. Further studies by Bownes et al. (2013) illustrated that *P. crassipes* productivity and responses to *C. aquaticum* herbivory are nutrient mediated, showing that under high nutrient conditions (typical in South Africa), compensatory growth of *P. crassipes* was significantly improved and the efficacy of biomass removal was reduced in *C. aquaticum*.

In the context of biological control, for an agent to be successful it must show the ability to reduce the invasiveness of the target weed (Moran et al., 2021); under predicted eCO_2 and climate change conditions, the agent must retain its efficacy, or have improved impact on its host to remain successful. In this study, the efficacy of the phloem-feeding by *M. scutellaris* and chewing herbivory of *C. aquaticum* had varying degrees of impact even under eCO_2 conditions. Yet, of the two agents representing two distinct feeding guilds, this study suggests that *M. scutellaris* will be the more effective biocontrol agent under predicted eCO_2 conditions. The enhanced impact on assimilation rates of *M. scutellaris* under eCO_2 shows the potential for substantial control of *P. crassipes*, provided the population densities of the agent reach sufficient levels, as seen in field studies of native and the introduced ranges (Sosa et al., 2007; Fitzgerald & Tipping, 2013; Coetzee et al., 2021). Due to enhanced compensatory growth of *P. crassipes* under eCO_2 conditions and the well-documented negative impacts of eCO_2 on the population dynamics of chewing insects (Bezemer & Jones, 1998; Stiling & Cornelissen, 2007), it is unlikely that *C. aquaticum* will provide a lower level of control compared to that predicted by phloem-feeding agents such as *M. scutellaris*.

AUTHOR CONTRIBUTIONS

Matthew Keenan Paper: Data curation (equal); formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead). **Tomas Righetti:** Investigation (equal); methodology (supporting); writing – original draft (supporting). **Sarah Raubenheimer:** Formal analysis (supporting); methodology (supporting); writing – review and editing (supporting). **Julie Coetzee:** Conceptualization (lead); supervision (lead); writing – review and editing (lead). **Alejandro Sosa:** Funding acquisition (equal); supervision (supporting); writing – review and editing (supporting). **Martin Patrick Hill:** Conceptualization (equal); funding acquisition (lead); supervision (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

Data available on request from the authors

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REFERENCES

Barton K (2020) MuMIn: Multi-Model Inference. *R package* v.*1*.43.17. https://CRAN.R-project.org/package=MuMIn.

Baso NC, Coetzee JA, Ripley BS & Hill MP (2021) The effects of elevated atmospheric CO_2 concentration on the biological control of invasive aquatic weeds. *Aquatic Botany 170*: 103348.

Bernacchi CJ, Singsaas EL, Pimentel C, Portis Jr AR & Long SP (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell & Environment 24*: 253–259.

Bezemer TM & Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2 quantitative analyses and guild effects. *Oikos 82*: 212–222.

Bowes G (1996) Photosynthetic responses to changing atmospheric carbon dioxide concentration. *Photosynthesis and the Environment*, Vol. *5* (ed. by NR Baker), pp. 387–407. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bownes A, Hill MP & Byrne MJ (2010) Evaluating the impact of herbivory by a grasshopper, *Cornops aquaticum* (Orthoptera: Acrididae), on the competitive performance and biomass accumulation of water hyacinth, *Eichhornia crassipes* (Pontederiaceae). *Biological Control 53*: 297–303.

Bownes A, Hill MP & Byrne MJ (2013) The role of nutrients in the responses of water hyacinth, *Eichhornia crassipes* (Pontederiaceae) to herbivory by a grasshopper *Cornops aquaticum* Brüner (Orthoptera: Acrididae). *Biological Control 67*: 555–562.

Byrne M, Hill MP, Robertson M, King A, Jadhav A et al. (2010) Integrated management of water hyacinth in South Africa: development of an integrated management plan for water hyacinth control, combining biological control, herbicidal control and nutrient control, tailored to the climatic regions of South Africa. *Report to the Water Research Commission 454*: 40–44.

Coetzee JA & Hill MP (2012) The role of eutrophication in the biological control of water hyacinth, *Eichhornia crassipes*, in South Africa. *BioControl 57*: 247–261.

Coetzee JA, Jones RW & Hill MP (2014) Water hyacinth, *Eichhornia crassipes* (Pontederiaceae), reduces benthic macroinvertebrate diversity in a protected subtropical lake in South Africa. *Biodiversity and Conservation 23*: 1319–1330.

Coetzee JA, Hill MP, Byrne MJ & Bownes A (2011) A review of the biological control programmes on *Eichhornia crassipes* (C. Mart.) Solms (Pontederiaceae), *Salvinia molesta* DS Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *African Entomology 19*: 451–468.

Coetzee JA, Hill MP, Julien MH, Center TD & Cordo HA (2009) *Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae). *Biological Control of Tropical Weeds using Arthropods* (ed. by R Muniappan, GVP Reddy & A Raman), pp. 183–210. Cambridge University Press, New York, NY, USA.

Coetzee JA, Bownes A, Martin GG, Miller BE, Smith R et al. (2021) A review of the biocontrol programmes against aquatic weeds in South Africa. *African Entomology 29*: 935–964.

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

Diez JM, D'Antonio CM, Dukes JS, Grosholz ED, Olden JD et al. (2012) Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment 10*: 249–257.

DeLucia EH, Nabity PD, Zavala JA & Berenbaum MR (2012) Climate change: resetting plant-insect interactions. *Plant Physiology 160*: 1677–1685.

Duursma RA (2015) 'Plantecophys' an R package for analysing and modelling leaf gas exchange data. *PloS One 10*: e0143346.

Ehleringer JR & Cerling TE (2002) C3 and C4 photosynthesis. *Encyclopedia of Global Environmental Change*, Vol. *2* (ed. by HA Mooney & JG Canadell), pp. 186–190. John Wiley & Sons, Chichester, UK.

Farquhar GD & Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology 33*: 317–345.

Fitzgerald D & Tipping PW (2013) Effect of insect density and host plant quality on wing-form in *Megamelus scutellaris* (Hemiptera: Delphacidae). *Florida Entomologist 96*: 124–130.

Gezie A, Assefa WW, Getnet B, Anteneh W, Dejen E & Mereta ST (2018) Potential impacts of water hyacinth invasion and management on water quality and human health in Lake Tana watershed, Northwest Ethiopia. *Biological Invasions 20*: 2517–2534.

Gosset DR & Norris WE (1971) Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, *Eichhornia crassipes* (Mart.) Solms. *Hydrobiologia 38*: 15–28.

Guerestein PG & Hildebrand JG (2008) Roles and effects of environmental carbon dioxide in insect life. *Annual Review of Entomology 53*: 161–178.

Hartig F (2022) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. *R package* v.*0*.4.5. https://CRAN.R-project.org/package=DHARMa.

Heard TA & Winterton SL (2000) Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *Journal of Applied Ecology 37*: 117–127.

Hellmann JJ, Byers JE, Bierwagen BG & Dukes JS (2008). Five potential consequences of climate change for invasive species. *Conservation Biology 22*: 534–543.

Hoveka LN, Bezeng BS, Yessoufou K, Boatwright JS & Van der Bank M (2016) Effects of climate change on the future distributions of the top five freshwater invasive plants in South Africa. *South African Journal of Botany 102*: 33–38.

Hunter MD (2001) Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Agricultural and Forest Entomology 3*: 153–159.

Larson KC & Whitham TG (1991) Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. *Oecologia 88*: 15–21.

Larson KC & Whitham TG (1997) Competition between gall aphids and natural plant sinks: plant architecture affects resistance to galling. *Oecologia 109*: 575–582.

Lavoie B & Oberhauser KS (2004) Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environmental Entomology 33*: 1062–1069.

Lefcheck JS (2016) 'piecewiseSEM': Piecewise structural equation modeling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution 7*: 573–579.

Lincoln DE, Couvet D & Sionit N (1986) Response of an insect herbivore to host plants grown in carbon dioxide enriched atmospheres. *Oecologia 69*: 556–560.

Liu JZ, Ge YM, Zhou YF & Tian GM (2010) Effects of elevated CO_2 on growth and nutrient uptake of *Eichhornia crassipe* under four different nutrient levels. *Water, Air, & Soil Pollution 212*: 387–394.

Masifwa WF, Twongo T & Denny P (2001) The impact of water hyacinth, *Eichhornia crassipes* (Mart) Solms on the abundance and diversity of aquatic macroinvertebrates along the shores of northern Lake Victoria, Uganda. *Hydrobiologia 452*: 79–88.

Miller BE, Coetzee JA & Hill MP (2019) Chlorophyll fluorometry as a method of determining the effectiveness of a biological control agent in post-release evaluations. *Biocontrol Science and Technology 29*: 1118–1122.

Moran VC, Zachariades C & Hoffmann JH (2021) Implementing a system in South Africa for categorizing the outcomes of weed biological control. *Biological Control 153*: 104431.

Newman JA, Gibson DJ, Parsons AJ & Thornley JHM (2003) How predictable are aphid population responses to elevated CO_2? *Journal of Animal Ecology 72*: 556–566.

Oberholster PJ & Ashton PJ (2008) *State of the Nation Report: An Overview of the Current Status of Water Quality and Eutrophication in South African Rivers and Reservoirs*. Parliamentary Grant Deliverable, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa.

Oberholzer IG & Hill MP (2001) How safe is the grasshopper *Cornops aquaticum* for release on water hyacinth in South Africa? *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated of Control of Water Hyacinth* (ed. by MH Julien, MP Hill, TD Center & D Jianging), pp. 82–88. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W et al. (2014) *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. by R Pachauri & L Meyer), pp. 151. Intergovernmental Panel on Climate Change (IPCC), Geneva, Switzerland.

Poff NL, Brinson MM & Day JW (2002) *Aquatic Ecosystems and Global Climate Change - Potential Impacts on Inland Freshwater and Coastal Wetland Ecosystems in the United States*. Pew Center on Global Climate Change, Arlington, VA, USA.

Raubenheimer SL & Ripley BS (2022) CO$_2$-stimulation of savanna tree seedling growth depends on interactions with local drivers. *Journal of Ecology 110*: 1090–1101.

R Core Team (2021) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reddy KR, Agami M & Tucker JC (1990) Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes* (Mart.) Solms) plants. *Aquatic Botany 37*: 355–365.

Reeves JL (2017) Climate change effects on biological control of invasive plants by insects. *CAB International Reviews 12*: 1.

Robinson EA, Ryan GD & Newman JA (2012) A meta-analytical review of the effects of elevated CO$_2$ on plant–arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytologist 194*: 321–336.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C & Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature 421*: 57–60.

Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO$_2$: the gas exchange perspective. *Photosynthesis Research 39*: 351–368.

Schädler M, Roeder M, Brandl R & Matthies D (2007) Interacting effects of elevated CO$_2$, nutrient availability and plant species on a generalist invertebrate herbivore. *Global Change Biology 13*: 1005–1015.

Schneider C, Rasband W & Eliceiri K (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods 9*: 671–675. https://doi.org/10.1038/nmeth.2089

Shabbir A, Dhileepan K, Khan N & Adkins SW (2014) Weed–pathogen interactions and elevated CO$_2$: growth changes in favour of the biological control agent. *Weed Research 54*: 217–222.

Shabbir A, Dhileepan K, Zalucki MP & Adkins SW (2019) Biological control under a changing climate: the efficacy of the parthenium weed stem-galling moth under an atmosphere enriched with CO$_2$. *Biological Control 139*: 104077.

Silveira-Guido A & Perkins DB (1975) Biology and host specificity of *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae), a potential biological control agent for water hyacinth. *Environmental Entomology 4*: 400–404.

Sosa AJ, Remes Lenicov de AM, Mariani R & Cordo HA (2004) Redescription of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a candidate for biological control of water hyacinth. *Annals of the Entomological Society of America 97*: 271275.

Sosa AJ, Lenicov AMDR & Mariani R (2007) Species of *Megamelus* (Hemiptera: Delphacidae) associated with Pontederiaceae in South America. *Annals of the Entomological Society of America 100*: 798–809.

Sosa AJ, Lenicov AMDR, Mariani R & Cordo HA (2005) Life history of *Megamelus scutellaris* with description of immature stages (Hemiptera: Delphacidae). *Annals of the Entomological Society of America 98*: 66–72.

Soti PG & Volin JC (2010) Does water hyacinth (*Eichhornia crassipes*) compensate for simulated defoliation? Implications for effective biocontrol. *Biological Control 54*: 35–40.

Spencer W & Bowes G (1986) Photosynthesis and growth of water hyacinth under CO$_2$ enrichment. *Plant Physiology 82*: 528–533.

Stiling P & Cornelissen T (2007) How does elevated carbon dioxide (CO$_2$) affect plant–herbivore interactions? A field experiment and meta-analysis of CO$_2$ mediated changes on plant chemistry and herbivore performance. *Global Change Biology 13*: 1823–1842.

Sun Y & Ge F (2011) How do aphids respond to elevated CO$_2$? *Journal of Asia-Pacific Entomology 14*: 217–220.

Thomson LJ, MacFadyen S & Hoffmann AA (2010) Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control 52*: 296–306.

Thuiller W, Albert C, Araujo MB, Berry PM, Cabeza M et al. (2008) Predicting global change impacts on plant species' distributions: future challenges. *Perspectives in Plant Ecology, Evolution and Systematics 9*: 137–152.

Toft JD, Simenstad CA, Cordell JR & Grimaldo LF (2003) The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets. *Estuaries 26*: 746–758.

Venturi G (2020) Thermal Physiology Components of the Grasshopper *Cornops aquaticum* (Brüner, 1906) and its Impact on Establishment in South Africa. PhD Dissertation, University of the Witswaterrand, South Africa (https://hdl.handle.net/10539/31007).

Villamagna AM & Murphy BR (2010) Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshwater Biology 55*: 282–298.

Villiers SD & Thiart C (2007) The nutrient status of South African rivers: concentrations, trends and fluxes from the 1970s to 2005. *South African Journal of Science 103*: 343–349.

Walther GR, Post E, Convey P, Menzel A, Parmesan C et al. (2002) Ecological responses to recent climate change. *Nature 416*: 389–395.

Welter SC (1993) Responses of plants to insects: eco-physiological insights. *International Crop Science*, Vol. *I* (ed. by DR Buxton, R Shibles, RA Forsberg, BL Blad, KH Asay, GM Paulsen & RF Wilson), pp. 773–778. Crop Science Society of America, Madison, WI, USA.

Welter SC (1989) Arthropod impact on plant gas exchange. *Insect-Plant Interactions*, Vol. *1* (ed. by Bernays EA), pp. 135–164. CRC Press, Boca Raton, FL, USA.

White AC, Rogers A, Rees M & Osborne CP (2016) How can we make plants grow faster? A source–sink perspective on growth rate. *Journal of Experimental Botany 67*: 31–45.

Zavala JA, Casteel CL, DeLucia EH & Berenbaum MR (2008) Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. *Proceedings of the National Academy of Sciences of the USA 105*: 5129–5133.

Zhang S, Li X, Sun Z, Shao S, Hu L et al. (2015) Antagonism between phytohormone signalling underlies the variation in disease susceptibility of tomato plants under elevated CO$_2$. *Journal of Experimental Botany 66*: 1951–1963.

Ziska LH & Dukes JS (2011) *Weed Biology and Climate Change*. Wiley-Blackwell, Oxford, UK.

Ziska LH & George KATE (2004) Rising carbon dioxide and invasive, noxious plants: potential threats and consequences. *World Resource Review 16*: 427–447.

Zvereva EL, Lanta V & Kozlov MV (2010) Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia 163*: 949–960.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Mean (± SE) total population density (number per mesocosm) of *Megamelus scutellaris* when exposed to ambient or elevated CO_2 conditions (400 or 800 p.p.m.). The means were not significantly different (generalized linear model: $F_{1,20} = 1.83$, $P = 0.2$).

Table S1. Mean (± SE) maximum rate (μmol CO_2 m^{-2} s^{-1}) of Rubisco carboxylase activity (V_{cmax}) and photosynthetic electron transport (J_{max}) of *Pontederia crassipes* as affected by CO_2 (400 and 800 p.p.m.) and insect herbivory by *Cornops aquaticum* and *Megamelus scutellaris.*

Table S2. Mean (± SE) root biomass (g), shoot biomass (g), dead biomass (g), and root weight:shoot weight ratio of *Pontederia crassipes* as affected by CO_2 (400 and 800 p.p.m.) and insect herbivory by *Cornops aquaticum* and *Megamelus scutellaris.*

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