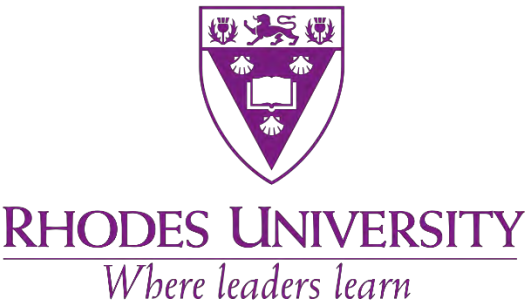
The Effects of Elevated CO_2 on Feeding Guild Responses of Biological Control Agents of *Pontederia crassipes* Mart. (Pontederiaceae)



THESIS

Submitted in fulfilment of the requirements for the degree

MASTERS OF SCIENCE

at Rhodes University

By

Matthew Keenan Paper

November 2021

Abstract

Elevated CO_2 (eCO_2) and rising global temperatures have the potential to alter plant-insect interactions with important implications for plant community structure and function. Previous studies on plant-insect interactions have shown that eCO_2 will affect insect feeding guilds differently, impacting negatively, positively or having very little effect. The implications of this on the global invasive plant biological control programme is largely unknown.

This study investigates the response of one of the world's most invasive aquatic plants, *Pontederia* (= *Eichhornia*) *crassipes* Mart. (Pontederiaceae), to predicted eCO_2 conditions of 800 ppm and how this may affect the feeding response of two biological control agents representing different feeding guilds; the leaf chewing *Cornops aquaticum* Brüner (Orthoptera: Acrididae) and the phloem-feeding *Megamelus scutellaris* Berg (Hemiptera: Delphacidae). A factorial eCO_2 x feeding impact study was conducted at the Rhodes University Elevated CO_2 Facility in the Eastern Cape Province of South Africa over 13 weeks in the growing season of 2019. The effect of insect herbivory by *C. aquaticum* and *M. scutellaris* at two atmospheric CO_2 concentrations, representing current and future predicted concentrations (400 ppm and 800 ppm) on *P. crassipes* was examined through both biomass and ecophysiological measures.

Assimilation rates, C:N ratio, total dry weight and relative growth rate of *P. crassipes* were unaffected by eCO_2 conditions, and plants experienced no CO_2 fertilization in eutrophic water conditions representative of South African waterways. Effects of eCO_2 on insect herbivory varied depending on the feeding guild. *Pontederia crassipes* showed compensatory growth responses when exposed to *C. aquaticum* herbivory regardless of CO_2 treatment, but chewing herbivory damage remained constant, and the agent maintained efficacy. *Pontederia crassipes* showed down-regulation of photosynthesis when exposed to *M. scutellaris* due to eCO_2-related feeding responses by *M. scutellaris* increasing substantially through a significant (30%) increase in adult population density under eCO_2 conditions.

These results indicate that the plant-insect interactions that underpin biological control programmes for *P. crassipes* should remain successful under future CO_2 conditions. Phloem-feeding insect damage (*M. scutellaris*) was significantly greater than chewing damage in this study, suggesting that invasive plant biological control programmes will need to shift focus away from the charismatic chewing insect herbivores and onto the often-neglected phloem-feeding biological control agents due to their overwhelmingly positive response to eCO_2.

Table of Content

Acknowledgements

I am very grateful to Rhodes University and the Centre for Biological Control (CBC) Water Weeds Program for the funding and support of this project. I would like to thank the South African Sugarcane Research Institute (SASRI) for providing the founding population of *C. aquaticum* used in this thesis. I would like to thank Prof. Brad S. Ripley for the support and use of the Rhodes University Elevated CO_2 Facility (RUECF) and associated equipment. I would like to sincerely thank my supervisor Prof. Julie A. Coetzee, without whom this thesis would not be possible. Your bountiful knowledge, excitement for science and ability to make mountains into manageable molehills encouraged me to persevere and create something that I am truly proud of. I would also like to thank my co-supervisor Prof. Martin P. Hill, your support, kind words, and inspiring passion for your craft has driven my curiosity for science since the first day I stepped into the CBC. I would like to offer a special thanks to Tomas Righetti, whom I would never have met if not for this thesis. Tomas, thank you for the hours spent counting insects and measuring plants. I really look forward to many more years of friendship. I would also like to sincerely thank Esther Mostert, who helped so much in the field. Your critical thinking and kind spirit made working in the field an absolute pleasure. I would like to thank my family for the unyielding support and absolute conviction in my ability. Lastly, I would like to thank Sarah Raubenheimer for the love and kindness throughout this experience. I am so grateful for the hours spent reading and re-reading countless drafts, for the support when things became difficult and for celebrating the successes with me along the way. Without your support and patience, this would have been a far more daunting task.

Chapter 1: General introduction and literature review

1.1 Problem statement

Anthropogenically driven increase in atmospheric CO_2, and the subsequent global climate change phenomenon has prompted significant research on ecological relationships across the globe (Solomon et al. 2009; IPPC 2014). Elevated CO_2 (eCO_2) influences plant-insect interactions worldwide, changing plant quality and resource allocation, which has cascading effects on herbivores (Sage 1994; Bezemer & Jones 1998). In the case of the biological control of alien invasive plants (the topic of this thesis), which uses arthropod biological control agents, the relationship between plant and insect is paramount and is the premise on which the control of the plant is deemed effective. Alterations in plant physiology due to future predicted eCO_2 could have major ramifications for the success of biological control programmes (Bezemer & Jones 1998; Hellmann et al. 2008), affecting the outcome of control depending on the plant-insect interaction through which control is achieved (Bezemer & Jones 1998, Whittaker 1999). A better understanding of how insects of different feeding guilds respond to eCO_2 and plant changes will help guide future biological control programmes of current and future invasive alien plants and the selection and formation of future biological control strategies.

1.2 Global climate change

Global climate change and the rates of average global temperature rise recorded in the last 50 years have more than doubled when compared to any other point in human history (IPCC 2014). The current rise in atmospheric CO_2 over the relatively short period of post-industrial society is unprecedented (Ehleringer & Cerling 2002), with a record rise in CO_2 at a rate of ±1ppm/yr (Field et al. 2014). The continual rise in anthropogenically driven atmospheric CO_2 has begun to create irreversible changes to climates across the globe; these changes and the associated impacts are predicted to extend on a millennial time scale (Solomon et al. 2009). The Intergovernmental Panel on Climate Change (IPCC), dedicated to improving our understanding of climate change and its impacts on future generations,

has predicted that by 2100, atmospheric CO_2 levels will be higher than 800 ppm if anthropogenic CO_2 continues to increase on the current trajectory (Meehl et al. 2007).

Trends of increasing "greenhouse gas" emissions, such as atmospheric CO_2, are linked to rising mean global temperatures (Field et al. 2014). The presence of greenhouse gases within the atmosphere is a naturally occurring phenomenon; however, the rate at which anthropogenically driven increases in these heat-trapping gases are of great concern (Jansen et al. 2007). The associated increase in mean global temperature put forward by the IPCC (IPCC 2014) for the "business as usual" model, where no significant decline in anthropogenically derived CO_2 occurs, is thought to increase from anything between 1.1 to 6.4°C by 2100 (IPCC 2014). This rate of increase in mean global temperature is unprecedented in human history and is unusual even in the geological time scale (Jansen et al. 2007).

Increases in mean global temperatures by as little as 2-3°C will have drastic impacts on drought-prone resource-poor countries (Houérou et al. 1996; Stringer et al. 2009; Dube et al., 2013). Those most likely to be negatively impacted by such climatic changes are impoverished marginal populations with little adaptive capacity to drastic change (IPCC 2007). The African continent has been highlighted as vulnerable due to the large majority of its population having low adaptability and high sensitivity to predicted changes (Callaway 2004; Stringer et al. 2009; IPCC 2014). In particular, South Africa suffers from a range of socio-economic and environmental issues, which have been compounded by climate change (Stringer et al. 2009). This has driven the necessity to develop predictive models on climate change impacting southern African ecosystems and such systems' resilience to change.

Anthropogenically derived CO_2 and the associated rise in mean global temperatures are directly linked to the burning of fossil fuels from the development of industrial levels of commercial agriculture, globalisation, and deforestation. Increases from current ambient CO_2 concentrations of 400 ppm to the 800 – 1200 ppm predicted by the IPCC are inevitable and will continue even if immediate action is taken to reduce the human CO_2 load (Meehl et al. 2007). The consequences of eCO_2 and associated climate change will be far-reaching and highly variable across the globe; however,

rapid change in ecosystem function services is expected (Reeves et al. 2017). Climate change is thus a phenomenon that is unlikely to be resolved for centuries to come, and the extent of its impact on natural and managed systems is still largely unknown (Reeves et al. 2017).

1.2.1 Effects of climate change on plants

Greenhouse gases are naturally occurring in the atmosphere, and atmospheric carbon in the form of CO_2 drives primary production on Earth, forming a key component of plant metabolism (Arora et al. 2013; Richardson et al. 2013). Atmospheric CO_2 is currently 415 ppm compared to 400 ppm 20 years ago (Lan et al. 2020; NOAA 2021), and continuing anthropogenic derived increases will have major effects on primary production across the globe, impacting the basic biochemical processes of plant species (Cornelissen 2011). The effect of eCO_2 on plant processes will be species-specific; however, those species limited by current atmospheric CO_2 will benefit significantly from the increases in CO_2 availability (Ehleringer & Björkman 1977).

The impacts of eCO_2 will vary across species, and increases in atmospheric CO_2 have been shown to increase biomass production in some agriculturally important species. However, the stimulation response varies in intensity and longevity depending on the photosynthetic pathway utilised by the plant (Poorter & Navas 2003). This potentially beneficial short-term response to eCO_2 will be accompanied by possible negative changes in climate, such as extreme changes in temperature and rainfall events ranging in severity (Houérou et al. 1996).

Climate change will not be selective nor simple in its impact on plant communities, as benefits will be felt by both valuable and undesirable species (Ziska & George 2004). The effects of increased atmospheric CO_2 and subsequent climate change will change community structure, species ranges and interactions, and therefore ecosystem structure and function (Walther et al. 2002; Root et al. 2003; Hellmann et al. 2008). Due to the adaptive ability of invasive species, atmospheric eCO_2 will have a disproportionally positive effect on the frequency and efficiency of biological invasions, opening avenues of invasion as yet inaccessible and providing novel environments (Ziska 2003; Hellmann et al.

2008; Ziska & Dukes 2011). Study of the impact of long-term exposure to eCO_2 and increased temperature on invasive species can provide practical insight into the management and control of these species in the future.

1.2.2 Effects of eCO_2 & rising temperature on C_3 photosynthesis

Changes in atmospheric CO_2 concentrations and temperature will have a range of effects on C_3, C_4 and CAM carbon fixing mechanisms of plants, all of which will respond differently to these changes (Ehleringer & Cerling 2002). The effects of a changing climate will result in alterations in the utilisation of atmospheric CO_2 as well as water use efficiency of photosynthesis (Pearcy & Björkman 1983). The C_3 photosynthetic mechanism, so named because of the three-carbon molecule first produced by the mechanism, is widely representative and occurs in all taxonomic plant groups and represents 80% of terrestrial productivity (Lloyd & Farquhar 1994; Roy & Saugier 2001; Ehleringer & Cerling 2002). The C_4 mechanism, creatively named from the four-carbon molecule it produces, is found in more advanced taxa such as monocotyledons, including most grasses, sugarcane, wheat and maize (Ehleringer & Cerling 2002). The C_4 photosynthetic process is far more robust and has evolved additional morphological and biochemical features during epochs of drought and low concentrations of atmospheric CO_2 (Ehleringer & Cerling 2002; Sage 2004).

The C_3 mechanism is a multiple-step process that combines ribulose biphosphate (RuBP) to the substrate CO_2 using the enzyme RuBP carboxylase/oxygenase (Rubisco) and forms two molecules of phosphoglycerate (3C molecule) (Ehleringer & Cerling 2002). However, Rubisco can also metabolise RuBP using O_2 as a substrate forming a molecule each of phosphoglycerate and phosphoglycolate (the 2C molecules) (Ehleringer & Cerling 2002). The latter oxygenation process is considered more energetically expensive and results in the formation of CO_2 through photorespiration (Ehleringer & Cerling 2002). The balance of carboxylation/oxygenation of Rubisco is a function of the CO_2:O_2 balance in the atmosphere, and thus the efficiency of the C_3 mechanism increases within rising CO_2 (Ehleringer

& Cerling 2002). Interestingly, the sensitivity of Rubisco to O_2 is also dependent on temperature, with oxygenation or photorespiration increasing with rising temperature conditions (Ehleringer et al. 2002).

Many studies have shown that under future eCO_2 concentrations, the efficacy of the Rubisco enzymes in C_3 plants may improve, and the assimilation rates of CO_2 over that of O_2 are enhanced (Sage 1994; Ehleringer et al. 2002). This occurs when C_3 plants are grown under eCO_2 conditions and experience an increased rate of photosynthesis and improved water use efficiency (Robinson et al. 2012). Physiological improvements in C_3 plants may increase assimilation rates, biomass production, and reproduction rates; however, increases will be governed by the associated climatic changes in temperature (Idso et al. 1987). Erhlinger & Cerling (2002) showcase which conditions favour each of the photosynthetic pathways, whereby the C_3 mechanisms benefit from high atmospheric CO_2 but lower temperatures, while the C_4 pathway benefits from higher temperatures (Erhlinger & Cerling 2002) (Figure 1.1.).

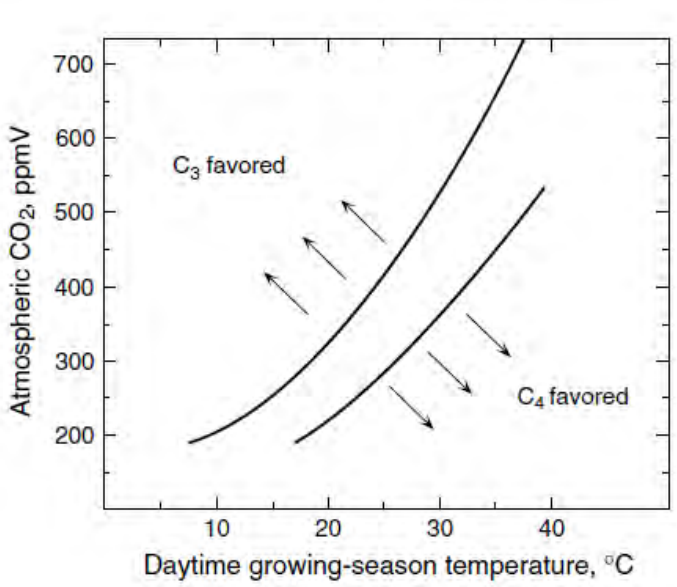


Figure 1.1: The modelled photosynthetic light-use efficiency for C_3 and C_4 plants as a function of atmospheric CO_2 concentrations and temperature (°C). Image from Ehleringer & Cerling (2002), modified from Ehleringer et al. (1997).

The positive CO_2 "fertilisation" benefits experienced by various C_3 plants in future climates can differ from species to species (Song et al. 2020) and are governed by environmental factors, whereby photosynthetic patterns generally follow one of two paths, depending on resource availability (Korner 2006; Reich et al. 2006). In some, the net photosynthetic rate increases per unit leaf area at eCO_2 and remains such for the duration of the plants' growth cycle (Huber et al. 1984; Spencer & Bowes et al. 1986). In an experiment on *Fagus sylvatica* L. (Fagaceae)*, Quercus petraea* Matt. (Fagaceae)*, Carpinus betulus* L. (Betulaceae)*, Acer campestre* L. (Sapindaceae), and *Tilia platyphyllos* Scop. (Malvaceae) in mature deciduous forests, Switzerland (47°28'N, 7°30'E), an increase in the net assimilation rates was maintained even after eight years of eCO_2 treatment, with no recorded acclimation response (Bader et al. 2010). This permanent improvement in photosynthetic efficiency is largely due to alterations to the CO_2:O_2 balance and the reduction of O_2 effects on Rubisco (Tolbert 1980; Erhlinger & Cerling 2002). The more common response to eCO_2 is a temporary enhancement in net photosynthetic rate per leaf area, resulting in the rapid accumulation of biomass (Spencer & Bowes 1986), followed by a reduction in the responsiveness of the photosynthetic apparatus (an acclimation) to nominal levels equal to, or below, that of plants at ambient levels (Spencer & Bowes 1986; Bowes 1991; Erhlinger & Cerling 2002). An elevated CO_2 experiment on red mangrove *Rhizophora mangle* (Rhizophoraceae) reported stimulated root:shoot ratios, relative growth rates and net assimilation rates in responses to eCO_2 conditions in the initial phases of growth (Farnworth et al. 1996). Yet, photosynthetic stimulation was reduced over time by the acclimation of photosynthetic mechanisms to eCO_2 (Farnworth et al. 1996; Ainsworth & Rogers 2007).

The acclimation response of C_3 plants is seen as a mechanism for the plant to rectify an imbalance within the system, maintain the maximum growth output, and improve resource use efficiency (Bowes 1991; Sage & Coleman 2001). However, plant acclimation responses to eCO_2 conditions vary greatly from species to species and can be altered by the environmental conditions in which the plants are grown (Erhlinger & Cerling 2002; Korner 2006; Reich et al. 2006). Thus, the interactions between eCO_2, increasing ambient temperatures and resource availability will

significantly influence the growth of many plant species under future predicted climates (Sage 1994; Jifon & Wolfe 2002; Andresen et al. 2016). When resources are not limited, ambient temperature increases are predicted to enhance photosynthetic responses to eCO_2 in C_3 plants, reducing acclimation response in some species (Long 1991; Gifford 1995; Bowes et al. 1996; Wang et al. 2012). In a meta-analysis by Wang et al. (2012), C_3 plants, which represent over 80% of all plant species, experienced an enhancement in net photosynthetic rate as a result of increasing temperatures. Similarly, Wang et al. (2012) found that legumes, nitrogen-fixers using the C_3 pathway, experienced improved assimilation rates when grown at higher temperatures and eCO_2 conditions, indirectly indicating the mediation effect of nutrient limitations on photosynthetic stimulation and potential acclimation phenomenon of C_3 plants.

1.2.3 eCO_2 and rising temperature on plant-insect interactions

One of the predominant effects of eCO_2 is the subsequent change to plant-insect interactions. eCO_2 does not directly affect an insect's behaviour or physiology but rather alters plant biochemical processes, indirectly affecting insect herbivores (Lincoln et al. 1984; Bezemer & Jones 1998; Stiling & Cornelissen 2007). Any changes in insect herbivore performance, developmental duration and abundance at eCO_2 are linked to changes in plant assimilation rates and water use efficiency, affecting the C:N balance and impacting whole plant metabolism (Lincoln et al. 1984; Fajer et al. 1989; Bezemer & Jones 1998). Generally, plant quality declines in foliar nitrogen, with a reciprocal increase in carbon-rich secondary metabolites; however, not all plant species respond similarly to eCO_2 (Fajer et al. 1989, 1991; Johnson & Lincoln 1991; Lincoln et al. 1993; Roth et al. 1994; Roth & Lindroth et al. 1995; Hunter 2001). Roth & Lindroth (1994) showed that eCO_2 led to a general decline in foliar nitrogen and increased carbon-rich tannins in *Betula papyrifera* Marsh. (Betulaceae) but not *Pinus strobus* L. (Pinaceae). Further studies by Roth et al. (1998) and Agrell et al. (2002) on *B. papyrifera* (Betulaceae), *Populus tremuliodes* Michx. (Salicaceae), and *Acer saccharum* Marshall (Sapindaceae) found that all species responded with increased foliar tannins, while *P. tremuliodes* also responded with an increase

in phenolic glycosides. Generally, a decline in foliar nitrogen reduces plant palatability in most plant species and is a predictable outcome under eCO_2 conditions (Zvereva & Kozlov 2006; Stiling & Cornelissen 2007; Robinson et al. 2012). The reduction in foliar nitrogen can be mediated with the addition of fertiliser regimes (Robinson et al. 2012). This fits well with Korner's (2006) suggestion of "decoupled" states whereby systems have abundant resources other than carbon, providing non-limiting scenarios to eCO_2 responses, such as recently disturbed, polluted or naturally nutrient-rich systems, as well as those treated with mineral fertilisers.

Insect herbivory responses to eCO_2 conditions vary considerably from species to species; however, the general insect response to plants grown under eCO_2 conditions can be viewed through the lens of insect feeding guild responses (Lincon et al. 1984; Bezemer & Jones 1998; Hunter 2001; Schädler et al. 2007). The feeding guild responses to eCO_2 conditions follow a general pattern of compensatory feeding by various mobile defoliators, where increased feeding occurs as a means to overcome poor leaf quality and/or secondary chemical defences (Bezemer & Jones 1998; Whittaker 1999; Hunter 2001; Zavala et al. 2013; Zavala et al. 2017). A long-running study by Brooks & Whittaker (1997) found compensatory feeding responses in third instar nymphs of green dock beetle *Gastrophysa viridula* De Geer (Coleoptera: Chrysomelidae) whilst feeding on *Rumex obtusifolius* L. (Polygonaceae) at eCO_2 due to reductions in plant quality. Significant negative impacts accompanied this response, affecting relative growth rates, egg number and egg weight in second and third generations compared to insects at ambient CO_2 concentrations (Brooks & Whittaker 1997).

Recent studies have suggested that declines in foliar quality may not fully describe many insects responses to eCO_2 conditions, but rather that secondary metabolites and the various triggered pathways potentially play a significant role in plant-insect interactions under eCO_2, more so than that of changes in the C:N balance (Zavala et al. 2013; Zavala et al. 2017). The review by Zavala et al. (2013) found that eCO_2 mediated plant-insect responses by affecting secondary metabolite production. Insect herbivores must contend with a diverse array of allelochemicals produced by plants, and under eCO_2 conditions, potential photosynthetic stimulation effects can alter this relationship, affecting the

nutritional quality and secondary metabolism of plants and, consequently, the suitability of plant material to insect herbivores (Long et al. 2004; Zavala et al. 2013).

The developmental responses of defoliating insects are highly variable and depend mainly on the insect and plant species studied (Traw et al. 1996; Coviella & Trumble 1999; Bezemer & Jones 1998). In many cases, the ability of insects to compensate for nitrogen deficiencies does decline but may not be as dramatic as has been suggested (Hunter 2001). For example, Lindroth (1995) tested the response of various saturniid moths feeding on *B. papyrifela* at eCO_2, and the customary reduction in foliar nitrogen (23%) was noted, as well as a two-fold increase in carbon-rich tannins and a change in C:N ratio from 12.7 to 28.1. Despite this decline in plant quality, first instar larvae only suffered marginally higher mortality rates, while later instars experienced only slight increases in feeding rates and declines in developmental rates (Lindroth 1995).

For other feeding guilds such as phloem-feeders, population-level responses are seen, where insect populations respond positively to eCO_2 growth conditions (Bezemer & Jones 1998; Coviella & Trumble 1999; Stiling & Cornelissen 2007; Robinson et al. 2012), with increases in growth rate and population size across generations (Awack 1997; Docherty et al. 1997). In a study by Ryalls et al. (2017), the pea aphid *Acyrthosiphon pisum* Mordvilko (Homoptera: Aphididae) feeding on *Medicago sativa* L. (Fabaceae) at eCO_2 experienced greater fecundity, longevity, colonisation success and intrinsic rate of increase at ambient temperatures. The ability of phloem-feeders to gain such benefits from eCO_2 conditions is largely unknown (Ryalls et al. 2016). In the case of aphids, the most widely studied phloem-feeder group, the mechanisms governing eCO_2 responses are thought to be mediated by changes in amino acid concentration, amino-acid composition (Sun & Ge 2011) and air temperature (Robinson 2012; Facey et al. 2014). Newman's (2003; 2004) studies on aphid population dynamics found that when nitrogen is not limiting, aphids benefit from eCO_2 conditions; however, the effect is reduced by increasing air temperature. This suggests that although benefits gained from eCO_2 conditions improve population responses, such as an increase in fecundity over a shorter time frame

(Docherty et al. 1997), it may come at a cost and eventually be hampered by predicted increases in global temperatures (Newman 2003; 2004).

Climate changes associated with increasing atmospheric CO_2 are known to directly affect insect population dynamics through the effects on fecundity, larval development rates, and adult survivability (Messenger 1959; Andrewatha & Birch 1984). Although changes in many climatic factors such as humidity, rainfall, solar radiation intensity, and ultraviolet light levels are involved in altering conditions beyond species tolerance (Hunter 2001; Newman 2005; Stireman et al. 2005; Asshoff & Hättenschwiler 2006), most studies essentially communicate the effect of changes in ambient temperature as the major driver of changes in insect physiology, behaviour and population dynamics (Robinet & Roques 2010). While thermal plasticity plays a role in insects being able to adjust to ambient climatic conditions, physiological responses are very sensitive to ambient temperature changes (Beck 1983), making them quick to react to increases in temperature (Logan et al. 2003). Slight increases in ambient temperature can result in a rapid change in metabolic rate (Gillooly et al. 2001) and increases in growth rate, but a decline in body size (Atkinsons 1994). The benefits of global increases in temperature on insects will be complex and may be species-specific or even population-specific (Musolin et al. 2010).

Increases in global temperatures may positively affect insect population developmental rates; however, benefits will only be possible if thermal threshold limits are not met (Robinet & Roques 2010). For example, warmer mean temperatures allowed the spruce beetle *Dendroctonus rufipennis* Kirby (Coleoptera: Scolytidae) to double its developmental rate, requiring half the time to reproduce, which has contributed to the unprecedented damage spruce forests in the US (Berg et al. 2006). Multivoltine species are expected to experience significant benefits from reproductive cycle increases, adding one or more generations per year (Virtanen & Neuvonen 1999; Ayres & Lombardero 2000; Kiritani 2006; Jönsson et al. 2009). For example, the fall webworm *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) has experienced a shift in the number of generations produced per year as a result of climate change, shifting from a bivoltine to a multivoltine life cycle (Gomi et al. 2007).

Increases in ambient temperatures will also have ramifications on insects surviving winters and range expansion due to changes in minimal temperature threshold limits (Robinet & Roques 2010). The best and most studied example of this is the pine processionary moth *Thaumetopuea pityocampa* Den & Schiff (Lepidoptera: Notodontidae), which has expanded its range to higher latitudes and altitudes from its original distribution in the Mediterranean (Battisti et al. 2005). The moth's larval development is restricted by minimum lethal temperatures of -16°C and temperature thresholds that allow for night-time feedings, such as day temperatures higher than 9°C and nest night temperatures higher than 0°C (Battisti et al. 2005). Climatic models of the mid-1990s reported that the area south of the Paris Basin, France, was a climatic barrier to larval development that restricted the expansion of *T. pityocampa* northwards (Robinet & Roques 2010). Due to climatic changes in day and night-time temperatures, this region no longer acts as a climatic barrier and the moth's expansion, which has crossed this area, has increased by 5.6 km per year (Robertina et al. 2007).

1.3 Plant invasions and control

Invasive alien plant species, exotic species that are the main drivers of biodiversity loss, are an increasing threat to biodiversity worldwide (Walther et al. 2009; Weber 2017; CBD 2022). The potential and consequences of such invasions are likely to be further exacerbated by anthropogenically driven climate change through increases in atmospheric CO_2 (Ziska & George 2004; Hellmann et al. 2008). Rapidly rising atmospheric CO_2 and other driving factors such as nitrogen availability and novel landscape formation can enhance alien plant species invasive potential within exotic ranges (Bradley et al. 2010; Gonzalez et al. 2010). Regional alterations to climate are expected to alter biodiversity and ecosystem structure, potentially exposing historically isolated regions to invasion by alien invasive plant species (Walther et al. 2002; Hellmann et al. 2008). This necessitates a full understanding of how invasive plants will respond to such changes effectively to manage them in the future.

The discussion of invasive species was proposed during the mid 1800's by Charles Darwin (cited text corresponds to Darwin 1996) and more formally in the late 1950s by Elton (1958), with the interest in biological invasions has grown along with the magnitude and the extent of these invasions (Richardson 2007). The main points debated range from what constitutes an invasive plant and how to effectively predict invasive traits to the biogeographic distributions and possible impacts that are associated with invasions (Roy 1990; Alpert et al. 2000; Richardson et al. 2000; Daehler et al. 2003). In the case of alien plants, the factors constituting the description of invasive potential are those that show patterns of growth that allow for rapid colonisation and spread within a disturbed region and cause negative impacts on ecosystem function and possibly on human welfare (Dukes & Mooney 2004; Le Maitre 2011). There is a general rule of thumb that for an exotic plants to be a successful invader, it must be a better coloniser than native species, allowing more successful development in disturbed regions; show more successful reproduction, with short juvenile periods and short seed crop development (Rejmánek & Richardson 1996); and be a superior survivor, to better compete in a range of climates and habitats (Rejmánek & Richardson 1996; Sutherland 2004).

Alien or exotic plants, introduced either intentionally or accidentally through human activity, occur worldwide. These species range from economically essential crops such as maize and wheat to garden variety plant species. In the cases where alien or exotic species become damaging to the local environment, impacting human health and well-being or negatively affecting economic productivity, they are termed invasive plants (Lodge et al. 2006; Richardson et al. 2000). Whether accidental or intentional, the aggressive establishment of alien invasive species causes negative impacts on the invaded habitat and the local economy and human health (Shackleton et al. 2007; Vaz et al. 2017). When an alien plant species dominate an ecosystem, it will potentially influence the performance of native species and their population dynamics (Vilà & Weiner 2004). Consequently, impacts of invasive species can disturb regimes beyond the range of variation to which native species are adapted, resulting in community changes and ecosystem shifts (Mack & D'Antonio 1998). For example, in Chehalis River, Washington, USA, the invasive aquatic plant, *Myriophyllum aquaticum* (Haloragaceae),

when forming the characteristic dense mats, significantly reduces dissolved oxygen content of sites dominated by the plant and alters invertebrate and fish assemblages to those more tolerant of degraded habitats (Kuehne et al. 2016). This has negative cascading effects, impacting whole ecosystem function (Brooks & Esque 2002).

1.3.1 The enemy release hypothesis and biological control

The invasiveness of an exotic species in a novel environmental range is commonly attributed to the mechanism proposed by the enemy release hypothesis, which has also been referred to as the ecological release hypothesis (Blossey 2011; McCallum & McCullum 2014). The enemy release hypothesis states that a plant species introduced to a novel or exotic range will experience an ecological release from strong top-down regulation from co-evolved herbivores and other natural enemies, increasing distribution, abundance and competitiveness of the exotic species (Williamson & Griffiths 1996; Keane & Crawley 2002; Mitchell & Power 2003). The key assumptions of the enemy release hypothesis are that natural enemies are an important top-down regulator of plant species populations and that the pressures from these natural enemies are more impactful on the native species associated with them than compared to the now “released” exotic species (Keane & Crawley 2002). In the absence of such associated top-down pressures, the exotic species can more readily redirect resources towards improving competitiveness (Keane & Crawley, 2002).

Biological control uses the mechanism outlined by the enemy release hypothesis and the concept that very few invasive species are ever invasive within their native range, with much of the invasive potential controlled by a series of co-evolved herbivores (McFadyen 1998). The practice of biological control is fundamentally the deliberate introduction of the natural enemy of a specific invasive plant species, be it an invertebrate such as insects, or pathogen, to reduce the effects of 'ecological release' and to bring about the control of these plants to the point of non-invasive status (Zachariades et al. 2017). The biological control agent works by inhibiting plant production through

above and below-ground tissue damage, reducing seed loads by damaging reproductive tissue, affecting resource allocation by placing pressure on plant defences or impacting whole-plant photosynthetic activity (Coetzee et al. 2007). The selection of a biological control agent involves rigorous host specificity testing, and as a result, biological control agents are highly specialised (Zachariades et al. 2017). As such, the method of biological control is deemed one of the most effective, sustainable and long-term methods of controlling invasive alien plants, with minimal to no risk of damaging non-target species (Reeves 2017; Zachariades et al. 2017). Biological control is also environmentally safe and cost-effective (van Wilgen et al. 2004; Arp et al. 2017). The strict practice of host specificity testing has resulted in very few examples of shifts in agent specificity, and in these cases, researchers were aware of the potential shift but deemed it necessary to control the invasive plant (van Wilgen et al. 2004). Biological control of invasive plant species has been used in 130 countries with great success, with over 550 biological control agents having been released to control invasions (Winston et al. 2014; Schwarzländer et al. 2018).

1.3.2 Successes and challenges to biological control in South Africa

In a South African context, there has been significant success in the control of invasive plants (Hill & Coetzee 2017; Zachariades et al. 2017), specifically noxious aquatic plants, through the use of biological control. First used in 1913 to control invasive *Opuntia monocantha* Haw. (Cactaceae) using the cochineal biological control agent, *Dactylopius ceylonicus* Costa (Hemiptera: Pseudococcidae) (Moran et al. 2013), it has since developed into a prominent method of controlling invasive plants, alongside mechanical and herbicidal control. Since the first release of the cochineal insect, over 103 agents have been used as biological control agents for over 47 invasive plant species, making South Africa one of the top five most active users of biological control practices (Cock et al. 2010; Moran & Hoffmann 2015).

South Africa's water scare status has made the management of invasive aquatic plants paramount in securing this resource for future generations. The alterations of South Africa's water

bodies through modified hydrological flow and eutrophication have created aquatic systems prone to invasion by exotic aquatic plants, and biological control has become a leading tool in managing these systems (Hill & Coetzee 2017; Coetzee et al. 2021). Of the 'Big Bad Five' aquatic invasive plants in South Africa, which include *P. crassipes, Pistia stratiotes* L. (Araceae) (water lettuce), *Salvinia molesta* D.S. Mitch. (Salviniaceae) (giant salvinia), *Myriophyllum aquaticum* (Vell. Conc.) Verd. (Haloragaceae) (parrot's feather), and *Azolla filiculoides* Lam. (Azollaceae) (red water fern) (Henderson & Cilliers 2002; Hill 2003); only *P. crassipes* is considered not under complete control; however, the introduction of nine biological control agents has significantly reduced the socio-economic impact of the plant in South Africa (Klein 2011; Coetzee et al. 2011; Paterson et al. 2016). In South African aquatic systems, one of the great biological control success stories is the introduction of the biological control agent *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) for the control of *A. filiculoides* in 1998. *Stenopelmus rufinasus* was so successful in South Africa that it effectively eradicated *A. filiculoides* at historically invaded sites in just several months after release, bringing *A. filiculoides* under complete control in South Africa in just a few years (Hill & McConnachie 2009; McConnachie et al. 2003; McConnachie et al. 2004).

Not all agents are as successful in controlling their host plant, as is the case in *P. crassipes* biological control. Four factors contribute to the difficulties of sustainable biological control of *P. crassipes* which were outlined 20 years ago by Hill & Olckers (2001) and can still be applied to current programmes (Hill & Coetzee 2017). These were the overuse of harmful herbicides, which has deleterious effects on agent populations, eutrophic water conditions found throughout South Africa's waterways which allowed for compensatory growth of the invasive plant, the variable climate throughout South Africa and the cold winters associated with the more temperate regions; and wind fetch. Much of the biological control research on persistent invasive aquatic plants like *P. crassipes* has been aimed at understanding these factors' influence on biological control efficacy in aquatic systems, resulting in more effective control under variable climates and alteration of release methods to overcome the ever-present lag-phase (Hill & Coetzee et al. 2017). The lag-phase in biological control

refers to the period in which warmer spring or early summer temperatures allow for rapid regrowth of invasive plant species, which creates a window of time in which biological control is left ineffective due to an inadequate number of agents to combat regrowth effectively; this pattern is seen predominately in *P. crassipes* biological control efforts (Hill & Cilliers 1999; Miller et al. 2021). Whilst many successes have been achieved through biological control, improved implementation of research and integration of biological control into other control methods are needed to truly showcase the sustainability and long-term success of the biological control programme (Zachariades et al. 2017).

1.3.3 The impact of the rapidly changing world on the efficacy of biological control

Invasive aquatic plants in South Africa are considered to be "back-seat" invaders, requiring a disturbance to establish (e.g. freshwater artificial impoundments and eutrophication), but once established, the plant continues to be invasive even after the disturbance has been removed (Hill & Coetzee 2017). The invasive potential of alien aquatic plants is positively affected by the presence of nitrate- and phosphate-rich inputs into freshwater systems due to urban, industrial and agricultural infrastructure (Coetzee & Hill 2012). In South Africa, freshwater eutrophication is well above permitted international standards and is one of the key factors affecting aquatic plants' invasive potential (Coetzee & Hill 2012; Matthews & Bernard 2015). The biological control effort in South Africa is greatly affected by the eutrophication of its water systems, and the efficacy of some of the programme's most successful agents, such as *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae), *N. eichhornia* (Coleoptera: Brachyceridae) and *N. bruchi* (Coleoptera: Brachyceridae) are negatively impacted by eutrophic water (Centre & Dray 2010; Coetzee et al. 2012) as highlighted by the findings of laboratory studies (Heard & Winterton 2000; Coetzee et al. 2007).

Predicted eCO_2 conditions and climate change, specifically rising ambient temperatures, in conjunction with eutrophic conditions, indicate that invasive alien plants will benefit from these changes but could hamper the associated biological control programme. Liu et al. (2010) showed that production of *P. crassipes* will increase significantly at eCO_2 conditions predicted for the next 100 years

and in water nutrient conditions equal to that of South African waterways (Oberholster & Ashton 2008). The invasive potential of *P. crassipes* will also be enhanced by rising ambient temperatures, extending the invasive range through increased accessibility to previously climatically incompatible regions in South Africa (Hoveka et al. 2016) and the rest of the world (Kriticos & Brunel 2016). Many plant species will respond differently to changes in eCO_2 and rising ambient temperature conditions, and predictions of the interactions of these factors are sparse (Baso et al. 2021).

It is also difficult to predict the future success of insect biological control agent responses to eCO_2 and temperature without adequate testing as they vary from species to species (Reeves et al. 2017). In some cases, growth responses of invasive plants may outstrip that of biocontrol population dynamics and feeding under predicted conditions, whilst in others, biocontrol may experience significant stimulation that outpaces the growth of the plant (Reeves et al. 2017; Baso et al. 2021).

1.4 Study system – *Pontederia crassipes* in South Africa

Water resource management is one of the most important political, social and economic issues facing governing bodies and development planners across the world, especially in water scarce countries such as South Africa (Walter et al. 2011; Falkenmark 2013; Donnenfeld, Crookes & Hedden 2018). A significant threat to water security is alien invasive plants, affecting water quantity and quality (Görgens & van Wilgen 2004; Chamier et al. 2012). The success of South Africa's biological control initiative allowed for the diversification of the programme in 1973 into the biological control of aquatic plants, first initiated against the country's most troublesome aquatic plant, *P. crassipes* (Cilliers 1991). The efficacy of these programmes has been greatly hindered by cold winter temperatures and the high nutrient levels (eutrophication) of South Africa's river systems, which allows for the explosive growth of the plant in the summer months (Hill & Cilliers 1999; Hill & Coetzee 2017). Eutrophic water is caused predominantly by raw sewage, factory effluent and agricultural run-off. One of the courses of action recommended by Hill & Olckers (2001) was to release the correct 'suite' of biological control agents for *P. crassipes* to control the plant (Oberholzer & Hill 2001). At present, nine biological control

agents have been released to control *P. crassipes* with ranging success (Hill & Coetzee 2017; Coetzee et al. 2021), emphasising the difficulty in controlling this aquatic plant and requiring further research.

1.4.1 The invasive plant, *Pontederia crassipes*

Pontederia crassipes has been recognised as a noxious aquatic plant in approximately 55 countries across five of the six habitable continents and is even considered invasive in parts of its native range (Parsons et al. 2001; Lowe et al. 2000; Jafari 2010). The invasive aquatic plant has a native range that extends across most of western and central South America (Lowe et al. 2000), including swathes of the Amazon Basin. The predominant mode of introduction of the plant is through the global ornamental trade network, with the majority of introductions occurring in botanical gardens and private homes (Martin & Coetzee 2011). The species was first introduced to South Africa in the early 1900s as an ornamental plant, after which *P. crassipes* became South Africa's most troublesome aquatic weed (Cilliers 1991).

Pontederia crassipes is a free-floating or rooted, herbaceous, perennial aquatic plant that ranges in size from 1 cm to 1 m in height (Penfound & Earle 1948). The waxy green leaves consist of a swollen petiole (2-5 cm thick) and an approximately 15 cm wide leaf blade that is roughly round, ovoid or kidney-shaped (Penfound & Earle 1948).

Figure 1.2 A flowering *Pontederia crassipes* plant. Image credited to the Centre for Biological Control, Water Weeds Program.

The submerged roots develop at the base of each leaf and form a dense feathery mat, usually 20-60 cm long, although they can extend up to 300 cm long in nutrient-rich conditions (Julien 2008). *Pontederia crassipes* reproduces both sexually and asexually. Sexual reproduction produces a spike-like inflorescence that develops 8-15 flowers (Penfound & Earle 1948). Each flower consists of 6 mauve-purple lobes, with the central lobe having a bright yellow, diamond-shaped pattern surrounded by a deeper purple (Mulcahy 1975) (Figure 1.2). Seeding bodies can produce up to 450 seeds, which may remain viable in the seed bank for more than 15 years (Parsons et al. 2001). Asexual reproduction frequently occurs in *P. crassipes* and involves the development of a 10-50 cm stolon or horizontal stem, which can establish multiple ramets or daughter plants (Penfound & Earle 1948; Mulcahy 1975). *Pontederia crassipes* can rapidly expand its population and can double its biomass under favourable conditions every 6-14 days, primarily through vegetative growth (Lodge & Keller 2009).

Pontederia crassipes has been shown to reduce dissolved oxygen content and light penetration beneath the dense mats, increase sedimentation rates and alter pH levels within the water column

(see review Villamagna & Murphy 2010). In the study by Toft et al. (2003), regions with *P. crassipes* were associated with an average dissolved oxygen content of less than 5 mg L^{-1}, significantly lower than regions with native flora, while the study by Masifwa, et al. (2001) found an inverse relationship between dissolved oxygen beneath *P. crassipes* mats and the distance to open water on Lake Victoria, Uganda. Within freshwater systems across the globe, *P. crassipes* causes extensive ecological and socio-economic impacts (Villamagna & Murphy 2010). Coetzee et al. (2014) demonstrated the negative impact of dense mats of *P. crassipes*, where benthic macroinvertebrate diversity was significantly reduced in water systems within the Enseleni Nature Reserve KwaZulu-Natal Province, South Africa. In comparison, Gezie et al. (2018) documented potential negative impacts on water quality and human health that *P. crassipes* has on communities of the Lake Tana watershed, Northwest Ethiopia.

1.4.2 Biological control of *Pontederia crassipes*

The past difficulty in controlling the plant in South Africa has led researchers to develop an extensive array of biological control agents, accumulating a suite of nine agents (Hill & Cilliers 1999; Coetzee et al. 2011). The suite of biological control agents includes: the two weevil species, *N. eichhorniae* and *N. bruchi*; a moth, *Niphograpta albiguttalis* Warren (Lepidoptera: Crambidae); a mite, *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae); a leaf sucking mirid, *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) and the newly discovered cryptic species *Eccritotarsus eichhorniae* Henry (Hemiptera: Miridae) (Henry 2017; Paterson et al. 2019); a pathogenic fungus, *Cercospora piaropi* Tharp. (Mycosphaerellales: Mycosphaerellaceae) (Hill & Cilliers 1999); a grasshopper, *Cornops aquaticum* (Brüner) (Orthoptera: Acrididae), and a true bug *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) (Coetzee et al. 2011). The extensive biological control efforts on *P. crassipes* have allowed for a notable reduction in the ecological and socio-economic impacts of the plant, and in some cases, *P. crassipes* has been brought under complete control solely through biological control, while

in other areas, alternative methods of control such as herbicide application are required less frequently (Hill & Cilliers 1999; Coetzee et al. 2011).

Arp et al. (2017) investigated the cost to benefit ratio of the control of *P. crassipes* on the Vaalharts Irrigation Scheme and showed that based on an Et:Ew (transpiration rates of *P. crassipes* vs evaporation rates of the water body) of 1.38 at only 25% *P. crassipes* coverage, resulted in a 54 million (South African Rand) annual net opportunity cost of water loss (R/m^3) to the country (Arp et al. 2017). This was based on a conservative evapotranspiration rate and percentage coverage (Arp et al. 2017). The annual net opportunity cost was shown to be well above the appraised value of an integrated control programme, which included direct costs (annual surveys, herbicides and travel) and indirect costs (developing control agents), which was valued at R1.6 million (Arp et al. 2017). The above example shows the necessity for invasive species control programmes in South Africa.

Yet even with this wide array of biological control agents, control of the plant is inconsistent across the country. The factors that govern successful control of the majority of aquatic invasive plants ring especially true for *P. crassipes*, namely, eutrophic water conditions and variable climate throughout South Africa and herbicide application (Hill & Olckers 2001). These factors have led researchers to release additional agents that are better suited to the diverse array of conditions in South Africa (such as *M. scutellaris* in 2014), and has emphasised variation in release strategies outside of classical and augmentative control (Hill & Coetzee et al. 2017). The two most recently released agents, *C. aquaticum* and *M. scutellaris*, have the potential to be the most effective agents, given the extensive damage they inflict on *P. crassipes*.

1.5 Study organisms

1.5.1 *Cornops aquaticum*

The neotropical semi-aquatic grasshopper *Cornops aquaticum* Brüner (Orthoptera: Acrididae: Tetrataeniini) is native to South America, ranging from southern Mexico to Central Argentina and Uruguay (Adis et al. 2007). *Cornops aquaticum* adults are typically about 2.4–3.4 cm in length (from

head to wing tip), with the adult males being on average smaller than females (Adis et al. 2008). Adults are bright green with a distinct, broad black stripe on either side, running from the eye to the tip of the wing (Figure 1.3a), while the nymphs range from 0.6 to 3 cm and are mottled in green-blue and orange-red (Figure 1.3b) (Ferreira & Vasconcellos-Neto 2001*)*.

Cornops aquaticum feeds and oviposits on floating aquatic plants within the family Pontederiaceae, predominantly on *P. crassipes* (Ferreira & Vasconcellos-Neto 2001). Females insert eggs in an egg packet into the young petioles of *P. crassipes* (Oberholzer & Hill 2001). Once in place, the oviposition hole is covered with a protective plug made up of a foamy substance (Oberholzer & Hill 2001). A female produces up to 30-70 eggs per packet and seven packets in her lifetime (Oberholzer & Hill 2000). The eggs hatch within 25-30 days, and the nymphs begin feeding on the leaf surface, immediately scarifying the leaves (Oberholzer & Hill 2001). The nymphal period lasts approximately 50 days (Oberholzer & Hill 2001).

The insect was first considered as an additional biological control agent against *P. crassipes* in South Africa in the mid-1990s, due to the extent of its feeding damage documented in field reports from its native range and the potential climatic suitability of the agent to South African climates (Perkins 1974; Adis et al. 2007). The initial culture of *C. aquaticum* was collected on a survey of Isla Terra Nova on the Amazon River in 1995 (Coetzee et al*.* 2011), with subsequent cultures collected from *P. crassipes* in Brazil in 1995, and later from Trinidad and Venezuela in 1996, and Mexico in 1997 (Oberholzer & Hill 2001). Host specificity testing by Oberholzer & Hill (2001) reported that *C. aquaticum* was oligophagous within the family Pontederiaceae, but it preferentially selected *P. crassipes* over other native species. *Cornops aquaticum* remained in quarantine for 15 years before its release in 2011. Prior to release, extensive studies on the agent's efficacy in damaging *P. crassipes* under South African conditions were performed, showing the insects' ability to damage *P. crassipes* even under high nutrient conditions, and populations of *C. aquaticum* may benefit from eutrophic water conditions experienced in South Africa (Bownes et al. 2010; Bownes et al. 2011; Bownes et al. 2013a). Bownes et al. (2013a) found bottom-up regulation of nutrients on *C. aquaticum* populations,

showing increasing fecundity and shifting of sex ratios towards more female-biased under high nutrient levels.

Cornops aquaticum was first released in 2011 in three provinces in South Africa, representing variable nutrient and climatic conditions that were thought to match those of the native range of the insect: Gauteng, KwaZulu-Natal and Mpumalanga (Coetzee et al. 2011). To date, *C. aquaticum* has failed to established at any sites in South Africa (Coetzee et al. 2021). The lack of establishment in invaded sites of *P. crassipes* across the country suggests a narrow thermal tolerance (Coetzee et al. 2021), with a preference for tropical conditions such as those from the host range. Venturi (2020) reported a critical thermal minimum, the point at which an insect loses locomotory function, of 6.15 ± 0.12 ºC, which is substantially higher than other *P. crassipes* biological control agents established in South Africa. It has been suggested that the extended period in quarantine, from 1995 to 2011 and the resulting ±80 generations of *C. aquaticum* may have resulted in a severe genetic bottleneck in the population, potentially causing the low-temperature sensitivity (Adis et al. 2008).

Figure 1.3: Adult *Cornops aquaticum* resting on a damaged *Pontederia crassipes* leaf, image sourced from CBC archives (a). An early instar of *Cornops aquaticum*, image credited to César Favacha.

1.5.2 *Megamelus scutellaris*

The newest agent to be released in South Africa, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), was released in 2013 in South Africa to control *P. crassipes* in the cool Highveld regions

of the country as it was potentially more cold-tolerant (Tipping et al. 2008; Hill & Coetzee 2017). The insect is native to South America, found throughout Brazil, Argentina, Peru and Uruguay (Sosa et al. 2007). During initial field surveys for testing host range, *M. scutellaris* was found to only occur on *P. crassipes* (Sosa et al. 2004), and subsequent host specificity testing confirmed it to be host-specific to *P. crassipes* (Tipping et al. 2011). The plant-hopper was released in South Africa in 2013 (Hill & Coetzee 2017) and in Florida in 2014 (Tipping et al. 2014).

Megamelus scutellaris is a multivoltine species that produces multiple, overlapping generations per year (Tipping et al. 2011). *Megamelus scutellaris* is light brown and cream with symmetrical mottled patterning throughout their life cycle, with the colouration darkening as the insect matures (Sosa et al. 2005) (Figure 1.4). Adults exhibit a wing dimorphism with a long-winged dispersal macropterous and a short-winged, non-flying brachypterous form (Fitzgerald & Tipping 2013). Adults mate near the base of the petiole, just above the water surface and the upper parts of the lamina (Sosa et al. 2005; Tipping et al. 2011). Females oviposit in the upper portion of the petiole and the lamina of *P. crassipes* tissue, causing minute vertical scars at the point of oviposition (Tipping et al. 2011). Eggs are small and milky white when laid and yellowish-white, forming darkened eye spots before hatching (Sosa et al. 2005). Nymphal emergence is temperature-dependent but generally emerge after 7 to 15 days (Sosa et al. 2005). Nymphs develop through five instars and feed on the surfaces of the lamina and petioles, feeding directly and in large part from the phloem and to a lesser extent the xylem (Hernández et al. 2011). Although the thermal range of *M. scutellaris* indicated that it was not climatically suitable to cold, higher elevation regions of South Africa, with a lower thermal threshold of 11.4°C (May & Coetzee et al. 2013), it has overwintered for a number of seasons in the coldest known invaded site of *P. crassipes*, the Kubusi River, Eastern Cape Province, South Africa (Miller et al. 2021). Furthermore, it has survived in the warmest known biological control site in the country on the Nseleni River, KwaZulu Natal Province (Coetzee et al. 2021).

Figure 1.4: Two brachypterous adult *Megamelus scutellaris* on a *Pontederia crassipes* leaf. Image credited to Julie Coetzee.

Whilst *M. scutellaris* feeding is not apparent, it directly inhibits photosynthesis and nutrient flow within the plant (Zvereva et al. 2010), and observing this damage before plant senescence is difficult unless through photosynthesis measurement proxies (Zvereva et al. 2010; Miller et al. 2019). Nevertheless, both *C. aquaticum* and *M. scutellaris* effectively reduce plant biomass and increase plant mortality of the plant (Bownes et al. 2010; Zvereva et al. 2010; Tipping et al. 2011). One of the critical factors affecting the control of *P. crassipes* in South Africa is the notably high nutrient levels in invaded water bodies (Coetzee et al. 2011). Both *C. aquaticum* and *M. scutellaris* damage are density-dependent, and if significant population densities are reached, the damage dealt to *P. crassipes* through herbivory may be significant (Bownes et al. 2013a; Bownes et al. 2013b; Coetzee et al. 2021). The ability to sustain high levels of damage in eutrophic systems coupled with the potential synergy between the various chewing and phloem-feeding agents (Coetzee et al. 2021), combined with both *C. aquaticum* and *M. scutellaris* strong dispersal strategies, suggest that they may be crucial in combating *P. crassipes* infestations in South Africa under current CO_2 concentrations. However, the impact that future eCO_2 concentrations predicted by the IPCC (2014) will have on the efficacy of

biological control agents, representing two distinct feeding guilds, is poorly understood and will have implications on future biological control strategies and programs

1.6 Aims of the study

Invasive alien plants are an increasing threat to biodiversity and ecosystem function worldwide, and the invasive potential and consequences of such invasions are likely to be further exacerbated by increases in anthropogenically derived atmospheric CO_2 (Ziska & George 2004; Hellmann et al. 2008). *Pontederia crassipes* is an ideal study species to test the impact of eCO_2 on invasive potential due to its extensive invasion on a global scale. The invasive potential of *P. crassipes* is expected to increase with climate change and rises in atmospheric CO_2, particularly under the high nutrient conditions typical of South African waterways (Spencer & Bowes 1986; Liu et al. 2010). Provided other resources essential to photosynthesis are not limiting, eCO_2 should lead to stimulation in plant performance, a response that is undesirable in plants that are already successful invaders. This potential increase in invasive potential necessitates a firm understanding of how *P. crassipes* and other invasive plant species will respond to such shifts in fundamental resources to guide effective management strategies in future climates, particularly those involving the use of biological control.

Studies have shown mixed results in the response of *P. crassipes* to eCO_2, with some showing *P. crassipes* responding positively to eCO_2 when nutrients are abundant (Liu et al. 2010), and acclimation to high CO_2 concentrations in others (Spencer & Bowes 1986), leaving uncertainty in the prediction of how this noxious plant may perform in the future. Furthermore, few studies to date (Diaz et al. 2012; Shabbir et al. 2019; Baso et al. 2021; for further examples, see review by Sun et al. 2020), and none on *P. crassipes*, have examined the effects of CO_2 changes on insect biological control agents and host plant interactions (Sun et al. 2020). This study provides a novel examination of a highly invasive aquatic plant and its biological control agents, representing distinct feeding guilds, under future CO_2 simulations.

This study aimed to assess how eCO_2 conditions and insect herbivory by two distinct insect feeding guilds would impact *P. crassipes* invasive potential under changing atmospheric CO_2 conditions. To enlightened control of *P. crassipes* under eCO_2 requires answers to four main questions: (1) Will the direct effects of eCO_2 on *P. crassipes* accentuate the plant's invasive potential in South African waterways? (2) Will insect herbivory by *C. aquaticum* and *M. scutellaris* alter or moderate the effects of eCO_2 on *P. crassipes*? (3) Do the effects of eCO_2 on the plant have knock-on effects on insect feeding or demographics? (4) Which of the studied biocontrol agents (representative of feeding guild) will most successfully control *P. crassipes* under eCO_2? To help answer these questions, the photosynthetic rates and growth of *P. crassipes* plants exposed to either ambient or eCO_2 (800 ppm) conditions were examined to determine the level of eCO_2 stimulation we might expect to see in an aquatic weed such as *P. crassipes* under the RCP 8.5 projection scenario. To determine how direct plant responses will be influenced by insect herbivory and the efficacy of biological control under future CO_2 conditions, a subset of *P. crassipes* plants were exposed to biological control agent herbivory by either (1) *C. aquaticum*, a chewing, semi-aquatic grasshopper or (2) *M. scutellaris*, a phloem-feeding planthopper. Particular focus is given to the differences between insect feeding guild responses in order to guide the selection of biological control agents in the future.

Chapter 2: Methods and Materials

2.1 Rhodes University elevated CO_2 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 facility consists of 16 decagonal open-top chambers (OTCs); each is 3m in diameter and 2.8m tall (Figure 2.1). Each OTC is covered with F-Clean polyethene sheeting that allows for 94% of solar radiation at visible and near-visible wavelengths to enter. The OTCs are ventilated with a 3-phase fan; airflow is distributed evenly via a 34cm diameter diffuser running the entire circumference of the chamber, positioned 1m above the ground, perforated with 300 one cm holes. The airflow speed entering the diffusers is regulated via a fan speed controlled by Senlan SB150 variable speed drives that respond to variations in temperature and CO_2 concentration. Temperatures are monitored via CS215 temperature/humidity sensors (Campbell Scientific), and data are processed via a CR6 data-logger (Campbell Scientific) and Logger-net software (Campbell Scientific). Data-logger and logger-net software use proportional-integral-derivative (PID) procedures to control fans speeds according to the temperature differentials. CO_2 concentrations within the OTCs are measured by open-path CO_2 analysers (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 (Burkert, Germany). Additional sensors monitor solar energy (ES2 sensor, Delta-T Devices, Ltd.), wind speed (Delta-T Devices, Ltd.), rainfall (RG2 rain gauge, Delta-T Devices, Ltd.).

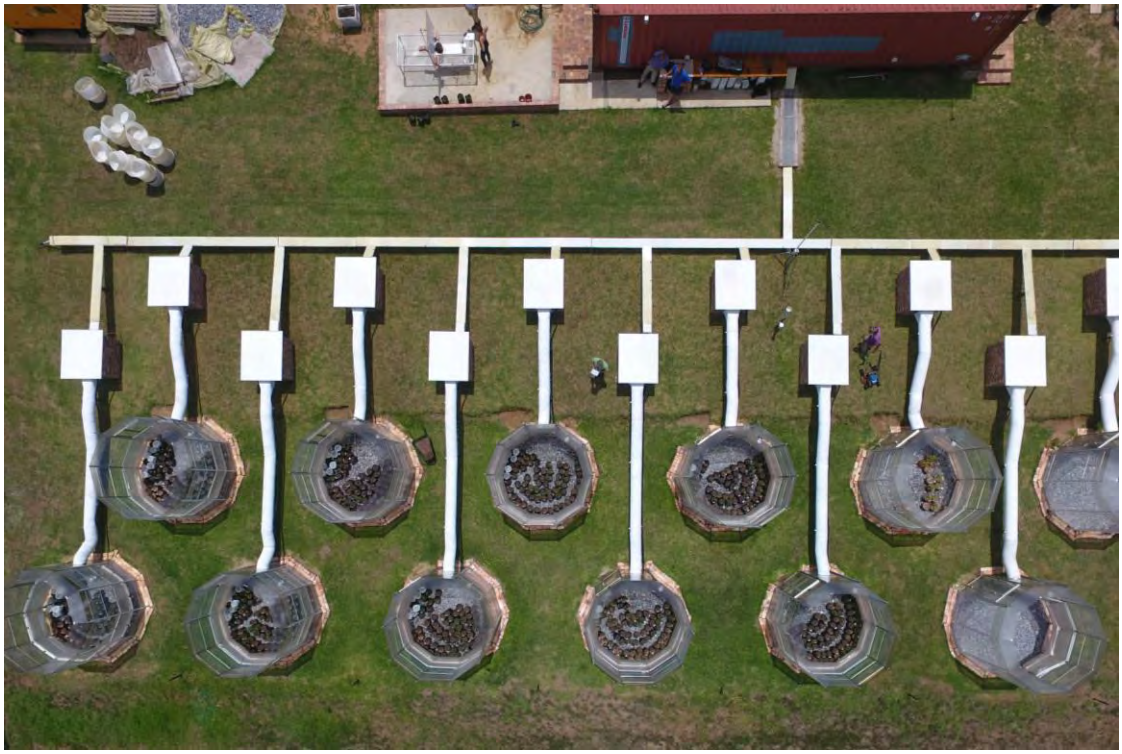


Figure 2.1: Ariel view of the Rhodes University Elevated CO_2 Facility. The facility consists of 16 decagonal open-top chambers (OTCs); each is 3m in diameter and 2.8m tall. Image credited to RUECF.

2.2 Experimental design

The effect of eCO_2 on herbivory to *P. crassipes* was measured by exposing test plants grown at two atmospheric CO_2 concentrations to herbivory by either *C. aquaticum* or *M. scutellaris.* Test plants were collected from the Centre for Biological Control (CBC) Mass Rearing Facility at Rhodes University, Makhanda, South Africa in December 2018 (mid-summer), and the experiment was conducted at the Rhodes University Elevated CO_2 Facility. Four OTCs were used for this experiment; two were set at an atmospheric CO_2 concentration of 400 ppm (ambient) and two at an atmospheric CO_2 concentration of 800 ppm (elevated). One hundred and ninety-two mature *P. crassipes* plants were grown in 20L cylindrical mesocosms (30cm diam x 33cm) in sets of 4. These plants were grown in the chambers for two months from the 15th December 2018 to 19th February 2019; at a nutrient concentration of 10 mg N/L, using the nutrient medium Culterra Multisol® 6.1.3. (44) Foliage and 1.1 mg Fe/L (13% Fe Chelate)

to replicate nutrient conditions found in South African waterways (Villiers & Thiart 2007). Nutrients were added every 14 days. Due to the prolonged drought experienced in the greater Makana region, the nutrient media were not replaced, but rather water levels were topped with local municipal water, and nutrients added. Nutrient concentrations were monitored during the experimental period to maintain them at a constant level.

The feeding impact study was conducted over 13 weeks from 19th February to 22nd May 2019. Before starting the feeding study, all dead material and daughter plants were removed from each mesocosm, leaving the four mature plants of approximately equal weight and size per mesocosm. The total wet weight of all plants was measured using an electronic balance with a maximum capacity of 20 kg. Mesocosms within each chamber were divided into three groups of six containers; an insect-free control, a *C. aquaticum* herbivory treatment, and an *M. scutellaris* herbivory treatment. Mesh sleeves covered all the mesocosms to prevent insect escapees and prevent any unwanted herbivore damage to the controls. Six first instar nymphs of *C. aquaticum* and 60 first instar nymphs of *M. scutellaris* were introduced to their respective treatment mesocosms to represent high field densities of biological control agents. Due to the difficulty of identifying sex in nymphs of both species, nymphs were randomly selected, assuming a 1:1 sex ratio. In total, 72 mesocosms were used for this experiment.

The *C. aquaticum* nymphs used in this experiment were reared from adult *C. aquaticum* provided by the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KZN (29°42'22.3"S 31°02'43.1"E) and were reared at the CBC's Waainek Mass Rearing Facility. The *M. scutellaris* nymphs used in the experiment were collected from the CBC Mass Rearing Facility, from the rearing population for the CBC's *P. crassipes* biological control programme.

2.3 Data collection

2.3.1 Plant characteristics

In order to determine the combined effect of CO_2 and herbivory on *P. crassipes* the invasive potential of *P. crassipes*, the growth parameters and plant assimilation rates were recorded every 14 days, while various plant growth parameters and destructive measurements were recorded at the beginning and at the end of the experiment. Leaf gas exchange, referring to plant carbon assimilation rates, intercellular CO_2 and leaf stomatal conductance, was measured using a portable photosynthesis system with a blue-red LED light source (LI-6400; Li-Cor, Inc., Lincoln, NE, USA) on the adaxial surface of the 2nd leaf of a representative plant, *viz*. a single plant selected and remeasured from each mesocosm; the second leaf refers to the second most recent leaf to develop from the whorl of the plant. Carbon assimilation rate (µmol CO_2. m^{-2}. s^{-1}), stomatal conductance (mol H_2O. m^{-2}. s^{-1}) and intercellular [CO_2] (Ci) (µmol CO_2) from the same plants from all treatments were measured using the same photosynthesis system on the 3rd, 6th, 10th and 13th week. The stomatal limitation was calculated using the difference between the *P. crassipes* assimilation rate (A) achieved at growth atmospheric CO_2 (Ca), and the growth intercellular CO_2 (Ci) achieved for that Ca (Ripley et al. 2007).

In addition, the number of leaves, flowers and ramets produced as well as the petiole length (cm), leaf length (cm) and breadth (cm) of the 2nd leaf of the representative plants were measured. Plant growth parameters were measured at the beginning of the experiment and every two weeks thereafter for 13 weeks. At the end of the experimental period, all insects were collected and frozen at -5°C for morphometric and gut bacteria analysis and the total wet weight of *P. crassipes* in each mesocosm was re-measured. Plant 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 2nd and 4th 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 isotopic analysis and sent to the UP Stable Isotope Laboratory at the Mammal Research Institute (MRI) at the University of Pretoria for analysis. Isotopic analysis was done using an

elemental analyzer (Flash EA 1112 Series) coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany). All remaining material was placed in a drying oven at 60°C and dried 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:

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

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).

2.3.2 Feeding impact quantification

In order to assess whether insect herbivory moderates the effects of eCO_2 on *P. crassipes*, and to determine the knock-on effects of eCo_2 on feeding impact, the difference 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 for each agent, respectively. The abaxial and adaxial surfaces of the 2nd, 3rd, and 4th leaf of the representative plant from each *C. aquaticum* treatment mesocosm were placed on a 1mm x 1mm laminated piece of graph paper, and photographed every 3 weeks from the 1st week of the study. Imaging software, Image J® (Schneider et al. 2012), was used to quantify the feeding damage of *C. aquaticum* by calculating the area of feeding scars (mm^2) 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*. *Megamelus scutellaris* damage is difficult to

measure unless fluorometry or leaf gas exchange tools are used to analyse assimilation rates (Miller et al. 2019). Thus, leaf gas exchange measurements were taken for all representative plants for all treatments over the experimental period to compare the impact of biological control agents. To further understand the impact of eCO_2 on insect demographic responses, the entire populations of *C. aquaticum* and *M. scutellaris* were collected from each mesocosm and frozen. The population densities for adults and nymphs were recorded, and individuals were placed in 98% ethanol for storage.

2.3.3 Statistical analysis

Statistical analyses were conducted in R studio (R version 3.5.3 (2019-03-11). Normality and homogeneity of data were tested using Shapiro-Wilks and Bartlett Tests. Photosynthetic ACi curves were fitted and analysed using the CRAN *plantecophys* package (Duursma, 2015). Differences in CO_2 assimilation rate of *P. crassipes* between insect and CO_2 treatments were analysed using the differences in V_{cmax} and J_{max}. Variable interactions were analysed using linear mixed-effects models fitted using the R package lme4 (Fitting Linear Mixed-Effects Models, Bates et al. 2015) to determine the relationship between the response variables and the fixed effects of CO_2 and insect herbivory treatments and the potential random effects in the response variables as a result of the OTCs split design. If a random chamber effect was found to be null (error code *isSingular*), an interaction linear regression model (lm in the R base package) was used on the selected variable. 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 2017). In the case of poor diagnostics from the DHARMa package, data were logged or sqrt transformed and re-run accordingly. As count data, *M. scutellaris* adult population density data were analysed using a non-parametric Wilcoxon Signed Ranks Test (R base package). Evaluating the slope and elevation of the relationship between *C. aquaticum* chewing damage or *M. scutellaris* population density on response variables

measured between 400 and 800 ppm was calculated using the package smatr (Standardised Major Axis Estimation and Testing Routines, Warton & Weber 2002).

Chapter 3: Results

3.1 Plant physiological responses to eCO_2 and insect herbivory

Maximum rate of Rubisco carboxylase activity (V_{cmax}) (µmol CO_2 $m^{-2}s^{-1}$) of *P. crassipes* was unaffected by eCO_2 in the absence of insect herbivory (t_7 = 0.828, *P* = 0.44) (Figure 3.1.1A). When exposed to insect herbivory, eCO_2 led to 49% lower V_{cmax} in the presence of *M. scutellaris* (t_6 = -2.07, *P* = 0.08) and a 35% lower V_{cmax} in the presence of *C. aquaticum* (t_8 = -1.73, *P* = 0.12) in comparison to insect herbivory at aCO_2. The effect of insect herbivory on V_{cmax} was only notable at 800 ppm, where V_{cmax} was 30% lower for plants under *C. aquaticum* (*C. a*) herbivory and 48% lower in plants under *M. scutellaris* (*M. s*) herbivory when compared to control treatments at eCO_2 (*C.* a: t_{10} = -1.47, *P* = 0.17; *M. s*: t_{10} = -2.15, *P* = 0.06). There was no significant difference in V_{cmax} between those exposed to *C. aquaticum* and *M. scutellaris*, regardless of CO_2 treatment. Maximum rate of photosynthetic electron transport (J_{max}) of *P. crassipes* was unaffected by eCO_2 in the absence of insect herbivory (t_7 = 1.27, *P* = 0.24) (Figure 3.1.1A). J_{max} decreased at eCO_2 in the presence of both *M. scutellaris* (t_{21} = -2.08, *P* < 0.08) and *C. aquaticum* (t_{21} = -1.76, *P* = 0.09) (Figure 3.1.1B & C). The effect of insect herbivory on J_{max} was only significant in plants grown at 800 ppm, with J_{max} being 37% lower for plants under *C. aquaticum* herbivory and 61% lower in plants under *M. scutellaris* herbivory when compared to control treatments (*C. a*: t_{10} = -1.39, *P* = 0.19; *M. s:* t_{10} = -2.19, *P* < 0.05). Similar reductions were seen at 400 ppm (36% and 60% lower with *C. aquaticum* and *M. scutellaris* herbivory), although these declines were not significant at the 95% level, but were significant at the 90% level (*C. a*: t_8 = -1.76, *P* = 0.09, *M. s*: t_6 = -2.083, *P* = 0.08). There was no significant difference between those exposed to *C. aquaticum* or *M. scutellaris,* regardless of CO_2 treatment.

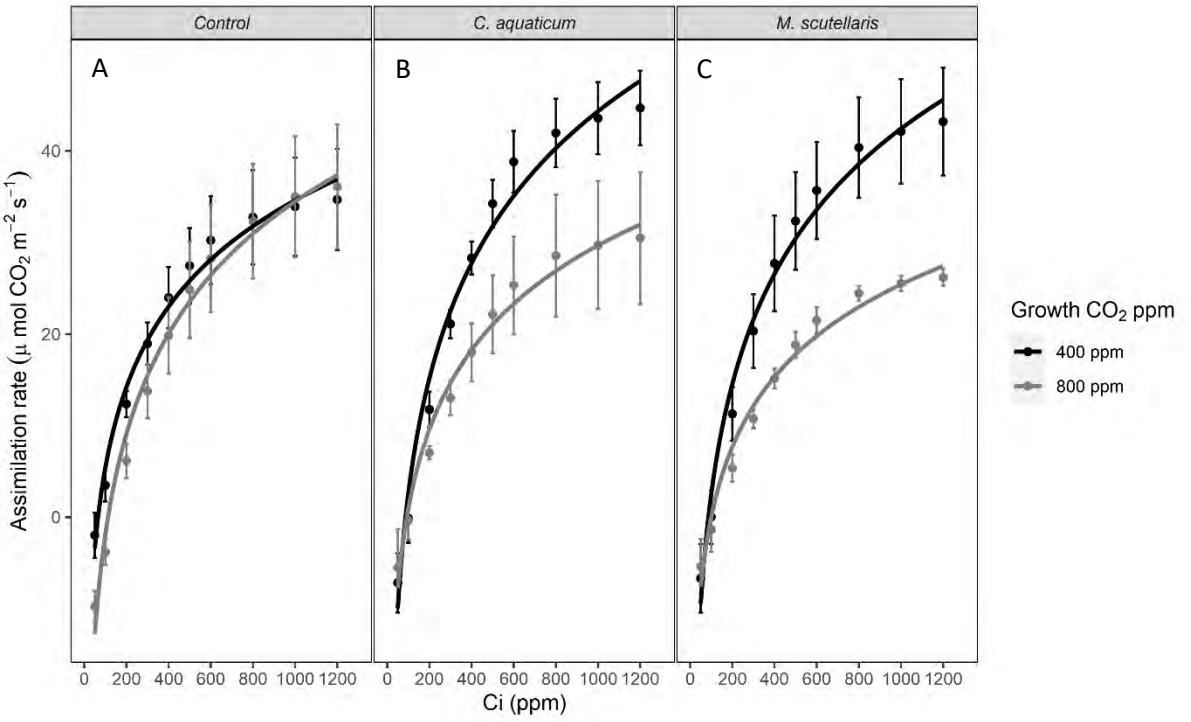


Figure 3.1.1: The carbon assimilation rates (µmol CO_2 $m^{-2}s^{-1}$) of *Pontederia crassipes* as affected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory of either *Cornops aquaticum* or *Megamelus scutellaris* (presence/absence). Assimilation rates are a factor of the maximum rate of Rubisco carboxylase activity (µmol CO_2 $m^{-2}s^{-1}$) (Vcmax) and the maximum rate of photosynthetic electron transport (µmol CO_2 $m^{-2}s^{-1}$) (Jmax). Error bars represent mean ± S.E.

Table 3.1.1: Summary of the mean ± s.e. of the maximum rate of Rubisco carboxylase activity (V_{cmax}; µmol CO_2 $m^{-2}s^{-1}$), the maximum rate of photosynthetic electron transport (J_{max}; µmol CO_2 $m^{-2}s^{-1}$), and stomatal limitation (L_s; %) of *Pontederia crassipes* as affected by CO_2 (400 ppm and 800 ppm) and insect herbivory by *Cornops aquaticum* and *Megamelus scutellaris*. The lower-case lettering indicates significant differences between both insect and CO_2 treatments.

	400 ppm			**800 ppm**		
	Control	*C. aquaticum*	*M. scutellaris*	Control	*C. aquaticum*	*M. scutellaris*
V_{cmax}	89.11 ±36.58 $_a$	113.07 ±26.63 $_a$	108.72 ±45.01 $_a$	106.86 ±25.64 $_a$	73.84 ±43.24 $_{ab}$	55.95 ±24.16 $_b$
J_{max}	216.34 ±153.07$_{ab}$	330.86 ±145.93$_a$	329.17 ±180.30$_a$	334.64 ±117.15$_a$	210.80 ±174.31$_{ab}$	129.47 ±65.15$_b$

Intercellular carbon (Ci) of *P. crassipes* was significantly affected by eCO_2 in the absence of insect herbivory, with a 99% increase in Ci as a result of eCO_2 conditions (t_{11} = 25.22, $P < 0.0005$). Ci of *P. crassipes* exposed to herbivory was significantly impacted, showing an 96% and 52% increase at 800 ppm when compared to 400 ppm (*C. a*: t_{10} = 13.37, $P < 0.005$, *M. s*: t_6 = 4.26, $P < 0.005$). Ci of plants exposed to herbivory by *C. aquaticum* and *M. scutellaris* was not affected at 400 ppm (*C. a*: t_{17} = 0.07, $P = 0.95$, *M. s*: t_{17} = -0.76, $P = 0.46$). When plants were grown at 800 ppm, Ci was similar for plants under *C. aquaticum* herbivory (t_{10} = -0.32, $P = 0.75$), and 27% lower in plants under *M. scutellaris* herbivory (t_{10} = -5.44, $P < 0.0005$) when compared to controls. Ci of plants was similar in both *M. scutellaris* and *C. aquaticum* herbivory treatments at 400 ppm, and was 25% lower under *M. scutellaris* herbivory treatments when compared to *C. aquaticum* herbivory treatments at 800 ppm.

Stomatal conductance (G_{st}) of *P. crassipes* was not affected by eCO_2 in the absence of insect herbivory (t_{11} = -1.79, $P = 0.10$). G_{st} of plants was significantly lower (72% lower) in plants exposed to *C. aquaticum* (*C. a*: t_{10} = -3.52, $P < 0.05$), while G_{st} was significantly lower (91%) in plants exposed to *M. scutellaris* in the 90th percentile (*M. s*: t_{10} = -2.28, $P < 0.06$) at 800 ppm when compared to 400 ppm (Figure 3.1.2). *Cornops aquaticum* herbivory did not affect G_{st} at 400 ppm, however G_{st} was significantly lower in plants exposed to *M. scutellaris* herbivory when compared to controls (*C. a*: t_{17} = 1.01, $P = 0.33$, *M. s*: t_{17} = 2.10, $P < 0.05$). When plants were grown at 800 ppm, G_{st} was 10% lower for plants under *C. aquaticum* herbivory (t_{10} = -0.65, $P = 0.53$), and 39% in plants under *M. scutellaris* herbivory (t_{10} = -2.60, $P < 0.03$) in comparison to controls. G_{st} was similar in plants under *C. aquaticum* and *M. scutellaris* herbivory treatments at 400 ppm but was 32% lower under *M. scutellaris* herbivory treatments when compared to *C. aquaticum* herbivory treatments at 800 ppm.

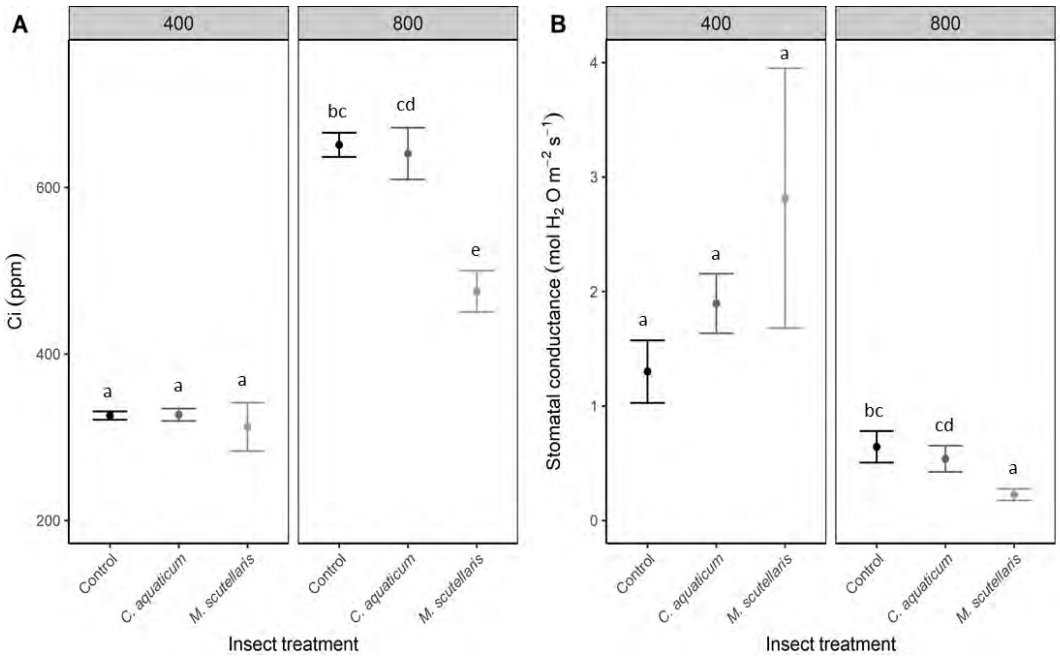


Figure 3.1.2: Intercellular CO_2 (Ci) (A) and stomatal conductance (G_{st}) (B) of *Pontederia crassipes* as effected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory by either *Cornops aquaticum* or *Megamelus scutellaris* (presence/absence). The lower-case lettering indicate significant differences between both insect and CO_2 treatments.

C:N ratio of *P. crassipes* was unaffected by eCO_2 in the absence of insect herbivory (t_{20} = 0.31, P = 0.76) (Figure 3.1.3A), and under *C. aquaticum* herbivory treatments (t_{21} =1.17, P = 0.26), whilst under *M. scutellaris* herbivory, C:N was significantly greater when grown at 800 ppm (t_{21} = 3.50, P < 0.0001). *Cornops aquaticum* herbivory significantly increased C:N ratio at 400 ppm by 29% (t_{33} = 4.16, P < 0.001), while *M. scutellaris* herbivory had no effect (t_{33} = -0.20, P = 0.85) when compared to control treatments. Similarly, at 800 ppm the C:N ratio of plants under *C. aquaticum* herbivory treatments and *M. scutellaris* herbivory treatments were 38% and 14% higher compared to control herbivory treatments (*C. a*: t_{29} = 4.39, P < 0.001, *M. s*: t_{29} = 1.67, P = 0.11) (Figure 3.1.3A).

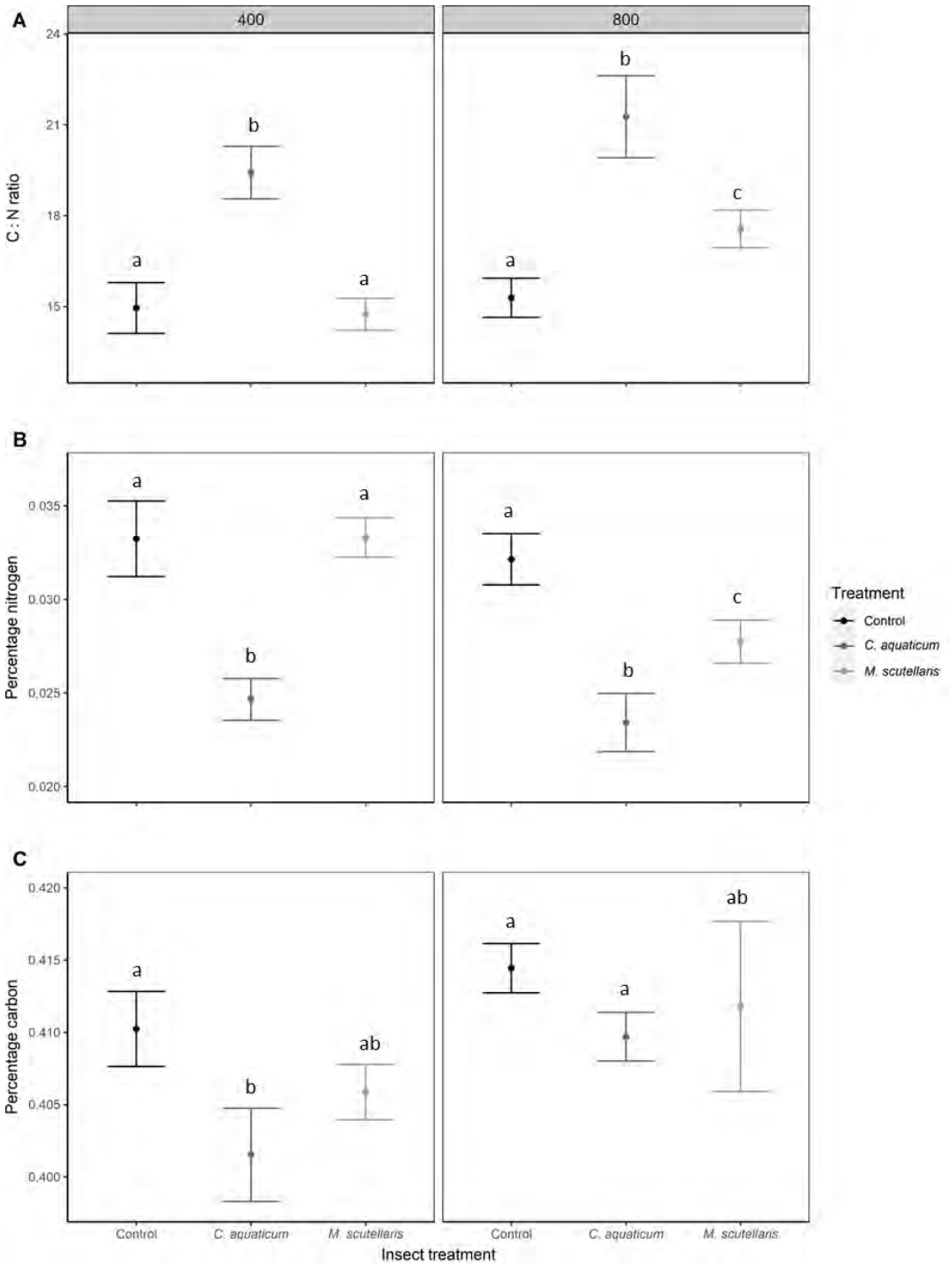


Figure 3.1.3: C:N ratio (A), percentage nitrogen (B) and percentage carbon (C) of the 4th leaf of *Pontederia crassipes* as affected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory of either *Cornops aquaticum* or *Megamelus scutellaris* (presence/absence). Error bars represent mean ± S.E. C.N. ratio in delta notation using a per mille scale and the standard equation: $\delta X\ (‰) = (R_{sample}/R_{standard})-1$, (where X= ^{15}N or ^{13}C and R represents $^{15}N/^{14}N$ or $^{13}C/^{12}C$ respectively). The lower-case lettering indicate significant differences between both insect and CO_2 treatments.

Inverse to the C:N ratio trends, the percentage nitrogen of the 4th leaf of *P. crassipes* was significantly reduced by exposure to *C. aquaticum* herbivory (25% lower; $t_{33} = -4.04$, $P < 0.01$) but not *M. scutellaris* herbivory ($t_{33} = -3.31$, $P = 0.76$) when compared to control treatments, at 400 ppm (Figure 3.1.3B). Percentage nitrogen for plants grown at 800 ppm was 27% lower for plants under *C. aquaticum* herbivory and 17% lower in plants under *M. scutellaris* herbivory when compared to control treatments (*C. a*: $t_{29} = -4.47$, $P < 0.001$, *M. s*: $t_{29} = -2.26$, $P < 0.05$). *Cornops aquaticum* herbivory significantly decreased percentage nitrogen in comparison to *M. scutellaris* (19% and 15% lower at 400 and 800 ppm, respectively). Percentage carbon was unaffected by eCO_2 in the absence of insect herbivory ($t_{20} = 1.30$, $P = 0.21$) (Figure 3.1.3C). Percentage carbon was also not statistically different between insect herbivory treatments and control treatments and 400 ppm and 800 ppm, with the exception of *C. aquaticum* herbivory treatments at 400 ppm ($t_{33} = -1.17$, $P < 0.05$). Percentage carbon of plants was similar between insect herbivory treatments at 400 ppm and 800 ppm.

C:N ratio of the 4th leaf of *P. crassipes* was not correlated with *C. aquaticum* herbivory at 400 ppm ($R^2(n = 10) = 0.18$, $P = 0.17$) and 800 ppm treatments ($R^2(n = 9) = 0.16$, $P = 0.23$) (Table 3.1.2). Similarly, there was no relationship between the C:N ratio of the 4th leaf of *P. crassipes* and *M. scutellaris* adult population density at either CO_2 treatments ($R^2(n = 10) = 0.026$, $P = 0.63$; $R^2(n = 9) = 0.051$, $P = 0.50$).

Percentage nitrogen of the 4th leaf was not correlated with *C. aquaticum* herbivory intensity regardless of CO_2 treatment (400 ppm: $R^2(n = 10) = 0.111$, $P = 0.29$, 800 ppm: $R^2(n = 9) = 0.211$, $P = 0.16$). The percentage nitrogen of *P. crassipes* was not correlated with *M. scutellaris* adult population density at either 400 ppm or 800 ppm (400 ppm: $R^2(n = 10) = 0.048$, $P = 0.52$, 800 ppm: $R^2(n = 9) = 0.00001$, $P = 0.99$).

Percentage carbon of the 4th leaf of *P. crassipes* was not correlated with *C. aquaticum* herbivory at 400 ppm and 800 ppm treatments (400 ppm: $R^2(n = 10) = 0.0008$, $P = 0.93$, 800 ppm: $R^2(n = 9) = 0.176$, $P = 0.20$) or with *M. scutellaris* adult population density at 400 ppm ($R^2(n = 10) = 0.243$, $P = 0.12$). However, percentage carbon of *P. crassipes* increased significantly with *M. scutellaris* adult

population density at 800 ppm (R^2(n = 9) = 0.459, $P < 0.05$) (Figure 3.1.4). There was no statistical difference in slope or elevation between 400 ppm and 800 ppm for the relationship between percentage carbon and *M. scutellaris* adult population (Likelihood ratio stat = 0.0001, $P = 0.99$; Wald stat = 0.046, $P = 0.83$).

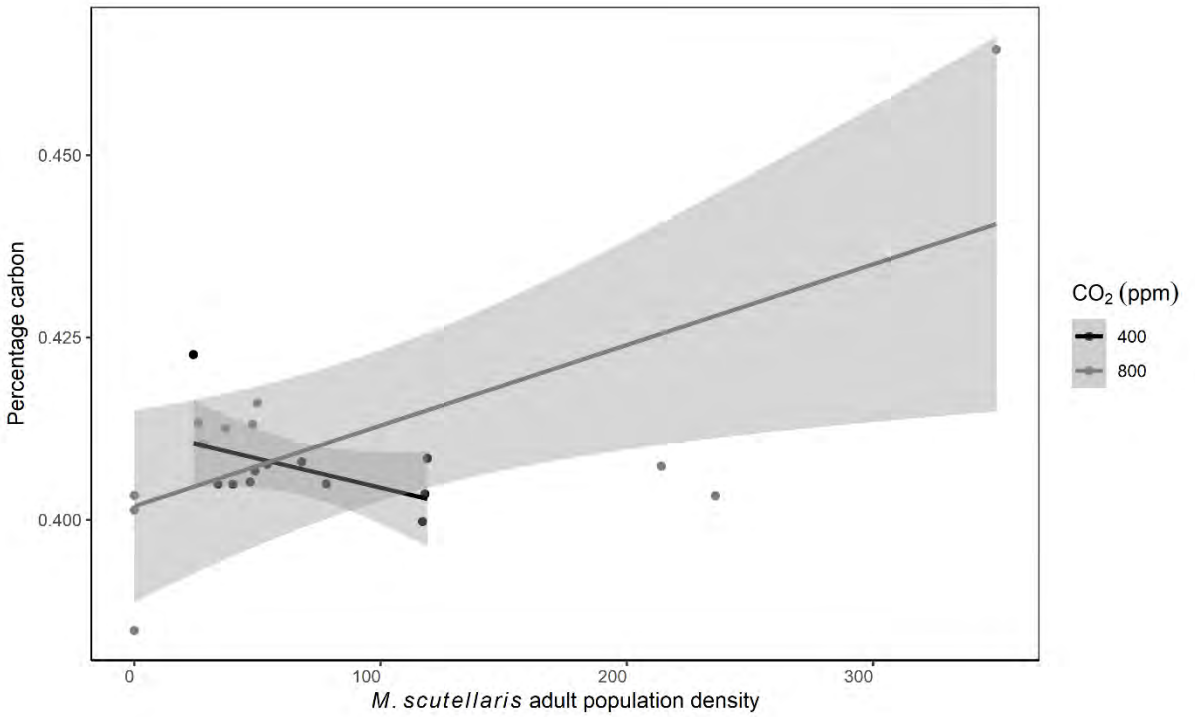


Figure 3.1.4: Percentage carbon of *Pontederia crassipes* vs *Megamelus scutellaris* adult population density as affected by CO_2 conditions. Bands indicate a 95% confidence interval.

Table 3.1.2: Chi-squared test summary of Standardized Major Axis Estimation and Testing Routines analysis on the effects of CO_2 (400 ppm & 800 ppm), *Cornops aquaticum* chewing herbivory and *Megamelus scutellaris* adult population density on C:N ratio, percentage N and percentage carbon of Pontederia crassipes. Bold indicates significance at P < 0.05.

Response	***Insect factor***	***CO_2***	***Equation, R^2 and P-value***	***Effect of eCO_2 on slope***	***Effect of eCO_2 on elevation***
C:N ratio	*C. aquaticum* chewing herbivory	400	y = 4.81*x + 17.28, R^2 = 0.181, *P* = 0.17	Likelihood ratio stat = 0.72, *P* = 0.396	Wald stat = 4.355, *P* = **0.04**
		800	y = 6.43*x + 19.38, R^2 = 0.156, *P* = 0.23		
	M. scutellaris population density	400	y = 0.006*x + 13.946, R^2 = 0.026, *P* = 0.63	Likelihood ratio stat = 3.003, *P* = 0.08	Wald stat = 4.991, *P* = **0.03**
		800	y = 0.004*x + 17.214, R^2 = 0.051, *P* = 0.50		
Percentage nitrogen	*C. aquaticum* chewing herbivory	400	y = -0.005*x + 0.027, R^2 = 0.111, *P* = 0.29	Likelihood ratio stat = 0.331, *P* = 0.57	Wald stat = 2.79, *P* = 0.10
		800	y = -0.009*x + 0.026, R^2 = 0.211, *P* = 0.16		
	M. scutellaris population density	400	y = -2.17e-05*x + 0.035, R^2 = 0.048, *P* = 0.52	Likelihood ratio stat = 5.30, *P* = **0.02**	Wald stat = 2.526, *P* = 0.11
		800	y = -1.026e-07*x + 0.028, R^2 = 1.036e-05, *P* = 0.99		
Percentage carbon	*C. aquaticum* chewing herbivory	400	y = -0.001 *x + 0.402, R^2 = 0.0008, *P* = 0.93	Likelihood ratio stat = 2.547, *P* = 0.11	Wald stat = 0.649, *P* = 0.42
		800	y = -0.009*x + 0.412, R^2 = 0.176, *P* = 0.20		
	M. Scutellairs population density	400	y = -8.013e-05 *x + 0.412, R^2 = 0.243, *P* = 0.12	Likelihood ratio stat = 0.0001, *P* = 0.99	Wald stat = 0.046, *P* = 0.83
		800	y = 0.0001*x + 0.402, R^2 = 0.459, *P* = **0.022**		

3.2 Plant growth responses to eCO_2 and insect herbivory

Relative growth rate was not affected by eCO_2 in the absence of insect herbivory ($t_{20} = 0.24$, $P = 0.81$), or by *C. aquaticum* and *M. scutellaris* herbivory respectively (*C. a*: $t_{29} = 1.45$, $P = 0.16$, *M. s*: $t_{33} = 1.48$, $P = 0.16$) (Figure 3.2.1A). When compared to control treatments, insect herbivory did not affect RGR at 400 ppm, but exposure to both *C. aquaticum* and *M. scutellaris* herbivory led to 4% increases when grown at 800 ppm (*C. a*: $t_{29} = 2.41$, $P < 0.05$, *M. s*: $t_{33} = 2.52$, $P < 0.05$).

Total dry biomass of *P. crassipes* was unaffected by eCO_2 in the absence of insect herbivory ($t_{20} = 0.19$, $P = 0.85$), but increased significantly in the presence of herbivory, with total biomass being 20% greater for plants under *C. aquaticum* herbivory and 19% greater in plants under *M. scutellaris* herbivory suggesting compensatory growth (*C. a*: $t_{29} = 2.54$, $P < 0.05$, *M. s*: $t_{29} = 2.466$, $P < 0.05$) (Figure 3.2.1B). No significant difference was observed between control plants and plants exposed to insect herbivory treatments at 400 ppm (*C. a*: $t_{32} = 1.019$, $P = 0.316$, *M. s*: $t_{32} = 0.005$, $P = 0.16$). Total dry biomass of plants under *M. scutellaris* herbivory was 3% higher than plants under *C. aquaticum* herbivory at 400 ppm and 1% lower at 800 ppm.

There was no correlation between relative growth rate of *P. crassipes* and *C. aquaticum* chewing herbivory (400 ppm: $R^2(n = 10) = 0.072$, $P = 0.40$, 800 ppm: $R^2(n = 9) = 0.113$, $P = 0.31$) or *M. scutellaris* adult population density (400 ppm: $R^2(n = 10) = 0.130$, $P = 0.0.28$, 800 ppm: $R^2(n = 9) = 0.0009$, $P = 0.93$) at either CO_2 treatment (Table 3.2.1). There was also no relationship between total dry biomass of *P. crassipes* and *C. aquaticum* chewing herbivory (400 ppm: $R^2(n = 10) = 0.065$, $P = 0.42$, 800 ppm: $R^2(n = 9) = 0.100$, $P = 0.34$) or *M. scutellaris* adult population density (400 Ppm: $R^2(n = 10) = 0.124$, $P = 0.29$, 800 ppm $R^2(n = 9) = 0.003$, $P = 0.867$) at 400 ppm and 800 ppm treatments (Table 3.2.1).

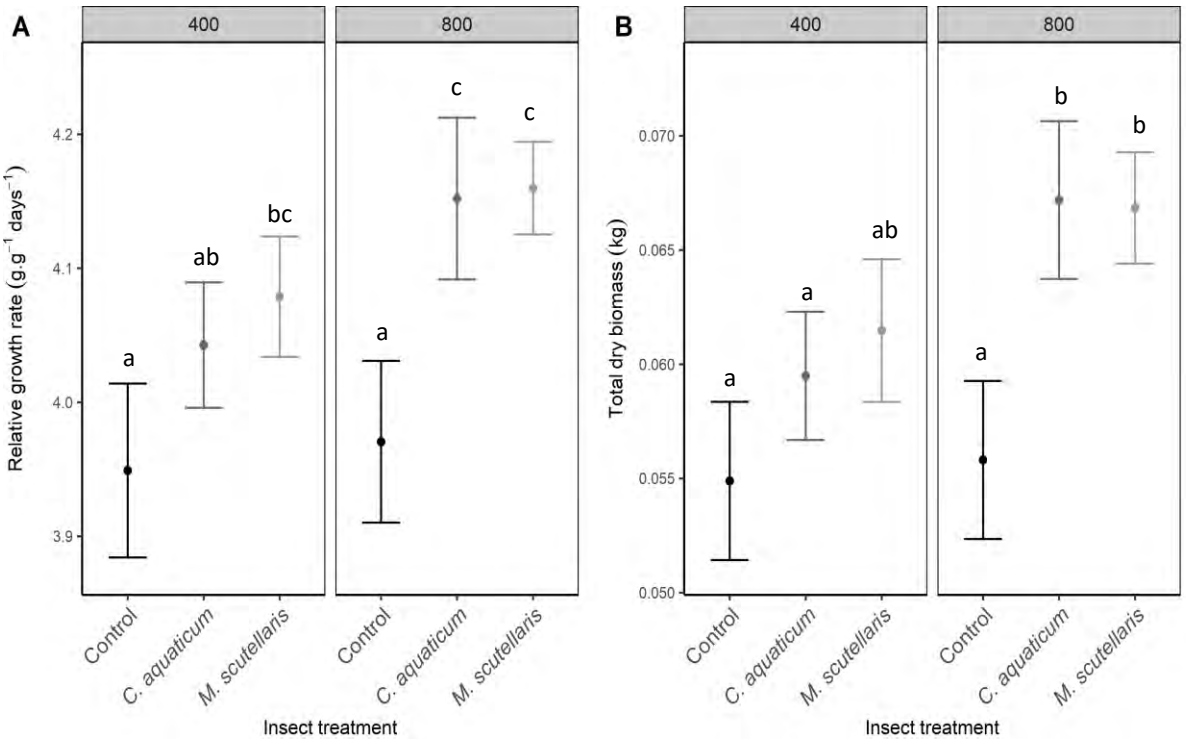


Figure 3.2.1: Relative growth rate (A) and total dry biomass (B) of *Pontederia crassipes* as affected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory by either *Cornops aquaticum* or *Megamelus scutellaris* (presence/absence). The lower-case lettering indicate significant differences between both insect and CO_2 treatments.

Table 3.2.1: Summary of Standardized Major Axis Estimation and Testing Routines analysis on the effects of CO_2 (400 ppm & 800 ppm), *Cornops aquaticum* chewing herbivory and *Megamelus scutellaris* adult population density on relative growth rate ($g.g^{-1}.days^{-1}$) and total dry biomass (kg) of *Pontederia crassipes*. Bold indicates significance at P < 0.05.

Response	***Insect factor***	***CO_2***	***Equation, R^2 and P-value***	***Effect of eCO_2 on slope***	***Effect of eCO_2 on elevation***
Relative growth rate ($g.g^{-1}.day^{-1}$)	*C. aquaticum* chewing herbivory	400	y = -0.163 *x + 4.115, R^2 = 0.072, *P* = 0.40	Likelihood ratio stat = 0.1527, *P* = 0.70	Wald stat = 3.625, *P* = 0.06
		800	y = 0.243 *x + 4.081, R^2 = 0.113, *P* = 0.31		
	M. scutellaris population density	400	y = -0.001*x + 4.193, R^2 = 0.130, *P* = 0.28	Likelihood ratio stat = 10.1, *P* = **0.001**	Wald stat = 1.502, *P* = 0.22
		800	y = 2.888e-05 *x + 4.157, R^2 = 0.0009, *P* = 0.93		
Total dry biomass (kg)	*C. aquaticum* chewing herbivory	400	y = -0.010*x + 0.064, R^2 = 0.065, *P* = 0.42	Likelihood ratio stat = 0.0804, *P* = 0.7768	Wald stat = 4.396, *P* = **0.036**
		800	y = 0.013*x + 0.063, R^2 = 0.100, *P* = 0.34		
	M. scutellaris population density	400	y = -0.0001*x + 0.070, R^2 = 0.124, *P* = 0.288	Likelihood ratio stat = 9.904, *P* = 0.002	Wald stat = 1.375, *P* = 0.241
		800	y = 3.892e-06*x + 0.067, R^2 = 0.003, *P* = 0.867		

Dry dead biomass was unaffected by eCO_2 in the absence of herbivory (t_{20} = 0.453, *P* = 0.66) (Figure 3.2.2A). Exposure to *C. aquaticum* chewing herbivory had no significant effect on dry dead biomass at 400 ppm (t_{32} = 0.20, *P* = 0.84), but this increased significantly by 17% at 800 ppm (t_{29} = 2.17, *P* < 0.05) when compared to control treatments. The impact of *M. scutellaris* on dry dead biomass was unaffected by eCO_2 conditions (t_{20} = -0.169, *P* < 0.87), however, the exposure to sap-sucking insect herbivory led to a 37% increase in dry dead biomass at 400 ppm (t_{32} = 2.62, *P* < 0.05) and a 21% increase at 800 ppm (t_{29} = 2.37, *P* < 0.05) when compared to control treatments. The impact of insect feeding guild was noticeable for *M. scutellaris,* which was 21% greater than that of *C. aquaticum* at 400 ppm and 6% greater at 800 ppm

Dry shoot biomass was unaffected by eCO_2 in the absence of herbivory (t_{20} = 0.938, *P* = 0.36) or in the presence of *M. scutellaris* herbivory (t_{20} = 0.878, *P* = 0.39), but was 14% higher at eCO_2 in those plants exposed to *C. aquaticum* herbivory (t_{21} = 2.15, *P* < 0.05) (Figure 3.2.2B). Exposure to insect herbivory at 400 ppm (*C. a*: t_{32} = 0.202, *P* = 0.84; *M. s*: t_{32} = -1.075, *P* = 0.29) had no effect on dry shoot biomass. However, dry shoot biomass of *P. crassipes* exposed to 800 ppm was 17% higher for plants under *C. aquaticum* herbivory (t_{29} = 2.17, *P* < 0.05), and 8% lower in plants under *M. scutellaris* herbivory when compared to control treatments (t_{29} = -1.04, *P* = 0.31). Differences in the impact of insect feeding guild were apparent at 800 ppm, where *C. aquaticum* had a 27% greater effect on dry shoot biomass relative to the effect of *M. scutellaris*.

Dry root biomass was unaffected by eCO_2 in the absence of herbivory (t_{20} = -0.52, *P* = 0.61) or in the presence of *C. aquaticum* herbivory (t_{21} = -0.315, *P* = 0.76), but was 10% higher at eCO_2 in plants exposed to *M. scutellaris* herbivory (t_{21} = 1.48, *P* = 0.16) (Figure 3.2.2C). There was no effect of insect herbivory at 400 ppm, but at 800 ppm *C. aquaticum* herbivory led to a 25% increase in dry root biomass (t_{29} = 1.64, *P* = 0.11), and *M. scutellaris* herbivory led to significant increases of 46% (t_{29} = 2.99, *P* < 0.01). Dry root biomass was similar between insect herbivory treatments at 400 ppm and 16% greater under *M. scutellaris* herbivory treatments than *C. aquaticum* herbivory treatments at 800 ppm.

Dry dead biomass of *P. crassipes* was not correlated with *C. aquaticum* chewing herbivory (400 ppm: R^2(n = 10) = 0.046, *P* = 0.50, 800 ppm: R^2(n = 9) = 0.090, *P* = 0.37) or *M. scutellaris* adult density (400 ppm: R^2(n = 10) = 0.098, *P* = 0.35, 800 ppm: R^2(n = 9) = 0.008, *P* = 0.79) at 400 ppm and 800 ppm treatments (Table 3.2.2)

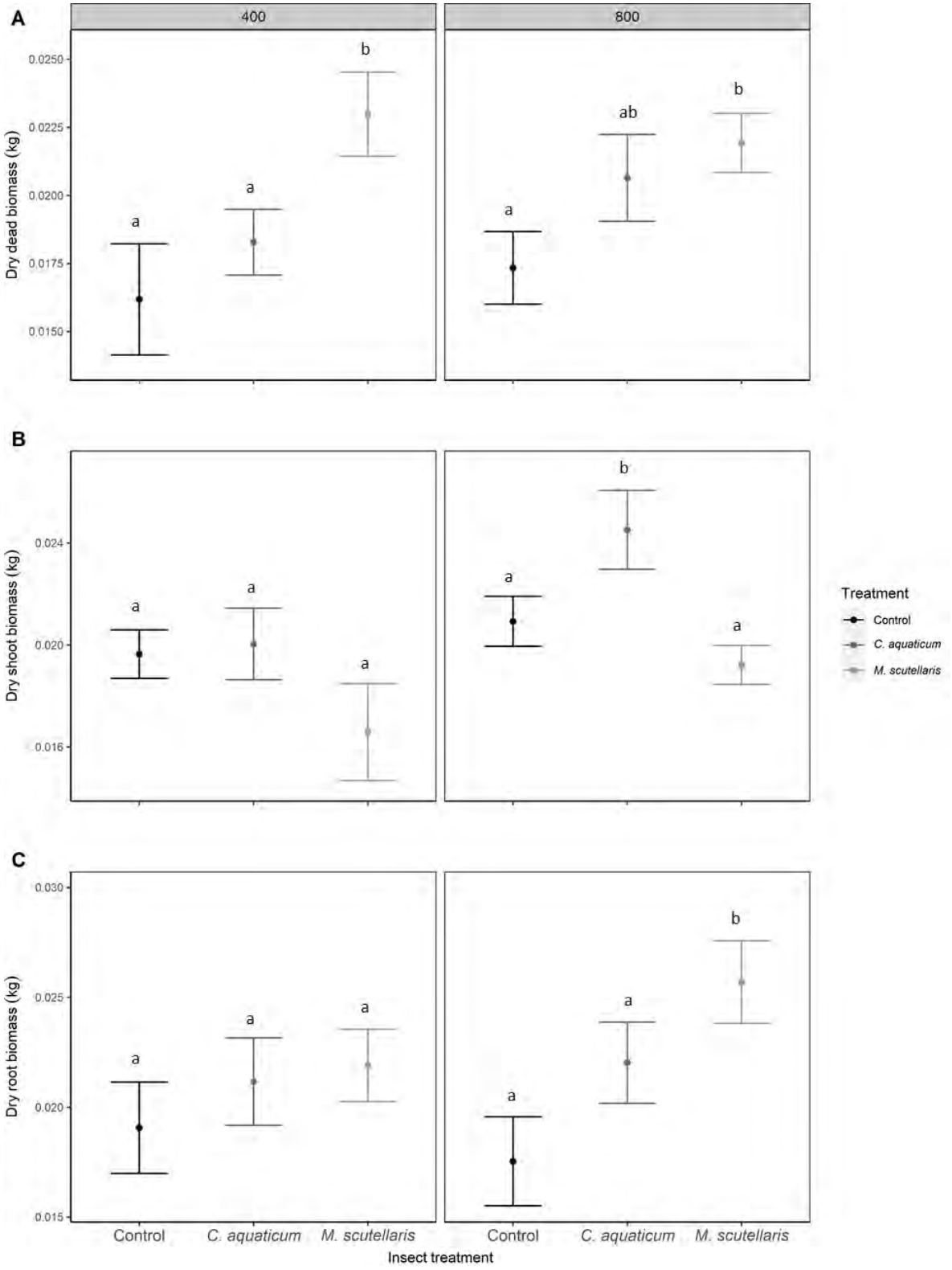


Figure 3.2.2: Dry dead biomass (kg) (A), dry shoot biomass (kg) (B), and dry root biomass (kg) (C) of *Pontederia crassipes* as affected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory of either *Cornops aquaticum* or *Megamelus scutellaris* (presence/absence). The lower-case lettering indicate significant differences between both insect and CO_2 treatments.

The dry shoot biomass of *P. crassipes* decreased significantly as *M. scutellaris* adult population density increased at 400 ppm, (R^2(n = 10) = 0.640, *P* = 0.003, Likelihood ratio stat = 21.12, *P* < 0.0001) (Figure 3.2.3, Table 3.2.2). Dry shoot biomass was not correlated with *M. scutellaris* adult population density at 800 ppm treatments, where *P. crassipes* dry shoot biomass was maintained regardless of *M. scutellaris* density (R^2(n = 9) = 0.027, *P* = 0.63). Dry shoot biomass of *P. crassipes* was not correlated with *C. aquaticum* chewing herbivory at 400 ppm and 800 ppm treatments (400 ppm: R^2(n = 10) = 0.172, *P* = 0.18, 800 ppm: R^2(n = 9) = 0.012, *P* = 0.76).

Dry root biomass of *P. crassipes* was not correlated with *C. aquaticum* chewing herbivory (400 ppm: R^2(n = 10) = 0.039, *P* = 0.54, 800 ppm: R^2(n = 9) = 0.011, *P* = 0.176) or *M scutellaris* adult population density (400 ppm: R^2(n = 10) = 0.011, *P* = 0.76, 800 ppm: R^2(n = 9) = 0.038, *P* = 0.57) at 400 ppm and 800 ppm treatments (Table 3.2.2).

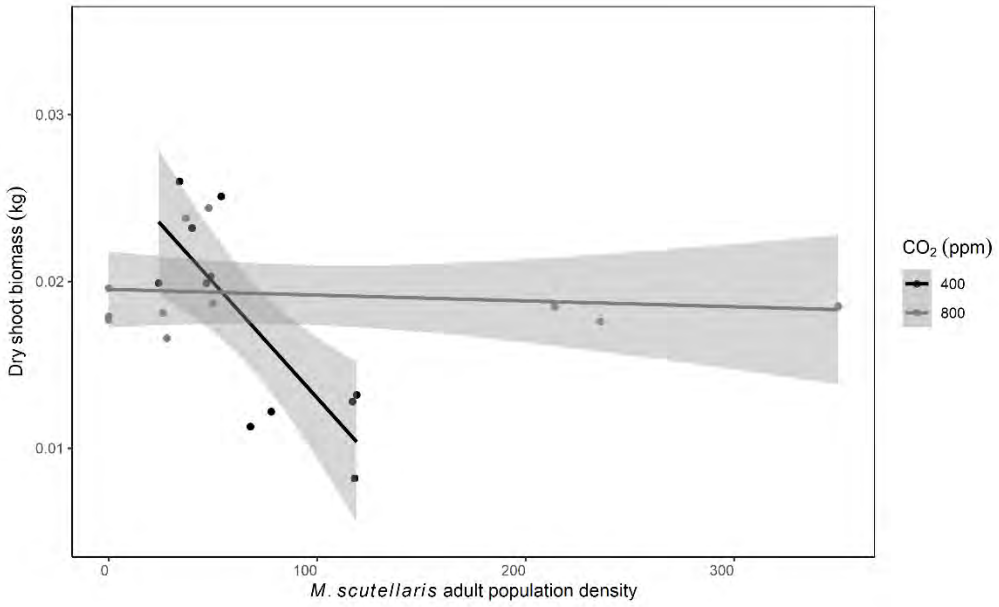


Figure 3.2.3: Dry shoot biomass of *Pontederia crassipes* vs *Megamelus scutellaris* adult population density. Bands indicates significance at P < 0.05.

Root:shoot length was not affected by eCO_2 in the absence of herbivory (t_{20} = 1.33, *P* = 0.20). Exposure to *C. aquaticum* chewing had no significant effect on root to shoot length ratio at 400 ppm (t_{32} = -0.85, *P* = 0.40) or at 800 ppm (t_{29} = -0.76, *P* = 0.45) when compared to control treatments (Figure 3.2.4A). The impact of *M. scutellaris* was also not affected by eCO_2 conditions (t_{20} = 1.60, *P* = 0.12), however, the exposure to sap-sucking herbivory led to a 38% decrease in root to shoot length at 400 ppm (t_{32} = -2.14, *P* < 0.05) and a 62% decrease at 800 ppm (t_{29} = -2.33, *P* < 0.05) when compared to control treatments. The impact of insect feeding guild was noticeable for *M. scutellaris,* which was 27% lower than that of *C. aquaticum* at 400 ppm and 51% at 800 ppm

Root length was unaffected by eCO_2 in the absence of herbivory (t_{20} = 1.17, *P* = 0.26). Exposure to *C. aquaticum* and *M. scutellaris* herbivory at 400 ppm (t_{33} = 0.425, *P* = 0.68; t_{33} = 0.29, *P* = 0.77) and 800 ppm (t_{29} = -0.03, *P* = 0.97; t_{32} = 0.95, *P* = 0.35) had no impact on root length when compared to control treatments. *Cornops aquaticum* herbivory was unaffected by eCO_2 conditions (t_{21} = 0.697, *P* = 0.49), however, *M. scutellaris* was significantly affected by eCO_2 conditions, with exposure to phloem-feeding resulting in a 12% increase in root length at eCO_2 (t_{21} = 2.28, *P* < 0.05) (Figure 3.2.4B). Differences in the impact of insect feeding guild were only marginally notable at 800 ppm, where *M. scutellaris* had a 5% greater effect on root length relative to the effect of *C. aquaticum*.

Shoot length was unaffected by eCO_2 in the absence of herbivory (t_{20} = -0.71, *P* = 0.48). Exposure to *C. aquaticum* chewing had no significant effect on shoot length at 400 ppm (t_{32} = 1.43, *P* = 0.17) and at 800 ppm (t_{29} = 0.70, *P* = 0.38) when compared to control treatments. The impact of *M. scutellaris* was not affected by eCO_2 conditions (t_{20} = 0.31, *P* = 0.76), however, the exposure to phloem-feeding led to a 21% increase in shoot length at 400 ppm (t_{32} = 3.15, *P* < 0.005) and a 31% increase at 800 ppm (t_{29} = 3.59, *P* < 0.005) compared to control treatments (Figure 3.2.4C). The insect feeding guild impact was not as noticeable for shoot length, as *C. aquaticum* herbivory resulted in a decline in shoot length by 9% at 400 ppm and 18% at 800 ppm relative to *M. scutellaris.*

Table 3.2.2: Summary of Standardized Major Axis Estimation and Testing Routines analysis on the effects of CO_2 (400 ppm & 800 ppm), *Cornops aquaticum* chewing herbivory *and Megamelus scutellaris* adult population density on dry dead biomass (kg), dry shoot biomass (kg) and dry root biomass (kg) of *Pontederia crassipes*. Bold indicates significance at P < 0.05.

Response	***Insect factor***	***CO_2***	***Equation, R^2 and p-value***	***Effect of eCO_2 on slope***	***Effect of eCO_2 on elevation***
Dry dead biomass (kg)	*C. aquaticum* chewing herbivory	400	y = 0.003*x + 0.017, R^2 = 0.046, *P* = 0.50	Likelihood ratio stat = 0.189, *P* = 0.664	Wald stat = 3.685, *P* = 0.055
		800	y = 0.006*x + 0.019, R^2 = 0.090, *P* = 0.37		
	M. scutellaris population density	400	y = 4.332e-05*x + 0.019, R^2 = 0.098, *P* = 0.35	Likelihood ratio stat = 9.481, *P* = **0.002**	Wald stat = 0.294, *P* = 0.588
		800	y = -2.769e-06*x + 0.022, R^2 = 0.008, *P* = 0.79		
Dry shoot biomass (kg)	*C. aquaticum* chewing herbivory	400	y = -0.0076*x + 0.023, R^2 = 0.172, *P* = 0.18	Likelihood ratio stat = 0.002, *P* = 0.97	Wald stat = 0.378, *P* = 0.54
		800	y = -0.002*x + 0.025, R^2 = 0.012, *P* = 0.76		
	M. Scutellaris population density	400	y = -0.0001*x + 0.027, R^2 = 0.640, *P* = **0.003**	Likelihood ratio stat = 21.12, *P* = **4.32e-06**	Wald stat = 0.964, *P* = 0.33
		800	y = -3.483*x + 0.020, R^2 = 0.027, *P* = 0.63		
Dry root biomass (kg)	*C, aquaticum* chewing herbivory	400	y = -0.005*x + 0.024, R^2 = 0.039, *P* = 0.54	Likelihood ratio stat = 0.117, *P* = 0.73	Wald stat = 1.328, *P* = 0.25
		800	y = 0.009*x + 0.019, R^2 = 0.176, *P* = 0.20		
	M. scutellaris population density	400	y = -1.776*x + 0.023, R^2 = 0.011, *P* = 0.76	Likelihood ratio stat = 5.705, *P* = **0.017**	Wald stat = 0.171, *P* = 0.68
		800	y = 1.014*x + 0.025, R^2 = 0.038, *P* = 0.57		

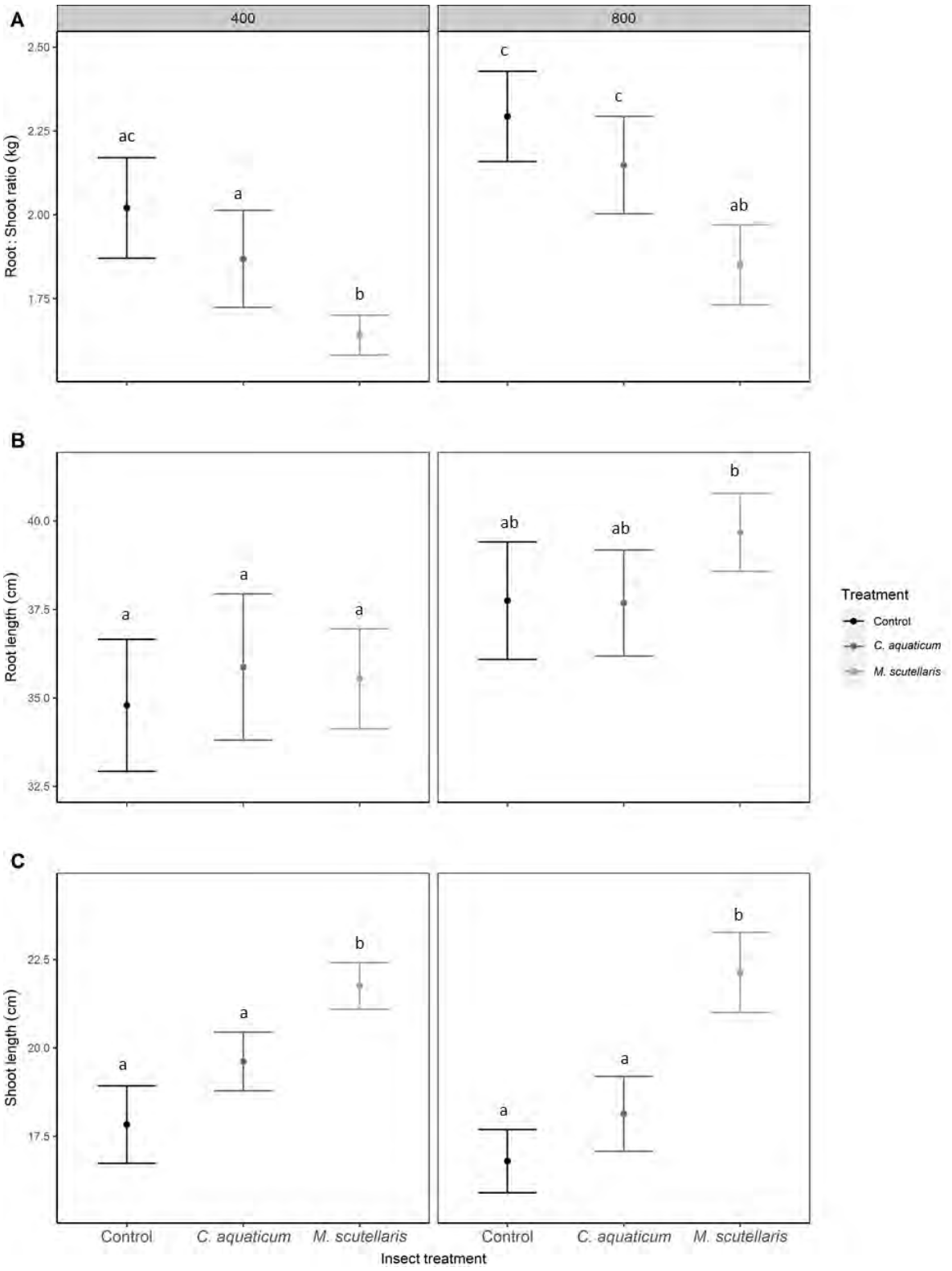


Figure 3.2.4: Root length to shoot length ratio (cm) (A), root length (cm) (B), and shoot length (cm) (C) of *Pontederia crassipes* as affected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory of either *Cornops aquaticum* or *Megamelus scutellaris* (presence/absence). The lower-case subscript indicates a significant difference between CO_2 treatments.

Root:shoot length of *P. crassipes* was not correlated with *C. aquaticum* chewing herbivory (400 ppm: R^2(n = 10) = 0.006, *P* = 0.80, 800 ppm: R^2(n = 9) = 0.29, *P* = 0.09) or *M. scutellaris* adult population density (400 ppm: R^2(n = 10) = 0.02, *P* = 0.63, 800 ppm: R^2(n = 9) = 0.097, *P* = 0.35) at 400 ppm and 800 ppm treatments (Table 3.2.3). Root length of *P. crassipes* was also not correlated with *C. aquaticum* chewing herbivory (400 ppm: R^2(n = 10) = 0.06, *P* = 0.41, 800 ppm: R^2(n = 9) = 0.09, *P* = 0.35) or *M. scutellaris* adult population density (400 ppm: R^2(n = 10) = 0.03, *P* = 0.61, 800 ppm: R^2(n = 9) = 0.016, *P* = 0.71) at 400 ppm and 800 ppm treatments (Table 3.2.3). Similarly, shoot length of *P. crassipes* was not correlated with *C. aquaticum* chewing herbivory (R^2(n = 10) = 0.072, *P* = 0.40, R^2(n = 9) = 0.200, *P* = 0.17) or *M. scutellaris* adult population density (R^2(n = 10) = 1.17e-05, *P* = 0.99, R^2(n = 9) = 0.078, *P* = 0.41) at 400 ppm and 800 ppm treatments (Table 3.2.3).

Table 3.2.3: Summary of Standardized Major Axis Estimation and Testing Routines analysis on the effects of CO_2 (400 ppm & 800 ppm), *Cornops aquaticum* chewing herbivory and *Megamelus scutellaris* adult population density on root length to shoot length ratio (cm), root length (cm) and shoot length (cm) of *Pontederia crassipes*. Bold indicates significance at P < 0.05.

Response	***Insect factor***	***CO_2***	***Equation, R^2 and p-value***	***Effect of eCO_2 on slope***	***Effect of eCO_2 on elevation***
Root: shoot length (cm)	*C. aquaticum* chewing herbivory	400	y = -0.155*x + 1.938, R^2 = 0.007, *P* = 0.79	Likelihood ratio stat = 0.030, *P* = 0.86	Wald stat = 0.0009, *P* = 0.98
		800	y = -0.942*x + 2.426, R^2 = 0.294, *P* = 0.09		
	M. scutellaris population density	400	y = 0.0002*x + 1.617, R^2 = 0.02, *P* = 0.63	Likelihood ratio stat = 3.458, *P* = 0.06	Wald stat = 0.901, *P* = 0.34
		800	y = -0001*x + 1.944, R^2 = 0.097, *P* = 0.35		
Root length (cm)	*C. aquaticum* chewing herbivory	400	y = -7.004*x + 38.992, R^2 = 0.068, *P* = 0.41	Likelihood ratio stat = 0.777, *P* = 0.38	Wald stat = 0.219, *P* = 0.64
		800	y = -5.679*x + 39.351, R^2 = 0.099, *P* = 0.35		
	M. scutellaris population density	400	y = 0.005*x + 34.943, R^2 = 0.027, *P* = 0.61	Likelihood ratio stat = 0.024, *P* = 0.88	Wald stat = 3.382, *P* = 0.066
		800	y = -0.004*x + 40.030, R^2 = 0.02, *P* = 0.71		
Shoot length (cm)	*C. aquaticum* chewing herbivory	400	y = -2.892*x + 20.903, R^2 = 0.071, *P* = 0.40	Likelihood ratio stat = 0.143, *P* = 0.71	Wald stat = 0.025, *P* = 0.88
		800	y = 5.698*x + 16.461, R^2 = 0.20, *P* = 0.17		
	M. scutellaris population density	400	y = 5.256*x + 21.744, R^2 = 1.17e-05, *P* = 0.99	Likelihood ratio stat = 2.344, *P* = 0.13	Wald stat = 0.224, *P* = 0.64
		800	y = 0.009*x + 21.346, R^2 = 0.078, *P* = 0.41		

3.3 Biological control agent responses to eCO_2

3.3.1 *Cornops aquaticum* chewing response to eCO_2

Leaf area was not affected by eCO_2 in the absence of herbivory (t_{16} = -1537.8, P = 0.09), nor was it affected by eCO_2 in the presence of *C. aquaticum* chewing herbivory (t_{12} = -232.1, P = 0.63) over time. But, as expected, *Cornops aquaticum* herbivory did significantly reduced the leaf area of *P. crassipes* when compared to control leaves, with 71% reductions at 400 ppm and 65% reductions at 800 ppm (400 ppm: t_{13} = -3494.4, P < 0.001, 800 ppm: t_{15} = -2198.7, P < 0.01) (Figure 3.3.1A). The leaf area damage ratio as impacted by *C. aquaticum* herbivory (the predicted leaf area vs the estimated area damaged by *C. aquaticum*) did not differ between CO_2 treatments (X^2 = 1.782, P = 0.18) Figure 3.3.1B).

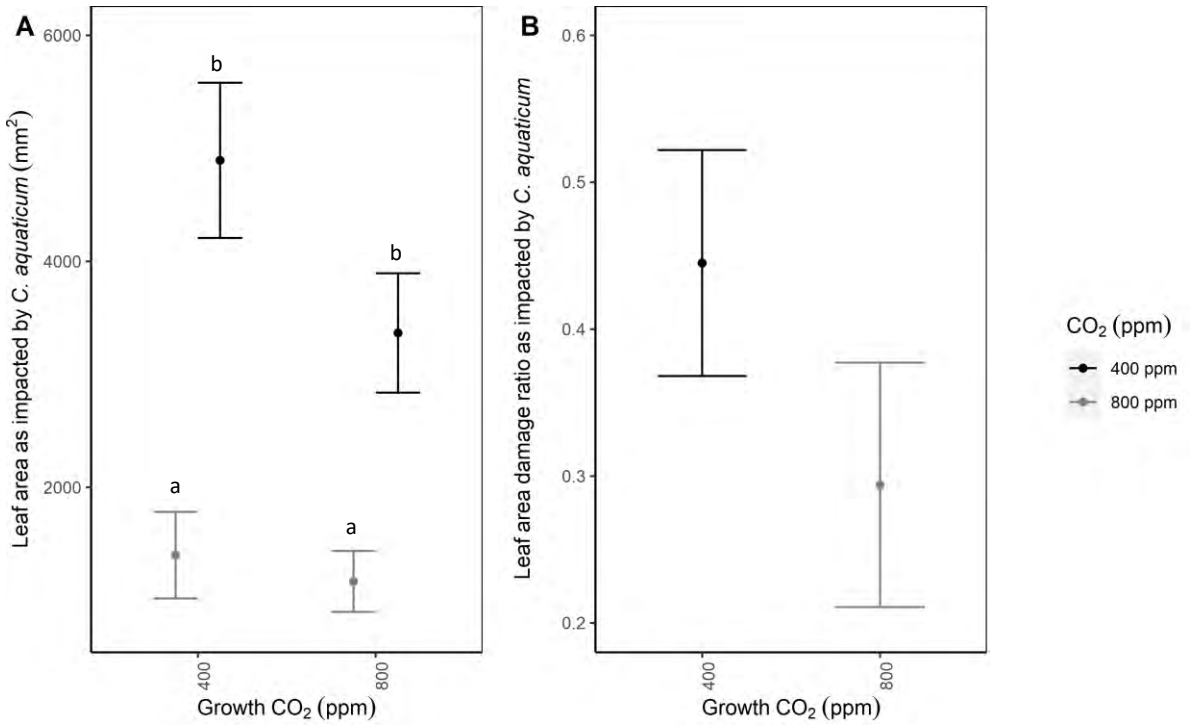


Figure 3.3.1: Leaf area (mm^2) (A) and leaf area damage ratio (B) as impacted by *C. aquaticum* of the 4th leaf of *Pontederia crassipes* (the ratio of predicted leaf area if unaffected by *C. aquaticum* vs estimated damage measured) as affected by CO_2 conditions (400 ppm & 800 ppm) and insect herbivory of *Cornops aquaticum* (presence/absence). Leaf area damage ratio. Lower-case letters indicate significant differences between CO_2 treatments.

3.3.2 *Megamelus scutellaris* population response to eCO_2

Megamelus scutellaris adult population density was 30% greater at 800 ppm than 400 ppm, albeit the difference was not statistically significant (W_{20} = 75, P = 0.36) (Figure 3.3.2A). *Megamelus scutellaris* female weight was significantly less at 800 ppm (W_{16} = 93.5, P < 0.05) (Figure 3.3.2B).

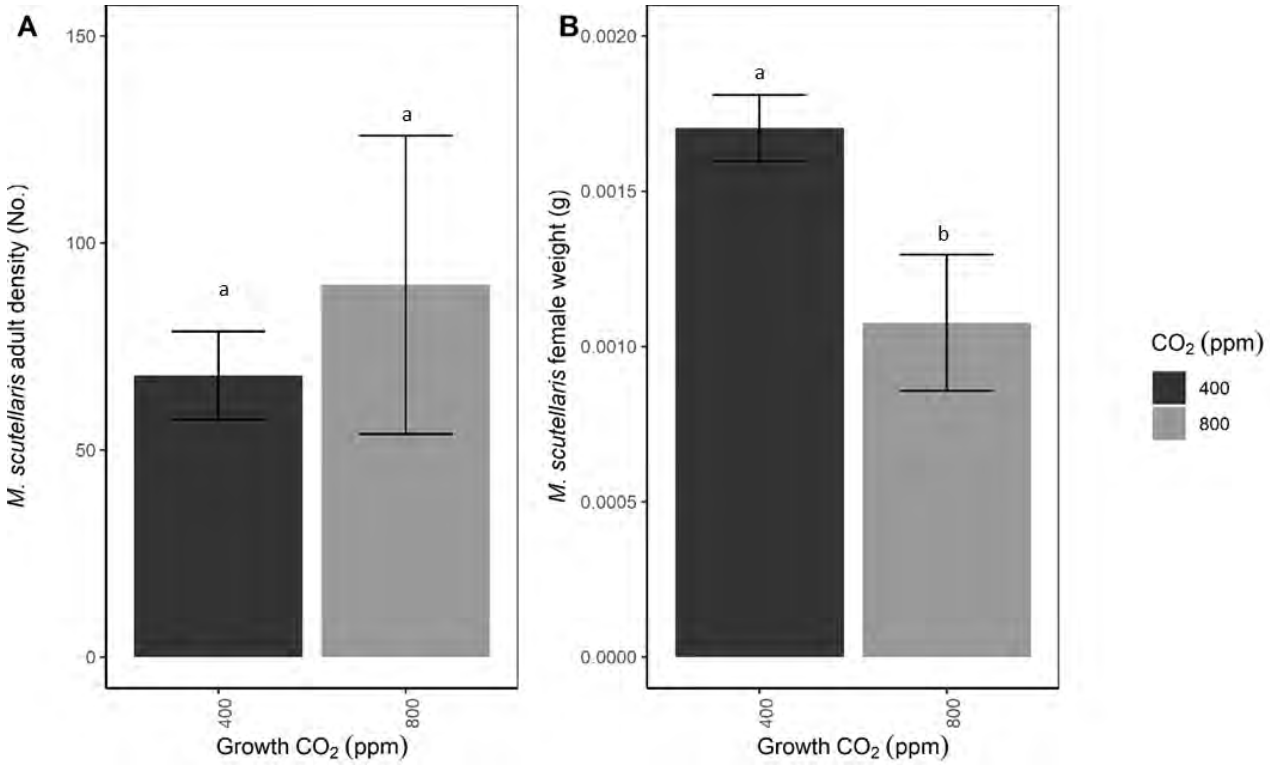


Figure 3.3.2: Mean adult population density (A) and adult female body weight (B) of *Megamelus scutellaris* on *Pontederia crassipes* as affected by CO_2 conditions (400 ppm & 800 ppm). Error bars represent the mean ± S.E. Lower-case letters indicate significant differences between CO_2 treatments.

Chapter 4: Discussion

4.1 Synthesis

The impact of rising atmospheric CO_2 on insect feeding guilds is well studied (see meta-analyses by Bezemer & Jones 1998; Stiling & Cornelissen 2007; Robinson et al. 2012); however, the effects of consistent eCO_2 on the efficacy of biological control agents to control robust invasive plants is less so. This study used *P. crassipes* and the associated biological control agents representing two feeding guilds, the leaf chewing *C. aquaticum* and the phloem-feeding *M. scutellaris*, as a model to identify the impacts of eCO_2 on plant-insect interactions within a biological control context. As expected, in the absence of insect herbivory, *P. crassipes* experienced photosynthetic acclimation, which translated into a lack of photosynthetic upregulation as expected in C_3 plants. However, net assimilation rates were higher at eCO_2 due to higher intercellular CO_2 concentrations (Ci), even under predicted declines in stomatal conductance (G_{st}), a typical eCO_2 response in C_3 plants. The only discernible growth response was an enhancement in the allocation to root biomass under eCO_2 conditions, a response common in C_3 plants as resources are often allocated to tissue needed to acquire the most limited resource (Chapin et al. 1987). Coinciding with the lack of CO_2 fertilization effect, there was no firm evidence to suggest an enhancement in the invasive potential of *P. crassipes* under future eCO_2 conditions. However, factors such as the potential for temperature limitation to plant growth and nutrient accessibility within experimental pots may have been hidden or even mitigated the potential growth responses of *P. crassipes* to eCO_2. Open, eutrophic water systems coupled with the predicted increases in global temperatures (IPCC 2014) may provide more ideal growth conditions for invasive species such as *P. crassipes* to take advantage of eCO_2 conditions (Hellmann et al. 2008; Thomas et al. 2010).

This study shows that *P. crassipes*, under aCO_2 conditions and in the presence of insect herbivory by both chewing and phloem-feeding herbivores, experienced a notable up-regulation of the photosynthetic capacity and alterations in the allocation of resources to plant compartments,

while at eCO_2 plants experience a notable down-regulation response when exposed to phloem-feeding by *M. scutellaris*. Under eCO_2 conditions, *P. crassipes* experienced stimulated compensatory growth responses and allocation to plant compartments based on insect feeding guild. In eCO_2 conditions, the chewing herbivory by *C. aquaticum* lead to a slight increase in C:N ratio, dead biomass, which is indicative of an increase in *P. crassipes* turnover rate, and enhanced compensatory growth of leaf biomass, whilst the phloem-feeding herbivory of *M. scutellaris* resulted in reduced foliar nitrogen and enhanced compensatory growth of root biomass in *P. crassipes*. The compensatory growth of *P. crassipes* under eCO_2 shows an allocation of resources to lost leaf biomass in the case of *C. aquaticum* and a reallocation of resources to counter the reduction in foliar nitrogen due to *M. scutellaris* herbivory affecting whole plant sink-source relations.

Plant responses to eCO_2 had mixed effects on insect feeding responses and population dynamics. eCO_2 conditions did not result in the hypothesised compensatory feeding of *C. aquaticum*, whilst *M. scutellaris* did experience the predicted enhancement in population density, measured as an increase in adult population density, under eCO_2 conditions (Bezemer & Jones 1998; Robinson et al. 2012). The evidence from this study suggests that of the two agents tested, *M. scutellaris* will be the more effective biological control agent under predicted eCO_2 conditions. Under eCO_2, the enhanced population density and improved impact on plant assimilation rates experienced by *M. scutellaris*, coupled with the reduced impact of *C. aquaticum* due to enhanced compensatory growth of *P. crassipes*, suggest that the phloem-feeding *M. scutellaris* and phloem-feeders, in general, may be better suited as biological control agents of *P. crassipes* under predicted eCO_2 conditions.

4.2 The impact of eCO_2 on productivity and invasive potential of *Pontederia crassipes*

When monitoring the impact of eCO_2 on plant physiology, the use of leaf gas exchange provides an accurate, real-time estimation of plant performance under various stress conditions (von Caemmerer & Farquhar 1981). *Pontederia crassipes* photosynthetic capacity at eCO_2 was equal to that of plants grown at aCO_2 levels, showing similar maximum Rubisco carboxylation (V_{cmax}) and electron transport

(J_{max}), which is indicative of the photosynthetic acclimation phenomenon (Sage 1994; Ainsworth & Rogers 2007). This response has been seen for *P. crassipes* before by Larigauderie et al. (1986), who found that under eCO_2 conditions of 6000 µmol(CO_2) mol^{-1} (or 6000 ppm), *P. crassipes* experienced declines of up to 15% in photosynthetic capacity relative to plants grown at ambient levels when measured at ambient conditions, whilst maximum assimilation rates were similar. The study by Lariguauderie et al. (1986) would be considered a short-term eCO_2 study by modern standards of eCO_2 experimentation but did illustrate acclimation of *P. crassipes* after only four weeks of growth at eCO_2 conditions representative of exceedingly high future concentrations. By having an extended growth period at high eCO_2 before the start of the study, *P. crassipes* acclimation realistically portrayed the potential physiological responses of this invasive plant to future CO_2 conditions. Many factors contribute to the acclimation phenomenon seen in a variety of plants when grown under eCO_2 conditions, and the phenomenon is still not fully understood (Sage 1994; Sage et al. 1989; Ziska et al. 1991; van Oosten et al. 1994; Nie et al. 1995; Osborne et al. 1997; Korner 2006; Smith & Keenan 2020). The results of this study are inconclusive in terms of the mechanisms for the acclimation observed here in *P. crassipes*. It has been suggested that the most likely drivers of this response are biochemical changes at the leaf level (Gunderson and Wullschleger 1994) brought about by limitations to source-sink relationships (Arp et al. 1991; White et al. 2016), limitations in resources necessary for photosynthesis limiting the plant's capacity to utilise abundant CO_2 (Ainsworth & Long 2005), temperature limitations through prolonged cold spells reducing the performance and growth of the plant (Tyndall 1982; Owens & Madsen 1995), and stomatal limitations associated with eCO_2 (Sellers et al. 1996; Medlyn et al. 2001; Ainsworth & Rogers 2007; Pastore et al. 2019).

Photosynthetic acclimation is thought to be a result of reduced carboxylation efficiency or reduced Rubisco activity within the leaf (Sage 1994; Drake et al. 1996; Ainsworth et al. 2003; Long et al. 2004). The reduction in Rubisco activity is considered a mechanism to mediate the balance between Rubisco and other processes limiting photosynthesis at eCO_2, such as electron transport capacity and/or light-harvesting (Sage et al. 1989; Sage 1990; Stitt 1991; Makino & Mae 1999; Tissue et al.

1993). The decline in these supplementary processes is potentially caused by alterations in sink-source relationships, with sources referring to the tissues providing a net uptake of resources from the environment, while sinks are tissues that result in a net internal use of the acquired resources (Burnett et al. 2016). Plant growth is directly controlled by exchanges between sinks and sources of resources (Arp 1991; White et al. 2016), which are fundamental in controlling plant growth (Arp 1991; White et al. 2016). In the case of carbon, at eCO_2, an increase in carbon availability enables higher carbohydrate products, such as starches within the plant (Griffin and Seemann 1996; Moore et al. 1999; Morison & Lawler 1999; Burnett et al. 2016). If there are insufficient carbon sinks (e.g. roots, shoots and daughter plants) to keep up with the higher production of carbon source products such as carbohydrates, there will most likely be a reduction in net photosynthetic rate to balance the source-sink activity (Thomas & Strain 1991), moderating the stimulating effects of eCO_2 (Bryant et al. 1998; Rogers et al. 1998; Ainsworth et al. 2003). In this study, *P. crassipes* declined in the total number of plants and displayed marginal reductions (non-significant decline by 15%) in dry biomass root:shoot ratio as well as some increase in root length (by 13%) under eCO_2 conditions. This interesting increase in root length may have also resulted from eCO_2 negative effect on nitrogen uptake within the root system of C_3 and C_4 species (Bassirirad et al. 2001). Plants will allocate resources to compartments where uptake is limited (Arp 1991; White et al. 2016). The small proportion of this increase to the root compartment may have overcome the nitrogen hampering effect of eCO_2, however, proving inadequate as a sink for excess carbon required to overcome photosynthetic acclimation.

Resource limitations of the surrounding environment can also limit a plant's capacity to respond to eCO_2 (Ainsworth & Long 2005; Korner 2006). *Pontederia crassipes* growth is directly proportional to nutrient concentration, particularly nitrogen and phosphorus (Gosset & Norris 1971; Reddy et al. 1990; Heard & Winterton 2000). Acclimation of assimilation rates in plants has been shown to correspond with increases in C:N ratios and a decline in foliar nitrogen under acute acclimation due to the accumulation of starches within the leaves (Drake et al. 1997; Moore et al. 1999; Teng et al. 2006). In this study, foliar C:N ratios of *P. crassipes* were only 1% higher in plants grown under eCO_2

conditions, with no significant increase in carbon content due to eCO_2 and no notable dilution of foliar nitrogen. The similar foliar C:N content at aCO_2 and eCO_2 conditions is likely the result of high nitrogen input preventing limitations to the plant, which mitigates acclimation in C_3 plants to some extent when not limited (Irigoyen et al. 2014). The nutrient conditions in this experiment were designed to replicate typical South African systems, where nutrient levels are orders of magnitude higher than the prescribed international standards (Villiers & Thiart 2007; Oberholster & Ashton 2008).

Thermal thresholds of *P. crassipes* are critical in the growth and regrowth of the plant under stressors due to the tropical climatic preferences of the plant (Knipling et al. 1970; Kumar et al. 1985), and plant responses to eCO_2 are expected to be higher when corresponding temperatures are high (Long 1991). When thermal conditions become unfavourable, below what is considered the thermal optima, the productivity of *P. crassipes* declines (Knipling et al. 1970; Kumar et al. 1985). During the experimental period, the ambient mean minimum temperatures declined to 7.4°C and peaked at 18.4°C. Ambient mean daily temperatures, calculated as an average of daily minima and maxima, ranged between 13.0°C and 25.4°C for the experimental period. South Africa has considerable variation in climatic conditions, and the growth of *P. crassipes* responds with declines in growth and concurrent dormancy phases during the cold winter periods and rapid growth at the onset of spring (Hill & Olckers 2001). Current temperate climatic conditions found in South Africa and specifically in the Eastern Cape may inhibit the potential stimulation response of eCO_2 in *P. crassipes* and may contribute to the acclimation of the carbon assimilation mechanisms at eCO_2 observed in this study. Under future global climate change, the increasing temperatures expected to occur in southern Africa may contribute to the increased invasive potential of *P. crassipes* (Hoveka et al. 2016).

Stomatal restrictions are a common response by many plants under eCO_2 conditions, which are intrinsically linked to assimilation rates (Sellers et al. 1996; Medlyn et al. 2001; Ainsworth & Rogers 2007; Pastore et al. 2019) and water use efficiency in plants (Pastore et al. 2019; Zhang et al. 2019; Mathias & Thomas 2021). Under eCO_2 conditions and in the absence of herbivory, *P. crassipes* experienced a 13% decline in stomatal conductance, however, this “diffusal limitation” is not ascribed

as the driver of acclimation in many plant species (Irigoyen et al. 2014), especially in the case of an aquatic plant such as *P. crassipes*. Studies on alfalfa (*Medicago sativa* L. cv. Aragón) have shown that with declines in stomatal conductance of 48%, stomatal aperture still allowed for the adequate inflow of CO_2 into the leaves for stimulated growth (Aranjuelo et al. 2005). In this study, intercellular carbon concentrations (Ci) vs external carbon concentrations (Ca) of *P. crassipes* plants grown under eCO_2 matched those of plants grown at aCO_2, regardless of Ca concentrations. This suggests that Ci increases within the leaf under eCO_2 conditions even when stomata experience restrictions in aperture, as shown in this study.

The invasive potential of noxious alien plants may 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, no firm evidence supports an increase in the invasive potential of *P. crassipes* under eCO_2 conditions alone. However, factors such as current temperature limitations on growth (Knipling et al. 1970; Kumar et al. 1985; Long 1991; Hill & Olckers 2001) and nutrient accessibility due to space restriction or lack of flow (Poorter et al. 2012) may have mitigated the potential enhancements in growth expected in this study.

4.3 Effect of eCO_2 on the impact of biological control agents

4.3.1 The impact of eCO_2 and insect herbivory on *Pontederia crassipes*

The impact of eCO_2 conditions on plant-insect interactions has prompted significant work on the direct effects of eCO_2 on plant physiology and the indirect cascading effects this will have on insect herbivory (Sage & Coleman 2001; Robinson et al. 2012; Reeves 2017). The same cannot be said for how insect biological control herbivory may mitigate the stimulating effects of eCO_2 on plant physiology, especially in an aquatic environment (Baso et al. 2021). Under current aCO_2 conditions, and when nutrients are not limited, *P. crassipes* can sufficiently tolerate herbivory based on herbivore density, improved growth and biomass renewal (Soti & Volin 2010). In this study, herbivory by *C. aquaticum* at eCO_2 led to notable down-regulation of the photosynthetic capacity of *P. crassipes*. It must be noted

that eCO_2 conditions still allowed for higher Ci within *P. crassipes*, providing greater CO_2 substrate for photosynthesis, shown by the marginal increase in net assimilation rate at eCO_2. As a result, *P. crassipes* experienced an increase in turnover and compensatory growth response in leaf biomass 18% whilst under eCO_2 conditions, and a marginal 2% increase in C:N ratio through increased carbon accumulation in the leaves. This suggests that eCO_2 conditions accentuated *P. crassipes'* ability to tolerate chewing herbivory, allowing for enhanced renewal and compensatory growth to leaf biomass compartments lost through *C. aquaticum* herbivory. 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. (2013b) 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 reduced in *C. aquaticum*. The study suggested that the biological control effort would be vastly improved if nutrient concentrations in invaded South African water bodies were reduced (Bownes et al. 2013a, 2013b).

Under aCO_2 conditions, phloem-feeding herbivory may effectively reduce the assimilation rates of many plant species (Welter 1993; Welter 2019; Zvereva et al. 2010; White et al. 2016). In this study, eCO_2 conditions exacerbated this pattern of phloem-feeders. Herbivory by *M. scutellaris* resulted in a substantial decline in photosynthetic capacity, greater than that by *C. aquaticum*, as well as marked declines in net assimilation rates at eCO_2. *Pontederia crassipes* foliar nitrogen was negatively impacted by *M. scutellaris* herbivory at eCO_2, by 15%. Feeding by phloem-feeders acts as nutrient sinks, removing metabolites directly from the phloem and xylem and interrupting source to sink transport flow of nutrients within the plant (Larson and Whitham 1991, 1997; see meta-analysis Zvereva et al. 2010). In this study, a net increase in C:N ratio was observed as a result of nitrogen dilution in those plants impacted by *M. scutellaris* phloem-feeding at eCO_2. Root biomass is considered a carbon sink, and the eCO_2-enhanced interruption by *M. scutellaris* resulted in enhanced compensatory growth of

root biomass by 15%, suggesting the *P. crassipes* was allocating resources to nutrient access in an attempt to overcome this form of herbivory.

4.3.2 Effect of eCO_2 on insect herbivore responses

In this study, plant responses to eCO_2 had mixed effects on insect feeding responses and population dynamics. Elevated CO_2 conditions did not result in the hypothesised enhanced feeding response in *C. aquaticum* outlined by the compensatory feeding hypothesis (Lincoln et al. 1986; Bezemer & Jones 1998; Schädler et al. 2007; Robinson et al. 2012). Rather, feeding by *C. aquaticum* remained similar between aCO_2 and eCO_2 conditions. This has been attributed to the marginal change in the C:N ratio due to high nutrient conditions of this experiment; the accustomed acclimation of *P. crassipes* photosynthetic capacity (Larigauderie et al. 1986; Spencer & Bowes 1986); and the further down-regulation of photosynthetic capacity due to *C. aquaticum* herbivory.

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 and population trends (81% increase in adult density) of many phloem-feeders under eCO_2 conditions (Bezemer & Jones 1998; Coviella & Trumble 1999; Hunter 2001; Stiling & Cornelissen 2007; Robinson et al. 2012).

4.3.3 The efficacy of biological control agents at eCO_2

Elevated CO_2 conditions will alter plant biochemistry, resulting in cascading effects on biological control agents' impacts and their efficacy in controlling invasive plants in future predicted climates (Zvereva & Kozlov 2010; DeLucia et al. 2012). In the context of biological control, for an agent to be successful, it must show the ability to maintain ‘complete’ or ‘substantial’ control on the host plant (Hoffmann 1995; McFadyen 1998); 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 chewing herbivory of *C. aquaticum* and phloem-feeding by *M. scutellaris* will continue to have varying degrees of success even under eCO_2 conditions. Yet, of the two agents representing two distinct feeding guilds, the evidence here suggests that *M. scutellaris* will be the more effective biological control agent under predicted eCO_2 conditions. Under eCO_2, the enhanced population density and impact on assimilation rates of *M. scutellaris* show the potential for substantial control of *P. crassipes* if population densities of the agent reach sufficient numbers (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 the same level of control as that predicted by phloem-feeding agents such as *M. scutellaris*.

4.4 Conclusion

Biological control is a well-supported tool in invasive plant management, being both environmentally sustainable and more economically viable than other control options (McFadyen 1998). Under current atmospheric CO_2 conditions, agent efficacy has proven to be more than adequate in controlling invasive populations of some of the world's most problematic aquatic plants (Hill & Coetzee 2017). However, future increases in anthropogenically derived atmospheric CO_2 will directly impact plant photosynthesis and other aspects of plant metabolism across the globe (Bowes et al. 1996; Sage et al. 1994), and it is suggested that eCO_2 conditions will have a disproportionally positive effect on invasive plant species (Ziska 2003; Hellmann et al. 2008; Ziska & Dukes 2011). eCO_2 will thus impact plant-insect interactions and has been discussed in detail in the past (Lincoln et al. 1984; Bezemer & Jones 1998; Stiling & Cornelissen 2007; Robinson et al. 2012), yet the literature is lacking on the responses of insect biological control agents to host plants grown under eCO_2 conditions (Shabbir et al. 2019). This knowledge gap has thus called into question the efficacy of current biological control agents under future conditions (Thomson et al. 2010).

Previously theorised plant-insect interactions under eCO_2 conditions largely held true for a typical aquatic plant, such as *P. crassipes,* and its biological control agents. However, eCO_2 did illicit some species-specific responses. As predicted, eCO_2 resulted in acclimation of photosynthetic capacity in *P. crassipes* (Larigauderie et al. 1986; Spencer & Bowes 1986), yet *P. crassipes* still experienced slight increases in net assimilation rates as a result of eCO_2 increasing Ci. This had cascading effects on the invasive potential of the aquatic plant and the biological control effort. *P. crassipes* under eCO_2 conditions and *C. aquaticum* herbivory experienced a slight decline in net assimilation rates and considerable compensatory growth of leaf biomass in response to biomass removal by the agent. On the other hand, herbivory by *M. scutellaris* under eCO_2 resulted in extensive declines in photosynthetic capacity, net assimilation rates, and notable compensatory growth of root biomass. Contrary to the majority of the literature (Bezemer & Jones 1998; Stiling & Cornelissen 2007; Cornelissen 2011; Robinson et al. 2012), *C. aquaticum* did not experience the compensatory feeding responses to eCO_2 conditions experienced by many mobile chewing insects. *Megamelus scutellaris* experienced significant increases in population density due to eCO_2 conditions, which is in line with the response of model phloem-feeders to eCO_2 (Bezemer & Jones 1998; Hunter 2001; Robinson et al. 2012).

In their meta-analysis, Stiling & Cornelissen (2005) cited that mobile chewing insects represent 41% of all biological control agents globally, specifically the order Coleoptera; while the majority of studies on plant-insect interactions of phloem-feeders under eCO_2 utilise the Aphididae as model species. This thesis expands biological control agent selection (van den Bosch 1971; Colfer & Rosenheim 2001) outside of the charismatic mobile chewing insects as well as provides some novel insight into eCO_2 response of phloem-feeders representing the Delphacidae.

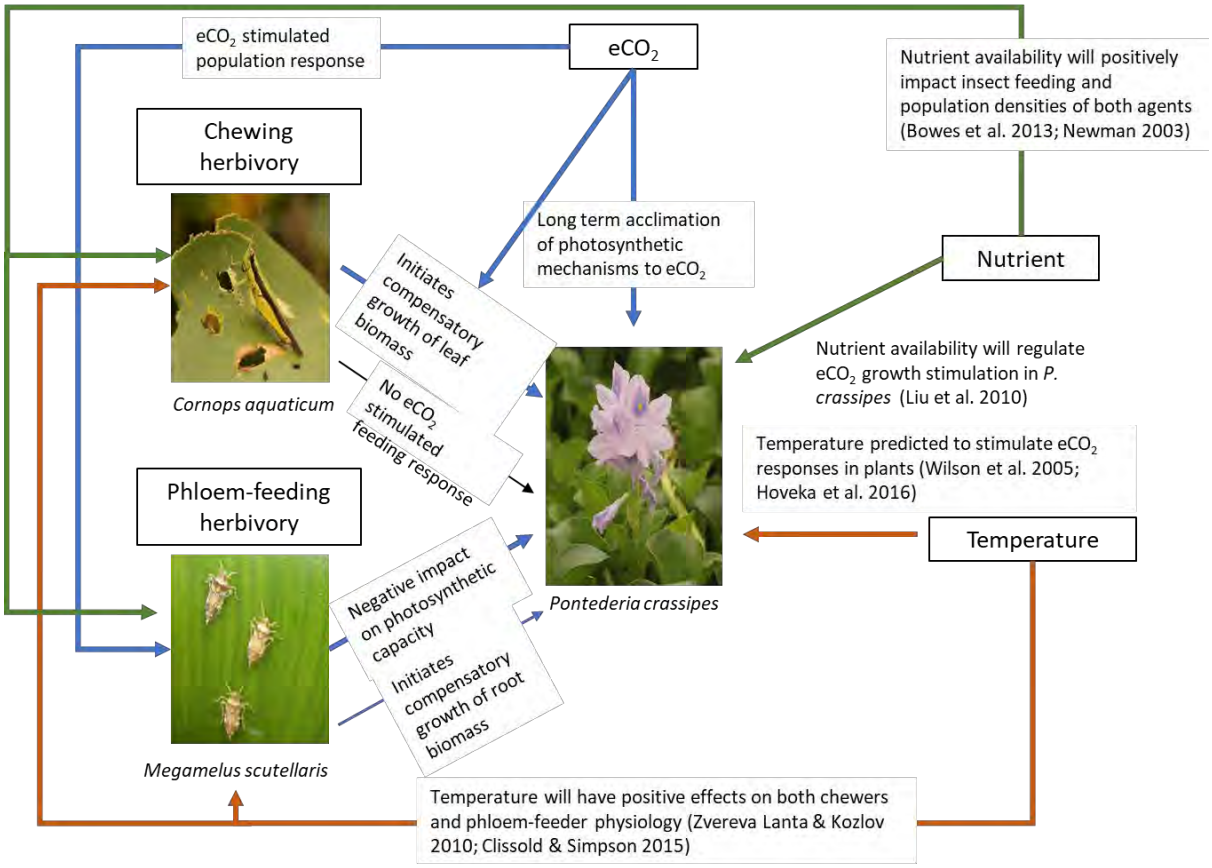


Figure 4.4.1: A conceptual model to illustrate the complexities and interactions affecting plant-insect interactions under changing conditions, illustrating the influence of eCO_2 from this study and the influence of temperature and nutrients from the literature on plant-insect interactions for *Pontederia crassipes* and two of its biological control agents, *Cornops aquaticum* and *Megamelus scutellaris,* representing chewing and phloem feeding respectively. Arrow thickness designates force of effect. Blue arrows indicate the eCO_2 effect, orange indicates the increasing temperature effect, and green arrows indicate nutrient effects on plant-insect interactions

In attempting to understand the impact of eCO_2 on biological control plant-insect interactions, one must also consider the potential drivers of invasions and the factors that would affect biological control in future climates, namely elevated ambient temperature (Wilson et al. 2007; Hellmann et al. 2008) and nutrients (Hill & Cilliers 1999; Liu et al. 2010). Here, I developed a conceptual model to illustrate the complexities and interactions affecting plant-insect interactions under these conditions

(Figure 4.4.1). Using data from this study, I attempt to expand the understanding of the impact of eCO_2 and relate this study to the extensive literature on the impacts of growth conditions on biological control. The findings of this study has implications for the biological control programme and insect-plant interactions under eCO_2 conditions. By understanding the impact of feeding guild responses to eCO_2 in a biological control context, a new facet of study on the future efficacy of the agent has been suggested here. If the biological control programme across all systems is to remain effective in a changing world, the impacts of eCO_2 and predicted climate change need to be considered when testing future agents.

Reference list

Adis, J., Bustorf, E., Lhano, M.G., Amedegnato, C. and Nunes, A.L., 2007. Distribution of Cornops grasshoppers (Leptysminae: Acrididae: Orthoptera) in Latin America and the Caribbean Islands. *Studies on Neotropical Fauna and Environment*, *42*(1), pp.11-24.

Adis, J., Sperber, C.F., Brede, E.G., Capello, S., Franceschini, M.C., Hill, M., Lhano, M.G., Nunes, A.L. and Polar, P., 2008. Morphometric differences in the grasshopper *Cornops aquaticum* (Bruner, 1906) from South America and South Africa. *Journal of Orthoptera Research*, *17*(2), pp.141-147.

Agrell, J., Kopper, B., McDonald, E.P. and Lindroth, R.L., 2005. CO_2 and O_3 effects on host plant preferences of the forest tent caterpillar (*Malacosoma disstria*). *Global Change Biology*, *11*(4), pp.588-599.

Ainsworth, E.A. and Long, S.P., 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist*, *165*(2), pp.351-372.

Ainsworth, E.A. and Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell & Environment*, *30*(3), pp.258-270.

Ainsworth, E.A., Davey, P.A., Hymus, G.J., Osborne, C.P., Rogers, A., Blum, H., Nösberger, J. and Long, S.P., 2003. Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO_2Enrichment (FACE). *Plant, Cell & Environment*, *26*(5), pp.705-714.

Andresen, L.C., Müller, C., De Dato, G., Dukes, J.S., Emmett, B.A., Estiarte, M., Jentsch, A., Kröel-Dulay, G., Lüscher, A., Niu, S. and Peñuelas, J., 2016. Shifting impacts of climate change: Long-term patterns of plant response to elevated CO_2, drought, and warming across ecosystems. *Advances in Ecological Research*, *55*, pp.437-473.

Aranjuelo, I., Pérez, P., Hernández, L., Irigoyen, J.J., Zita, G., Martínez-Carrasco, R. and Sánchez-Díaz, M., 2005. The response of nodulated alfalfa to water supply, temperature and elevated CO_2: photosynthetic downregulation. *Physiologia Plantarum*, *123*(3), pp.348-358.

Arora, R.A.M.E.S.H. and Dhawan, A.K., 2013. Climate change and insect pest management. *Integrated Pest Management.* Scientific Publisher*,* India, pp.44-60.

Arora, V.K., Boer, G.J., Friedlingstein, P., Eby, M., Jones, C.D., Christian, J.R., Bonan, G., Bopp, L., Brovkin, V., Cadule, P. and Hajima, T., 2013. Carbon–concentration and carbon–climate feedbacks in CMIP5 Earth system models. *Journal of Climate*, *26*(15), pp.5289-5314.

Arp, R.S., Fraser, G.C.G. and Hill, M.P., 2017. Quantifying the economic water savings benefit of water hyacinth (*Eichhornia crassipes*) control in the Vaalharts Irrigation Scheme. *Water SA*, *43*(1), pp.58-66.

Arp, W.J., 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant, Cell & Environment*, *14*(8), pp.869-875.

Asshoff, R. and Hättenschwiler, S., 2006. Changes in needle quality and larch bud moth performance in response to CO_2 enrichment and defoliation of treeline larches. *Ecological Entomology*, *31*(1), pp.84-90.

Atkinson, D., 1994. Temperature and organism size: a biological law for ectotherms? *Advances in Ecological Research*, *25*, pp.1-58.

Aurand, D., 1982. *Nuisance aquatic plants and aquatic plant management programs in the United States Vol 2, South Eastern Region*. The Mitre Corporation, McLean.

Ayres, M.P. and Lombardero, M.J., 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *Science of the Total Environment*, *262*(3), pp.263-286.

Bader, M.K.F., Siegwolf, R. and Körner, C., 2010. Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. *Planta*, *232*(5), pp.1115-1125.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J. and Good, J.E., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), pp.1-16.

Baso, N.C., Coetzee, J.A., Ripley, B.S. and Hill, M.P., 2021. The effects of elevated atmospheric CO_2 concentration on the biological control of invasive aquatic weeds. *Aquatic Botany*, *170*, p.103348.

Bassirirad, H., Gutschick, V.P. and Lussenhop, J., 2001. Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO_2. *Oecologia*, *126*(3), pp.305-320.

Bates, D., Kliegl, R., Vasishth, S. and Baayen, H., 2015. Parsimonious mixed models. *arXiv preprint arXiv:1506.04967*.

Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A. and Larsson, S., 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications*, *15*(6), pp.2084-2096.

Beck, S.D., 1983. Insect thermoperiodism. *Annual Review of entomology*, *28*(1), pp.91-108.

Berg, E.E., Henry, J.D., Fastie, C.L., De Volder, A.D. and Matsuoka, S.M., 2006. Spruce beetle outbreaks on the Kenai Peninsula, Alaska, and Kluane National Park and Reserve, Yukon Territory: relationship to summer temperatures and regional differences in disturbance regimes. *Forest Ecology and Management*, *227*(3), pp.219-232.

Bezemer, T.M. and Jones, T.H., 1998. Plant-insect herbivore interactions in elevated atmospheric CO_2 quantitative analyses and guild effects. *Oikos*, pp.212-222.

Blossey, B. and Hunt, T.R., 1999. Mass rearing methods for *Galerucella calmariensis* and *G. pusilla* (Coleoptera: Chrysomelidae), biological control agents of *Lythrum salicaria* (Lythraceae). *Journal of Economic Entomology*, *92*(2), pp.325-334.

Blossey, B., 2011. Enemy release hypothesis. In *Encyclopedia of Biological Invasions*. University of California Press. Los Angeles, California, pp.193-196.

Bowes, G., 1991. Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. *Plant, Cell & Environment*, *14*(8), pp.795-806.

Bowes, G., 1996. Photosynthetic responses to changing atmospheric carbon dioxide concentration. In Baker, N.R. (ed.) *Photosynthesis and the Environment*, Springer, Dordrecht. pp. 387-407.

Bowes, G., Vu, J.C., Hussain, M.W., Pennanen, A.H. and Allen, L.H., 1996. An overview of how rubisco and carbohydrate metabolism may be regulated at elevated atmospheric [CO_2] and temperature. *Agricultural and Food Science*, *5*(3), pp.261-270.

Bownes, A., Hill, M.P. and Byrne, M.J., 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*(3), pp.297-303.

Bownes, A., Hill, M.P. and Byrne, M.J., 2013a. Nutrient-mediated effects on *Cornops aquaticum* Brüner (Orthoptera: Acrididae), a potential biological control agent of water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae). *Biological Control*, *67*(3), pp.548-554.

Bownes, A., Hill, M.P. and Byrne, M.J., 2013b. 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*(3), pp.555-562.

Bownes, A., King, A. and Nongogo, A., 2011, September. Pre-release studies and release of the grasshopper *Cornops aquaticum* in South Africa–a new biological control agent for water hyacinth, *Eichhornia crassipes.* In *XIII International Symposium on Biological Control of Weeds. Suráfrica* (pp. 3-13).

Bradley, B.A., Blumenthal, D.M., Wilcove, D.S. and Ziska, L.H., 2010. Predicting plant invasions in an era of global change. *Trends in Ecology & Evolution*, *25*(5), pp.310-318.

Brooks, G.L. and Whittaker, J.B., 1998. Responses of multiple generations of *Gastrophysa viridula*, feeding on *Rumex obtusifolius*, to elevated CO_2. *Global Change Biology*, *4*(1), pp.63-75.

Brooks, M.L. and Esque, T.C., 2002. Alien plants and fire in desert tortoise (*Gopherus agassizii*) habitat of the Mojave and Colorado deserts. *Chelonian Conservation and Biology*, *4*, pp.330-340.

Bryant, J., Taylor, G. and Frehner, M., 1998. Photosynthetic acclimation to elevated CO_2 is modified by source: sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. *Plant, Cell & Environment*, *21*(2), pp.159-168.

Burnett, A.C., Rogers, A., Rees, M. and Osborne, C.P., 2016. Carbon source–sink limitations differ between two species with contrasting growth strategies. *Plant, Cell & Environment*, *39*(11), pp.2460-2472.

Callaway, J.M., 2004. The benefits and costs of adapting to climate variability and climate change. *The Benefits of Climate Change Policies*, pp.111-157.

Center, T.D. and Dray Jr, F.A., 2010. Bottom-up control of water hyacinth weevil populations: do the plants regulate the insects? *Journal of Applied Ecology*, *47*(2), pp.329-337.

Chamier, J., Schachtschneider, K., Le Maitre, D.C., Ashton, P.J. and Van Wilgen, B.W., 2012. Impacts of invasive alien plants on water quality, with particular emphasis on South Africa. *Water SA*, *38*(2), pp.345-356.

Chapin, F.S., Bloom, A.J., Field, C.B. and Waring, R.H., 1987. Plant responses to multiple environmental factors, *Bioscience*, *37*(1), pp.49-57.

Chytrý, M., Maskell, L.C., Pino, J., Pyšek, P., Vilà, M., Font, X. and Smart, S.M., 2008. Habitat invasions by alien plants: a quantitative comparison among Mediterranean, subcontinental and oceanic regions of Europe. *Journal of Applied Ecology*, *45*(2), pp.448-458.

Cilliers, C.J., 1991. Biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae), in South Africa. *Agriculture, Ecosystems & Environment*, *37*(1-3), pp.207-217.

Cock, M.J., van Lenteren, J.C., Brodeur, J., Barratt, B.I., Bigler, F., Bolckmans, K., Cônsoli, F.L., Haas, F., Mason, P.G. and Parra, J.R.P., 2010. Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? *BioControl*, *55*(2), pp.199-218.

Coetzee, J.A. and Hill, M.P., 2012. The role of eutrophication in the biological control of water hyacinth, *Eichhornia crassipes*, in South Africa. *BioControl*, *57*(2), pp.247-261.

Coetzee, J.A., Byrne, M.J. and Hill, M.P., 2007. Impact of nutrients and herbivory *by Eccritotarsus catarinensis* on the biological control of water hyacinth, *Eichhornia crassipes*. *Aquatic Botany*, *86*(2), pp.179-186.

Coetzee, J.A., Jones, R.W. and Hill, M.P., 2014. Water hyacinth, *Eichhornia crassipes* (Pontederiaceae), reduces benthic macroinvertebrate diversity in a protected subtropical lake in South Africa. *Biodiversity and Conservation*, *23*(5), pp.1319-1330.

Coetzee, J.A., Hill, M.P., Byrne, M.J. and 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*(1), pp.451-468.

Coetzee, J.A., Bownes, A., Martin, G.G., Miller, B.E., Smith, R., Weyl, P.S.R. and Hill, M.P. 2021, A review of the biocontrol programmes against aquatic weeds in South Africa. *African Entomology* (in press).

Colfer, R.G. and Rosenheim, J.A., 2001. Predation on immature parasitoids and its impact on aphid suppression. *Oecologia*, *126*(2), pp.292-304.

Convention on Biological Diversity (2022) Decision VI/23* of the Conference of the Parties to the CBD, Annex, Footnote to the Introduction. https://www.cbd.int/invasive/terms.shtml. Accesssed March 1, 2022.

Cornelissen, T., 2011. Climate change and its effects on terrestrial insects and herbivory patterns. *Neotropical Entomology*, *40*, pp.155-163.

Coviella, C.E. and Trumble, J.T., 1999. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conservation Biology*, *13*(4), pp.700-712.

Crookes, C., Hedden, S. and Donnenfeld, Z., 2018. A delicate balance: Water scarcity in South Africa. *ISS Southern Africa Report*, *2018*(13), pp.1-24.

Darwin, C., 1996. The Origin of Species. Oxford University Press, London.

DeLucia, E.H., Nabity, P.D., Zavala, J.A. and Berenbaum, M.R., 2012. Climate change: resetting plant-insect interactions. *Plant Physiology*, *160*(4), pp.1677-1685.

Diaz, R., Manrique, V., He, Z. and Overholt, W.A., 2012. Effect of elevated CO_2 on tropical soda apple and its biological control agent *Gratiana boliviana* (Coleoptera: Chrysomelidae). *Biocontrol Science and Technology*, *22*(7), pp.763-776.

Diez, J.M., D'Antonio, C.M., Dukes, J.S., Grosholz, E.D., Olden, J.D., Sorte, C.J., Blumenthal, D.M., Bradley, B.A., Early, R., Ibáñez, I. and Jones, S.J., 2012. Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment*, *10*(5), pp.249-257.

Docherty, M., Wade, F., Hurst, D., Whittaker, J. and Lea, P., 1997. Responses of tree sap-feeding herbivores to elevated CO_2. *Global Change Biology*, *3*(1), pp.51-59.

Drake, B.G., Gonzàlez-Meler, M.A. and Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Biology*, *48*(1), pp.609-639.

Drake, B.G., Peresta, G., Beugeling, E. and Matamala, R., 1996. *Long-term elevated CO_2 exposure in a Chesapeake Bay wetland: ecosystem gas exchange, primary production, and tissue nitrogen*. Academic Press, San Diego, pp.197-214.

Dube, T. and Phiri, K., 2013. Rural livelihoods under stress: The impact of climate change on livelihoods in South Western Zimbabwe. *American International Journal of Contemporary Research*, *3*(5), pp.11-25.

Dukes, J.S. and Mooney, H.A., 2004. Disruption of ecosystem processes in western North America by invasive species. *Revista Chilena de Historia Natural*, *77*(3), pp.411-437.

Duursma, R.A., 2015. Plantecophys-an R package for analysing and modelling leaf gas exchange data. *PloS One*, *10*(11), p.e0143346.

Ehleringer, J. and Björkman, O., 1977. Quantum yields for CO_2 uptake in C_3 and C_4 plants: dependence on temperature, CO_2, and O_2 concentration. *Plant Physiology*, *59*(1), pp.86-90.

Ehleringer, J.R., and Cerling, T.E., 2002. C3 and C4 photosynthesis. In Mooney, H.A. and Canadell, J.G. (eds.), *Encoclopedia of Global Environmental Change Vol 2*. Wiley and Sons, New York, pp.186–190.

Elton, C.S., 1958. The reasons for conservation. In Elton, C.S. (ed.), *The ecology of invasions by animals and plants*. Springer, Boston, pp.143-153.

Engelbrecht, F., Adegoke, J., Bopape, M.J., Naidoo, M., Garland, R., Thatcher, M., McGregor, J., Katzfey, J., Werner, M., Ichoku, C. and Gatebe, C., 2015. Projections of rapidly rising surface temperatures over Africa under low mitigation. *Environmental Research Letters*, *10*(8), p.085004.

Facey, S.L., Ellsworth, D.S., Staley, J.T., Wright, D.J. and Johnson, S.N., 2014. Upsetting the order: how climate and atmospheric change affects herbivore–enemy interactions. *Current Opinion in Insect Science*, *5*, pp.66-74.

Fajer, E.D., Bowers, M.D. and Bazzaz, F.A., 1989. The effects of enriched carbon dioxide atmospheres on plant—insect herbivore interactions. *Science*, *243*(4895), pp.1198-1200.

Falkenmark, M., 2013. Adapting to climate change: towards societal water security in dry-climate countries. *International Journal of Water Resources Development*, *29*(2), pp.123-136.

Farnworth, E.J., Ellison, A.M. and Gong, W.K., 1996. Elevated CO_2 alters anatomy, physiology, growth, and reproduction of red mangrove (*Rhizophora mangle* L.). *Oecologia*, *108*(4), pp.599-609.

Ferreira, S.A. and Vasconcellos-Neto, J., 2001. Host plants of the grasshopper *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae) in the wetland of Poconé, MT, Brazil. *Neotropical Entomology*, *30*, pp.523-533.

Field, C.B. and Barros, V.R. (eds.), 2014. *Climate change 2014–Impacts, adaptation and vulnerability: Regional aspects*. Cambridge University Press, Cambridge, pp.668.

Fitzgerald, D. and Tipping, P.W., 2013. Effect of insect density and host plant quality on wing-form in *Megamelus scutellaris* (Hemiptera: Delphacidae). *Florida Entomologist*, pp.124-130.

Gezie, A., Assefa, W.W., Getnet, B., Anteneh, W., Dejen, E. and Mereta, S.T., 2018. Potential impacts of water hyacinth invasion and management on water quality and human health in Lake Tana watershed, Northwest Ethiopia. *Biological Invasions*, *20*(9), pp.2517-2534.

Gifford, R.M., 1995. Whole plant respiration and photosynthesis of wheat under increased CO_2 concentration and temperature: long-term vs. short-term distinctions for modelling. *Global Change Biology*, *1*(6), pp.385-396.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. and Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science*, *293*(5538), pp.2248-2251.

Gomi, T., 2007. Seasonal adaptations of the fall webworm *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) following its invasion of Japan. *Ecological Research*, *22*(6), pp.855-861.

Gorgens, A.H.M. and Van Wilgen, B.W., 2004. Invasive alien plants and water resources in South Africa: current understanding, predictive ability and research challenges: Working for Water. *South African Journal of Science*, *100*(1), pp.27-33.

Gossett, D.R. and Norris, W.E., 1971. Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, *Eichhornia crassipes* (Mart.) Solms. *Hydrobiologia*, *38*(1), pp.15-28.

Griffin, K.L. and Seemann, J.R., 1996. Plants, CO_2 and photosynthesis in the 21st century. *Chemistry & Biology*, *3*(4), pp.245-254.

Gunderson, C.A. and Wullschleger, S.D., 1994. Photosynthetic acclimation in trees to rising atmospheric CO_2: a broader perspective. *Photosynthesis Research*, *39*(3), pp.369-388.

Hartig F., 2018. Package 'DHARMa.' http://florianhartig.github.io/DHARMa/. https://cran.r-project.org/web/packages/DHARMa/DHARMa.pdf. Accessed 12 January 2021.

Heard, T.A. and Winterton, S.L., 2000. Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *Journal of Applied Ecology*, *37*(1), pp.117-127.

Hellmann, J.J., Byers, J.E., Bierwagen, B.G. and Dukes, J.S., 2008. Five potential consequences of climate change for invasive species. *Conservation Biology*, *22*(3), pp.534-543.

Henderson, L. and Cilliers, C.J., 2002. Invasive Aquatic Plants Plant Protection Research Institute Handbook No. 16. Paarl Printers, Cape Town, pp.1-88.

Henry, T.J., 2017. Description of a cryptic new species of the plant bug genus *Eccritotarsus* (Heteroptera: Miridae: Bryocorinae) from Peru, a new biocontrol agent of water hyacinth, *Eichhornia crassipes* (Pontederiaceae). *Proceedings of the Entomological Society of Washington*, *119*(3), pp.398-407.

Hernández, M.C., Brentassi, M.E., Sosa, A.J., Sacco, J. and Elsesser, G., 2011. Feeding behaviour and spatial distribution of two planthoppers, *Megamelus scutellaris* (Delphacidae) and *Taosa longula* (Dictyopharidae), on water hyacinth. *Biocontrol Science and Technology*, *21*(8), pp.941-952.

Hill, M. P., and C. J. Cilliers., 1999. A review of the arthropod natural enemies, and factors that influence their efficacy, in the biological control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae), in South Africa. In Olckers, T. and Hill, M.P. (eds.), Biological Control of Weeds in South Africa (1990-1998). *African Entomology Memoir 1*, pp.103-112.

Hill, M.P. and Coetzee, J., 2017. The biological control of aquatic weeds in South Africa: Current status and future challenges. *Bothalia-African Biodiversity & Conservation*, *47*(2), pp.1-12.

Hill, M.P. and McConnachie, A.J., 2009. *Azolla filiculoides*. *Biological control of tropical weeds using arthropods*, Cambridge University Press. Cambridge, UK, pp.74-87

Hill, M.P. and Olckers, T. 2001. Biological control initiatives against water hyacinth in South Africa: constraining factors, success and new courses of action. In Julien, M.H., Hill, M.P., Center, T. and Ding, J. (eds.), *Proceedings of the Meeting of the Global Working Group for the Biological Control and Integrated Control of Water Hyacinth Proceedings 102*. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia. pp.33–38.

Hill, M.P., 2003. The impact and control of alien aquatic vegetation in South African aquatic ecosystems. *African Journal of Aquatic Science*, *28*(1), pp.19-24.

Hoffmann, J.H., 1995. *Biological control of weeds: the way forward, a South African perspective*. Proceedings of the British Crop Protection Council Symposium: Weeds in a Changing World. British Crop Protection Council, Farnham, pp.77-89.

Hoveka, L.N., Bezeng, B.S., Yessoufou, K., Boatwright, J.S. and 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*, pp.33-38.

Huber, S.C., Rogers, H.H. and Mowry, F.L., 1984. Effects of water stress on photosynthesis and carbon partitioning in soybean (*Glycine max* [L.] Merr.) plants grown in the field at different CO_2 levels. *Plant Physiology*, *76*(1), pp.244-249.

Hunter, M.D., 2001. Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Agricultural and Forest Entomology*, *3*(3), pp.153-159.

Idso, S.B., Kimball, B.A., Anderson, M.G. and Mauney, J.R., 1987. Effects of atmospheric CO_2 enrichment on plant growth: the interactive role of air temperature. *Agriculture, Ecosystems & Environment*, *20*(1), pp.1-10.

Irigoyen, J.J., Goicoechea, N., Antolín, M.C., Pascual, I., Sánchez-Díaz, M., Aguirreolea, J. and Morales, F., 2014. Growth, photosynthetic acclimation and yield quality in legumes under climate change simulations: an updated survey. *Plant Science*, *226*, pp.22-29.

Jafari, N., 2010. Ecological and socio-economic utilization of water hyacinth (*Eichhornia crassipes* Mart Solms). *Journal of Applied Sciences and Environmental Management*, *14*(2).

Jansen, E., Overpeck, J., Briffa, K.R., Duplessy, J.C., Joos, F., Masson-Delmotte, V., Olago, D., Otto-Bliesner, B., Peltier, W.R., Rahmstorf, S., Ramesh, R., Raynaud, D., Rind, D., Solomina, O., Villalba, R. and Zhang, D. (eds.), 2007. *Palaeoclimate Chapter 6*, Cambridge University Press, Cambridge, pp.433-97.

Jifon, J.L. and Wolfe, D.W., 2002. Photosynthetic acclimation to elevated CO_2 in *Phaseolus vulgaris* L. is altered by growth response to nitrogen supply. *Global Change Biology*, *8*(10), pp.1018-1027.

Johnson, R.H. and Lincoln, D.E., 1991. Sagebrush carbon allocation patterns and grasshopper nutrition: the influence of CO_2 enrichment and soil mineral limitation. *Oecologia*, *87*(1), pp.127-134.

Julien, M., 2008. Plant biology and other issues that relate to the management of water hyacinth: a global perspective with focus on Europe 1. *EPPO Bulletin*, *38*(3), pp.477-486.

Keane, R.M. and Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, *17*(4), pp.164-170.

Lodge, D.M., Lewis, M.A., Shogren, J.F. and Keller, R.P., 2009. Introduction to biological invasions: biological, economic and social perspectives. In Keller, R.P., Lodge, D.M., Lewis, M.A., and Shogren, J.F. (eds.), *Bioeconomics of Invasive Species: Integrating Ecology, Economics, Policy, and Management*, Oxford University Press, Oxford, pp.1-24.

Kiritani, K., 2006. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. *Population Ecology*, *48*(1), pp.5-12.

Klein, H., 2011. A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in South Africa. *African Entomology*, *19*(1), pp.515-549.

Knipling, E.B., West, S.H. and Haller, W.T., 1970. Growth characteristics, yield potential, and nutritive content of Water Hyacinths. *Proceedings, Soil and Crop Science Society of Florida* Vol. 30, pp.51-63.

Kolar, C.S. and Lodge, D.M., 2000. Freshwater nonindigenous species: interactions with other global changes. *Invasive Species in a Changing World*, pp.3-30.

Körner, C., 2006. Plant CO_2 responses: an issue of definition, time and resource supply. *New Phytologist*, *172*(3), pp.393-411.

Kowarik, I., 1995. Time lags in biological invasions with regard to the success and failure of alien species. *Plant invasions: general aspects and special problems*, SPB Academic Publishing, Amsterdam. pp.15-38.

Kriticos, D.J. and Brunel, S., 2016. Assessing and managing the current and future pest risk from water hyacinth, (*Eichhornia crassipes*), an invasive aquatic plant threatening the environment and water security. *PloS one*, *11*(8), p.e0120054.

Kumar, L., 1985. Studies on vegetative reproduction rate of water hyacinth and water chestnut. *Indian Journal of Agricultural Research*, *19*, pp.54-58.

Lan, X., Hall, B.D., Dutton, G., Mühle, J. and Elkins, J.W., 2020. Atmospheric composition. In Dunn, R.J.H., Stanitski, D.M., Gobron, N., Willet, K.M., (eds.), *State of the Climate in 2018, Chapter 2: Global Climate Special Online Supplement to the Bulletin of the American Meteorological Society*, 101(8), pp. 70-75.

Larigauderie, A., Roy, J. and Berger, A., 1986. Long term effects of high CO_2 concentration on photosynthesis of water hyacinth (*Eichhornia crassipes* (Mart.) Solms). *Journal of Experimental Botany*, *37*(9), pp.1303-1312.

Larson, K.C. and Whitham, T.G., 1991. Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. *Oecologia*, *88*(1), pp.15-21.

Larson, K.C. and Whitham, T.G., 1997. Competition between gall aphids and natural plant sinks: plant architecture affects resistance to galling. *Oecologia*, *109*(4), pp.575-582.

Le Houérou, H.N., 1996. Climate change, drought and desertification. *Journal of Arid Environments*, *34*(2), pp.133-185.

Le Maitre, D.C., De Lange, W.J., Richardson, D.M., Wise, R.M. and Van Wilgen, B.W., 2011. The economic consequences of the environmental impacts of alien plant invasions in South Africa. In Pimentel D., (ed.), *Biological invasions: Economic and environmental costs of alien plant, animal, and microbe species*, CRC Press Inc, Baco Raton, pp.295-323.

Lincoln, D.E., Couvet, D. and Sionit, N., 1986. Response of an insect herbivore to host plants grown in carbon dioxide enriched atmospheres. *Oecologia*, *69*(4), pp.556-560.

Lincoln, D.E., Fajer, E.D. and Johnson, R.H., 1993. Plant-insect herbivore interactions in elevated CO_2 environments. *Trends in Ecology & Evolution*, *8*(2), pp.64-68.

Lincoln, D.E., Sionit, N. and Strain, B.R., 1984. Growth and feeding response of *Pseudoplusia includens* (Lepidoptera: Noctuidae) to host plants grown in controlled carbon dioxide atmospheres. *Environmental Entomology*, *13*(6), pp.1527-1530.

Lindroth, R.L., Arteel, G.E. and Kinney, K.K., 1995. Responses of three saturniid species to paper birch grown under enriched CO_2 atmospheres. *Functional Ecology*, pp.306-311.

Liu, J.Z., Ge, Y.M., Zhou, Y.F. and Tian, G.M., 2010. Effects of elevated CO_2 on growth and nutrient uptake of *Eichhornia crassipe* under four different nutrient levels. *Water, Air, & Soil Pollution*, *212*(1), pp.387-394.

Lloyd, J. and Farquhar, G.D., 1994. 13 C discrimination during CO_2 assimilation by the terrestrial biosphere. *Oecologia*, *99*(3), pp.201-215.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle, P.B., Smith, M., Andow, D.A. and Carlton, J.T., 2006. Biological invasions: recommendations for US policy and management. *Ecological Applications*, *16*(6), pp.2035-2054.

Logan, J.A., Régnière, J. and Powell, J.A., 2003. Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and the Environment*, *1*(3), pp.130-137.

Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? *Plant, Cell & Environment*, *14*(8), pp.729-739.

Long, S.P., Ainsworth, E.A., Rogers, A. and Ort, D.R., 2004. Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review Plant Biology*, *55*, pp.591-628.

Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M., 2000. *100 of the world's worst invasive alien species: a selection from the global invasive species database Vol. 12*. Invasive Species Specialist Group, Auckland, pp.11.

Mack, M.C. and D'Antonio, C.M., 1998. Impacts of biological invasions on disturbance regimes. *Trends in Ecology & Evolution*, *13*(5), pp.195-198.

Mäenpää, M., Riikonen, J., Kontunen-Soppela, S., Rousi, M. and Oksanen, E., 2011. Vertical profiles reveal impact of ozone and temperature on carbon assimilation of *Betula pendula* and *Populus tremula*. *Tree Physiology*, *31*(8), pp.808-818.

Makino, A. and Mae, T., 1999. Photosynthesis and plant growth at elevated levels of CO_2. *Plant and Cell Physiology*, *40*(10), pp.999-1006.

Martin, G.D. and Coetzee, J.A., 2011. Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA*, *37*(3).

Masifwa, W.F., Twongo, T. and 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*(1), pp.79-88.

Mathias, J.M. and Thomas, R.B., 2021. Global tree intrinsic water use efficiency is enhanced by increased atmospheric CO_2 and modulated by climate and plant functional types. *Proceedings of the National Academy of Sciences*, *118*(7).

Matthews, M.W. and Bernard, S., 2015. Eutrophication and cyanobacteria in South Africa's standing water bodies: A view from space. *South African Journal of Science*, *111*(5), pp.1-8.

May, B. and Coetzee, J., 2013. Comparisons of the thermal physiology of water hyacinth biological control agents: Predicting establishment and distribution pre-and post-release. *Entomologia Experimentalis et Applicata*, *147*(3), pp.241-250.

McCallum, M.L. and McCallum, J.L., 2014. Ecological release of an exotic species upon suppression of its invasive predator: A five-year case study, with notes on other species, and the life history of the Mediterranean gecko, *Hemidactylus turcicus*. *Journal of North American Herpetology*, pp.21-27.

McConnachie, A.J., De Wit, M.P., Hill, M.P. and Byrne, M.J., 2003. Economic evaluation of the successful biological control of *Azolla filiculoides* in South Africa. *Biological Control*, *28*(1), pp.25-32.

McConnachie, A.J., Hill, M.P. and Byrne, M.J., 2004. Field assessment of a frond-feeding weevil, a successful biological control agent of red waterfern, *Azolla filiculoides*, in southern Africa. *Biological control*, *29*(3), pp.326-331.

McFadyen, R.E.C., 1998. Biological control of weeds. *Annual Review of Entomology*, *43*(1), pp.369-393.

Medlyn, B.E., Barton, C.V.M., Broadmeadow, M.S.J., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S.B., Kellomäki, S., Laitat, E. and Rey, A., 2001. Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. *New Phytologist*, *149*(2), pp.247-264.

Meehl, G.A., Covey, C., Delworth, T., Latif, M., McAvaney, B., Mitchell, J.F., Stouffer, R.J. and Taylor, K.E., 2007. The WCRP CMIP3 multimodel dataset: A new era in climate change research. *Bulletin of the American Meteorological Society*, *88*(9), pp.1383-1394.

Messenger, P.S., 1959. Bioclimatic studies with insects. *Annual Review of Entomology*, *4*(1), pp.183-206.

Miller, B.E., Coetzee, J. and Hill, M., 2019. Chlorophyll fluorometry as a method of determining the effectiveness of a biological control agent in post-release evaluations. *Biocontrol Science and Technology*, *29*(11), pp.1118-1122.

Miller, B.E., Coetzee, J.A. and Hill, M.P., 2021. Mind the gap: the delayed recovery of a population of the biological control agent *Megamelus scutellaris* Berg. (Hemiptera: Delphacidae) on water hyacinth after winter. *Bulletin of Entomological Research*, *111*(1), pp.120-128.

Mitchell, C.E. and Power, A.G., 2003. Release of invasive plants from fungal and viral pathogens. *Nature*, *421*(6923), pp.625-627.

Mooney, H.A. and Saugier, B., 2001. *Terrestrial global productivity*. Academic Press, San Diego, pp.575.

Moore, B.D., Cheng, S.H., Sims, D. and Seemann, J.R., 1999. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. *Plant, Cell & Environment*, *22*(6), pp.567-582.

Moran, V.C. and Hoffmann, J.H., 2015. The fourteen international symposia on biological control of weeds, 1969–2014: delegates, demographics and inferences from the debate on non-target effects. *Biological Control*, *87*, pp.23-31.

Moran, V.C., Hoffmann, J.H. and Zimmermann, H.G.A.R.C., 2013. 100 years of biological control of invasive alien plants in South Africa: history, practice and achievements: news & views. *South African Journal of Science*, *109*(9), pp.1-6.

Morison, J.I.L. and Lawlor, D.W., 1999. Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant, Cell & Environment*, *22*(6), pp.659-682.

Mulcahy, D.L., 1975. The reproductive biology of *Eichhornia crassipes* (Pontederiaceae). *Bulletin of the Torrey Botanical Club*, pp.18-21.

Musolin, D.L., Tougou, D. and Fujisaki, K., 2010. Too hot to handle? Phenological and life-history responses to simulated climate change of the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae). *Global Change Biology*, *16*(1), pp.73-87.

Newman, J.A., 2004. Climate change and cereal aphids: the relative effects of increasing CO_2 and temperature on aphid population dynamics. *Global Change Biology*, *10*(1), pp.5-15.

Newman, J.A., 2005. Climate change and the fate of cereal aphids in Southern Britain. *Global Change Biology*, *11*(6), pp.940-944.

Newman, J.A., Gibson, D.J., Parsons, A.J. and Thornley, J.H.M., 2003. How predictable are aphid population responses to elevated CO_2? *Journal of Animal Ecology*, *72*(4), pp.556-566.

Nie, G., Hendrix, D.L., Webber, A.N., Kimball, B.A. and Long, S.P., 1995. Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO_2 concentration in the field. *Plant Physiology*, *108*(3), pp.975-983.

Oberholster, P.J. and Ashton, P.J., 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. Pretoria: Council for Scientific and Industrial Research (CSIR)*.

Oberholzer, I.G. and Hill, M.P., 2001. How safe is the grasshopper *Cornops aquaticum* for release on water hyacinth in South Africa? In Julien, M.H., Hill, M.P., Center, T.D. and Jianging D. (eds.), *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated of Control of Water Hyacinth*. Australian Centre for International Agricultural Research (ACIAR), Cranberra, Australia, pp.82–88.

Osborne, C.P., Drake, B.G., LaRoche, J. and Long, S.P., 1997. Does long-term elevation of CO_2 concentration increase photosynthesis in forest floor vegetation? (Indiana strawberry in a Maryland forest). *Plant Physiology*, *114*(1), pp.337-344.

Owens, C.S. and Madsen, J.D., 1995. Low temperature limits of water hyacinth. *Journal of Aquatic Plant Management*, *33*, pp.63-68.

Parmesan, C. and Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), pp.37-42.

Parsons, W.T., Parsons, W.T. and Cuthbertson, E.G., 2001. *Noxious weeds of Australia*. CSIRO Publishing, Melbourne, pp.139.

Pastore, M.A., Lee, T.D., Hobbie, S.E. and Reich, P.B., 2019. Strong photosynthetic acclimation and enhanced water-use efficiency in grassland functional groups persist over 21 years of CO_2 enrichment, independent of nitrogen supply. *Global Change Biology*, *25*(9), pp.3031-3044.

Paterson, I.D., Coetzee, J.A., Weyl, P., Griffith, T.C., Voogt, N. and Hill, M.P., 2019. Cryptic species of a water hyacinth biological control agent revealed in South Africa: host specificity, impact, and thermal tolerance. *Entomologia Experimentalis et Applicata*, *167*(7), pp.682-691.

Paterson, I.D., Mangan, R., Downie, D.A., Coetzee, J.A., Hill, M.P., Burke, A.M., Downey, P.O., Henry, T.J. and Compton, S.G., 2016. Two in one: cryptic species discovered in biological control agent populations using molecular data and crossbreeding experiments. *Ecology and Evolution*, *6*(17), pp.6139-6150.

Pearcy, R.W., Björkman, O., Caldwell, M.M., Keeley, J.E., Monson, R.K. and Strain, B.R., 1987. Carbon gain by plants in natural environments. *BioScience*, *37*(1), pp.21-29.

Penfound, W.T. and Earle, T.T., 1948. The biology of the water hyacinth. *Ecological Monographs*, pp.447-472.

Perkins, B.D., 1974. Arthropods that stress water hyacinth. *PANS Pest Articles & News Summaries*, *20*(3), pp.304-314.

Poff, N.L., Brinson, M.M. and Day, J.W., 2002. *Aquatic ecosystems and global climate change*. Pew Center on Global Climate Change, Arlington, pp.1-36.

Poorter, H. and Navas, M.L., 2003. Plant growth and competition at elevated CO_2: on winners, losers and functional groups. *New Phytologist*, *157*(2), pp.175-198.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J. and Postma, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, *39*(11), pp.839-850.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reddy, K.R., Agami, M. and Tucker, J.C., 1990. Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes* (Mart.) Solms) plants. *Aquatic botany*, *37*(4), pp.355-365.

Reeves, J.L., 2017. Climate change effects on biological control of invasive plants by insects. *CAB International Reviews*, *12*(10.1079).

Reich, P.B., Hungate, B.A. and Luo, Y., 2006. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon *dioxide. Annual Review of Ecology Evolution and Systematics*, *37*, pp.611-636.

Rejmanek, M. and Richardson, D.M., 1996. What attributes make some plant species more invasive? *Ecology*, *77*(6), pp.1655-1661.

Richardson, A.D., Keenan, T.F., Migliavacca, M., Ryu, Y., Sonnentag, O. and Toomey, M., 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology*, *169*, pp.156-173.

Richardson, D.M., Allsopp, N., D'antonio, C.M., Milton, S.J. and Rejmánek, M., 2000. Plant invasions–the role of mutualisms. *Biological Reviews*, *75*(1), pp.65-93.

Richardson, D.M., Pyšek, P., Simberloff, D., Rejmánek, M. and Mader, A.D., 2008. Biological invasions–the widening debate: a response to Charles Warren. *Progress in Human Geography*, *32*(2), pp.295-298.

Riikonen, J., Holopainen, T., Oksanen, E. and Vapaavuori, E., 2005. Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO_2 and O_3 in the field. *Tree Physiology*, *25*(5), pp.621-632.

Robinet, C. and Roques, A., 2010. Direct impacts of recent climate warming on insect populations. *Integrative Zoology*, *5*(2), pp.132-142.

Robinson, E.A., Ryan, G.D. and Newman, J.A., 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*(2), pp.321-336.

Rogers, A., Fischer, B.U., Bryant, J., Frehner, M., Blum, H., Raines, C.A. and Long, S.P., 1998. Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under Free-Air CO_2 Enrichment. *Plant Physiology*, *118*(2), pp.683-689.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. and Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants. *Nature*, *421*(6918), pp.57-60.

Roth, S., Lindroth, R.L., Volin, J.C. and Kruger, E.L., 1998. Enriched atmospheric CO_2 and defoliation: effects on tree chemistry and insect performance. *Global Change Biology*, *4*(4), pp.419-430.

Roth, S.K. and Lindroth, R.L., 1994. Effects of CO_2-mediated changes in paper birch and white pine chemistry on gypsy moth performance. *Oecologia*, *98*(2), pp.133-138.

Roy, J., 1990. In search of the characteristics of plant invaders. In di Castri, F., Hansen, A. J. and Debussche M. (eds.), *Biological invasions in Europe and the Mediterranean Basin,* Springer, Dordrecht. pp.335-352.

Ryalls, J.M., Hartley, S.E. and Johnson, S.N., 2017. Impacts of silicon-based grass defences across trophic levels under both current and future atmospheric CO_2 scenarios. *Biology Letters*, *13*(3), p.20160912.

Ryalls, J.M., Moore, B.D., Riegler, M., Bromfield, L.M., Hall, A.A. and Johnson, S.N., 2017. Climate and atmospheric change impacts on sap-feeding herbivores: a mechanistic explanation based on functional groups of primary metabolites. *Functional Ecology*, *31*(1), pp.161-171.

Sage, R.F. and Coleman, J.R., 2001. Effects of low atmospheric CO_2 on plants: more than a thing of the past. *Trends in Plant Science*, *6*(1), pp.18-24.

Sage, R.F., 1990. A model describing the regulation of ribulose-1, 5-bisphosphate carboxylase, electron transport, and triose phosphate use in response to light intensity and CO_2 in C_3 plants. *Plant Physiology*, *94*(4), pp.1728-1734.

Sage, R.F., 1994. Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. *Photosynthesis Research*, *39*(3), pp.351-368.

Sage, R.F., 2004. The evolution of C_4 photosynthesis. *New Phytologist*, *161*(2), pp.341-370.

Sage, R.F., Sharkey, T.D. and Seemann, J.R., 1989. Acclimation of photosynthesis to elevated CO_2 in five C_3 species. *Plant Physiology*, *89*(2), pp.590-596.

Daehler, C.C., 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), pp.183-211.

Schädler, M., Roeder, M., Brandl, R. and Matthies, D., 2007. Interacting effects of elevated CO_2, nutrient availability and plant species on a generalist invertebrate herbivore. *Global Change Biology*, *13*(5), pp.1005-1015.

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), pp.671-675.

Schwarzländer, M., Hinz, H.L., Winston, R.L. and Day, M.D., 2018. Biological control of weeds: an analysis of introductions, rates of establishment and estimates of success, worldwide. *BioControl*, *63*(3), pp.319-331.

Sellers, P.J., Bounoua, L., Collatz, G.J., Randall, D.A., Dazlich, D.A., Los, S.O., Berry, J.A., Fung, I., Tucker, C.J., Field, C.B. and Jensen, T.G., 1996. Comparison of radiative and physiological effects of doubled atmospheric CO_2 on climate. *Science*, *271*(5254), pp.1402-1406.

Shabbir, A., Dhileepan, K., Zalucki, M.P. and Adkins, S.W., 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*, p.104077.

Shackleton, C.M., McGarry, D., Fourie, S., Gambiza, J., Shackleton, S.E. and Fabricius, C., 2007. Assessing the effects of invasive alien species on rural livelihoods: case examples and a framework from South Africa. *Human Ecology*, *35*(1), pp.113-127.

Smith, N.G. and Keenan, T.F., 2020. Mechanisms underlying leaf photosynthetic acclimation to warming and elevated CO_2 as inferred from least-cost optimality theory. *Global Change Biology*, *26*(9), pp.5202-5216.

Solomon, S., Plattner, G.K., Knutti, R. and Friedlingstein, P., 2009. Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Sciences*, *106*(6), pp.1704-1709.

Song, W.K., Byeon, S.Y., Lee, H., Lee, M.S., Ryu, D., Kang, J.W., Han, S.H., Oh, C.Y. and Kim, H.S., 2020. Species-specific morphological and physiological characteristics and progressive nitrogen limitation under elevated CO_2 concentration. *iForest-Biogeosciences and Forestry*, *13*(4), p.270.

Sosa, A.J., Lenicov, A.M.D.R. and Mariani, R., 2007. Species of *Megamelus* (Hemiptera: Delphacidae) Associated with Pontederiaceae in South America. *Annals of the Entomological Society of America*, *100*(6), pp.798-809.

Sosa, A.J., Lenicov, A.M.D.R., Mariani, R. and Cordo, H.A., 2004. Redescription of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a candidate for biological control of water hyacinth. *Annals of the Entomological Society of America*, *97*(2), pp.271-275.

Sosa, A.J., Lenicov, A.M.D.R., Mariani, R. and Cordo, H.A., 2005. Life history of *Megamelus scutellaris* with description of immature stages (Hemiptera: Delphacidae). *Annals of the Entomological Society of America*, *98*(1), pp.66-72.

Soti, P.G. and Volin, J.C., 2010. Does water hyacinth (*Eichhornia crassipes*) compensate for simulated defoliation? Implications for effective biocontrol. *Biological control*, *54*(1), pp.35-40.

Spencer, W. and Bowes, G., 1986. Photosynthesis and growth of water hyacinth under CO_2 enrichment. *Plant Physiology*, *82*(2), pp.528-533.

Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B. and Osman, R.W., 2002. Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences*, *99*(24), pp.15497-15500.

Stiling, P. and 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*(9), pp.1823-1842.

Stireman, J.O., Dyer, L.A., Janzen, D.H., Singer, M.S., Lill, J.T., Marquis, R.J., Ricklefs, R.E., Gentry, G.L., Hallwachs, W., Coley, P.D. and Barone, J.A., 2005. Climatic unpredictability and parasitism of caterpillars: implications of global warming. *Proceedings of the National Academy of Sciences*, *102*(48), pp.17384-17387.

Stitt, M., 1991. Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell & Environment*, *14*(8), pp.741-762.

Stringer, L.C., Dyer, J.C., Reed, M.S., Dougill, A.J., Twyman, C. and Mkwambisi, D., 2009. Adaptations to climate change, drought and desertification: local insights to enhance policy in southern Africa. *Environmental Science & Policy*, *12*(7), pp.748-765.

Sun, Y. and Ge, F., 2011. How do aphids respond to elevated CO_2? *Journal of Asia-Pacific Entomology*, *14*(2), pp.217-220.

Sun, Y., Ding, J., Siemann, E. and Keller, S.R., 2020. Biocontrol of invasive weeds under climate change: progress, challenges and management implications. *Current Opinion in Insect Science*, *38*, pp.72-78.

Teng, N., Wang, J., Chen, T., Wu, X., Wang, Y. and Lin, J., 2006. Elevated CO_2induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. *New Phytologist*, *172*(1), pp.92-103.

Thomas, C.D., 2010. Climate, climate change and range boundaries. *Diversity and Distributions*, *16*(3), pp.488-495.

Thomas, R.B. and Strain, B.R., 1991. Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiology*, *96*(2), pp.627-634.

Thomson, L.J., MacFadyen, S. and Hoffmann, A.A., 2010. Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control*, *52*(3), pp.296-306.

Thuiller, W., Albert, C., Araujo, M.B., Berry, P.M., Cabeza, M., Guisan, A., Hickler, T., Midgley, G.F., Paterson, J., Schurr, F.M. and Sykes, M.T., 2008. Predicting global change impacts on plant species' distributions: future challenges. *Perspectives In Plant Ecology, Evolution and Systematics*, *9*(3-4), pp.137-152.

Tipping, P.W., Center, T.D. and Dray, F.A., 2008. Proposed field release of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) for control of waterhyacinth *Eichhornia crassipes* Mart. *Solms)*

(Pontederiales: Pontederiaceae), Petition submitted to the Technical Advisory Group for Biological Control Agents of Weeds, U.S.A., pp.1-34.

Tipping, P.W., Center, T.D., Sosa, A.J. and Dray, F.A., 2011. Host specificity assessment and potential impact of *Megamelus scutellaris* (Hemiptera: Delphacidae) on water hyacinth *Eichhornia crassipes* (Pontederiales: Pontederiaceae). *Biocontrol Science and Technology*, *21*(1), pp.75-87.

Tipping, P.W., Sosa, A., Pokorny, E.N., Foley, J., Schmitz, D.C., Lane, J.S., Rodgers, L., Mccloud, L., Livingston-Way, P., Cole, M.S. and Nichols, G., 2014. Release and establishment of *Megamelus scutellaris* (Hemiptera: Delphacidae) on water hyacinth in Florida. *Florida Entomologist*, *97*(2), pp.804-806.

Tissue, D.T., Thomas, R.B. and Strain, B.R., 1993. Long-term effects of elevated $CO_2$and nutrients on photosynthesis and rubisco in loblolly pine seedlings. *Plant, Cell & Environment*, *16*(7), pp.859-865.

Toft, J.D., Simenstad, C.A., Cordell, J.R. and Grimaldo, L.F., 2003. The effects of introduced water hyacinth on habitat structure, invertebrate assemblages, and fish diets. *Estuaries*, *26*(3), pp.746-758.

Tolbert, N.E., 1980. Photorespiration. In Stumpf, P. Ê. and Conn, Å. Å. (eds.), *Metabolism and Respiration,* Academic Press, London, pp.487-523.

Traw, M.B., Lindroth, R.L. and Bazzaz, F.A., 1996. Decline in gypsy moth (*Lymantria dispar*) performance in an elevated CO_2 atmosphere depends upon host plant species. *Oecologia*, *108*(1), pp.113-120.

Tyndall, R.W., 1982. *Nuisance aquatic plants and aquatic plant management programs in the United States, Southwestern Region Vol 1,* The Mitre Corporation*,* McLean.

Ulén, B.M. and Weyhenmeyer, G.A., 2007. Adapting regional eutrophication targets for surface waters—influence of the EU Water Framework Directive, national policy and climate change. *Environmental Science & Policy*, *10*(7-8), pp.734-742.

Van Den Bosch, R., 1971. Biological control of insects. *Annual Review of Ecology and Systematics*, *2*(1), pp.45-66.

Van Oosten, J.J., Wilkins, D. and Besford, R.T., 1994. Regulation of the expression of photosynthetic nuclear genes by $CO_2$is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO_2? *Plant, Cell & Environment*, *17*(8), pp.913-923.

Van Wilgen, B.W., De Wit, M.P., Anderson, H.J., Le Maitre, D.C., Kotze, I.M., Ndala, S., Brown, B. and Rapholo, M.B., 2004. Costs and benefits of biological control of invasive alien plants: case studies from South Africa: Working for Water. *South African Journal of Science*, *100*(1), pp.113-122.

Vaz, A.S., Kueffer, C., Kull, C.A., Richardson, D.M., Vicente, J.R., Kühn, I., Schröter, M., Hauck, J., Bonn, A. and Honrado, J.P., 2017. Integrating ecosystem services and disservices: insights from plant invasions. *Ecosystem Services*, *23*, pp.94-107.

Venturi, G., 2020. *Thermal physiology components of the grasshopper Cornops aquaticum (Brüner, 1906) and its impact on establishment in South Africa*. PhD thesis, https://hdl.handle.net/10539/31007, University of the Witswaterrand, South Africa,

Vila, M. and Weiner, J., 2004. Are invasive plant species better competitors than native plant species? – evidence from pair-wise experiments. *Oikos*, *105*(2), pp.229-238.

Villamagna, A.M. and Murphy, B.R., 2010. Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshwater Biology*, *55*(2), pp.282-298.

Villiers, S.D. and 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*(7-8), pp.343-349.

Virtanen, T. and Neuvonen, S., 1999. Performance of moth larvae on birch in relation to altitude, climate, host quality and parasitoids. *Oecologia*, *120*(1), pp.92-101.

Von Caemmerer, S.V. and Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, *153*(4), pp.376-387.

Walter, J., Essl, F., Englisch, T. and Kiehn, M., 2005. Neophytes in Austria: habitat preferences and ecological effects. *Neobiota*, *6*, pp.13-25.

Walter, T., Kloos, J. and Tsegai, D., 2011. Options for improving water use efficiency under worsening scarcity: Evidence from the Middle Olifants Sub-Basin in South Africa. *Water SA*, *37*(3), pp.357-370.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F., 2002. Ecological responses to recent climate change. *Nature*, *416*(6879), pp.389-395.

Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukat, Z., Bugmann, H. and Czucz, B., 2009. Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution*, *24*(12), pp.686-693.

Wang, D., Heckathorn, S.A., Wang, X. and Philpott, S.M., 2012. A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. *Oecologia*, *169*(1), pp.1-13.

Warton, D.I. and Weber, N.C., 2002. Common slope tests for bivariate errors-in-variables models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, *44*(2), pp.161-174.

Weber, E., 2017. *Invasive plant species of the world: a reference guide to environmental weeds*. CAB International Publishing, Wallingford, pp.583.

Welter, S.C., 1993. Responses of Plants to Insects: Eco-physiological Insights. *International Crop Science Vol I*, Crop Science Society of America, Madison, pp.773-778.

Welter, S.C., 2019. Arthropod impact on plant gas exchange. In Bernays, E.A., (eds.), *Insect-plant interactions*, CRC Press, Baco Raton, pp.135-164.

Whisenant, S.G., 1990. Postfire population dynamics of *Bromus japonicus*. *American Midland Naturalist*, pp.301-308.

White, A.C., Rogers, A., Rees, M. and Osborne, C.P., 2016. How can we make plants grow faster? A source–sink perspective on growth rate. *Journal of Experimental Botany*, *67*(1), pp.31-45.

Whitehead, P.G., Wilby, R.L., Battarbee, R.W., Kernan, M. and Wade, A.J., 2009. A review of the potential impacts of climate change on surface water quality. *Hydrological Sciences Journal*, *54*(1), pp.101-123.

Whittaker, J.B., 1999. Impacts and responses at population level of herbivorous insects to elevated CO_2. *European Journal of Entomology*, *96*, pp.149-156.

Williamson, M. and Griffiths, B., 1996. *Biological invasions*. Chapman & Hall, London, pp.115-149.

Wilson, R.J., Davies, Z.G. and Thomas, C.D., 2007. Insects and climate change: processes, patterns and implications for conservation. In Stewart, A.J.A., New, T.R. and Lewis, O.T. (eds.), *Insect Conservation Biology. Proceedings of the Royal Entomological Society's 22nd Symposium,* CAB International Publishing, Wallingford*,* pp. 245-279.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cock, M.J. and Julien, M.H., 2014. *Biological control of weeds: A world catalogue of agents and their target weeds*, CAB International Publishing, Morgantown, pp.838.

Zachariades, C., Paterson, I.D., Strathie, L.W., Van Wilgen, B.W. and Hill, M.P., 2017. Assessing the status of biological control as a management tool for suppression of invasive alien plants in South Africa. *Bothalia-African Biodiversity & Conservation*, *47*(2), pp.1-19.

Zavala, J.A., Gog, L. and Giacometti, R., 2017. Anthropogenic increase in carbon dioxide modifies plant–insect interactions. *Annals of Applied Biology*, *170*(1), pp.68-77.

Zavala, J.A., Nabity, P.D. and DeLucia, E.H., 2013. An emerging understanding of mechanisms governing insect herbivory under elevated CO_2. *Annual Review of Entomology*, *58*, pp.79-97.

Zhang, Q., Ficklin, D.L., Manzoni, S., Wang, L., Way, D., Phillips, R.P. and Novick, K.A., 2019. Response of ecosystem intrinsic water use efficiency and gross primary productivity to rising vapor pressure deficit. *Environmental Research Letters*, *14*(7), p.074023.

Ziska, L.H., 2003. Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. *Journal of Experimental Botany*, *54*(381), pp.395-404.

Ziska, L.H. and Dukes, J.S., 2011. *Weed Biology and Climate Change*, Wiley-Blackwell, Oxford, pp.235.

Ziska, L.H. and George, K.A.T.E., 2004. Rising carbon dioxide and invasive, noxious plants: potential threats and consequences. *World Resource Review*, *16*(4), pp.427-447.

Ziska, L.H., Hogan, K.P., Smith, A.P. and Drake, B.G., 1991. Growth and photosynthetic response of nine tropical species with long-term exposure to elevated carbon dioxide. *Oecologia*, *86*(3), pp.383-389.

Zvereva, E.L. and Kozlov, M.V., 2006. Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: a meta-analysis. *Global Change Biology*, *12*(1), pp.27-41.

Zvereva, E.L., Lanta, V. and Kozlov, M.V., 2010. Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia*, *163*(4), pp.949-960.