The past, present and future of cactus invasions in South Africa in response to rising atmospheric CO_2 and climate change

by

Nicolaas Venter

2326379

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted by me before for any other degree, diploma or examination at any university or tertiary institution.

28th day of August 2023 at Johannesburg, South Africa.

Supervisor

Prof. Marcus Byrne (University of the Witwatersrand)

DEDICATION

This PhD is dedicated to my late Aunt, Cheryl Avis, who along with my Uncle Ted Avis, provided unwavering support and encouragement when I first embarked upon my career as a botanist at Rhodes University.

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ABSTRACT

Cactaceae originate from the Americas and over the past 250 years have been introduced into South Africa and elsewhere for agricultural and horticultural purposes. Numerous species, including useful taxa, have become important invasive weeds that have serious socio-economic and environmental impacts. Fortunately, management strategies, in particular biological control, have been successful in controlling certain species. However, with rising atmospheric CO_2 invasive cacti are likely to pose a renewed threat, whereby evidence shows that cactus species are responsive to CO_2 fertilisation, which is likely to increase their vigour, mainly through improved water use efficiency (*WUE*). Additionally, studies show that plant quality declines with increasing CO_2 which in general has negative effects on their arthropod herbivores. This study sought to determine the effect of CO_2 on two functionally different invasive cactus species and how they may respond to predicted increases in atmospheric CO_2. *Opuntia stricta* (a succulent shrub) is an obligate CAM photosynthetic species that invades grasslands and savannas across semi-arid to subtropical environments whereas *Pereskia aculeata* (a scrambling woody vine with well-developed leaves) is a CAM-cycling photosynthetic species that invades forest type habits across subtropical environments. Plants were grown at three CO_2 concentrations that represented pre-industrial (sub-ambient - 250 ppm), current (ambient - 400 ppm) and future (elevated - 600 ppm) atmospheric CO_2 conditions. Plants were also subject to suboptimal and optimal watering treatments for the duration of the experiments to determine the ameliorative effect of CO_2 on productivity in response soil moisture deficits. In addition, an experiment was conducted on *O. stricta* to test the effects of the three CO_2 concentrations on plant quality and the subsequent effect on the fitness and efficacy of its insect biological control agent, *Dactylopius opuntiae*. Growth and productivity of *O. stricta* and *P. aculeata* responded positively to increasing CO_2, however the responses varied with CO_2 concentration. Increasing atmospheric CO_2 concentration from date of introduction to present possibly facilitated *O. stricta* invasion whereas this was less likely for *P. aculeata*. In both species *WUE* increased with increasing CO_2. Under suboptimal watering, there was partial amelioration of productivity at 600

ppm CO_2, but the plant traits that benefitted varied according to species. Plant quality declined for both species, most notably cladode nitrogen (N) content decreased, and carbon/nitrogen ratios (C/N) increased. When *D. opuntiae* were exposed to *O. stricta* grown at elevated CO_2 (only tested on well-watered plants), *D. opuntiae* fitness was reduced which in turn reduced the rates of plant mortality due to the insect damage. Using the *WUE* results from *O. stricta*, a mechanistic species distribution model (SDM) created here predicted greater increases in its potential distribution in South Africa under climate change relative to the SDM that did not include *WUE* as a predictor variable. This suggests that improved *WUE* under future CO_2 concentrations can offset the effect of declining rainfall in certain regions of South Africa. Overall, these results suggest that *O. stricta* and *P. aculeata* will show more vigorous growth and are also likely to expand their ranges into regions where rainfall currently limits their distribution. This expanded distribution may be further facilitated by reduced biocontrol agent efficacy as host plant quality declines. These findings suggest that management of these two species and other invasive cacti is likely to become more challenging with increasing atmospheric CO_2 and climate change.

RATIONALE

There are 33 listed invasive cactus species in South Africa (Kaplan et al. 2017), but this number is undoubtedly higher owing to emerging species that have not yet been assessed. One of the main environmental impacts of these cacti is their ability to form dense infestations which can have negative effects on agricultural productivity and biodiversity (Shackleton et al. 2017). Historical events such as early and repeated introductions, their attractiveness to the horticultural industry, combined with their value as agriculture fodder, and fruit production have encouraged humans to move cacti around the world (Novoa et al. 2015; Kaplan et al. 2017). Characteristics such as vegetative and sexual reproduction, inter-species hybridisation, phenotypic plasticity and polyploidy have created a speciose family with many growth forms (Arakaki et al. 2007; Novoa et al. 2015). Additionally, their water use efficient crassulacean acid metabolism (CAM) photosynthetic physiology well adapts them to invading arid and semi-arid environments (Zimmermann et al. 2009). However, rising atmospheric CO_2 and climate change are expected to influence their invasion (De Cortazar and Nobel, 1990; Nobel, 1991; Nobel and Israel, 1994).

Due to anthropogenic activities, atmospheric CO_2 concentrations have increased since the industrial revolution (ca. 1750s) and continues to increase (IPCC, 2022). Increased CO_2 has a positive effect on cacti productivity and is suggested to increase their productivity globally (De Cortazar and Nobel, 1990; Nobel, 1991; Nobel and Israel, 1994). While this may undoubtably have negative implications for management strategies such as herbicide treatment, where efficacy is reduced (Cowie et al. 2020), and mechanical removal, biological control which is the most successful management tool to date may be most impacted. Despite notable biocontrol successes, particularly on *Opuntia monacantha* Haw. and *O. stricta* (Haw.) Haw. using *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae) and *D. opuntiae* (Cockerell), respectively (Paterson et al. 2011a), increases in atmospheric CO_2 may negate these successes. Elevated CO_2 alters the interactions between a plant and its arthropod herbivore, and much of this can be attributed to changes in host plant quality. Plants tend to become more efficient with increases in atmospheric CO_2, whereby they

incorporate less N in their tissue which increases the C/N ratios (Robinson et al. 2012). Despite numerous studies on many plant-arthropod interactions under elevated CO_2, a review of the literature shows that this has not yet been tested on cactus weeds or their biological control agents. Furthermore, one should be cautious when making inferences from existing studies because only a few of the arthropod families that are used for biological control of invasive weeds in South Africa are represented (Robinson et al. 2012), with the literature being dominated by agricultural and horticultural plants and their pests.

Rising CO_2 and other greenhouse gases are contributing to increases in global temperatures, driving climate change (IPCC, 2022). One of the major consequences of this is altered rainfall patterns. Within South Africa these changes are predicted to be significant, with certain regions projected to experience declines in mean annual rainfall (Engelbrecht, 2019; IPCC, 2022). Despite these regions becoming less climatically suitable for cacti in the future, increases in plant photosynthetic efficiency with elevated CO_2 may serve to mitigate this effect, such that these regions may still be at risk of invasion by cacti in the future. This range expansion is mostly predicted due to increases in plant *WUE* in response to rising CO_2. Although native plants may in turn respond positively to elevated CO_2, evidence suggests that weedy plants are more stimulated by elevated CO_2 (Belote et al. 2004; Manea and Leishman, 2011; Ziska et al. 2010; Ziska and George, 2004), and therefore cacti may maintain a competitive edge over the co-occurring native plants.

Owing to the invasive nature of Cactaceae species, in South Africa and globally (Novoa et al. 2015), it is useful that we understand how elevated CO_2 and the associated changes to climate will affect these prolific invaders. Studies on the direct effects of elevated CO_2 are limited to only a few cactus species, with the majority conducted on the agricultural nature of *Opuntia ficus-indica* (L.) Mill. (Nobel, 1991; Cui et al. 1993; Nobel et al. 1994; Cui and Nobel, 1994; Nobel and Israel, 1994; Owen et al. 2015). Of these studies, none were performed in the context of invasion and the consequences for management of a weed. By investigating the effects of CO_2 on important invasive cactus species, this study will attempt to understand how these invaders respond to past, present and future CO_2 concentrations. While an understanding of how this group of plants will react

directly to increasing CO_2 is filling an important knowledge gap, the implications for their future invasion and management are equally important. Thereby, looking at the direct effects of CO_2 and water on two functionally different cactus species, *Opuntia stricta* (Haw.) Haw. (a succulent shrub) and *Pereskia aculeata* Mill. (a scrambling woody vine) and the indirect effects thereof on their management, these findings will help us make informed decisions about the future of cacti invasions in South Africa and globally.

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GLOSSARY OF TERMS AND ABBREVIATIONS

A – Net photosynthesis, net CO_2 uptake or net CO_2 exchange (µmol CO_2 m^{-2} s^{-1}) which includes cellular respiration.

aCO_2 – refers to ambient CO_2 concentrations (400 ppm) used in this study to correspond to current atmospheric CO_2 concentration which is currently ~ 420 ppm (August 2023).

CAM – Crassulacean acid metabolism

CCM – Carbon concentrating mechanism. Refers to a component of the photosynthetic metabolism of CAM and C_4 photosynthesis.

CO_2 uptake – refers to net photosynthesis or net CO_2 exchange (*A*) and used to refer to night-time CAM photosynthesis and CAM daytime C_3 photosynthesis in Chapter 2.

eCO_2 – refers to elevated CO_2 concentrations (600 ppm) used in this study which is expected to correspond to predicted mid-century to late century atmospheric CO_2 concentrations based on RCP4.5.

C_i/C_a – the ratio of intercellular CO_2 concentration to ambient CO_2 concentration.

Elevated CO_2 – refers to CO_2 concentration above ambient, usually for studies that use different experimental elevated CO_2 concentrations or referring future CO_2 concentrations.

g_{ST} – Leaf stomatal conductance (mol H_2O m^{-2} s^{-1})

Habitat suitability – predicted suitability of a habitat for an organism based on the climatic envelope developed by the species distribution model (SDM). Habitat suitability is used interchangeably with ‘distribution’ in this study.

IAP – Invasive alien plant

IPCC – Intergovernmental Panel on Climate Change

Long-term watering regimes – plants were given the same quantity of water weekly throughout the experiment, either suboptimal or optimal watering amounts.

MAP – Mean annual precipitation

NEM:BA – South African National Environmental Management: Biodiversity Act no. 10 of 2004

Optimal watering / well-watered / ambient-water – refers to the 400 ml wk^{-1} watering treatment used in this study. It also refers to ideal/optimal rainfall/soil moisture for the species investigated in this study and others.

Plant productivity or productivity – this refers to photosynthesis and or biomass accumulation and in the context in this study.

PPFD – Photosynthetic photon flux density (µmol m^{-2} s^{-1}), which is a measure of how much photosynthetically active radiation (PAR) the plant receives.

RCP – Representative concentration pathway adopted by the IPCC.

sCO_2 – refers to sub-ambient CO_2 concentration (250 ppm) used in this study to correspond to pre-industrial (ca. 1750s) atmospheric CO_2 concentrations of ~ 280 ppm.

SDM – Climate envelope species distribution model or referred to as 'model' in this study.

Suboptimal watering / reduced watering – refers to the 200 ml wk^{-1} watering treatment used in this study. It also refers to below optimal rainfall/soil moisture for the species investigated in this study.

VPD – Vapour pressure deficit (kPa)

Water/ing regime – refers to the long-term watering treatments implemented in this study.

WUE – Water use efficiency. The ratio of photosynthesis to stomatal conductance or transpiration rate (A/g_{ST} or A/E).

CHAPTER ONE

General Introduction

1.1 Cacti invasion overview

Members of the Cactaceae originate from the Americas (with the exception of *Rhipsalis baccifera* (J.S.Muell.) Stearn which also occurs in the Old World) and are serious invaders globally (Zimmerman et al. 2009; Novoa et al. 2015). South Africa has a long history of cactus invasions, and at present, of the estimated 400 species introduced into the country (Walters et al. 2011), 33 are NEM:BA (National Environmental Management: Biodiversity Act no. 10 of 2004) listed invasive species (Kaplan et al. 2017). Introductions of cactus species into South Africa started over 250 years ago, with the introduction of *O. ficus-indica* (Zimmerman and Moran, 1991). Invasive cacti have serious socio-economic and environmental impacts, which include habitat degradation, damage to non-coevolved animals and the high cost of their management (Kaplan et al. 2017). Despite this, both invasive and non-invasive cacti species have important socio-economic benefits. Owing to their drought resistance, they are useful fruit and fodder plants in marginal lands, for both subsistence and commercial farmers (Brutsch and Zimmermann, 1993). Additionally, the horticultural trade, which is regarded as one of the main routes for invasion, also brings economic benefits. Management of invasive cacti in South Africa goes as far back as the late 1800s, when both mechanical and chemical control of *Opuntia* species was attempted (Zimmerman and Moran 1991). But it was only in 1913, with the intentional introduction of the biological control agent, *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae), a scale insect, to control *O. monocantha*, that infestations were reduced to negligible levels (Zimmerman et al. 2009). However, owing to *Opuntia's* vegetative and sexual reproduction, and a physiology adapted to both mesic and xeric habitats (Novoa et al. 2015), subsequent invasions by additional species require control. Moreover, due to increasing atmospheric CO_2 concentrations and associated changes in global climate patterns, biological control of cactus species may be hindered in the future.

1.2 Invasive cacti in South Africa

All biomes in South Africa, except for the desert biome, have been invaded by one or more species of cactus. These invasive cacti belong to 11 genera consisting of 33 species according to the NEM:BA list of 2017. Fourteen of these species belong to the *Opuntia* genus (mostly with flattened segmented cladodes, growing as prostrate shrubs to small trees), followed by six species of *Cylindropuntia* (Engelm.) F.M.Knuth (cylindrical segmented stems, mainly growing shrubs) (Fig. 1.1). Of these listed species, only two are grown for their fruit: *O. ficus-indica* (sweet prickly pear) and *Hylocereus undatus* (Haworth) D.R. Hunt (dragon fruit) (Paterson et al. 2011a). Spineless varieties of *O. ficus-indica* and *O. robusta* J.C.Wendl. ex Pfeiff. are grown as supplement fodder for livestock (Kaplan et al. 2017). This early introduction and translocation for agricultural purposes, with the exception of *H. undatus* which is a more recent introduction (Paterson et al. 2011a), possibly provided the propagule pressure for the widespread invasion of the two species in South Africa (Fig. 1.1). The remaining invasive species were likely ornamental horticultural escapees (Walters et al. 2011) as they have little to no agricultural value. Despite socio-economic benefits, both agriculturally important and non-important species have negative socio-economic and environmental impacts.

Cacti reproduce sexually and vegetatively resulting in large dense monocultures that exclude competing flora and animals, particularly livestock. The spines and glochids can cause severe injury to animals, particularly when the cladodes are eaten (Shackleton et al. 2007). Within rangelands, invasions result in the loss of productivity and grazing, for both commercial and subsistence farmers (Lloyd and Reeve, 2014). At the peak of the *O. ficus-indica* invasion in the mid-1900s, more than 900 000 ha were invaded, predominantly in the Eastern Cape Province and the Karoo (Annecke and Moran, 1978). Biodiversity and tourism were also impacted such as in the Skukuza region of the Kruger National Park, South Africa, where approximately 30 000 ha were invaded by *O. stricta* by the mid-1990s (Foxcroft and Hoffmann, 2000). Because of this long history of invasions, management of invasive cacti in South Africa dates to the 1890s with the early attempts to control *O. ficus-indica* (Zimmermann and Moran, 1991).

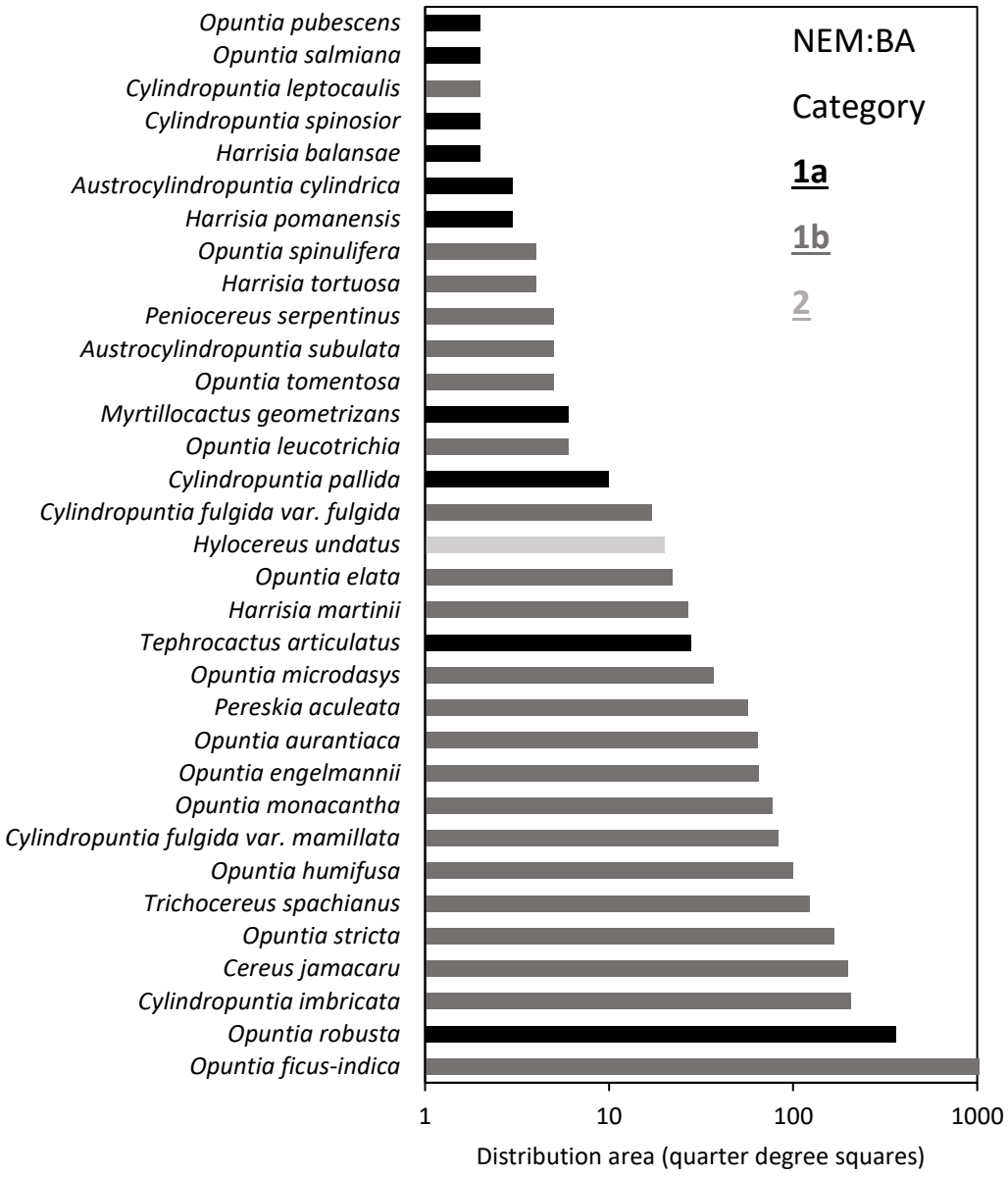


Figure 1.1: Number of quarter degree squares (QDS) occupied by 33 invasive cactus species in South Africa. Species bars are shaded according to NEM:BA category. QDS measure approximately 25 x 25 km. Data redrawn from Kaplan et al. (2017).

1.3 Biological control of cacti in South Africa

Because of the historical extent of *Opuntia* invasions in South Africa, it was the first invasive taxon to be regulated under the Agricultural Pests Act no. 11 of 1911 (Zimmermann et al. 2009). Biocontrol agents from three insect groups: *Dactylopius* spp. (Costa) (cochineal scale insects; Hemiptera: Dactylopiidae),

Hypogeococcus sp. (Costa) (mealybug; Hemiptera: Pseudococcidae) and *Cactoblastis cactorum* (Berg) (cactus moth: Lepidoptera: Pyralidae), have been responsible for the substantial or complete control of 12 cactus species (Paterson et al. 2011a; Kaplan et al. 2017; Sutton et al. 2018). Of these, eight are under substantial control (limited additional management required) and four are under complete control (no additional management required). Much of this success can be attributed to the fact that Cactaceae originate from the New World (except *R. baccifera*), and therefore the oligophagous cactophage insects used for control do not attack functionally similar yet distantly related plants in their introduced ranges (Paterson et al. 2011a). Attempts to extend cactus biological control in South Africa have, however, been hindered by a few economically significant conflict-of-interest species, such as *O. ficus- indica*, *O. rubusta* and *H. undatus*, but this resistance has mostly been countered by analyses that indicate the cost of their invasions outweigh their economic benefits (Shackleton et al. 2007; van Wilgen and Richardson, 2014). Nevertheless, when cacti invasions are not managed (with a combination of mechanical removal, chemical treatment and biocontrol), their lack of natural enemies, mechanical defences, prolific sexual and vegetation reproduction and high *WUE* allows them to spread rapidly. Additionally, their CAM photosynthetic physiology is an adaptive trait which may serve to enhance their competitiveness, particularly in low rainfall environments.

1.4 CAM photosynthesis

Crassulacean acid metabolism (CAM) photosynthesis is found in at least 38 plant families representing over 400 genera (~ 6% terrestrial plants and 6% aquatic plants) with more than 60 independently evolved origins (Black and Osmond, 2003; Hultine et al. 2019). Evolution of CAM is thought to primarily to be driven by aridity whereby plants are exposed to high daytime temperatures and low relative humidities (Hultine et al. 2019). Alternatively, studies suggest selection for CAM coincided with low atmospheric CO_2 about 20 - 30 million years ago (Edwards and Ogburn, 2011). CAM differs mainly from C_3 and C_4 photosynthesis by temporally separating photosynthetic processes between day and night

(Fig. 1.2). CAM plants open their stomata at night (phase I) to allow CO_2 to diffuse into the mesophyll cells when the evapotranspirative demand is low due to lower vapour pressure deficits (VPD). Similar to C_4 photosynthesis, CAM also uses the carbon concentration mechanism (CCM) to concentrate CO_2 around the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which catalyses the assimilation of CO_2 into sugars for growth (Raven et al. 2008). CO_2 within the mesophyll cell is converted to HCO_3^- (bicarbonate) by carbonic anhydrase (CA) and then phosphoenolpyruvate carboxylase (PEPCase) uses the bicarbonate to carboxylate phosphoenolpyruvate (PEP) to form oxaloacetate (OAA), a 4-carbon acid which is then converted to malate (Winter and Smith, 2021). PEPCase has a higher affinity for CO_2 than RuBisCO which allows CO_2 to be scavenged at low concentrations, making it most efficient at lower intercellular CO_2 concentrations. Malate is then transported to the vacuoles and stored as malic acid. During this stage, pH within the tissue decreases (more acidic) (Winter and Smith, 2021; Grey et al. 2022). In the absence of light energy to drive the reaction, stored energy in the form of carbohydrates is liberated to perform these tasks. Nocturnal acid accumulation and dark phase stomatal opening are defining characteristics of CAM photosynthesis. During the light part of the day (phase III), with the stomata closed, malate exits the vacuole and is decarboxylated by NADP-malic enzyme or by PEP carboxykinase (depending on the species) to release CO_2 at high concentrations within the mesophyll cells (analogous to the bundle sheath cells in C_4 plants), which is subsequently fixed in the Benson-Calvin Cycle using RuBisCO (Winter and Smith, 2021). Thus, similar to C_4 photosynthesis, CAM is a CCM that concentrates CO_2 around RuBisCO (Raven et al. 2008). During this phase, pH within the vacuoles increases (less acidic) (Winter and Smith, 2021; Grey et al. 2022). Therefore, nocturnal opening of stomata under conditions of low VPD/high humidity results in high *WUE* (the ratio of photosynthesis to stomatal conductance/transpiration rate) in CAM plants (Drennan and Nobel, 2000; Winter and Smith, 2021). Under optimal conditions, such as high rainfall/soil moisture and low VPD/high humidity, some CAM plants can open their stomata during the light phase (phases II and IV) and fix atmospheric CO_2 directly via the Benson-Calvin Cycle, much like typical C_3 plants. Using C_3 during the day can be advantageous because the C_3 photosynthetic metabolism is energetically more efficient than CAM, whereas

CAM requires additional energy for the decarboxylation of malate (de Mattos and Luttge, 1999; Maxwell et al. 1999; Winter and Smith, 2021). However, if exposed to higher VPD during the day when performing C_3-type photosynthesis, more water is lost through transpiration compared to nocturnal stomatal opening, which can lower overall *WUE*. CAM expression may be constitutive (or obligate) whereby CAM is always expressed, or facultative, whereby plants express C_3 photosynthesis and CAM is expressed in a reversable manner only when the plant is stressed (Winter, 2019). Most known CAM species are constitutively CAM (Winter, 2019).

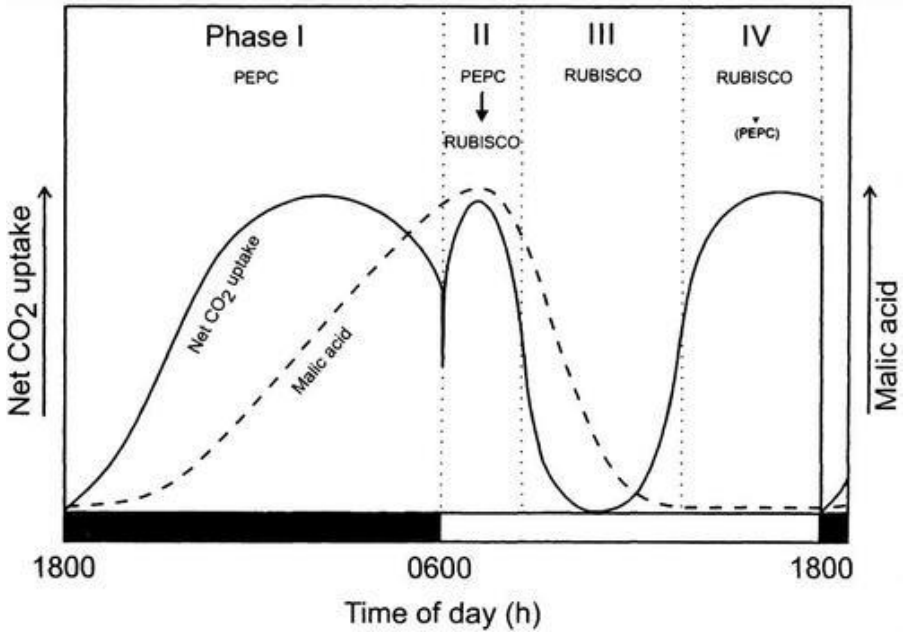


Figure 1.2: Generalised daily patterns of CO_2 fixation and malic acid levels in an obligate CAM plant under ideal conditions. The CAM photosynthesis cycle over a 24-hour day is divided into four phases. Stomata are open during phases I, II and IV and closed during phase III (from Keeley and Rundel, 2003).

CAM-cycling, sometimes referred to as CAM-idling, although the distinctions are not always very clear, is C_3-like, whereby there is no detectable nocturnal net CO_2 exchange (Nobel and Hartsock, 1987; Martin and Wallace, 2000; Winter, 2019). During drought when stomatal conductance is reduced and intercellular CO_2 concentrations decline, nocturnally respired CO_2 can be fixed into malate (Martin and Wallace, 2000; Herrera et al. 2009; Rayder and Ting, 1981). During the day, this malate is decarboxylated to release the nocturnally

fixed CO_2, much like typical CAM photosynthesis, theoretically contributing to plant survival during periods of water stress (Rayder and Ting, 1981). It is unclear as to whether CAM-cycling significantly enhances fitness of these species, particularly in drier habitats, and therefore are mainly restricted to mesic habitats (Martin and Wallace, 2000).

Obligate CAM expression is the major pathway of carbon assimilation most cacti species, including the *Opuntia* which have a daily cycle that typically consists of four phases (Fig. 1.2), depending on environmental conditions. However, prior to the obligate CAM expression becoming the major pathway, seedlings of *Opuntia* species may express the facultative CAM pathway (Winter et al. 2011; Winter et al. 2008). CAM-cycling is present in certain species from the *Pereskia* genus, a basal cactus lineage that has a woody stem and well-developed leaves (Edwards et al. 2005). Depending on the authors, CAM-cycling is thought to be strongly expressed (Rayder and Ting, 1981) or not expressed at all (Nobel and Hartsock, 1987; Martin and Wallace, 2000).

This photosynthetic flexibility of CAM can serve to enhance cactus productivity under both suboptimal and optimal conditions. Obligate CAM cactus species can remain productive when water-stressed or exposed to high VPD and, moreover, under favourable conditions, can increase productivity by initiating daytime (phases II and IV) C_3 photosynthesis. Although the adaptive advantage of CAM-cycling is less well understood (Martin and Wallace, 2000; Herrera, 2009) maintaining a positive carbon balance via fixing respired CO_2 is likely to act as a survival mechanism that extends the lifetime of the plant during dry seasons or periods of water stress.

1.5 CO_2 and climate change

Climate change is primarily driven by the increased emission of greenhouse gases (GHGs) through anthropogenic activities (Anderson et al. 2016) which coincided with the start of the industrial revolution in the 1750s (IPCC, 2014). CO_2 is the largest contributor to GHG emissions and is mainly released from the burning of fossil fuels (Montzka et al. 2011) and deforestation (Longobardi et al. 2016).

Atmospheric CO_2 concentrations are currently 421 ppm (August 2023) (www.co2.earth) compared to pre-industrial concentrations of ~ 280 ppm (IPCC, 2014). This is an increase of 33% in two and a half centuries, a rate of increase not observed in geological history (Prentice et al. 2001). Methane (CH_4) is the second most abundant GHG (Montzka et al. 2011) and while it is 25 times more potent as a GHG compared to CO_2, it occurs at much lower atmospheric concentrations, which currently stand at 1.92 ppm (October 2022) (Lan et al. 2023). Other important GHGs include nitrous oxide (N_2O), ozone-depleting substances, hydrofluorocarbons, sulphur hexafluoride (SF6) and perfluorocarbons (Montzka et al. 2011). A consequence of these GHG, in particular CO_2, is rising global temperatures (1.06 ˚C increase since 1880) which have already altered the global climate and is predicted to become more severe in the near future (IPCC, 2022; Anderson et al. 2016).

Predicted changes to global climate are largely dependent on the representative concentrations pathways (RCP) scenarios used in the climate models. RCPs are GHG concentration trajectories based on future anthropogenic emissions under different mitigation efforts to reduce GHG emissions, in particular CO_2. Based on these different RCP mitigation scenarios, changes to radiative forcing (difference between the amount of energy entering and leaving the atmosphere) are used to calculate changes to average global temperatures (IPCC, 2014). Relative to the period 1986–2005, the increase of global mean surface temperature by the end of the 21st century (2081–2100) is likely to be 0.3°C to 1.7°C under RCP2.6, 1.1°C to 2.6°C under RCP4.5, 1.4°C to 3.1°C under RCP6.0 and 2.6°C to 4.8°C under RCP8.5. Thus, by using different RCP scenarios for future climate predictions, climate scientists can account for uncertainties surrounding future GHG emissions and ultimately provide best to worst case climate predictions and how this may affect the ecosystem in question.

Depending upon the RCP scenario, climate change models predict further increased temperatures, greater shifts in precipitation patterns, increased drought frequency and intensity, and extreme weather events. Although increasing CO_2 is mostly uniform in the atmosphere, the effects on temperature and weather are manifested across different spatial scales. Within South Africa, annual average temperatures have already increased 1.5 times the global average of 0.65 ˚C over

the last 50 years (Ziervogel et al. 2014) and temperature increases could be twice as high as the global average during the late 21st century (Englebrecht, 2019). A low mitigation scenario (RCP8.5) predicts greater increases in temperature relative to a high mitigation scenario (RCP4.5) (Engelbrecht, 2019), which may be expected due to the relationship of atmospheric CO_2 and its effect on temperature. Increased frequencies of extreme rainfall events have already been observed (Ziervogel et al. 2014) although high mitigation (RCP4.5) and low mitigation (RCP8.5) scenarios predict a similar frequency of these events (Engelbrecht, 2019). Mean annual precipitation (MAP) is mainly predicted to increase in the east and decrease in west of South Africa, with both RCP4.5 and RCP8.5 predicting similar broad patterns although at regional scales there are differences between the two RCP scenarios (Engelbrecht, 2019; IPCC, 2022).

These changes to climate are predicted to have far reaching effects on socio-economic and environmental systems in South Africa (IPCC, 2022), yet only a few studies have investigated the effect of climate change on invasive weeds in South Africa (Richardson et al. 2000; Taylor and Kumar, 2014; Hoveka et al. 2016; Sintayehu et al. 2020). Richardson et al. (2000) predicted declines in distribution of certain weed species in South Africa under climate change, including *O. ficus-indica*. This is in contrast to Nobel (1991) who predicted an increase in *O. ficus-indica* productivity across its distribution, including South Africa. Nobel (1991) attributed this increase in productivity to higher minimum temperatures and improved *WUE* in response to elevated atmospheric CO_2 concentrations. Thus, while some studies have investigated the effects of climate change on weeds in South Africa, these studies have not included the interaction of elevated CO_2 and MAP, however they do acknowledge the role of improved photosynthetic efficiency under elevated CO_2 (Richardson et al. 2000; Parker-Allie et al. 2009).

1.6 CAM photosynthesis and elevated CO_2

Studies across a range of CAM photosynthetic species exposed to approximately double ambient CO_2 concentrations show an increase in biomass productivity of ~ 35% (Drennan and Nobel, 2000). Similarly, studies investigating the effect of elevated CO_2 on different plant functional groups within C_3 and C_4 species, report increases in biomass that range between ~ 20% to ~ 58% and 0% to ~ 33%, respectively (Poorter, 1993, Wand et al. 1999, Wang et al. 2012). 24 hour net CO_2 uptake for CAM plants is enhanced under elevated CO_2, along with changes to the daily CO_2 uptake pattern. Most obligate CAM species show an increase in daytime (phases II and IV) CO_2 uptake, which is often suppressed or very low under ambient CO_2. This increases the proportion of C_3 photosynthesis to net carbon gain (Nobel and Hartsock, 1986). Energetically more efficient daytime CO_2 uptake may be favoured under elevated CO_2 (see Section 1.4) (de Mattos and Luttge, 1999; Maxwell et al. 1999). Factors that may stimulate daytime CO_2 uptake are increased intercellular CO_2 concentrations (as is the case under elevated CO_2) and CO_2 saturation of the enzyme PEPcase during the night-time phase (Drennan and Nobel, 2000).

Cactus species *O. ficus-indica*, *H. undatus*, *Ferocactus acanthodes* (Lemaire), *Stenocereus queretaroensis* (Weber) Buxb. and *Cylindropuntia imbricata* Haw. (DC.) all exhibited significant increases in daytime CO_2 uptake under elevated CO_2 (Nobel and Hartsock, 1986; Cui et al. 1993; Cui and Nobel, 1994; Raveh et al. 1995; Nobel, 1996; Yu et al. 2019). There are no elevated CO_2 studies conducted on CAM-cycling species, such as *Pereskia*, but owing to their C_3-like photosynthesis, *Pereskia* are likely to improve productivity, particularly at higher CO_2 concentrations due to C_3 photosynthesis saturating at higher CO_2 concentrations relative to C_4 and CAM pathways. The magnitude of this response can be altered by abiotic stresses such as high temperatures, water availability and nutrient stress, however, elevated CO_2 should partially ameliorate the effects of these stresses.

Hylocereus undatus and *Ananas comosus* (L.) Merr. (Bromeliaceae) showed a decline in CO_2 uptake when grown at very high temperatures and ambient CO_2. However, under elevated CO_2 the relative decline in CO_2 uptake was considerably

lower (Raveh et al. 1995; Zhu et al. 1999). Moreover, these studies showed that the optimal growth temperature at elevated CO_2 was higher than that for ambient CO_2. Similarly, when *H. undatus*, *C. imbricata* and *O. ficus-indica* were exposed to water stress at elevated CO_2, the decline in CO_2 uptake was less apparent and or delayed when compared to ambient CO_2 (Raveh et al. 1995; Yu et al. 1995; Nobel and Israel, 1994), a response largely attributed to improved *WUE*. Additionally, photosynthetic nitrogen use efficiency increased due to lower investment in cladode N (Cui and Nobel, 1994). Therefore, elevated CO_2 is likely to partially ameliorate the negative effects of elevated temperature and decreased precipitation, as predicted for future climate scenarios (IPCC, 2022), and depending on the species, higher temperatures may in fact be beneficial. Additionally, CAM productivity could be sustained in lower nutrient environments under elevated CO_2.

1.7 Elevated CO_2 and plant-arthropod interactions

Elevated CO_2 can increase photosynthetic efficiency, whereby plants can invest fewer resources in photosynthetic functions but still achieve similar or higher rates of photosynthesis relative to ambient CO_2 concentrations (Cui and Nobel 1994; Sun and Ge, 2011). This reduced investment, or reallocation of resources elsewhere, can alter plant traits resulting in plants of a lower quality for herbivores (Cui and Nobel, 1994; Sun and Ge, 2011 Lincoln et al. 1993; Stiling and Cornelissen, 2007; Robinson et al. 2012). On average, leaf N content declines, increasing C/N ratios, and defence increases (e.g., chemical and mechanical) which in turn can prolong arthropod developmental times and reduce their fecundity. While some arthropod herbivores attempt to compensate for lower host plant quality by increasing relative consumption rates (biomass of ingested food / biomass of arthropod x time), this for the most part does not appear to mitigate the effects of reduced nutritional quality (Lincoln et al. 1993; Stiling and Cornelissen, 2007; Robinson et al. 2012). Most of these effects are summarised by Stiling and Cornelissen (2007) and Robinson et al. (2012) who conducted meta-analyses to report effect sizes of the effects of elevated CO_2 on host plants and the subsequent effect on their arthropod hosts. Despite each publication reporting different effect

sizes of elevated CO_2 relative to ambient conditions, the effect on host plant and herbivores showed similar trends for most variables reported. When reporting the effect sizes here, the values in parentheses correspond to the Stiling and Cornelissen (2007) and the Robinson et al. (2012) publications, respectively.

Host plants grown under elevated CO_2 exhibited larger biomass (38 & 25%), decreased N (-16 & -15%), increased C/N ratios (27 & 19%), and increased concentrations of tannins (30 & 22%). Increases in starch (45 & 50%), soluble sugars (14 & 8%) and total non-structural carbohydrates (18 & 39%) were also observed. Nitrogen-based secondary metabolites decreased (-16%; Robinson et al. 2012), but total phenolics (9 & 19%) and flavonoids increased (27%; Robinson et al. 2012), while plant terpenoid concentrations decreased (-15 & -13%). Although there was no significant decrease in the specific leaf area (SLA), there was an increase in leaf toughness (8 & 11%) and specific leaf weight (27 & 18%). Overall, these changes to leaf traits enhance efficiency and resilience to disturbance and natural enemies.

Based on Stiling and Cornelissen (2007) and Robinson et al. (2012) herbivorous arthropods are in general negatively affected when exposed to host plants grown at elevated CO_2. Decreases in arthropod relative growth rates (-8.3 & -4.5%), conversion efficiency (-19 & -14.5%) and pupal weight (-5 & -5.5%) were reported (Stiling and Cornelissen, 2007; Robinson et al. 2012). Increases in relative consumption rates (16.5 & 14%), development times (3.8 & 3.5%) and total consumption (9.2 & 22%) also contributed to negative impacts on herbivores. This indicates that under elevated CO_2, herbivores can inflict more damage by consuming more plant material to compensate for lower quality forage, although this is likely mitigated by faster plant growth rates and or less investment in the plant organs that are fed on.

The effect of rising atmospheric CO_2 on invasive weeds is predicted to reduce biocontrol agent efficacy in the future (Ziska and George, 2004; Reeves, 2017). This prediction is mostly inferred from studies related to agricultural systems (Scherm et al. 2000; Vil`a et al. 2007). Compared to leaf feeders/chewers, Hemiptera (phloem feeders) generally respond positively to plants grown at elevated CO_2 (Robinson et al. 2012). However, the studies that showed this trend

have largely been conducted using aphids which are mobile and can alter their feeding behaviour by seeking out areas on the plants that have higher nutritional value (Hughes and Bazazz, 2001; Sun and Ge, 2011; Robinson et al. 2012). There has not been any similar/equivalent studies on the genus *Dactylopius* which are sessile sap feeders, nor on host plants that express the CAM photosynthetic pathway.

1.8 Study aims and objectives

Studies on the effect of rising atmospheric CO_2 concentrations and climate change on species of Cactaceae have predominantly focused on the tree-like *O. ficus-indica*, and none of the studies have investigated this in the context of invasion (Drennan and Nobel, 2000; Gomez-Casanovas et al. 2007; Guevara et al. 2003). Although inferences could be made from the findings of the existing studies on the invasive potential of cactus weeds in South Africa, these studies do not represent the diverse functional groups and growth forms of these cactus weeds.

Opuntia stricta is a widespread NEM:BA Cat. 1b semi-arid to sub-tropical succulent shrub invader (Kaplan et al. 2017; Hoffmann et al. 2020; Paterson et al. 2021). It is the third most widespread *Opuntia* species after *O. ficus-indica* and *O. robusta* (Kaplan et al. 2017) (Figs. 1.1 & 1.2). Almost half the invasive cacti in south Africa belong to the *Opuntia* genus (Kaplan et al. 2017) and thus it would be useful to understand how *O. stricta* with a different growth form to the tree-like *O. ficus-indica* may respond to elevated CO_2. Furthermore, the biocontrol agent *D. opuntiae* has been successful in suppressing *O. stricta* and is present at most sites where *O. stricta* occurs, indicating *O. stricta* is likely at biotic equilibrium with its biocontrol agent (Moran et al. 2021). Additionally, *O. stricta's* long history of invasion and its widespread distribution that encompasses climatic extremes at either end of the spectrum likely represents its realised niche (environmental equilibrium) in South Africa. These factors need to be considered when trying to make predictions about its future distribution in response to rising CO_2 and climate change (Gallien et al. 2012; Barbet-Massin et al. 2018).

Pereskia aculeata is a NEM:BA Cat. 1b scrambling woody vine (liana) with well-developed leaves that invades mainly coastal forests/thickets in sub-tropical environments (Fig. 1.2) (Paterson et al. 2011a; King et al. 2021). *Pereskia aculeata* is the only known C_3-like (CAM-cycling) invasive cactus in South Africa and understanding how this differs to obligate CAM is useful as there are no equivalent studies that have investigated the effects of elevated CO_2 on this genus. Unlike *O. stricta*, biocontrol has been less effective on *P. aculeata*, the reasons for which are not clear (King et al. 2021). Moreover, *O. stricta* and *P. aculeata* differ in their rainfall (and temperature) requirements, whereby *P. aculeata* is mainly restricted to mesic environments. Thus, being less adapted to semi-arid environments like most other invasive cactus species, incorporating it in this study adds a continuum of invasive cactus functional types. This may help give a broader understanding as to how invasive cactus species within South Africa may respond to rising atmospheric CO_2 and the interactions with abiotic and biotic factors such as climate and herbivory in the form of insect biocontrol.

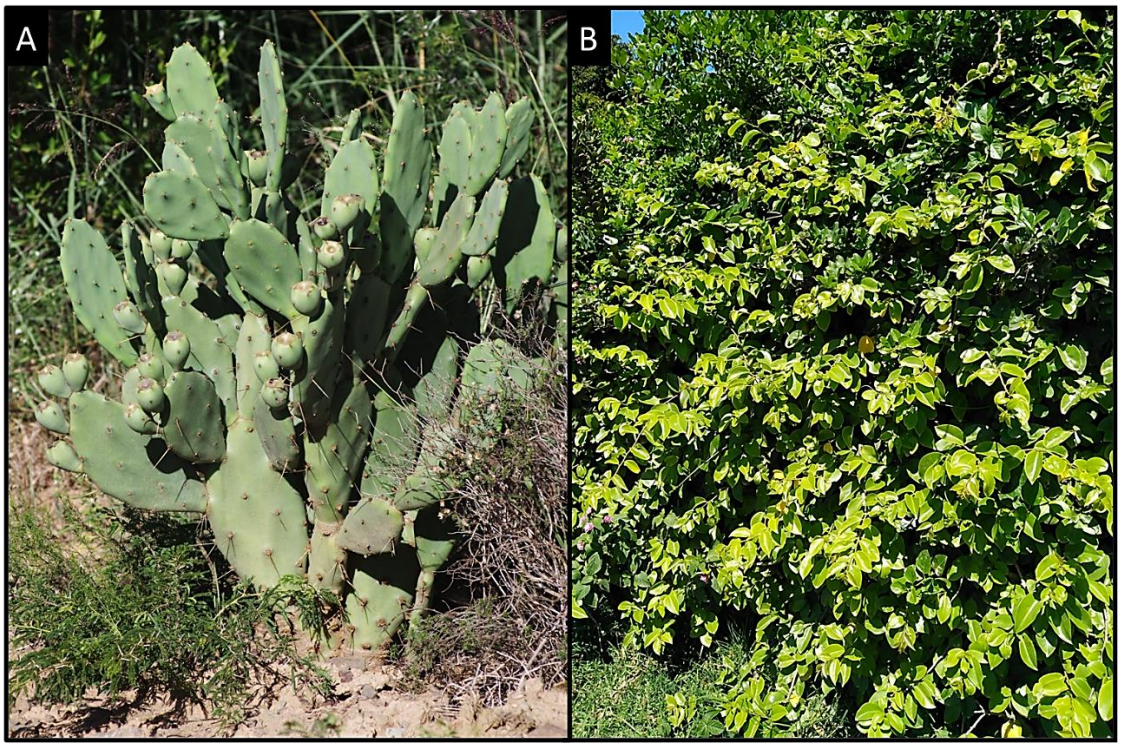


Figure 1.3: A) *Opuntia stricta* growing in the Eastern Cape Province, South Africa and B) *Pereskia aculeata* scrambling up indigenous coastal vegetation in Coffee Bay, Eastern Cape Province, South Africa. (Images: Nic Venter).

Therefore, the primary aim of this study is to determine the effect of past, present and future atmospheric CO_2 and rainfall on the invasive vigour and the subsequent implications for the management of two functionally different cactus species: *Opuntia stricta*, a sub-tropical to semi-arid succulent shrub invader and *Pereskia aculeata*, a sub-tropical woody vine invader and (Fig. 1.3).

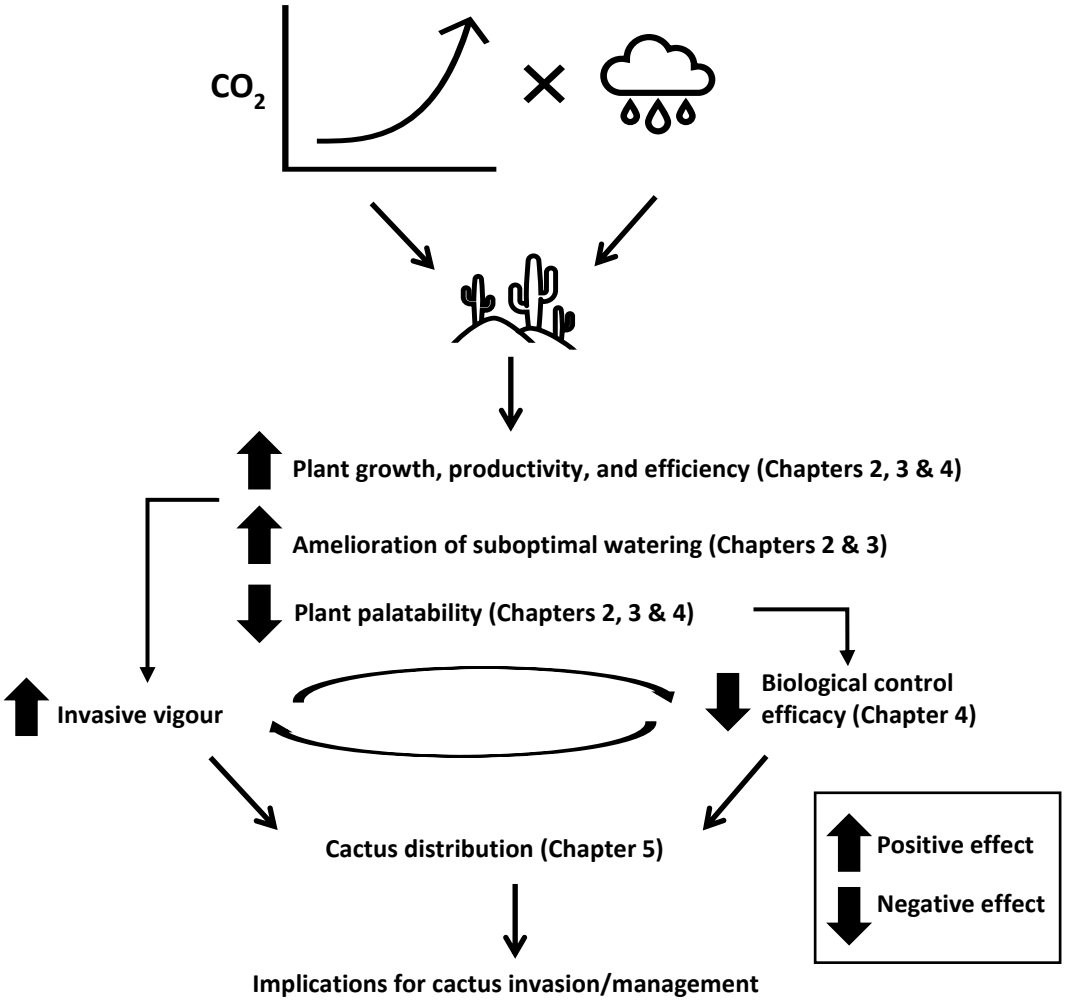


Figure 1.4: Conceptual framework indicating links and feedbacks between the four research chapters and the predicted effects on plants traits, vigour, biological control, and distribution.

In order to address the primary aim of this study, *O. stricta* and *P. aculeata* were grown at three different CO_2 concentrations: 250 ppm (pre-industrial; sub-ambient - sCO_2), 400 ppm (current; ambient - aCO_2) and 600 ppm

(RCP4.5 ca. 2080; elevated - eCO_2) using two watering regimes to simulate the optimal and suboptimal rainfall for either species. Pre-industrial CO_2 concentrations were included for three reasons 1) to determine if historical increases in atmospheric CO_2 may have contributed their invasions observed to date, 2) projecting beyond 600 ppm may be impractical given the RCP projections and 3) using temporally relevant CO_2 concentrations to predict trends. Atmospheric CO_2 concentrations ranged between 221 and 277 ppm during the last million years (Higgins et al. 2015) and thus exposing these cacti to CO_2 concentrations beyond 600 ppm (increase of more than 630 ppm) may be physiologically impractical thereby potentially diminishing the relevance of the study. The following four objectives addressed the main aim of the study (summarised in Fig. 1.4):

1. To determine conserved or diverging effects of increasing CO_2 concentrations on the invasive vigour of *O. stricta* and *P. aculeata*, plant growth rates, biomass, cladode traits, resource allocation and the physiology that underlies these responses were measured on propagated potted plants that developed under their respective CO_2 growth concentrations over a period of five to eight months (Chapters 2, 3 & 4).
2. To determine if increasing CO_2 ameliorates the effect of low soil moisture on *O. stricta* and *P. aculeata*, two watering treatments were implemented over the duration of the experiment to emulate a gradient of suboptimal and optimal rainfall for either species. This was done in combination with Objective 1 (Chapters 2 & 3).
3. To determine the effect increasing CO_2 on plant quality and subsequent biological control efficacy, *O. stricta* plants were grown under the three CO_2 concentration for 70 days and then inoculated with *D. opuntiae* to assess the impact on insect fitness and the subsequent effect of their herbivory on plant growth and traits for a further four months (Chapter 3). In addition to this, plant quality parameters were measured in Chapters 1 and 3 to support the conclusions from this objective.
4. To determine if *WUE* moderates or amplifies the distribution range of *O. stricta* under elevated CO_2 and climate change. *WUE* calculated from physiological measurements conducted on *O. stricta* in Chapter 2 were

used to create a mechanistic species distribution model which was compared to a non-mechanistic species distribution model (Chapter 4).

CHAPTER TWO

The effects of CO_2 and water on the growth, physiology, and resource allocation for the invasive cactus *Opuntia stricta*

Preface

Using *O. stricta*, this chapter aimed to address Objectives 1 and 2 by assessing invasive vigour and amelioration of suboptimal watering. *WUE* measured in this chapter was subsequently used to address Objective 4 which sought to determine if *WUE* moderates or amplifies the distribution range of cactus under elevated CO_2 and future climate change scenarios.

2.1 Abstract

Opuntia stricta is a widespread invasive cactus species in South Africa that invades grassland and savanna type ecosystems, and despite successful management using biocontrol, infestations still persist in certain regions. Moreover, CAM photosynthetic species have been shown to respond positively to elevated CO_2 by increasing biomass and improving *WUE,* and therefore observed increases in atmospheric CO_2 concentrations may increase its invasive vigour. *Opuntia stricta* plants were grown at three CO_2 concentrations: sub-ambient (sCO_2 - 250 ppm), ambient (aCO_2 - 400 ppm) and elevated (eCO_2 - 600 ppm) CO_2 to assess the effects on plants traits and photosynthetic physiology. Plants were also subject to suboptimal and optimal watering treatments over the duration of the experiment to emulate two rainfall scenarios to determine the effect of elevated CO_2 on amelioration of soil moisture deficits. Plants showed an increase in biomass from sCO_2 to aCO_2, but not from aCO_2 to eCO_2, and with each increase in CO_2, *WUE* increased by ~ 37 %. At eCO_2, plants allocated resources to forming spines and increased basal cladode biomass at the expense of increasing overall size and biomass, with a similar trend observed at suboptimal watering,

indicating some amelioration of lower soil moisture levels on productivity. Increased spine formation could improve defence while larger basal cladodes may buffer against environmental stresses, thereby enhancing fitness and invasive vigour. Additionally, with improved *WUE*, *O. stricta* has the potential to expand into lower rainfall regions in the future. Although these effects may manifest gradually, if ignored, *O. stricta* and other invasive cacti species have the potential to become significantly more problematic and challenging to manage in the future.

Keywords: CAM, CO_2 exchange, defence, elevated CO_2, nitrogen content, stable isotopes, resource allocation, water use efficiency

2.2 Introduction

The Cactaceae is a family of approximately 1922 species from 130 genera (Novoa et al. 2015), many of which are significantly important agricultural and invasive species, yet there are few studies that have investigated the effects of elevated CO_2 on their productivity and biology. Extensive keyword literature searches have revealed only five cactus species where the effects of elevated CO_2 with or without additional abiotic treatment/s have been investigated. Of these studies, most have focused on *O. ficus-indica* owing to its importance as a fruit crop and fodder plant (Nobel, 1991). However, due to its high productivity under various climatic conditions, it is also a widespread invasive alien plant (IAP) in South Africa and elsewhere (Zimmermann et al. 2009; Novoa et al. 2015; Kaplan et al. 2017). The remaining studies investigated three species: *H. undatus* (fruit crop) (Raveh et al. 1995; Weiss et al. 2010), *Selenicereus megalanthus* (K.Schum. ex Vaupel) Moran (fruit crop) (Weiss et al. 2010) and *Ferocactus acanthodes* (Torr. & Gray) Britt. & Rose (horticulture) (Nobel and Hartsock, 1986). Of these species, only *O. ficus-indica* is included in the 33 listed invasive cacti in South Africa (Kaplan et al. 2017). There are many more studies on CAM photosynthetic species from other families, some that also share the same form of obligate CAM photosynthesis to the aforementioned cacti species (Drennan and Nobel, 2000).

Based on these studies it appears that obligate CAM photosynthesis is responsive to elevated CO_2, and therefore is predicted to increase the vigour of invasive cacti in South Africa.

Under favourable environmental conditions (high soil moisture, high rainfall, lower vapour pressure deficits), CAM plants may exhibit enhanced daytime phase II and IV CO_2 uptake, which can greatly contribute to their overall productivity (Drennan and Nobel, 2000). Although daytime C_3 photosynthesis is energetically more efficient than CAM due to lower ATP requirements for metabolism (Cui and Nobel, 1994; Osmond et al. 1979; Herrera, 2009; Skrzypek et al. 2013), the trade-off is a decrease in *WUE* due to higher transpiration rates exhibited during the daytime (higher vapour pressure deficit during the day relative to the night), hence conditions need to be favourable to initiate daytime C_3 photosynthesis. However, as atmospheric CO_2 rises, similar to C_3 and C_4 plants, CAM plants become more *WUE* enabling them to augment or simply initiate daytime C_3 photosynthesis (Cui et al. 1993; Cui and Nobel, 1994; Nobel et al. 1994). While the consequence of this is decreased daytime *WUE*, the higher *WUE* exhibited at night-time tends to offset this additional day-time water loss (Cui et al. 1993). Doubling of atmospheric CO_2 showed an overall increase in *WUE* of ~ 40%, 52% and 27% for *O. ficus-indica*, *Agave deserti* Engelm. (Asparagaceae) and *A. comosus* respectively, despite lower *WUE* during day-time CO_2 uptake relative to night-time CO_2 uptake (Cui et al. 1993; Graham and Nobel, 1996; Zhu et al. 1999). This greater *WUE* is also accompanied by more biomass accumulation (Cui et al. 1993, Cui and Nobel, 1994, Graham and Nobel, 1996).

CAM plants are adapted to arid and semi-arid environments that experience seasonal rainfall conditions (Cushman, 2001; Herrera, 2009). This adaptation to these environments is largely due to their high *WUE*, small stomatal size and or density and succulence (Luo and Nobel, 1993; Drennan and Nobel, 2000). Despite having these adaptive traits to persist in these environments, productivity can be significantly reduced under low soil moisture conditions relative to non-water stressed plants (Luo and Nobel, 1993; Graham and Nobel, 1996; Drennan and Nobel, 2000). However, under elevated CO_2, CAM species such as *O. ficus-indica*, *H. undatus* and *A. deserti* can partially mitigate the effects of progressive drought on productivity relative to ambient CO_2 (Nobel and Israel,

1994; Raveh et al. 1995; Graham and Nobel, 1996; Drennan and Nobel, 2000). Relative to plants at grown ambient CO_2, plants at grown at elevated CO_2 can maintain higher CO_2 uptake during drought and therefore CO_2 uptake can be maintained at soil moisture levels that are too low for the same plants grown at ambient CO_2 to remain productive. This is largely attributed to improved *WUE* (increased CO_2 uptake and lower stomatal conductance) which maintains higher cladode water content which enable plants to continue CO_2 uptake further into a drought. Additionally, soil moisture is conserved which can extend the period for growth (Raveh et al. 1995, Graham and Nobel, 1996). Additionally, under elevated CO_2 water storage capacity is increased whereby *O. ficus-indica* shows a threefold increase in basal cladode biomass (Cui et al. 1993). Luo and Nobel (1993) suggested that larger *O. ficus-indica* basal cladodes store more water and carbohydrates and are subsequently buffered from environmental stresses.

Unlike C_3 and C_4 photosynthetic species which have distinctly different carbon 13 isotopic ($\delta^{13}C$) fractionation signals due to their characteristic photosynthetic metabolisms, obligate CAM photosynthetic plants can utilise both C_3 and C_4-type pathways (Winter and Smith, 2021). However, CAM $\delta^{13}C$ values are generally more reflective of C_4 (~ -12‰) than C_3 (~ -27‰) photosynthetic plants, which is due to the majority of CO_2 fixation being performed by PEPcase (phase I vs phases II and IV) (Winter and Holtum, 2002; Skrzypek et al. 2013). However, depending on the contribution of either daytime and or night-time CO_2 uptake to carbon gain, $\delta^{13}C$ in CAM plants can range between -9 to -23‰ (Winter and Holtum, 2002; Herrera, 2013). According to Winter and Holtum (2002), CAM plants with $\delta^{13}C$ values of -13‰ to -14‰ obtain ~ 71% to 77% of their carbon at night, whereas $\delta^{13}C$ values around -19‰ indicate ~ 44% of carbon gained at night. Skrzypek et al. (2013) showed that *Agave havardiana* Trel. and *Opuntia engelmannii* Salm-Dyck. had more negative $\delta^{13}C$ values at higher altitude relative to low altitude, which was attributed to less extreme climate i.e., lower VPD (as function of temperature and humidity) and higher rainfall. This also suggests a greater contribution of C_3 vs CAM at a higher altitude where conditions are more favourable for daytime C_3 photosynthesis. Although not shown in the literature, changes to the CAM phases, particularly increased

daytime C_3 contribution to CO_2 uptake as observed under elevated CO_2, should also elicit similar changes to tissue $\delta^{13}C$ as observed by Skrzypek et al. (2013).

CO_2 at current atmospheric concentrations (420 ppm: August 2023) is too low to saturate PEPcase and or RuBisCO and therefore a CO_2 is a limiting substrate for photosynthesis. Subsequently, plants need to invest in limited resources (e.g., N) to remain productive and competitive (Ziska et al. 2001). However, as CO_2 rises, plants can invest in less N, a major component of RuBisCo, and still maintain or achieve higher photosynthetic rates, while being more *WUE*, a trend evident in *O. ficus-indica* grown at elevated CO_2 (Cui and Nobel, 1994; Nobel et al. 1994). Nitrogen is one of the main (and to a lesser extent phosphorus, depending on the habitat) growth limiting nutrients in most terrestrial environments (Ainsworth et al. 2003; Fisher et al. 2012). Being more nitrogen-use efficient can improve a plants competitive ability, which has important implications for cacti that tend to invade marginal landscapes (Shackleton et al. 2007). In addition to less investment in N, improved photosynthetic productivity with increasing CO_2 allows additional carbon to be allocated elsewhere such as carbon-based secondary compounds (phenolics and terpenoids) which can increase plants defences against herbivores (Lincoln et al. 1993; Penuelas et al. 1997). Thus, less N (less protein) and potentially increased carbon-based defence increases the C/N ratios, which consequently reduce the nutritional quality of plant tissue for herbivores (Lincoln et al. 1993; Penuelas et al. 1997; Robinson et al. 2012). Overall, this possibly creates a positive feedback whereby plants become more photosynthetically efficient and less nutritious for herbivores, thus contributing to overall increased vigour under future elevated CO_2 scenarios. This has implications for the management of invasive cacti whereby invasiveness could be exacerbated while effects of insect biological control could be weakened.

Based upon existing elevated CO_2 cactus studies, inferences could be made as to the invasive potential of cactus weeds, however cactus growth forms can vary significantly (Novoa et al. 2015). *Opuntia ficus-indica* is larger and more tree-like whereas *O. stricta* is small and shrub-like. These different growth forms could have significant effects on how either plant responds to elevated CO_2 (de Graaf et al. 2006; Stiling et al. 2007; Robinson et al. 2012). Furthermore, invasive

cacti tend to have extensive distributions that encompass a wide range of climatic conditions, such as mean annual precipitation (Kaplan et al. 2017), which can affect their invasive vigour, both at current and future CO_2 concentrations. Studies that include the effect of water on cacti to assess the ameliorative effect of elevated CO_2 have only examined plants grown under well-watered conditions and then exposed to progressive short-term droughts (Nobel and Israel, 1994; Raveh et al. 1995), as opposed to being grown under different long-term watering regimes that emulate different mean annual rainfall. Drought stressing plants that have developed under well-watered conditions can give insight into the ability of plants to mitigate short-term drought events, and the mechanism/s employed i.e., drought escape, drought avoidance, drought survival, and drought tolerance (Lawlor, 2013). However, *Opuntia* have long-lived cladodes and are unlikely to alter their morphological traits during short-term drought and thus will not properly reflect how populations growing across a rainfall gradient may respond physiologically. *Opuntia* that develop at lower soil moisture levels under eCO_2 may show improved acclimation to these conditions, giving greater insight into their ability to ameliorate the effects of reduced soil moisture on productivity and growth. Such responses will also give better insight when trying to predict changes in distribution under future climate scenarios. Thus, understanding how *O. stricta* will respond under future climates, such as changes to mean annual rainfall along with the interaction of CO_2 needs to reflect both optimal and suboptimal conditions.

Of the listed invasive cacti taxa in South Africa, 14 (plus numerous varieties thereof e.g., *O. engelmannii* varieties) are *Opuntia* (Kaplan et al. 2017), which highlights the need to understand how *Opuntia* species may respond to rising atmospheric CO_2 concentrations and climate change. *Opuntia stricta* is the most widespread non-agriculturally significant *Opuntia* species in South Africa, and thus the main aim of this study is to investigate the combined effects of CO_2 and rainfall on its invasive vigour and the implications for future management. *Opuntia stricta* plants were grown under three CO_2 concentrations corresponding to pre-industrial (250 ppm), current (400 ppm), and future (600 ppm) CO_2 concentrations using two watering regimes: suboptimal and optimal. To address Objective 1 of the study, plant growth rates, biomass, cladode traits, resource

allocation and photosynthetic productivity were measured to assess changes to *O. stricta* invasive vigour. Objective 2 was addressed by growing the plants under the two different long-term watering regimes to determine if increasing CO_2 ameliorated the effects of suboptimal watering on the traits measured in Objective 1. By addressing Objective 1 and 2, changes to resource allocation, photosynthetic efficiency and amelioration of suboptimal watering should give insights into the effects of historical increases in CO_2 on this weed's invasive vigour and importantly how projected increases in atmospheric CO_2 and climate change may influence its future invasiveness.

2.3 Methods

2.3.1 Plant material and experimental set-up

Opuntia stricta daughter cladodes were obtained from the Agricultural Research Council - Plant Health and Protection (ARC-PHP) Roodeplaat, Gauteng, South Africa (-25.607, 28.352). The daughter cladodes ranged in mass from 50 – 90 grams (15 – 20 cm long). The bottom third of the cladodes were planted vertically in 20 cm (3 litre) pots containing ~ 2 kg of air dried sandy-loam soil. After potting, plants were moved into one of the three climate-controlled growth chambers (Conviron PGV40, Winnipeg, Canada) each with a different CO_2 concentration: 250 ppm (pre-industrial; sub-ambient - sCO_2), 400 ppm (current; ambient - aCO_2) and 600 ppm (RCP6.5 ~ ca. 2080; elevated - eCO_2). Ambient temperatures within the chambers were programmed to change at approximately 1 °C / hour between the day-time maximum of ± 27 °C (11:00 to 16:30) and the night-time minimum of ± 20 °C (23:00 to 05:00). To obtain the desired range of VPD of 0.82 kPa (dark) to 1.25 kPa (light), relative humidity was maintained at 65%. Light was supplied by overhead fluorescent tubes (Philips Master TL5 HO 54W/840, 4000 Kelvin) and halogen bulbs (Philips 55W GLS A55 E27, 2800 Kelvin). Lights turned on at 05:00 and the intensity increased to its peak at 09:30 and thereafter decreased from 16:30 till 19:00, at which time the lights turned off (Fig. S2.1). Plants were positioned in such a manner to achieve a photosynthetic

photon flux density (PPFD) of approximately 1000 µmol m^{-2} s^{-1} at the top of the plants. Only one of the three chambers could scrub incoming CO_2 (using soda lime) to create sub-ambient CO_2 levels (~ 250 ppm), therefore plants were not moved between chambers. Temperature, humidity, VPD and [CO_2] were logged every five minutes (point measures) during most of the experiment (see Table S3.1). To verify conditions within the three chambers were similar and being maintained correctly, a LI-6400XT portable open path infrared gas analyser (LICOR Biosciences, Lincoln, Nebraska, USA) was used fortnightly to obtain spot readings for the following parameters: temperature, RH, VPD, PPFD and [CO_2] (this data was not recorded). Six grams of slow release granular 7:1:3 fertiliser (N = 95 g kg^{-1}; P = 14 g kg^{-1}; K = 41 g kg^{-1}) was added to each pot on day 30.

Each chamber (CO_2 concentration) contained 20 plants split into 10 suboptimal water treatment plants (soil water potential ≤ 0.5 MPa) and 10 optimal water treatment plants (soil water potential > 0.5 MPa). The suboptimal watering treatment plants were given 200 ml wk^{-1} which corresponded to ~ 6 mm rainfall per week (6 mm x 52 weeks = ~ 312 mm mean annual precipitation: MAP) and the optimal watering treatment plants were given 400 ml wk^{-1} (12 mm x 52 weeks = ~ 624 mm MAP). The following formula: volume water = π $radius^2$ of pot x height of water on soil surface, was used to calculate the weekly quantity of water required to simulate the two MAPs. Plants were watered at the start of each week for the duration of the experiment. Prior to watering, soil water content was measured using a HS2 HydroSense II (Campbell Scientific, Logan, Utah, USA). The experiment was conducted over an 8-month period (initiated 24th Jan 2018, concluded 1st October 2018; 250 days).

2.3.2 Plant growth and biomass parameters

Plant growth parameters were measured monthly for the duration of the experiment. These included: plant height, number of cladodes and cladode area (length x width ellipse formula). At harvest, the following parameters were assessed: cladode hardness, spine traits, specific leaf (cladode) area (SLA) and the

above and below ground biomass. SLA was calculated as: area/dry mass (cm^2 g^{-1}). Cladode hardness was measured by vertically attaching a blunt needled with a known surface area (0.1385 mm^2) on a balance stage and the detached cladode was gently pressed onto the needle and when the needle pierced the cladode, the force required to do so was recorded. Hardness was calculated as g force mm^{-2}. All spines per plant were removed oven dried (60 °C for a week), counted and weighed. Total spine mass, number of spines, mean spine mass and spine density (grams spines by one side cladode area) were calculated. Dry plant biomass was obtained by separately oven drying (60 °C for a week) the daughter cladodes (new growth), the basal cladode and roots. Whole plant weekly water use was calculated from water remaining in the soil at the end of the week (derived from SWC) over the final dry biomass of the plants (ml mg^{-1}).

2.3.3 Gas exchange 24-hour cycles and cladode pH

Opuntia species are obligate CAM plants and thus understanding the effect of the CO_2 and water treatments required gas exchange measurements to be conducted over a 24-hour cycle. Measurements were performed after 7.5 months of growth. Gas exchange measurements were conducted on two occasions which started on the Wednesday morning (day 231) (48 hours after watering) and continued for 24 hours until Thursday morning (day 232) and then repeated the following Wednesday (day 238) and Thursday (day 239). Plants were randomly selected (random number table) within each chamber, measuring two plants of either watering treatment and then sequentially moving through all the chambers repeating the measurements on two plants of either watering treatment for the 24-hour period. Net CO_2 and H_2O gas exchange measurements were conducted using a LI-6400XT infrared gas analyser with the 2 × 3 cm clear top LI-COR cuvette. A clear top cuvette was used such that the light cycle programmed in the chambers and the associated PPFD was tracked and therefore reflected in the gas exchange measurements. The cuvette could not be positioned horizontally on the cladode (cladodes grow vertically) and thus maximum PPFD for gas exchange measurements was ~ 800 µmol m^{-2} s^{-2} (Fig. S2.1). To conduct gas exchange

measurements that reflected the actual ambient conditions experienced by the plants within the growth chambers, the temperature and VPD within the cuvette were not pre-programmed. However, incoming CO_2 was programmed according to the chamber CO_2 concentration. Data were logged after 2 - 3 minutes once cuvette conditions stabilised in response to being opened prior to enclosing a section of the cladode. Using the equations of von Caemmerer and Farquhar (1981), net photosynthesis (*A*, also referred to as CO_2 uptake or CO_2 exchange in this chapter) and stomatal conductance to water vapour (g_{ST}) were calculated. Intrinsic water use efficiency (*WUE*) was calculated as the ratio of net photosynthesis to stomatal conductance (A/g_{ST}: μmol CO_2 mol^{-1} H_2O).

Cladode pH was measured to monitor the 24-hour malic acid cycle and how this was affected by CO_2 treatments. This provides additional photosynthetic productivity data in support of the gas exchange measurements. Cladode pH was measured using a micro pH probe with a 1.7 mm diameter hypodermic tip (Orion™ 9863BN Micro pH Electrode: Thermo Fisher Scientific, Waltham, MA USA 02451) attached to an Orion 3 Star Benchtop pH Meter (Thermo Fisher Scientific, Waltham, MA USA 02451). The pH probe was calibrated using pH 4, 7 and 10 buffer solutions. This procedure to assess cladode pH was preferred because it was less destructive than removing cladode cores. Using a pin, a small hole was made in a standardised position on a cladode and the needle was inserted about 2 -3 mm into the cladode to obtain pH values. pH measurements were conducted two weeks before (day 217) the gas exchange measurements. Measurements commenced at 10 am and were repeated every four hours, until 10 pm when the pH probe broke, thus a full 24-hour curve was not completed. Five plants per water treatment were sampled at each CO_2 concentration for each sampling event, however, because the probe broke, pH results were grouped by CO_2 treatment and did not include watering treatment as an effect.

2.3.4 Cladode pigments

At the end of the experiment, cladode tissue was extracted from one daughter cladode per plant using a 4 mm diameter punch. The epidermal/cortex layers on

either side of the cladode were separated from the vascular bundle and internal pith. The epidermal/cortex samples from both sides of the cladodes were placed in separate test tubes containing a known volume of 96% analytical grade ethanol, crushed, and incubated in the dark at 25 ˚C for 72 h. A 3 ml aliquot was then transferred to a standard 4.5 ml cuvette and light absorptance was measured using a spectrophotometer (Thermo Scientific™ GENESYS 20). Absorptance wavelengths of 664 nm, 649 nm and 470 nm were used to measure chlorophyll a, b and c (carotenoids), respectively. Pigment contents (mg m^{-2}) were calculated using the equations as described by Lichtenthaler (1987). The pigment results from both sides of cladode were averaged for each plant.

2.3.5 Cladode element and stable isotope analyses

Three small subsamples of oven dried cladode tissue were removed from an individual plant, crushed and homogenised. A subsample of 1 mg ± 0.1 of the crushed homogenised tissue was weighed into 6 x 4 mm tin capsules (D1006 Capsules. Elemental Microanalysis, Okehampton, United Kingdom). Weighed samples were then analysed for C and N and $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) stable isotope ratios. Analyses of samples were conducted according to the methods presented in Section 3.3.5 (Chapter 3). Cladode C and N contents were used to calculate C/N ratios.

2.3.6 Statistical analyses

All statistical analyses were performed using RStudio (R Core Team, 2022). Mean annual precipitation (MAP) for South African *O. stricta* distribution records were extracted from the BIO12 Baseline (MAP in mm) raster from Worldclim using functions from the *'raster'* package (Fick and Hijmans, 2017). To control for repeated measures, linear mixed-effects models were used to analyse the linear and interactive effects of time, CO_2 concentration, and watering treatment on plant growth (cladode area) (individual plant was the random effect). This was

conducted using the '*lmer'* function from the *'lme4'* package (Bates et al. 2015). Linear models (*lm*) were used to analyse the linear and interactive effects of CO_2 and water on final data (i.e., measurements made at final harvest) and therefore individual plant was not treated as a random effect (no repeated measures). The '*Anova'* function from the *'car'* package (John et al. 2020) was used to calculate analysis-of-variance tables for the *lmer* and *lm* models. Tukey post-hoc analyses were conducted for all models using functions from the *'multcomp'* package. Data were transformed where necessary to improve the distribution of the residuals. Principal component analyses (PCA) of plants traits were conducted using the '*psych*' package (Revelle, 2022).

2.4 Results

2.4.1 Plant growth rates

Opuntia stricta growth rates (cladode area) were affect by the linear effects of time ($F_{1,357}$ = 234; P < 0.001), CO_2 ($F_{2,356}$ = 11.4; P < 0.001) and water ($F_{1,357}$ = 34; P < 0.001) (Fig. 2.1). There was no significant interaction of time x CO_2 x water ($F_{5,354}$ = 0.23; P = 0.8), indicating similar growth trends amongst treatments for the duration of the experiment. Water treatment within CO_2 treatments influenced cladode area with increasing CO_2 concentrations, whereby the effect of water treatment on cladode area was greatest for sCO_2 and smallest for eCO_2 (Fig. 2.1). The initial growth rates increased across all treatments (CO_2 and water) until day ~ 91 and then plateaued. These initial growth rates differed by CO_2 ($F_{2,57}$ = 6.5; P = 0.003) and water ($F_{1,58}$ = 55; P < 0.001) but there was no interaction of CO_2 and water ($F_{5,54}$ = 0.5; P = 0.6) (Fig. 2.2).

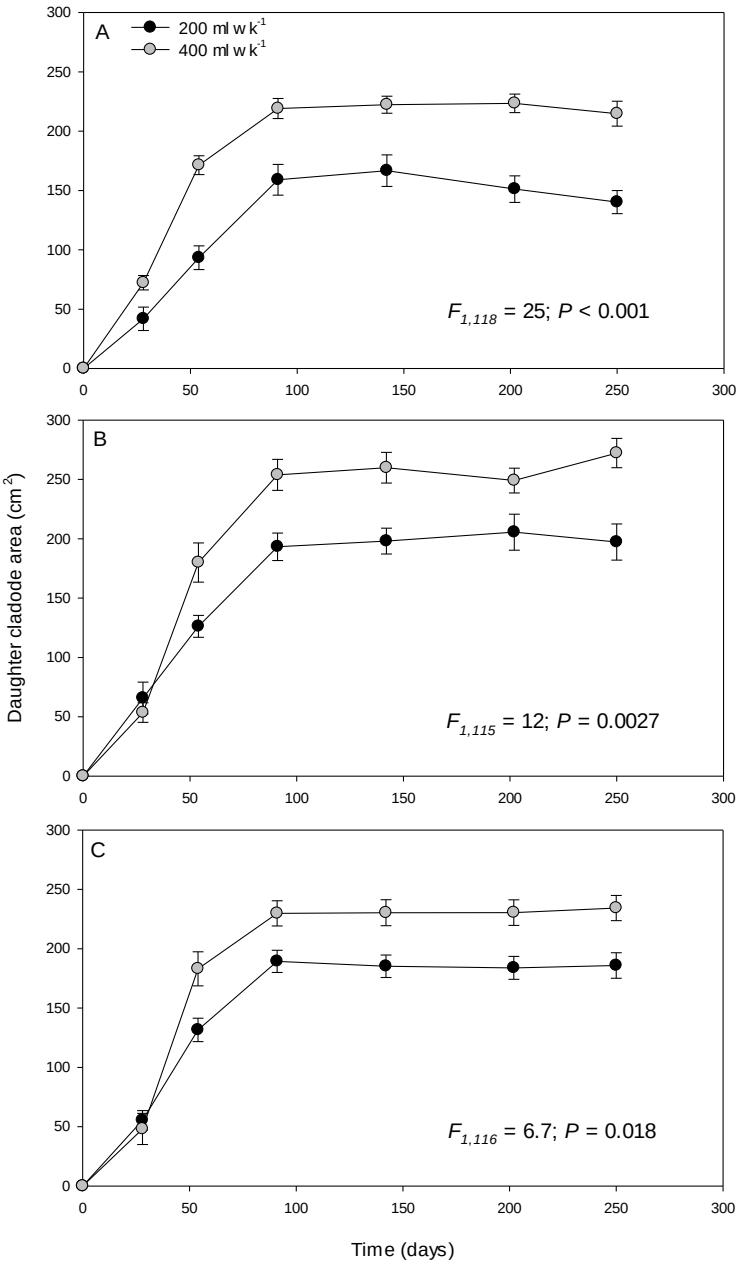


Figure 2.1: *Opuntia stricta* daughter cladode (new growth) area measured over time for plants grown at three CO_2 concentrations: (A) 250 (sCO_2), (B) 400 (aCO_2) and (C) 600 (eCO_2) ppm, with two water treatments: 200 ml week^{-1} and 400 ml week^{-1}. n = 10 plants for each data point (mean ± SE). Statistics reported in each graph are the effect of water treatment. Full interaction: time x CO_2 x water ($F_{2.356}$ = 0.22; P = 0.8).

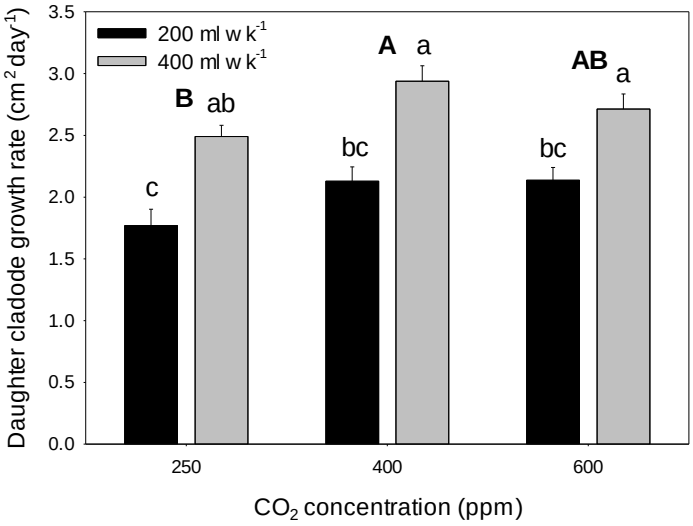


Figure 2.2: *Opuntia stricta* daughter cladode (new growth) area growth rate for the initial 91 days for plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml week^{-1} and 400 ml week^{-1}. n = 10 plants for each bar (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase bold letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

2.4.2 Plant biomass and cladode traits

The effects of CO_2 and water treatment were significant for daughter cladode mass, total spine mass, average spine mass, total mass (Fig. 2.3 B, C, D & F; Table 2.1), number of spines and spine density (Fig. 2.4 E & F; Table 2.1). Basal cladode mass (Fig. 2.3 A; Table 2.1) and cladode hardness were only affected by CO_2 whereas the water treatment only had a significant effect on root mass and root/shoot ratios (Fig. 2.3 E; Table 2.1). Neither treatment influenced plant height nor the number of cladodes (Fig. 2.4 C & D; Table 2.1).

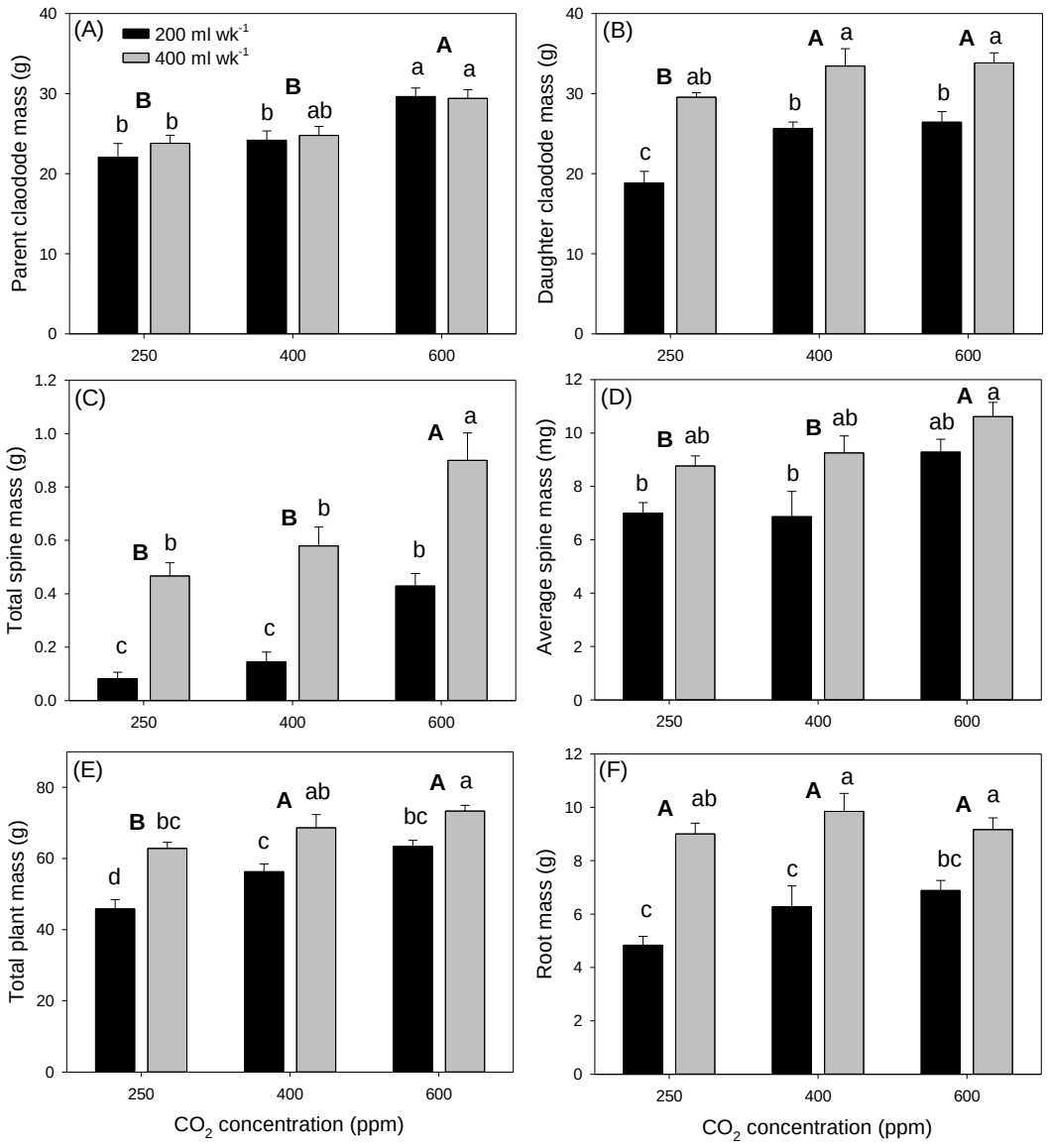


Figure 2.3: Biomass parameters measured on *Opuntia stricta* plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml week^{-1} and 400 ml week^{-1}. n = 10 plants for each bar (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase bold letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

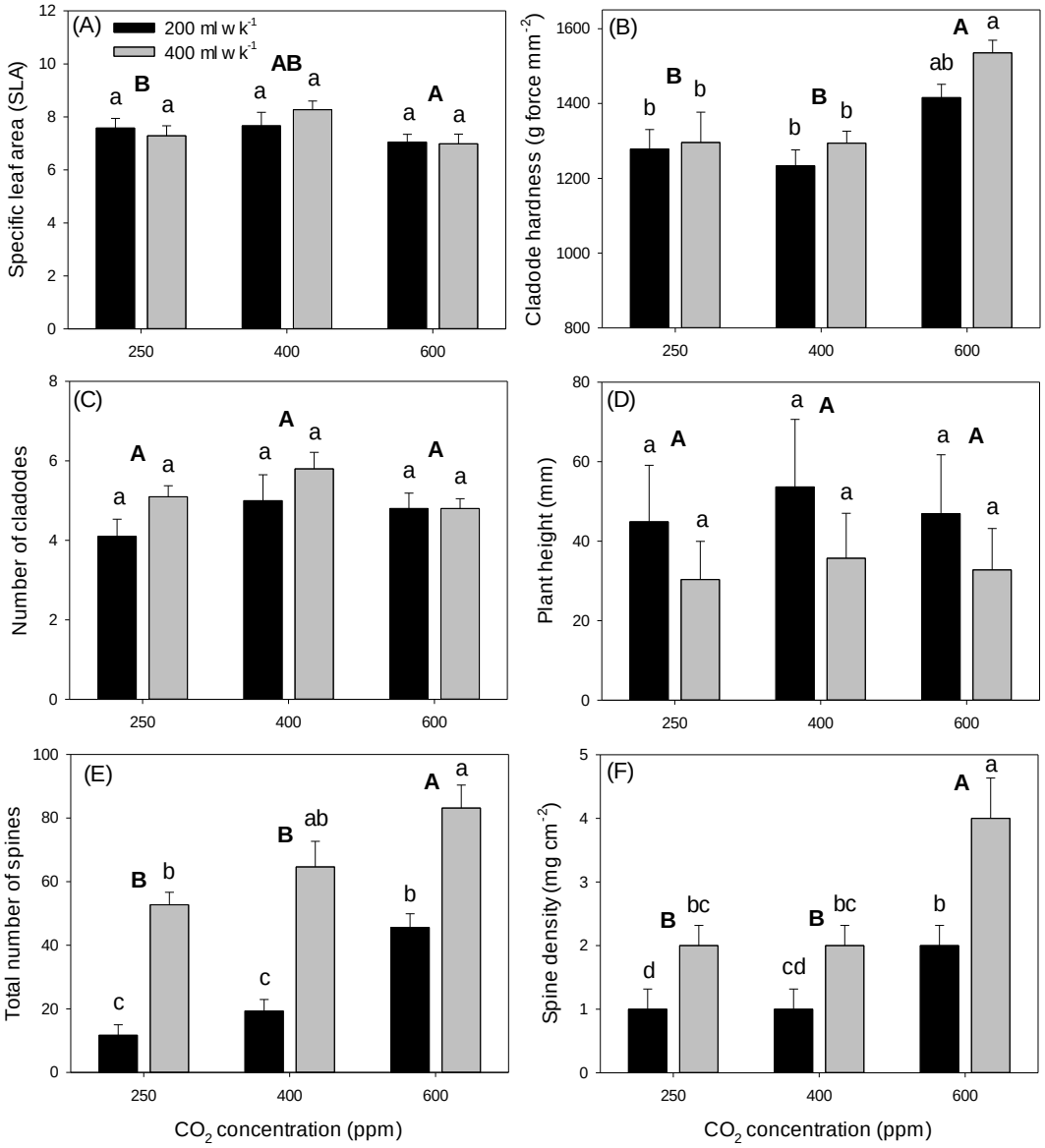


Figure 2.4: Plant traits measured on *Opuntia stricta* plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml week^{-1} and 400 ml week^{-1}. n = 10 plants for each bar (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

2.4.3 Cladode pigments

CO_2 had a significant effect on chlorophyll b and c which decreased with increasing CO_2 while the opposite was observed for chlorophyll a/b ratio (Fig. 2.5 B, C & E; Table 2.1). Watering treatment had no effect on cladode pigments but there was a significant interaction of CO_2 and water treatment on chlorophyll a and total chlorophyll (Fig. 2.5 A & E; Table 2.1).

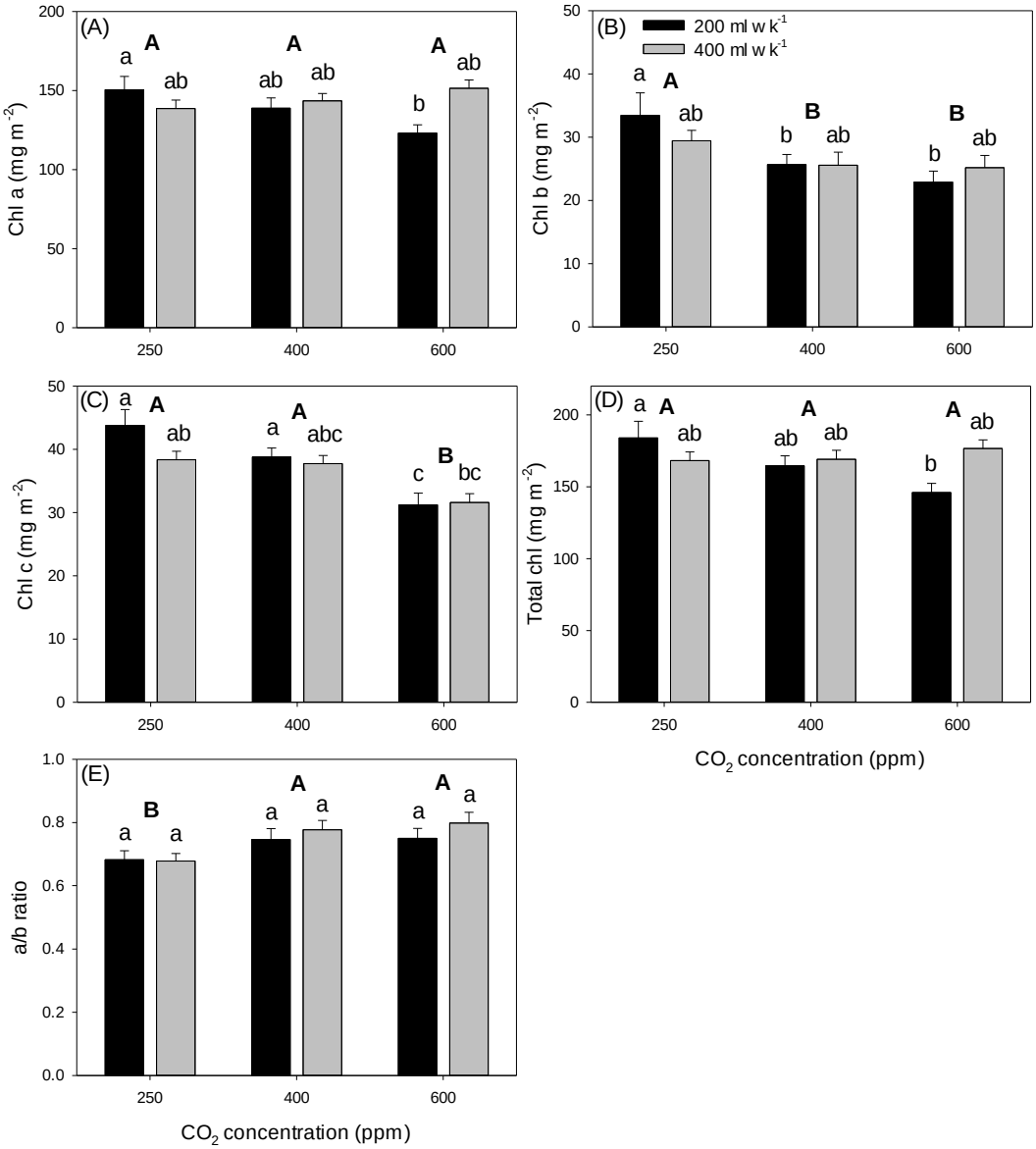


Figure 2.5: Cladode pigments measured on *Opuntia stricta* plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml $week^{-1}$ and 400 ml $week^{-1}$. n = 10 plants for each bar (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase bold letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

2.4.4 Cladode element concentrations

Cladode C and C/N ratios were significantly higher with the 200 ml week^{-1} water treatment (Fig. 2.6 A & C; Table 1). CO_2 had a significant effect on N and C/N ratios whereby N decreased with increasing CO_2 while C/N ratios increased (Fig. 2.6 B & C; Table 2.1). Cladode C was not significantly affected by CO_2 (Fig. 2.6 A; Table 2.1).

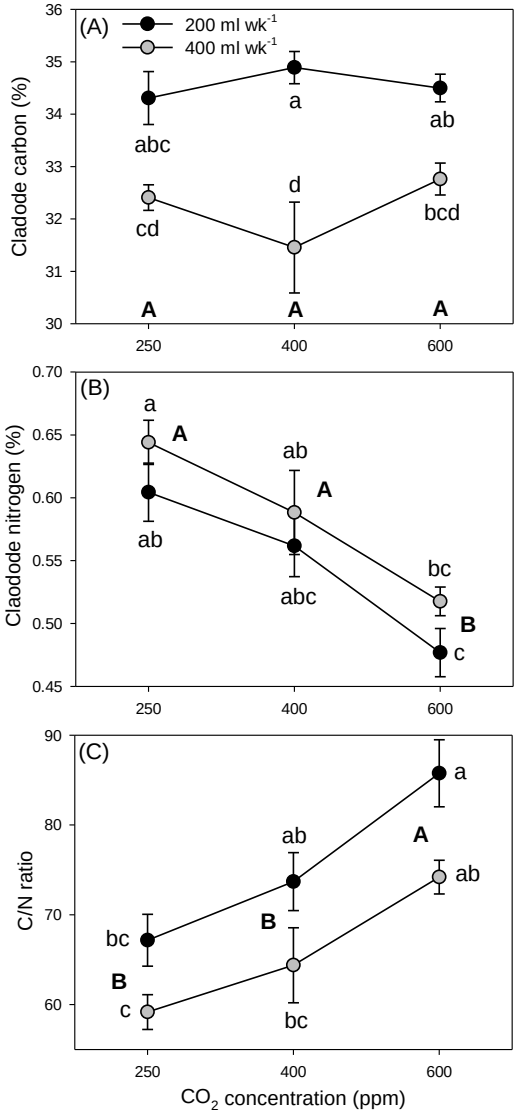


Figure 2.6: Cladode element concentrations measured on *Opuntia stricta* plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml week^{-1} and 400 ml week^{-1}. n = 10 plants for each data point (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase bold letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

Table 2.1: Summary of general linear model statistics for *Opuntia stricta* traits measured at final harvest. Plants were grown at 250 (sCO_2), 400 (aCO_2) and, 600 ppm (eCO_2) CO_2 concentrations with two watering treatments: 200 ml wk^{-1} and 400 ml $week^{-1}$. Bold results indicate significance ($P < 0.05$).

		F_{stat}: *P* - value		
***Opuntia stricta* traits**	**Units**	**CO_2**	**Water**	**CO_2 x water**
Height	mm	$F_{2,93}$= 0.8; *P* = 0.5	$F_{1,94}$= 0.4; *P* = 0.5	$F_{5,90}$= 0.01; *P* = 0.1
Daughter cladode mass	g	**$F_{2,93}$= 12; *P* < 0.001**	**$F_{1,94}$= 60; *P* < 0.001**	$F_{5,90}$= 0.88; *P* = 0.4
Root mass	g	$F_{2,93}$= 2.95; *P* = 0.06	**$F_{1,94}$= 159; *P* < 0.001**	$F_{5,90}$= 1.7; *P* = 0.2
Basal cladode mass	g	**$F_{2,93}$= 16; *P* < 0.001**	$F_{1,94}$= 0.5; *P* = 0.5	$F_{5,90}$= 0.3; *P* = 0.7
Total spine mass	g	**$F_{2,93}$= 23; *P* < 0.001**	**$F_{1,94}$= 77; *P* < 0.001**	$F_{5,90}$= 0.3; *P* = 0.8
Average spine mass	mg	**$F_{2,93}$= 7.5; *P* < 0.01**	**$F_{1,94}$= 14; *P* < 0.001**	$F_{5,90}$= 0.4; *P* = 0.7
Total mass	g	**$F_{2,93}$= 18; *P* < 0.001**	**$F_{1,94}$= 46; *P* < 0.001**	$F_{5,90}$= 1.1; *P* = 0.3
Total number of spines		**$F_{2,93}$= 17; *P* < 0.001**	**$F_{1,94}$= 88; *P* < 0.001**	$F_{5,90}$= 0.3; *P* = 0.7
Spine density	$g\ m^{-2}$	**$F_{2,93}$= 17; *P* < 0.001**	**$F_{1,94}$= 30; *P* < 0.001**	$F_{5,90}$= 0.06; *P* = 0.9
Total number of cladodes		$F_{2,93}$= 1.9; *P* = 0.16	$F_{1,94}$= 3; *P* = 0.09	$F_{5,90}$= 0.8; *P* = 0.5
Total cladode area	cm^2	**$F_{2,93}$= 12; *P* < 0.001**	**$F_{1,94}$= 48; *P* < 0.001**	$F_{5,90}$= 0.8; *P* = 0.4
Cladode hardness	g force mm^{-2}	**$F_{2,93}$= 11; *P* < 0.001**	$F_{1,94}$= 2.7; *P* = 0.1	$F_{5,90}$= 0.54; *P* = 0.6
Specific leaf area*	$cm^2\ g^{-1}$	**$F_{2,93}$= 3.2; *P* < 0.05**	$F_{1,94}$= 0.07; *P* = 0.8	$F_{5,90}$= 0.76; *P* = 0.48
Root / shoot		$F_{2,93}$= 0.8; *P* = 0.43	**$F_{1,94}$= 29; *P* < 0.001**	$F_{5,90}$= 1.2; *P* = 0.3
Cladode Chl a	$mg\ m^{-2}$	$F_{2,123}$= 1.4; *P* = 0.2	$F_{1,124}$= 0.8; *P* = 0.4	**$F_{5,120}$= 10; *P* < 0.001**
Cladode Chl b	$mg\ m^{-2}$	**$F_{2,123}$= 7.4; *P* = 0.001**	$F_{1,124}$= 0.05; *P* = 0.83	$F_{5,120}$= 1.2; *P* = 0.3
Cladode carotenoids	$mg\ m^{-2}$	**$F_{2,123}$= 22; *P* < 0.001**	$F_{1,124}$= 0.7; *P* = 0.42	$F_{5,120}$= 2.4; *P* = 0.1
Cladode Chl a / Chl b	$mg\ m^{-2}$	**$F_{2,123}$= 5.5; *P* < 0.01**	$F_{1,124}$= 0.1; *P* = 0.34	$F_{5,120}$= 0.36; *P* = 0.7
Cladode total Chl	$mg\ m^{-2}$	**$F_{2,123}$= 3.7; *P* = 0.024**	$F_{1,124}$= 0.49; *P* = 0.5	**$F_{5,120}$= 8.8; *P* < 0.001**
Cladode carbon (C)	%	$F_{2,57}$= 0.48; *P* = 0.62	**$F_{1,58}$= 37; *P* < 0.001**	$F_{5,54}$= 2; *P* = 0.15
Cladode nitrogen (N)	%	**$F_{2,57}$= 16; *P* < 0.001**	$F_{1,58}$= 3.7; *P* = 0.058	$F_{5,54}$= 0.06; *P* = 0.94
Cladode C / N		**$F_{2,57}$= 15; *P* < 0.001**	**$F_{1,58}$= 315; *P* < 0.001**	$F_{5,54}$= 0.17; *P* = 0.84
$\delta^{13}C$	‰	**$F_{2,57}$= 421; *P* < 0.001**	$F_{1,58}$= 0.04; *P* = 0.85	$F_{5,54}$=1.4; *P* = 0.25
$\delta^{15}N$	‰	**$F_{2,57}$= 8.9; *P* < 0.001**	$F_{1,58}$= 0.02; *P* = 0.89	$F_{5,54}$=0.9; *P* = 0.4

* Specific leaf area refers to cladodes

2.4.5 PCA analysis of plant resource allocation patterns

PCA analysis of biomass and plants traits grouped by CO_2 treatment showed a strong association of increasing basal biomass and defence (cladode hardness, spines by area, total spine mass and average spine mass) and decreasing nutritional quality (cladode N and C/N ratios) with eCO_2 (Fig. 2.7). Daughter cladode and root mass parameters are similarly associated across both aCO_2 and eCO_2 treatments. This indicates that beyond 400 ppm, plants no longer invest in additional growth with the exception of basal biomass and N, but rather allocate resources to mechanical defence. The same PCA grouped by water treatment shows that 400 ml wk^{-1} watering is associated with greater plant biomass and enhanced defence traits (Fig. 2.8). Despite the significant effect of CO_2 fertilisation on most plant traits, the effect sizes are largely dependent on the water treatment. The same PCA grouped by CO_2 x water (Fig. 2.9) shows a similar trend to that observed in Figure 2.7 (grouped by CO_2) but highlighting the importance of water (Fig. 2.8; grouped by water treatment) for the associations. Nevertheless, the stronger association of the 200 ml wk^{-1} treatment at eCO_2 (compared to the same watering treatment at the sCO_2 and aCO_2 treatments) with enhanced mechanical defence traits indicates that eCO_2 partially ameliorates the effect of reduced water.

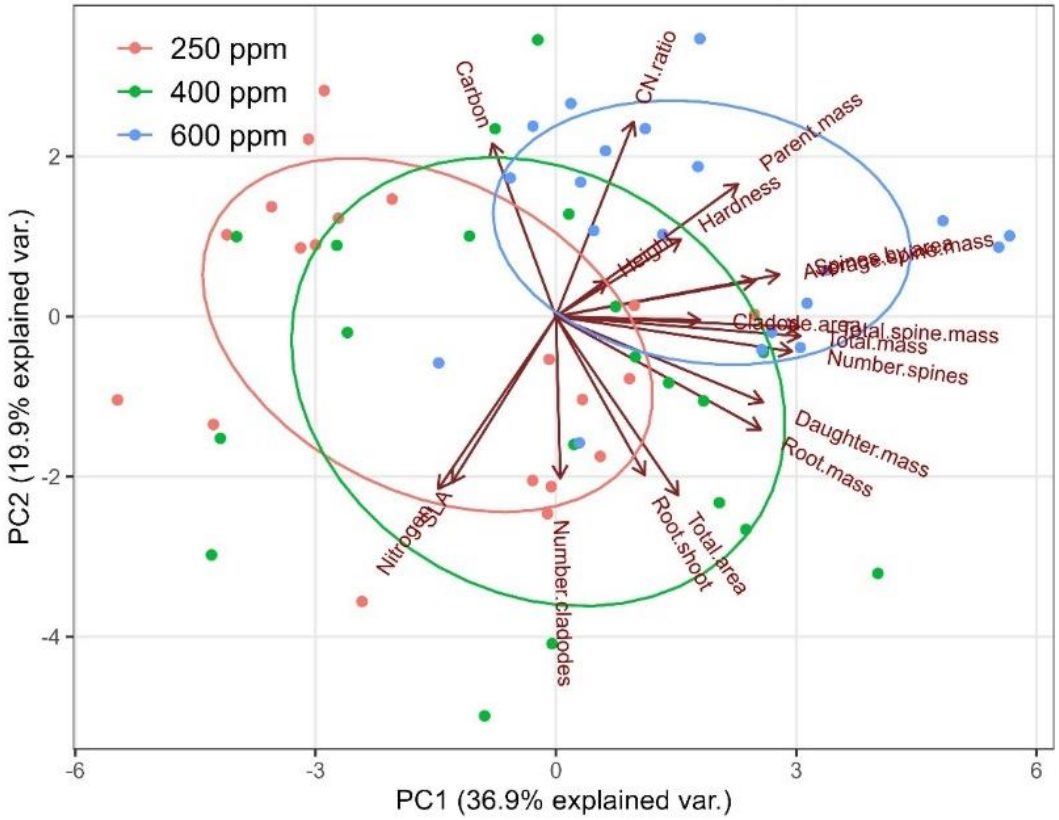


Figure 2.7: Principal component analysis (PCA) of 18 *Opuntia stricta* biomass and cladode traits grouped by CO_2 concentration. Longer vectors (variables) have a larger influence on the PC ordination, vectors that that form a shallow angle to one another show a positive correlation, vectors at 90° are not well correlated to one another and vectors at 180° to one another infer a negative correlation. The two overlapping variables are 'Spines by area' and 'Average spine mass'.

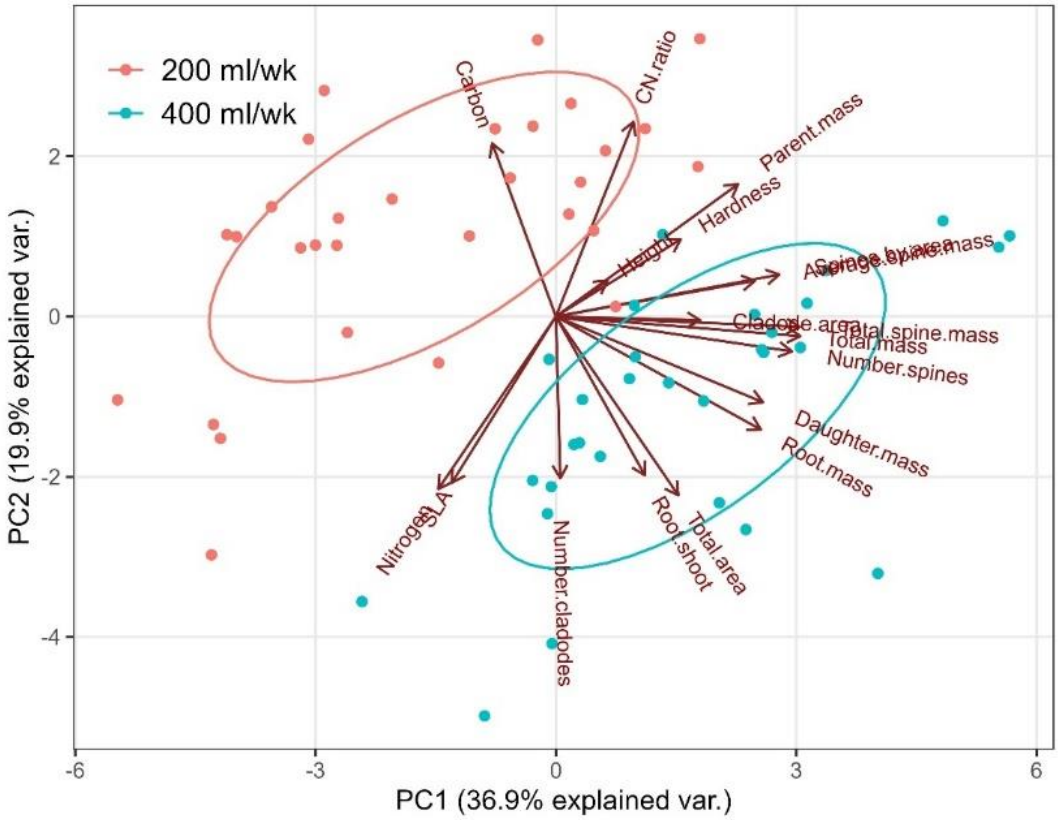


Figure 2.8: Principal component analysis (PCA) of 18 *Opuntia stricta* biomass and cladode traits for plants grown at three CO_2 concentrations grouped by watering treatments: 200 ml/wk (ml wk^{-1}) and 400 ml/wk (ml wk^{-1}). Longer vectors (variables) have a larger influence on the PC ordination, vectors that that form a shallow angle to one another show a positive correlation, vectors at 90° are not well correlated to one another and vectors at 180° to one another infer a negative correlation. The two overlapping variables are 'Spines by area' and 'Average spine mass'.

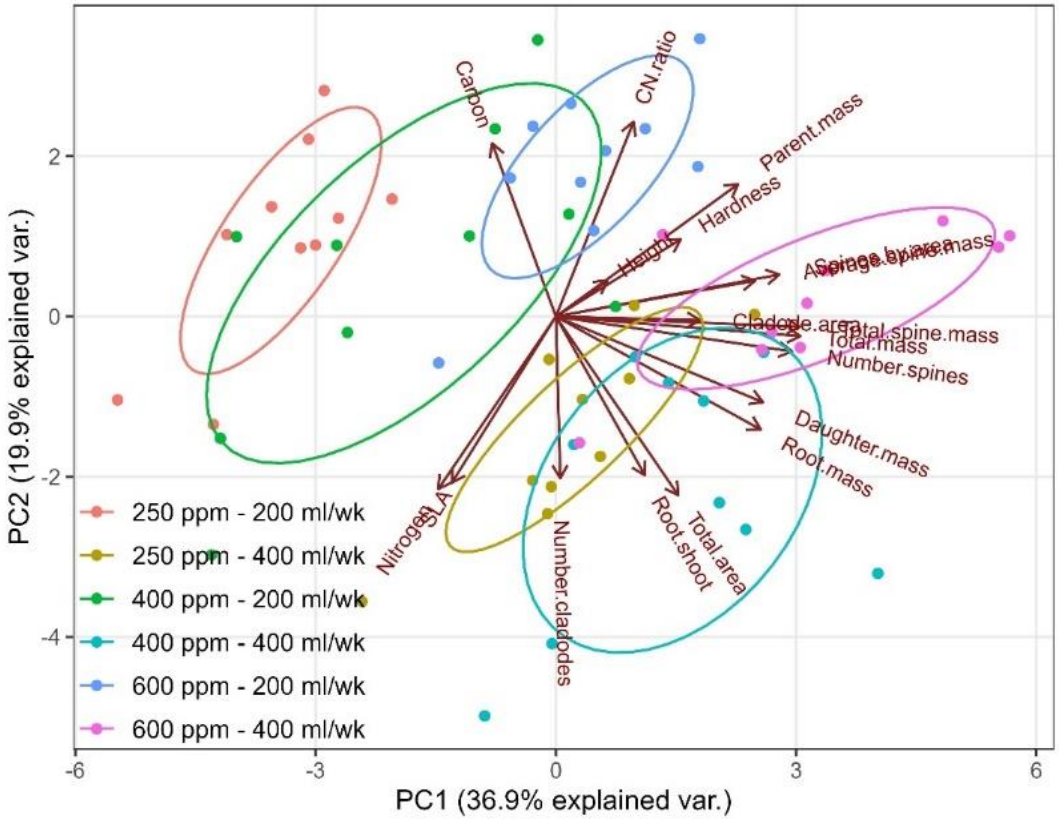


Figure 2.9: Principal component analysis (PCA) of 18 growth and biomass traits for *Opuntia stricta* grouped by CO_2 and watering treatments. Longer vectors (variables) have a larger influence on the PC ordination, vectors that that form a shallow angle show a positive correlation to one another, vectors at 90° are not well correlated and vectors at 180° infer a negative correlation. The two overlapping variables are 'Spines by area' and 'Average spine mass'.

2.4.6 Gas exchange and cladode pH

Day-time CO_2 uptake (05:00 to 17:00) was predominately negative, indicating only respiration was detected for all CO_2 concentrations and water treatments, with the expectation of the 600 ppm (both water treatments) that exhibited conventional C_3 photosynthesis that started at about 16:00 (late afternoon), and was not observed for the 250 and 400 ppm plants (Fig. 2.10 A, C & E). This daytime C_3 photosynthesis in the 600 ppm plants was accompanied by increased stomatal conductance under both watering treatments (Fig. 2.10 F). Interestingly, the 600 ppm plants showed a sharp increase in respiration prior to the initiation of

their late afternoon C_3 photosynthesis (Fig. 2.10 E). Maximum night-time CO_2 uptake was 12.64 μmol m^{-2} s^{-1} (200 ml wk^{-1}) and 12.02 μmol m^{-2} s^{-1} (400 ml wk^{-1}) for 250 ppm plants, 14.63 μmol m^{-2} s^{-1} (200 ml wk^{-1}) and 13.78 μmol m^{-2} s^{-1} (400 ml wk^{-1}) for 400 ppm plants and 13.92 μmol m^{-2} s^{-1} (200 ml wk^{-1}) and 18.57 μmol m^{-2} s^{-1} (400 ml wk^{-1}) for 600 ppm plants. Furthermore, CO_2 uptake was higher for the 200 ml $week^{-1}$ treatment compared to the 400 ml wk^{-1} treatment at 250 and 400 ppm, whereas both water treatments at 600 ppm showed similar CO_2 uptake rates. However, there were not enough individual data points at each time point to fully resolve these curves for statistical analyses.

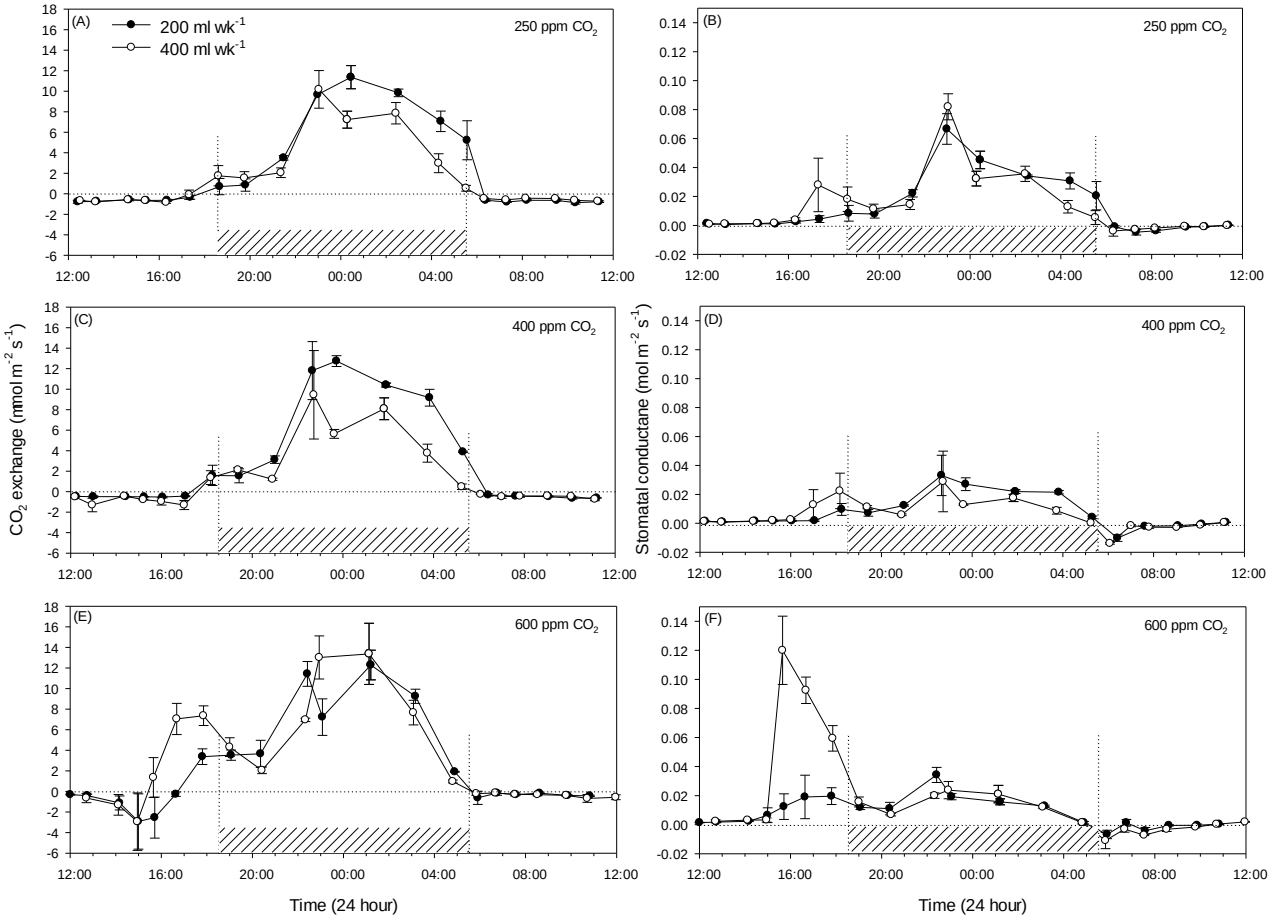


Figure 2.10: *Opuntia stricta* 24-hour CO_2 exchange (photosynthesis) and stomatal conductance (g_{ST}) for plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml $week^{-1}$ and 400 ml $week^{-1}$. n = 1- 3 per data point (mean ± SE). Gas exchange measurements were performed between days 231 to 239 of the experiment which lasted a total of 250 days. Night-time is denoted by the hatched block (extended vertically by the dotted lines for visual purposes) between 18:30 and 05:30.

Late afternoon CO_2 exchange (between 16:00 and 17:00) was negative for 250 and 400 ppm treatments (only respiration), whereas 600 ppm CO_2 uptake was 1.55 μmol m^{-2} s^{-1} (SE ± 0.893) and 5.26 μmol m^{-2} s^{-1} (SE ± 1.24) for 200 ml $week^{-1}$ and 400 ml $week^{-1}$ water treatments, respectively (Fig. 2.11 A; Table 2.2). While the 600 ppm 200 ml $week^{-1}$ treatment CO_2 uptake was not different to the 250 and 400 ppm treatments, when the two negative CO_2 uptake values measured between 16:00 and 16:30 were excluded from this time-period, CO_2 uptake increased to 2.54 μmol m^{-2} s^{-1} (SE ± 1.0), thus showing significance. CO_2 and water treatment had a significant effect on daytime g_{ST} (Table 2.2). Stomatal conductance was significantly highest for the 600 ppm 400 ml $week^{-1}$ treatment (Fig. 2.11 B). Despite the 600 ppm 200 ml $week^{-1}$ treatment demonstrating similar g_{ST} to the 250 and 400 ppm plants, these 600 ppm plants could achieve positive CO_2 uptake rates, thus indicating greater *WUE* (A/g_{ST}), although this could not be calculated for 250 and 400 ppm plants due to the negative CO_2 uptake (respiration).

Table 2.2: Summary of general linear model statistics for *Opuntia stricta* gas exchange and water use parameters. Plants were grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations with two watering treatments: 200 ml wk^{-1} and 400 ml $week^{-1}$. Bold results indicate significance ($P < 0.05$).

		F_{stat}: P - value		
***Opuntia stricta* traits**	**Units**	**CO_2**	**Water**	**CO_2 x water**
Daytime CO_2 exchange	μmol m^{-2} s^{-1}	**$F_{2,37}$= 21; P < 0.001**	$F_{1,38}$= 3.1; P = 0.08	**$F_{5,44}$= 4.7; P = 0.016**
Daytime stomatal conductance (g_{ST})	mol m^{-2} s^{-1}	**$F_{2,37}$= 20; P < 0.001**	**$F_{1,38}$= 13; P = 0.001**	$F_{5,44}$= 1.9; P = 0.16
Night-time *WUE*	μmol CO_2 mol^{-1} H_2O	**$F_{2,104}$= 39; P < 0.001**	$F_{1,105}$= 2.6; P = 0.1	$F_{5,101}$= 0.58; P = 0.55
Whole plant weekly water use	ml mg^{-1}	**$F_{2,297}$= 43; P < 0.001**	**$F_{1,298}$= 97; P < 0.001**	$F_{5,294}$= 3.0; P = 0.05

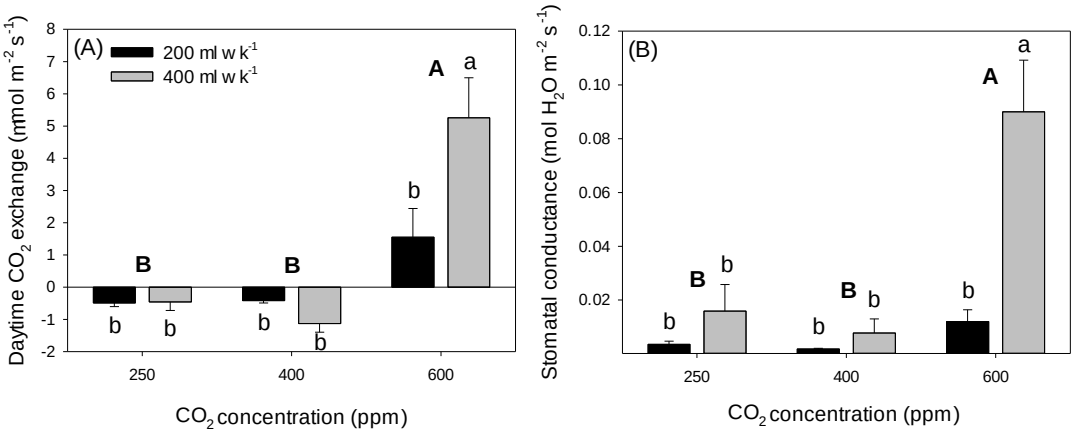


Figure 2.11: *Opuntia stricta* daytime A) CO_2 exchange (*A*) and B) stomatal conductance measured between 16:00 and 18:00 (late afternoon). Plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: 200 ml $week^{-1}$ and 400 ml $week^{-1}$. n = 6 - 9 (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

CO_2 treatment had a significant effect on night-time *WUE* (Fig. 2.12 A; Table 2.2) and although the *WUE* for the 200 ml $week^{-1}$ treatments at 400 and 600 ppm were slightly higher than the associated 400 ml $week^{-1}$ treatment, they were not different (Fig. 2.12, Table 2.2). On average, night-time *WUE* was 200 (SE ± 24.1), 316 (SE ± 46.2) and 509 (SE ± 66.1) μmol CO_2 mol^{-1} H_2O for the 250, 400 and 600 ppm treatments, respectively. This indicates in improvement in *WUE* of ~ 37.3 % with each increase in CO_2 concentration.

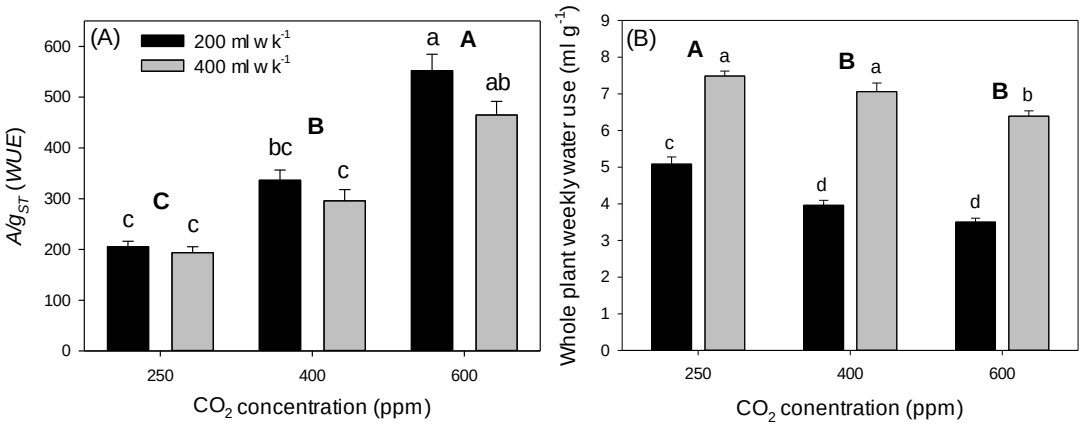


Figure 2.12: *Opuntia stricta* A) night-time water use efficiency (*WUE*: µmol CO_2 mol^{-1} H_2O) measured between 18:00 and 05:00 and B) whole plant water use measured over the duration of the experiment (includes soil evaporation as soil was exposed). Plants were grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: 200 ml $week^{-1}$ and 400 ml $week^{-1}$. n = 18 - 19 (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

Due to the curvilinear trend in night-time CO_2 exchange and the intervals between individual gas exchange measurements, it was difficult to ascertain whether overall CO_2 uptake productivity differed by CO_2 treatment. Cladode pH was lowest in the morning (10:00) for 600 ppm plants followed by 400 ppm and then 250 ppm plants ($F_{2,105} = 24$; $P < 0.001$) (Fig. 2.13). Using cladode pH as an indication of night-time CAM productivity, it was shown that because pH was lowest for 600 ppm plants, followed by 400 and then 250 ppm plants. By 14:00 (time: $F_{2,105} = 206$; $P < 0.001$) pH had increased to similar values across all CO_2 treatments.

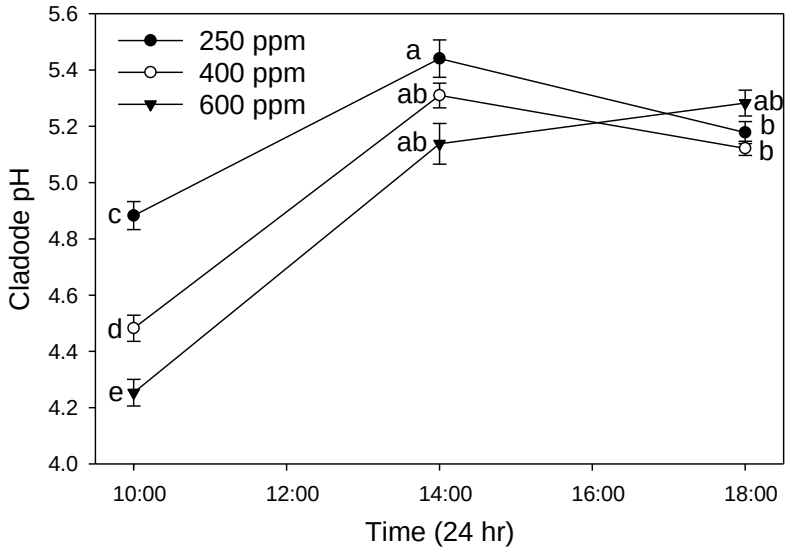


Figure 2.13: *Opuntia stricta* diurnal cladode pH measured on *Opuntia stricta* plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml $week^{-1}$ and 400 ml $week^{-1}$. n = 10 plants for each data point (mean ± SE). pH measurements were made on day 217 of the experiment which lasted a total of 250 days. Different lowercase letters indicate significant differences across time and CO_2 treatments ($P < 0.05$). Watering treatment was not included in the interaction (see Methods Section 2.3.3).

2.4.7 Cladode stable isotopes

CO_2 had a significant effect on cladode $\delta^{13}C$ which was lowest for 600 ppm (both water treatments), whereas 250 and 400 ppm did not differ from one another (Fig. 2.14 A; Table 2.1). Similarly, $\delta^{15}N$ declined with increasing CO_2 treatment (Fig. 2.14 B; Table 2.1). Water treatment had no effect on $\delta^{13}C$ or $\delta^{15}N$.

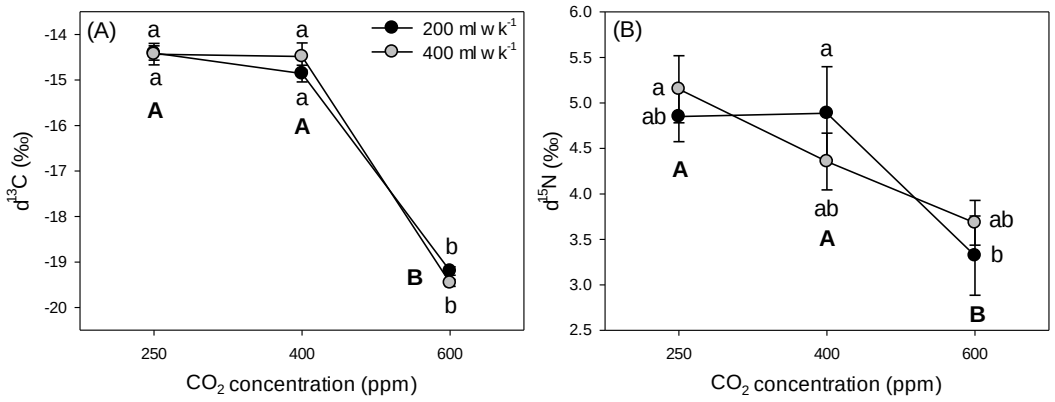


Figure 2.14: Opuntia stricta cladode stable isotope values for A) $\delta^{13}C$ and B) $\delta^{15}N$ measured on *Opuntia stricta* plants grown at three CO_2 concentrations: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm, with two water treatments: 200 ml $week^{-1}$ and 400 ml $week^{-1}$. n = 10 plants for each data point (mean ± SE). Different lowercase letters indicate significant differences across CO_2 and water treatments; uppercase bold letters indicate significant differences between CO_2 concentrations ($P < 0.05$).

2.5 Discussion

Opuntia stricta growth and productivity was responsive to different CO_2 concentrations, however these responses to increasing CO_2 were not always linear, indicating resources were differentially allocated to specific traits in response to different CO_2 concentration thresholds. Growth rates and total plant biomass responded to an increase in CO_2 of 150 ppm (sCO_2 to aCO_2) indicating *O. stricta* growth at historical CO_2 concentrations was likely inhibited. However, between aCO_2 and eCO_2 (200 ppm increase), growth rates and total plant biomass did not differ. This contrasts with *O. ficus-indica* that has been shown to accumulate additional biomass linearly with a 150 ppm increase in CO_2 from 370 to 520, and 520 to 750 ppm (Cui et al. 1993; Nobel et al. 1994; Nobel and Israel, 1995). Instead, *O. stricta* plants grown at eCO_2 allocated carbon resources to cladode hardness, spine traits and basal cladode biomass at the expense of faster growth rates, increasing size, daughter cladode biomass and subsequent cladode photosynthetic area. The different strategies of each species may be related to their growth forms, where *O. stricta* which grows as a shrub, invests in physical

defence at eCO_2 as opposed to growing larger (taller) as observed for *O. ficus-indica*.

Because *Opuntia* cladodes are long-lived photosynthetic structures that require significant resource investment, increasing hardness and spine formation can improve their physical defences against herbivores, subsequently increasing cladode longevity (Poorter and Bongers, 2006; Kitajima and Poorter, 2010). Similar to that seen in *O. ficus-indica* (Luo and Nobel, 1993), *O. stricta* basal biomass increased significantly, suggesting greater organ storage capacity for carbohydrates, water and structural support, which could improve *O. stricta's* ability to buffer against environmental stresses (Luo and Nobel, 1993). Importantly, the additional resources generated at eCO_2 to allocate to these traits was achieved with cladode areas similar to sCO_2 and aCO_2, indicating improved photosynthetic productivity at eCO_2.

While it was not possible to ascertain from the night-time CO_2 exchange pattern whether total CO_2 uptake differed statistically between the three CO_2 treatments, the lower morning (10 am) pH values with increasing CO_2 suggested eCO_2 plants had the highest night-time CAM productivity. The pattern of positive CO_2 uptake observed in the late afternoon for eCO_2 but not for sCO_2 or aCO_2 plants has been observed for other CAM species (Cui et al. 1993; Cui and Nobel, 1994; Nobel and Hartsock, 1986; Nobel, 1996). Although gas exchange measurements on sCO_2 and aCO_2 plants did not indicate daytime CO_2 uptake (phases I and IV), the cladode $\delta^{13}C$ values (~ -14‰) suggest that approximately 20% of carbon may have been acquired from daytime C_3 photosynthesis (Winter and Holtum, 2002). However, the significantly lower cladode $\delta^{13}C$ (~ -19‰) of eCO_2 plants suggests that approximately 50 to 60% of carbon was potentially acquired from daytime C_3 photosynthesis, thus contributing to greater overall photosynthetic productivity. Therefore, the increased contribution of daytime C_3 photosynthesis to carbon gain probably explains the additional carbon resources that were allocated to cladode defence and basal cladode biomass.

Although photosynthetic productivity was enhanced at eCO_2 due to the augmentation of daytime C_3 photosynthesis, this came at the expense of additional water loss via transpiration during this period. Despite ~ 37% higher night-time

WUE of eCO_2 plants relative to aCO_2, whole plant weekly water use did not differ, although this result may be an artifact of only measuring SWC once a week as soil would have dried through evaporation between watering, even though plants may have used water at different rates. Only with a doubling of CO_2, did eCO_2 whole plant water use improve by ~ 20% relative to sCO_2, despite a ~ 60% higher *WUE*, a response similar to that observed for other CAM species, whereby a doubling of CO_2 was sufficient to offset daytime water loss (Cui et al. 1993; Graham and Nobel, 1996; Zhu et al. 1999). Nevertheless, for the same amount of water, plants grown at eCO_2 could achieve significantly greater productivity relative to sCO_2 and aCO_2 plants.

Numerous *O. stricta* traits were negatively affected when plants received half the amount of water (200 ml wk^{-1} vs 400 ml wk^{-1}) for the duration of the experiment. However, the magnitude of the effect sizes generally decreased with increasing CO_2 concentration, indicating the ameliorative effect of CO_2 fertilisation on water limitations, which was most notable at eCO_2. Although 600 ppm CO_2 did not completely mitigate the effect of 50% less watering (200 ml wk^{-1}) on all plant traits, basal cladode biomass and spine traits increased and did not differ from sCO_2 and aCO_2 plants that received 400 ml $week^{-1}$. This partial amelioration was likely due to the high night-time *WUE* that facilitated daytime C_3 photosynthesis. Although 200 ml wk^{-1} plants CO_2 uptake rates were lower than 400 ml wk^{-1} treat plants, $\delta^{13}C$ values were the same, indicating a large contribution of carbon gain from daytime C_3 photosynthesis (Winter and Holtum, 2002). Night-time CO_2 uptake was similar for both water treatments at eCO_2, whereas at sCO_2 and aCO_2, the 200 ml $week^{-1}$ treatment CO_2 uptake was higher than the 400 ml week, but this was not reflected in the growth traits measured here. Diel gas exchange was conducted two days following watering, and the 200 ml wk^{-1} treatments at sCO_2 and aCO_2 probably had high initial CO_2 uptake rates that declined rapidly as the soil dried over the subsequent days. The 400 ml wk^{-1} treatment plants likely maintained lower but consistent CO_2 uptake for longer. At eCO_2, CO_2 uptake rates were probably more consistent throughout the week, driven by improved *WUE* and conservation of soil moisture. These results suggest that under future climate scenarios, eCO_2 could to some extent ameliorate the

effects of reduced soil moisture (lower rainfall) on productivity through improved *WUE* (Donohue et al. 2013).

In addition to improved *WUE*, eCO_2 plants invested in significantly less N, indicating improved photosynthetic nitrogen use efficiency (*NUE*). However, Cui and Nobel (1994) showed that when N declined in *O. ficus-indica*, total chlorophyll content also declined but this trend was not as clear for *O. stricta*. While it is difficult to directly compare chlorophyll contents across cactus studies due to differences in the units used, when chlorophyll content is expressed by area, *A. deserti* (CAM species) chlorophyll contents were 800 and 640 mg m^{-2} under ambient CO_2 and elevated CO_2 respectively, approximately 75% higher than the values observed in this experiment. This indicates that *O. stricta* chlorophyll a and b contents are comparatively low and subsequently *O. stricta* has an inherently high *NUE*. The significant decline in chlorophyll b with a doubling of CO_2 does not explain the difference in N between aCO_2 and eCO_2. Instead, there was a significant decline in carotenoids (18%) between aCO_2 and eCO_2, which correlates to the observed decline in N. Carotenoids are important pigments for plant function, including photosynthesis, however a trend of declining carotenoid concentrations with rising CO_2 has observed for species from different families, indicating plants may be less reliant on carotenoids with increasing CO_2 (Loladze et al. 2019). Similar to improved *WUE* with increasing CO_2, investing less in nitrogen and photosynthetic pigments implies that *O. stricta* can be more competitive in low nutrient environments and potentially expand its distribution into lower nutrient environments while maintaining greater productivity, thereby increasing its invasive range.

2.5.1 Conclusion

The increase in *O. stricta* growth rates and biomass from sCO_2 to aCO_2 suggests that observed increases in atmospheric CO_2 from time of introduction of the weed into South Africa to the present day may have contributed to its invasive success. However, at 400 ppm *O. stricta* reached an apparent size limit and thereafter as CO_2 increased, additional resources acquired from increased photosynthetic

productivity were instead allocated to traits that improved physical defence, organ storage capacity and structural support. At eCO_2, *WUE* likely reached a threshold which allowed greater metabolic flexibility whereby *O. stricta* could fix CO_2 during the day using C_3 photosynthesis in addition to CAM photosynthesis. Furthermore, the improved *WUE* at eCO_2 which allowed this metabolic flexibility meant that *O. stricta* could partially ameliorate the effects of suboptimal watering. Thus, with increasing atmospheric CO_2, *O. stricta* could become a greater socio-ecological threat to livelihoods, particularly subsistence livestock farmers in areas where it is already considered problematic, additionally it could expand its climatic niche with rising atmospheric CO_2 and potentially establish in lower rainfall environments that are currently unsuitable.

2.6 Supplementary data

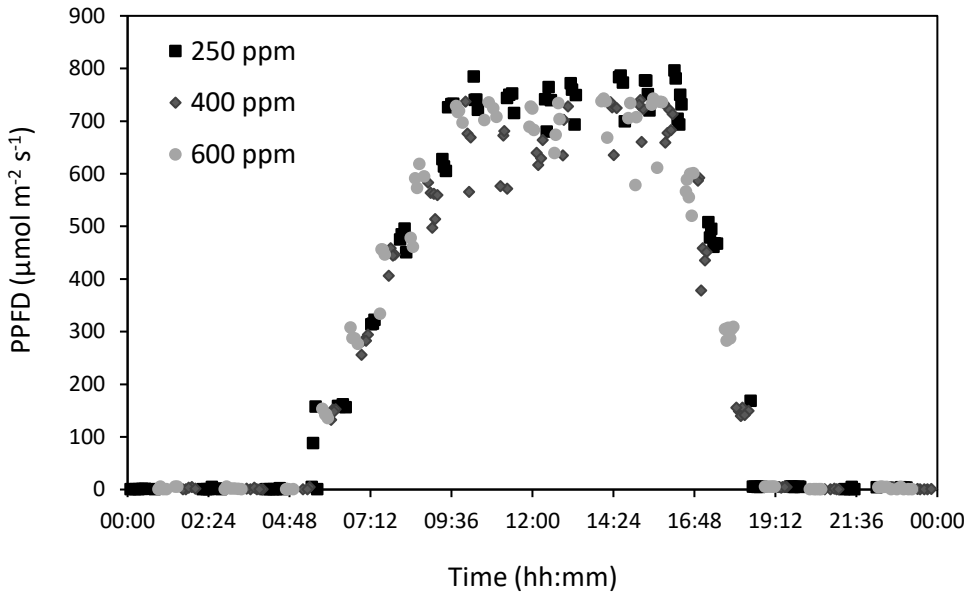
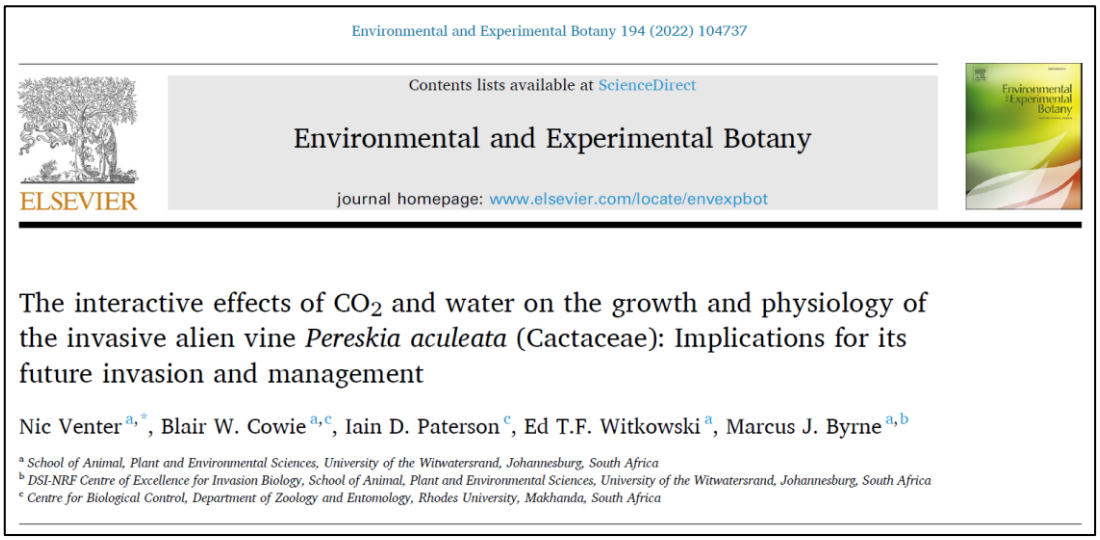


Figure S2.1: Photosynthetic photon flux density (PPFD) as measured by the PAR sensor within the 2 x 3 cm clear top cuvette (LI-6400XT) during gas exchange measurements of *Opuntia stricta* grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and, 600 (eCO_2) ppm CO_2. Light period: 05:00 to 19:00. Each curve consists of 81 to 98 data points and includes both water treatments. Due to the angular position of the cuvette, maximum PPFD measured here was lower than the maximum growth PPFD of ~ 1000 µmol m^{-2} s^{-1}.

CHAPTER THREE

The interactive effects of CO_2 and water on the growth and physiology of the invasive alien vine *Pereskia aculeata* (Cactaceae): implications for its future invasion and management



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The interactive effects of CO_2 and water on the growth and physiology of the invasive alien vine *Pereskia aculeata* (Cactaceae): Implications for its future invasion and management

Nic Venter [a,*], Blair W. Cowie [a,c], Iain D. Paterson [c], Ed T.F. Witkowski [a], Marcus J. Byrne [a,b]

[a] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa
[b] DSI-NRF Centre of Excellence for Invasion Biology, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa
[c] Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa

Preface

Using *P. aculeata,* this chapter aimed to address Objectives 1 and 2 by assessing invasive vigour and amelioration of suboptimal watering. Unlike *D. opuntiae* that has established across most of *O. stricta's* invaded range, the two insect biocontrol agents for *P. aculeata*: *Phenrica guerini* and *Catorhintha schaffneri* have shown low establishment rates, the reasons for which are not properly understood (King et al. 2021). Therefore, changes to host plant quality under elevated CO_2 are unlikely to influence their overall efficacy, instead this may largely be driven by unrelated factors such as climate incompatibility of the insects and or predation. Thus, predicting their future impact based on host plant quality is currently unwarranted.

The final MS Word document submitted for publication was used here, modified slightly to maintain a similar formatting to the rest of the thesis. No changes were made to the text other than assigning new heading numbers.

3.1 Abstract

Pereskia aculeata Miller (Cactaceae) is a primitive leafy cactus indigenous to Central and South America that has become a problematic invasive alien plant elsewhere in the world. In South Africa the plant invades established forests, clearings, thickets and plantations along the southern and eastern higher rainfall regions of the country. *Pereskia aculeata* is a woody vine (liana) that scrambles up existing vegetation competing for resources, often causing canopies to collapse under its weight. Similarly, to other invasive vines, mechanical and chemical control is challenging due to the plant growing intertwined with indigenous vegetation and therefore, biological control using specialist insect herbivores is considered essential for its management. Increasing atmospheric carbon dioxide (CO_2) concentrations are expected to favour weeds, however this response is likely to be influenced by projected changes to rainfall patterns. *Pereskia aculeata* were grown from truncheons for five months at three CO_2 concentrations: 250 (sub-ambient), 400 (ambient) and 600 (elevated) ppm under two watering regimes, reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}), to simulate two annual rainfall scenarios of ~520 mm (below present optimal) and ~1040 mm (present optimal), respectively. Elevated CO_2 (eCO_2) increased growth rates, biomass accumulation and reduced leaf nutritional quality (nitrogen content and C:N ratios), but only for ambient-water treatment plants. Nevertheless, higher photosynthetic rates under eCO_2 improved water use efficiency for both water treatments, which was greatest for the reduced-water treatment plants. Management of *P. aculeata* in South Africa is already extremely challenging and with rising atmospheric CO_2, *P. aculeata* has the potential to become more problematic, particularly in optimal rainfall areas. Furthermore, with improvements in water use efficiency, *P. aculeata* has the potential to expand into

lower rainfall areas, although it is unlikely to pose the same risk as those plants invading higher rainfall sites.

Keywords: Biological control; climate change; ecophysiology; elevated CO_2; herbivory; invasion biology; liana; sub-ambient CO_2; water use efficiency

3.2 Introduction

The *Pereskia* genus is considered ancestral to all other cacti and primarily differs by having woody stems and true leaves but also possessing spines and areoles typical of cacti (Edwards et al., 2005). Unlike other *Pereskia* species which are shrubs/trees, *P. aculeata* is categorised as a liana (woody vine) due to its scrambling growth form (semi-scandent habit) (Leuenberger, 1986). It has a wide native distribution which includes the Caribbean and large parts of Central and South America (Leuenberger, 1986). *Pereskia aculeata* was first recorded in South Africa in 1858 and has since become naturalised and invasive in many parts of South Africa and elsewhere around the world (McGibbon, 1858; Moran and Zimmermann, 1991). Within South Africa, the fruits produce viable seeds that are dispersed by frugivorous animals, enabling *P. aculeata* to colonise new habitats (Campbell, 1988), however asexual reproduction from fragments and vegetative growth leads to high densities of plants in suitable habitats (Paterson et al., 2011a). Whereas most invasive cactus species invade lower rainfall regions in South Africa, *P. aculeata* invasions are more noticeable along the southern and eastern part of the country (Eastern Cape and KwaZulu-Natal provinces) (Paterson et al., 2011b). It is most destructive in higher rainfall forests and coastal vegetation where it occurs in high densities, scrambling up existing trees and shrubs, competing for resources such as light and water, resulting in the eventual mortality of the affected vegetation (Moran and Zimmermann, 1991, Paterson et al., 2011a). Severe invasions of *P. aculeata* can smother forest canopies causing them to collapse under its weight. *Pereskia aculeata* is currently present in 60 quarter-degree grid cells, the 12th most widespread cactus in South Africa (Kaplan et al., 2017). As with many invasive alien vines or lianas, management of

P. aculeata remains challenging due to its vegetative propagation and the way it entangles itself in other vegetation rendering mechanical and herbicidal treatments ineffective. Due to this, the only viable long-term method of control has been biological control (hereafter biocontrol) (Moran and Zimmermann, 1991). Two insect species have been released to date in South Africa, namely the leaf chewing beetle *Phenrica guerini* Bechyné (Chrysomelidae: Coleoptera) in 1991, and the stem-wilting bug *Catorhintha schaffneri* Brailovsky & Garcia (Coreidae: Hemiptera) in 2014 (Klein, 1999; Paterson et al., 2014a,b). The efficacy of these two agents has been variable (Moran et al., 2021), and although the reasons behind this are not fully understood, the vigorous growth of the four South African invasive genotypes (Egbon et al., 2020) may be a contributing factor.

Carbon dioxide is an important greenhouse gas and increases in atmospheric CO_2 have been linked to increases in global temperatures which has altered the Earth's climate (Anderson et al., 2016). Prior to the industrial revolution in the mid 1800's, atmospheric CO_2 ranged between 270 and 280 ppm (IPCC, 2014). At present, atmospheric CO_2 concentrations measure ~419 ppm (July 2021) (www.co2.earth), which is an increase of ~33% in ca. 250 years, a rate of increase not seen in at least the last 20 000 years (Prentice et al., 2001). In addition to being a greenhouse gas, atmospheric CO_2 is also one of the main substrates for photosynthesis. However, despite increases in atmospheric CO_2, photosynthesis is still inhibited (i.e. not fully CO_2 saturated) at current atmospheric CO_2, and further CO_2 enrichment can have significant direct effects on plant physiology, growth and chemistry (Ziska et al., 2001). These effects are not uniform, and the magnitude of the effect is dependent on the plant's photosynthetic pathway (i.e. C_3, C_4 or CAM photosynthesis), plant functional type (i.e. annual, forb, shrub, succulent, tree, liana etc.), and the interaction of abiotic factors (i.e. light, temperature, water, nutrients etc.) (Florides and Christodoulides, 2009; Robinson et al., 2012; Stiling and Cornelissen, 2007; Wang et al., 2012). While most Cactaceae species use the obligate CAM photosynthetic pathway (Nobel and Hartsock, 1987), *P. aculeata* utilises CAM-cycling (Martin and Wallace, 2000; Rayder and Ting, 1981). Much like the conventional C_3 photosynthetic pathway, in CAM-cycling there is no nocturnal stomatal opening as seen in obligate CAM species, but there are diel changes in enzymes associated

with CAM photosynthesis. It is suggested that the CO_2 generated by respiration is recycled through the CAM pathway, and under conditions of severe water-stress this response becomes magnified (Rayder and Ting, 1981). Whether this contributes to carbon gain or improves water use efficiency (*WUE:* the ratio of photosynthesis to plant water loss) remains unclear and the adaptive advantage of CAM-cycling is not well understood (Edwards and Diaz, 2006; Herrera, 2013, 2009). *Pereskia* species appear to lie on a photosynthetic continuum, neither possessing 'typical' CAM nor 'typical' C_3 photosynthesis, making it difficult to conclude how they may respond to elevated CO_2 (eCO_2). However, both photosynthetic pathways are stimulated by eCO_2 (Ainsworth and Rogers, 2007; Drennan and Nobel, 2000; Wang et al., 2012), indicating that an intermediate of the two photosynthetic pathways is likely to benefit from eCO_2 (Herrera, 2009). Photosynthetic pathway notwithstanding, at a functional level, research has demonstrated that sub/tropical lianas respond positively to eCO_2, (Granados and Körner, 2002; Hättenschwiler and Körner, 2003; Zotz et al., 2006), with some authors suggesting that rising atmospheric CO_2 is to some extent responsible for the observed increase in sub/tropical liana abundance (Schnitzer and Bongers, 2011). While it could be argued that rising atmospheric CO_2 affects plants in a community uniformly and the complexities of these competitive interactions negate the direct effects of eCO_2 (Marvin et al., 2015; Ramesh et al., 2017), evidence suggests that the relative competitive advantage of weeds increases compared to that of co-occurring non-weed species (Belote et al., 2004; Manea and Leishman, 2011; Ziska et al., 2010; Ziska and George, 2004).

The increase in atmospheric CO_2 is ubiquitous but the effects of climate change are experienced at regional levels (IPCC, 2014). Within South Africa, rainfall is projected to shift from west to east, with certain models projecting an 80% increase in mean annual precipitation (MAP) in the extreme eastern parts of the country, while less rainfall is projected along the west coast and adjacent interior (Lumsden et al., 2009). While the interaction of eCO_2 and unaltered or increased rainfall is likely to have a positive effect on plant growth, the interaction of eCO_2 and reduced rainfall is less clear. Evidence suggests that eCO_2 largely ameliorates the negative effects of reduced soil moisture on plants through improved *WUE*, however this is not always apparent, particularly under more

severe water stress (Ainsworth and Rogers, 2007; Nackley et al., 2018; Oliveira et al., 2016; Reich et al., 2014; Wang et al., 2017).

Research investigating the effects of eCO_2 and climate change on Cactaceae species has primarily been focused on *Opuntia ficus-indica* (L.) Miller, an agriculturally significant species (Drennan and Nobel, 2000; Gomez-Casanovas et al., 2007; Guevara et al., 2003). However, numerous cactus species (including *O. ficus-indica*) are prevalent as invasive alien plants of global significance, and South Africa is the global hotspot for cacti invasion (Kaplan et al., 2017). Unlike most invasive cacti, *P. aculeata* differs in growth form, physiology and environmental requirements which are largely in contrast to other invasive cacti. Thus, it is important to understand how *P. aculeata* may respond to rising atmospheric CO_2 levels and predicted changes to climate. *Pereskia aculeata's* invasive success is largely dependent on vegetative growth and understanding what conditions either promote or impede its growth is important when trying to predict its invasion potential. Therefore, this study sought to determine 1) the effects of CO_2 and water on its historical, current, and future growth and physiology, 2) if these results infer invasion potential from introduction to present and into the future and 3) the implications for its subsequent management.

3.3 Methods

3.3.1 Plant material, experimental setup, and growth chamber conditions

Pereskia aculeata truncheons were obtained from the Kariega Biocontrol Mass Rearing Facility (Eastern Cape province, South Africa) operated by the Centre for Biological Control (CBC), Rhodes University, Makhanda. *Pereskia aculeata* plants were propagated from 20 cm x 2 cm wood truncheons placed in 17 cm diameter (2.3 litres) pots containing ~1.5 kg of loam soil. Sixteen plants were placed in each of the three walk-in Conviron plant growth chambers (Conviron PGV40, Winnipeg, Canada) with the following CO_2 levels; 250 ppm (pre-industrial; sub-ambient - sCO_2), 400 ppm (current; ambient - aCO_2) and 600 ppm (RCP6.5 ~ca. 2080; elevated - eCO_2). Air temperature within the Convirons were

programmed to change at approximately 1 °C / hour between the day-time maximum of 27 °C (11:00 to 16:30) and the night-time minimum of 20 °C (23:00 to 05:00). To maintain vapour pressure deficits (VPD) between 0.82 kPa and 1.25 kPa, relative humidity was maintained at 65%. Light was supplied by overhead fluorescent tubes (Philips Master TL5 HO 54W/840, 4000 Kelvin) and halogen bulbs (Philips 55W GLS A55 E27, 2800 Kelvin). Lights turned on at 05:00 and the intensity increased to its peak at 09:30 and thereafter decreased from 16:30 till 19:00, at which time the lights turned off (Fig. S3.1). Plants were positioned in such a manner to achieve a photosynthetic photon flux density (PPFD) of approximately 400 µmol m^{-2} s^{-1} at the top of the plants, similar to conditions experienced naturally in the field, particularly in sub-canopy environments. Only one of the three Conviron chambers was capable of scrubbing (soda lime) incoming CO_2 to create sub-ambient CO_2 levels (250 ppm), therefore plants were not moved between chambers. Temperature, humidity, VPD and [CO_2] were logged every five minutes for most of the duration of the experiment (Table S1). To verify conditions within the three chambers were similar and being maintained correctly, a LI-6400XT portable open path infrared gas analyser (LICOR Biosciences, Lincoln, Nebraska, USA) was used fortnightly to obtain spot readings for the following parameters: temperature, RH, VPD, PPFD and [CO_2] (this data was not recorded). Six grams of slow release granular 7:1:3 fertiliser (N = 95 g kg^{-1}; P = 14 g kg^{-1}; K = 41 g kg^{-1}) was added to each pot on day 60. The experiment lasted five months (initiated 16th Jan 2018, concluded 12th June 2018; 147 days), at which time the plants had outgrown the available chamber space.

3.3.2 Watering treatment

The mean annual rainfall (MAP) value was extracted for each *P. aculeata* location (occurrence) record to develop a watering treatment that simulated the MAP range experienced by *P. aculeata* across its invaded range. *Pereskia aculeata* occurrence records (n=198) for South Africa were obtained from the Global Biodiversity Information Facility (GBIF; www.gbif.org) and The South African Plant Invaders Atlas (SAPIA) (Henderson and Wilson, 2017). The MAP value for each record

was extracted from the Bioclim_12 Raster for South Africa using RStudio (see *Section 2.7*). MAP at *P. aculeata* sites ranged between 440 and 1170 mm, with a mean of 983 mm (SD = 172) and a median of 1061 mm. 78% of *P. aculeata* sites experience between 900 to 1200 mm MAP, with the remaining 22% of sites experiencing between 400 to 900 mm MAP. Using these data, within each CO_2 treatment, the plants were split into either reduced-water or ambient-water treatments which simulated ~520 mm MAP and ~1040 mm MAP, respectively. Using the following formula: volume water = π radius^2 of pot x height of water on soil surface, the weekly quantity of water required to simulate the two MAP's was calculated. The reduced-water treatment plants were given 200 ml wk^{-1} (soil water potential < 0.5 MPa) and the ambient-water treatment plants were given 400 ml wk^{-1} (soil water potential > 0.5 MPa). Plants were watered at the start of each week for the duration of the experiment.

3.3.3 Plant growth and biomass parameters

Shoot (branch) lengths were measured on each plant (n = 8 per CO_2 x water treatment) on four occasions over the duration of the experiment (days: 36, 63, 100 and 147). At the end of the experiment (day 147), plants were harvested for above and below ground dry biomass (oven dried at 60 °C for five days) (excluding the truncheon). Relative growth rate (RGR) was calculated using the total dry mass of the plants (excluding the truncheon): RGR = ln(dry mass)/time. Plants did not produce any flowers or fruit during the experiment.

3.3.4 Leaf traits

Upon concluding the experiment (day 147), six fully developed leaves from each plant (n = 8 per CO_2 x water treatment) were randomly selected to measure leaf thickness, leaf water content (LWC), leaf area and specific leaf area (SLA). Immediately after removal of a leaf, thickness was measured using digital callipers, then weighed to obtain the fresh mass, photographed for leaf area

analysis and oven dried at 60 °C for three days. LWC was calculated as: (fresh mass – dry mass)/fresh mass. Leaf area (cm^2) was analysed using ImageJ software (Ver. 1.48, Wayne Rasband, National Institute of Health, USA). SLA was calculated as: area/dry mass ($cm^2\ g^{-1}$).

Prior to destructive sampling upon conclusion of the experiment (day 147), leaf pigments were estimated *in situ* using a SPAD 502+ Chlorophyll Meter (Minolta, Osaka 542, Japan) on 30 random fully developed leaves per plant and averaged to attain a single value per plant. This was done to better represent the range of leaf pigments on the whole plant and to avoid biased sampling. Following this, standard curves of SPAD (unitless value) to pigments were constructed for each CO_2 x water treatment (Table S3.2). To construct the curve, SPAD was measured in situ on 15 to 20 leaves per treatment (selected from the eight treatment plants) to obtain a range of SPAD values of low to high, after which the measured area was removed using a 5 mm diameter (0.196 cm^2) leaf punch (leaf disc). Leaf discs were placed in test tubes containing 96% analytical grade ethanol, crushed and incubated in the dark at 25 °C for 72 hours. A 3 ml aliquot was transferred to a standard 4.5 ml cuvette and absorptance was measured using a spectrophotometer (Thermo Scientific™ GENESYS 20). Absorptance wavelengths of 664 nm, 649 nm and 470 nm were used to measure chlorophyll a, b and carotenoids, respectively. Following this, the leaf material was oven dried and the pigment contents ($mg\ g^{-1}$) were calculated using the equations as described by Lichtenthaler, (1987). Using the averaged SPAD value measured *in situ* per treatment plant, pigments were then read off the standard curves to obtain total chlorophyll, chlorophyll a, b, chlorophyll a/b ratios and carotenoids for each plant.

3.3.5 Stable isotope ratios, carbon and nitrogen concentrations

Using the same *P. aculeata* leaves from the leaf trait measurements (*Section 2.4*), a subsample from each of the six oven dried leaves per plant (n = 8 per CO_2 x water treatment) was removed and bulk ground. A subsample was removed from the bulk ground mixture and ± 1.25 mg was weighed in 6 x 4 mm tin capsules

(D1006 Capsules. Elemental Microanalysis, Okehampton, United Kingdom). These samples were then used to determine the stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$), carbon (C) and nitrogen (N) concentration of each plant per treatment. Samples for isotopic analysis were combusted at 1020 °C using an elemental analyser (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), housed at the UP Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria, Pretoria, South Africa. Two laboratory running standards (Merck Gel: $\delta^{13}C$ = -20.26‰, $\delta^{15}N$=7.89‰, C%=41.28, N%=15.29) & (DL-Valine: $\delta^{13}C$ = -10.57‰, $\delta^{15}N$=-6.15‰, C%=55.50, N%=11.86) and a blank sample were run after every 11 unknown samples. Data corrections were done using the values obtained for the Merck Gel & DL- Valine during each run. The standard deviations of the nitrogen and carbon values for the DL-Valine standard provided the ± error for the sample $\delta^{15}N$ and $\delta^{13}C$ values. The carbon and nitrogen ratios for the secondary (NIST) and lab running (Merck & DL-Valine) standards were calibrated using the following PRIMARY standards: IAEA-CH-3 (Cellulose), IAEA-CH-6 (Sucrose), IAEA-CH-7 (Polyethylene foil), IAEA N-1 & IAEA N-2 (Ammonium sulphate), IAEA NO-3 (Potassium nitrate). All results are referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen isotope values. Results are expressed in delta (δ) notation using a per mille (‰) scale using the standard equation:

$$\delta X(‰) = [(R_{sample}-R_{standard})/R_{standard}-1]$$

where X= ^{13}C or ^{15}N and R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively.

3.3.6 Gas exchange: diurnal cycle

Net CO_2 and H_2O gas exchange measurements were performed on *P. aculeata* plants on days 119, 120, 126 and 127 of the experiment (± 4 months into the experiment) to obtain photosynthetic productivity as realised under the growth chamber ambient conditions (e.g. diurnal light, temperature and VPD cycle) and the long-term CO_2 and water treatments. Measurements were conducted within

two days of being watered and commenced at 04:30 (light cycle started at 05:00) and continued throughout the day until about 19:30 (light cycle ended at 19:00). Pre-and post-light measurements were done to ascertain if there was any nocturnal CO_2 uptake. Measurements were performed by sequentially cycling through the three CO_2 treatments (chambers), measuring a reduced-water and then an ambient-water treatment plant within each CO_2 treatment. Of the 8 plants per CO_2 x water treatment, 6 – 7 plants were selected, and repeat measured over the course of the day with 43 - 45 gas exchange data points collected per CO_2 x water treatment over the four days. Statistical analysis was only performed on 37 - 38 data points per CO_2 x water treatment due to all the plants demonstrating similar respiration rates in the dark and low light (PPFD < 1 µmol m^{-2} s^{-1}) (see *Section 3.3*). Gas exchange measurements were conducted using a LI-6400XT infrared gas analyser with a 2 × 3 cm clear top LI-COR cuvette. Cuvette height was maintained to sample leaves which were exposed to a PPFD of ~400 µmol m^{-2} s^{-1}, which was approximate PPFD experienced at the canopy of the plants (as per the experimental design). To conduct gas exchange measurements that reflected the actual ambient conditions experienced by the plants within the growth chambers, the temperature and VPD within the cuvette was not pre-programmed, however, incoming CO_2 was set according to the chamber CO_2 level. Data were logged after 2 - 3 minutes once cuvette conditions stabilised in response to being opened prior to enclosing the leaf (2^{nd} or 3^{rd} fully expanded leaf). Using the equations of von Caemmerer and Farquhar, (1981), net photosynthesis (*A*), stomatal conductance to water vapour (g_{ST}), transpiration (*E*) and intercellular [CO_2] (C_i) values were calculated. C_i/Ca was calculated as the ratio of intercellular [CO_2] (C_i) to ambient [CO_2] and intrinsic water use efficiency (*WUE*) was calculated as the ratio of net photosynthesis to stomatal conductance (A/g_{ST}).

3.3.7 Statistical analyses

All statistical analyses were performed using RStudio (R Core Team, 2018). Mean annual precipitation (MAP) for South African *P. aculeata* distribution records were extracted using functions from the *'raster'* package. The BIO_12

Baseline (mean annual rainfall in mm) raster from WorldClim was used to extract MAP (Fick and Hijmans, 2017). To control for repeated measures, linear mixed-effects models were used to analyse the linear and interactive effects of CO_2, time and water on plant growth (shoot length) (individual plant was the random effect). This was conducted using the *lmer* function from the *'lme4'* package (Bates et al., 2015). Linear models (*lm*) were used to analyse the linear and interactive effects of CO_2 and water on final data (i.e. measurements made at end of the experiment) and therefore individual plant was not treated as a random effect (no repeated measures). The *Anova* function from the *'car'* package (John et al., 2020) was used calculate analysis-of-variance tables for the *lmer* and *lm* models. Tukey post-hoc analyses was conducted for all models using functions from the *'multcomp'* package.

3.4 Results

3.4.1 Plant growth and biomass parameters

The interaction of time, CO_2 and water treatment on total shoot length was not significant ($F_{11,165} = 0.16$; $P = 0.85$), due to the similar initial growth rates up until day 100 (Fig. 3.1). For the ambient-water treatments, elevated CO_2 (eCO_2) differed to sub-ambient CO_2 (sCO_2), but not ambient CO_2 (aCO_2), with the same trend evident across the CO_2 levels for the reduced-water treatment plants (Fig. 3.1). Within each CO_2 treatment, the effect of the water treatment was only significantly different by day 147 for the sCO_2 and eCO_2 treatments (Fig. 3.1).

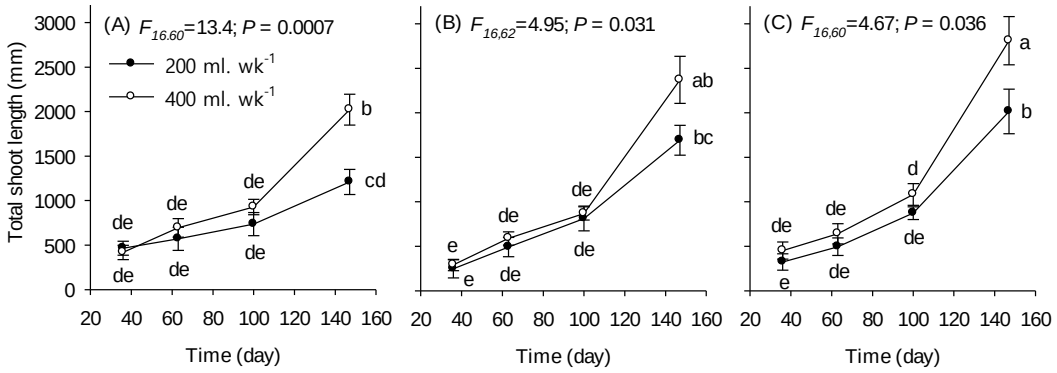

Figure 3.1: Total shoot length measured over time for *Pereskia aculeata* plants grown at three [CO_2]: (A) 250 (sCO_2), (B) 400 (aCO_2) and (C) 600 (eCO_2) ppm CO_2, with two water treatments: reduced-water (200 ml wk^{-1}) (●) and ambient-water (400 ml wk^{-1}) (○). n = 5 - 8 plants for each data point (mean ± SE). Different lowercase letters indicate significant differences across all (A – C) CO_2 treatments ($P < 0.05$). Full interaction: CO_2 x time x water ($F_{11,165} = 0.16$; $P = 0.85$). Shoot length increase was zero at day 0.

The interaction of CO_2 and water treatment had a significant effect on total plant biomass ($F_{5,42} = 5.43$; $P = 0.008$) (Fig. 3.2). Ambient-water treatment plants grown at eCO_2 produced 35.2% and 41.5% more biomass than sCO_2 and aCO_2 plants, respectively. For the reduced-water treatment, eCO_2 biomass differed to sCO_2, but not aCO_2. Within each CO_2 treatment, the water treatment had a significant effect on total biomass, with the ambient-water plants accumulating 39% (sCO_2), 31% (aCO_2) and 46% (eCO_2) more biomass than the reduced-water treatment plants. Above ground biomass followed a similar trend to total biomass, except that the reduced-water treatment plants were not different between CO_2 treatments (Table 3.1). The eCO_2 ambient-water treatment had the greatest root biomass, while the sCO_2 and aCO_2 treatments were not different to one another (Table 3.1). Root:shoot ratios did not differ across all treatments (Table 3.1). Ambient-water treatment plants at eCO_2 had the greatest relative growth rates (RGR), which were 32% greater than the reduced-water treatment plants at sCO_2 (Table 3.1).

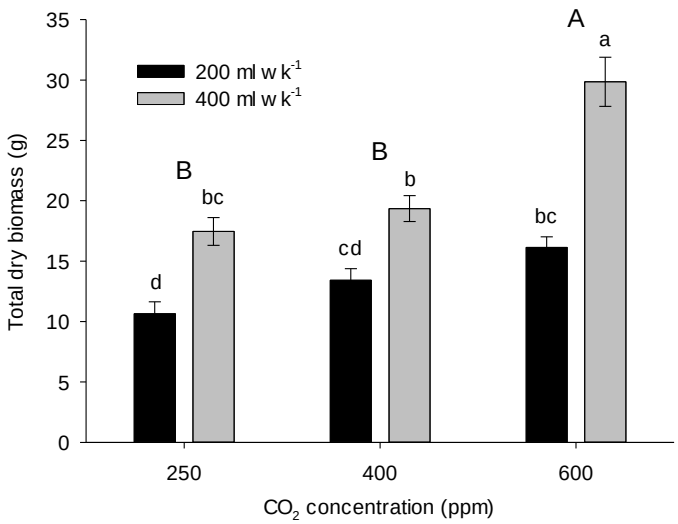

Figure 3.2: Total dry biomass, measured after 147 days for *Pereskia aculeata* plants grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}). n = 8 plants for each data point (mean ± SE). Different lowercase letters indicate significant differences ($P < 0.05$) across all treatments ($F_{5,42} = 5.43$; $P = 0.008$) and different uppercase letters indicate significant differences for the linear effect of [CO_2] ($F_{2,45} = 25.75$; $P < 0.001$). Water treatment linear effect: $F_{1,46} = 69.74$; $P < 0.001$.

Table 3.1: Growth parameters (mean ± SE) measured after 147 days for *Pereskia aculeata* plants grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}). n = 8 per mean. Different superscript lowercase letters indicate significant differences within a parameter ($P < 0.05$) and different uppercase letters (Linear effects: CO_2) indicate significant differences across the three CO_2 concentrations of 250, 400 and 600 ppm, respectively. See Table S3 for the full summary of the linear and interactive effects of CO_2 and water (F_{stat} and *P-values*).

	Units	250 ppm (sCO_2)		400 ppm (aCO_2)		600 ppm (eCO_2)		F_{stats}; *P-value*	Linear effects	
	ml wk^{-1}	200	400	200	400	200	400	CO_2 x water	CO_2	water
Shoot biomass	g	9.66 ^{d} ± 0.878	16.03 ^{bc} ± 0.928	12.35 ^{cd} ± 0.744	17.97 ^{b} ± 0.985	14.18 ^{bc} ± 1.04	27.13 ^{a} ± 2.02	$F_{5,42}$=5.38; **P=0.0083**	**P<0.001** B, B, A	**P<0.001**
Root biomass	g	0.98 ^{b} ± 0.175	1.43 ^{b} ± 0.299	1.07 ^{b} ± 0.153	1.38 ^{b} ± 0.236	1.96 ^{ab} ± 0.248	2.73 ^{a} ± 0.402	$F_{5,42}$=0.38; P=0.685	**P<0.001** B, B, A	**P=0.029**
Root:shoot		0.10 ^{a} ± 0.013	0.088 ^{a} ± 0.013	0.091 ^{a} ± 0.016	0.078 ^{a} ± 0.016	0.138 ^{a} ± 0.014	0.105 ^{a} ± 0.020	$F_{5,42}$=0.258; P=0.773	P=0.068 A, A, A	P=0.15
Relative growth rate (RGR)	g g^{-1} day^{-1}	0.0158 ^{d} ± 0.0005	0.0193 ^{bc} ± 0.0005	0.0175 ^{cd} ± 0.0005	0.020 ^{b} ± 0.0005	0.0188 ^{bc} ± 0.0005	0.0231 ^{a} ± 0.0005	$F_{5,42}$=1.50; P=0.235	**P<0.001** B, B, A	**P<0.001**

3.4.2 Leaf traits

Ambient-water treatment plants grown at eCO_2 had the lowest chlorophyll content, foliar nitrogen, SLA, $\delta^{13}C$ and the highest C:N ratios (Table 3.2). SLA was highest for sCO_2 ambient-water treatment plants and lowest for eCO_2 ambient-water treatment plants. Leaf thickness for both water treatments at eCO_2 was significantly greater than all the remaining treatments. Leaf water content of eCO_2 ambient-water treatment plants was significantly lower than the reduced-water treatment plants grown at sCO_2 and aCO_2 (Table 3.2). $\delta^{13}C$ differed between the two water treatments at eCO_2 but not for the sCO_2 and aCO_2 treatments.

Table 3.2: Leaf traits (mean ± SE) measured after 147 days for *Pereskia aculeata* plants grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}). n = 8 per mean. Different superscript lowercase letters indicate significant differences within a trait ($P < 0.05$) and different uppercase letters (Linear effects: CO_2) indicate significant differences across the three [CO_2] of 250, 400 and 600 ppm, respectively. See Table S4 for the full summary of the linear and interactive effects of CO_2 and water (F_{stat} and *P-values*).

	Units	250 ppm (sCO_2)		400 ppm (aCO_2)		600 ppm (eCO_2)		F_{stats}; *P-value*	*Linear effects*	
	ml wk^{-1}	200	400	200	400	200	400	CO_2 x water	CO_2	water
Leaf area	cm^2	22.0 ^{a} ± 1.71	22.9 ^{a} ± 1.16	23.8 ^{a} ± 1.72	23.9 ^{a} ± 2.31	24.9 ^{a} ± 2.52	27.7 ^{a} ± 1.66	$F_{5,42}$=0.26; *P*=0.77	*P*=0.15 A, A, A	*P*=0.44
Specific leaf area (SLA)	cm^2 g^{-1}	154.2 ^{b} ± 4.45	174.0 ^{a} ± 6.13	151.2 ^{b} ± 3.51	138.5 ^{b} ± 5.89	131.6 ^{b} ± 3.38	104.0 ^{c} ± 3.06	$F_{5,42}$=13.35; *P*<0.001	***P*<0.001** A, B, C	*P*=0.081
Leaf thickness	mm	0.532 ^{b} ± 0.022	0.526 ^{b} ± 0.02	0.605 ^{b} ± 0.021	0.606 ^{b} ± 0.032	0.799 ^{a} ± 0.05	0.844 ^{a} ± 0.042	$F_{5,42}$=0.31; *P*=0.73	***P*<0.001** B, B, A	*P*=0.63
Leaf water content	%	89.77 ^{ab} ± 0.53	91.15 ^{a} ± 0.5	90.38 ^{a} ± 0.27	89.64 ^{ab} ± 0.47	88.90 ^{ab} ± 0.81	87.66 ^{b} ± 0.63	$F_{5,42}$=2.96; *P*=0.063	***P*<0.001** A, A, B	*P*=0.67
Total chlorophyll	mg g^{-1}	9.84 ^{bc} ± 0.53	10.52 ^{ab} ± 0.68	12.98 ^{a} ± 0.64	10.03 ^{bc} ± 0.48	7.70 ^{c} ± 0.87	4.91 ^{d} ± 0.35	$F_{5,42}$=5.57; *P*=0.0072	***P*<0.001** A, A, B	***P*=0.002**
Chlorophyll a	mg g^{-1}	6.707 ^{b} ± 0.47	7.052 ^{b} ± 0.45	8.784 ^{a} ± 0.45	6.619 ^{b} ± 0.32	4.761 ^{c} ± 0.48	3.212 ^{c} ± 0.23	$F_{5,42}$=5.34; *P*=0.0086	***P*<0.001** A, A, B	*P*=0.001
Chlorophyll b	mg g^{-1}	3.13 ^{b} ± 0.12	3.47 ^{ab} ± 0.23	4.19 ^{a} ± 0.19	3.41 ^{ab} ± 0.16	2.94 ^{bc} ± 0.4	1.70 ^{d} ± 0.12	$F_{5,42}$=6.63; *P*=0.0031	***P*<0.001** A, A, B	***P*=0.003**
Chlorophyll a:b		2.13 ^{a} ± 0.046	2.03 ^{abc} ± 0.001	2.09 ^{ab} ± 0.016	1.94 ^{bc} ± 0.003	1.70 ^{d} ± 0.086	1.90 ^{c} ± 0.002	$F_{5,42}$=10.15; *P*=0.0003	***P*<0.001** A, A, B	*P*=0.62
Carotenoids	mg g^{-1}	1.85 ^{a} ± 0.054	1.20 ^{bc} ± 0.101	1.49 ^{b} ± 0.073	1.15 ^{cd} ± 0.041	1.03 ^{de} ± 0.079	0.78 ^{e} ± 0.033	$F_{5,42}$=4.62; *P*=0.015	***P*<0.001** A, A, B	***P*<0.001**
Foliar nitrogen	%	2.56 ^{a} ± 0.14	2.30 ^{a} ± 0.16	2.62 ^{a} ± 0.15	2.12 ^{a} ± 0.14	2.13 ^{a} ± 0.17	1.46 ^{b} ± 0.07	$F_{5,42}$=1.11; *P*=0.337	***P*<0.001** A, A, B	***P*<0.001**
Foliar carbon	%	33.68 ^{a} ± 0.62	33.00 ^{a} ± 0.84	34.74 ^{a} ± 0.6	34.23 ^{a} ± 0.51	35.35 ^{a} ± 0.70	35.03 ^{a} ± 0.78	$F_{5,42}$=0.176; *P*=0.84	*P*=0.15 A, A, A	*P*=0.78
Foliar C:N		15.65 ^{b} ± 0.7	17.7 ^{b} ± 1.08	15.88 ^{b} ± 0.93	19.36 ^{b} ± 1.02	20.50 ^{b} ± 2.1	28.60 ^{a} ± 1.6	$F_{5,42}$=2.86; *P*=0.068	***P*<0.001** B, B, A	***P*<0.001**
$\delta^{13}C$	‰	-27.2 ^{a} ± 0.86	-27.54 ^{a} ± 0.42	-26.53 ^{a} ± 0.37	-28.67 ^{ab} ± 0.26	-30.56 ^{b} ± 0.60	-33.29 ^{c} ± 0.32	$F_{5,42}$=2.94; *P*=0.064	***P*<0.001** A, A, B	***P*<0.001**

3.4.3 Gas exchange: diurnal cycle

Net photosynthesis (*A*) was highest for plants grown at eCO_2 and did not differ between the two water treatments (Fig. 3.3 C; Table 3.3). In contrast, the water treatments yielded differences in *A* of plants grown at sCO_2 and aCO_2 (Fig. 3.3 A-

B; Table 3.3). Stomatal conductance (g_{ST}) and transpiration (E) were similar across all CO_2 and water treatments, aside from the ambient-water treatment plants at sCO_2 which showed the highest and most variable g_{ST} and E (Fig. 3.3 D-F; Table 3.3). Intercellular [CO_2] (C_i) increased with increasing CO_2 treatments and water treatments only elicited differences in C_i for the plants grown at eCO_2 (Fig. 3.3 G-I; Table 3.3). Across all CO_2 x water treatments C_i/C_a ratios ranged between 0.58 and 0.68 (Table 3.3). Intrinsic water use efficiency (*WUE*) was lowest for sCO_2 and highest for eCO_2 plants (Fig. 3.3 J-L; Table 3.3). Unlike the sCO_2 and aCO_2 plants where the water treatment had no influence on *WUE*, this was not the case for eCO_2 plants, as the *WUE* of reduced-water treatment plants was 16.6 % higher than ambient-water treatment plants. Gas exchange measurements conducted in the dark showed no CO_2 uptake for plants in either of the CO_2 x water treatments.

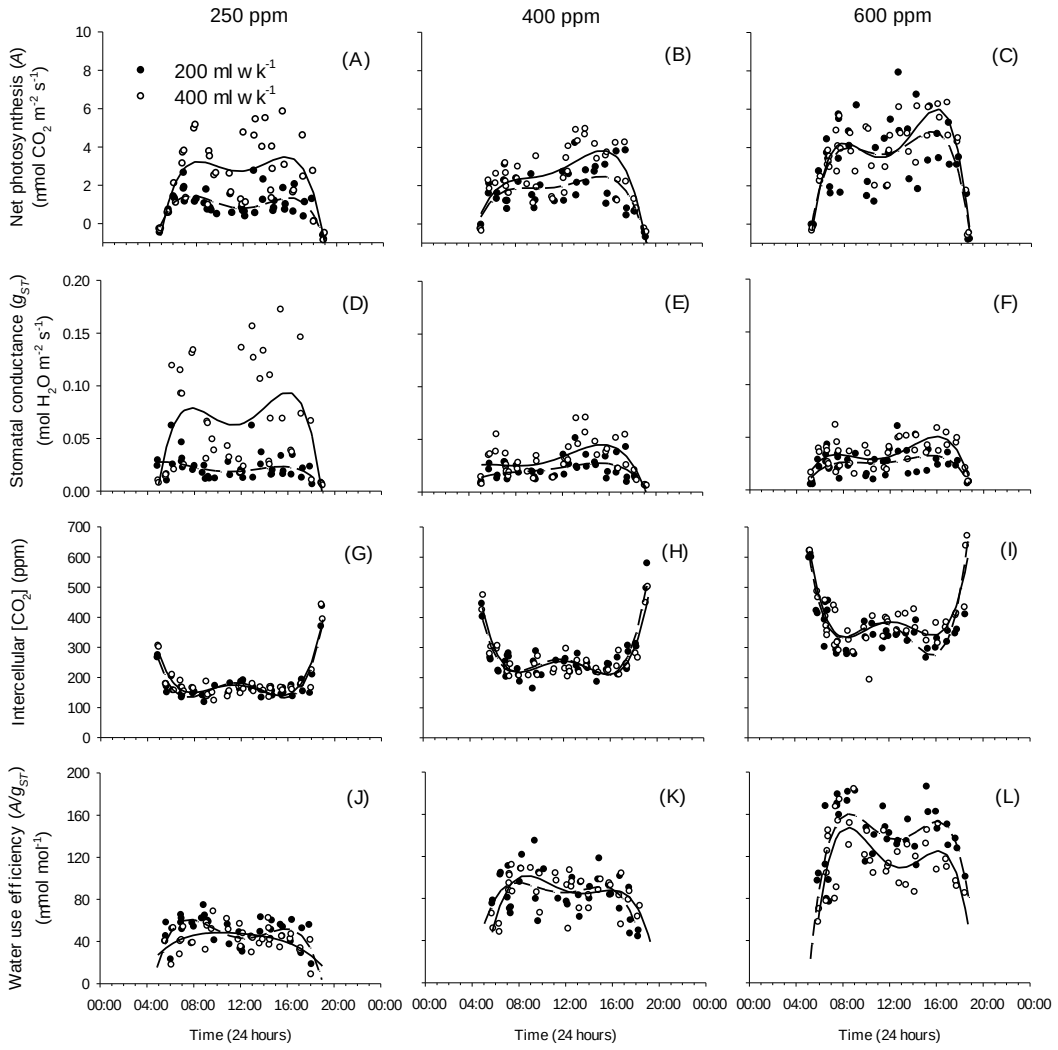

Figure 3.3: *Pereskia acuelata* diurnal gas exchange trends for (A-C) net photosynthesis (*A*), (D-F) stomatal conductance (g_{ST}), (G-I) intercellular [CO_2] (C_i) and (J-L) intrinsic water use efficiency (*WUE*: A/g_{ST}) grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}). Light period: 05:00 to 19:00. Gas exchange data was collected on days 119,120, 126 and 127 of the 147-day experiment. There was no measurable night-time CO_2 uptake. Graphs includes all the data points, but statistical analyses exclude data collected within the first and last 30 mins of the day when light intensity was at its lowest and all plants responded similarly. 4th order polynomial lines fitted to the data for graphic purposes only (dashed line: 200 ml wk^{-1}, solid line: 400 ml wk^{-1}).

Table 3.3: Gas exchange parameters (mean ± SE) measured on *Pereskia aculeata* plants grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}). Gas exchange data was collected on days 119,120, 126 and 127 of the 147-day experiment. Statistics exclude data collected between 05:00 to 05:30 and 19:00 to 19:30 when light intensity was at its lowest and all plants responded similarly (see Fig. 2.3). n = 37 – 38 data points per mean (n = 6 – 7 plants). Different superscript lowercase letters indicate significant differences within a trait ($P < 0.05$) and different uppercase letters (Linear effects: CO_2) indicate significant differences across the three [CO_2] of 250, 400 and 600 ppm, respectively. See Table S5 for the full summary of the linear and interactive effects of CO_2 and water (F_{stat} and *P-values*).

	Units	250 ppm (sCO_2)		400 ppm (aCO_2)		600 ppm (eCO_2)		F_{stats}; *P-value*	*Linear effects*	
	ml wk^{-1}	200	400	200	400	200	400	CO_2 x water	CO_2	water
Net photosynthesis (*A*)	µmol CO_2 m^{-2} s^{-1}	1.11 ^{c} ± 0.097	2.79 ^{b} ± 0.24	1.91 ^{c} ± 0.14	2.69 ^{b} ± 0.18	3.73 ^{a} ± 0.25	4.06 ^{a} ± 0.2	$F_{5,219}$=5.4; *P*=0.005	***P*<0.001** B, B, A	***P*<0.001**
Stomatal conductance (g_{ST})	mol H_2O m^{-2} s^{-1}	0.022 ^{b} ± 0.002	0.071 ^{a} ± 0.007	0.022 ^{b} ± 0.001	0.033 ^{b} ± 0.002	0.027 ^{b} ± 0.002	0.036 ^{b} ± 0.002	$F_{5,219}$=19.2; *P*<0.001	***P*<0.001** A, B, B	***P*<0.001**
Transpiration (*E*)	mol H_2O m^{-2} s^{-1}	0.575 ^{b} ± 0.045	1.37 ^{a} ± 0.15	0.573 ^{b} ± 0.048	0.809 ^{b} ± 0.072	0.618 ^{b} ± 0.054	0.860 ^{b} ± 0.066	$F_{5,219}$=7.6; *P*<0.001	***P*<0.001** A, B, B	***P*<0.001**
Intercellular [CO_2] (C_i)	µmol CO_2 mol^{-1} or ppm	157.1 ^{d} ± 3.16	163.1 ^{d} ± 2.9	242.8 ^{c} ± 5.2	239.2 ^{c} ± 4.58	342.8 ^{b} ± 7.46	370.5 ^{a} ± 8.58	$F_{5,219}$=3.3; *P*=0.038	***P*<0.001** C, B, A	*P*=0.051
C_i/C_a		0.64 ^{a} ± 0.012	0.68 ^{a} ± 0.012	0.62 ^{ab} ± 0.013	0.61 ^{b} ± 0.012	0.58 ^{b} ± 0.012	0.64 ^{ab} ± 0.013	$F_{5,219}$=2.6; *P*=0.097	*P*=0.074 A, B, B	*P*=0.63
Water use efficiency (*WUE*) (A/g_{ST})	µmol CO_2 mol^{-1} H_2O	49.4 ^{d} ± 1.91	42.4 ^{d} ± 1.79	84.5 ^{c} ± 3.13	85.5 ^{c} ± 2.78	139.2 ^{a} ± 4.34	117.9 ^{b} ± 4.46	$F_{5,219}$=5.1; *P*=0.006	***P*<0.001** C, B, A	***P*=0.002**

3.5 Discussion

These results demonstrate that *P. aculeata's* growth response to a stepwise increase of CO_2 from 250 to 600 ppm is largely influenced by water. When receiving sufficient water (ambient-water treatment), there was a non-linear increase in biomass accumulation as opposed to a linear increase when receiving less water (reduced-water treatment). The most pronounced effect on growth parameters was the increase of 200 ppm from aCO_2 to eCO_2 ppm for ambient-water treatment plants, which demonstrated faster growth rates and greater biomass accumulation. This contrasts with the findings of Granados and Korner

(2002) who found an overall stronger effect on liana biomass when increasing CO_2 from sub-ambient to ambient levels. Thus, for *P. aculeata* it appears that an increase of 150 ppm from sCO2 to aCO2 is not sufficient to elicit a relative improvement in growth for both water treatments. Atmospheric CO_2 is therefore, unlikely to have had a significant influence on *P. aculeata's* historical invasion success in South Africa from 1850's to present. However, from 400 to 600 ppm CO_2, the increase in growth for ambient-water plants and improvement in photosynthetic efficiency, particularly *WUE* for both water treatments, indicates that CO_2 may become a significant driver in *P. aculeata's* future invasion success.

Pereskia aculeata grown at eCO_2 a showed significantly greater *WUE* compared to sCO_2 and aCO_2 treatments. This improvement was largely due to increased photosynthetic rates, a response similarly shown by other tropical liana species under eCO_2 (Marvin et al., 2014). Within the eCO_2 treatment, the reduced-water treatment plants were able to improve their *WUE* by 16.6 % over their ambient-water counterparts, a trend not shown for the sCO_2 and aCO_2 treatments. While gas exchange measurements did not show a significant decline in stomatal conductance, using $\delta^{13}C$ as a long-term average to elucidate the mechanism for this *WUE* response, it is likely that greater stomatal limitations were imposed in response to lower soil moisture, thereby affecting CO_2 influx and H_2O efflux (Ehleringer, 1990). The higher (less negative) $\delta^{13}C$ values indicate less photosynthetic discrimination of the heavier ^{13}C isotope was likely due to reduced CO_2 supply into the leaf (Edwards, 2006; Ehleringer, 1993, 1990; Farquhar et al., 1989). This reduction in CO_2 supply via stomatal limitations as inferred by $\delta^{13}C$ is supported by the lower intercellular [CO_2] (C_i) measured on reduced-water treatment plants. While it appears that eCO_2 ameliorated the effects of a reduced water supply by improving *WUE*, there was a trade-off, whereby reduced-water treatment plants had to invest in 57% more chlorophyll to compensate for the effect of lower CO_2 supply on photosynthesis. Therefore, despite having higher photosynthetic rates compared to plants grown at aCO_2, the only notable effect on productivity measured here for eCO_2 plants with reduced-water was an increase in leaf thickness. Based on the different responses of the two water treatments under eCO_2, it appears that eCO_2 only partially ameliorated the effects of reduced water on *P. aculeata*, which may be anticipated given its origin, evolving in sub/tropical

environments that experience higher rainfall (Houghton, 1929). Nevertheless, the measured improvement in growth and physiological parameters under eCO_2 is likely to have important consequences for *P. aculeata* invasions in the future.

The scrambling nature of *P. aculeata* essentially limits its invasions to habitats that have suitable vegetation with the appropriate architecture to support it, such as thickets and forests (Paterson et al., 2011a), and thus under eCO_2 it is unlikely to become more problematic beyond these vegetation types. However, within these habitat types, under current climatic conditions and atmospheric CO_2, *P. aculeata* is most abundant at sites that experience between 900 to 1200 mm MAP. Therefore, at these optimal rainfall sites, increased growth rates (RGR) and biomass accumulation under eCO_2 could render *P. aculeata* plants more vigorous and damaging. Moreover, with no significant increases in leaf area and transpiration rates, unlike that seen for functionally similar liana's (Marvin et al., 2014), whole plant water use will likely remain similar. Thus, improvements in *WUE*, particularly that observed for plants grown with reduced-water, may facilitate *P. aculeata* invasions into less optimal rainfall areas (~500 to ~900 mm MAP), increasing its realised invasive range. Naturally, these predictions are dependent on complex interactions, with temperature being an important climatic variable that affects plant distributions and productivity (Sage and Kubien, 2007; Suggitt et al., 2019).

Temperatures are predicted to increase across southern Africa (Van Oldenborgh et al., 2013), and if the temperature increase is beyond a plant's thermal optimal, it will have negative effects on productivity (Sage and Kubien, 2007; Wang et al., 2012). Although elevated temperature was not included as an interactive effect in this study, other research indicates that the interaction of eCO_2 and mild temperature increases can stimulate plant productivity and improve further photosynthetic efficiency (i.e. *WUE*) (Robinson et al., 2012; Wang et al., 2012; Woodward, 1988). Conversely, elevated temperatures increase the air vapour pressure deficit which can accelerate soil moisture evaporation and increasing the risk of drought stress (Grossiod et al 2020). While we cannot be certain of the relative contribution of eCO_2 and temperature to plant productivty, Donohue et al (2013) showed that improved *WUE* with rising CO_2 is likely to disproportionally benefit plants in warm arid regions, such as South Africa.

Although the effect of CO_2 on *P. aculeata* plant traits were evaluated in this study, neither of the two biocontrol agents (*P. guerini* and *C. schaffneri*) were included in this study to determine the subsequent effect of changes to plant traits on their fitness. Nevertheless, based on the observed declines in foliar nitrogen and increased foliar C:N ratios of plants grown at eCO_2 (ambient-water treatment), the leaf chewing beetle, *P. guerini*, is likely to be negatively affected (Bezemer et al., 1998; Robinson et al., 2012; Stiling and Cornelissen, 2007; Baso et al., 2021). Nitrogen concentration is positively correlated to the ability of an insect to convert ingested plant tissue into body mass (Mattson Jr, 1980) and reduced nitrogen concentration may therefore negatively affect the development and overall performance (fitness) of biocontrol agents, such as *P. guerini* (see Cowie et al., 2019). While the literature indicates that insect folivores are hindered by declines in leaf nutrient quality (Robinson et al., 2012; Cowie et al., 2019; Baso et al., 2021), the effect of eCO_2 on *P. aculeata* and the subsequent implications on its biocontrol agents warrants further investigation.

3.5.1 Conclusion

In conclusion, increasing atmospheric CO_2 appears unlikely to have been a major contributor to the invasion success of *P. aculeata* in South Africa from time of introduction (1850's) to present day. However, when grown under eCO_2 with sufficient water, *P. aculeata* showed increased growth rates, biomass accumulation, *WUE*, C:N ratios and reduced foliar nitrogen. This is likely to increase *P. aculeata's* invasive potential in optimal rainfall sites by rendering it more vigorous and damaging, and potentially less nutritious to biocontrol agent/s, further hindering the management of this invasive alien plant. Furthermore, eCO_2 only partially ameliorated the negative effect of lower soil moisture on productivity, whereby plants had improved *WUE* but growth rates, biomass accumulation, C:N ratios and foliar nitrogen were not dissimilar to plants grown at aCO_2 and plants grown at sCO_2 with sufficient water. Despite this reduced vigour, improved *WUE* could allow it to expand into lower rainfall regions where it is less common than present. Lastly, this study also highlights the importance of

including different watering treatments as to not overestimate the potential invasive vigour of invasive plants under elevated CO_2 and future climate scenarios.

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Author statement

Nic Venter: Conceptualization, Methodology, Investigation, Visualisation, Writing - original draft, Data curation, Formal analysis.

Blair Cowie: Conceptualization, Methodology, Visualisation, Writing - review & editing.

Iain Paterson: Conceptualization, Resources, Writing - review & editing.

Ed Witkowski: Conceptualization, Resources, Writing - review & editing.

Marcus Byrne: Conceptualization, Supervision, Resources, Writing - review & editing.

Declaration of Competing Interest: None.

3.6 Supplementary data

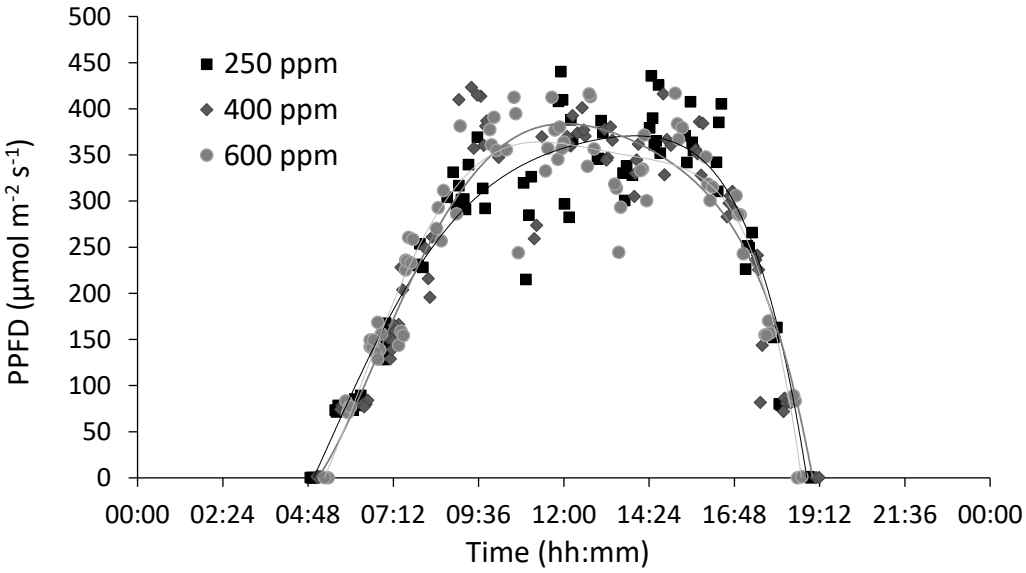


Figure S3.1: Photosynthetic photon flux density (PPFD) as measured by the PAR sensor within the 2 x 3 cm clear top cuvette (LI-6400XT) during gas exchange measurements of *Pereskia aculeata* grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm CO_2. These curves represent the approximate PPFD cycle that the plant canopies were exposed to throughout the entire experiment. Light period: 05:00 to 19:00. Each curve consists of either 88 or 89 data points and includes both water treatments. 5^{Th} order polynomials were fitted to each curve.

Table S3.1: Mean (± SD) [CO_2], temperature, relative humidity, and air vapour pressure deficit (VPD) for each of the three chambers. Data were logged at 5 minute intervals over the majority of the five month experiment. Photosynthetic photon flux density (PPFD) was not logged.

Chamber CO_2	CO_2 (ppm)	Temperature (°C)	Relative humidity (%)	VPD (kPa)
250	260.31 ± 41.22	23.515 ± 2.759	66.41 ± 5.55	0.82 ± 0.19
400	401.27 ± 30.18	23.516 ± 2.755	64.13 ± 5.71	0.87 ± 0.19
600	599.42 ± 41.89	23.426 ± 2.659	65.05 ± 5.22	0.84 ± 0.18

Table S3.2: Standard curves of SPAD+ (Single Photon Avalanche Diode) to extracted leaf pigments (mg g^{-1}) from *Pereskia aculeata* for each CO_2 x water treatment. Plants were grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}).

	250 ppm (sCO_2)		400 ppm (aCO_2)		600 ppm (eCO_2)	
	200 ml wk^{-1}	400 ml wk^{-1}	200 ml wk^{-1}	400 ml wk^{-1}	200 ml wk^{-1}	400 ml wk^{-1}
Chlorophyll a	$y = 0.0022x^2 - 0.0058x$; $R^2 = 0.897$	$y = 0.002x^2 + 0.0353x$; $R^2 = 0.8852$	$y = 0.0014x^2 + 0.0676x$; $R^2 = 0.5792$	$y = 0.0033x^2 - 0.0297x$; $R^2 = 0.7067$	$y = 0.0011x^2 + 0.0402x$; $R^2 = 0.8311$	$y = 2E\text{-}06x^2 + 5E\text{-}05x$; $R^2 = 0.9227$
Chlorophyll b	$y = 0.0003x^2 + 0.0386x$; $R^2 = 0.8567$	$y = 0.001x^2 + 0.0165x$; $R^2 = 0.8283$	$y = 0.0004x^2 + 0.0481x$; $R^2 = 0.5687$	$y = 0.0016x^2 - 0.0104x$; $R^2 = 0.6483$	$y = 0.0014x^2 - 0.0119x$; $R^2 = 0.6805$	$y = 0.0004x^2 + 0.0211x$; $R^2 = 0.7566$
Total chlorophyll (a + b)	$y = 0.0025x^2 + 0.0328x$; $R^2 = 0.8988$	$y = 0.003x^2 + 0.0518x$; $R^2 = 0.8881$	$y = 0.0018x^2 + 0.1157x$; $R^2 = 0.5793$	$y = 0.0049x^2 - 0.0401x$; $R^2 = 0.6948$	$y = 0.0011x^2 + 0.0916x$; $R^2 = 0.8133$	$y = 0.0012x^2 + 0.0591x$; $R^2 = 0.7713$
Carotenoids	$y = 0.0006x^2 - 0.0073x$; $R^2 = 0.8723$	$y = 1E\text{-}06x^2 + 0.0327x$; $R^2 = 0.3587$	$y = 0.0002x^2 + 0.0136x$; $R^2 = 0.6511$	$y = 0.0003x^2 + 0.0083x$; $R^2 = 0.5373$	$y = 7E\text{-}05x^2 + 0.0172x$; $R^2 = 0.6936$	$y = -5E\text{-}05x^2 + 0.02x$; $R^2 = 0.4236$

Table S3.3: Summary of the linear and interactive effects of CO_2 and water treatment (F_{stat} and *P-values*) on *Pereskia aculeata* growth parameters. Plants were grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}).

		F_{stat}; *P-value*		
	Units	CO_2	water	CO_2 x water
Total biomass	g	$F_{2,45}$=25.75; **P<0.001**	$F_{1,46}$=69.74; **P<0.001**	$F_{5,42}$=5.44; **P=0.008**
Shoot biomass	g	$F_{2,45}$=21.32; **P<0.001**	$F_{1,46}$=68.68; **P<0.001**	$F_{5,42}$=5.38; **P=0.0083**
Root biomass	g	$F_{2,45}$=11.24; **P<0.001**	$F_{1,46}$=5.08; **P=0.029**	$F_{5,42}$=0.38; P=0.685
Root:shoot		$F_{2,45}$=2.86; P=0.068	$F_{1,46}$=2.15; P=0.15	$F_{5,42}$=0.258; P=0.773
Relative growth rate (RGR)	g g^{-1} day^{-1}	$F_{2,45}$=24.07; **P<0.001**	$F_{1,46}$=72.87; **P<0.001**	$F_{5,42}$=1.50; P=0.235

Table S3.4: Summary of the linear and interactive effects of CO_2 and water treatment (F_{stat} and *P-values*) on *Pereskia aculeata* leaf traits. Plants were grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}).

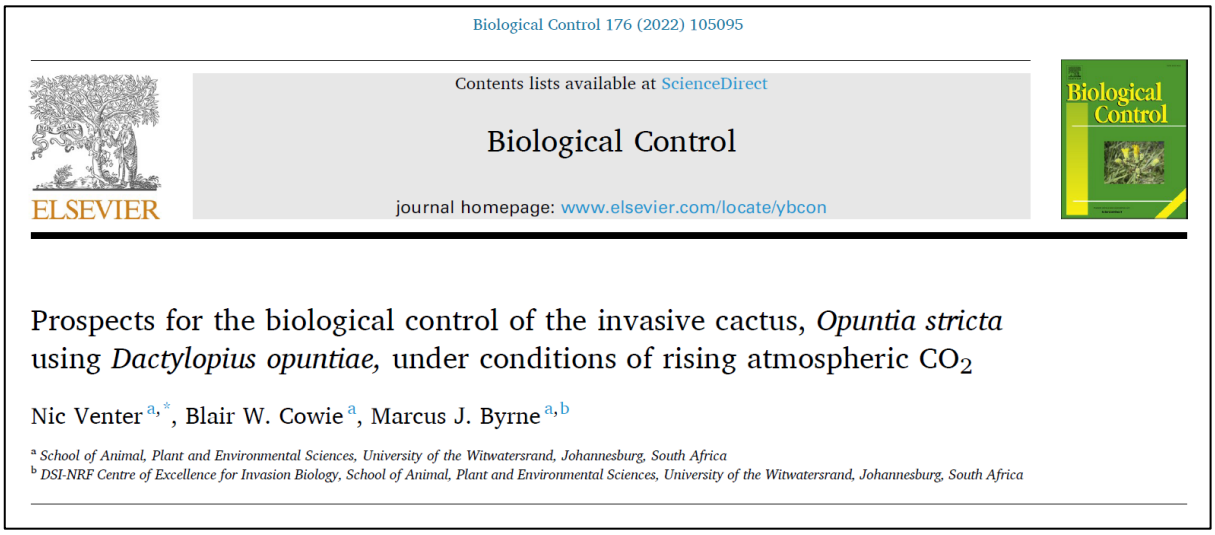
		F_{stat}; *P-value*		
	Units	CO_2	water	CO_2 x water
Leaf area	cm^2	$F_{2,45}$=1.98; *P*=0.15	$F_{1,46}$=0.62; *P*=0.44	$F_{5,42}$=0.26; *P*=0.77
Specific leaf area (SLA)	$cm^2\ g^{-1}$	$F_{2,45}$=49.08; ***P*<0.001**	$F_{1,46}$=3.19; *P*=0.081	$F_{5,42}$=13.35; ***P*<0.001**
Leaf thickness	mm	$F_{2,45}$=49.08; ***P*<0.001**	$F_{1,46}$=0.23; *P*=0.63	$F_{5,42}$=0.31; *P*=0.73
Leaf water content	%	$F_{2,45}$=49.08; ***P*<0.001**	$F_{1,46}$=0.23; *P*=0.67	$F_{5,42}$=2.96; *P*=0.063
Total chlorophyll	$mg\ g^{-1}$	$F_{2,45}$=36.63; ***P*<0.001**	$F_{1,46}$=11.33; ***P*=0.002**	$F_{5,42}$=5.57; ***P*=0.0072**
Chlorophyll a	$mg\ g^{-1}$	$F_{2,45}$=47.58; ***P*<0.001**	$F_{1,46}$=11.81; ***P*=0.001**	$F_{5,42}$=5.34; ***P*=0.0086**
Chlorophyll b	$mg\ g^{-1}$	$F_{2,45}$=22.90; ***P*<0.001**	$F_{1,46}$=9.70; ***P*=0.003**	$F_{5,42}$=6.63; ***P*=0.0031**
Chlorophyll a:b		$F_{2,45}$=27.43; ***P*<0.001**	$F_{1,46}$=0.24; *P*=0.62	$F_{5,42}$=10.15; *P*<0.001
Carotenoids	$mg\ g^{-1}$	$F_{2,45}$=43.85; ***P*<0.001**	$F_{1,46}$=55.24; ***P*<0.001**	$F_{5,42}$=4.62; ***P*=0.015**
Foliar nitrogen	%	$F_{2,45}$=12.31; ***P*<0.001**	$F_{1,46}$=16.86; ***P*<0.001**	$F_{5,42}$=1.11; *P*=0.337
Foliar carbon	%	$F_{2,45}$=1.98; *P*=0.15	$F_{1,46}$=0.077; *P*=0.78	$F_{5,42}$=0.176; *P*=0.84
Foliar C:N		$F_{2,45}$=21.12; ***P*<0.001**	$F_{1,46}$=17.76; ***P*<0.001**	$F_{5,42}$=2.86; *P*=0.068
$\delta^{13}C$	‰	$F_{2,45}$=49.95; ***P*<0.001**	$F_{1,46}$=17.20; ***P*<0.001**	$F_{5,42}$=2.94; *P*=0.064

Table S3.5: Summary of the linear and interactive effects of CO_2 and water treatment (F_{stat} and *P-values*) on *Pereskia aculeata* gas exchange parameters. Plants were grown at three [CO_2]: 250 (sCO_2), 400 (aCO_2) and 600 (eCO_2) ppm, with two water treatments: reduced-water (200 ml wk^{-1}) and ambient-water (400 ml wk^{-1}).

	Units	F_{stat}; *P-value*		
	ml wk^{-1}	CO_2	water	CO_2 x water
Net photosynthesis (*A*)	µmol CO_2 m^{-2} s^{-1}	$F_{2,222}$=49.88; ***P*<0.001**	$F_{1,223}$=30.30; ***P*<0.001**	$F_{5,219}$=5.4; ***P*=0.005**
Stomatal conductance (g_{ST})	mol H_2O m^{-2} s^{-1}	$F_{2,222}$=15.68; ***P*<0.001**	$F_{1,223}$=58.84; ***P*<0.001**	$F_{5,219}$=19.2; ***P*<0.001**
Transpiration (*E*)	mol H_2O m^{-2} s^{-1}	$F_{2,222}$=6.84; ***P*=0.001**	$F_{1,223}$=39.77; ***P*<0.001**	$F_{5,219}$=7.6; ***P*<0.001**
Intercellular [CO_2] (C_i)	µmol CO_2 mol^{-1} or ppm	$F_{2,222}$=506; ***P*<0.001**	$F_{1,223}$=3.85; *P*=0.051	$F_{5,219}$=3.3; ***P*=0.038**
C_i/C_a		$F_{2,222}$=2.35; *P*=0.097	$F_{1,223}$=0.22; *P*=0.63	$F_{5,219}$=2.6; *P*=0.074
Water use efficiency (*WUE*) (A/g_{ST})	µmol CO_2 mol^{-1} H_2O	$F_{2,222}$=274; ***P*<0.001**	$F_{1,223}$=10; ***P*=0.002**	$F_{5,219}$=5.1; ***P*=0.006**

CHAPTER FOUR

Prospects for the biological control of the invasive cactus, *Opuntia stricta* using *Dactylopius opuntiae*, under conditions of rising atmospheric CO_2



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Prospects for the biological control of the invasive cactus, *Opuntia stricta* using *Dactylopius opuntiae,* under conditions of rising atmospheric CO_2

Nic Venter [a,*], Blair W. Cowie [a], Marcus J. Byrne [a,b]

[a] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa
[b] DSI-NRF Centre of Excellence for Invasion Biology, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

Preface

Using *O. stricta*, this chapter aimed to address Objective 3 by assessing the effect of increasing CO_2 on plant quality and subsequent biological control efficacy. Results from this chapter were also used to address Objective 1 as it measured *O. stricta* invasive vigour.

The final MS Word document submitted for publication was used here, modified slightly to maintain a similar formatting to the rest of the thesis. No changes were made to the text other than assigning new heading numbers.

4.1 Abstract

Opuntia stricta var. stricta (Haworth.) Haworth (Cactaceae) is an important invasive alien plant (IAP) in South Africa, that invades both disturbed and undisturbed habitats mainly within grasslands and savannas. Fortunately, substantial control has been achieved using the biological control agent, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), a sap sucking scale insect. With rising atmospheric CO_2 concentrations, many plants alter their growth traits, most notably leaf nutritional quality which in turn affects their arthropod herbivores. Therefore, we tested whether *D. opuntiae* fitness and its subsequent efficacy as a biological control agent is altered by *O. stricta* plants grown at elevated CO_2. Using walk-in Conviron growth chambers, *O. stricta* was grown at three CO_2 concentrations; 250 (sub-ambient – sCO_2). 400 (ambient - aCO_2) and 600 ppm (elevated – eCO_2). After 70 days of growth, plants were inoculated with 20 first instar *D. opuntiae* crawlers and we subsequently measured parameters to assess their fitness. Plant traits were measured regularly throughout the 6.5-month experiment. *Dactylopius opuntiae* fitness index was significantly lowered on eCO_2 plants which slowed the rate of herbivory. *Dactylopius opuntiae* fitness index was negatively correlated to cladode carbon/nitrogen ratios indicating reduced plant quality was a potential driver. However, despite reduced *D. opuntiae* fitness and slowed rates of damage, *O. stricta* growth at eCO_2 was still inhibited by the insect, and final plant biomass did not differ from aCO_2 plants, unlike that observed for the control plants. Furthermore, irrespective of CO_2 treatment, all plants succumbed to *D. opuntiae* herbivory with plants grown at eCO_2 taking approximately three weeks longer than sCO_2 and aCO_2 plants to die. Despite mortality of *O. stricta* plants grown at eCO_2 due to *D. opuntiae* feeding, the long-term potential cumulative effects of reduced agent fitness and their subsequent efficacy may hinder the management of this IAP with rising atmospheric CO2 concentrations.

Keywords: Arthropod-plant interactions; climate change; cochineal; elevated CO_2; host plant quality; invasive alien plants (IAP)

4.2 Introduction

Native to Central America, the southeastern United States, and the Caribbean, *Opuntia stricta* var. *stricta*, is a NEM:BA (National Environmental Management: Biodiversity Act no. 10 of 2004) Category 1b (taxon must be controlled wherever possible, no trade or planting thereof) invasive succulent shrub found in southern Africa and 20 other countries globally (Novoa et al. 2015; Hoffmann et al. 2020). *Opuntia stricta* was introduced into South Africa as an ornamental (year unknown) and as of 2017 was the fifth most widespread invasive cactus in the country. Its presence has been recorded in ± 180 quarter-degree grid cells (QDS) across all nine provinces, where it mainly invades savanna and dry grassland type habitats (Kaplan et al. 2017; Hoffmann et al. 2020; Paterson et al. 2021). Like other invasive cacti, *O. stricta* invades both disturbed and undisturbed habitats, displacing native vegetation, reducing productivity, and causing damage to wildlife and livestock due to its spines and glochids (Kaplan et al. 2017; Shackleton et al. 2017; Hoffmann et al. 2020). Long distance dispersal is facilitated by frugivorous animals and, large, dense impenetrable thickets often result from its vegetative reproduction (Kaplan et al. 2017; Hoffmann et al. 2020). Interestingly, it was not considered a major problem until the 1990s, and up until then the cactus biological control (hereafter: biocontrol) programme exploited only two cactophagous agents which were used to control *Opuntia ficus-indica* (L.) Miller. *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) achieved only moderate control of *O. stricta*, while damage by the cochineal scale insect *Dactylopius opuntiae* 'ficus-indica' lineage (also referred to as a biotype) was deemed trivial (Hoffmann et al. 1999; Zachariades, 2021). With an observed increase in *O. stricta* spread, particularly within the Kruger National Park (KNP), the *D. opuntiae* 'stricta' lineage (hereafter *D. opuntiae*), which was successful in Australia, was released in 1997 (Hoffmann et al. 1999). Effective control using this lineage was achieved in the KNP and has been repeated across various climatic zones in South Africa (Moran et al. 2021; Paterson et al. 2021). However, *O. stricta* is still widespread and at certain sites dense infestations continue to persist (Hoffmann et al. 2020). Less effective control is largely attributed to climate, such as colder and higher rainfall regions that reduce *D. opuntiae's*

efficacy. Additionally, hybridisation of the two *D. opuntiae* lineages ('stricta' and 'ficus-indica') at sites where *O. ficus-indica* may be abundant reduces the host specificity of subsequent generations on the less abundant *Opuntia* species (Hoffmann et al. 2002; Paterson et al. 2011a). While these effects on *D. opuntiae* efficacy are relatively well accepted, the effects of rising atmospheric carbon dioxide (CO_2) on host plant quality may add further complexity to the agent's effectiveness as a biocontrol agent.

Atmospheric CO_2 has increased from ~ 270 ppm (pre-industrial revolution) to current CO_2 concentrations of ~ 416 ppm (CO_2 Earth, 2022). Increases in atmospheric CO_2 and other greenhouse gasses (methane, nitrous oxide, chlorofluorocarbons etc.) have an impact on global temperatures which are driving global climate change (Anderson et al. 2016). Increased frequency of intense extreme weather events such as heat waves, droughts and floods etc. are a consequence of climate change but due to the spatial and temporal nature of these events, it can be difficult to predict the effect on arthropod-plant interactions (Reeves, 2017). However, the indirect effects of elevated CO_2 (eCO_2) on herbivorous arthropod performance are fairly well understood (Bezemer and Jones, 1998; Zavala et al. 2013). While there is considerable variation amongst feeding guilds and orders, in general arthropods respond negatively when exposed to plants grown at eCO_2 (Robinson et al. 2012). Declines in host plant quality are most often cited as the main reason for negative arthropod reactions. Host plant quality can be affected by numerous allocation responses (primary and secondary metabolites), but the most notable are changes to the carbon (C) and nitrogen (N) balance within the plant tissue (Stiling and Cornelissen, 2007; Robinson et al. 2012). Decreases in N relative to C as observed for eCO_2 studies, causes a N dilution effect (less protein), thus higher C/N ratios in plant tissue results in decreased plant nutritional quality (Lincoln et al. 1986). In addition to changes in allocation patterns, plants generally respond to eCO_2 by accumulating more biomass (Stiling and Cornelissen, 2007; Robinson et al. 2012). Naturally these plant responses to eCO_2 depend on numerous factors, which include but are not limited to plant functional group, growth form and the interaction of biotic and abiotic factors such as temperature, light, water, and nutrient availability (Zvereva & Kozlov, 2006; Stiling and Cornelissen, 2007; Robinson et al. 2012).

Field studies suggest that IAP's are favourably stimulated by eCO_2 relative to co-occurring non-weed species, and therefore will have a competitive advantage (Belote et al. 2004; Manea and Leishman, 2011; Ziska et al. 2010; Ziska and George, 2004). Coupled with the negative effect of eCO_2 on host plant quality and subsequent arthropod performance, it is predicted that the management of invasive alien plant species (IAP) using classical biocontrol may be adversely affected in the future (Ziska and George, 2004; Reeves 2017). However, these assumptions are mostly inferred from arthropod-plant studies conducted within agricultural systems (Baso et al. 2021). Therefore, there is a need to investigate this effect within an IAP biocontrol context, particularly as the agent/target IAP relationships are specialist associations. Of the studies that have focused on weed biocontrol, results remain variable, with both positive and negative effects on the biocontrol agents' performance and efficacy (Johns and Hughes, 2002; Johns et al. 2003; Diaz et al. 2012; Reeves et al. 2015; Henriksen et al. 2018; Baso et al. 2021).

Dactylopius opuntiae is an important IAP management tool that has been very successful in controlling the majority of *Opuntia stricta* (also *O. humifusa*) invasions in South Africa (Moran et al. 2021). Furthermore, no studies in either agricultural or biocontrol related fields have investigated the interaction of *D. opuntiae* herbivory and eCO_2 on any cacti species. Therefore, the main aim of the study was to determine if *D. opuntiae* will remain effective in controlling *O. stricta* plants in future eCO_2 scenarios. By exposing *D. opuntiae* to *O. stricta* plants grown at three different CO_2 levels representing pre-industrial (sub-ambient CO_2), present day (ambient CO_2) and future scenarios (elevated CO_2) we sought to determine 1) if changes to *O. stricta* traits would alter *D. opuntiae* fitness and 2) if these observed outcomes would affect its performance (feeding damage) as a biocontrol agent. These results will help inform the future usage of *D. opuntiae* in managing *O. stricta,* and more broadly other *Dactylopius* species and lineages thereof used against other invasive cacti species.

4.3 Methods

4.3.1 Plant material, experimental setup, and growth chamber conditions

Opuntia stricta is a succulent multi-branched shrub growing up to 2 m from a single stem. Cladodes are bluish green, elliptically shaped (± 23 x 10 cm), with one (± 4 cm long), or no spines per areole (Henderson, 2020). Plants were propagated from cladodes ranging in wet mass from 50 – 90 grams (15 – 20 cm long) obtained from several parental plants maintained at the Agricultural Research Council - Plant Health and Protection (ARC-PHP) Roodeplaat, Gauteng, South Africa (-25.607, 28.352). Ninety-six cladodes were individually planted upright in 17 cm diameter (2.3 litre volume) pots containing ~ 1.5 kg of sandy-loam soil, with a third of the cladode buried in the soil. Thirty-two potted plants were randomly selected and moved into each of three walk-in Conviron growth rooms (Conviron PGV40, Winnipeg, Canada) with the following CO_2 concentrations; ~ 250 ppm (sub-ambient – sCO_2 – pre-industrial concentration), 400 ppm CO_2 (ambient – aCO_2 – current concentration) and 600 ppm CO_2 (elevated - eCO_2 – RCP6.5 ~ ca. 2080) (IPCC, 2014). The Conviron growth chambers were located at the University of the Witwatersrand, Johannesburg, South Africa (-26.191, 28.031). Temperature and relative humidity used throughout the experiment were chosen to closely replicate those observed in Skukuza, Kruger National Park, Mpumalanga Province, South Africa (see introduction), where the largest and most dense *O. stricta* infestations were successfully controlled upon release of *D. opuntiae*. Air temperature was programmed to change at ± 1 °C / hour between the day-time maximum of 27 °C (11:00 to 16:30) and the night-time minimum of 20 °C (23:00 to 05:00) with an average temperature of 23.5 °C. Vapour pressure deficits (VPD) were maintained between 0.82 kPa and 1.25 kPa by using a relative humidity of 65%. Light was supplied by overhead fluorescent tubes (Philips Master TL5 HO 54W/840, 4000 Kelvin) and halogen bulbs (Philips 55W GLS A55 E27, 2800 Kelvin). The light cycle started at 05:00 and the intensity increased to ± 1000 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (at the top of the plants) at 09:30 and thereafter decreased from 16:30 till 19:00, at which time the light cycle ended.

Plants were not cycled between chambers as only one of the three Conviron chambers could scrub incoming CO_2 (using soda lime) to create sub-ambient CO_2 levels (250 ppm). Temperature, humidity, VPD and [CO_2] were logged every five minutes for the duration of the experiment (Table S4.1). In addition, spot measurements of Conviron conditions were conducted fortnightly using a LI-6400XT portable open path infrared gas analyser (LICOR Biosciences, Lincoln, Nebraska, USA) to verify that temperature, RH, VPD and PPFD within the three chambers were similar and [CO_2] was being maintained correctly. Six grams of slow release granular 7:1:3 fertiliser (N = 95 g kg^{-1}; P = 14 g kg^{-1}; K = 41 g kg^{-1}) was added to each pot on day 60. The experiment was conducted over a 6.5-month period (initiated 24th Jan 2018, concluded 6th August 2018; 194 days). Plants were adequately watered (400 ml per week; soil water potential > 0.5 MPa) during the entire experiment.

4.3.2 *Dactylopius opuntiae* inoculation and development

Seventy days after planting, *O. stricta* had grown to a suitable size within all CO_2 treatments (parental cladodes produced fully developed daughter cladodes) and were inoculated with *D. opuntiae* 'stricta' biotype. *Dactylopius opuntiae* 'stricta' lineage were obtained from the ARC-PHP (see Section 4.3.1). Sixteen of the 32 plants per CO_2 treatment were each inoculated with 20 neonate first instars (nymphal crawlers). Crawlers were randomly collected from a culture of *D. opuntiae* females reared under similar conditions to the experimental conditions (ambient CO_2 concentration grown plants). Hand inoculations were performed by gently picking up individual crawlers using a fine No.1 paint brush and placing them on the rooted parental cladode. Insect inoculated plants (herbivory treatment) were monitored twice a day for the first three days to ensure the transferred crawlers had not been injured and had consequently dispersed on their plant. Thereafter herbivory treatment plants were monitored regularly to observe when crawlers matured into gravid females (intermediate development stages were not assessed). Males are winged and can disperse hence we were unable to assess male development for sex ratios due to the uncaged (open) experimental

design. Additionally, males are small and are mostly hidden in the wax coverings of the females. The number of gravid females (evidence of crawler production) that developed per treatment plant was counted and the 'settling proportion' was calculated (No. gravid females / No. inoculated crawlers). The time to F_1 crawler emergence was monitored for each parental generation female and the average crawler emergence time for all females per treatment plant was calculated and expressed as 'time to female maturity' (development time) in days from the initial inoculation at day 70 (counted as time 0 from crawler inoculation). Thereafter, it was not possible to track subsequent generations and 'settling' and 'time to female maturity' could only be calculated from the parental generation. Female mass and number of progeny was obtained by sampling mature (gravid) F_1/F_2 generation individuals ~ 113 days after inoculation. Not all treatment plants were sampled for gravid females as some plants did not have any or only a few mature F_1 females by day 113, while other plants were beginning to succumb to herbivory. Therefore, we collected more than one female for some plants, while for other plants we did not collect any. This was done as we did not want to interfere with the final effect of herbivory as females are sessile due to their stylets being permanently inserted in the plant. In total, we removed 23 females per CO_2 treatment. In situ females were considered gravid when there was evidence of crawlers emerging from the wax coating. Once removed, females were weighed (wax coating removed) and then individually placed in an Eppendorf tube blocked with cotton wool to allow air flow and to stop emerging crawlers from escaping (see Rule and Hoffmann, 2018). Crawlers emerge for up to a week after removal and subsequently die, dry out and remain preserved indefinitely in the Eppendorf tube. The number of crawlers produced per female was later counted. Using these data, the Female Fitness Index (FI) was calculated for the 23 sampled females per CO_2 treatment: FI = settling proportion x (no. progeny / time to female maturity) (Mathenge et al. 2010). The remaining 16 control plants per CO_2 treatment were kept free of *D. opuntiae* throughout the experiment by removing unwanted crawlers by hand.

4.3.3 *Opuntia stricta* response to CO_2 and herbivory

During the 6.5 month experiment the following parameters were measured on each plant on seven occasions (days 0, 28, 54, 90, 142, 166 and 194): plant height, number of new growth cladodes, number of detached cladodes in response to *D. opuntiae* herbivory and cladode area. Plant height was measured from the top of the soil to the highest cladode and cladode area was calculated using an ellipse formula for area using the length and width. At termination of the experiment biomass and plant traits were measured. Above ground biomass, below ground (roots) and spines were harvested, oven dried at 60 °C for a week and weighed. Specific leaf (cladode) area (SLA) was calculated as follows: cladode area/dry biomass. Cladode area used for SLA were taken from cladode area measurements made on day 142 (June month) prior to the herbivory treatment plants succumbing (cladode detachment) to *D. opuntiae* feeding.

Chlorophyll a, b and carotenoid analyses were conducted at the end of the experiment so as not to interfere with the insects and thus was only performed on control plants (herbivory treatment plants were dead). Using a 4 mm punch, cladode tissue was extracted and the epidermal /cortex layer on either side of the cladode were separated from the vascular bundle and internal pith. The epidermal/cortex samples from either side of the cladodes were placed in separate test tubes containing 96% analytical grade ethanol, crushed, and incubated in the dark at 25 °C for 72 hours. A 3 ml aliquot was transferred to a standard 4.5 ml cuvette and absorptance was measured using a spectrophotometer (Thermo Scientific™ GENESYS 20). Absorptance wavelengths of 664 nm, 649 nm and 470 nm were used to measure chlorophyll a, b and carotenoids, respectively. Following this, the leaf material was oven dried, and the pigment contents (mg m^{-2}) were calculated using the equations as described by Lichtenthaler, (1987).

Samples for cladode carbon (C) and nitrogen (N) concentration analysis were obtained by randomly removing from 2 to 3 small pieces of dried cladodes per plant (control and herbivory treatments). This cladode material was then crushed and homogenised. A subsample was removed from the bulk ground mixture and ± 1.25 mg was weighed in 6 x 4 mm tin capsules (D1006 Capsules.

Elemental Microanalysis, Okehampton, United Kingdom). Analysis of cladode tissue C and N concentrations were conducted according to the methods of Venter et al. (2022).

4.3.4 Statistical analyses

All statistical analyses were performed using RStudio (RStudio Team, 2022). General linear models were used to analyse 1) *D. opuntiae* crawler settling proportions and time to female maturity (development), 2) the interactive effects of CO_2 and treatment (control and herbivory) on *O. stricta* plant traits and 3) the relationships between *D. opuntiae* traits and cladode C, N and C/N ratios. The lmer function in the 'lme4' package (Bates et al. 2015) was used to analyse *D. opuntiae* female gravid mass, number of offspring and female fitness index (FI) as multiple measures were conducted per replicate plant (see Methods section 2.2) and thus plant individual was treated as a random effect. To control for repeat measures, a glmer model with a binomial error distribution was used to test the interactive effects of time (days) and CO_2 on the percentage of attached cladodes in response to *D. opuntiae* herbivory. The Anova function from the 'car' package (John et al. 2020) was used calculate analysis-of-variance tables for the models. Using functions from the 'multcomp' package (Hothorn et al. 2008), Tukey post-hoc tests were conducted for all models except the glmer model where a Type II Wald chi-square post hoc test was performed. Data were transformed where necessary to improve the distribution of the residuals.

4.4 Results

4.4.1 *Dactylopius opuntiae* development

Dactylopius opuntiae crawlers inoculated on plants grown at eCO_2 showed a ~ 63% reduction in the proportion of crawlers that developed into gravid females (settling) relative to aCO_2 plants (Fig. 4.1A; $F_{2,45}$= 19; $P < 0.001$). Time to female

maturity (development time) at eCO_2 was delayed by ~ 14 days relative to plants grown at aCO_2 (Fig. 4.1B; $F_{2,45}$= 5.6; P < 0.01). Female gravid mass (Fig. 4.1C; $F_{2,66}$= 1.95; P = 0.15) and number of offspring (Fig. 4.1D; $F_{2,66}$= 0.65; P = 0.53) did not differ between the three CO_2 treatments. Female fitness index (FI) was lowest on plants grown at eCO_2 (0.371 ± 0.034) which was different to both sCO_2 (0.858 ± 0.124) and aCO_2 (1.069 ± 0.0986) (Fig. 4.1E; $F_{2,66}$= 23; P < 0.001) treatments which were not statistically different from each other.

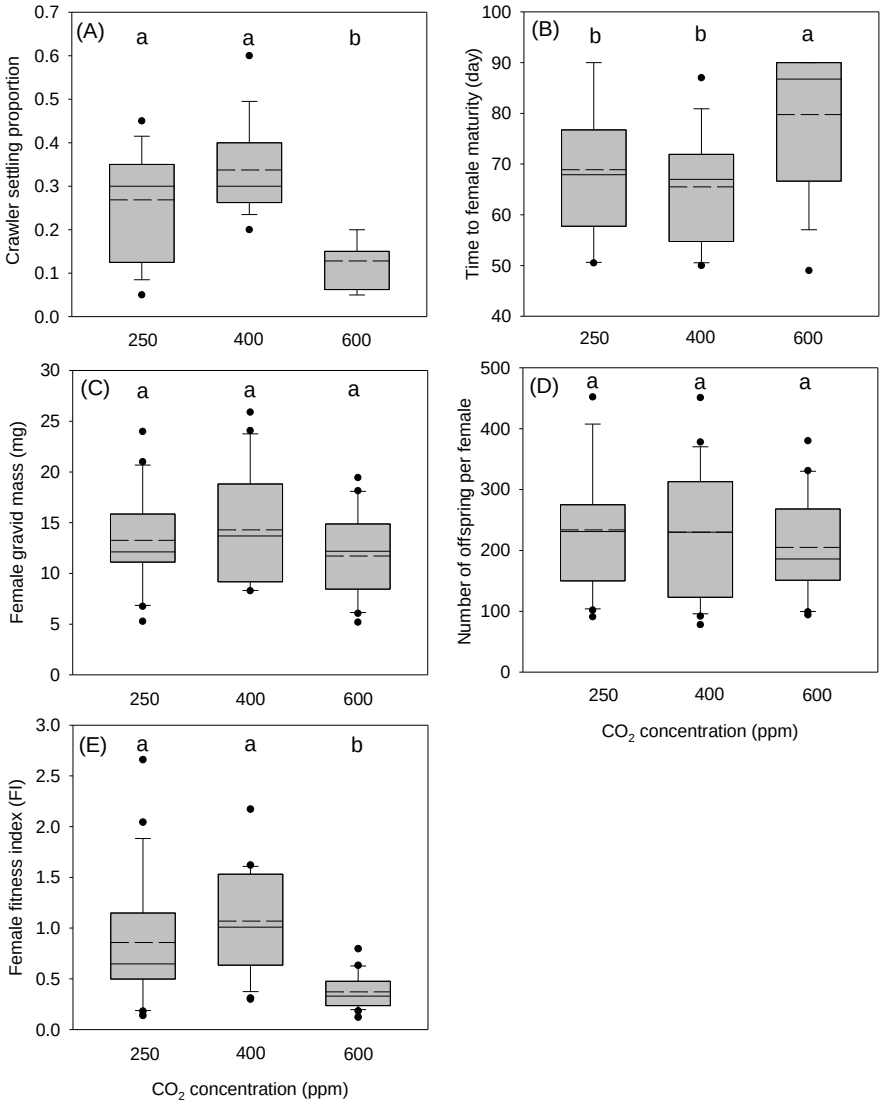

Figure 4.1: The biocontrol agent, *Dactylopius opuntiae* A) crawler settling proportion, B) time to female maturity, C) female gravid mass, D) number of offspring per female and E) female fitness index (FI) when reared on *Opuntia stricta* plants grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Boxplots show median (——), mean (– – –), 10th, 25th, 75th and 90th percentiles and points (•) outside whiskers denote outliers. Different lowercase letters indicate significant differences between CO_2 treatments (P < 0.05). n=16 (A & B) and n=23 (C - E).

4.4.2 Effects of *Dactylopius opuntiae* herbivory and CO_2 on *Opuntia stricta*

There was no interactive effect of time and CO_2 ($\chi^2_{(2)}$= 2.0; P = 0.37; n=429)) on the percentage of attached cladodes, but rather the main effects of time ($\chi^2_{(1)}$= 97; $P < 0.001$) and CO_2 ($\chi^2_{(2)}$= 58; $P < 0.001$) were significant (Fig. 4.2). This indicates that while all plants dropped cladodes through time because of *D. opuntiae* feeding, this was dependant on the CO_2 treatment. Plants grown at aCO_2 showed the fastest rate of cladode detachment, followed by sCO_2 and then eCO_2. All plants exposed to the biocontrol agent subsequently died, with the eCO_2 plants taking approximately three weeks longer than sCO_2 and aCO_2 ppm plants (Fig. 4.2). None of the control plants dropped cladodes.

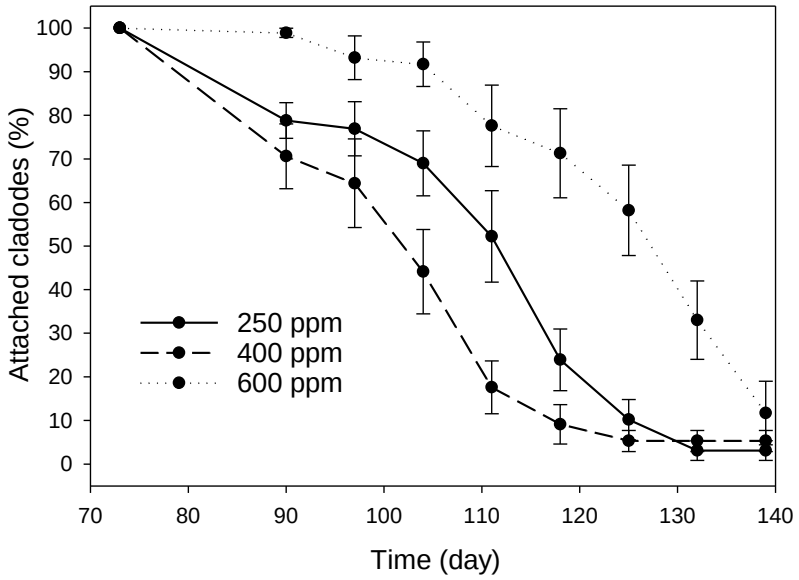

Figure 4.2: Mean (± SE) percentage of *Opuntia stricta* cladodes remaining attached to the basal (parental) cladode over time (days) after inoculation with the biocontrol agent *Dactylopius opuntiae* on plants grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Each plant was inoculated with 20 first instar crawlers on day 0 (day 70 since planting) and then left to continue their development for 140 days. n=16.

Similarly, there were no interactive effects of CO_2 and *D. opuntiae* herbivory on carbon (C) or nitrogen (N) or C/N ratios, rather the main effects of CO_2 and herbivory treatments on these parameters (Fig. 4.3A-C; Table 4.2). This indicates that changes observed for herbivory treatment plants were the result of *D. opuntiae* feeding and not plant induced CO_2 responses. Herbivory resulted in a

significant reduction in cladode C and N at sCO_2 when compared to control plants, but not at aCO_2 and eCO_2 (Fig. 4.3A&B; Table 4.2). Cladode N and C/N ratios for eCO_2 control plants were higher than sCO_2 and aCO_2, and similarly for herbivory treatment plants (Fig. 4.3C).

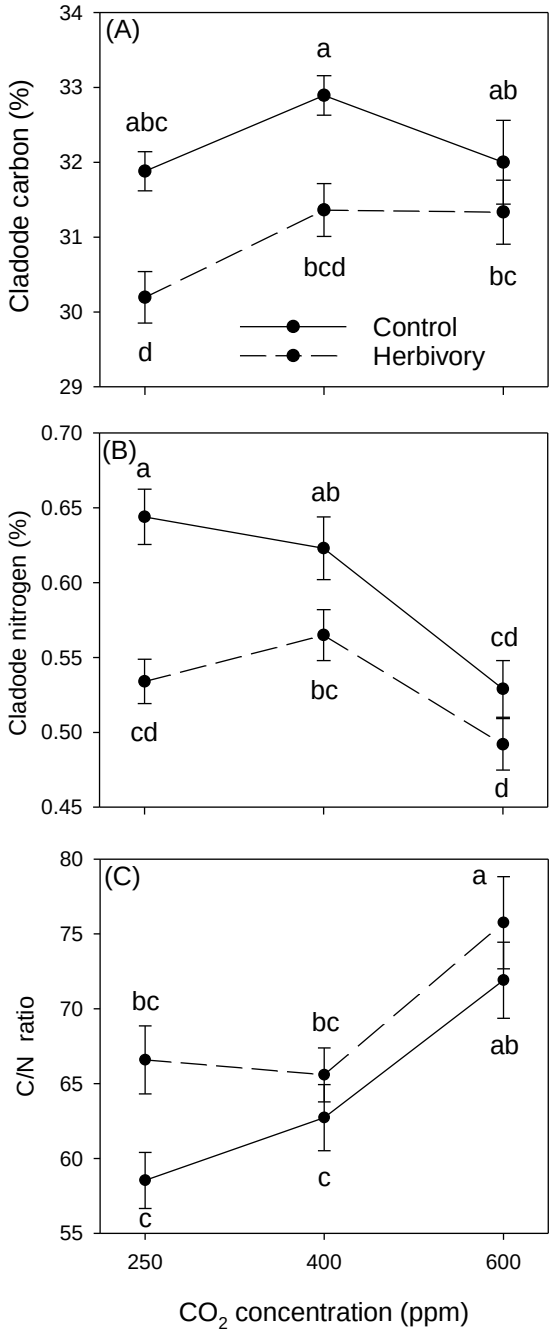

Figure 4.3: Mean (± SE) *Opuntia stricta* cladode A) carbon concentration, B) nitrogen concentration and C) carbon/nitrogen ratios (C/N) measured on control plants and those subjected to herbivory by the biocontrol agent *Dactylopius opuntiae*. Plants were grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Different lowercase letters indicate significant differences ($P < 0.05$). n=16.

Dactylopius opuntiae female fitness index (FI) was negatively correlated with increasing cladode C/N ratios (Fig. 4.4) but not for C (P = 0.35; Adj. R^2 = -0.003; n=29) or N (P = 0.22; Adj. R^2 = 0.02; n=29;). The proportion of *D. opuntiae* crawlers that settled was not correlated to either cladode C (P = 0.9; Adj. R^2 = -0.02; n=48), N (P = 0.58; Adj. R^2 = -0.015; n=48) or C/N ratios (P = 0.07; Adj. R^2 = 0.05; n=48). *Dactylopius opuntiae* time to female maturity (development) was positively correlated to cladode C (P = 0.02; Adj. R^2 = 0.09; n=48) as well as C/N ratios (P = 0.005; Adj. R^2 = 0.13; n=48) but not N (P = 0.6; Adj. R^2 = -0.02; n=48).

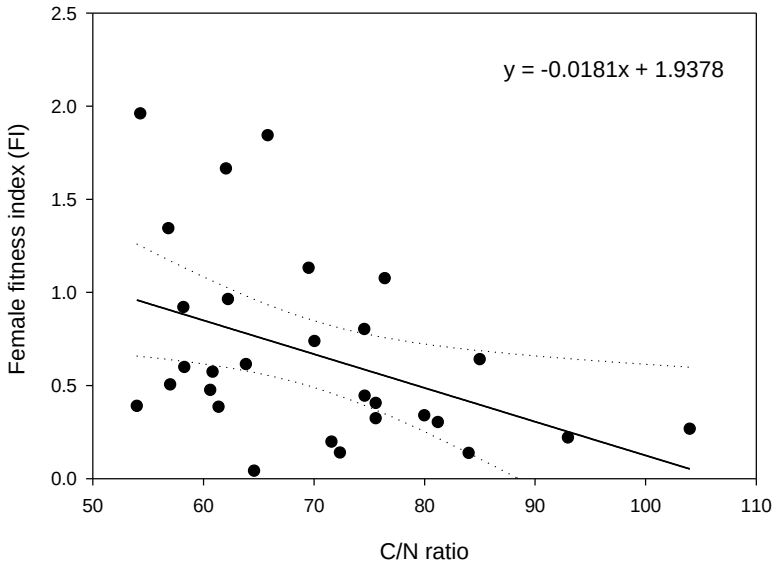

Figure 4.4: *Dactylopius opuntiae* female fitness index (FI) plotted against *Opuntia stricta* cladode C/N ratios (P = 0.021; Adj. R^2 = 0.15; n = 29) for plants grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Dashed lines denote the 95% confidence intervals (C.I.).

Opuntia stricta total biomass was highest for eCO_2 control plants followed by aCO_2 and then sCO_2 whereas biomass did not differ between herbivory treatment plants grown at eCO_2 and aCO_2 and between aCO_2 and sCO_2 plants (Fig. 4.5; Tables 4.1 & 4.2). Control plants grown at eCO_2 had higher daughter cladode mass, root mass, basal cladode mass, spine mass and spines by area when compared to both sCO_2 and aCO_2 control plants (Tables 4.1 & 4.2). Herbivory

treatment plants grown at eCO_2 had a higher spine mass, spines by area, total cladode area, specific leaf area, root/shoot ratios and lower specific leaf area (SCA) when compared to both sCO_2 and aCO_2 herbivory treatment plants (Tables 4.1 & 4.2). The effect of CO_2 treatment on photosynthetic pigments was significant for Chl b, carotenoids and Chl a/b ratios whereby sCO_2 and eCO_2 control plants differed (Tables 4.1 & 4.2). CO_2 treatment had no significant effect on Chl a or the total Chl (Tables 4.1 & 4.2).

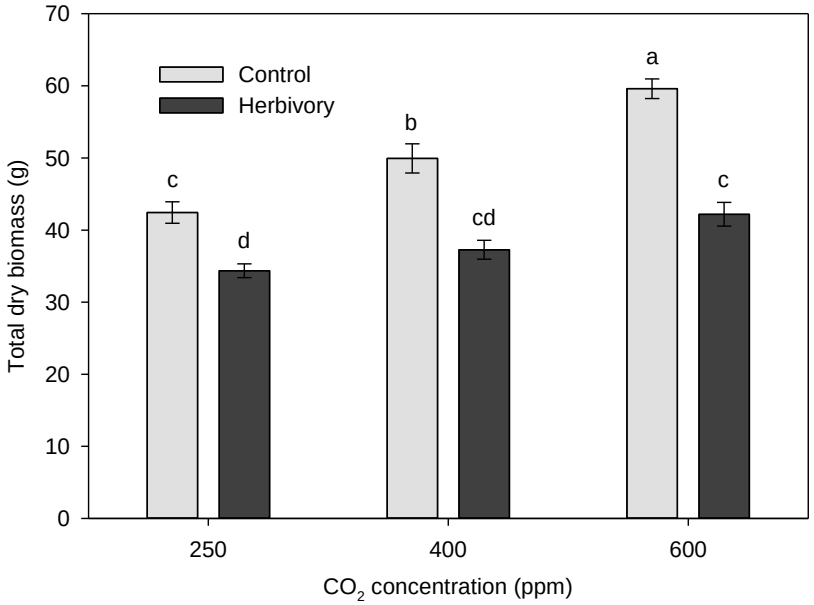

Figure 4.5: Mean (± SE) *Opuntia stricta* total dry biomass measured on control plants and those subjected to herbivory by the biocontrol agent *Dactylopius opuntiae*. Plants were grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Different lowercase letters indicate significant differences between groups ($P < 0.05$). n=16.

Table 4.1. *Opuntia stricta* traits measured at final harvest for control plants and those subjected to herbivory by the biocontrol agent *Dactylopius opuntiae*. Plants were grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Herbivory treatment plants were inoculated with 20 1st instar *D. opuntiae* after 70 days of plant growth and final harvest was conducted after a further 126 days (total 196 days). Different lowercase letters indicate significant differences across groups ($P < 0.05$). Mean ± SE; n=16.

		250 ppm (sCO_2)		**400 ppm (aCO_2)**		**600 ppm (eCO_2)**	
***Opuntia stricta* traits**	**Units**	**Control**	**Herbivory**	**Control**	**Herbivory**	**Control**	**Herbivory**
Height	mm	228.13 ^{a} ± 4.88	98.75 ^{b} ± 3.04	229.38 ^{a} ± 6.78	99.69 ^{b} ± 3.94	232.19 ^{a} ± 6.11	98.13 ^{b} ± 2.37
Daughter cladodes mass	g	21.71 ^{bc} ± 0.76	20.42 ^{c} ± 0.85	25.68 ^{b} ± 1.12	22.02 ^{bc} ± 1.09	29.89 ^{a} ± 1.08	23.69 ^{bc} ± 0.96
Root mass	g	5.52 ^{c} ± 0.23	1.79 ^{e} ± 0.16	6.71 ^{b} ± 0.39	1.97 ^{de} ± 0.15	7.98 ^{a} ± 0.16	2.92 ^{d} ± 0.24
Basal cladode mass	g	14.86 ^{bcd} ± 0.73	12.0 ^{d} ± 0.42	17.05 ^{b} ± 0.76	13.11 ^{cd} ± 0.6	20.97 ^{a} ± 0.6	15.15 ^{bc} ± 1.1
Spine mass	g	0.35 ^{bc} ± 0.05	0.17 ^{c} ± 0.03	0.50 ^{b} ± 0.04	0.18 ^{c} ± 0.02	0.76 ^{a} ± 0.05	0.43 ^{b} ± 0.06
Total mass	g	42.45 ^{c} ± 1.5	34.38 ^{d} ± 0.97	49.94 ^{b} ± 2.0	37.27 ^{cd} ± 1.3	59.6 ^{a} ± 1.4	42.2 ^{c} ± 1.6
Spines by area*	g m^{-2}	9.54 ^{b} ± 1.42	4.09 ^{c} ± 0.69	10.93 ^{b} ± 1.13	3.73 ^{c} ± 0.51	17.13 ^{a} ± 1.39	10.81 ^{b} ± 1.75
# Cladodes		4.8 ^{a} ± 0.25	4.4 ^{a} ± 0.33	5.6 ^{a} ± 0.27	3.5 ^{a} ± 0.6	5.2 ^{a} ± 0.33	4.2 ^{a} ± 0.31
Total cladode area**	cm^2	190 ^{a} ± 6.37	22.57 ^{c} ± 10.4	236 ^{a} ± 10.3	11.69 ^{c} ± 5.26	229 ^{a} ± 12.3	124.1 ^{b} ± 22.7
SLA June	cm^2 g^{-1}	8.82 ^{c} ± 0.3	10.81 ^{ab} ± 0.35	9.25 ^{bc} ± 0.28	11.46 ^{a} ± 0.69	7.62 ^{c} ± 0.23	8.98 ^{c} ± 0.35
Root:shoot		0.130 ^{a} ± 0.002	0.053 ^{c} ± 0.005	0.133 ^{a} ± 0.003	0.054 ^{c} ± 0.004	0.134 ^{a} ± 0.002	0.069 ^{b} ± 0.005
Cladode Chl a	mg m^{-2}	145.5 ^{a} ± 4.5	n/a	139.4 ^{a} ± 6.3	n/a	133.1 ^{a} ± 8.5	n/a
Cladode Chl b	mg m^{-2}	41.75 ^{a} ± 2.27	n/a	37.1 ^{ab} ± 4.55	n/a	26.65 ^{b} ± 2.73	n/a
Cladode carotenoids	mg m^{-2}	47.23 ^{a} ± 4.14	n/a	33.84 ^{b} ± 3.13	n/a	28.84 ^{b} ± 2.74	n/a
Cladode Chl a / Chl b		3.62 ^{b} ± 0.198	n/a	4.68 ^{ab} ± 0.55	n/a	5.55 ^{a} ± 0.46	n/a
Cladode total Chl	mg m^{-2}	185.23 ^{a} ± 5.81	n/a	174.76 ^{a} ± 8.47	n/a	159.73 ^{a} ± 9.17	n/a

*Spines by area: mass of spines by one-sided cladode area. **Total cladode area: one-sided area.

Table 4.2: Summary of general linear model statistics for *Opuntia stricta* traits measured at final harvest for control plants and those subjected to herbivory by the biocontrol agent *Dactylopius opuntiae*. Plants were grown at 250 (sCO_2), 400 (aCO_2) and 600 ppm (eCO_2) CO_2 concentrations. Herbivory treatment plants were inoculated with 20 first instar *D. opuntiae* after 70 days of plant growth and final harvest was conducted after a further 126 days (total 196 days). Results in bold type indicate significance ($P < 0.05$).

			F_{stat}: P- value	
***Opuntia stricta* traits**	**Units**	**CO_2**	**Herbivory**	**CO_2 x Herbivory**
Height	mm	$F_{2,93}$= 0.04; P = 0.96	**$F_{1,94}$= 631; P < 0.001**	$F_{5,90}$= 0.08; P = 0.92
Daughter cladodes mass	g	**$F_{2,93}$= 17; P < 0.001**	**$F_{1,94}$= 21; P < 0.001**	**$F_{5,90}$= 3.1; P = 0.049**
Root mass	g	**$F_{2,93}$= 30; P < 0.001**	**$F_{1,94}$= 550; P < 0.001**	**$F_{5,90}$= 4.3; P = 0.016**
Basal cladode mass	g	**$F_{2,93}$= 21; P < 0.001**	**$F_{1,94}$= 50; P < 0.001**	$F_{5,90}$= 2.1; P = 0.13
Spine mass	g	**$F_{2,93}$= 33; P < 0.001**	**$F_{1,94}$= 61; P < 0.001**	$F_{5,90}$= 1.9; P = 0.16
Total mass	g	**$F_{2,93}$= 35; P < 0.001**	**$F_{1,94}$= 108; P < 0.001**	**$F_{5,90}$= 4.8; P = 0.01**
Spines by area	g m^{-2}	**$F_{2,93}$= 21; P < 0.001**	**$F_{1,94}$= 40; P < 0.001**	$F_{5,90}$= 0.25; P = 0.78
# Cladodes		$F_{2,93}$= 0.08; P = 0.92	**$F_{1,94}$= 13; P < 0.001**	$F_{5,90}$= 2.4; P = 0.1
Total cladode area	cm^2	**$F_{2,93}$= 17; P < 0.001**	**$F_{1,94}$= 1258; P < 0.001**	**$F_{5,90}$= 11; P < 0.001**
SLA June	cm^2 g^{-1}	**$F_{2,93}$= 15; P < 0.001**	**$F_{1,94}$= 33; P < 0.001**	$F_{5,90}$= 0.63; P = 0.53
Root / shoot		**$F_{2,93}$= 4.7; P = 0.012**	**$F_{1,94}$= 633; P < 0.001**	$F_{5,90}$= 2.41; P = 0.1
Cladode Chl a	mg m^{-2}	$F_{2,45}$= 0.9; P = 0.43	n/a	n/a
Cladode Chl b	mg m^{-2}	**$F_{2,45}$= 5.4; P = 0.008**	n/a	n/a
Cladode carotenoids	mg m^{-2}	**$F_{2,45}$= 7.8; P = 0.001**	n/a	n/a
Cladode Chl a / Chl b	mg m^{-2}	**$F_{2,45}$= 5; P = 0.01**	n/a	n/a
Cladode total Chl	mg m^{-2}	$F_{2,45}$= 2.6; P = 0.08	n/a	n/a
Cladode carbon (C)	%	**$F_{2,93}$= 3.83; P = 0.025**	**$F_{1,94}$= 16; P < 0.001**	$F_{5,90}$= 1.03; P = 0.36
Cladode nitrogen (N)	%	**$F_{2,93}$= 13; P < 0.001**	**$F_{1,94}$= 21; P < 0.001**	$F_{5,90}$= 2.1; P = 0.13
Cladode C/N		**$F_{2,93}$= 13; P < 0.001**	**$F_{1,94}$= 6.5; P < 0.013**	$F_{5,90}$= 0.67; P = 0.51

Pigment analyses (n/a) were not performed on herbivory treatment plants due to mortality. See methods.

4.5 Discussion

Our results demonstrate that the fitness of the biocontrol agent *Dactylopius opuntiae* is significantly reduced when reared on *O. stricta* grown at eCO_2 (600 ppm) relative to those reared on sCO_2 (250 ppm) and aCO_2 (400 ppm) plants. The lower fitness index (FI) measured here is primarily the result of increased

nymphal mortality (crawler settling) and delayed development (time to female maturity). Conversely, gravid female mass and number of offspring did not differ between the three CO_2 levels, which were similar to other non-eCO_2 *D. opuntiae* studies (conducted at ambient CO_2) (Volchansky et al. 1999; Rule and Hoffmann, 2018). Therefore, despite being equally fecund as the sCO_2 and aCO_2 treatments, the effect of increased nymphal mortality and delayed development time at eCO_2 ultimately translated into slower rates of herbivory damage (detached cladodes) and delayed plant mortality. These results imply that the rise in atmospheric CO_2 observed since the industrial revolution until present day is unlikely to have affected *O. stricta* traits enough to have altered *D. opuntiae* fitness. However, in the next ca. 80 years, the increase in atmospheric CO_2 is likely to produce changes to *O. stricta* traits that will reduce *D. opuntiae* fitness.

Dactylopius opuntiae development time is correlated to both cladode C concentrations and C/N ratios while the FI is correlated to C/N ratios, but neither development time nor FI were correlated to cladode N concentrations. Nitrogen concentrations showed no significant correlation to the herbivory treatment plants due to the extraction of available N through herbivory, resulting in similar final N concentrations at all three CO_2 levels. Moreover, the eCO_2 control plants had significantly less N than the sCO_2 and aCO_2 control plants, indicating a lower initial starting concentration prior to *D. opuntiae* inoculation. Thus, using the control plants as the starting N concentration, herbivory at eCO_2 reduced N by ± 0.04%, as opposed to ± 0.11% and ± 0.06% for sCO_2 and aCO_2 herbivory treatments, respectively. These results further indicate an available (practical) cladode N concentration threshold of ± 0.5% for *D. opuntiae* and below that it is unable to utilise the remaining cladode N. Nitrogen concentrations measured on *O. ficus-indica* plants under eCO_2 ranged between ± 1 to 2.5% (Cui and Nobel 1994; Nobel et al. 1994), compared to ± 0.55% for *O. stricta*, which is probably the effect of species and/or soil fertilisation. If the latter, invaded habitats that contain higher soil nutrients may benefit *D. opuntiae* by increasing cladode N concentration. However, evidence suggests that increasing soil nitrogen under eCO_2 does not always translate into higher leaf N concentrations, and C/N ratios do not alter in favour of herbivores under these conditions (Robinson et al. 2012). Therefore, results here suggest that the N concentration dilution effect (lower

protein) may have contributed to the decline in *D. opuntiae* fitness (Lincoln et al. 1986). Additionally, the significant relationship of plant C concentration to *D. opuntiae* development times infers carbon-based defence compounds may have contributed to reduced plant nutritional quality (Bryant et al. 1983). Cladode C and N concentrations notwithstanding, declines in plant quality, both nutritional and mechanical/physical are almost certainly a result of an interaction of numerous plant traits (Stiling and Cornelissen 2007; Robinson et al. 2012). *Opuntia stricta* cladode hardness increases significantly with eCO_2 (Venter unpubl.) which may influence the proportion of crawlers that are able to settle, (by inserting their delicate stylets through the cladode's cuticle and epidermal layers), thus contributing to increased mortality rates at a vulnerable life stage.

The magnitude of the response of *D. opuntiae* fitness to *O. stricta* grown at elevated CO_2 supports the idea that specialist/monophagous herbivores (such as biocontrol agents) are more susceptible to CO_2 induced changes in their host plants (Stiling and Cornelissen, 2007). This supports Johns and Hughes (2002) and Diaz et al. (2012) who showed strong detrimental effects on their respective biocontrol agents' development when reared on host weeds grown under eCO_2. However, despite reduced *D. opuntiae* fitness and concomitant slower rates of damage (reduced herbivory) to *O. stricta* shown here, the plant's physiology remained unable to compensate for herbivory. The growth advantages gained with each stepwise increase in CO_2 was eventually negated by herbivory whereby total biomass of eCO_2 plants was the same as the aCO_2 herbivory plants (similarly for sCO_2 vs. aCO_2). This response to eCO_2 is in contrast with that found in three terrestrial and four aquatic weeds, where plant growth was not affected by agent herbivory at eCO_2, even when feeding damage increased (Johns and Hughes 2002; Johns et al. 2003; Diaz et al. 2012; Baso et al. 2021). The absence of a significant interaction of CO_2 and herbivory on *O. stricta* cladode C, N and C/N ratios indicates that the plant's resource allocation is primarily influenced by CO_2. Therefore, changes to these resources in response to herbivory are due to *D. opuntiae* feeding and/or effects linked to feeding damage and are not an induced compensatory response by *O. stricta*. This may explain an inherent susceptibility of *O. stricta* physiology to *D. opuntiae* feeding and the plant's inability to mitigate for this, even at reduced agent levels. Unfortunately, due to

experimental limitations we did not test the interactive effect of temperature and CO_2 which may add further complexity to this relationship (Johns and Hughes, 2002; Johns et al. 2003; Reeves et al. 2015). Their research shows both positive and negative effects of combined elevated temperature and CO_2 on biological control agent development and subsequent efficacy.

Due to the variety of functional groups of organisms tested in the literature, it is difficult to draw clear conclusions on the potential changes to *D. opuntiae's* efficacy as a biocontrol agent in response to combined elevated temperature and CO_2. At a plant level, based on *O. stricta's* photosynthetic physiology (CAM photosynthesis), evidence suggests that its productivity is likely to benefit from the combination of rising temperature and CO_2 (Drennan and Nobel, 2000). Similarly, *D. opuntiae's* development time (time to maturity) is accelerated at higher temperatures (± 40 days at ~ 27 °C ± 1 at ambient CO_2 vs. ± 65 days at ~ 23.5 °C at ambient CO_2) (see Githure et al. 1999; Volchansky et al. 1999; Rule and Hoffmann, 2018). Assuming no further decline in *O. stricta* plant quality in response to combined elevated temperature and CO_2 (see Robinson et al. 2012), *D. opuntiae* development rates will likely benefit from higher average temperatures. However, development rates are only one component of fitness, and if survival of crawlers (settling) and number of offspring does not increase at higher temperatures under eCO_2, the net effect may still be a lower fitness (index) relative to aCO_2. Naturally, this warrants further investigation but predicted warmer temperatures may favour *D. opuntiae* disproportionally over the effects of changes to *O. stricta* plant quality shown under eCO_2, particularly in temperate regions where the current biocontrol efficacy is not as good as sub-tropical regions in South Africa (Moran et al. 2021).

4.5.1 Conclusion

The relative decline in *O. stricta* cladode nutritional quality in response to eCO_2 significantly reduced *D. opuntiae* fitness. Longer development times and higher nymphal mortality resulted in slower rates of damage and delayed plant mortality. While the cumulative effects of reduced agent fitness over the long-term may

render *D. opuntiae* less effective with rising atmospheric CO_2, this is somewhat conjectural without the inclusion of other a/biotic interactions. However, the results here indicate that *D. opuntiae* efficacy was diminished at eCO_2, and under future CO_2 scenarios *D. opuntiae* may be less effective as a management tool, which could necessitate additional intervention.

CRediT authorship contribution statement

Nic Venter: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft. **Blair W. Cowie**: Methodology, Investigation, Writing - review & editing. **Marcus J. Byrne**: Methodology, Supervision, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4.6 Supplementary data

Table S4.1: Mean (± SD) [CO_2], temperature, relative humidity and air vapour pressure deficit for each of the three chambers. Data were logged every 5 minutes for the duration of the 6.5-month experiment. Photosynthetic photon flux density (PPFD) was not logged.

Chamber CO_2	CO_2 (ppm)	Temperature (°C)	Relative humidity (%)	VPD (kPa)
250	262.01 ± 41.80	23.529 ± 2.759	65.94 ± 4.95	0.83 ± 0.18
400	398.78 ± 49.31	23.530 ± 2.756	63.85 ± 6.26	0.87 ± 0.20
600	597.26 ± 48.08	23.467 ± 2.688	64.93 ± 4.61	0.85 ± 0.17

CHAPTER FIVE

Opuntia stricta mechanistic climate change distribution model

Preface

Chapter 5 addressed Objective 4 to determine if changes to water use efficiency (*WUE*) potentially moderates or amplifies the distribution range of cactus under elevated CO_2 and future climate change. *WUE* calculated from the physiological measurements conducted on *Opuntia stricta* in Chapter 2 was used to create a mechanistic climate change species distribution model that included *WUE* as a term which was compared to a conventional climate change species distribution model.

WUE data collected on *P. aculeata* from Chapter 3 was not included in this analysis due to *P. aculeata's* reliance on specific vegetations types for invasion.

5.1 Abstract

Species distributions models (SDMs) are frequently used tools to predict the dynamics of invasive weeds under future climate scenarios. Based on these SDMs, invasive weeds are generally predicted to increase their distribution ranges under climate change. However, these models do not include the effect elevated CO_2 concentrations will have on weeds photosynthetic efficiency. With increasing atmospheric CO_2, plant water use efficiency (*WUE*) improves, potentially facilitating establishment into environments where rainfall is deemed unsuitable based on the weed's current physiological responses. Results from Chapter 2 showed *Opuntia stricta* significantly improved *WUE* under elevated CO_2 (600 ppm), and while this is finding helped explain its invasive vigour, extrapolating this response to a landscape level may help determine if improved *WUE* could influence future invasion dynamics. Future climate scenarios predict declines in mean annual rainfall in the west of South Africa, and if *O. stricta* 's future

distribution is modelled based on its current climate requirements, these regions would be deemed unsuitable in the future. However, the effect of elevated CO_2 on *WUE* should ameliorate the effect of lower rainfall, thereby allowing establishment in these environments. To evaluate this, a relatively simple approach was used whereby the Africlim RCP4.5 mean annual precipitation (MAP) raster was converted, whereby cell MAP values were variably adjusted upwards to account for greater *O. stricta WUE.* This converted Africlim RCP4.5 was used to create a mechanistic climate change Maxent SDM (mechanistic SDM) that incorporated the effect of elevated CO_2 on *O. stricta WUE*. Results showed that the *O. stricta* predicted future distribution in South Africa is greater with the inclusion of *WUE* as a variable in the mechanistic SDM, compared to the climate change SDM (future SDM) that did not include *WUE* as a variable. Relative to the future SDM, the mechanistic SDM (habitat suitability score > 0.7) predicts a further 5 000 km^2, mainly in the south-western region of South Africa, that could be at risk of *O. stricta* invasion due to greater *WUE* under elevated CO_2.

Keywords: Climate change, climate envelope, elevated CO_2, environmental niche, habitat suitability, Maxent, mean annual precipitation, species distribution model, water use efficiency

5.2 Introduction

Climate envelope species distribution models (SDMs) are used to predict the probability of suitable habitat/distribution of an organism in a geographic area of interest. Using known observations (location occurrence data) of an organism and associated climatic variables, the model identifies its climatic niche which is used to infer/predict climatically suitable habitats (habitat suitability). Species distribution models can be particularly useful when trying to predict the 'new occurrences' of rare organisms beyond their known location using restricted occurrence data (Williams et al. 2009; Zhang et al. 2020). With the increase in invasive species due to globalisation, SDMs have become a useful tool to predict

invasion risk (Barbet-Massin et al. 2018). SDMs can be used to create a climate envelope from the organism's native range and then projected onto the geographic region that is being invaded. Alternatively, when native range distribution data is scarce (low sampling effort, uncertain native distribution, or taxonomic issues), using location data of an emerging invasive species from the invaded range, SDMs can be used to predict potential range expansion in the invaded geographic space.

Species distribution models are also useful tools when trying to predict changes to species distribution in response to climate change (Franklin et al. 2012; Liu et al. 2020). Similar to the aforementioned modelling techniques, SDMs create a current climate niche for an organism and then project this onto the same geographic space using predicted climate scenarios (e.g., Worldclim RCP4.5 future ensemble models). However, SDMs cannot incorporate the mechanisms that underly biological processes, and hence cannot predict shifts beyond the environmental niche in which the model was developed. In other words, species biological responses to current climatic conditions are not always representative of their future biological responses (Rodriguez et al. 2019). This is notable for autotrophs such as terrestrial plants that respond directly to changes in CO_2 concentration, whereby their physiological response to current CO_2 concentrations is not indicative of how they may respond to projected atmospheric CO_2 concentrations. With rising atmospheric CO_2 plants can improve physiological efficiency, most notably photosynthetic water use efficiency (*WUE*: the ratio of photosynthesis to stomatal conductance) (Keenan et al. 2013; Dusenge et al. 2018). At higher CO_2 concentrations, plants can reduce stomatal conductance thereby decreasing water loss via transpiration and still maintain equal or higher intercellular CO_2 concentrations and concomitant photosynthetic rates (Keenan et al. 2013; Dusenge et al. 2018). Essentially, plants can achieve higher productivity while using the same amount of water, or alternatively achieve similar productivity using less water, or somewhere in between. This has been shown in warm arid regions where rainfall is one of the factors constraining plant productivity as opposed to higher rainfall regions where other resources may limit productivity (e.g., nutrients, light, temperature) (Donohue et al. 2013; Bradie and Leung, 2016). Observed greening over the past few decades in warm arid regions

globally was concluded to be a consequence of improved *WUE* in response to rising atmospheric CO_2 (Donoghue et al. 2013). Consequently, modelling future distributions of plants using current biological responses is likely to underestimate subtle changes to their distribution, particularly in warm arid regions, unless they include increased *WUE* in response to rising atmospheric CO_2.

Climate change scenarios generally predict changes to rainfall patterns and mean annual precipitation (MAP) (IPPC, 2022). This suggests that using SDMs that model future distributions on current climate envelopes would likely show a decrease in habitat suitability for the regions where rainfall may fall below the lower MAP threshold for a plant. Naturally the effect size of this will depend on the importance (contribution) of MAP in explaining a plant's realised niche. Thus, if MAP is a significant contributor to the plant's distribution, including *WUE* as a mechanism in the SDM, reductions in suitable habitats may be less significant with declining MAP due to the mitigating effect of improved *WUE*. Of the literature examined on plant SDMs for future climate scenarios, no studies have included changes to *WUE* as a predictor variable. Several authors have discussed the implications of increased *WUE* in response to increasing CO_2 when predicting future distributions of plants, however, this does not appear to have been explored further (Booth et al. 2013; Mosocha and Dube, 2018). Therefore, there is a need to include changes to *WUE* into SDMs to improve model predictions of plants distributions with climate change.

Elevated CO_2 and climate change are predicted to favour invasive weeds, potentially increasing their spread, impact on ecosystems and livelihoods (Reeves, 2017; Sun et al. 2020; Clements and Jones, 2021). This prediction is supported by numerous SDM studies that have shown projected increases in invasive weed distributions with climate change (Sheppard, 2013; Sheppard et al. 2014; Thapa et al. 2018; Fang et al. 2021; Shabani et al. 2020; Kriticos et al. 2010; Clements and Jones, 2021), however, none of these studies have included the potential effects of changes to *WUE* on the outcomes. This is relevant because elevated CO_2 studies on invasive weeds have concluded that greater productivity is largely attributed to improved *WUE* (Ziska et al. 2019). Thus, there appears to be a disconnect between studies that conduct SDMs on weeds and the observed direct effects of elevated CO_2 on their physiological efficiency, which could alter their realised

habitat niche. Therefore, more accurate predictions to their distributions may show escalating invasions which has implications for management strategies that based on the status quo.

Opuntia stricta WUE was ~ 37% higher when grown at 600 ppm relative to 400 ppm (Chapter 2), which theoretically should broaden its realised habitat niche in the future, allowing it to persist in regions that may experience declines in MAP. Within South Africa, rainfall is predicted to increase in the central interior and along the east coast, whereas the opposite is predicted for western interior, northeastern parts, and the winter rainfall region of the southwestern Cape (Engelbrecht, 2019). Therefore, the aim of this chapter is to determine if elevated CO_2 induced changes in *O. stricta WUE* are incorporated as an additional predictor variable in a climate change SDM, will this act to this broaden the weeds predicted distribution relative to a SDM that does not include *WUE* as a predictor variable. The objective of this study was not to produce a model that predicts the future distribution of *O. stricta* per se, but rather to test if the inclusion of a biological mechanism would influence the outcome of such a climate change SDM. If in regions where future MAP is predicted to be at the lower limit of *O. stricta's* realised rainfall requirements, will improved *WUE* allow it to persist or expand its range relative to the climate change SDM based on its current biological response?

5.3 Methods

5.3.1 Species distribution model assumptions

Species distribution models rely on assumptions which are sometimes violated when modelling invasive species (Gallien et al. 2012; Barbet-Massin et al. 2018). Species distribution models rely on the assumption that an organism is at equilibrium with its environment (it has reached all suitable habitats), however unoccupied areas may not be unfavourable habitats but rather that the invasive organism has not had sufficient time to arrive there (dispersal limitations) (Mainali et al. 2015; Hattab et al. 2017). To try meet this assumption, using

distribution data from the organism's native range to model its climate envelope which can be projected onto the introduced geographic space. However due to climate niche shifts, a phenomenon that can occur when an organism is exposed to a new environment, invasive species can occupy climatic niches that differ to their native range (Early and Sax, 2014), the mechanisms of which are not clearly understood (Bates and Bertelsmeier, 2021). Additionally, species within their native range encounter different biotic interactions which cannot easily be accounted for in SDMs (Vaclavik and Meentemeyer, 2012; Mainali et al. 2015). Thus, consideration needs to be given to the invasive organism in question when constructing SDMs that attempt to predict changes to their distribution under future climate change scenarios.

Opuntia stricta's date of introduction into South Africa is not cited in the literature but it has undoubtably been present in South Africa for at least a century (*O. ficus-indica* was introduced in the 18th century) (Zimmermann and Moran, 1991). Thus, being established in South Africa for an extended period and having an extensive distribution, it is expected to be near to equilibrium with its environment, thereby accounting for dispersal limitations and climatic niche shifts. Moreover, given the success of its biological control agents, *Dactylopius opuntiae* and to a lesser extent, *Cactoblastis cactorum*, it is expected that the agents have had sufficient time to reach biotic equilibrium with *O. stricta* (Moran et al. 2020). Additionally, unlike *O. ficus-indica* which has been extensively translocated for agricultural purposes, *O. stricta* has little to no agricultural significance and non-horticultural dispersal should largely be non-anthropogenic (Paterson et al. 2011a). Therefore, its widespread distribution in South Africa is assumed to be largely representative of its realised environmental niche and using occurrence data from South Africa should be adequate to meet the assumptions of an SDM.

5.3.2 *Opuntia stricta* occurrence records in South Africa

Opuntia stricta has a widespread distribution within South Africa (Fig. 5.1). Presence only occurrence data (decimal degree GPS coordinate data) was collated

from different sources, which includes personal observations, South African Plant Invaders Atlas (SAPIA), Global Biodiversity Information Facility (GBIF) (https://www.gbif.org/) and iNaturlist (https://www.inaturalist.org/). Where possible, iNaturlist images were vetted personally to ensure correct identification. Occurrence records date from the 1990s to present (16th January 2023). Duplicate records were removed which resulted in 304 *O. stricta* presence records.

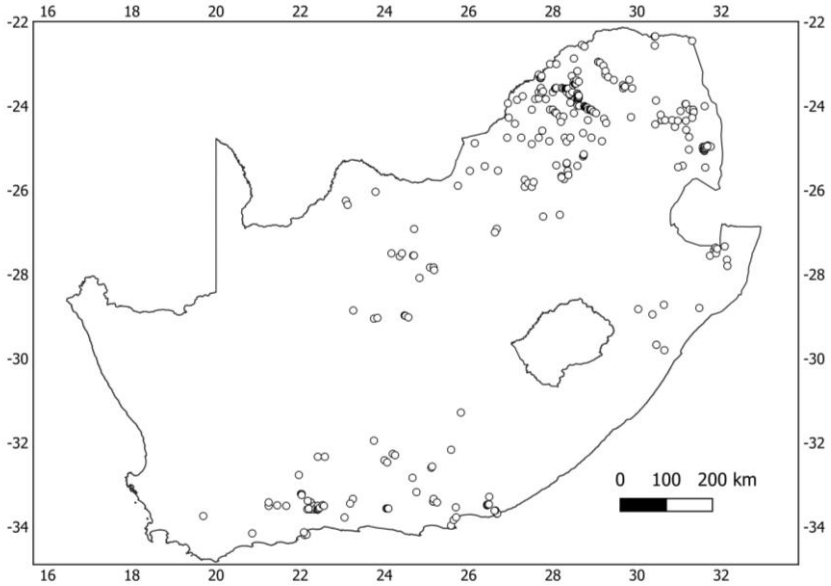

Figure 5.1: *Opuntia stricta* occurrence records (○) for South Africa based on various sources including personal observations, South African Plant Invaders Atlas (SAPIA), Global Biodiversity Information Facility (GBIF) and iNaturalist (n = 304 records). Values on the xy axes denote decimal degrees of longitude and latitude respectively.

5.3.3 Environmental predictors

Africlim annual and seasonal environmental predictor climate data were downloaded from https://www.york.ac.uk/environment/research/kite/resources/ which included 21 high resolution (30 second = ~ 1 km^2) baseline climate and projected climate RCP4.5 mid-century ensemble models. Baseline models are averaged for the period 1961–1990 and mid-century RCP4.5 predictions for the period 2041-2070. All rasters have the same spatial resolution and use the WGS84 geographic coordinate system.

Table 5.1: Summary of Africlim climatic variables (from Platts et al. 2015).

Code	Description	Abbrev.	Units
BIO1	Mean annual temperature	MAT	°C
BIO2	Mean diurnal range in temp	MDRT	°C
BIO3	Isothermality	ISO	°C
BIO4	Temperature seasonality	TSEAS	°C
BIO5	Max temp warmest month	MTWM	°C
BIO6	Min temp coolest month	MTCM	°C
BIO7	Annual temperature range	ATR	°C
BIO10	Mean temp warmest quarter	MTWQ	°C
BIO11	Mean temp coolest quarter	MTCQ	°C
PET	Potential evapotranspiration	PET	mm
BIO12	Mean annual rainfall	MAP	mm
BIO13	Rainfall wettest month	RWM	mm
BIO14	Rainfall driest month	RDM	mm
BIO15	Rainfall seasonality	RSEAS	mm
BIO16	Rainfall wettest quarter	RWQ	mm
BIO17	Rainfall driest quarter	RDQ	mm
MI	Annual moisture index	MI	-
MIMQ	Moisture index moist quarter	MIMQ	-
MIAQ	Moisture index arid quarter	MIAQ	-
DM	Number of dry months	DM	Months
LLDS	Length of longest dry season	LLDS	Months

5.3.4 Climate variable pre-selection

All analyses were conducted using RStudio (R Core Development Team, 2022) (ver. 4.2.2), unless otherwise specified. Multicollinearity analysis using the '*usdm*' package was conducted on the 21 Africlim variables with the accompanying *O. stricta* location data to reduce the number of variables that showed collinearity. Variance inflation factor (VIF) analyses using the 'vifstep' and 'vifcor' functions were performed to provide an objective means of reducing the number of predictor variables. However, climate variable selection did not rely solely on VIF analysis, instead climate variables that could be biologically

important for *O. stricta* distribution were also considered (Merow et al. 2013). *Opuntia stricta* grows predominantly in the summer rainfall regions and, therefore, seasonality variables were selected for the model to account for their distribution. Thus, based on VIF analysis and these biological considerations, the four climate variables selected to conduct the SDMs included mean annual temperature (BIO1 - MAT), temperature seasonality (BIO4 - TSEAS), mean annual precipitation (BIO12 - MAP) and rainfall seasonality (BIO15 – RSEAS).

5.3.5 Current SDM and projected future SDM (non-mechanistic)

Maxent (maximum entropy) tends to outperform other presence-only correlative SDMs such as random forest, boosted regression trees etc. (Valavi et al. 2022), particularly when dealing with complex interactions between responses and predictor variables, and smaller sample sizes (Fourcade et al. 2014), and thus Maxent was selected for this study. Additionally, as stated, the objective of the study was not to produce a model that predicts the future distribution of *O. stricta* per se, but rather to test if the inclusion of a biological mechanism could influence the outcome of the models.

Maxent was implemented using the '*dismo*' (ver. 1.3-9), '*sp*' (ver. 1.6-0) and '*SDMtune*' packages (ver. 1.2.0). The Maxent default of 10 000 background points or pseudo-absences (Barbet-Massin et al. 2012) were randomly generated and 20% of the location data was withheld for testing. The Maxent model was trained using the default 500 iterations. The importance of each Africlim environmental variable used to train the Maxent model were ranked according to percent contribution and permutation importance. Authors argue as to which is the best variable ranking classification, percent contribution or permutation importance, however, in most studies, permutation importance is suggested to be the most reliable variable importance ranking metric (Searcy et al. 2016). The trained Maxent model (climatic envelope) was then used to predict the current habitat suitability ('current SDM') of *O. stricta* using the using the 'cloglog' output (Phillips et al. 2017) and exported as a GeoTIFF format raster where each pixel (~ km^2) represents a predicted habitat suitability score (scale: 0 – 1). The

climatic envelope produced by the maxent model trained using current climatic variables (BIO1, BIO4, BIO12 and BIO15) was then projected onto Africlim ensemble RCP4.5 variables (BIO1, BIO4, BIO12 and BIO15) ('future SDM') and exported as a GeoTIFF format raster where each pixel (~ km^2) represents a predicted habitat suitability score (scale: 0 – 1). Model GeoTIFF's were imported into QGIS ver. 3.28.3 (QGIS Development Team, 2022) to create the maps.

5.3.6 Model evaluation

Model evaluation was conducted on the current SDM to assess how well the four Africlim baseline variables performed in producing a climate envelope based on *O. stricta* occurrence data to predict spatial patterns of *O. stricta* habitat suitability. Model evaluation was conducted using the True Skills Statistic (TSS) and Area Under the Curve (AUC). TSS is measure of agreement between the distribution data (observed) and modelled distribution. Scores ≤ 0 indicate randomness while 1 indicates perfect agreement. Additionally, the TSS model score and testing score should be similar to show good model validation (Allouche et al. 2006). AUC is a measure of model performance and predictive accuracy but should be evaluated using two metrics. Firstly, AUC training scores of approximately 0.5 indicates that model performance is equal to a random prediction, > 0.8 indicates good predictive performance and 1 indicates a perfect model. However, to assume high trainings scores, the training and testing scores should be similar which indicates good discrimination between known presence (occurrence data) and background data points (pseudo-absences). Therefore, a high training score needs to be accompanied by an almost equally high testing score. (Jiménez-Valverde, 2012; Komac et al. 2016).

5.3.7 Water use efficiency mechanistic SDM

Chapter 2 investigated the effects of different growth CO_2 concentrations (sub-ambient, ambient, and elevated CO_2) and watering treatments (soil moisture) on

O. stricta growth and productivity. While total biomass did not significantly differ between ambient (400 ppm) and elevated (600 ppm) CO_2 treatments, plants grown at 600 ppm did show significantly improved night-time (phase I) *WUE*. *WUE* was ~ 37% higher for *O. stricta* plants grown at 600 ppm relative to 400 ppm plants for both watering treatments (200 ml wk^{-1} and 400 ml wk^{-1}). However, the significantly higher stomatal conductance required to facilitate C_3 photosynthesis as demonstrated in the late afternoon (daytime) resulted in lower *WUE* relative to night-time *WUE*. This likely reduced the overall *WUE* of well-watered (optimal water) plants (400 ml wk^{-1}: ~ 624 mm MAP) grown at 600 ppm. Nevertheless, studies conducted on other CAM photosynthetic species such as *O. ficus-indica*, *Agave deserti* and *Ananas comosus* under well-watered elevated CO_2 conditions indicate improved diel *WUE* of 40%, 52% and 27%, respectively, relative to ambient CO_2 (Cui et al. 1993; Graham and Nobel, 1996; Zhu et al. 1999). Conversely, *O. stricta* plants grown at 600 ppm and subjected to the suboptimal 200 ml wk^{-1} (~ 312 mm MAP) treatment demonstrated daytime C_3 photosynthesis, and while these photosynthetic rates were lower than the optimal watering treatment, stomatal conductance values were similar to 250 and 400 ppm plants that showed no daytime C_3 photosynthesis (only respiration). Thus, the 200 ml wk^{-1} plants overall *WUE* was likely to be representative of their night-time *WUE*. Using these data and considering important ecological assumptions and uncertainties, a mechanistic model was developed based on the following principles:

1) For regions that experience higher/optimal MAP, alternative resource limitations become more important for plant establishment than water, so improvements in *WUE* are unlikely to significantly alter the habitat suitability under future climate scenarios (Donohue et al. 2013; Bradie and Leung, 2016; Hatfield and Dold, 2019). Therefore, improved *WUE* at elevated CO_2 for well-watered (optimal) *O. stricta* plants will have low contribution to the mechanistic model at higher rainfall regions and uncertainties regarding the change to diel *WUE* because of daytime C_3 photosynthesis becomes less important.
2) For regions that experience lower/suboptimal MAP, water becomes the dominant limiting factor for plant establishment and small changes in

WUE are likely to have a significant effect on their habitat suitability under future climate scenarios (Donohue et al. 2013; Bradie and Leung, 2016; Hatfield and Dold, 2019). Therefore, improved *WUE* of *O. stricta* at elevated CO_2 will have greater contribution to the mechanistic model at lower rainfall regions as these represent the lower MAP threshold which limits their establishment.

3) Climate change is predicted to alter rainfall frequency and intensity which will affect soil aridity and run-off. It is suggested that that increased *WUE* from rising atmospheric CO_2 will mitigate increased evaporative demand, but some researchers argue that climate change will dominate over the effect of *WUE* on soil evaporation (Dai et al. 2018). Therefore, the mechanistic model was adapted accordingly to account for these uncertainties.

Mid to late-century (2041 – 2070) RCP4.5 climate change predictions should coincide with predicted atmospheric CO_2 concentrations of between 500 to 600 ppm (IPCC, 2022), therefore, the increase in *WUE* for *O. stricta* measured at 600 ppm (Chapter 2) was presumed to reflect its future physiological response and subsequent climatic niche for this period. The current lower MAP threshold for *O. stricta* is ~ 200 mm per annum and optimal rainfall is ~ 500 to 600 mm per annum with the frequency of *O. stricta* occurrence declining thereafter, indicating MAP is no longer the primary factor that governs its distribution (Fig. 5.2). Thus, in regions where the future predicted MAP falls slightly below *O. stricta's* current threshold of ~ 200 mm per annum, shifts in its future climatic niche should allow establishment due to improved *WUE* via CO_2 fertilisation. Because the future SDM model is based on *O. stricta's* current climate envelope whereby the lower MAP threshold for occurrence is ~ 200 ml per annum, but improved *WUE* assumes it could establish in habitats that have lower MAP in the future, upward adjusting the future MAP values should account for changes to *WUE*. Based on this principle, increasing the lower *O. stricta* threshold of the MAP raster variables should account for shifts in its climatic niche. Therefore, by increasing the MAP cell values on the BIO12 RCP4.5 raster, this acts as the mechanism to incorporate improved *O. stricta WUE* under eCO_2. To achieve this, a variable conversion factor (exponential distribution function) was applied to each cell

value of the RCP4.5 BIO12 raster. A conservative variable conversion factor was applied to account for uncertainties of climate change (soil aridity, runoff etc.) that could not be included in the model as a predictor variable. Additionally, other potential interactive effects of future climate, such as elevated temperatures on *O. stricta* physiology and subsequent *WUE* that could not be included in Chapter 4 experiments were also considered. Therefore, for example, the exponential distribution function applied a conversion of 1.173 for a MAP of 170 mm, (below *O. stricta's* current threshold but assumed to be within its future threshold due to improved *WUE*), which increased RCP4.5 BIO12 170 mm MAP cell values to 199.42 mm (equivalent to a 17.3% increase). Thus, regions that experience 170 mm per annum in the future will be suitable habits for *O. stricta* due to improved *WUE* with elevated CO_2 (Fig. 5.3). Because improved *WUE* is likely to be less important for *O. stricta's* distribution at higher MAP, the conversion factor for MAP was set at 1.0239 for 500 mm MAP (new 512), 1.013 for 600 mm MAP (new 607.9), 1.0072 for 700 mm MAP (new 705), 1.0034 for 800 mm MAP (new 803.2), 1.0022 for 900 MAP (new 902) and 1.0012 for 1000 mm MAP (new 1001.2) (Fig. 5.3). Because water is assumed to no longer be the main limitation to *O. stricta* establishment at higher MAP values, increasing MAP in the BIO12 raster above their optimal of ~ 500 mm MAP will have a negative impact on the climate change SDM. This concept of increasing *O. stricta's* lower MAP threshold for the BIO12 RCP4.5 raster is illustrated in Figure 5.4. Figure 5.4A shows the regions in blue where *O. stricta* does not establish based on its current lower MAP threshold of < 200 mm per annum (baseline BIO12), whereas Figure 5.4B shows an increase (shift to the east) in the regions for the same threshold of < 200 mm per annum under future MAP predictions (BIO12 RCP4.5). This increase in the regions that fall below the *O. stricta* MAP threshold is due to predicted declines in MAP for BIO12 RCP4.5. Figure 5.4C shows that if the *O. stricta* lower MAP threshold is lowered from ~ 200 mm to ~ 170 mm for the RCP4.5 BIO12 raster to account for improved *WUE*, the regions that fall below the threshold decrease (shift to the west). This results in a similar expanse of MAP suitable establishment regions for *O. stricta* compared to current MAP regions.

Using the same methods used in Section 5.3.5, the climate envelope produced by the Maxent model trained using current climatic variables (BIO1, BIO4, BIO12 and BIO15) was projected onto Africlim ensemble RCP4.5 variables BIO1, BIO4 and BIO15, but BIO12 was substituted with the converted BIO12 raster to produce the future mechanistic model ('mechanistic SDM). The mechanistic SDM was exported as a GeoTIFF format raster where each pixel (~ km^2) represents a predicted habitat suitability score (scale: 0 – 1).

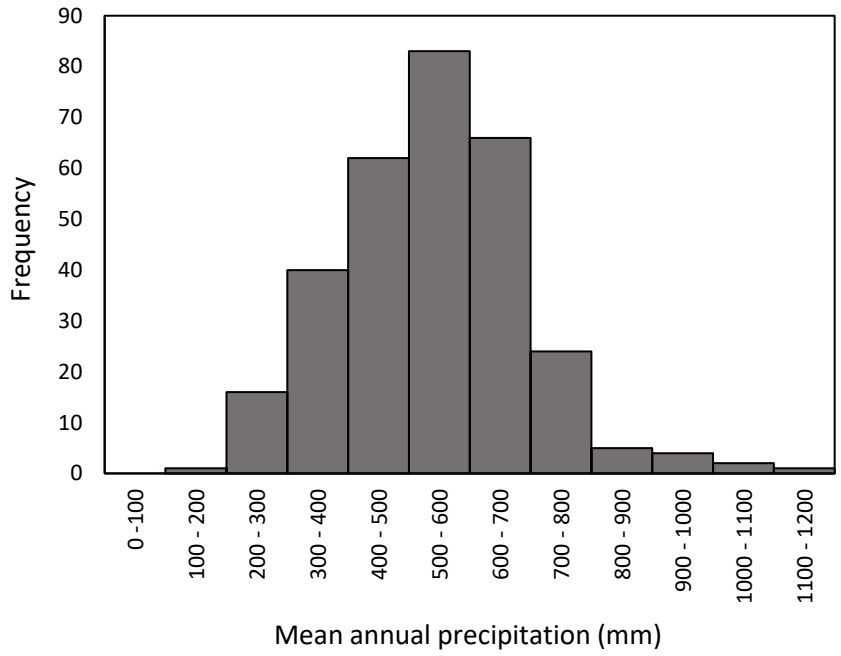

Figure 5.2: Frequency of *Opuntia stricta* occurrence records (n = 304) from South Africa grouped by rainfall (MAP) using Africlim BIO12 (MAP) baseline raster.

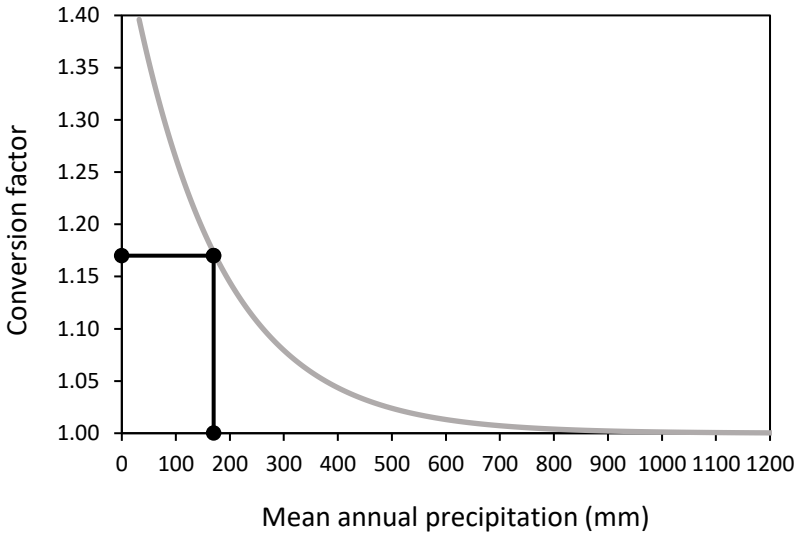

Figure 5.3: The variable conversion factor applied to the mean annual precipitation (MAP) of the RCP4.5 BIO12 raster using an exponential distribution function. E.g., the vertical line that intercepts the x-axis at 170 mm per annum corresponds to a conversion factor of 1.17 on the y-axis which equals ~ 200 mm per annum.

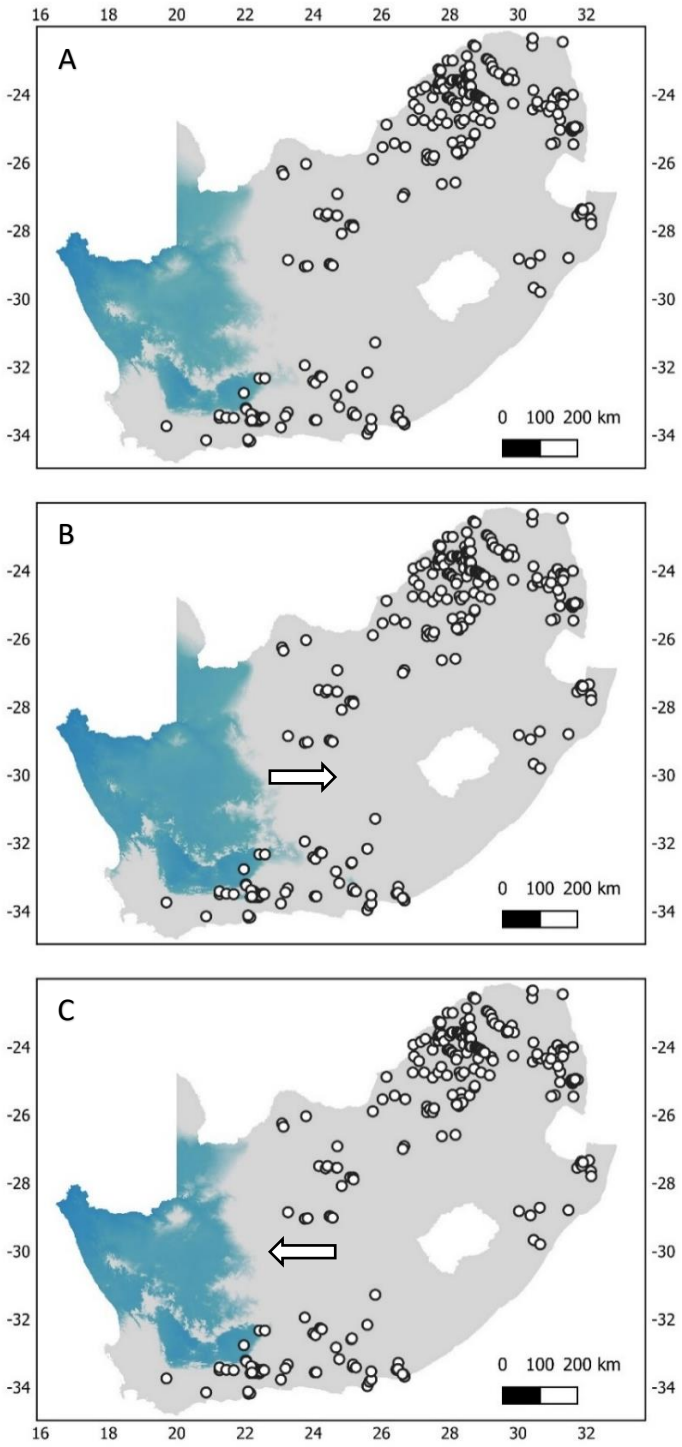

Figure 5.4: Blue regions indicate where MAP in South Africa is A) < 200 mm per annum using the Africlim BIO12 MAP baseline raster, and is assumed to be limiting to *Opuntia stricta* establishment, B) predicted to be < 200 mm per annum using the Africlim RCP4.5 BIO12 MAP raster indicating the regions unsuitable for *O. stricta* establishment will increase and C) < 170 mm per annum using the Africlim RCP4.5 BIO12 MAP raster to account for improved water use efficiency by *O. stricta* under elevated CO_2 predicting regions unsuitable for establishment to reduce relative to B (see Methods 5.3.7). The white arrows indicate the direction in which the blue regions expand or contract. Circles represent *O. stricta* distribution and values on the xy axes represent decimal degrees of longitude and latitude, respectively.

5.3.8 Model comparisons

To visually evaluate the spatial differences in predicted habitat suitability between the three models (current SDM, future SDM and mechanistic SDM), the current SDM map was deducted from the future SDM map, and the future SDM map was deducted from the mechanistic SDM map using the raster calculator function in QGIS. The '*cell stats'* function in the '*raster'* package in RStudio was used to extract the total number of raster cells (~ km^2) containing the following habitat suitability scores: > 0.5, > 0.6, > 0.7> 0.8 and > 0.9 for either model.

5.4 Results

5.4.1 Climate variable selection

Mean temperature (BIO1) and temperature seasonality (BIO4) VIF scores were < 2 which showed low collinearity with the four variables (Table 2). Mean annual precipitation (BIO12) and precipitation seasonality (BIO 15) had VIF scores of 7.54 and 5.31, respectively, indicating higher collinearity. However, the Maxent algorithm is robust to issues of predictor collinearity and can account for these correlated variables in the model (Feng et al. 2019).

5.4.2 Environmental variable importance

Percent contribution and permutation importance was highest for MAT. MAP was ranked second by permutation importance and third by percent contribution (Table 5.2). This indicates that temperature is an important climatic variable that describes *O. stricta* distribution in South Africa, followed by MAP and TSEAS.

Table 5.2: Maxent variable importance ranking of the Africlim climatic variables used to train the current model. MAT (BIO1), MAP (BIO12), TSEAS(BIO4) and PSEAS (BIO15).

Africlim variable	Percent contribution (%)	Permutation importance (%)
MAT	37.88	42.18
MAP	22.57	26.48
TSEAS	33.74	19.09
PSEAS	5.81	12.25

5.4.3 Model evaluation

The TSS score for the Maxent model was 0.636 indicating a good model and model validation score of 0.562 indicating good model fit (Allouche et al. 2006). The AUC training score was 0.885 and the testing score was 0.839 indicating a good predictive model. These scores show that the model trained using the four selected Africlim variables based on the presence only *O. stricta* location data, fitted a relatively accurate habitat suitability model of its current distribution. Therefore, the current SDM is suitable to use for the future SDM and the mechanistic SDM.

5.4.4 Model extrapolation – current, future and mechanistic SDMs

The current SDM (Fig. 5.5 A) shows the highest habitat suitability for *O. stricta* is mainly located -26 S, 26 E (north-east of South Africa) and below -32S and between 20E to 28E (Eastern Cape and southern Cape region), which is largely representative of its current distribution (Fig. 5.1). The future SDM (Figs. 5.5 B & 5.6 B) shows a general increase in habitat suitability in the north-east and northern central region but a decline in habitat suitability in the Eastern Cape and southern Cape. The mechanistic SDM shows similar habitat suitability to the future model, however, the declines in habitat suitability are less apparent in the western half of South Africa. Deducting the future SDM from the mechanistic SDM shows regions in the Eastern Cape and southern Cape where habitat suitability is predicted to improve by up to 30% with the inclusion of *WUE* in the model (Fig. 5.6 C).

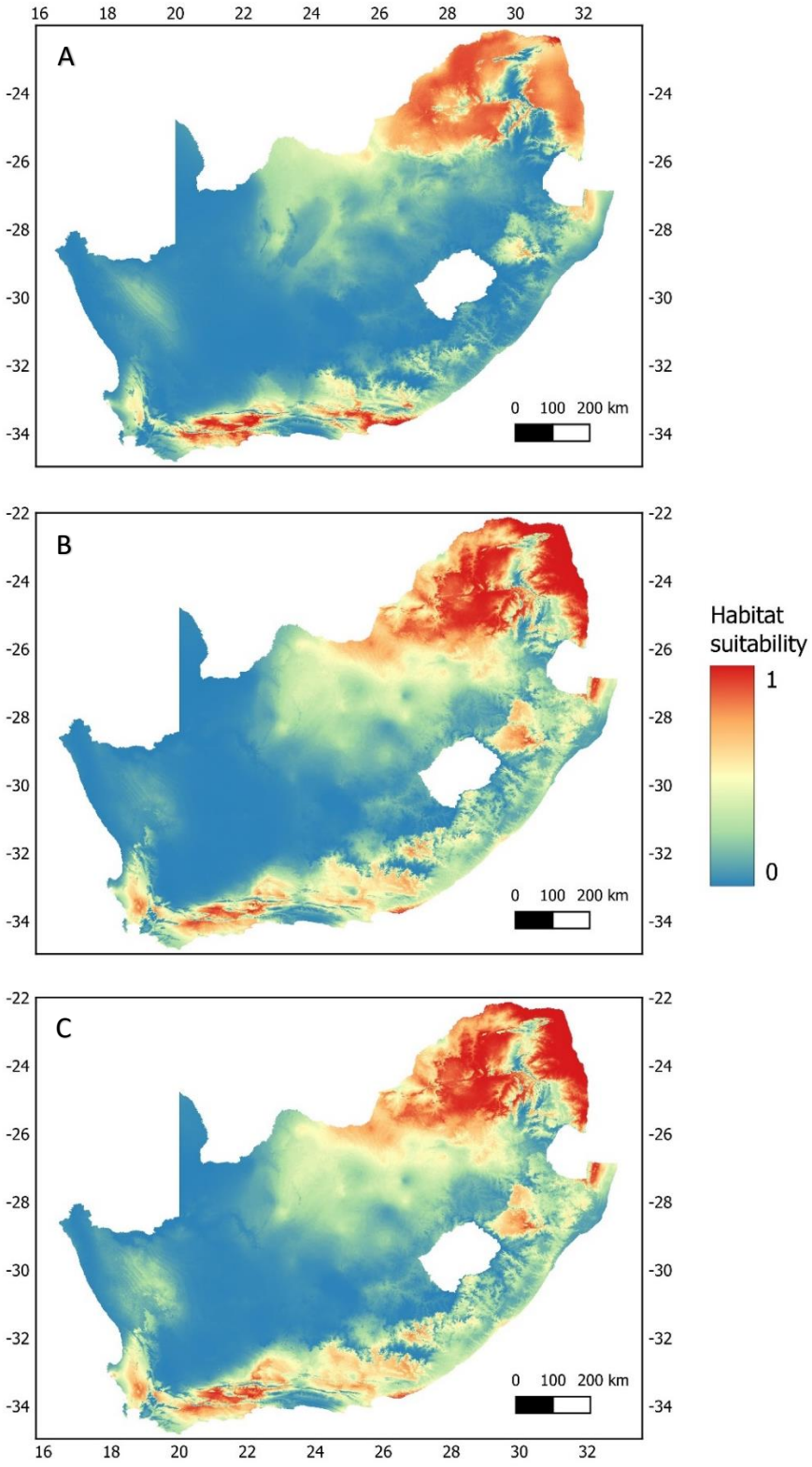

Figure 5.5: Maxent predicted habitat suitability for *Opuntia stricta* in South Africa for A) current SDM, b) future SDM and c) mechanistic SDM. High habitat suitability scores are expressed by warmer colours (red), and low habitat suitability scores are expressed by cooler colours (blue). Values on the xy axes denote decimal degrees of longitude and latitude, respectively.

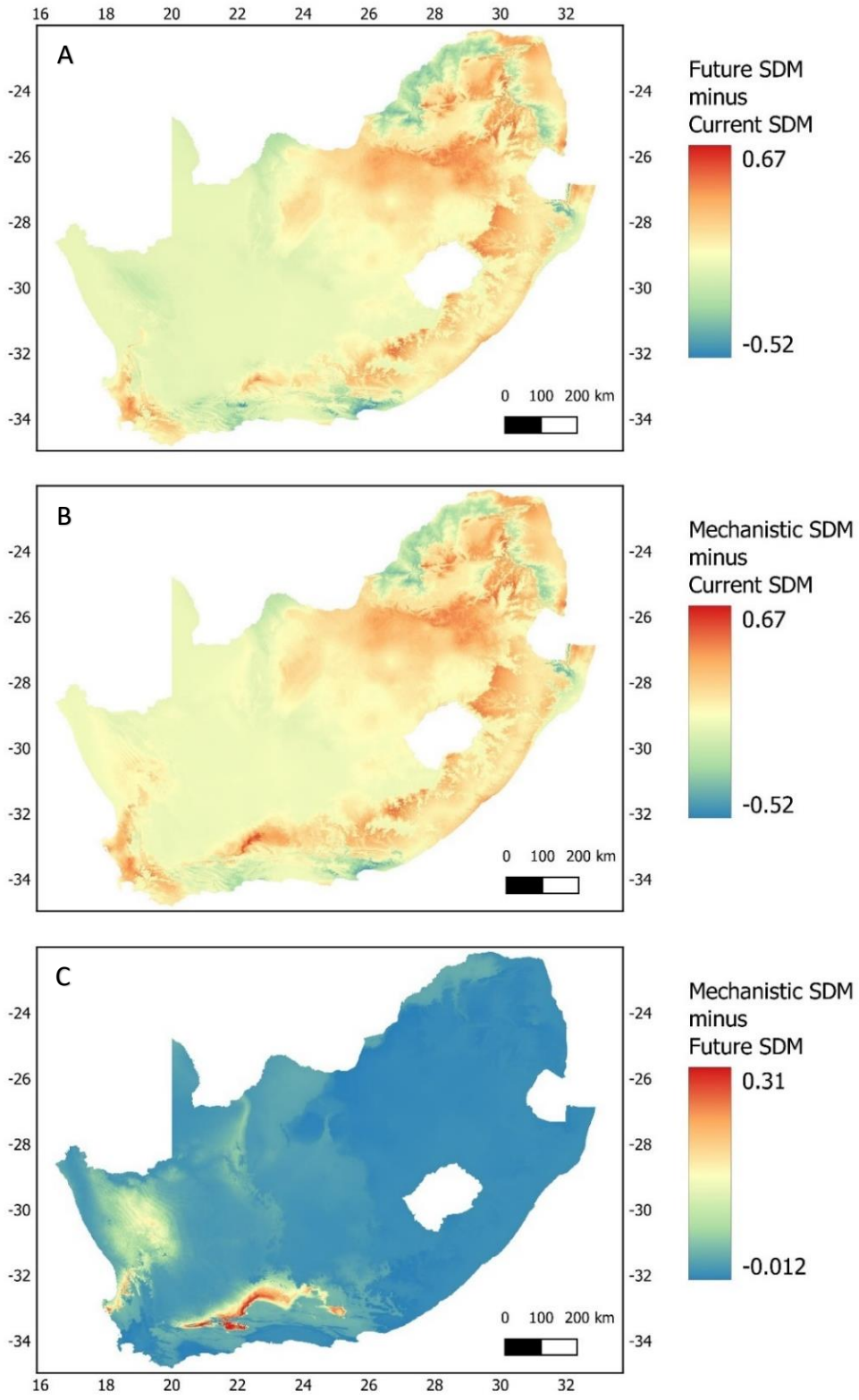

Figure 5.6: Predicted shifts in habitat suitability for *Opuntia stricta* in South Africa for the A) future SDM minus the current SDM, B) mechanistic SDM minus the Current SDM and C) mechanistic SDM minus the future SDM. Warmer colours (red) indicate regions where habitat suitability probability will increase whereas cooler colours (blue) indicate where the habitat suitability probability will decrease or show no change. Values on the xy axes denote decimal degrees of longitude and latitude, respectively.

Both the future SDM and the mechanistic SDM showed an increase in the area (km^2) of predicted suitable habitat for *O. stricta* in South Africa under the RCP4.5 climate change scenario. However, the increase in area was greater for the mechanistic SDM which was most notable for habitat suitability probabalities scores ranging between 0.5 to 0.8 (Figs. 5.7 & 5.8). Relative to the future SDM, the mechanistic SDM predicted further increases in the area of suitable habitat of 3.63% (habitat suitability > 0.5), 4.01% (habitat suitability > 0.6), 2.55% (habitat suitability > 0.7), 2.13% (habitat suitability > 0.8) and 0.39% (habitat suitability > 0.9) (Figs. 5.7 & 5.8).

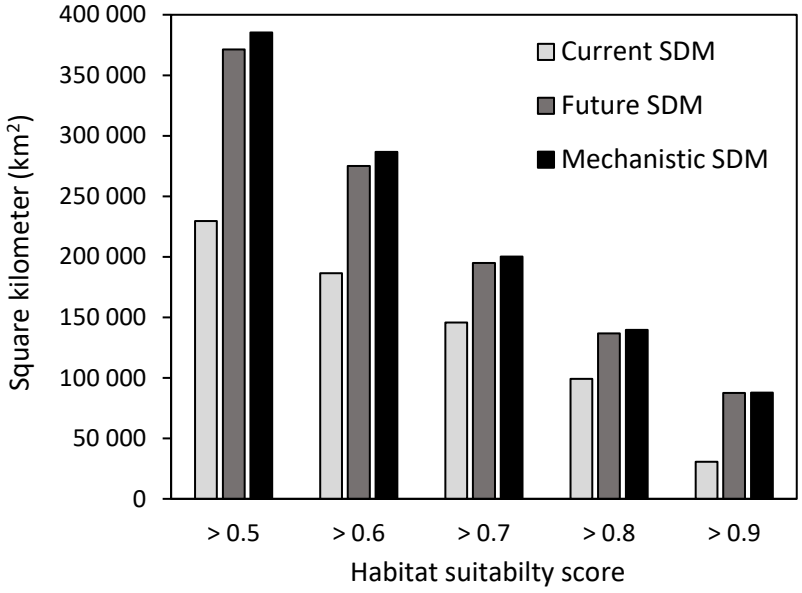

Figure 5.7: Predicted total area (~ km^2) with increasing habitat suitability for *Opuntia stricta* in South Africa for the three models: current SDM, future SDM and the mechanistic SDM.

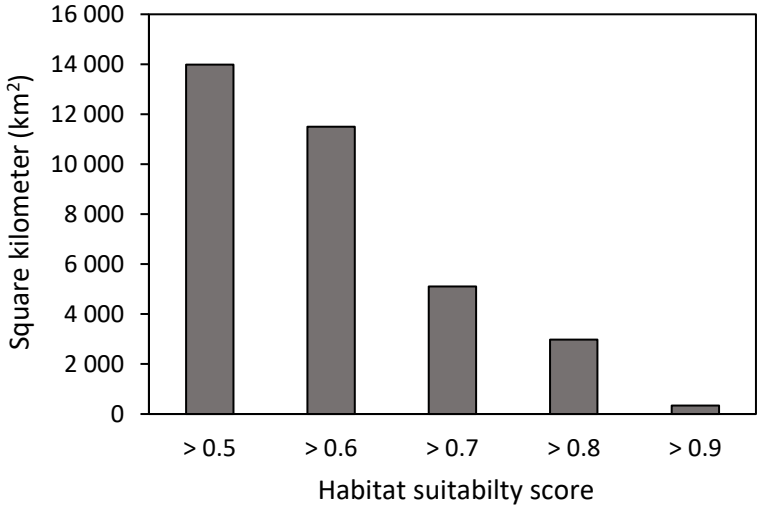

Figure 5.8: The difference in habitat suitability between the mechanistic SDM and the future SDM, indicating the increase in the area (~ km^2) predicted to be suitable for *Opuntia stricta* in South Africa.

5.5 Discussion

Using *O. stricta's* current climate envelope to project into the future (AD 2041 to 2070), the non-mechanistic future SDM (future SDM) does not have sufficient information to accommodate changes to plants physiological efficiency that is expected to take effect at higher atmospheric CO_2 concentrations. Thus, the climate change mechanistic SDM (mechanistic SDM) developed here demonstrated that the inclusion of greater *O. stricta WUE*, as shown in Chapter 2, resulted in a greater predicted distribution relative to future SDM. The mechanistic SDM was based on the assumption that improved *WUE* would be more important in regions where rainfall limits *O. stricta* productivity (Donohue et al. 2013: Hatfield and Dold, 2019). For regions where MAP is predicted to fall below *O. stricta's* lower MAP threshold (~ 200 mm per annum), the future SDM using *O. stricta's* current climate envelope showed reduced habitat suitability, however, the inclusion of improved *WUE* as a variable in the mechanistic SDM partially ameliorated this affect. Despite using a conservative variable conversion factor to adjust for *O. stricta WUE* (e.g., ~ 17.3% at 170 mm per annum = 200 mm) in the mechanistic SDM, there was a notable increase in predicted habitat

suitability. For *O. stricta* habitat suitability probabilities > 0.7, the future SDM predicted a total area encompassing ~ 195 000 km^2 in South Africa, whereas for the same probability, the mechanistic SDM predicted ~ 200 000 km^2. However, for habitat suitability probabilities > 0.9, the difference between models was negligible. This is expected because improved *WUE* is less likely to be the main factor that drives establishment in regions that experience optimal rainfall (~ 500 mm per annum), and other factors (e.g., light, nutrients, temperatures etc.) are likely to limit establishment (Hatfield and Dold, 2019), which are accounted for in mechanistic SDM. Furthermore, the mechanistic SDM did not account for the effect of ambient growth temperature on *WUE* (temperature was not included as a variable in the Chapter 2 experiments). However, based on Zhu et al. (1999) and other studies on different photosynthetic types, marginal changes to *WUE* require large differences in temperature at both ambient and elevated CO_2 (Eamus, 1991; Hatfield and Dold, 2019), and therefore the influence of temperature should not significantly alter the mechanistic SDM output presented here.

While non-correlative mechanistic SDMs are widely used in the literature, they require detailed prior knowledge of the organism's biology, life-history traits, environmental thresholds, explicit environmental predictors and cannot be implemented using standard SDMs such as Maxent, Random Forest etc. (Hijmand and Graham, 2006; Kearney and Porter, 2009; Buckley et al. 2010; Evans et al. 2015; Conradie et al. 2020; Kearney and Porter, 2020; Schouten et al. 2020). However, improved *WUE* under elevated CO_2 is a conserved response amongst different plant functional types, almost irrespective of other interactions, and is frequently the most cited change in plant physiological efficiency under elevated CO_2 (Prior et al. 2011; Hatfield and Dold, 2019). Thus, including additional predictor variables into this model will add unnecessary complexity that may do little to improve the model. Therefore, adjusting the climate predictor variables, in particular MAP, to account for *WUE* under future climate scenarios and elevated CO_2, reduces the model's perceived complexity and makes its accessible to non-experts.

Numerous studies use SDMs to assess invasion risk of weeds under future climate change (Sheppard, 2013; Sheppard et al. 2014; Thapa et al. 2018; Fang et al. 2021; Shabani et al. 2020; Kriticos et al. 2010; Clements and Jones, 2021),

however based on the results of this study, their predicted invasion risks could be underestimated, particularly in regions where MAP limits establishment (Donohue et al. 2013; Hatfield and Dold, 2019). Despite MAP being the second/third most important contributor to the climate envelope produced by the current SDM for *O. stricta*, the effect of *WUE* on predicted habitat suitability as shown by the mechanistic SDM was significant, even with a conservative change in *WUE*. Therefore, for invasive species that are mainly limited by MAP, predicting future distributions using non-mechanistic SDMs and subsequent risk may be more significant with the inclusion of *WUE* in the models.

5.5.1 Conclusion

Opuntia stricta distribution is expected to increase significantly under future climate change scenarios. However, this predicted distribution is potentially underestimated in regions where rainfall may fall below *O. stricta* 's current lower threshold of ~ 200 mm per annum if changes to *WUE* are not accounted for. Improved *WUE* under rising atmospheric CO_2 will ameliorate the effects of suboptimal rainfall, resulting in a broader climatic niche for *O. stricta*, thereby increasing the probability of establishment in regions that are currently deemed unsuitable based on the weeds current photosynthetic physiologically. For example, the mechanistic SDM showed a substantial increase in climatic habitat suitability in regions, such as parts of the Karoo, where non-mechanistic future SDM predicted lower suitability. While this change may appear trivial at a national scale, it does highlight that these regions may be at greater risk. Based on *O. stricta's* response, other invasive cacti in South Africa are likely to respond similarly, potentially expanding their distributions further than may be reported by models that do not include *WUE* as variable. Finally, this mechanistic SDM approach can be applied to most weeds where the literature has reported the effects of elevated CO_2 on their *WUE*, thus negating the need to perform these *WUE* experiments.

CHAPTER SIX

Synthesis and conclusion

6.1 Study context

Cacti are prevalent as invasive weeds in South Africa, and numerous other countries globally, where they have detrimental effects on socio-ecological and environmental systems, from degraded to pristine habitats (Novoa et al. 2015; Shackleton et al. 2017). Despite being amongst the most important alien invasive species globally (Weber, 2003), there has been no research investigating the effect of rising atmospheric CO_2 concentrations and the interaction of abiotic and biotic factors on their invasive vigour, unlike that seen for other invasive weed species (Belote et al. 2004; Ziska and George, 2004; Ziska et al. 2010; Manea and Leishman, 2011; Baso et al. 2021). Other elevated CO_2 studies have demonstrated that invasive weeds tend to outperform native plants, indicating weeds are more responsive to CO_2 fertilisation and are likely to become more problematic in the future (Belote et al. 2004; Manea and Leishman, 2011; Ziska and George, 2004). Elevated CO_2 studies on cacti have mainly been restricted to *O. ficus-indica* which has been shown to respond positively to elevated CO_2, indicated by increased growth. However, these studies have been conducted in the context of agricultural productivity (Nobel, 1991; Cui et al. 1993; Nobel and Israel, 1994; Nobel et al. 1994; Cui and Nobel, 1994) rather than invasive vigour and range expansion. Given *O. ficus-indica's* invasive nature, inferences could be made as to how it and other invasive cacti may also respond to rising atmospheric CO_2 and climate change. However, given the variety of functional types of invasive cacti and growth forms (Novoa et al. 2015), using a one-size-fits-all approach might not accurately reflect species specific responses to elevated CO_2 as a driver. This represents a gap in the knowledge of an important group of invasive weeds. In an attempt to address this, this study sought to determine the effects of CO_2 (and other abiotic and biotic factors) on the invasive vigour of two functionally different species, *Opuntia stricta* and *Pereskia aculatea*.

6.2 Implications for the invasion of cacti under elevated CO_2 and climate change scenarios

The first objective of the study was to determine the effect of increasing CO_2 concentrations on the invasive vigour of *O. stricta* and *P. aculeata*, measured by plant growth, traits, and the physiology underlying them. *Opuntia stricta* and *P. aculeata* both responded positively to increasing CO_2, but their responses varied according to the CO_2 concentration. With an increase in CO_2 of 150 ppm between sCO_2 and aCO_2, *Opuntia stricta*, showed a significant increase in growth rates, biomass and *WUE*. This suggests that *O. stricta's* invasion was likely facilitated by rising atmospheric CO_2 over the last 200 years. For the same increase in CO_2, *P. aculeata* showed no increase in growth rates, and biomass, although it did exhibit a significant increase in *WUE*. While the conclusion drawn in Chapter 3 stated that the increases in atmospheric CO_2 concentrations from time of introduction (~ ca. 1858) to present were unlikely to have acted as a significant factor that in promoting *P. aculeata's* invasion and its subsequent distribution observed to date, this significant increase in *WUE* may have facilitated the establishment of *P. aculeata* populations at the boundary of their range.

With an increase in CO_2 of 200 ppm between aCO_2 and eCO_2, *O. stricta* either showed an increase in total biomass (Chapter 4) or no increase in total biomass (Chapter 2), despite a significant increase in night-time *WUE*. These different outcomes with regards to biomass may have been due to different experimental durations and slightly different pot sizes: Chapters 2 and 4 experiments lasted 6.5 months (2.3 litre pots) and 8 months (3 litre pots), respectively. However, biomass is not the only important metric of invasive potential, instead other traits can improve fitness and facilitate invasion. Across both experiments (Chapters 2 & 4), *O. stricta* significantly increased resource allocation to mechanical defence (spine formation) and basal cladode biomass, indicating a conserved response under eCO_2, irrespective of experimental duration and pot size. While spines serve as a mechanical defence, research has shown that spines and glochids on different cacti species, including *O. stricta* also act as dew harvesters and direct condensed water to the areoles where it is absorbed into the cladode (Malik et al. 2016; Masrahi, 2020). This effect was however, not

considered in the present study. Under eCO_2, *O. stricta* increased photosynthetic productivity, particularly daytime C_3 photosynthesis, however it appears the additional resources acquired were allocated to spine formation and basal cladode biomass in favour of size and total biomass. In contrast, between aCO_2 and eCO_2, growth rates, total biomass and photosynthesis rose sharply for *P. aculeata*. This indicates that a CO_2 concentration threshold was encountered between aCO_2 and eCO_2 whereby *P. aculeata* became significantly more productive, despite a more modest increase in *WUE* relative to that seen from sCO_2 to aCO_2. This suggests that *P. aculeata* productivity is somewhat inhibited at current atmospheric CO_2 concentrations, but as CO_2 concentrations rise *P. aculeata* is likely to become more problematic than it already is. Therefore, when additional resources became available under eCO_2 from improved photosynthetic productivity, unlike *P. aculeata, O. stricta* limited investment in biomass and size, instead allocating resources to traits that may buffer against abiotic and biotic stresses. These outcomes reflect their different growth forms, whereby *O. stricta* which grows as a shrub, and is at greater risk from physical disturbance, whereas *P. aculeata* need to invest in size to access resources such as light at the top of forest canopies (Paterson et al. 2011a)

The second objective of the study was to determine if increasing CO_2 ameliorates the effect of low soil moisture on *O. stricta and P. aculeata* productivity. Two watering treatments were implemented for the entire duration of the CO_2 experiments to emulate suboptimal and optimal rainfall for either species. Under eCO_2, neither *O. stricta* nor *P. aculeata* could fully ameliorate the effects of suboptimal watering on their growth rates or total biomass. There were however subtle amelioration effects, whereby, similar to the optimal watering treatment at eCO_2, *O. stricta* maintained investment in spine formation and basal biomass, while investing in less cladode N but still demonstrating modest daytime C_3 photosynthesis and significantly improved *WUE* at night and during the day (Chapter 4). *Pereskia aculeata* increased leaf thickness, reduced chlorophyll a content which was likely facilitated by increased photosynthesis and *WUE* (Chapter 2). While it is difficult to determine which species was more efficient at ameliorating the effects of suboptimal watering under eCO_2, both species improved photosynthetic efficiency and invested in traits to improve fitness. Thus,

under future climate scenarios where rainfall may be predicted to decline in South Africa, *O. stricta* and *P. aculeata* could maintain or enhance their invasive vigour.

Biological control is the most successful management tool to control invasive cactus in South Africa (Paterson et al. 2021). However, observed changes to host plant quality under elevated CO_2 can have a negative effect on arthropod herbivores (Robinson et al. 2012; Sun et al. 2020). Therefore, the third objective was to determine the effect increasing CO_2 on plant quality and the subsequent impact on cactus biological control efficacy. This was directly tested on *O. stricta* plants which were grown under the three CO_2 concentrations (only well-watered treatment) and then exposed to their bicontrol agent, *D. opuntiae*. *Dactylopius opuntiae* fitness was significantly reduced when exposed to plants grown at eCO_2, which was correlated to increasing cladode C/N ratios driven by reduced N content. This resulted in slower rates of feeding damage and delayed mortality for *O. stricta* plants. This potentially has serious ramifications for *O. stricta* and other invasive cacti species that are controlled using one of the four *Dactylopius* species. Numerous cacti species, including *O. stricta*, are under substantial or complete control using *Dactylopius* species (Moran et al. 2021; Paterson et al. 2021). *Opuntia ficus-indica* invasions in South Africa are under substantial control due to *D. opuntiae* (Moran et al. 2021), however, similar to *O. stricta*, cladode N contents have been shown to decline under elevated CO_2 (Cui and Nobel, 1994; Nobel et al. 1994; Gomez-Casanovas et al. 2007). While these *Opuntia* species only represent two of the eight *Opuntia* species that are controlled using *Dactylopius* species in South Africa (Moran et al. 2021), a trend of declining N content with elevated CO_2 is evident. Assuming the remaining six *Opuntia* species respond similarly, the future biocontrol efficacy of *Dactylopius* species may be hindered. Additionally, elevated CO_2 is suggested to hinder the ability of the biocontrol agent, *C. cactorum* to detect *O. stricta* plants (Stange et al. 1995). Although the effect of plant quality on *P. aculeata* biocontrol agents was not directly tested, declines in leaf N, and increases in C/N ratios and leaf thickness may negatively affect the two biocontrol agents currently used to control its invasion. In particular, the leaf chewing beetle *Phenrica guerini's* fitness may be hindered if *P. aculeata* leaves thicken under eCO_2 as shown in Chapter 3, resulting in the leaves being more resilient to herbivory (Wright and

Cannon, 2001; Wang et al. 2023). Naturally, the interaction of climate change on plant host quality and biocontrol agent development may affect these plant-arthropod interactions, and thus future work in this field would need to include additional abiotic parameters.

Increasing atmospheric CO_2 is driving climate change resulting in rising temperatures and altered rainfall patterns which are expected to increase the vigour and distribution of invasive weeds (Reeves, 2017; Ziska et al. 2019; Sun et al. 2020; Clements and Jones, 2021; IPCC, 2022). In addition to the effects of climate change, CO_2 directly impacts weed vigour by improving productivity, as observed for *P. aculeata* and *O. stricta* in this study. However, increases in weed distributions from climate change are likely to be compounded by the effect of improved *WUE* (Hatfield and Dold, 2019; Ziska et al. 2019). Changes to invasive weed *WUE* are not incorporated into SDMs, despite SDMs being an important tool for making predictions as to how invasive weeds will respond to climate change. Therefore, the fourth objective of this study was to create a mechanistic SDM that accounted for expected changes to *WUE* in response to projected atmospheric CO_2 concentrations. (Chapter 5). *WUE* calculated from physiological measurements conducted on *O. stricta* in Chapter 2 were used as a predictor variable in a mechanistic SDM which was then compared to a non-mechanistic SDM. Using a Maxent SDM (non-mechanistic SDM), *O. stricta* range is predicted to expand under the RCP4.5 scenario, which is largely a result of increases in mean annual temperatures. However, the mechanistic SDM predicts increased range expansion in the west of South Africa, where the non-mechanistic predicted lower habitat suitability. Thus, in regions that are predicted to be unsuitable due to declines in rainfall, particularly in the west of South Africa (Engelbrecht, 2019; IPCC, 2022), the effect of improved *WUE* under eCO_2 could offset the effect of lower rainfall on potential establishment. Although the effects of improved *WUE* in higher rainfall regions is likely to increase cactus vigour, their distribution in these regions is mainly driven by other resource limitations (Hatfield and Dold, 2019). Therefore, the effects of improved *WUE* under elevated CO_2 is likely to only increase the distribution of cactus into regions where rainfall is the main limitation to establishment. Moreover, this spread of *O. stricta* will likely be further exacerbated by the potential reduction in *D. opuntiae* biocontrol efficacy

as shown in Chapter 4, thus creating a positive feedback situation, reinforcing the weed's invasive vigour. Therefore, improvements in *O. stricta* efficiency under elevated CO_2 which serve to enhance its invasive vigour, also act as a defence mechanism whereby the impact of its biocontrol agents are reduced.

However, it could be argued that increasing temperatures (IPCC, 2022) and atmospheric N deposition (Tylianakis et al. 2008) could serve to mitigate the effects of reduced plant quality on *O. stricta* biocontrol fitness and subsequent efficacy under elevated CO_2. *Dactylopius opuntiae* development time is accelerated at higher temperatures (Githure et al. 1999; Volchansky et al. 1999; Rule and Hoffmann, 2018), and N deposition could increase the plant N content and decrease the C/N ratios of *O. stricta* cladodes, subsequently improving host plant quality. While it is difficult to determine if higher temperatures would improve *D. opuntiae* fitness, as measured in Chapter 3, if 1st instar mortality rates remain high at higher temperatures, long-term fitness will still be reduced. Additionally, while N fertilisation does increase plant biomass under elevated CO_2, plants still appear to maintain the same C/N ratios (Robinson et al. 2012). Therefore, while the results here suggest that the biocontrol efficacy of *D. opuntiae* on *O. stricta* will be reduced with rising atmospheric CO_2, this effect size could vary depending on the interplay of these abiotic drivers.

6.3 Limitations and future recommendations

Like most studies, there were limitations to this study, particularly as it aimed to address feedbacks in complex systems where multiple drivers are acting simultaneously (Tylianakis et al. 2008). The most notable of these was the absence of accompanying temperature treatments, which was largely due to access to additional CO_2 enabled growth chambers. However, the effects of temperature are well cited in the literature, and the interactive effects of temperature on CO_2 and water were thoroughly addressed in this study using the available literature. Other important aspects that could enhance or diminish the effects of elevated CO_2 on *O. stricta's* and *P. aculeata's* invasive vigour were beyond the scope of this study and could not be addressed. This was largely due to

the prohibitive costs of running long-term experiments and equipment sharing time-constraints. These include the effects of eCO_2 on 1) sexual reproduction and 2) acclimation of photosynthesis to eCO_2. Firstly, only a few studies report the effects of elevated CO_2 on fruit quality, with both positive and negative results, and these studies are limited to fruits for human consumption (Sun et al. 2012; Fischer et al. 2022). Thus, it would have been interesting to determine if *O. stricta* and *P. aculeata* fruit quality improved under eCO_2, which would improve their attractiveness to frugivorous dispersal agents, compounding invasive vigour. Additionally, Novoa et al. (2016) reported that invasive cacti with bigger seeds are more prone to be invasive, adding an additional interesting question as to whether seed size increases under eCO_2. Secondly, studies indicate that plants can acclimate to elevated CO_2, whereby photosynthesis declines and subsequent growth also declines over time, although this is mostly reported for C_3 annual species (Sage, 1989; Sage et al. 1994; Heineke et al. 1999; Moore et al. 1999). Although Drennan and Nobel (2000) concluded that CAM plants, including *O. ficus-indica,* do not acclimate to elevated CO_2, which is attributed to succulence, it would be useful to test this on *O. stricta* and *P. aculeata* to determine whether their increases in invasive vigour would be maintained long-term. Therefore, future work should attempt to address the interactive effect of temperature and elevated CO_2 on invasive cacti and additionally, other aspects that may contribute or hinder invasive vigour should be considered.

Despite these limitations, the strengths of the study include adhering to ecologically appropriate CO_2 concentrations. Elevated CO_2 studies on invasive weeds tend to use future CO_2 concentrations (Belote et al. 2004; Ziska et al. 2010; Ziska and George, 2004) that are unlikely to be encountered in this century, let alone the next century. While this is not a criticism as such, it does have the potential to report large effects, and hence diminish the predictive power of the study by being less ecologically appropriate. Moreover, studies that have investigated the effect of pre-industrial CO_2 concentrations on invasive weeds are uncommon, with only one study encountered that explained the effect of pre-industrial and future CO_2 (720 ppm) concentrations on six herbaceous C_3 weeds (Ziska, 2003). This is largely due to the cost and technical considerations of scrubbing CO_2.

However, based on the expertise and experience gained during this study, it is recommended that future investigations on invasive weeds should rather implement smaller incremental increase in CO_2 treatments of 50 or 100 pm i.e., 400, 450, 500 ppm or 400, 500, 600 ppm, with the number of CO_2 treatments essentially dictated by the available CO_2 enabled growth facilities. In doing so, CO_2 treatments will not go beyond predicted atmospheric CO_2 concentrations and, importantly, weed response thresholds that may not be resolved with large stepwise CO_2 increases could be resolved using smaller increments of increasing CO_2. Additionally, to address the effects of past CO_2 concentrations on invasive vigour, analysing cactus $\delta^{13}C$ isotopic signatures and C/N ratios on herbarium specimens could determine 1) if photosynthetic productivity has increased with rising atmospheric CO_2 whereby historical samples should demonstrate more negative $\delta^{13}C$ values indicating an increased contribution of day-time C_3 photosynthesis to carbon gain, and 2) if C/N ratios increased indicating declines in host plant quality, subsequently affecting the relationship with their biocontrol agents. These results would help validate the observations made in these CO_2 experiments and support the conclusion drawn from these results. Nevertheless, despite these limitations and proposed questions, the outcome of this study has and will make a significant contribution to the understanding of how invasive cacti, and potentially invasive species from different families, should respond to elevated CO_2 and climate change.

6.4 Conclusion

Biotic invasions are cited as one of the five major causes of global environmental change, among rising atmospheric CO_2, nitrogen deposition, climate change and land use change (Tylianakis et al. 2008). Therefore, understanding the effects of drivers such as elevated CO_2 and climate change on invasive weeds is informative, considering these drivers will only serve to compound the negative effects of invasive weeds on socio-environmental systems. This study proposes that the management of *O. stricta* and *P. aculeata,* and by extension other invasive cactus species in South Africa, and potentially globally, will be impacted

by rising atmospheric CO_2 and climate change. If left unchecked, increases in invasive cacti vigour in response to rising atmospheric CO_2 and climate change could result in a tipping point whereby an ecological threshold is encountered leading to potentially irreversible alternate unstable ecological states (Barney et al. 2013; Reynolds and Aldridge, 2021).

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