How will increasing temperatures affect reproduction in tropical tree species?

Georgina Anna Werkmeister

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Rationale for submitting the thesis in alternative format

This thesis has been submitted following the University of Leeds Faculty of Environment protocol for an alternative style of doctoral thesis including published material. The work undertaken to investigate the research question of this thesis was novel in its approach and area of interest, and the findings offer unique and exciting insights. We hope that it could potentially inspire greater interest in (and provide possible methods for investigation of) tropical tree reproduction under high temperature. Dissemination of this work to a larger audience through publication is therefore key. The research question was investigated through three major experimental campaigns that naturally formed three manuscripts (the first is under second review following initial review and revisions; the second is published; and the third is ready for submission to a journal), which have become the central (data) chapters of this thesis.

The data chapters therefore follow the progress of the work, from the initial experiment, heating inflorescences of a single Cerrado species; through the development and testing of an improved whole-tree heating methodology; to the final, larger-scale experiment, heating entire individuals of two Cerrado species. These data chapters are preceded by an introductory chapter which reviews current knowledge on the impacts of high temperatures on plant reproduction and potential field methodologies for environmental manipulation. Finally, they are drawn together by a discussion chapter, which considers the results of the thesis chapters as a whole and places them in a wider context.

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Thesis Abstract

The Brazilian Cerrado biome – regarded as the world's most biodiverse savannah, housing many endemic species – is crucial for maintaining South America's water, food, and energy supplies, and providing carbon storage and climate regulation services. Yet, already heavily fragmented, what remains of the Cerrado's poorly protected native vegetation potentially faces additional threats from steadily increasing temperatures. To fully comprehend the risks posed by higher temperatures, we must better understand their potential impacts on plant growth and development, one of the most temperature-sensitive stages being sexual reproduction. Important for maintaining genetic variation, recruitment, and dispersal ability of a population, continued reproduction is essential for long-term persistence and, if necessary, migration. Although high temperatures can limit reproductive success by altering the development and function of male and female reproductive tissues (stamens and pistils) and gametophytes (pollen grains and egg sacs), investigations of such impacts in wild – particularly tropical – species are rare. This thesis comprises the first direct investigations of high-temperature impacts on woody Cerrado species *in situ*. It describes the development, testing, and utilisation of two novel methodologies for *in situ* passive heating (namely targeted inflorescence heating chambers and whole-tree heating structures) and their effects on viable pollen and fruit production. Contrary to expectation, heating inflorescences did not affect pollen viability (analysed through pollen staining and *in vitro* germination) and whole-tree heating had a positive impact, suggesting high thermal limits in the studied species. Conversely, both inflorescence and whole-tree heating reduced fruit production of hand-pollinated flowers in one common and widespread species, indicating a higher temperature sensitivity of female than male reproductive development (unusual in species studied to date). Though initial, the insights gained through this work are unique. It highlights the need for more extensive investigation of these important processes and undervalued tropical ecosystems, and provides a possible framework for future study.

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Abbreviations

AbsH	Absolute humidity
AC	Alternating current
A_{net}	Net photosynthesis rate
A_{opt}	Optimum rate of photosynthesis
ASA	Acrylonitrile styrene acrylate
BRT	Brasilia time
C3S	Copernicus Climate Change Service
CO_2	Carbon dioxide
CV	Coefficient of variation
DSH	Diameter at stump height (30cm)
GHG	Greenhouse gas
GPP	Gross primary production
INMET	Instituto Nacional de Meteorologia (Brazilian meteorological service)
IPCC	Intergovernmental Panel on Climate Change
IR	Infrared
LED	Light emitting diode
OTC	Open top chamber
PCD	Programmed cell death
PVC	Polyvinyl chloride
Q_{10}	Proportional change in respiration rate given a 10°C increase in leaf temperature
R	Respiration rate
R_{25}, R_{35}, R_{45}	Respiration rates at leaf temperatures of 25, 35, and 45°C
RCP	Representative Concentration Pathway
RH	Relative humidity
ROS	Reactive oxygen species
SSP	Shared Socioeconomic Pathway
T_{max}	Maximum temperature for photosynthesis
T_{opt}	Optimum temperature for photosynthesis
T_{span}	Temperature range over which photosynthesis is at least 80% of A_{opt}
UNEMAT	Universidade do Estado de Mato Grosso (University of Mato Grosso)
UTC	Coordinated Universal Time
UV	Ultraviolet
VPD	Vapour pressure deficit
WTHS	Whole-tree heating structure

Chapter 1

Introduction

1.1 Background

Anthropogenic climate change is one of the foremost drivers of global ecosystem change (Bowler et al., 2017; IPCC, 2021; Higgins et al., 2023), and climate warming in particular represents an immediate threat to Earth's ecosystems (Bellard et al., 2012; Pecl et al., 2017; Singh et al., 2021). Unless we can swiftly and drastically curb the emission of greenhouse gases (GHGs), global mean surface temperatures are predicted to rise by 2.1 to 5.7°C by 2100 (compared to pre-industrial levels) based on intermediate to high GHG emissions scenarios (or Shared Socioeconomic Pathways referred to as SSP2-4.5 and SSP5-8.5 respectively; IPCC, 2021). Temperature has a profound impact on living organisms at all levels, from molecules and cells to whole ecosystems (Hasanuzzaman et al., 2013; Bozinovic and Pörtner, 2015). It constrains the biochemistry and physiology of metabolism, growth, development, and reproduction within species-specific thermal thresholds, which partly determine a species' ecological niche (Bozinovic and Pörtner, 2015). Furthermore, temperature influences phenology, behaviour, demographic processes, and species interactions, and therefore affects the ecology, structure, and functioning of ecosystems (Butt et al., 2015; Piao et al., 2019; Williams et al., 2022). Increasing temperatures will likely reduce the suitability of current habitat for many individuals and species (taking them outside of their ecological niches; Parmesan, 2006), meaning they will have to acclimate or adapt to new environmental conditions, or migrate to more suitable areas (Jump and Peñuelas, 2005; Feeley et al., 2012). Species that cannot adapt or migrate at the speed of environmental change will experience population declines, range reductions, and possibly local extirpation or global extinction (Cahill et al., 2013). In this way climate change has already been linked to global change and biodiversity loss (Parmesan, 2006) with consequences for ecosystem health and services, including those that support human livelihoods and food security, and feedback on the global climate (Pecl et al., 2017).

Carbon sequestration through photosynthesis (measured as gross primary production; GPP) is particularly important for reducing the effect of GHG emissions on climate, and tropical forests and savannahs together account for 60% of the world's terrestrial GPP

(Beer et al., 2010). However, plant species in the tropics are likely to already be experiencing temperatures close to their upper thermal thresholds (Sentinella et al., 2020; Doughty et al., 2023), and – although species' responses vary substantially – increasing temperatures have led to a general trend of warm adapted species migrating to higher latitudes and altitudes (Freeman et al., 2018; Rubenstein et al., 2023); for example mangrove species expanding further northward (Cavanaugh et al., 2014). Nevertheless, due to their sessile nature, plant species generally struggle more than animals to disperse and migrate to more suitable environments – especially at a rate that tracks that of environmental change – making them more vulnerable to local extinction (Corlett and Westcott, 2013; McNichol and Russo, 2023). Loss of species (whether through migration or extirpation) will likely have the greatest impact in the tropics as there are no warmer latitudes from which species can move to replace those being lost. Overall, the structure and functioning of tropical communities and ecosystems into the future will be determined by complex species-specific responses to environmental change and interactions with – and additional impacts on – co-evolved species (of plants, animals, and fungi; Jump and Peñuelas, 2005; Corlett and Westcott, 2013; Barlow et al., 2018). It is therefore important to better understand how temperature shapes the current distributions of species in the tropics, and how these will be affected by predicted increases in temperature.

1.1.1 The Brazilian Cerrado

While the importance of tropical forests for global biodiversity and service provisioning is generally well known, other tropical biomes such as savannas and grasslands – many of which were historically considered degraded landscapes – are routinely undervalued and therefore provided far less protection (Murphy et al., 2016; Williams et al., 2022). However, tropical savannahs account for roughly an eighth of global land cover (Scholes and Archer, 1997), a quarter of global terrestrial GPP (Beer et al., 2010), and potentially a seventh of the global terrestrial carbon sink (Grace et al., 2006), and thus contribute significantly to moderating the impact of GHG emissions on the global climate. One such savannah biome is the Cerrado biome of Brazil, which is the most biodiverse savannah on Earth (Sano et al., 2019), with over 12,000 native species of flora, around 35% of which are endemic (Mendonça et al., 2008; Forzza et al., 2012).

Figure 1.1 *Map of Brazil highlighting the Cerrado biome and the field site near Nova Xavantina in the state of Mato Grosso, where the experiments within this thesis were carried out.*

The Cerrado biome (Figure 1.1) reaches from the edge of the Amazon Forest biome in the north-west to the Atlantic Forest biome in the south-east, and from the Caatinga in the north-east to the Chaco (both seasonally dry tropical forest biomes) and Pantanal (wetland biome) in the south-west (Vieira et al., 2019). It comprises a complex mixture of vegetation types, ranging from grasslands to shrublands, woodlands, and dry forest, with varying levels of openness, relief, and substrate properties (Ribeiro and Walter, 2008; Pereira and Fernandes, 2022). The Cerrado climate is predominantly classified as Aw (according to the Köppen system), characterised by marked dry and wet seasons (occurring roughly from April to September and October to March respectively; Peel et al., 2007; Silva et al., 2008; Alvares et al., 2013). Annual precipitation ranges from 600 mm in the east to 2,000 mm in the west (averaging around 1430 mm; Sano et al., 2019), while annual mean temperature ranges from 18°C in the south-east to 27°C in the north-east (with mean maximum temperatures ranging from 24 to 33°C; Silva et al., 2008). Originally covering approximately 2 million km^2 (Ratter et al., 1997), the Cerrado has been undervalued as a natural ecosystem and disproportionately targeted for conversion to pasture and agricultural land (Garcia and Ballester, 2016; Strassburg et al., 2017), facing faster rates of deforestation than the Brazilian Amazon (Klink and Machado, 2005). As a result, less than half of the Cerrado's native vegetation remains intact (Beuchle et al., 2015; Parente et al., 2021), much of which is highly fragmented (Carvalho et al., 2009) and poorly protected, with only 6.5% located in officially protected areas (Françoso et al., 2015). This makes it even more sensitive to further land use change and the possible impacts of climate change. The region is already exhibiting noticeable warming and drying (Hofmann et al., 2021; Marengo et al., 2022), and a recent analysis of regional climate models predicts temperature increases of between 2.8 and 6.6°C across the Cerrado biome by 2100 (compared to 2006) according to intermediate to high GHG emissions scenarios (rising by 0.03 to 0.07°C per year respectively; Ferreira et al., 2023).

Because it contains such high levels of species richness and endemism, while also being under considerable threat from anthropogenic action, the Cerrado has been classified as a biodiversity 'hotspot' (Myers et al., 2000), and therefore a significant target for biodiversity conservation. Furthermore, the Cerrado is socioeconomically important, with many native species having cultural, nutritional, or ethnopharmacological uses (Guilhon-Simplicio and Pereira, 2011; Simon et al., 2013; Valli and Bolzani, 2019; Alves and Loeuille, 2022; Arruda et al., 2022; Williams et al., 2022; Passos, 2023). It also contains the headwaters of multiple major rivers and contributes to many of Brazil's major hydrographic basins (Lima, 2011), making it essential for providing water and hydroelectric energy to much of South America (Cuartas et al., 2022; Salmona et al., 2023). Furthermore, intact Cerrado vegetation captures and stores carbon, protects soil from erosion, and regulates local climate conditions (through water recycling and regional cooling; Grace et al., 2006; Anache et al., 2018; Rodrigues et al., 2022). Continued degradation or loss of natural Cerrado vegetation will likely enhance climate warming and drying in this region, and increase the likelihood of fires (Spera et al., 2016; P.S. Silva et al., 2019; Rodrigues et al., 2022), with further repercussions for vegetation, biodiversity, and ecosystem health, and therefore the ecosystem services it provides. It is therefore clearly important to better understand how the remaining vegetation of the Cerrado may be impacted by climate change.

Species distribution models (also known as ecological niche models) can be used to map current – and predict future – species ranges, to then identify key species or areas for protection or conservation effort (Araújo and New, 2007; J.M.C. da Silva et al., 2019). Models must be fed by data, typically occurrence records and bioclimatic data associated with occurrence locations. For example, Alves and Loeuille (2022) used occurrence data to model and predict the current and potential future distributions of *Eremanthus* species – an overexploited genus comprised of tree and shrub species endemic to Brazil and Bolivia but occurring to the greatest extent in the Cerrado – and Simon et al. (2013) similarly modelled current and future distributions of 110 Cerrado species of economic and cultural importance. Interestingly, both of these studies predicted significant reductions in the extent of suitable habitat for their studied species within the Cerrado biome by 2070/2080 (Simon et al., 2013; Alves and Loeuille, 2022). For many species, however, occurrence records are lacking, inaccurate, or outdated, and models can be greatly improved by the addition of other types of data. This can include physiological trait data, such as thermal thresholds for photosynthesis, seed germination, or seedling growth, which can be gathered from field observation or experimentally (as in Feng et al., 2018; Ribeiro et al., 2019; Ferreira et al., 2022). Nevertheless, data on thermal limits for the vast majority of Cerrado species are still absent. Direct investigation of the impacts of temperature on native Cerrado species is required to better predict the effects of increasing temperatures on species in their current ranges, and on the availability of suitable habitat in the future. Additionally, this may further highlight the importance of keeping climate warming to a minimum.

1.1.2 Studying reproduction

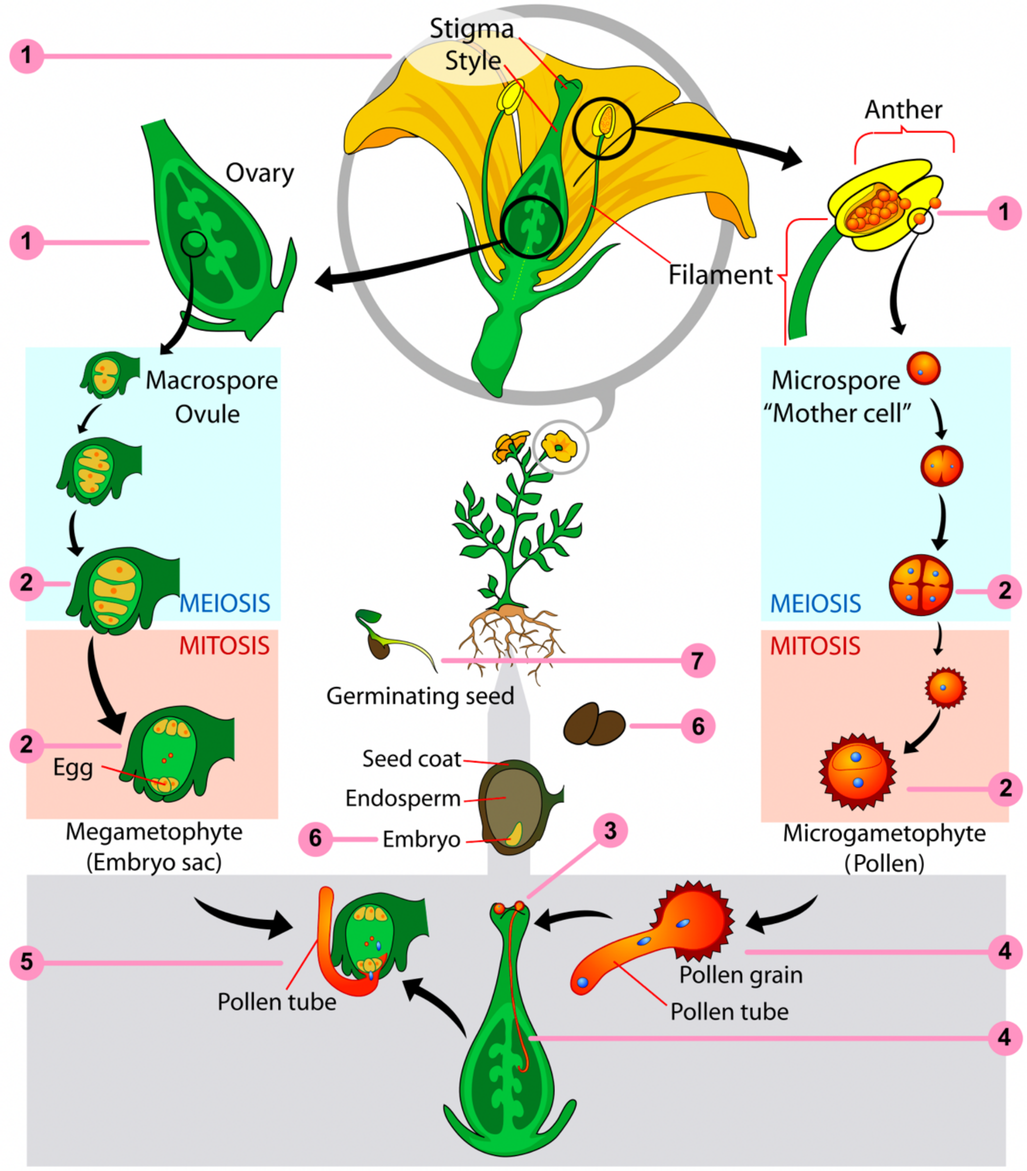
There are a number of key plant processes – for example photosynthesis, respiration, primary and secondary metabolism, and within-plant signalling – that are known to be affected by high temperatures, potentially impacting plant growth and development, and consequently survival (Bita and Gerats, 2013). One process that has received far less attention than others is reproduction, and yet sexual reproduction is thought to be one of the most sensitive stages in a plant’s life cycle to temperature stress (Hedhly et al., 2009; Zinn et al., 2010). The regeneration niche of a species is defined as the environmental conditions that sexually mature plants require to successfully produce, disperse, and germinate seeds, and establish seedlings (Grubb, 1977). This often differs from the ecological niche necessary for the survival of adult individuals, which is more often considered in the modelling of species distributions (Bykova et al., 2012; Rosbakh et al., 2018). However, when considering the long-term persistence of a species, the ability of individuals to reproduce and recruit is no less important than adult survival, and even more crucial to its ability to disperse and migrate to new areas in the face of environmental change.

When attempting to predict the impacts of increasing temperatures on ecosystems (such as those of the Cerrado), it is therefore necessary to better consider reproduction and the regeneration niche, and incorporate them into ecological models (Bykova et al., 2012; Chefaoui et al., 2019). Borghetti et al. (2021), for example, combined climate forecasts with experimental data on seed germination temperatures (gathered through data mining) of 73 Cerrado species (from both open savannah and more forested vegetation formations) to predict the future extent of suitable environments for seed germination (and therefore potential recruitment), and how variables including fire, rainfall, and temperature seasonality might impact future recruitment and the extent of the two vegetation types. Similar but smaller-scale studies have been carried out on individual Cerrado species – for example *Dipteryx alata* (Ribeiro et al., 2019) and *Apuleia leiocarpa* (Ferreira et al., 2022) – in which experiments to test the thermal limits of seed germinability and seedling vigour were carried out as part of the study and the results incorporated into distribution models, with both examples detecting a reduction in suitable habitat in the future. Nonetheless, the vast majority of Cerrado species remain entirely unstudied.

Study of the regeneration niche has traditionally focused more on the post-fertilisation stages of reproduction – from seed production to seedling establishment (Rosbakh et al., 2018) – and none of the aforementioned studies consider the impacts of temperature on floral development and the essential processes and stages leading up to the production of viable seeds (or seed dispersal). Yet, many elements of reproductive development – especially in the earlier stages – are influenced by environmental conditions and constrained particularly by temperature (Hedhly, 2011; Sage et al., 2015). To predict the future of Cerrado vegetation more accurately, more comprehensive data therefore needs to be gathered on the ecological requirements (particularly thermal limits) of species at all life stages, including throughout the various stages of reproduction (discussed below).

1.1.3 Sensitivity of reproduction to temperature

The processes of plant reproduction and recruitment are heavily influenced by temperature (Zinn et al., 2010; Walck et al., 2011; Sage et al., 2015; Lohani et al., 2020). High temperatures can affect the timing or extent of flowering or fruiting (Nerd et al., 2002; Albrigo and Galán Saúco, 2004; Sherry et al., 2007; Luo, 2011; Richardson et al., 2013), potentially limiting the production of viable seeds and reproductive success. Since the harvestable elements of many commercially grown species (crops) are products of reproduction (fruits and seeds) the effects of temperature on plant sexual reproduction – the main stages of which are depicted in Figure 1.2 – have been a focus of crop research for some time. Male reproductive development (culminating in the production of viable pollen) is generally considered to be the most sensitive process to high temperatures, possibly because male reproductive tissues are often more exposed to the environment than those that house the female gametophytes (Zinn et al., 2010; Hedhly, 2011; Sage et al., 2015; Pacini and Dolferus, 2016). Reductions in yield are therefore often attributed to reductions in pollen production or viability leading to fertilisation failure (Hedhly, 2011; Lohani et al., 2020). However, high temperatures have the potential to act at almost any stage of male or female reproductive development (before, during, or after pollination; Figure 1.2) through a multitude of possible mechanisms (many of which are discussed below) to negatively impact reproduction (reviewed in Sage et al., 2015; Lohani et al., 2020; Zhu et al., 2021).



① **Development of floral tissues** (female stigma, style, ovary; male anther, filament)
② **Development of viable gametophytes** (female embryo sacs; male pollen grains)
③ **Acceptance of pollen** by the stigma during **pollination**
④ **Pollen germination and pollen tube growth** through the style (towards the ovary)
⑤ **Fertilisation** of the egg cell
⑥ **Development of the embryo** into fruit and viable seed
⑦ **Germination** of the seed (and later seedling establishment)

Figure 1.2 *Major stages of angiosperm reproduction (adapted from Villarreal, 2012), all of which have the potential to be negatively impacted by high temperatures.*

1.1.3.1 Pre-pollination

Reproductive development in angiosperms begins with the initiation of the floral meristem, bud development and the differentiation of floral organs (Figure 1.2 stage 1; Alvarez-Buylla et al., 2010). These include the reproductive tissues, namely the male tissues or stamens (each consisting of an anther and filament) and the female tissues or pistil (one or more carpels made up of a stigma, style, and ovary; Starr and Taggart, 2001). Higher temperatures have been found to reduce (or sometimes increase; del Cacho et al., 2013) the number of flowers initiated (Nerd et al., 2002; Albrigo and Galán Saúco, 2004; Petrie and Clingeleffer, 2005), or to increase the number of buds or flowers aborted during development (Gross and Kigel, 1994; Guilioni et al., 1997; Erickson and Markhart, 2001), limiting reproductive potential. Temperature can also influence the timing of the initiation of flowering (Sherry et al., 2007; Cook et al., 2012; Richardson et al., 2013) as well as cause physiological changes (for example altering floral odour or nectar rewards; Scaven and Rafferty, 2013), which can alter interactions with pollinators, potentially limiting the dispersal of pollen and successful pollination (Hegland et al., 2009; Scaven and Rafferty, 2013; Gérard et al., 2020).

Floral organ development is followed by the synchronised initiation of meiosis to form male and female gametophytes in the anther and ovary respectively (Figure 1.2 stage 2; Pacini and Dolferus, 2016). Pollen mother cells in the anther undergo meiosis to form haploid tetrads (microsporogenesis) that release four uninucleate young microspores, each of which then undergo two successive mitotic divisions (pollen mitosis I and II, or microgametogenesis) to produce a mature tri-cellular pollen grain containing two sperm cells (Pacini and Dolferus, 2016; Hafidh et al., 2016). Mitosis II can occur in the anther tissues, though in many angiosperms pollen is released as bi-cellular grains and mitosis II takes place in the pollen tube after pollen germination on the stigma (Brewbaker, 1967). High temperatures during any stage of this process can have a negative impact on pollen development (Hedhly, 2011; Santiago and Sharkey, 2019), although microsporogenesis and the uninucleate stage are considered to be particularly sensitive to temperature (Giorno et al., 2013; Pacini and Dolferus, 2016; Raja et al., 2019; Iovane and Aronne, 2022). Reductions in viable pollen production have been reported in numerous species when grown at high temperatures (≥30°C), including rice, wheat, barley, and soybean (to name a few, reviewed in Lohani et al., 2020). Furthermore, even native tropical species (that might be expected to tolerate higher temperatures) such as custard apple *(Annona cherimola*) and dragon fruit (*Hylocereus polyrhizus*) demonstrate reduced pollen viability at growth temperatures of 30 and 35°C respectively (Higuchi et al., 1998; Lora et al., 2012; Chu and Chang, 2022).

Heat-induced losses in pollen viability may be due to temperature impacts on the developing pollen grains themselves, or the abnormal development of the anther tissues, and in particular the tapetum (the inner cell layer of the anther wall) that surrounds the pollen mother cells (Giorno et al., 2013; Pacini and Dolferus, 2016; Santiago and Sharkey, 2019). The tapetal cells play a vital role, secreting proteins, lipids, and secondary metabolites necessary for pollen development, as well as providing carbohydrates as a source of energy to the developing pollen (Hafidh et al., 2016; Tariq et al., 2023). They also undergo a controlled programmed cell death (PCD) which enables the release of further nutrients and essential components of the pollen grain (Parish and Li, 2010; Pacini and Dolferus, 2016). Tapetum development and the careful timing of PCD can be disrupted by heat stress – for example through the overproduction of reactive oxygen species (ROS) – causing pollen abnormalities or abortion (Raja et al., 2019; Tariq et al., 2023; Zhao et al., 2023).

Development of viable pollen also requires large amounts of energy, mainly supplied in the form of carbohydrates such as sucrose, some of which is also converted to starch and stored in the pollen grain to supply it with enough energy for post-dispersal processes such as germination and initial tube growth (Santiago and Sharkey, 2019; Liu et al., 2021). Pollen development therefore relies heavily on carbohydrate metabolism in vegetative tissues and translocation to the developing anthers and pollen (regulated by the tapetal cells; Ferguson et al., 2021; Liu et al., 2021). This can be affected by the impacts of temperature on vegetative tissues and photosynthesis, reducing the provision of carbohydrates, or on reproductive tissues, disrupting carbohydrate transport and partitioning (Hedhly, 2011).

Should viable pollen grains develop normally, abnormal anther development or high temperatures at anthesis can disrupt pollen grain dehydration (an important stage of pollen maturation; Pacini and Dolferus, 2019) or impair anther dehiscence (splitting open) and the release of the pollen grains (Raja et al., 2019; Jagadish, 2020). If viable pollen are released, viability declines after dispersal (at varying speeds; Dafni and Firmage, 2000; Brunet et al., 2019), and extreme weather conditions often exacerbate this decline (Sage et al., 2015). Although, pollen grains are considered to be much more tolerant to temperature stress after dispersal than during development (Hedhly, 2011;

Distefano et al., 2018). Nevertheless, high temperatures during development have also been shown to cause accelerated senescence of viable pollen grains, further reducing their longevity after dispersal (Iovane and Aronne, 2022).

Although the effect of high temperatures on female reproductive development has received less attention than male, evidence of the temperature sensitivity of pistil tissue and embryo sac development is accumulating (Hedhly, 2011; Lohani et al., 2020; Wang et al., 2021). In normal development, ovules develop from the wall of the ovary and inside each ovule a mother cell divides twice by meiosis to form four haploid megaspores (megasporogenesis), three of which then disintegrate (Starr and Taggart, 2001). This is followed by megagametogenesis, when the remaining megaspore undergoes mitosis three times without the division of cytoplasm, forming a cell with eight haploid nuclei. The nuclei then migrate and the cytoplasm divides to form six haploid cells (one of which will be the egg cell) and one diploid cell (the endosperm mother cell), which together constitute the embryo sac (female gametophyte; Starr and Taggart, 2001; Yadegari and Drews, 2004). Heat stress during the differentiation and development of pistil tissues and ovules can shorten the time to anthesis (resulting in underdeveloped tissues; Rodrigo and Herrero, 2002) or cause a variety of morphological and anatomical abnormalities (reviewed in Wang et al., 2021) such as desiccated or deformed stigmas, styles, or ovaries (Rodrigo and Herrero, 2002; Prasad and Djanaguiraman, 2014; Djanaguiraman, Perumal, Jagadish, et al., 2018), resulting in reduced viability or accelerated senescence of the female gametophyte. These impacts can also hinder the processes of pollination and fertilisation (discussed in the next section).

The negative effects of high temperature on female reproductive development have been demonstrated in a number of crop species such as tomato, rice, and wheat (Lohani et al., 2020; Wang et al., 2021), tropical natives such as custard apple (Higuchi et al., 1998; Lora et al., 2011), and other crop species often grown in the tropics including apricot, sweet-cherry, and peach (Rodrigo and Herrero, 2002; Hedhly et al., 2003; Hedhly et al., 2005; Nava et al., 2009). Furthermore, although male development is generally considered to be more sensitive to temperature stress (Hedhly, 2011; Sage et al., 2015), a higher sensitivity of female development has now been detected in some species, including peach (Kozai et al., 2004; Hedhly et al., 2005) and pearl millet (Gupta et al., 2015; Djanaguiraman, Perumal, Ciampitti, et al., 2018). However, the impacts of temperature are often studied in relation to pollen viability and final reproductive output (known to be affected and easily measurable), and studies directly comparing the sensitivities of male and female development are few.

1.1.3.2 During and post-pollination

For successful fertilisation to occur, a viable pollen grain must land, adhere, and rehydrate on a receptive stigma (Figure 1.2 stage 3); germinate and grow a pollen tube through the stigma and style to the ovary (Figure 1.2 stage 4); and fertilise the egg cell (and the endosperm mother cell) through the fusion of gametes (Figure 1.2 stage 5; Starr and Taggart, 2001; Dresselhaus and Franklin-Tong, 2013; Pacini and Dolferus, 2016). This relies on the careful coordination of the timing of male and female development, as well as the coordination of complex interactions between the pollen grain and the pistil tissues after pollination (Herrero, 2003; Erbar, 2003). Both the coordination of development and the interactions themselves can be disrupted by high temperatures, either acting prior to anthesis (during tissue and gametophyte development; discussed earlier) or acting during and after pollination. High temperatures can cause an asynchrony of development, for example by altering the timing of anther dehiscence (Sage et al., 2015; Djanaguiraman, Perumal, Jagadish, et al., 2018) or shortening the period of stigma receptivity, or pollen or ovule longevity (Lora et al., 2009; Lora et al., 2011; Hedhly, 2011). They can further disrupt male/female interactions by leading to diminished stigma receptivity (Hedhly et al., 2003; Carpenedo et al., 2020); reduced pollen adhesion, hydration, and germination on the stigma (Higuchi et al., 1998; Lora et al., 2012); retarded pollen tube growth within the style (Sukhvibul et al., 2000; Zhang et al., 2018); and ultimately failure of fertilisation (Saini et al., 1983; Snider et al., 2009).

Retardation of pollen tube growth itself can have a number of different heat-induced causes, including abnormalities in the development of the pollen grain (Djanaguiraman, Perumal, Jagadish, et al., 2018), direct impacts of temperature on the pollen tube (Snider, Oosterhuis and Kawakami, 2011), reduced levels of attractants in the style that direct the pollen tube to the ovary (Palanivelu and Preuss, 2006), or sugar starvation (Snider, Oosterhuis, Loka, et al., 2011). Pollen germination and tube growth are additional energy demanding process involved in reproduction. While some of this is provided by carbohydrate reserves (in the form of starch) in the pollen grain (Liu et al., 2021), much is provided by the style (as carbohydrates translocated from vegetative tissues; Ferguson et al., 2021), and can therefore again be limited by high temperature impacts on vegetative (source) tissues, or damage to the pistil tissues themselves (Hedhly, 2011; Ferguson et al., 2021).

Even successful fertilisation does not guarantee successful fruit and seed development. Persistent temperature stress during embryo development (Figure 1.2 stage 6) has been shown to increase fruit abortion (Young et al., 2004; Chu and Chang, 2020), reduce fruit or seed size and weight (Barnabás et al., 2008; Pagamas and Nawata, 2008), reduce seed quality (germinability and vigour; Keigley and Mullen, 1986; Hampton et al., 2013; Suriyasak et al., 2020), and affect offspring fitness (Johnsen et al., 2005). In addition, high temperatures can act after seed development and dispersal, reducing the viability and germination of healthy seeds (Figure 1.2 stage 7; Walck et al., 2011; Liu et al., 2015), and affecting the growth and survival of seedlings (Essemine et al., 2010). Furthermore, heat stress during development prior to anthesis can impact male and female reproductive fitness to an extent that might not prevent fertilisation, but instead later affect the development of fruits, the germinability of seeds produced, the fitness of seedlings, and phenological characteristics of the progeny (Higuchi et al., 1998; Aizen and Harder, 2007; Kochanek et al., 2010; Rosbakh et al., 2018). There are therefore many ways in which high temperatures can reduce the production of fruits and viable seeds, and affect seed germination (and seedling establishment), and therefore limit successful reproduction and recruitment.

1.1.4 Current knowledge in wild species

Overall, the available research – focussed particularly on crop species – clearly demonstrates that exposure to high temperatures at any stage (or indeed all stages) of sexual reproduction has the potential to greatly impact reproductive success and recruitment. However, crop species have been bred to grow and reproduce in optimum conditions, selecting for high yield (and related traits) often at the expense of natural resilience to high temperatures, temperature variation, and other abiotic stresses (Kapazoglou et al., 2023). Wild species may therefore show different responses to increasing temperatures, especially if they already experience substantial temperature variation in their natural environments. Nevertheless, climate warming is likely to expose many wild species to temperatures above those often experienced previously, and what impact this will have, particularly on reproduction, is not well understood (Zi et al., 2023). Available research on the reproductive responses of wild species to warming covers a wide range of climatic regions (from artic to tropical); vegetation types (such as heathland, grassland, forest, understory, savannah); growth forms (from herbaceous to woody); life cycles (from annual to perennial); and research methodologies (including *in situ* and *ex situ* experimental warming, transplant experiments, and observational study of natural ecological gradients; for example De Frenne et al., 2011; Bokhorst et al., 2011; Liu et al., 2012; De Frenne et al., 2013; del Cacho et al., 2013; Ratnaningrum et al., 2016; Drake et al., 2019; Chapman et al., 2021). Although, the variety of methodologies and breadth of studies makes it difficult to compare results or draw reliable conclusions.

Ecological gradients (such as elevational gradients) provide useful natural laboratories to study the influence of temperature on species traits (Malhi et al., 2010). Environmental manipulation experiments can complement such observations, allowing us to gain a more mechanistic understanding of the specific impacts of increasing temperatures on species (De Boeck et al., 2015; Frei et al., 2020). Furthermore, they allow the testing of responses to temperatures that may not exist naturally within a species observable range, providing novel insights into the impacts of future climate conditions, and important data for inclusion in (or validation of) ecological models (Cavaleri et al., 2015; Frei et al., 2020). Nevertheless, considering the global diversity of species and ecosystems, experimental warming studies on the reproductive responses of wild species are few. In fact, in a recently published global meta-analysis of the effects of warming on reproductive effort, Zi et al. (2023) found only 61 studies (covering 164 terrestrial plant species) that employed experimental manipulation.

Of such investigations, studies of Arctic and alpine tundra vegetation suggest that reproduction may be improved by warming (particularly in the winter) in colder regions, as earlier snow melt allows for a longer growing season, earlier flowering and a longer fruit and seed development period (Arft et al., 1999; Klady et al., 2011). However, warmer Arctic and alpine sites may respond with greater input into vegetative growth rather than reproductive development (Wookey et al., 1993; Arft et al., 1999), and many temperate species – particularly those that require a winter chilling period to transition from vegetative to reproductive development (Luedeling et al., 2009; Penfield et al., 2021) – may also demonstrate reduced reproduction given a warmer climate (Liu et al., 2012). Although, several temperate studies have detected both positive and negative changes in reproductive outputs of plant species in the same habitat, often in the same study site or experimental plot (Kudo and Suzuki, 2003; Hovenden et al., 2007; De Frenne et al., 2011; Liu et al., 2012; del Cacho et al., 2013; Jacques et al., 2015). Reproductive responses to ecosystem warming therefore vary both spatially and temporally, and are often species-specific (Lambrecht et al., 2007; Hovenden et al., 2007; Liu et al., 2012). Nevertheless, the cross-study meta-analysis by Zi et al. (2023) suggests that climate warming will lead to a global reduction in fruit production, along with a possible increase in seed mass (notably more so for nondominant species). This trend may result from an increased investment in seed quality over quantity, increased photosynthetic activity with warming providing more resources for seed investment, or longer growing seasons providing more time for investment in seed mass (Zi et al., 2023).

Although nominally a global analysis, the dataset of Zi et al. (2023) did not include any studies from the tropics, due to the lack of published research – particularly involving *in situ* experimental warming – on reproduction in tropical species (Feeley et al., 2016; Slot and Winter, 2016). This is perhaps partially due to the logistical difficulties of attempting environmental manipulation in diverse, and sometimes remote environments with extreme climatic conditions (De Frenne, 2015; Cavaleri et al., 2015). Nevertheless, species at lower latitudes could potentially respond quite differently to warming than those at higher latitudes as they may already be experiencing temperatures much closer to their physiological thermal limits (Doughty et al., 2023). This includes, in particular, their thermal limits for reproductive processes (such as seed germination; Sentinella et al., 2020), which, as already discussed in section 1.1.3, may be lower than those of other physiological processes (Hedhly, 2011). Furthermore, a very recent study of the widespread tropical tree *Muntingia calabura* demonstrated a reduction in the viability of pollen produced by flowers exposed to experimental daytime warming of 3.5°C (studying cut branches in controlled greenhouses; Slot et al., 2023). This provides the first direct indication (other than the work within this thesis, which was undertaken before this research was published) that reproduction of tropical woody species (other than crop species) may be negatively impacted by rising temperatures.

No direct investigations of the impacts of temperature on Cerrado species reproduction have been carried out (until this work). Nonetheless, some areas of the Cerrado are already experiencing localised warming as a result of vegetation fragmentation (Pongratz et al., 2006; Camargo et al., 2011) and have therefore been used to study the potential impacts of environmental change. In some instances, localised variations in temperature have been shown to alter reproductive phenology, affecting the synchrony and intensity of reproductive phenophases (Camargo et al., 2011; Athayde and Morellato, 2014; Vogado et al., 2016). These changes can potentially impact plant-animal interactions – such as with pollinators, seed dispersers, and seed predators – and in one example caused a reduction in fruit set through pollinator mismatch (Athayde and Morellato, 2014). However, fragmentation may impose additional pressures on plant species, such as limiting pollinator availability or reducing the genetic pool of individuals (leading to genetic drift), with detrimental effects on their reproductive output and success (Cunningham, 2000; Aguilar and Galetto, 2004; de Almeida et al., 2012; Athayde and Morellato, 2014; Melo et al., 2014; Franceschinelli et al., 2015), making the specific impacts of temperature difficult to distinguish. On the other hand, Vilela et al. (2018) monitored the flowering and fruiting of four woody species of Malphigiaceae in a continuous area of Cerrado for six years, finding that changes in reproductive phenology – which for some species correlated with changes in temperature and precipitation – altered interactions with herbivores and pollinators. This led in some species to changes (both positive and negative) in flower and fruit production, although complex and species-specific (Vilela et al., 2018).

Other research on climate impacts on reproduction in the Cerrado has focussed largely on the impacts of fire and temperature on seed germination and viability (Daibes et al., 2022), possibly as the thermal limits of germination are testable in a laboratory environment (given strong knowledge of the species-specific requirements for seed germination). Due to the high prevalence of fire in the Cerrado (Miranda et al., 2009), seeds of Cerrado species are thought to have a high tolerance to high temperatures and heat shocks (Ribeiro and Walter, 2008; Daibes et al., 2022). Yet, through the integration of their own seed germination experiments and thermal modelling, Correa et al. (2021) demonstrated a likely negative impact of climate warming on germination of seeds in the Cerrado soil seed bank into the future, even under optimistic warming predictions. Furthermore, similar smaller studies (already mentioned in section 1.1.2) have integrated seed experiments with distribution modelling (of individual Cerrado species; Ribeiro et al., 2019; Ferreira et al., 2022), predicting less suitable area for seed germination under warming, with potential consequences for species' ranges and persistence.

Altogether, the overall lack of knowledge about reproduction and the impacts of higher temperatures in wild species – particularly in the tropics – highlights the importance of further investigation, especially in such threatened ecosystems as the Cerrado. While studying natural variation is valuable (and generally requires less physical and monetary investment), integrating this with controlled heating experiments – particularly those carried out *in situ* – is necessary to elucidate the specific impacts of rising temperatures and detect possible thermal thresholds for successful reproduction in wild species (Rustad, 2008; Feng et al., 2018). This data is key to more accurately predicting the impacts that the anticipated environmental change will have on native species in their current ranges, and their ability to migrate to new areas with more favourable climate conditions if necessary (Corlett and Westcott, 2013).

1.1.5 Experimental methods for environmental manipulation

The effects of temperature on woody plant species are often studied using juvenile individuals (seedlings and saplings), which are grown – usually in pots, but sometimes planted out in experimental fields – in controlled environments such as growth chambers and greenhouses (Bolstad et al., 2003; Blessing et al., 2015; Smith and Dukes, 2017; Drake et al., 2019; Hara et al., 2021). However, juveniles and adults can respond differently to environmental change (Cavender-Bares and Bazzaz, 2000; Vitasse, 2013), and while potted or planted individuals (and growth chamber conditions) are easier to manipulate, *ex situ* study can further impact natural plant responses (Kawaletz et al., 2014). Study of mature individuals *in situ* in their natural habitat is therefore likely to provide more realistic data on the responses of established plant communities to environmental change. Nevertheless, such investigations are generally limited to short-stature communities of herbaceous and sometimes shrub species (Kudo and Suzuki, 2003; Hovenden et al., 2007; Lambrecht et al., 2007; Bokhorst et al., 2011; Carter et al., 2020), as environmental manipulation becomes increasingly difficult with increasing size (of the study organism).

Heating entire larger-stature individuals (such as adult trees) *in situ* can require significant inputs of time, physical effort, equipment, and money (Aronson and McNulty, 2009). For this reason, often only the specific organs of interest (such as branches, leaves, or inflorescences) are heated (targeted heating; Nakamura et al., 2010; Doughty, 2011; Slavković et al., 2016; Yamaguchi et al., 2016; Carter and Cavaleri, 2018). This can provide useful and often novel information on the physiological responses of the target organs – such as the photosynthetic responses of leaves of tall forest trees (Yamaguchi et al., 2016; Carter and Cavaleri, 2018) – with less investment of resources. However, it can also result in large variations in the temperatures experienced by target organs (Carter and Cavaleri, 2018) and may not always elicit the same responses as heating entire individuals (Medhurst et al., 2006), as complex and sometimes long-distance signalling pathways (involving the whole tree) are involved in sensing and responding to environmental change (Hasanuzzaman et al., 2013; Belhassine et al., 2019; Delker et al., 2022).

Whether attempting to warm entire individuals or target organs, many methods have been devised and employed globally to experimentally heat soil, air and vegetation *in situ* to investigate the effects of increasing temperatures on individual and ecosystem functioning (Aronson and McNulty, 2009; Chung et al., 2013; De Frenne, 2015). Typically, the methods of ecosystem warming fit into two categories: active, including the use of electrical heat resistance cables, infrared (IR) lamps, and active field chambers (Aronson and McNulty, 2009); and passive, such as greenhouses, plastic tents, and open top chambers (OTCs; Marion et al., 1997). Each method has advantages and disadvantages in relation to the environment in which they are employed, the goals of the experiment, installation and running costs, the scale of the experiment, and confounding factors (or artefacts) specific to each method. Therefore, many factors must be considered in an investigation before deciding on the most suitable warming method.

Passive warming methods are particularly useful in remote areas where there is often no access to an electric power source. The most commonly employed method for *in situ* warming experiments is the OTC (Aronson and McNulty, 2009), which is particularly useful for long-term or smaller-budget experiments due to its low construction and maintenance costs. Classic OTCs are hexagonal plexiglass chambers with inclined walls, which allow transmittance of shortwave solar radiation into the chamber and trap infrared (longwave) radiation, heating the enclosed vegetation (Marion et al., 1997). They also have an open top, which means precipitation can enter as normal and allows mixing of internal and external air. OTCs usually lie on the ground and heat small areas of low-stature vegetation, although modified OTCs have been employed to heat taller plant communities (up to 2 m tall) by up to 1.8°C (during the daytime; Welshofer et al., 2018; Coldren et al., 2019; Chapman et al., 2021). Furthermore, Miserere et al. (2019) used 2 m cuboid OTCs to heat whole olive trees (transplanted in a cleared field) by approximately 3°C during the daytime with the addition of solar heated rocks; although they also employed electric fans, making them not entirely passive.

Tents have also been used to heat larger vegetation. For example, Rodrigo and Herrero (2002) constructed polyethylene tents around whole apricot trees (in an orchard) to study the effects of warming on floral development, increasing mean temperatures by 3°C. Although, maximum temperature differentials between inside and outside the tents were high and they had to be manually opened daily to prevent overheating (Rodrigo and Herrero, 2002). Overall, there are few examples of passive heating methods being used on larger trees (outside of planted field conditions) or in tree canopies. Nevertheless, Yamaguchi et al. (2016) used large OTCs (open top acrylic boxes) built on scaffold towers in an experimental forest (in Japan) to heat tall canopy leaves, although by an average of only 1°C during the daytime. Furthermore, Doughty (2011) found that it was possible to heat branches of Amazon forest trees by approximately 2°C (during sunny periods) by placing rectangular pieces of black plastic below the branches, which absorbed incident shortwave radiation and emitted infrared.

However, all passive chambers suffer from limitations, in particular the inability to control the temperatures within a chamber. There is often high variability in the differential between control (normally ambient) and treatment (chamber) temperatures, and sometimes strong variation within – and between – treatment chambers themselves (Marion et al., 1997). Also, since passive methods generally rely on solar irradiance, heating only takes place during sunny periods. Passive chambers are therefore affected by cloud cover and nocturnal cooling, often leading to greater variation in diurnal temperature ranges than in actively heated experiments (Godfree et al., 2011). Although, additions such as water filled plastic pipes can be inserted into passive chambers to increase the thermal mass, which may reduce nocturnal cooling and stabilise the diurnal temperature range (Godfree et al., 2011). Nevertheless, passive heating structures can also alter wind patterns around vegetation (potentially making growing conditions more favourable); affect internal gas concentrations, precipitation, light levels, or relative humidity (RH); and potentially act as barriers to pollinators, wind pollination, and herbivory (Marion et al., 1997; De Boeck et al., 2012). It is therefore clear that many factors must be considered when designing a passive heating system in order to balance the production of a strong heating effect with the potential chamber impacts on variables other than temperature, as both the strength of heating and unwanted chamber effects tend to increase the more closed a chamber system becomes.

Due to the potential drawbacks of passive heating, a variety of active heating methods have also been developed to experimentally warm vegetation (Aronson and McNulty, 2009). Heat resistance cables, for example, are a common heat source used for on or below ground warming studies, and cables are usually placed in, on, or just above the soil (De Frenne, 2015). However, they have also been used by Nakamura et al. (2010) to heat the branches of tall forest trees by approximately 5°C (above control branch temperature), and Doughty (2011) used heat resistance wires folded in aluminium foil to heat leaves by roughly 2°C (above ambient air temperature). More recently, Slot et al. (2014) used flexible heat rope in a similar way to heat leaves by an average of 3°C, fixing it underneath target leaves using an infrared reflective frame, whereas Carter and Cavaleri (2018) developed a methodology using silicon heating pads to heat leaves (also by 3°C).

Infrared lamps are another common active heat source used for *in situ* warming experiments, potentially favoured for their less localised heating effect, which possibly provides a more realistic simulation of ecosystem warming (Aronson and McNulty, 2009). Although often employed at ground level (for example by Carter et al., 2020), Nakamura et al. (2016) used infrared heat lamps fixed to tall scaffolds built around forest trees (in an experimental forest in Japan) to warm tree canopies by approximately 1°C. Finally, active whole-tree chambers (of 5.5 m tall but easily extended; Medhurst et al., 2006) have been developed and employed in a number of studies to heat entire tall trees (in planted forests; Ryan, 2013; Drake et al., 2019).

The major benefit of active methods is having greater control over treatment temperatures, as heat sources can be connected to monitoring and control systems that can maintain a certain (and relatively constant) temperature differential between treatment and control plots (Aronson and McNulty, 2009). Active heating also enables heating at night, reducing diurnal variation in temperature. While active whole-tree chambers potentially allow the strongest control over treatment conditions (Medhurst et al., 2006), they can also suffer from similar limitations as passive chambers – such as inhibiting pollinator movement or affecting gas concentrations – and therefore every internal environmental variable must be carefully controlled. Furthermore, their rigid structure perhaps makes them unsuitable to set up in natural ecosystems with complex vegetation structure. Active systems also often require large amounts of electricity to power the heating elements and monitoring devices (De Frenne, 2015), which sometimes necessitates access to an AC power source (Kimball et al., 2018; Carter and Cavaleri, 2018). This is not often attainable for studies carried out in remote field sites (for instance for studying the reproductive responses of Cerrado trees *in situ*), yet a combination of portable power solutions could be utilised, for example solar panels and batteries (as in Doughty, 2011). Nevertheless, such equipment can lead to high start-up costs, requires sufficient electronics expertise, and can be inclined to malfunction under harsh environmental conditions (such as those of tropical savannahs).

Overall, there are a numerous possible methods for experimental manipulation of temperature that can be employed *in situ* to study the potential responses of natural vegetation to predicted climate warming. Whether targeted or whole-tree, active or passive, the feasibility of each approach depends predominantly on the characteristics of the study species (in particular its height) and environment (which may render certain methodologies completely impractical, for example through lack of access to electricity) as well as the overall objectives (for example studying reproduction as opposed to photosynthesis primarily requires the heating of inflorescences as opposed to leaves). However, it is clearly important to also consider the type of expertise available, and of course the research budget, which may limit the use of more costly – mainly active – methodologies.

1.2 Overview of the work

1.2.1 Aim and objectives

The overall aim of this thesis is to begin to understand the effects that increasing temperatures will have on the ability of tropical woody species to reproduce, in particular those in the heavily threatened yet understudied Cerrado biome of Brazil. To achieve this the objectives were:

1) to design and test novel methodologies for heating developing flowers of woody species *in situ* in remote areas;
2) to successfully employ these methodologies in an area of typical Cerrado vegetation to investigate the potential impacts of higher temperatures on the floral development and successful reproduction of native woody species *in situ*;
3) to evaluate the potential impacts of rising temperatures by quantifying any effects of the heating treatments on the production of viable pollen, fruits, or seeds of native Cerrado species.

1.2.2 Approach and strategy

This work first required the development of suitable methods, primarily one for heating the developing flowers of Cerrado trees *in situ* at the intended field site (Figure 1.1), which was an area of typical Cerrado vegetation located close to the Nova Xavantina campus of the University of Mato Grosso (UNEMAT) in Brazil (remaining the same through all chapters of this thesis). Simplified methods (not requiring the use of high-tech equipment) were also developed for analysing the viability of pollen samples (that were to be taken from study individuals in the field). These methodologies were designed and tested at the University of Leeds, further tested and developed in Brazil, and then implemented – with the collaboration of professors and students at UNEMAT – in the first direct investigation of the impacts of heating on Cerrado species reproduction (chapter two).

As previously discussed (in section 1.1.5), there are numerous advantages and disadvantages of targeted and whole-tree heating, and of active and passive methodologies, and many potential possibilities were first considered. Nevertheless, a targeted passive heating methodology was chosen and designed (to heat individual inflorescences *in situ*; described in chapter two and more fully in appendix A), inspired partially by the successful passive heating of branches of tropical forest species in Brazil by Doughty (2011). Passive heating was favoured in particular due to the lack of access to an electrical power source in the Cerrado field site, and the difficulties of employing portable power solutions such as solar panels in the harsh and open environment of the Cerrado (although solar irradiance would have been sufficient). Furthermore, passive heating was more achievable within the limitations imposed by the research budget, and achieving a strong heating effect (of ~3°C during the daytime, in line with mid-range climate projections; IPCC, 2021; Ferreira et al., 2023) passively, without imposing the confounding factors involved in closed chamber designs (previously discussed), was considered more likely through targeted heating than whole-tree heating. Reproductive output (in this instance considered in terms of viable pollen and fruit production) was also expected to vary significantly between individuals (Augspurger, 1983; Melo et al., 2014), and for that reason targeted heating was favoured as it allowed the comparison of treatment and control inflorescences on the same individual. Additionally, whole-tree heating was considered less practically achievable given the anticipated height of the study individuals, the complex nature of the Cerrado vegetation (as opposed to uniform fields in which whole-tree chambers had previously been employed), and the remoteness of the field site.

Nevertheless, following the first *in situ* study of Cerrado tree reproduction under heating (chapter two), the reliability of the results was questioned due to the physical connection – and therefore potential lack of independence – of treatment and control inflorescences on the same individual (discussed in chapter two). For this reason and given the levels of heating that were achieved through the targeted passive methodology, it was developed into a passive method for heating entire trees *in situ* in remote locations such as the Cerrado. This passive whole-tree heating methodology was tested and employed in a short-term demonstrative study of the photosynthetic and respirational acclimation of a Cerrado shrub species under heating (chapter three). Finally, it was utilised in a larger-scale whole-tree heating experiment to study the reproductive responses of two woody Cerrado species to heating (chapter four), supplemented by an initial investigation (in the previous year) of pollen viability in the study individuals under ambient conditions.

1.3 Thesis structure

As explained above, the chapters that make up this thesis follow the progression of my research, from the initial method development and experiments on one Cerrado species; to improving on the methodologies; and finally carrying out a larger-scale investigation over two years of pollen viability and fruit set in – and the impacts of heating on – two Cerrado species. The thesis is therefore formed of five chapters: the preceding introductory chapter (chapter one), outlining the reasoning for this research and current knowledge; the three data chapters (chapters two, three, and four), which are in the form of manuscripts at various stages of publication (see earlier pages on intellectual property and publications); and a final concluding chapter (chapter five). Each further chapter is outlined briefly below.

Chapter two: Impacts of higher daytime temperatures on viable pollen and fruit production in common Cerrado tree *Byrsonima pachyphylla* (Malpighiaceae). This chapter summarises the primary investigation, in which the newly developed targeted passive heating methodology was employed to heat individual inflorescences of Cerrado tree *Byrsonima pachyphylla* to study the potential impacts of higher daytime temperatures on its reproductive success.

Chapter three: A novel *in situ* passive heating method for evaluating whole-tree responses to daytime warming in remote environments. This chapter details the design and testing of the novel whole-tree passive heating methodology that was developed from the targeted heating methodology. It further demonstrates its use in a short-term investigation of the impacts of daytime warming on photosynthesis and respiration in Cerrado shrub *Erythroxylum suberosum*.

Chapter four: Contrasting responses of pollen and fruit to whole-tree heating in tropical savannah species. This chapter summarises the larger-scale investigation of reproduction in two woody Cerrado species (namely *Byrsonima pachyphylla* and *Davilla elliptica*), carried out over two years. This included (in the first year) an investigation of pollen viability in the study species under ambient conditions and (in the second year) a whole-tree heating experiment studying the impacts of daytime warming on Cerrado species reproductive success through the utilisation of the methodology detailed in chapter three.

Chapter five: Synthesis and conclusions. This chapter summarises and draws together the work of all three chapters; discusses the findings as a whole, their implications in a wider context, and potential limitations of the works; and suggests future directions for investigation.

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Chapter 2

Impacts of higher daytime temperatures on viable pollen and fruit production in common Cerrado tree *Byrsonima pachyphylla* (Malpighiaceae)

2.1 Abstract

The Brazilian Cerrado – a complex mixture of grassland and woodland – is considered a biodiversity hotspot that provides many ecosystem services. Already threatened by deforestation, it could experience temperature increases of 1–5°C by 2100, potentially impacting plant growth and function. Sexual reproduction is considered very sensitive to high temperatures, which are known to cause reductions in pollen viability and fruit set in a range of tropical crops. However, impacts on non-crop species, particularly larger woody species, are largely unknown. To investigate the impacts of higher daytime temperatures on native Cerrado species reproduction, we carried out direct *in situ* heating of developing inflorescences of common tree species *Byrsonima pachyphylla*. Novel passive heating chambers were positioned around inflorescences from an early bud stage until fruit maturation, raising daytime air temperatures by 3–4°C. We quantified the effects on pollen viability (through *in vitro* pollen germination and staining) and fruit set (of hand-pollinated flowers), finding no impact of heating on viable pollen production; however, fruit set was significantly reduced. This could have serious implications for future species recruitment and community structure, as well as insect and animal food chains, especially if representative of other Cerrado species. To the best of our knowledge this is the first *in situ* direct heating experiment on native Cerrado species, providing initial insights into the effects that increasing temperatures could have on future reproductive success, and demonstrating that fruit (and seed) production is an important factor to consider when quantifying the impacts of climate change on tropical ecosystems.

2.2 Introduction

Originally covering around 2 million km^2 (Ratter et al., 1997), the Cerrado (Brazilian savannah) is the second largest biome in South America after the Amazon rainforest. This tropical savannah extends from the South-eastern border of Amazonia across much of Brazil and comprises a range of vegetation types from dense grassland, to shrubland and woodland (Ratter et al., 1997). It is vital for maintaining large South American watersheds (Lima and Silva, 2007), and it contains such high levels of species richness and endemism, while facing significant anthropogenic pressure, that it is categorised as a ‘hotspot’ for biodiversity conservation (Myers et al., 2000; Klink and Machado, 2005). Historically undervalued and therefore understudied (Overbeck et al., 2015; Murphy et al., 2016), the Cerrado is less protected than the Amazon region, and remaining intact vegetation is under immediate threat from deforestation – driven by land use change for agriculture (Garcia and Ballester, 2016; Strassburg et al., 2017) – and potentially also from climate change (Velazco et al., 2019; Silva et al., 2019). According to the IPCC (Intergovernmental Panel on Climate Change), regional climate models predict temperature increases of between 1 and 5°C across the Cerrado biome by the end of this century (compared with pre-industrial levels) based on very low to high greenhouse gas emission scenarios (IPCC, 2013). Furthermore, significant temperature increases have already been detected in the Cerrado at both local and regional scales (Hofmann et al., 2021). However, our understanding of the effects of these increasing temperatures on Cerrado vegetation is severely limited.

While many aspects of plant growth and function can be impacted by higher than average temperatures (Hasanuzzaman et al., 2013), sexual reproduction is thought to be one of the most sensitive stages of plant development to temperature (Hedhly, 2011). Changes in temperature can alter the timing, extent and success of flowering and fruiting events (Butt et al., 2015; Sage et al., 2015), potentially taking species outside of their regeneration niches (the environmental conditions required to successfully produce, disperse and germinate seeds, and establish seedlings; Grubb, 1977). This can have repercussions for species persistence, plant-plant interactions, community structure and ecosystem functioning (Brooker, 2006; Vilela et al., 2018), not to mention interactions with plant pollinators, herbivores, frugivores, and human populations (Butt et al., 2015; Vilela et al., 2018; Rabeling et al., 2019). The impact that higher temperatures will have on reproduction in Cerrado vegetation is therefore an important area of research that could provide new insight into the future structure and composition of native plant communities, and perhaps strengthen the evidence base for keeping climate warming to a minimum.

In crop species, where the aim of cultivation is to produce fruits and seeds, the effects of temperature on reproduction have long been studied, and many stages from pre- to post-anthesis (flower opening) have been shown to be sensitive to temperature (reviewed in Sage et al., 2015; Lohani et al., 2020). High temperatures can reduce the number of flowers initiated per plant (Albrigo and Galán Saúco, 2004); affect floral tissue development (Djanaguiraman et al., 2018); or accelerate development, resulting in a shorter effective pollination period (Distefano et al., 2018) or an asynchrony between male and female development (Rodrigo and Herrero, 2002). They can also cause an increase in the number of buds, flowers or fruits aborted during development (Erickson and Markhart, 2001; Young et al., 2004), and overall reduce the quantity and quality of fruit and seed produced (Jagadish, 2020). Male reproductive development is widely considered to be the most vulnerable to high temperature stress (Giorno et al., 2013; Sage et al., 2015; Pacini and Dolferus, 2016; Santiago and Sharkey, 2019; Lohani et al., 2020), which can induce irregular development or degradation of male reproductive tissues (Bennici et al., 2019); disrupt microsporogenesis and pollen development (Wang et al., 2017); and cause pollen morphological abnormalities (for example of the exine wall; Djanaguiraman et al., 2018). High temperatures can also disrupt the supply and regulation of water, nutrients and metabolites to the anther tissues and developing pollen (Paupière et al., 2014; Pacini and Dolferus, 2016), and disrupt reactive oxygen species (ROS) homeostasis leading to oxidative damage (Djanaguiraman et al., 2018). These can lead to reductions in the quantity or viability of pollen produced or released at anthesis, and reduced thermotolerance of pollen grains post-anthesis (Paupière et al., 2014). Reductions in fruit set (the proportion of flowers that develop into fruit) at high temperatures have also been largely attributed to losses in pollen viability leading to fertilisation failure (Rosbakh et al., 2018). However, several studies highlight that high temperatures can impact almost all developmental stages of the male and female reproductive tissues and gametophytes, potentially reducing reproductive success (Giorno et al., 2013; Jagadish, 2020; Wang et al., 2021).

These impacts are far less well studied in non-crop species. Direct *in situ* investigation of plant responses to temperature are largely limited to short stature plant communities such as herbs and shrubs (Kudo and Suzuki, 2003; Hovenden et al., 2007; Lambrecht et al., 2007; Bokhorst et al., 2011; Elmendorf et al., 2012; Liu et al., 2012). Larger woody species are more often studied as young plants or grafts in controlled greenhouses, growth chambers or planted field experiments (Johnsen et al., 1995; Webber et al., 2005; Drake et al., 2019), as *in situ* environmental manipulation becomes increasingly difficult when investigating larger adult trees. However, such experiments would provide more reliable indications of the impacts of climate change on established communities (Chung et al., 2013). Nonetheless, higher temperatures have been shown to affect flowering and fruiting phenology or reproductive output in several temperate species, although impacts are complex and species-specific (Kudo and Suzuki, 2003; Hovenden et al., 2007; Lambrecht et al., 2007; De Frenne et al., 2011; Liu et al., 2012; del Cacho et al., 2013; Jacques et al., 2015; Marchin et al., 2015; Ma et al., 2022). A recent meta-analysis of simulated warming experiments suggests that global fruit production will decrease with climate warming, while seed mass may increase (Zi et al., 2023). However, no studies from the tropics were included in this analysis, and little is known about the potential effects of increasing temperatures on reproduction in tropical species.

Tropical research on reproductive responses to temperature has focused mainly on changes in phenology (for example Vilela et al., 2018; Numata et al., 2022). Furthermore, studies on native Cerrado species are largely limited to seed experiments (reviewed in Daibes et al., 2022) or those utilising ecological gradients, for example looking at edge effects on phenology and fruit set as the Cerrado landscape becomes increasingly fragmented (Camargo et al., 2011; Athayde and Morellato, 2014; Melo et al., 2014; Vogado et al., 2016). Nevertheless, as high temperatures negatively impact reproduction in several tropical tree crops (such as peach, mango, date palm, *Annona* and *Citrus* species; Sukhvibul et al., 2000; Hedhly et al., 2005; Nava et al., 2009; Lora et al., 2011; Lora et al., 2012; Slavković et al., 2016; Alves Rodrigues et al., 2018; Distefano et al., 2018; Shafqat et al., 2021; Liu et al., 2023), it might be expected that other tropical woody species, such as those native to the Cerrado, could also be negatively affected.

To investigate how the reproduction of woody Cerrado species will be affected by increasing temperatures, we carried out a direct heating experiment *in situ* on inflorescences of native tree species *Byrsonima pachyphylla* A.Juss. (Malpighiaceae) using novel passive heating chambers. To the best of our knowledge, this was the first direct heating experiment attempted *in situ* on any native Cerrado vegetation. We hypothesised that inflorescences exposed to higher temperatures would: (a) produce pollen of lower viability (analysed through two complementary techniques of pollen germination and staining); and (b) have lower percentages of fruit set (given the same pollination treatment).

2.3 Methods

2.3.1 Study site and species

This study was conducted between June and November 2019 in an area of Cerrado típico or ‘typical Cerrado’ (a subcategory of Cerrado *sensu stricto*; Ribeiro and Walter, 2008) in the Bacaba Municipal Park reserve (14°42'28.8" S, 52°21'03.9" W) in Nova Xavantina, Mato Grosso, Brazil. Typical Cerrado is the dominant vegetation type of the reserve (Marimon-Junior and Haridasan, 2005), comprised of tree and shrub woodland with 20–50% tree cover of average height 3–6 m (Ribeiro and Walter, 2008). The climate is classified as Aw (Köppen system; Peel et al., 2007), with a pronounced wet (mid-October to April) and dry season, with peak temperatures coinciding with the end of the dry season (August to October; Figure A3.5). The region is characterised by 1300–1500 mm annual precipitation and average monthly temperatures of 25°C (Brazilian Meteorological Service; Marimon et al., 2010), although it has experienced recent and rapid warming and heatwaves of increasing strength and frequency (Marimon et al., 2020; Tiwari et al., 2021). According to the long-term ERA5-Land dataset (Muñoz Sabater, 2019; data assimilation based dataset with 9 km resolution, see Appendix A1 for details), 2010–2020 mean annual daytime and night-time temperatures locally were 28.6 ± 0.2°C (*SD*) and 24.6 ± 0.2°C respectively.

Byrsonima pachyphylla (also known by synonym *Byrsonima crassa*; Francener, 2023) is a widespread tree of the Cerrado biome (Bridgewater et al., 2004) and among the ten most common species of the typical Cerrado vegetation in Bacaba Park (Mews et al., 2011). Its pollen and floral oils are collected by pollinators (predominantly bees; Boas et al., 2013); its fruits are consumed by animals, for example birds (Purificação et al., 2014) and to some extent humans (Passos, 2023); and its leaves and bark have medicinal properties (Sannomiya et al., 2005; Guilhon-Simplicio and Pereira, 2011), making it both socially and ecologically important. Flowering can occur throughout the dry season, but peaks in July and August (Silvério and Lenza, 2010). The fruits begin to develop soon after flowering, maturing in November. Flowers are zygomorphic with five yellow petals, oil glands at the base of the calyx, and a diameter of ~13 mm (Figure 2.1A); bisexual with ten stamens and three carpels; and borne on terminal, racemose inflorescences ~8 cm long (Figure 2.1C). Most flowers open in the morning around 06:00 and are receptive for one day (Boas et al., 2013), changing colour to orange and red on the days after anthesis. *B. pachyphylla* is self-incompatible and bee pollinated (Boas et al., 2013), with stigmas covered by a thin cuticle that prevents pollen from adhering and germinating unless broken by pollinators.

Figure 2.1 *Byrsonima pachyphylla, showing (A) individual flower; (B) developing inflorescence at the stage at which the heating chambers were set up; and (C) inflorescence during flowering with yellow buds nearing anthesis, yellow flowers open on the day of anthesis, and orange and red flowers that have opened on previous days.*

2.3.2 Heating experiment

Five healthy adult individuals of *B. pachyphylla* (Table A2.4) were chosen for the experiment, that were at least 20 m apart and had at least six separate inflorescences at the same, early developmental stage (Figure 2.1B; the earliest stage possible given the timing of *B. pachyphylla* flowering and delays initiating the experiment). Heating chambers were set up around three developing inflorescences per individual (T1–T3). Three further inflorescences were chosen per individual as unheated controls (C1–C3), each physically close to a heated (treatment) inflorescence (although not on the same branch) to reduce variation in microclimatic variables other than temperature between treatments and their corresponding controls. Heating chamber installation took place 12th–18th June, after which all five individuals were visited every day in anticipation of flowering.

2.3.3 Measurement of climatic variables

On each *B. pachyphylla* individual, temperature sensors (Maxim DS18B20+; accurate to ± 0.5°C) were placed at two treatment inflorescences and at their two corresponding control inflorescences, while temperature and relative humidity (RH) were measured together (using Adafruit DHT22 sensors; accurate to ± 0.5°C and ± 2–5% RH) at the third heated inflorescence and its corresponding control. Sensors on each tree were powered by a 12 V battery and an Arduino microcontroller recorded data to a memory card every minute. To reduce the effects of solar radiation on readings, temperature sensors were painted white and sheathed in white heat resistant cable sleeving, and all sensors were housed in custom radiation shields (see Figure A3.1B).

2.3.4 Heating methodology

Midday solar irradiance at the field site is high and repeatedly reached over 1,000 W · m^{-2} in 2019 (according to a weather station within the reserve; Spectrum Technologies, Inc.). To heat the treatment inflorescences *in situ*, we developed a novel, elevated open-topped chamber (~25 × 25 cm) with a telescopic support structure (Figure A3.1B), designed to passively heat the air surrounding the inflorescences (and developing flower buds) during sunny periods. Each chamber was comprised of two parts, a square base 75% covered with black polythene (part A; Figure A3.1A), and a transparent acetate upper section (part B; Figure A3.1A), with the top left completely uncovered. A detailed description and discussion of the heating methodology is included in supporting information (Appendix A1).

Between the initiation of heating and the end of flowering (approximately eight weeks), the control inflorescences experienced average daytime (06:30 – 18:30 local time; BRT = UTC -3) and night-time (18:30 – 06:30) air temperatures of 29.4 ± 1.5°C (*SD*) and 18.7 ± 2.1°C respectively, reaching mean maximum (daytime) temperatures of 36.7 ± 2.1°C (Table A2.1). Treatment chamber air temperatures correlated closely with controls (Figure 2.2A) but rose an average of 2.7 ± 1.0°C higher in the daytime (Table A2.1). The strongest and most consistent period of heating (peak heating) occurred between 09:00 and 17:00 each day, when treatment temperatures rose to 4.0 ± 1.4°C above controls (Table A2.1). Mean treatment temperatures were 32.1 ± 2.0°C in the daytime and 36.8 ± 2.5°C during peak heating, reaching mean maximum temperatures of 42.3 ± 2.9°C (Table A2.1). Control daytime and night-time RH was 50 ± 6% and 83 ± 11% respectively (Table A2.2). Treatment RHs also correlated closely with controls, but fell on average 8 ± 3% lower during peak heating (Figure 2.2B, Table A2.2), reflecting the pattern of temperature (Figure 2.2).

Following flowering, temperatures rose seasonally throughout August until the onset of the wet season (late-September; Figure A3.4A; Table A2.3), reaching mean daily maximums of 42.0 ± 2.4°C in September (controls). While these temperatures exceeded those experienced by the treatment inflorescences during flowering, the chambers continued to produce a strong heating effect (Table A2.3), despite increasing cloud cover towards the wet season (Figure A3.4A). Treatment inflorescences were therefore exposed to even higher maximum temperatures of 47.4 ± 2.5°C on average in September, during fruit development.

According to the ERA5-Land dataset since 2010 (see Appendix A1 for details) 2019 was a warmer than average year (for most months; Figure A3.5). Furthermore, the standard deviation around monthly mean daytime temperatures (interannual variation) over the last ten years was on average ± 0.9°C (and lower for June–September; Figure A3.5). Therefore, it is likely that the treatment chambers produced temperatures above those experienced by local individuals during the same period in previous years.

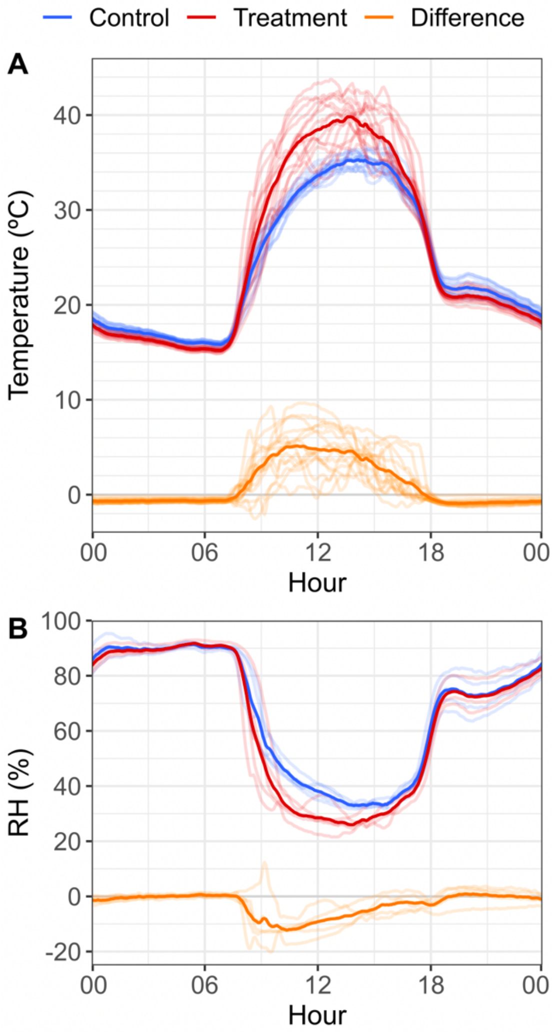

Figure 2.2 *Mean diurnal patterns of (A) temperature and (B) relative humidity (RH) for control and treatment inflorescences, with differences in temperature and RH calculated between the treatment inflorescences and their corresponding controls. Values averaged across every 5-minute interval of each day of recorded data, between the initiation of heating and the end of flowering. Faded lines show mean diurnal patterns for each inflorescence.*

2.3.5 Preparation and pollen sampling

To investigate the effect that heating during floral development had on *B. pachyphylla* pollen viability, pollen samples from treatment and control flowers were analysed using two complementary techniques: *in vitro* pollen germination, in which successfully germinated pollen grains are presumed to be viable; and differential staining of aborted and non-aborted pollen grains, in which the non-aborted pollen grains are presumed to be viable (Dafni and Firmage, 2000). Prior to experiment initiation, variations of the classic Brewbaker and Kwack (1963) pollen germination medium and germination conditions were tested and optimised for *B. pachyphylla* (Table A2.5), and a protocol for *in vitro* pollen germination was developed based on the hanging-drop method (Shivanna and Rangaswamy, 1992). *B. pachyphylla* pollen germinated well at 30°C (as in some other tropical species; Reddy and Kakani, 2007; Youmbi et al., 2011; Hebbar et al., 2018), and pre-hydration of pollen was not required. A modified version of Alexander’s staining solution (Peterson et al., 2010) was also optimised for *B. pachyphylla* pollen and local lab conditions (Table A2.6).

Flowering began on June 24th. Before 10:00 (local time) on each day from 3rd–19th July, pollen samples were collected from any of the 15 treatment and 15 control inflorescences (three treatment and three control inflorescences per individual) displaying flowers at anthesis. Two anthers per flower were taken for testing by pollen staining, and two for testing by *in vitro* pollen germination, from up to three newly opened flowers per inflorescence per day sampled. The aim was to collect repeat samples from each inflorescence on at least five different days, however variations in flowering meant 2–10 samples were taken per inflorescence (6 on average). Each treatment bud was exposed to heating for 14–35 days before pollen sampling at anthesis (25 on average).

2.3.6 In vitro pollen germination

Each sample (anthers) taken for analysis by *in vitro* pollen germination was macerated with the chosen germination medium (Table A2.5). 40 µl drops of each solution were transferred to petri dishes and inverted over moistened filter paper. The dishes were sealed with petroleum jelly and incubated in BOD incubation chambers at 30°C in the dark for 24 hours, always starting by 12:00 on the day of sample collection. Drops were then transferred to microscope slides and observed under an Eclipse E200 microscope (Nikon, Tokyo, Japan) paired with a Nikon DS-Fi2-U3 camera system at 100 × magnification. At least four images (unique microscopic fields) were taken per sample (Figure A3.6A), and all grains were counted per image (mean of 51). Pollen grains were classified as germinated if the pollen tube was at least equal to the diameter of the pollen grain (Dafni and Firmage, 2000). Sample pollen viability was estimated as the percentage of all grains in each image that had germinated, averaged over all images per sample.

2.3.7 Differential pollen staining

Each sample taken for analysis by pollen staining was macerated with the optimised staining solution (Table A2.6) and 30 µl of this mixture was transferred to a microscope slide, heated gently (protocol as in Peterson et al., 2010) and observed using the same microscope and camera system as above, in order to differentiate between aborted (cell walls stained blue/purple) and non-aborted (presumed viable; cell protoplasm stained orange/red) pollen grains (Figure A3.6B). At least four images were taken per sample, and all grains counted per image (mean of 134). Sample pollen viability was estimated as the percentage of all grains per image that stained orange/red (non-aborted), averaged over all images per sample. Image analysis (classification and counting of pollen grains) for stained and germinated samples was carried out manually using ImageJ software (Version 1.51; Rasband, 2018).

2.3.8 Hand-pollination and fruit set analysis

Every day during the flowering period (24th June – 9th August) all flowers at anthesis on all experimental inflorescences (treatments and controls) were hand-pollinated to provide them with an equal chance of pollination, recording the number of flowers pollinated. The pollen for this was collected each day from two newly-opened flowers on each of three non-experimental individuals of *B. pachyphylla* (six flowers in total), using the same individuals throughout the experiment. The pollen was mixed together (mixture on the first day showing 78% pollen viability analysed by staining) and applied vigorously using a thin paintbrush in order to break the stigmatic cuticle (necessary for fertilisation), while care was taken not to damage the flowers.

Following the flowering period, experimental inflorescences were contained within net bags (sold for agricultural fruit protection) to prevent the loss of fruits through falling or herbivory. Heating chambers remained in place. Mature fruits were collected and counted in November, and fruit set for each inflorescence was calculated as the percentage of pollinated flowers that went on to produce mature fruits.

2.3.9 Data analysis

Pollen samples with less than 200 pollen grains were excluded from the viability analysis due to low reliability. Percentage pollen viability results for each sample estimated by germination and staining were compared by linear regression to assess the reliability of the methods. For both the pollen germination and staining results, mean percentage pollen viability was calculated for each inflorescence (15 control and 15 treatment inflorescences) from repeat sample results, and used to calculate treatment-level results. Means for the control and treatment inflorescences on each tree (*n* = 5) were also calculated weighted by the number of samples taken per inflorescence. Percentage fruit set was calculated at the inflorescence level and simply averaged to estimate tree- and treatment-level results. Coefficients of variation (*CV*s) were calculated for tree-level pollen viability and fruit set control results to quantify spread in relation to their means.

As sample sizes were small and tree-level results were highly variable, paired Wilcoxon signed-rank tests were used to assess the significance of differences in pollen viability and fruit set between the treatment and control groups. As our hypotheses were directional – that heating would induce loss of pollen viability and loss of fruit set – we tested for these effects using one-tailed hypothesis tests. We repeated our analyses at the tree-level and inflorescence-level. Although inflorescences on the same tree may not be independent due to their physical attachment, uncertainty exists about the level of autonomy of branches on a tree (for example when considering carbon allocation; Auzmendi and Hanan, 2020). Additionally, Slavković et al. (2016) have demonstrated independent responses of separate inflorescences to multiple temperature treatments on one tree, which supports the idea that separate inflorescences on one individual can be considered as independent reproductive units. Therefore, we present the results considering both the inflorescences (*n* = 15) and trees (*n* = 5) as independent units. To check for an effect of length of heating on pollen viability of the treatment samples, we tested whether the slope of regression of pollen viability versus heating length differed from zero for each tree. All data analyses were carried out in R version 4.1.1 (R Core Team, 2021).

2.4 Results

2.4.1 Pollen viability

Pollen viability results were generally lower when estimated by pollen germination analysis than by staining (Figure 2.3); however, there was a strong linear relationship between the viability results of each sample estimated by the two methods ($R = 0.89$, $p < 2.2e^{-16}$; Figure A3.7), demonstrating their complementarity and increasing confidence in the pollen viability results when taken together.

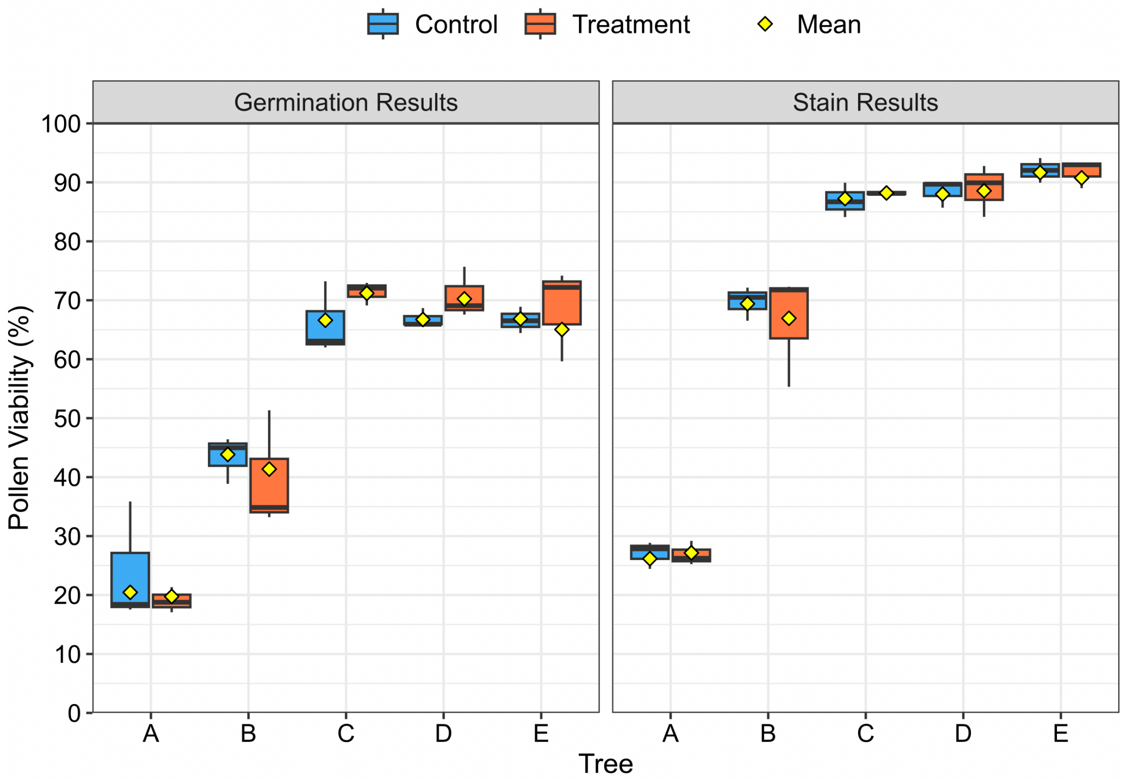

Figure 2.3 *Pollen viability for the three control and three treatment inflorescences on each tree (A–E) based on in vitro pollen germination (left) and pollen staining (right) analyses. Tree-level control and treatment means (yellow diamonds) were calculated from the inflorescence values, weighted by the number of repeat samples taken per inflorescence.*

Pollen viability results were influenced by the individual tree (Figure 2.3), with high *CV*s in the tree-level control results estimated by both the pollen germination and staining analysis methods (39% and 38% respectively). Pollen viability was on average 0.6 ± 2.1% (*SE*) higher in the treatment inflorescences than in their corresponding controls when tested by germination, and 0.4 ± 1.4% lower in the treatment inflorescences when tested by staining (Table 2.1). No significant impact of heating was found when comparing the treatment and control results (all p values > 0.5), whether estimated by germination or staining, and whether testing mean values for each inflorescence (n = 15) or each tree (n = 5). Treatment sample pollen viability showed a significant negative correlation ($p < 0.01$) with length of heating in one out of five trees (per viability analysis method); however, which tree differed depending on the method (tree B for germination, E for staining; Figure A3.8). All other relationships between length of heating and pollen viability varied in strength and direction depending on the tree and viability analysis method used (Figure A3.8), suggesting no overall impact.

Table 2.1 *Mean pollen viability and fruit set (% ± SE) for the treatment and control inflorescences, and the differences in results between the treatments and their corresponding controls.*

	Pollen viability analysed by		**Fruit set**
	Germination	**Staining**	
Controls	53.4 ± 4.8	72.8 ± 6.5	16.6 ± 4.1
Treatments	53.9 ± 5.8	72.5 ± 6.6	8.0 ± 3.3
Differences	0.6 ± 2.1	-0.4 ± 1.4	-8.5 ± 3.6

2.4.2 Fruit set

Fruit set was low in all inflorescences (maximum 52%) and also influenced by tree (Figure 2.4), showing a 90% *CV* in the tree-level control results. Fruit set was on average 8.5 ± 3.6% (*SE*) lower in the heated inflorescences than in their corresponding controls (Table 2.1). This difference was significant ($p = 0.01$) when analysed by Wilcoxon signed-rank tests considering the inflorescences independently ($n = 15$, $V = 89$, $R = 0.6$; one-tailed test), and when comparing the mean values for treatment and control fruit set per tree (not assuming independence of branches within an individual) although more marginally so ($p = 0.03$; $n = 5$, $V = 15$, $R = 0.9$; one-tailed test).

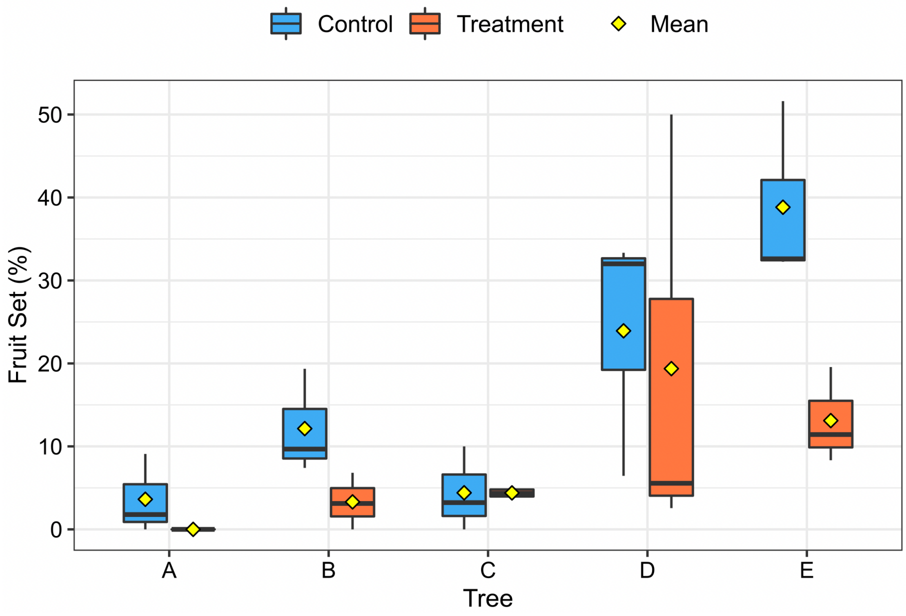

Figure 2.4 *Fruit set for the three control and three treatment inflorescences on each tree (A–E).*

2.5 Discussion

This study was the first direct investigation of the impacts of higher daytime temperatures on the reproductive success of any Cerrado species. Our novel passive heating chambers were effective in heating developing inflorescences and fruits of *B. pachyphylla* by 3–4°C during the day, in line with mid-range predictions of future warming across the Cerrado (IPCC, 2013). Furthermore, considering the extent of local interannual variation in temperature, and that 2019 was a particularly warm year (Figure A3.5), we likely exposed treatment inflorescences to daytime temperatures above those often experienced previously. However, contrary to our hypothesis, we found no impact of higher daytime temperatures during floral development on the viability of pollen produced by *B. pachyphylla*. Conversely, and perhaps more importantly, fruit production was significantly negatively affected by higher temperatures, which could have a direct impact on the success (or failure) of *B. pachyphylla* reproduction and recruitment in a future, hotter Cerrado.

2.5.1 Pollen viability

Pre-anthesis temperatures above 25°C have been shown to negatively impact the development of viable pollen in many species, including several tropical tree crops (Higuchi et al., 1998; Kozai et al., 2004; Nava et al., 2009; Lora et al., 2012; Distefano et al., 2018; Bennici et al., 2019). As control temperatures during bud development in our study often rose higher than this, it is clear that local individuals of *B. pachyphylla* are able to reproduce at temperatures above the threshold for many species previously studied. Furthermore, temperatures in the Cerrado rise strongly towards the start of the wet season (Figure A3.5; Table A2.3) – for example reaching daily maximum temperatures of ~ 42°C in September 2019 – and fires are also common (Miranda et al., 2009). Many native species are therefore physically and physiologically adapted to withstand extreme temperatures (Simon and Pennington, 2012; Araújo et al., 2021). It is worth noting that although *B. pachyphylla* may have evolved to flower in July to avoid exposure to higher temperatures during this more sensitive period of development, it might have also developed an effective heat stress response to deal with the extreme temperatures that come later in the year (Qu et al., 2013), which could have acted to maintain physiological homeostasis in the treatment inflorescences during pollen development (Rieu et al., 2017; Raja et al., 2019).

In any case, as the treatment temperatures did not impact pollen viability, male reproductive tissue and pollen grain development in *B. pachyphylla* appears resistant to temperature stress up to a very high threshold (> 40°C). Additionally, as Nova Xavantina is at the higher end of temperatures experienced within the Cerrado (Araújo et al., 2021), our results suggest that pollen viability of *B. pachyphylla* in other areas will not be affected by increasing daytime temperatures for some time. Sustained pollen viability could be advantageous for the reproductive success of *B. pachyphylla* – and nutrition of its associated pollinators (Boas et al., 2013; Yeamans et al., 2014) – into the future as temperatures increase, especially considering potential reductions in pollen availability due to further habitat fragmentation (Melo et al., 2014; Franceschinelli et al., 2015) or pollinator mismatches resulting from phenological changes (Rabeling et al., 2019; Gérard et al., 2020). However, while pollen viability was unaffected in our study, high temperatures can also impact pollen grain quality, potentially affecting seed viability and seedling fitness (Higuchi et al., 1998; Rosbakh et al., 2018). This study could therefore be expanded upon by pollinating flowers with pollen developed at high temperatures – in addition to control pollen, as used here – and comparing the fitness of seeds and seedlings produced, which might reveal delayed impacts of high temperature development on viable pollen and its progeny.

It is important to note that the initial stages of pollen development (namely microsporogenesis) have been identified as the most sensitive to temperature in many species (Hedhly, 2011; Sage et al., 2015), and some species initiate their pollen development months before anthesis (Szalay et al., 2019). While the treatment buds were heated from an early stage, we were unable to initiate the experiment prior to inflorescence development. It is therefore possible that heating began too late to impact the most sensitive stages of male development, although there is no previous research on the timing of bud developmental stages in *B. pachyphylla* or related species. However, microsporogenesis has been found to occur closer to anthesis in other tropical tree species, for example 10–6 days before anthesis in clementine (Distefano et al., 2018; Bennici et al., 2019). Additionally, racemose inflorescences (such as those of *B. pachyphylla*) develop successionally, meaning buds that opened later in the experiment were heated from an earlier developmental stage and were therefore more likely to be affected by the treatment. Nevertheless, we did not detect a significant overall impact of length of heating on treatment sample viability (Figure A3.8). Therefore we surmise that the late initiation of the heating treatment did not reduce its impact on pollen development, and instead that viable pollen development in *B. pachyphylla* is indeed resistant to very high temperatures.

2.5.2 Fruit set

Agreeing with our second hypothesis, we found a negative impact of higher daytime temperatures on fruit set in *B. pachyphylla*, as reported in a range of crop species (Sage et al., 2015; Lohani et al., 2020; Shafqat et al., 2021) and in keeping with the trend found in temperature manipulation studies from outside of the tropics (Zi et al., 2023). Although a lack of suitable viable pollen can lead to reduced fruit set (Knight et al., 2005), hand-pollination of our experimental flowers should have removed pollen limitation in our experiment. However, high temperatures during pollination have been shown to reduce fruit set in several species by affecting the germination of viable pollen on the stigma or growth of the pollen tube, preventing fertilisation (Sukhvibul et al., 2000; Distefano et al., 2018; Liu et al., 2023). While *B. pachyphylla* pollen germinated readily at 30°C *in vitro* (high for many studied species; Sage et al., 2015; Beltrán et al., 2019), experimental inflorescences regularly experienced temperatures higher than this during flowering – although more often and to a greater degree for the treatment inflorescences (Figure A3.3) – which may have limited *in vivo* pollen germination or tube growth, leading to the reduced fruit set. Nevertheless, some tropical species have shown continued (although reduced) pollen germination up to 42°C (coconut; Hebbar et al., 2018). Furthermore, pollen germination is thought to be less sensitive to temperature stress than pollen development (Higuchi et al., 1998; Distefano et al., 2018; Chu and Chang, 2022), which we have shown to be resistant to the treatment temperatures. This suggests that reduced fruit set in the treatment inflorescences was more likely a result of high temperature impacts on female development than pollen germination.

Although male reproductive development is widely considered to be more sensitive to temperature stress (Hedhly, 2011; Rosbakh et al., 2018), evidence of the sensitivity of female development is accumulating (Lohani et al., 2020; Wang et al., 2021). High temperatures can disrupt the timing of anthesis or the development of the pistil tissues, leading to underdeveloped embryo sacs, deformed structures, reduced stigma receptivity, reduced ovule longevity, altered levels of pollen tube attractants, imbalances in ROS and important hormones, and disruption of complex male/female interactions (Hedhly, 2011; Wang et al., 2021). Individually or collectively these can cause reductions in fruit and seed development, as observed in a number of woody tropical and sub-tropical crops (including apricot, peach, sweet-cherry and olive; Rodrigo and Herrero, 2002; Hedhly et al., 2003; Hedhly et al., 2005; Nava et al., 2009; Benlloch-González et al., 2018). High temperatures post-fertilisation can also impact fruit and seed set and the development of viable seeds (as seen in rapeseed and chili pepper; Young et al., 2004; Pagamas and Nawata, 2008), although the isolated effects are not well documented.

Having not performed microscopic investigation of experimental flowers throughout the experiment, we cannot know which stages of reproductive development were most affected to cause the observed reduction in fruit set. Nor can we comment on the impact of temperature on seed viability as attempts to analyse seeds were unsuccessful. Nevertheless, our results suggest that increasing temperatures will lead to a reduction in fruiting in *B. pachyphylla*, which could have serious repercussions for reproductive success and recruitment into the future, especially considering fruit set in the control inflorescences was already low (even given hand-pollination). This might also limit *B. pachyphylla*'s ability to disperse and migrate in the face of changing environmental conditions (McNichol and Russo, 2023), with potential consequences for species persistence, and knock-on effects for associated animal and insect species (Purificação et al., 2014; Butt et al., 2015; Rabeling et al., 2019). Furthermore, higher temperatures are likely to act both during and after fruit and seed production and dispersal, potentially affecting not only the quantity and quality of seed produced, but the longevity and germinability of seeds in the seed bank (well-studied in comparison; Daibes et al., 2022), and seedling establishment (Marimon et al., 2020; Nottingham et al., 2023), further exacerbating their effects on successful reproduction and recruitment.

As limited to one species, this study provides only an initial insight into the potential impacts of higher temperatures on Cerrado species reproduction. Less common species, those which flower and fruit at different times of year, or those that are common in different areas or vegetation types of the Cerrado may respond differently, and much further investigation is needed. Nevertheless, variation in species' reproductive responses to temperature will likely lead to changes in Cerrado community structure and species richness (Vilela et al., 2018). Understanding and integrating the impacts of temperature at all stages of growth and development into future ecological models is therefore important to help elucidate the possible consequences of higher temperatures on ecosystem structure and function into the future (Simon et al., 2013; Velazco et al., 2019; Correa et al., 2021; Ferreira et al., 2022).

2.5.3 Study methodology

It is important to consider that some aspects of our heating methodology may have influenced the results, for example the targeted heating of inflorescences, or sampling control and treatment inflorescences on the same tree. Both male and female reproductive development require large amounts of energy, relying mainly on foliar photosynthesis and metabolite transport, which can be disrupted by heat-induced reproductive tissue damage, or by high temperature impacts on vegetative tissues (Ferguson et al., 2021; Liu et al., 2021). As inflorescence heating did not impact pollen viability, we can infer that it did not damage reproductive tissues enough to disrupt metabolite transport during pollen development, and this was therefore unlikely the reason for the reduction in fruit set. Still, by leaving the remainder of each tree unheated we removed the potential effects of heat stress on vegetative tissues. However, heating entire trees *in situ* is challenging, and the experiment was designed to make pairwise comparisons between treatment and control inflorescences on the same tree due to the expectation of finding high variability in pollen viability and fruit set values at the tree level (Augspurger, 1983; Melo et al., 2014), which was indeed the case.

While we were unable to heat at night due to our choice of passive heating methodology, we expect that the addition of night-time heating would have caused further reductions in fruit set (Echer et al., 2014). It is however possible that this would have elicited a different response in pollen viability – which has been shown to be affected by high night-time temperatures alone (Djanaguiraman et al., 2013) – although we demonstrated a resilience in the development of *B. pachyphylla* pollen up to very high temperatures. Finally, while the RH in the treatment chambers was slightly reduced in the daytime, RH changes with temperature, and there is far less research on the impacts of RH alone on reproductive development (Pacini and Dolferus, 2016). Furthermore, the future climate of the Cerrado appears to be not only hotter but also drier (Hofmann et al., 2021), meaning these experiments may more realistically reflect the effects that future climate warming will have on reproduction in *B. pachyphylla*, and perhaps other Cerrado species.

In conclusion, this study provides an initial and important insight into how increasing temperatures might impact Cerrado species reproduction and recruitment in the future. While we observed no impact of higher daytime temperatures during floral development on viable pollen production in *B. pachyphylla*, we found a negative impact on fruit set, which could have serious repercussions for future species persistence as the climate of the Cerrado changes to become hotter and drier (Hofmann et al., 2021). Higher temperatures may affect not only fruit production, but also seed viability and germination, and seedling establishment (Rosbakh et al., 2018; Marimon et al., 2020; Daibes et al., 2022; Nottingham et al., 2023). Furthermore, this impact will likely be exacerbated in the future by localised heating due to land use change (Rodrigues et al., 2022) and increased fragmentation of suitable habitat leading to further reductions in fruit set (Melo et al., 2014), not to mention the impacts of climate change and fragmentation on pollinators themselves and the likelihood of plant-pollinator mismatches due to changes in reproductive phenology (Vilela et al., 2018; Rabeling et al., 2019; Gérard et al., 2020). Cerrado species already show low levels of fertility and fruit set (Athayde and Morellato, 2014; Melo et al., 2014; Montesinos and Oliveira, 2015), and – although only one species was studied here – if the relationship found between higher temperatures and fruit set is replicated in other Cerrado species, it could have significant implications for species richness, community assemblage and ecosystem function in a future, hotter climate. The effects of temperature on reproduction are therefore important factors to consider when quantifying the impacts of climate change on the Cerrado biome, and should be integrated to a greater degree when modelling future species distributions (Borghetti et al., 2021; Correa et al., 2021; Ferreira et al., 2022). Much could therefore be gained by expanding upon this study and investigating threshold temperatures for reproduction in other Cerrado species and areas, and indeed other tropical ecosystems.

2.6 References

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Chapter 3

A novel *in situ* passive heating method for evaluating whole-tree responses to daytime warming in remote environments

3.1 Abstract

Background: Many significant ecosystems, including important non-forest woody ecosystems such as the Cerrado (Brazilian savannah), are under threat from climate change, yet our understanding of how increasing temperatures will impact native vegetation remains limited. Temperature manipulation experiments are important tools for investigating such impacts, but are often constrained by access to power supply and limited to low-stature species, juvenile individuals, or heating of target organs, perhaps not fully revealing how entire or mature individuals and ecosystems will react to higher temperatures.

Results: We present a novel, modified open top chamber design for *in situ* passive heating of whole individuals up to 2.5 m tall (but easily expandable) in remote field environments with strong solar irradiance. We built multiple whole-tree heating structures (WTHSs) in an area of Cerrado around native woody species *Davilla elliptica* and *Erythroxylum suberosum* to test the design and its effects on air temperature and humidity, while also studying the physiological responses of *E. suberosum* to short-term heating. The WTHSs raised internal air temperature by approximately 2.5°C above ambient during the daytime. This increased to 3.4°C between 09:00 and 17:00 local time when thermal impact was greatest, and during which time mean internal temperatures corresponded closely with maximum ambient temperatures. Heating was consistent over time and across WTHSs of variable size and shape, and they had minimal effect on humidity. *E. suberosum* showed no detectable response of photosynthesis or respiration to short-term experimental heating, but some indication of acclimation to natural temperature changes.

Conclusions: Our WTHSs produced a consistent and reproducible level of daytime heating in line with mid-range climate predictions for the Cerrado biome by the end of the century. The whole-tree *in situ* passive heating design is flexible, low-cost, simple to build using commonly available materials, and minimises negative impacts associated with passive chambers. It could be employed to investigate the high temperature responses of many understudied species in a range of complex non-forest environments with sufficient solar irradiance, providing new and important insights into the possible impacts of our changing climate.

3.2 Background

The Cerrado (Brazilian savannah), is the second largest biome in South America (originally covering approximately 2 million km^2; Ratter et al., 1997), is considered a 'hotspot' for biodiversity conservation (Myers et al., 2000) and is essential for maintaining major South America watersheds (Lima and Silva, 2007). However, this important biome – like many significant non-forest woody ecosystems – has been historically undervalued and understudied (Overbeck et al., 2015; Murphy et al., 2016), and is experiencing the highest rates of land use change in Brazil (Garcia and Ballester, 2016). It is also under immediate threat from climate change (Velazco et al., 2019; Hofmann et al., 2021), with regional climate models predicting a temperature increase of between 1 and 5°C across the Cerrado biome by the end of this century (IPCC, 2013). Such increases in temperature have the potential to affect many aspects of plant growth and function (Hasanuzzaman et al., 2013), with repercussions for important plant-plant and plant-animal interactions (Brooker, 2006; Butt et al., 2015), making it essential to improve our understanding of high temperature responses of native vegetation. However, the majority of direct temperature manipulation experiments investigate low-stature species (such as tundra vegetation; Elmendorf et al., 2012), young saplings, or seedlings (for example Slot and Winter, 2017b; Fauset et al., 2019) that can be grown in moveable units and climate-controlled chambers. Far fewer study adult individuals or larger, woody species, especially *in situ*, although such investigations would help provide more accurate predictions of how established communities and natural ecosystems will respond to climate change.

Targeted heating of organs, for example of leaves or branches (Doughty, 2011; Slot et al., 2014; Carter and Cavaleri, 2018), is a common method for studying temperature effects on larger individuals. However, this can lead to high variation in temperatures experienced by target organs (Carter and Cavaleri, 2018), and may not realistically indicate the effects of climate warming, as temperature responses involve complex signals sometimes incorporating the whole plant (Qu et al., 2013; Hasanuzzaman et al., 2013). However, heating whole individuals typically requires greater inputs of time, equipment, and money, therefore few whole-tree *in situ* warming experiments have taken place (reviewed in Chung et al., 2013), with even fewer in the tropics (Aronson and McNulty, 2009; Cavaleri et al., 2015), likely due to the added difficulties of establishing experiments in often remote and harsh field environments.

In situ warming methods broadly fit into two categories: active, including using electrical heat resistance cables, infrared lamps, and active field chambers (Aronson and McNulty, 2009; Chung et al., 2013); and passive, including tents (Rodrigo and Herrero, 2002) and variations on open top chambers (OTCs; Marion et al., 1997). Although active methods can provide greater control over temperature and enable night-time warming (Aronson and McNulty, 2009), they can also overshoot target temperatures with detrimental effects (such as leaf-scorching; Carter and Cavaleri, 2018), and often require large amounts of electricity, necessitating access to AC power or a combination of portable power solutions, such as solar panels and batteries (as in Doughty, 2011). Equipment can lead to high start-up costs, require electronics expertise, and be at risk of theft or malfunctioning over long-term experiments in remote and harsh environments. Conversely, passive warming methods that utilise solar radiation are often more appropriate in remote field sites where power access is often limited, and when portable power systems are impractical or prohibitively expensive.

OTCs, the most commonly employed method for passive warming (Aronson and McNulty, 2009), are particularly useful in long-term or smaller-budget studies, having low construction and maintenance costs. Traditionally OTCs are short and hexagonal in design, heating small areas of low-stature vegetation (Marion et al., 1997). However, Chapman et al. (2021), Welshofer et al. (2018) and Coldren et al. (2019) each employed modified OTCs to heat taller plant communities (up to 1.2, 1.5 and 2 m tall respectively) by approximately 1.8°C during the daytime, and Miserere et al. (2019) used 2 m cuboid OTCs to heat whole olive trees (transplanted in a cleared field) by approximately 3°C during the daytime using solar heated rocks, although they also used electric fans. Chambers can also affect growing conditions by altering wind patterns; affecting internal gas concentrations, precipitation, light and humidity; and obstructing pollination and herbivory (Marion et al., 1997; Aronson and McNulty, 2009). However, OTCs traditionally have inclined sides or partially covered tops to aid heating, and uncovering the top completely (as in Coldren et al., 2019; Chapman et al., 2021) can reduce such impacts.

To investigate how Cerrado shrubs and trees growing *in situ* (possibly already reaching their upper thermal limits; Sentinella et al., 2020) will respond to daytime temperature increases they may experience by the end of this century, we developed a passive methodology for heating entire individuals of up to 2.5 m tall (larger than previously described methods and easily expandable) *in situ* by approximately 3°C during the daytime, in line with mid-range climate predictions for the Cerrado (IPCC, 2013). Our novel *in situ* whole-tree passive heating structure (hereon referred to simply as whole-tree heating structure or WTHS) was designed to work in areas of low-canopy savannah and transition woodland (or similar areas with strong solar radiation), be low-cost and simple to build, use commonly available materials, and minimise the aforementioned artefacts of passive chambers.

The objective of this study was to test and demonstrate the functioning of our novel WTHS in the field. To further illustrate its usefulness we also carried out a short-term experiment investigating the photosynthesis and leaf dark respiration responses of Cerrado shrub *Erythroxylum suberosum* A.St.-Hil. (Erythroxylaceae) to *in situ* heating. Respiration typically increases exponentially with temperature, approximately doubling with every 10°C, giving a Q_{10} (or proportional change in respiration rate given a 10°C temperature increase) of approximately 2.0 (Atkin and Tjoelker, 2003). Photosynthesis increases with temperature up to an optimum temperature (T_{opt}) at which the rate of photosynthesis peaks (A_{opt}) and then declines until zero at T_{max} (Sage and Kubien, 2007). Studies have suggested that tropical species are growing at temperatures close to their T_{opt} (Slot and Winter, 2017a) and if temperatures increase above these values, photosynthesis may be reduced while respiration may continue to increase, leading to a reduction in the carbon available for growth, and possibly in the carbon storage potential of tropical ecosystems (Slot et al., 2014). Acclimation of respiration (through a reduction in respiration rates at a given temperature or in Q_{10}; Atkin and Tjoelker, 2003) or of photosynthesis (through an increase in T_{opt} or A_{opt}; Slot and Winter, 2017a) could help to reduce this imbalance, but there is limited evidence on whether tropical species, particularly in non-forest ecosystems, are capable of this acclimation over long or short timescales. Our study on *E. suberosum* demonstrates the possibilities of using our WTHS to gain insight into these and other important and complex temperature responses of understudied woody species in the Cerrado and similar remote but ecologically important non-forest ecosystems, such as the tapia woodlands of Madagascar and other neotropical savannahs (Murphy et al., 2016).

3.3 Materials and methods

3.3.1 Field site

This study was conducted in the Bacaba Municipal Park (14°42'28.8" S, 52°21'03.9" W), Nova Xavantina, Brazil, where the dominant physiognomy is typical Cerrado (Cerrado *sensu stricto*; Marimon-Junior and Haridasan, 2005) comprised of tree and shrub woodland, with a discontinuous upper layer of vegetation (tree cover 20 – 50%, average height 3 – 6 m; Ribeiro and Walter, 2008). The climate classification is Aw under the Köppen system, with a pronounced dry season (May – September), annual precipitation of 1300 – 1500 mm, and average monthly temperature of 25°C according to the Nova Xavantina INMET (Brazilian Meteorological Service) climate station. In 2019, midday solar irradiance was 760 w/m^2 on average, reaching up to 1170 w/m^2, based on nearby WatchDog weather station data (Spectrum Technologies, Inc.).

3.3.2 Structural Design

3.3.2.1 Prototype whole-tree passive heating structure (WTHS)

Our WTHS (Figure 3.1) was developed to utilise solar radiation to passively heat whole individuals of woody species in semi-open environments like typical Cerrado. To test its functioning in a field situation, we built the prototype WTHS in a Cerrado location (Figure 3.1A) around a 2.4 m tall (23.8 cm diameter at 30 cm) adult individual of *Davilla elliptica* A.St.-Hil. (Dilleniaceae), a dominant woody species both locally (Marimon-Junior and Haridasan, 2005) and across much of the Cerrado biome (Ratter et al., 2003) with ethnopharmacological uses (Sousa et al., 2020). The individual was chosen due to its average size within the local population, but otherwise at random.

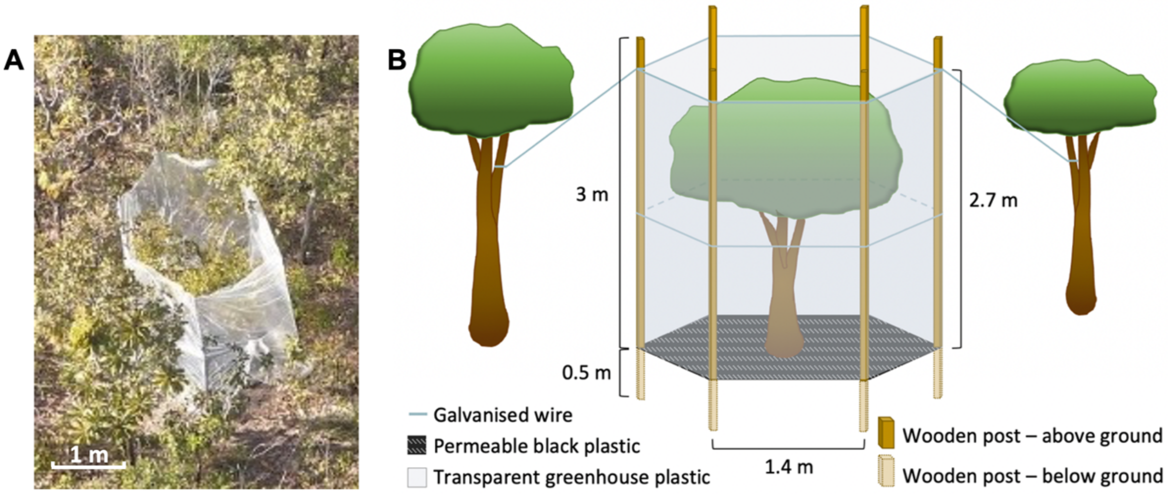

Figure 3.1 *The novel, whole-tree in situ passive heating structure (WTHS). (A) Drone image of the prototype built in the Cerrado around an individual of D. elliptica. (B) Diagram depicting shape, size and materials.*

The WTHS was designed with six sides (Figure 3.1) similar to traditional OTCs (Marion et al., 1997). Six 3.5 m long wooden posts (3 x 5 cm cross-section, with 30 mm U-shaped nails hit partially into them at heights of 1.8 and 3.4 m) were positioned 1.4 m apart around the chosen individual of *Davilla elliptica*, producing a hexagon with an overall perimeter of 8.4 m (Table 3.1) and diameter (from corner to corner) of 2.8 m. The posts were buried 50 cm deep, and galvanised steel wire was thread through the U-shaped nails at the top and middle of each post, joining the posts together to stabilise the structure and create the basic frame (Figure 3.1B). Translucent white polythene film designed for greenhouses (4 m wide, 100 µm thick with UV protection, locally known simply as 'plástico para estufa') was held at 2.7 m (30 cm higher than the tree) and attached to each post in turn using 8 mm staples, creating the sides of the WTHS. The final edge of the plastic was attached in a temporary manner using wire, allowing easy detachment of the lower half, enabling the opening of a 'door' into the WTHS when required for testing (as seen on a later WTHS in Figure 3.2B). Excess plastic at the bottom of the structure sides was covered with loose earth to seal the WTHS and prevent movement or tearing of the plastic on windy days. More wire was attached between the tops of the posts and nearby vegetation like guy ropes, providing extra stabilisation (Figure 3.1B). Black polythene sheeting (as used in construction) was spread out around the tree, across the whole base of the WTHS (Figure 3.1B), and holes were punched in it (taking care to avoid the root system) to allow movement of water to the soil underneath.

Table 3.1 *Measurements for the prototype and three four-sided WTHSs (S1 – S3).*

Whole-tree heating structure	**Length of side (cm)**						**Total perimeter length (cm)**	**Height (cm)**	**Cross-sectional area of base (m^2)**	**Internal volume of WTHS (m^3)**
	1	**2**	**3**	**4**	**5**	**6**				
Prototype	140	140	140	140	140	140	840	270	5.09	13.74
S1	160	165	160	165	-	-	650	210	2.58	5.41
S2	130	135	255	200	-	-	720	280	2.93	8.19
S3	210	260	175	185	-	-	830	310	4.19	12.99

Figure 3.2 *Four-sided WTHSs in situ in the Cerrado. (A) Aerial image of WTHSs S2 and S3 in situ in the Cerrado, with the position of control individuals C2 and C3 marked with umbrellas. (B) Photograph of WTHS S2 partially opened for taking measurements for the analysis of plant responses to temperature.*

The black plastic base of the WTHS was designed to function as a passive heat source during sunny periods, absorbing incident solar radiation and emitting it as thermal radiation, while polythene film sides allowed high transmittance of shortwave, but low transmittance of longwave radiation, reducing thermal radiative loss (Balocco et al., 2018). The sides were built perpendicular to the ground and the top left completely open (as in Coldren et al., 2019; Chapman et al., 2021), unlike in other OTC designs with inclined sides (Marion et al., 1997; Welshofer et al., 2018), or partially covered tops (Miserere et al., 2019). This design aimed to maximise light transmission into the WTHS; avoid rain shadowing; and permit air movement between the inside and outside, preventing overheating and reducing the impact of the WTHS on gas exchange of the enclosed tree, the natural movement of pollinators or herbivores, and on microclimatic variables other than temperature (such as humidity); all of which are classic disadvantages of using chambers.

3.3.2.2 Experimental WTHSs

To further demonstrate the function and possible uses of our WTHS, we conducted a short-term experiment on the gas exchange acclimation response of *E. suberosum* (a woody Cerrado shrub) to *in situ* heating. For this we built three more WTHSs (S1, S2 and S3) in the Cerrado, each around an individual of *E. suberosum* (T1, T2 and T3 respectively; size data in Appendix B: Figure B2.1). These WTHSs were built using the prototype design, except with only four sides (Figure 3.2), sized specifically to accommodate the smaller individuals (Table 3.1) or to encompass obstructing vegetation (WTHS S3). The plastic walls of each WTHS were erected at least 30 cm above the tallest branch of each individual.

3.3.3 Abiotic measurement

Temperature and relative humidity (RH) data were recorded every minute using DTH22 sensors (Adafruit, NYC, USA; accurate to ± 0.5°C and ± 2% RH) housed inside solar radiation shields (Windspeed Limited, Rhyl, UK), controlled by Arduino UNO (www.arduino.cc). To determine the impact of a WTHS on the internal microclimate, one sensor was placed at the centre of the WTHS, just above the crown of the enclosed individual (to avoid shading by the plant), while ambient temperature and RH were measured by a sensor placed above a nearby, similarly sized individual of the same species (acting as a control). Ambient air temperature, RH and solar irradiance were also measured every 15 minutes by the nearby WatchDog weather station.

Temperature and RH measurements for the prototype WTHS and nearby control individual of *D. elliptica* were taken continuously from June 9th to 27th 2020. Measurements taken inside the three four-sided WTHSs (S1 – S3) built around individuals of *E. suberosum* (T1 – T3) were compared with ambient readings taken at three corresponding control individuals (C1 – C3). One pair of individuals was monitored at a time and the sensors were rotated between the three WTHSs (and their corresponding controls) over three weeks from August 4th 2020 (during the heating experiment). Climate data collected while WTHSs were open (for testing) were removed before analysis.

3.3.4 Analysis of microclimate data

Temperature and RH measurements were averaged for every 15 minutes. Absolute water vapour content of the air (absolute humidity; AbsH) and vapour pressure deficit (VPD) were calculated from the temperature and RH data, according to the ideal gas law and Buck's improved equation for saturation vapour pressure (Buck Research Manual, 1996).

Mean diurnal patterns of internal and external temperature, RH and VPD were produced for each WTHS. Temperature and RH differences (between inside each WTHS and its corresponding control) were calculated for the daytime (06:30 to 18:30 local time), night-time (18:30 to 06:30), and between 09:00 and 17:00 each day (observed as the period of day with the strongest, most consistent levels of heating). We also compared temperature measurements with ambient temperatures recorded by the local weather station, and mean diurnal patterns of the relationships between them were calculated to enable estimation of temperatures for any WTHS on any day that direct measurement was not possible.

3.3.5 Investigating plant responses to short-term in situ heating

To explore the suitability of our WTHS for direct field study, we conducted an *in situ* temperature manipulation experiment (July 27th to August 23rd 2020) to assess whether photosynthesis and dark respiration of *E. suberosum* could acclimate to the higher daytime temperatures reached inside the WTHS on a short time scale. *E. suberosum* is a woody shrub species common to the study area (Marimon-Junior and Haridasan, 2005) and many parts of the Cerrado (Ratter et al., 2003). It was chosen due to this prevalence, its importance (having multiple medicinal uses; Restrepo et al., 2019), and because at the time of the study in the latter half of the dry season, the leaves of *D. elliptica* (the species enclosed in the prototype WTHS) were not healthy enough for studying metabolism. Six individuals of *E. suberosum* were chosen for the study based on their similar size of ~2 m tall and 3 cm DSH (diameter at stump height or 30 cm; Figure B2.1) and leaf health. Three of these were randomly selected to be heated (treatment individuals; T1, T2 and T3), and the other three were designated as controls (C1, C2 and C3).

On the first morning of the experiment, photosynthesis and respiration temperature response curves were measured for the first treatment individual (T1), before the first four-sided WTHS (S1) was constructed around it. Physiological measurements were always taken in the morning (between 08:00 and 11:30) using two LI-6400XT portable photosynthesis systems (LI-COR Biosciences Inc., Nebraska, USA); one with a fluorometer chamber head (6400-40; for photosynthesis measurements), and one with an LED chamber head (6400-02B; for respiration measurements). Physiological measurement methodology and analysis are described in detail in Appendix B. On the second morning, the same measurements were made for the first control individual (C1). This pattern was repeated for the remaining four trees (T2, C2, T3 and C3) and two WTHSs (S2 and S3). Each individual was re-measured 7, 14 and 21 days after the first measurements were made (and after heating began for the treatment individuals). The last set of respiration measurements (C3, day 21) was removed from the analysis as wind caused the leaf to rip during measurement.

3.4 Results

3.4.1 Impact of WTHS on microclimate

Internal air temperature inside each WTHS correlated closely with ambient temperature, but rose higher in the daytime with increased irradiance (Figure 3.3A). The mean difference between internal and ambient temperature (heating effect) during the daytime was 2.51°C (± 2.03°C *SD*) averaged across all four WTHSs, with little variation from day to day (*SD* between days of ± 0.38°C) or structure to structure (Figure 3.4B), although the strength of the heating effect varied between dawn and dusk. Each WTHS consistently produced and maintained a heating effect close to our target of 3°C between 09:00 and 17:00 (Figure 3.3A), averaging 3.42°C across all WTHSs (± 1.43°C *SD*; Figure 3.4A). Again this varied little from day to day (*SD* between days of ± 0.47°C) or structure to structure (Figure 3.4A), except in WTHS S3 where heating fell around 14:00 (Figure 3.3A), likely due to localised shading.

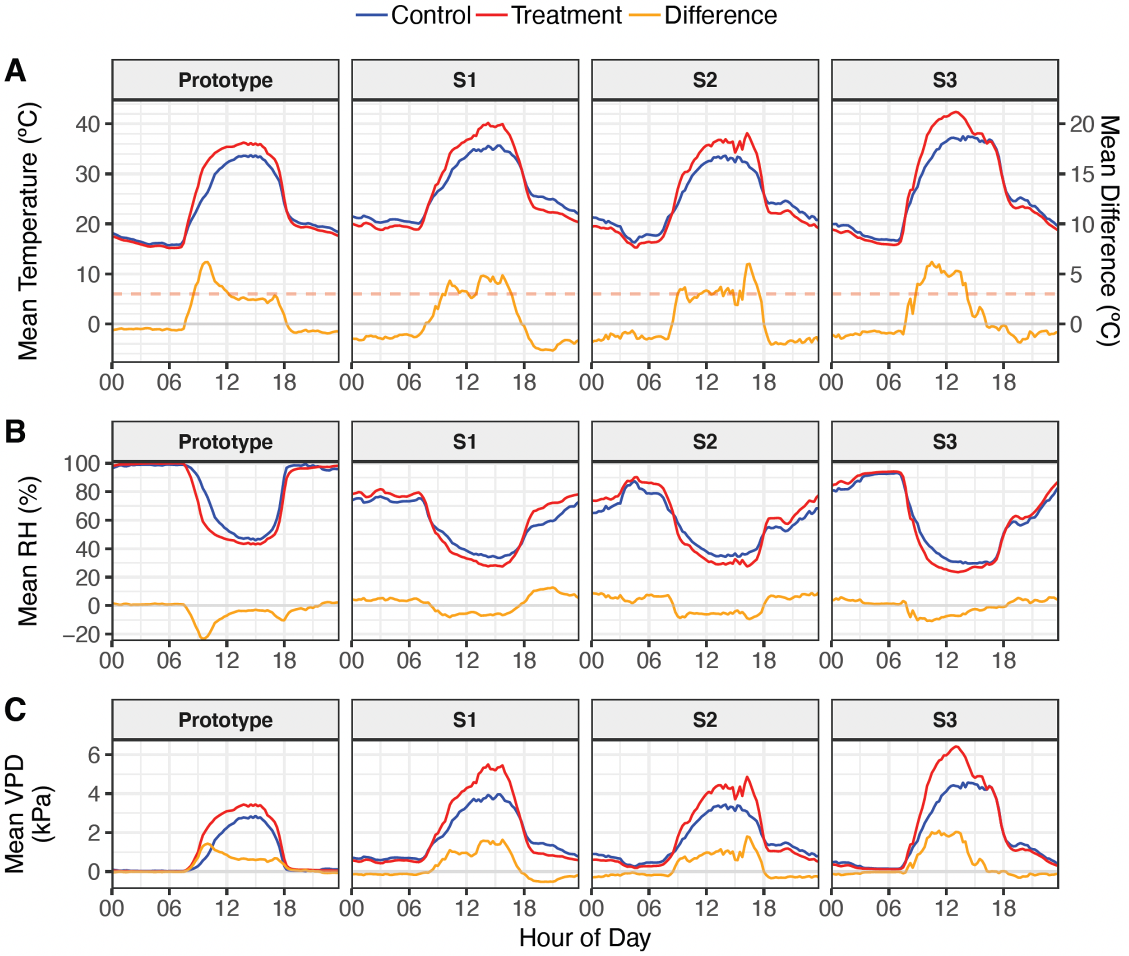

Figure 3.3 *Mean diurnal patterns of (A) temperature; (B) relative humidity (RH); and (C) vapour pressure deficit (VPD); calculated from data recorded at treatment individuals inside each of the four WTHSs (the prototype, S1, S2 and S3; red lines), and their relative controls (blue lines), with the differences calculated between the two (orange lines). In (A) temperature difference is given on the right-hand axis for greater definition, and the dashed line indicates the target of 3°C.*

Maximum temperature differences generated by the WTHSs each day ranged between 4.80 and 7.66°C depending on the day and the structure (Figure B2.2). The relationship between the minimum, mean and maximum temperatures recorded at the control individuals (between 09:00 and 17:00) was replicated very effectively within the WTHSs, approximately 3°C higher, and mean internal temperatures repeatedly corresponded with maximum external temperatures (Figure B2.2).

There was no heating effect at night (between 18:30 and 06:30; Figure 3.4C), and temperatures were on average 0.97°C (± 0.46°C) lower inside the WTHS than outside. This cooling effect was smaller in WTHS S3 than in the other four-sided WTHSs, and smallest in the prototype (Figure 3.4C), the largest WTHS (Table 3.1).

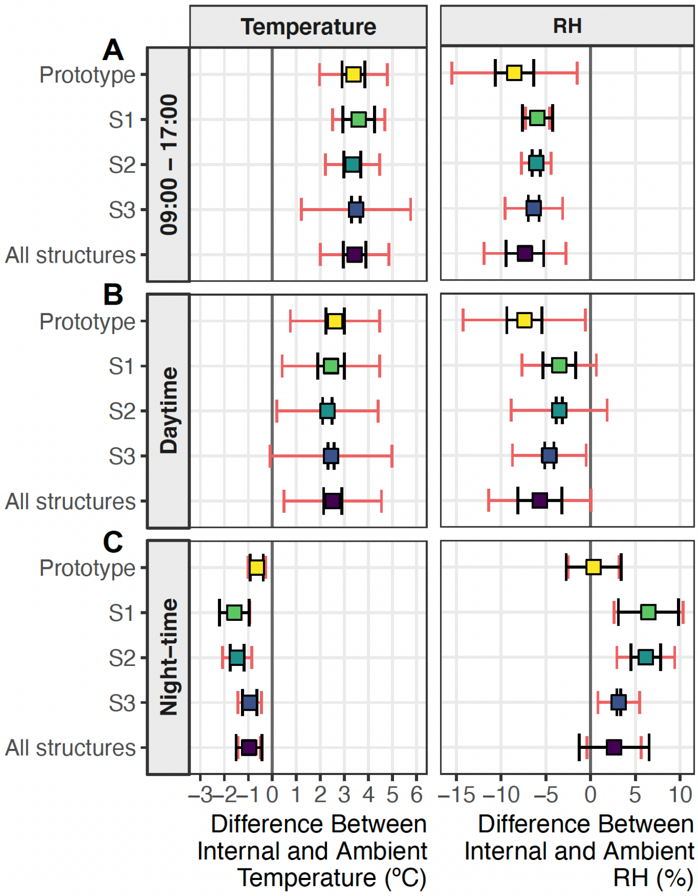

Figure 3.4 *Mean differences in temperature and RH between inside each WTHS (prototype, S1, S2, S3) and their relative controls during given time periods, including average values for all four WTHSs together. Mean differences were calculated for (A) 09:00 to 17:00 (period of strongest heating), (B) daytime (06:30 to 18:30), and (C) night-time (18:30 to 06:30). Red error bars are the average (over all days of measurement) of the daily standard deviation of the mean difference in temperature or RH. Black error bars are the standard deviation of the daily mean differences over all days of measurement.*

The diurnal patterns of RH reflected those of temperature, showing lower RH inside each WTHS than outside during the daytime (Figure 3.3B), with the biggest differences generated between 09:00 and 17:00 (when heating was strongest) when internal RH was on average 7.34% (± 4.57%) lower than ambient (Figure 3.4A). At night, internal RH of all four-sided WTHSs was higher than ambient RH (Figure 3.4C), particularly in S1 and S2 (6.46 and 6.16% higher on average respectively). High RH readings both inside and outside the prototype (Figure 3.3B) meant no difference could be detected. Diurnal patterns of VPD corresponded with those of temperature, showing larger increases in internal VPD than ambient when heating was strongest (Figure 3.3C). However, AbsH inside each WTHS remained close to ambient AbsH throughout the experiment (Figure B2.3).

3.4.2 Response of E. suberosum to short-term heating

From the initiation of heating of *E. suberosum*, the treatment individuals experienced mean daytime temperatures 2.35°C (± 2.13°C *SD*) above the controls (Figure 3.5A), increasing to 3.45°C (± 1.44°C) above between 09:00 and 17:00 (when the heating effect was strongest and most consistent). The RH was 3.81% lower on average during the daytime (± 4.37%; Figure 3.5A) inside the treatment WTHSs than outside, and this difference increased to 6.05% (± 2.09%) between 09:00 and 17:00. Over the course of the experiment (day 0 – 21) there was also a large increase in mean ambient daytime temperature of 6.74°C (Figure 3.5A).

T-tests comparing the photosynthesis and respiration parameter results (T_{max}, T_{opt}, T_{span}, A_{opt}, R_{25}, R_{35}, R_{45} and Q_{10}) of each leaf showed no significant differences ($p < 0.05$) between the treatment and control groups either before or after the initiation of heating. Over the course of the experiment however, maximum and optimum temperatures for photosynthesis (T_{max} and T_{opt}) showed increasing trends for both treatment and control groups (Figures 3.5B1 and 3.5B2), while respiration rates at given temperatures appeared to decrease (for example R_{45}; Figure 3.5B4) as did Q_{10} values for all individuals (except T2; Figure B2.4). Optimum photosynthesis rates (A_{opt}) showed no obvious trend (Figure 3.5B3).

Pearson correlation analysis of all results together showed no significant relationships ($p < 0.05$) between the photosynthesis parameter results of each leaf and temperatures experienced during the day or night prior to sampling. R_{25}, R_{35} and R_{45} results each showed a significant negative relationship with the mean temperature of the previous day ($R = -0.45$, $p = 0.027$; $R = -0.55$, $p = 0.005$; $R = -0.45$, $p = 0.027$; for R_{25}, R_{35} and R_{45} respectively).

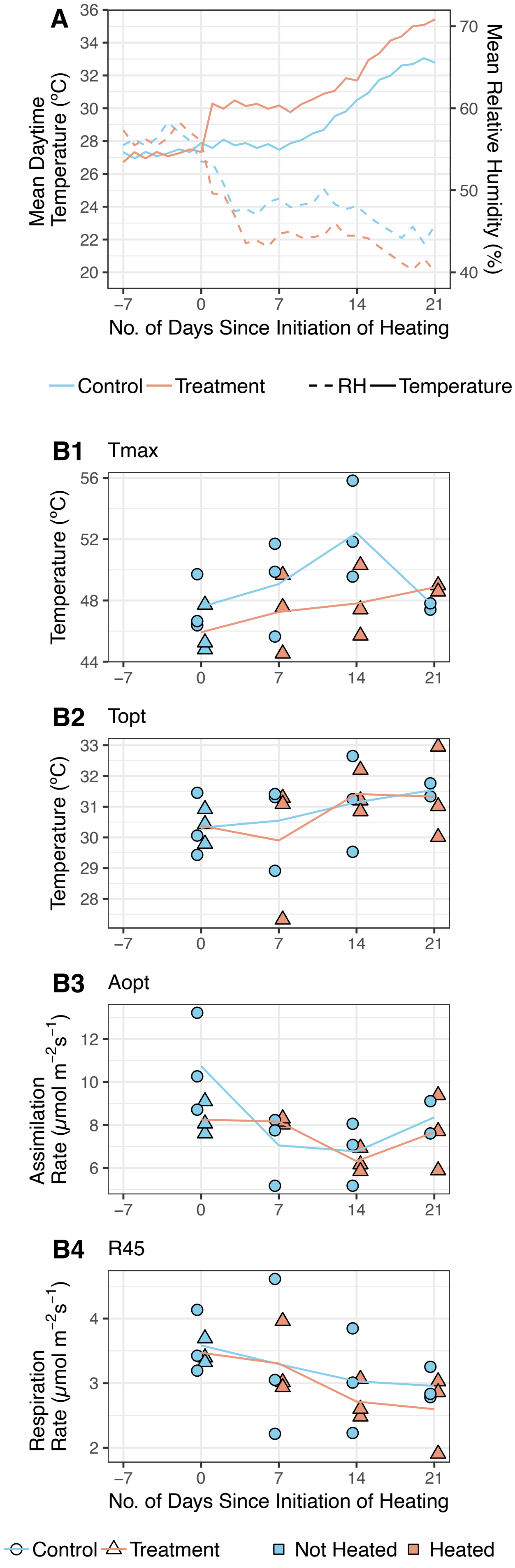

Figure 3.5 *Climatic conditions and example results from the heating experiment on E. suberosum. (A) Mean daytime (06:30 – 18:30) temperature and RH experienced by treatment and control groups in relation to the number of days before or after heating began. (B1–4) Results of the photosynthesis and respiration parameters (B1) Tmax, (B2) Topt, (B3) Aopt, and (B4) R45, estimated from leaf measurements taken at 0, 7, 14 and 21 days after experiment initiation for each individual. Each point represents the results from one leaf on one day. Lines show average results for treatment and control groups.*

3.5 Discussion

3.5.1 Impact of WTHS on microclimate

The results demonstrate that our novel whole-tree *in situ* passive heating structure (WTHS) provides a useful and flexible methodology for direct experimental warming, which can be used to investigate the effects of increasing daytime temperatures on woody plant species in semi-open field environments with strong solar irradiance. Air temperatures inside the WTHSs that we built and tested in the Brazilian Cerrado varied in accordance with ambient temperatures while producing a strong and reproducible heating effect of approximately 3.4°C above ambient air temperature when irradiance was high (between 09:00 and 17:00 local time), a temperature increase that could be experienced across the Cerrado by 2100 given moderate emissions scenarios (IPCC, 2013). Furthermore, mean air temperatures inside the WTHSs closely matched maximum ambient temperatures, meaning the WTHSs exposed treatment individuals to realistic temperatures for the area, which they were already experiencing at a lower frequency.

While the maximum temperature differences between inside and outside the WTHSs sometimes reached as high as 7.66°C, this often resulted from a delay in heating of the ambient environment (seen in the morning in the prototype; Figure 3.3A), and the difference fell to approximately 3°C once both internal and external environments reached their maximum daytime temperatures. Moreover, similar and sometimes more extreme spikes in temperature can also occur when employing active heating methods due to delays between the sensing of target temperatures and the deactivation of heating apparatus (Carter and Cavaleri, 2018).

Night-time air temperatures were slightly lower inside the WTHSs than outside (Figure 3.4C), however the inability to heat at night – sometimes accompanied by nocturnal cooling – is an unavoidable consequence of solar-powered passive heating methods (Marion et al., 1997). The extent of cooling was lower in the larger WTHSs (the prototype and S3; Table 3.1), suggesting larger structures (with larger openings) are favourable as they allow more mixing of internal (cooler) and external (warmer) air, even during still nights. To increase night-time temperatures in their passive OTCs in Australia, Godfree et al. (2011) used water filled plastic pipes to increase their thermal mass, and rocks could be used in a similar way. Although this method would likely be feasible in the Cerrado due to the high levels of solar irradiance, this addition could have reduced the heating effect produced by our WTHSs during the daytime (Godfree et al., 2011) and we wanted to retain as strong and long a period of daytime heating as possible. However, this addition could be tested in the future, or by those wishing to employ our WTHS in their own research. More reliable night-time heating would require active methods, which are often expensive, technically complicated, and impractical in remote field sites such as the Cerrado. Conversely, our WTHS was designed to be entirely passive to provide a low-cost, easy to scale up methodology that does not require advanced technical knowledge or costly, high-tech equipment to set up.

As stated in the methods, the straight sides and entirely open top of our WTHS were designed to minimise common undesirable impacts of chambers, such as changes to microclimate variables other than temperature, in particular humidity. Nonetheless, RH still decreased and VPD increased inside each WTHS during the daytime, particularly during peak heating. However, AbsH remained largely unaffected by the WTHSs, suggesting that changes in RH and VPD were driven by changes in temperature and are an unavoidable consequence of temperature manipulation. Furthermore, RH has decreased over the Cerrado in recent years and could continue to fall with increasing temperatures (Hofmann et al., 2021), meaning our WTHSs may simulate more realistic climate change scenarios.

3.5.2 Structural build and materials

The WTHS that we introduced here is simple to build and flexible in design. It can be modified to work with the locally available materials and equipment, as well as shaped to fit complex environments and different study species. Our initial, hexagonal design is appropriate for heating larger shrubs and trees as it can be efficiently expanded to encircle larger crown sizes. However, to surround *E. suberosum* — a small shrub species with a narrow growth form — it was more practical to erect four-sided WTHSs, saving materials, time and physical effort. For larger trees, if WTHSs need to be enlarged so there is more than 2 m between each post, more posts should be added to increase stability and reduce the likelihood of the plastic sides tearing in high winds (often experienced in the Cerrado).

Wooden posts were used to build the WTHS's frame for strength and stability, even in high winds. Square cross-section metal posts (1.5 x 1.5 cm) were initially tested because they were lightweight and easier to transport, but they didn't provide enough strength. However, if wood was not readily available, more robust metal posts could be a viable substitute, but would require testing first to ascertain their effect on internal temperature. We also plan to trial using locally sourced bamboo (an invasive species on nearby farms) in place of sawn wooden posts to reduce the cost and improve sustainability of the design. Using wood also allowed the use of U-shaped nails, which made attaching the wire frame easy and secure. Alternatives to wire for connecting the posts and securing the frame include various non-stretch cords (such as polyester or Kevlar rope), or wood or plastic poles for longer-term stability. Also, in areas where very hard ground impedes digging deep holes for the posts, reinforcing bar (rebar) can be hammered into the ground and fastened to the posts for additional support.

Since our WTHSs were built during the pronounced Cerrado dry season, permeability to water was not a principal concern. However, for use in wetter seasons or regions, the plain black polythene can be replaced with permeable alternatives to ensure the full movement of water, provided they remain black. Woven black polypropylene (designed for suppressing weeds in agricultural settings) is a low-cost and permeable alternative readily available in Brazil – known as 'ráfia de solo' – and often available in regions where agriculture is common, which we plan to test in the future.

3.5.3 Response of E. suberosum to short-term heating

The *in situ* heating of three individuals of *E. suberosum* provided no conclusive evidence of the higher daytime temperatures impacting photosynthesis, or of photosynthetic acclimation to short-term experimental heating, as no significant differences were found between the heated and control individuals for any of the parameters measured. It is possible that the period over which the individuals were heated (three weeks) was too short to develop any detectable response, although photosynthetic acclimation has previously been observed after only one week of heating (Smith and Dukes, 2017). The sample size may also have been too small (three leaves tested per group per week) to detect any significant differences. In addition, ambient air temperature increased dramatically over the course of the experiment (Figure 3.5A), which meant that in the latter half of the experiment, control individuals were experiencing temperatures as high as those produced inside the WTHSs roughly a week before. These high ambient temperatures could suggest that both the treatment and control individuals were already acclimated prior to the experiment to the higher temperatures produced. Interestingly, there was an increasing trend in T_{max} and T_{opt} (Figures 3.5B1 and 3.5B2) for both treatment and control groups which, although not statistically significant, suggests a possible acclimation to natural seasonal warming instead (Gunderson et al., 2010). To investigate this further our experiment could be expanded to study a greater number of individuals – and species – for a longer duration.

Although we found no significant effects of short-term experimental heating on respiration rates when comparing the treatment individuals to the controls, Reich et al. (2021) have shown that respiration rates may be more strongly controlled by night-time temperatures than daytime, and it is possible that stronger acclimation (upregulation of respiration) to the slightly cooler night-time temperatures experienced by the treatment individuals negated any acclimation (downregulation of respiration) to the higher daytime temperatures. Interestingly, the decreasing trends in both the estimated respiration rates of both groups (e.g. Figure 3.5B4) and the Q_{10} of most individuals (Figure B2.4) throughout the experiment, along with the significant negative relationships found between the estimated respiration rates of all individuals and the temperatures experienced the day prior to taking measurements, suggest a possible downregulation of respiration in both groups and acclimation to the naturally increasing ambient temperatures (Slot et al., 2014). However, deterioration in leaf health could also have contributed to these results as measurements were made towards the end of the growing season. Again, the results suggest the need for a longer and larger scale investigation in order to draw any reliable conclusions, which given more time would be achievable using our WTHS due to its low cost and versatile shape. Nevertheless, this study provides a clear demonstration of how our WTHS can be utilised in a range of *in situ* temperature manipulation experiments.

3.6 Conclusions

Overall, the consistent level of heating produced by our novel, whole-tree *in situ* passive heating structure (WTHS) – regardless of size, shape, or enclosed species – demonstrates that the design is flexible and can be adapted for different individuals and field situations, while still providing a reproducible daytime heating effect in line with mid-range climate predictions for the Cerrado biome by the end of this century. Production of a strong heating effect without the more traditional inclined walls or reduced openings decreases the likelihood of non-desirable chamber effects, and the lack of any electricity requirement means that the design can be widely used in long-term investigations, provided solar irradiance is sufficient. Low-cost and simple to build, our WTHS can be easily scaled up to apply one treatment scenario to multiple taller-stature individuals *in situ* (as we did with *E. suberosum*), particularly in complex environments that don't allow for construction of uniform chambers, providing new opportunities to gain vital insight into the high temperature responses of understudied shrub and tree species in remote environments.

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Chapter 4

Contrasting responses of pollen and fruit to whole-tree heating in tropical savannah species

4.1 Abstract

Predicting the impacts of global heating on species, ecosystems, and biodiversity requires thorough understanding of how higher temperatures affect plant physiology. Sexual reproduction is particularly sensitive to temperature extremes and associated impacts of heating on crop yields are well documented. However, such impacts remain poorly studied in the wild and experimental investigations rare, especially in tropical ecosystems. In South America, the Cerrado – the world's most biodiverse savannah – is rapidly warming, potentially threatening thousands of species as well as large-scale water supply and carbon storage services, yet direct investigations of Cerrado species' responses to heating are lacking. Here, we report the first *in situ* whole-tree heating experiment of tropical savanna tree species, aiming to provide insights into the impacts of increasing temperatures on their reproductive success. Contrary to expectation, pollen development was resilient to very high temperatures, and pollen viability demonstrated a positive response to heating. Nevertheless, fruit production was reduced at high temperature, with negative implications for future recruitment, species persistence, and the potential for migration to more favourable environments. The implied greater temperature sensitivity of female than male reproductive development (unusual in species studied to date) emphasises the urgent need for systematic research into heating impacts on tropical diversity.

4.2 Introduction

Anthropogenic climate change, in particular climate warming, is one of the most immediate challenges facing global ecosystems (Bellard et al., 2012; Scheffers et al., 2016), and has been linked to species range losses, distribution changes, local extinctions, and overall losses in biodiversity (Parmesan, 2006; Pecl et al., 2017; Freeman et al., 2018), with implications for ecosystem function and service provisioning (Tilman et al., 2014). Complex models are often employed to try to understand and predict the impacts of climate warming on organisms from the individual to global scales, which can help to target conservation efforts, or prepare for – and hopefully mitigate – future ecological issues (Elith and Leathwick, 2009). However, there are still large gaps in our understanding of how individual plants, species, or ecosystems will respond to increasing temperatures, and such models can be greatly enhanced by the incorporation of new data, such as that obtained experimentally (Luo et al., 2011; Feng et al., 2018; Schuwirth et al., 2019). While there is a focus in the literature on temperature impacts on photosynthesis and primary production (Tiwari et al., 2021) due to their direct relationships to the global carbon balance, other processes, such as reproduction, have received far less attention, although sexual reproduction is one of the most sensitive stages of plant development to temperature (Hedhly, 2011; Lohani et al., 2020). However, it is now being recognised that models should consider not only the (classic) ecological niche of adult individuals, but also the ecological requirements for successful reproduction, seed dispersal, seed germination, and seedling establishment (or the regeneration niche; Grubb, 1977; Rosbakh et al., 2018; Marimon et al., 2020). This would enable us to make better predictions of, for example, the extent of suitable habitat for species recruitment, and the impacts of climate warming on this habitat and species ranges as a whole (as in Borghetti et al., 2021; Ferreira et al., 2022).

Temperature impacts on reproduction have been extensively studied in crops (reviewed in Sage et al., 2015; Lohani et al., 2020; Zhu et al., 2021) as fruits and seeds are the commercial components of many crop species. High temperatures prior to anthesis (flowering) have been shown to alter the timing and extent of flowering (Albrigo and Galán Saúco, 2004), and cause the deformation, degeneration, or lack of function of the developing anther (male) and pistil (female) tissues and the developing gametophytes (pollen grains and embryo sacs; Kozai et al., 2004; Djanaguiraman, Perumal, Jagadish, et al., 2018; Raja et al., 2019; Bennici et al., 2019). They can also affect the timing of the development of floral tissues and gametophytes leading to underdevelopment at anthesis, or an asynchrony between male and female development (Rodrigo and Herrero, 2002; Herrero, 2003; Hedhly et al., 2009; Lora et al., 2011). Overall, this can affect pollen release and thermotolerance at anthesis, impact pollen-pistil interactions at pollination, and reduce fertilisation success (Fang et al., 2010; Song et al., 2015; Wang et al., 2021). After fertilisation, high temperatures can affect embryo development, and fruit and seed maturation, further affecting the quantity and quality of the seed produced (Albrigo and Galán Saúco, 2004; Pagamas and Nawata, 2008; Suriyasak et al., 2020). Male reproductive development is considered to be more sensitive to temperature stress than female (Zinn et al., 2010; Giorno et al., 2013; Pacini and Dolferus, 2016; Santiago and Sharkey, 2019), and reduced yields at high temperature are often attributed to reduced pollen viability and fertilisation failure (Vara Prasad et al., 2001; Slavković et al., 2016; Zhu et al., 2021). Furthermore, temperature effects on pollen fitness have been linked to reduced seed quality, in turn influencing seedling establishment (Rosbakh et al., 2018). Crop research has therefore revealed a very negative impact of high temperature stress on reproductive success.

In wild populations, reduced reproductive success has implications for species persistence, with possible repercussions for interacting species (flora and fauna), and ultimately community stability (Brooker, 2006; Butt et al., 2015; Vilela et al., 2018; Rabeling et al., 2019). Gaining a better understand of how reproduction in wild species will respond to higher temperatures is therefore clearly important, but these responses are often complex and species-specific (Hovenden et al., 2007; Lambrecht et al., 2007; Liu et al., 2012). Experimental warming studies are limited in number and predominantly focus on herbs and shrubs (Kudo and Suzuki, 2003; del Cacho et al., 2013; Jacques et al., 2015; Marchin et al., 2015) as experimental manipulation becomes progressively more difficult with increasing organism size (Chung et al., 2013). Nevertheless, a recent meta-analysis of studies on reproduction under experimental warming (Zi et al., 2023) has indicated a potential global decrease in fruit production as temperatures increase, although no studies from the tropics were included. Yet tropical species may already be growing at temperatures close to their thermal maxima (Sentinella et al., 2020; Araújo et al., 2021; Doughty et al., 2023), and it is unclear to what extent they might be able to acclimate and adapt to further warming (Rieu et al., 2017; Flores-Rentería et al., 2018). High temperatures negatively impact the reproduction of a number of tree crop species native to the tropics (including mango, *Annona*, and *Citrus* species; Sukhvibul et al., 2000; Lora et al., 2011; Lora et al., 2012; Distefano et al., 2018; Alves Rodrigues et al., 2018; Shafqat et al., 2021; Liu et al., 2023), and reproduction in wild species could potentially be affected in the same way, although rarely investigated until now (Slot and Winter, 2016). However, pollen viability (at anthesis) was recently shown to be reduced in cut branches of widespread tropical tree *Muntingia calabura* L. exposed to experimental daytime warming of 3.5°C (Slot et al., 2023), providing the first direct indication that reproduction in wild woody species may decline given rising temperatures.

Within tropical research, non-forest ecosystems such as the Brazilian Cerrado biome, have received far less attention (and protection) than forest ecosystems (Overbeck et al., 2015; Bispo et al., 2023). Yet the Cerrado – the world's most biodiverse savannah (Sano et al., 2019) – possesses such high levels of species richness and endemism, and is under such significant threat from anthropogenic action, it has been classed as a 'hotspot' for biodiversity conservation (Myers et al., 2000). It also provides vital ecosystem services such as carbon capture and storage, climate regulation, and water provisioning to large regions of Brazil (Klink and Machado, 2005; Lima and Silva, 2007; Sano et al., 2019; Rodrigues et al., 2022). Although known as savannah, the Cerrado is a complex mixture of grassland, shrubland, and woodland that originally covered approximately 2 million km^2 (Ratter et al., 1997). Sadly, less than half of this remains as native vegetation (Beuchle et al., 2015; Parente et al., 2021), and what remains could be under further threat from climate warming as regional climate models predict an increase of between 2.8 and 6.6°C across the Cerrado biome by 2100 based on moderate to high greenhouse gas emissions scenarios (Representative Concentration Pathways or RCPs of 4.5 and 8.5 respectively; Ferreira et al., 2023). With significant increases in temperature already recorded (Hofmann et al., 2021), it is important to understand how remaining vegetation will respond to further increases in temperature, and in particular – since it is a key process for both species survival and migration – how reproduction will be affected. Furthermore, investigation of the temperature sensitivities of reproduction in Cerrado species could provide insight into species inhabiting analogously extreme and understudied environments such as other tropical savannahs (Castro et al., 2020), and perhaps tropical forest species that are exposed to similar temperature regimes, for instance those present in the vast transition zone between the Cerrado and Amazon biomes (Marimon et al., 2006; Marimon et al., 2010).

Research on the effects of environmental variation on reproduction in Cerrado vegetation has mostly focussed on seeds and seedlings (Daibes et al., 2022) and investigations along ecological gradients, such as between the edge and centre of vegetation fragments (Camargo et al., 2011; Vogado et al., 2016). Fragmentation alters the environment by, for example, causing localised warming (Pongratz et al., 2006; Camargo et al., 2011). It has been shown to influence the phenology and intensity of flowering and fruiting in Cerrado species (Camargo et al., 2011; Vogado et al., 2016) and even reduce fruit set (the proportion of flowers that develop into fruits/seeds; Athayde and Morellato, 2014), including in common Cerrado tree *Byrsonima pachyphylla* A.Juss., one of the focal species of this study (Melo et al., 2014). Although generally linked to phenological changes and altered interactions with pollinators, the direct impacts of temperature on floral development and fruit set have rarely been considered. Furthermore, direct investigation of the effects of temperature on Cerrado species reproduction has heretofore been lacking. Recently however, Werkmeister et al. (in review; chapter two) studied the impacts of higher daytime temperatures (~3°C above ambient) on *B. pachyphylla* by heating developing inflorescences of adult individuals *in situ*. While they found a significant reduction in fruit set – a result which could have serious implications for reproductive success in the future – they found no impact of higher daytime temperatures on pollen viability, suggesting a resilience of pollen production to very high temperatures. Nevertheless, late initiation of their heating experiment (once flower buds were already partially developed) may have limited the potential impacts of heating on floral development, and their targeted heating methodology did not incorporate the effects that higher temperatures may have had on vegetative tissues. It is therefore plausible that heating entire trees (to include the vegetative tissues) from much earlier on (e.g. prior to inflorescence development) would produce a negative response in pollen viability (as in Slot et al., 2023) as well as fruit set (as in Werkmeister et al., in review).

Here we have expanded on those previous experiments by employing a novel whole-tree heating methodology (described in Werkmeister et al., 2022; chapter three) to better understand the impacts that increasing temperatures will have on reproduction (pollen viability and fruit set) in *B. pachyphylla* (Malpighiaceae), and an additional species *Davilla elliptica* A.St.-Hil. (Dilleniaceae). Both are woody species with medicinal properties (Sannomiya et al., 2005; Bonacorsi et al., 2011; Guilhon-Simplicio and Pereira, 2011; Sousa et al., 2020) that are common and widespread across the Cerrado (Marimon-Junior and Haridasan, 2005; Ratter et al., 2003). We exposed entire individuals of both species to higher temperatures from before reproductive structures were apparent until fruit maturation, to quantify the impact on pollen viability and fruit set. Furthermore, we carried out an initial investigation of pollen viability in both species (in the year prior to whole-tree heating) to gain an understanding of the levels of intra- and inter-specific variation in pollen viability in Cerrado species, and to enhance our interpretation of the results from the heating experiment. We hypothesised that both (a) viable pollen production and (b) fruit set would be reduced in the heated individuals.

4.3 Methods

4.3.1 Study site and species

The field site (14°42'30.1" S, 52°21'01.0" W) was located in an area of Cerrado *típico*, or ‘typical Cerrado’ in the Bacaba Municipal Park reserve in Nova Xavantina (Mato Grosso, Brazil), situated in a region of transition between the Cerrado biome and Amazon forest (Amazonia-Cerrado transition zone; Marimon-Junior and Haridasan, 2005; Araújo et al., 2021). All lab work was carried out at the Plant Ecology and Genetics laboratories at the nearby Nova Xavantina campus of the University of Mato Grosso (UNEMAT). Typical Cerrado is a classification of Cerrado *sensu stricto* (tree and shrub woodland) exhibiting 20–50% tree cover with an average height of 3–6 m (Ribeiro and Walter, 2008), and the dominant vegetation type in the reserve (Marimon-Junior and Haridasan, 2005). The climate – classified as Aw by the Köppen system (Peel et al., 2007) – exhibits pronounced wet (mid-October to April) and dry (May to October) seasons, with the hottest period occurring towards the end of the dry season (August to October). Average monthly temperatures are 25°C, and annual precipitation 1300–1500 mm (Marimon et al., 2010), although over recent decades the region has experienced rapid warming and more frequent extreme heatwaves (Araújo et al., 2021; Tiwari et al., 2021).

The investigation focussed on two native woody species, namely *Byrsonima pachyphylla* – also known by synonym *Byrsonima crassa* Nied. (Francener, 2023) – and *Davilla elliptica*. Common species of the typical Cerrado, both in the study area (Marimon-Junior and Haridasan, 2005; Mews et al., 2011) and across the Cerrado biome (occurring, respectively, in 60 and 58% of the 315 widespread Cerrado areas sampled by Ratter et al., 2003), they are also both ecologically and socioeconomically important. Each having many ethnopharmacological uses, they are potential sources for new medicines (Sannomiya et al., 2005; Bonacorsi et al., 2011; Guilhon-Simplicio and Pereira, 2011; Sousa et al., 2020); their pollen and – in the case of *B. pachyphylla* – floral oils are collected and consumed by pollinators, particularly bees (Martins, 2005; Boas et al., 2013); and their fruits and seeds are consumed by insects and animals, including humans (Purificação et al., 2014; Passos, 2023).

D. elliptica and *B. pachyphylla* both flower in the dry season. Flowering can occur throughout this period but *D. elliptica* often peaks in May/June, and the fruits mature in September/October (Silvério and Lenza, 2010). *D. elliptica* inflorescences are paniculate, terminal or axillary, with 3–37 flowers. Flowers are bisexual with ~90 stamens, two carpels, five yellow petals, and five sepals, two of which are larger and enclose the corolla until the day of flowering (Fraga, 2012). Flowers open around dawn on the day of anthesis (06:00 – 06:30; Gottsberger, 1977), the petals then fall off around 11:00 on the same day and the two largest sepals slowly close back over creating a capsule enclosing the ovary (Gottsberger, 1977), which may develop into one or two seeds (Fraga, 2012). The capsule opens again once seeds are mature.

B. pachyphylla flowering peaks in July/August and the fruits mature in October/November (Silvério and Lenza, 2010; and personal observation). *B. pachyphylla* inflorescences are terminal racemose, with ~30 flowers (personal observation). Flowers are bisexual with ten stamens, three carpels, five yellow petals, and five sepals (each with a pair of oil glands). They also have a thin stigmatic cuticle that must be broken by pollinators before pollen can adhere and germinate (Boas et al., 2013). Flowers also open at dawn and they are receptive for only one day, changing colour to orange then red on the days following anthesis. The fruits are fleshy globose drupes containing a single seed, which turn yellow/orange at maturity (Boas et al., 2013).

4.3.2 Heating methodology

To heat individuals of *D. elliptica* and *B. pachyphylla in situ*, we employed novel passive whole-tree heating structures (WTHSs), which were developed and tested prior to these experiments (Werkmeister et al., 2022; chapter three). These WTHSs, similar to large *in situ* open-top chambers (Figure C2.1), were designed to raise internal air temperatures by 2–3°C during the daytime, in line with mid-range climate predictions for the future of the Cerrado (Ferreira et al., 2023). Each WTHS was 3.5 m tall with a frame made of six (4 m) wooden posts buried 50 cm deep; six straight vertical sides made of translucent white polythene film (100 µm thick agricultural plastic designed for greenhouses; bought locally as 'plástico para estufa'); a permeable base of woven black polypropylene (used in agriculture for supressing weeds; bought locally as 'ráfia de solo'); and a 'roof' that was left entirely open. The black bases were included to function as passive heat sources, designed to absorb incident solar radiation during the daytime and emit thermal radiation, which would be largely trapped inside the WTHSs by the polythene sides. The large size, straight vertical sides, and open roof maximised light transmission into the WTHSs, and allowed mixing of air between the inside and outside, preventing overheating and reducing the impact of the WTHSs on variables other than temperature, such as internal humidity and gas concentrations.

4.3.3 Experimental design

We carried out this investigation in two parts: 1) a preliminary study in 2020 of the natural levels of pollen viability across individuals of *D. elliptica* and *B. pachyphylla*; and 2) a direct, *in situ*, whole-tree heating experiment in 2021, in which a subset of the individuals studied in 2020 were heated to quantify the impact of long-term higher daytime temperatures on viable pollen and fruit (or seed) production. For the preliminary study in 2020, 16 individuals of each species that were healthy and mature enough to reproduce were identified and tagged, and DSH (diameter at stump height or 30cm, commonly used as a measure for multi-stemmed woody Cerrado species) and height were measured. Tagged individuals of *D. elliptica* had a mean height of 2.5 m (±

0.5 *SD*) and DSH of 8.1 cm (± 1.8), while *B. pachyphylla* individuals had a mean height of 2.9 m (± 0.5) and DSH of 9.3 cm (± 0.1). Pollen from these individuals was collected (see section 4.3.4) and analysed (see section 4.3.5) to better understand the natural levels of pollen viability both within and between individuals and species. Of the 16 individuals tagged per species, six individuals were randomly selected to be treatment (heated) individuals in the following year's whole-tree heating experiment, leaving ten individuals per species as controls, although some treatment individuals were re-selected in 2021 due to local vegetation preventing the building of WTHSs around them. Mean height and DSH were equivalent between treatment and control groups for each species. Sample sizes of the treatment and control groups were unbalanced (6 treatment and 10 control individuals per species) as the labour-intensive nature of building the WTHSs *in situ* with a small team limited the number of treatment individuals possible, while the number of control individuals studied was maximised in order to reduce the influence of natural intraspecific variation in pollen viability and fruit set on the results.

For the whole-tree heating experiment, WTHSs (of average side length 2 m) were built in early 2021 around the six treatment individuals of both *B. pachyphylla* and *D. elliptica* (between the 14th February and 4th March, and the 1st and 12th of April respectively), before inflorescences had begun to develop (according to visual inspection). While *D. elliptica* flowers earlier than *B. pachyphylla*, WTHSs were built around *B. pachyphylla* first to capture all stages of floral development – which begins earlier in *B. pachyphylla* (personal observation) – in both species, although this led to *B. pachyphylla* being heated for longer overall. WTHSs remained in place until the end of the year (after fruit maturation). During flowering, pollen samples were collected and analysed (see sections 4.3.4 and 4.3.5) to compare viability between treatment and control groups in 2021 (in which the treatment individuals were heated), and between the years 2020 (in which no individuals were heated) and 2021. Flowers were also hand pollinated (see section 4.3.6) and mature fruits counted, to compare the proportion of fruits that developed between treatment and control individuals in 2021.

4.3.4 Pollen Sampling

In 2020 (the pre-treatment year) we aimed to collect pollen samples from all individuals of *D. elliptica* and *B. pachyphylla* and test their levels of viability through staining (see section 4.3.5). Pollen samples were collected from all 16 individuals of *D. elliptica* (between the 15th and 19th of June). Due to time constraints, pollen samples were only collected from 11 of the 16 individuals of *B. pachyphylla* (between the 23rd of June and 24th of July). No temporal repetitions were possible (all individuals were only sampled on one day). Nonetheless, to get a reliable value of individual-level pollen viability, six pollen samples were taken per individual: one from a flower on each side of the tree crown (North, East, South and West-facing) at approximately the same height (middle height of the tree crown); plus one from lower and one from higher on the South-facing side.

In 2021, following initiation of the whole-tree heating experiment, the field site was visited regularly to monitor flowering. *D. elliptica* began to flower in May, and pollen samples were collected from tagged individuals between the 6th May and 22nd June. Treatment individuals had therefore been growing at higher temperatures for between 24 and 71 days when samples were taken. *B. pachyphylla* pollen samples were collected between the 16th June and 20th July, therefore treatment individuals were sampled between 107 and 127 days after the initiation of heating (longer than *D. elliptica* due to the longer period of floral development, mentioned above). Individuals were sampled as they flowered, attempting to sample from both treatment and control individuals each day, although not always possible due to natural variation in flowering. To maximise the number of individuals sampled each day, only four samples were taken per individual (each day sampled). These were taken from one flower on each side (North, East, South and West-facing) at approximately the same height (middle of the crown). Each individual was sampled on multiple days, aiming for five temporal repeats within the study period, although achieving on average four for *B. pachyphylla* and three for *D. elliptica* individuals (which completed their flowering much faster).

4.3.5 Pollen viability

In a previous study – investigating the impacts of inflorescence heating on *B. pachyphylla* pollen – Werkmeister et al. (in review; chapter two) employed two complementary pollen viability analysis methods: differential staining of aborted and non-aborted pollen grains (using a simplified version of the classic Alexander stain; Peterson et al., 2010); and *in vitro* pollen germination (Shivanna and Rangaswamy, 1992). While germination is thought to provide a more accurate measure of pollen viability than staining (Dafni and Firmage, 2000), it is also more challenging. However, Werkmeister et al. (in review) found a significant and strong positive correlation between the viability of pollen samples analysed by the two methods, providing confidence that the staining method alone was reliable for sample comparisons, at least in *B. pachyphylla*. Therefore, to maximise the number of samples that could be processed each day in these experiments, only the staining method was used.

Pollen samples were collected early in the morning (before 10:00 local time; BRT = UTC - 3) on the day of anthesis. For each sample, all anthers of a single flower were removed using tweezers and placed into a 2 ml Eppendorf tube. Samples were taken to the nearby laboratories at UNEMAT, and each macerated with 400 µl of the staining solution (Table C1.1), which had been previously optimised for the study species and local laboratory conditions. 30 µl of each sample mixture were transferred to microscope slides and gently heated (following the protocol of Peterson et al., 2010). Slides were observed at 100 × magnification under an Eclipse E200 microscope (Nikon, Tokyo, Japan) paired with a Nikon DS-Fi2-U3 camera system. At least five images (of unique microscopic fields) were taken per sample in order to later count and classify pollen grains as aborted (cell walls stained blue/purple) or non-aborted (presumed to be viable; cell protoplasm stained orange/red). All pollen grains were counted per image, with *D. elliptica* and *B. pachyphylla* samples displaying means of 79 and 314 grains per image respectively. Pollen viability of a sample was estimated as the percentage of all grains per image that stained orange/red (non-aborted), averaged over all images per sample. Image analysis (pollen grain classification) was carried out manually using ImageJ software (Version 1.51; Rasband, 2018). During image analysis, samples were visually evaluated and those of poor quality or containing very few pollen grains were excluded from further analysis due to low reliability (excluding all 2021 samples from individual D2).

4.3.6 Hand pollination and fruit set

To study the impact of higher daytime temperatures on fruit set in 2021, three flowering branches/inflorescences (spread evenly around the middle height of the crown) were tagged on each experimental individual before flowering began. No pollen samples were taken from any of these tagged branches. These branches were checked every day during the flowering period and any newly-opened flowers were carefully hand-pollinated using a mixture of pollen that was collected fresh every morning (separately for each species). This mixture contained all anthers of two flowers from three non-experimental individuals (six flowers in total) mixed together in a 5 ml Eppendorf tube using a paintbrush. These individuals remained the same throughout. The pollen mixture was applied to the stigmas of each open experimental flower using the paintbrush, taking particular care to break the stigmatic cuticle present in the flowers of *B. pachyphylla* (Boas et al., 2013) without damaging any other flower parts.

Following hand-pollination of all tagged flowers (completed between the 6th May and 23rd June for *D. elliptica*, and the 21st June and 24th August for *B. pachyphylla*) the inflorescences were enclosed within net bags (locally used in agricultural fruit production) to prevent loss of fruits and seeds through falling or herbivory. On the 26th August an early count was made of any developing fruits, and early fruit set was calculated for each *B. pachyphylla* inflorescence as the percentage of pollinated flowers that clearly displayed initial fruit development. This could not be performed for *D. elliptica* as the capsules (formed by the sepals after flowering) persist even if empty, concealing the presence or absence of developing seeds. Once all fruits had matured (by end of October) they were collected and counted and final fruit set per inflorescence was calculated (as the percentage of pollinated flowers that developed into mature fruit). Fruit retention was also calculated for each inflorescence as the percentage of fruit at the early count that remained until the final count.

Unfortunately, storms in October (before fruit collection) broke many branches from the experimental individuals. At fruit collection, some *B. pachyphylla* inflorescences had fallen (although retained their labels), some fruits had fallen (from inflorescences) into the net bags, and many fruits – including those still attached – had started to decay due to moisture retained by the bags. The final fruit count therefore included all fruits that appeared fully developed and of a reasonable size, whether still attached or not, although we cannot be certain that all counted fruits had or would have reached full maturity. Too many branches and labels were lost from *D. elliptica* individuals (which have more delicate branches), therefore we present fruit set results for *B. pachyphylla* only. Furthermore, as seed size and weight are common indicators of seed quality

(Ambika et al., 2014), collected fruits and seeds were also weighed and measured. However, damage and premature decay caused by the storms meant that measurements were not reliable, and were therefore omitted.

4.3.7 Climate data collection

Maxim DS18B20+ temperature sensors (accuracy ± 0.5°C) were used to monitor the air temperatures to which the treatment and control individuals were exposed in 2021. They were controlled by Arduino microcontrollers which recorded data to memory cards every minute. The calibration and testing of these sensors was ongoing at the initiation of the whole-tree heating experiment, which began without any sensors in place. From the 17th March 2021, temperatures were measured at one treatment individual of *B. pachyphylla* by one sensor placed inside the WTHS (treatment temperature) and one outside (as control). The number of sensors in the field gradually increased (as more sensors were ready for deployment) until the 26th June when full sets of four sensors (positioned to the North, East, South, and West sides of each individual) were in place at each of nine individuals, including five *B. pachyphylla* and four *D. elliptica*, three of which were controls and six were treatments (details summarised in Table C1.2).

Relative humidity (RH) was recorded every five minutes by pre-calibrated Elitech RC-51H USB Data Loggers (accuracy ± 3–5%), which were installed at the North and South sides of two *B. pachyphylla* treatment individuals and one control at the start of July 2021. All sensors were mounted 1.5m from the ground. To reduce the effects of solar radiation on readings, temperature sensors were painted white and sheathed in white heat-resistant cable sleeving, and all sensors were contained in custom 3D-printed radiation shields covered on top with thin self-adhesive reflective insulation (Figure C2.2), which performed well when tested against the commercial weather station (data unpublished).

Long-term climate data was obtained from the data assimilation based ERA5-land dataset (9km resolution; Muñoz Sabater, 2019) for the closest grid reference to the Bacaba field site (14°43'12.0" S, 52°21'36.0" W). Hourly, 2m surface air temperature measurements from 2010 to end-2021 were used to compare the temperatures reached in our WTHSs to those of the previous year, and all years since 2010.

4.3.8 Data analysis

4.3.8.1 Climate data

Temperature measurements recorded (each minute) by our own sensors in 2021 were averaged over 5-minute intervals. These were then averaged across all sensors on each individual being measured, and then across all treatment and all control individuals. A 5-minute record of the heating effect produced by the WTHSs was estimated from these. Mean RH values (recorded every five minutes) were similarly calculated for each individual measured, and then for treatments and controls. Daily mean values of daytime (06:30 – 18:30 local time) and night-time (18:30 – 06:30) control and treatment temperature, heating effect, and RH were calculated. Mean values were also calculated for 09:00 – 17:00 each day (peak hours), during which the WTHSs were previously found to be most effective (Werkmeister et al., 2022; chapter three). These could then be used to investigate temperatures over specific periods of the experiment, such as pollen sampling. Unfortunately, although all structures were built as early as possible (by 5th March for *B. pachyphylla* and 13th April for *D. elliptica*) in an attempt to heat the individuals from the earliest stages of floral development, the permeable black plastic bases were mistakenly inserted into all of the structures at once on 21st of April, resulting in less effective heating prior to this. Separate results were therefore calculated for prior to base insertion.

To further investigate the effectiveness of the WTHSs, average diurnal patterns of treatment and control temperature and heating effect were calculated from the 5-minute temperature records, separately for the periods before and after the addition of the black plastic bases. For reliability, the post-bases diurnal pattern of heating (Figure C2.3) was calculated only from temperature data collected after the 26th June 2021 (from which the maximum number of sensors were in place; Table C1.2).

As the ERA5-land dataset is a hybrid of observed data and physics-based time-stepped modelling of the atmosphere, agreement with our recorded temperatures was close, although not exact. Therefore, to estimate temperatures for periods of 2021 when our sensors were not functioning and compare with other years, we used the ERA5-land data as control temperatures and estimated treatment temperatures for both *B. pachyphylla* and *D. elliptica* using the mean diurnal patterns of heating (pre- and post-base insertion). These allowed us to compare the temperatures experienced by the control and treatment individuals of both species during the experimental periods of 2020 and 2021, as well as during all previous years from 2010.

4.3.8.2 Pollen viability and fruit set

In 2020, mean pollen viability values for each individual were calculated from their sample results (up to six samples per individual, which were all collected on the same day). In 2021 (when individuals were sampled across multiple days) daily mean values of pollen viability were calculated for each individual on each day sampled. Individual-level results were calculated as an average of these, excluding days with only one sample (taken or remaining in the analysis) due to low reliability, which excluded individuals D8 and D9 from the 2021 analysis. By this point, all 2021 sample results from three *D. elliptica* individuals (2, 8 and 9) had been excluded from further analysis, leaving seven control individuals (and six treatments). Between 3 and 20 samples per individual remained in the analysis of the 2021 results (on average 14 samples per individual, collected over 4 days).

Individual-level fruit set (both early and final) and fruit retention results were calculated as a mean of the three branches studied on each individual (2021 only). For each species and each year measured, group-level (treatment or control) mean values of pollen viability and fruit set were calculated from individual-level results to examine differences between groups, both before (in 2020; only pollen viability) and after (in 2021) heating treatment was applied. Coefficients of variation (*CV*s) were calculated for each species and each group each year to quantify the spread of individual-level pollen viability and fruit set values in relation to their means.

Sample sizes within groups were small and some data distributions were skewed. Although remedied in most cases by applying the logit-transformation to the results, we chose instead to use non-parametric Wilcoxon tests on the untransformed data as they are more resilient to outliers, and to avoid issues of interpreting analyses of transformed data. For each species, unpaired Wilcoxon rank-sum tests were used to assess the significance of differences in individual-level pollen viability and fruit set results between the treatment and control groups, both in 2020 when no individuals were heated (pollen viability results only), and in 2021 when treatment individuals were heated. Paired Wilcoxon signed-rank tests were also used to assess the significance of changes in the pollen viability results of each individual between the two years for both the treatment and control groups.

4.4 Results

4.4.1 Preliminary study pollen viability

During the preliminary study in 2020 – in which all individuals experienced natural climate conditions – the mean percentage of viable pollen produced by *B. pachyphylla* was high (92 ± 1% *SE*; Table C1.3), showing low variation within (Figure 4.1) or between individuals (4% *CV*; Table C1.3). *D. elliptica* displayed lower pollen viability (mean of 70 ± 2%) and more variation, both within (Figure 4.1) and between individuals (13% *CV*; Table C1.3). There were no significant differences in pollen viability between the treatment and control groups ($p > 0.5$ for both species; Table C1.4).

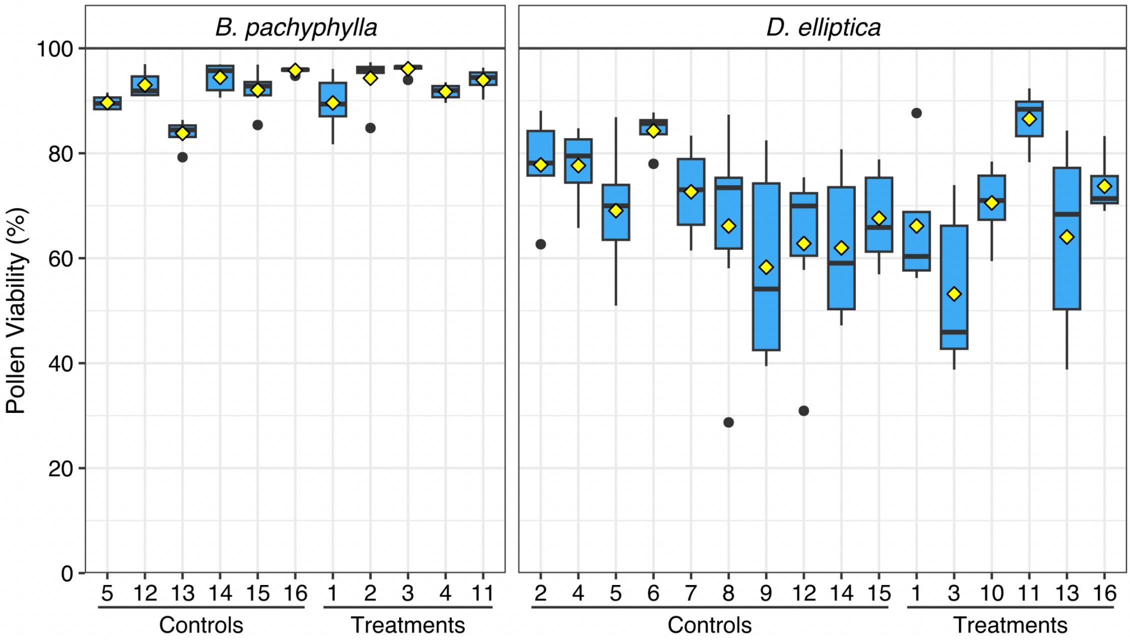

Figure 4.1 *Pollen viability of samples taken from each tagged individual (1–16) of each species for the pre-treatment year (2020), showing the division of individuals into control and treatment groups. Yellow diamonds denote mean values. Note that pollen viability did not differ between control and treatment groups for either species (p values > 0.5).*

4.4.2 Whole-tree heating experiment

4.4.2.1 Performance of the WTHSs

The passive WTHSs (previously described in Werkmeister et al., 2022; chapter three) performed as expected, steadily raising internal air temperature as solar irradiance increased during the daytime, and producing a strong and consistent level of heating during the peak hours (09:00 – 17:00; Figure 4.2A). From full-scale heating initiation until the end of flowering the WTHSs successfully raised air temperatures around treatment individuals by 2.2 ± 0.3°C (*SD*) during the daytime, rising to 3.0 ± 0.3°C during the peak hours, and reaching a mean daily maximum difference of 4.5 ± 0.6°C. This heating effect was consistent throughout the different stages of the experiment from the insertion of the black bases (Figure 4.2B; Table C1.5). Before the black bases were added, the WTHSs were producing a mean daytime heating effect of 1.4 ± 0.5°C (1.8 ± 0.7°C during the peak hours; Table C1.5), and the maximum daily heating effect was 4.1 ± 1.2°C. The WTHSs were therefore regularly producing higher temperatures, but for a shorter period of each day compared to the rest of the experiment. The WTHSs did not impact RH.

4.4.2.2 Experimental temperatures

Daytime air temperatures in 2021 remained relatively stable from the start of recording (in March) until August, increased steadily throughout August into September (reaching maximum control and treatment temperatures of approximately 44 and 49°C respectively, although no control sensors were recording on the hottest days), and decreased again towards the end of the dry season in October (Figure 4.2B). Treatment temperatures were consistently higher than control temperatures yet correlated closely with them throughout (Figure 4.2B; Table C1.5), displaying corresponding responses to natural climate variation. From the initiation of full-scale heating (black plastic bases added to all WTHSs; 21st of April) until the end of the flowering period (final hand-pollinations of *B. pachyphylla* flowers; 26th August; Figure 4.2B), the control individuals experienced mean daytime and night-time temperatures of 29.8 ± 2.3°C (*SD*) and 18.2 ± 2.2°C respectively. Mean peak hour temperatures were higher at 33.4 ± 2.6°C, reaching maxima of 36.6 ± 2.6°C. Treatment individuals experienced mean daytime and night-time temperatures of 31.9 ± 2.2°C and 18.3 ± 2.0°C respectively. Mean treatment temperatures during peak hours rose to 36.2 ± 2.6°C (comparable to maximum temperatures for controls) and reached maxima of 39.9 ± 2.4°C. Natural temperature variation (day-to-day and seasonal) during the pollination period of *B. pachyphylla* (Figure 4.2B) resulted in partial overlap of temperatures experienced by treatment and control individuals. Nevertheless, mean and (in particular) maximum temperatures experienced by treatment individuals on pollination days were still distinct from those experienced by the controls (Figure C2.4).

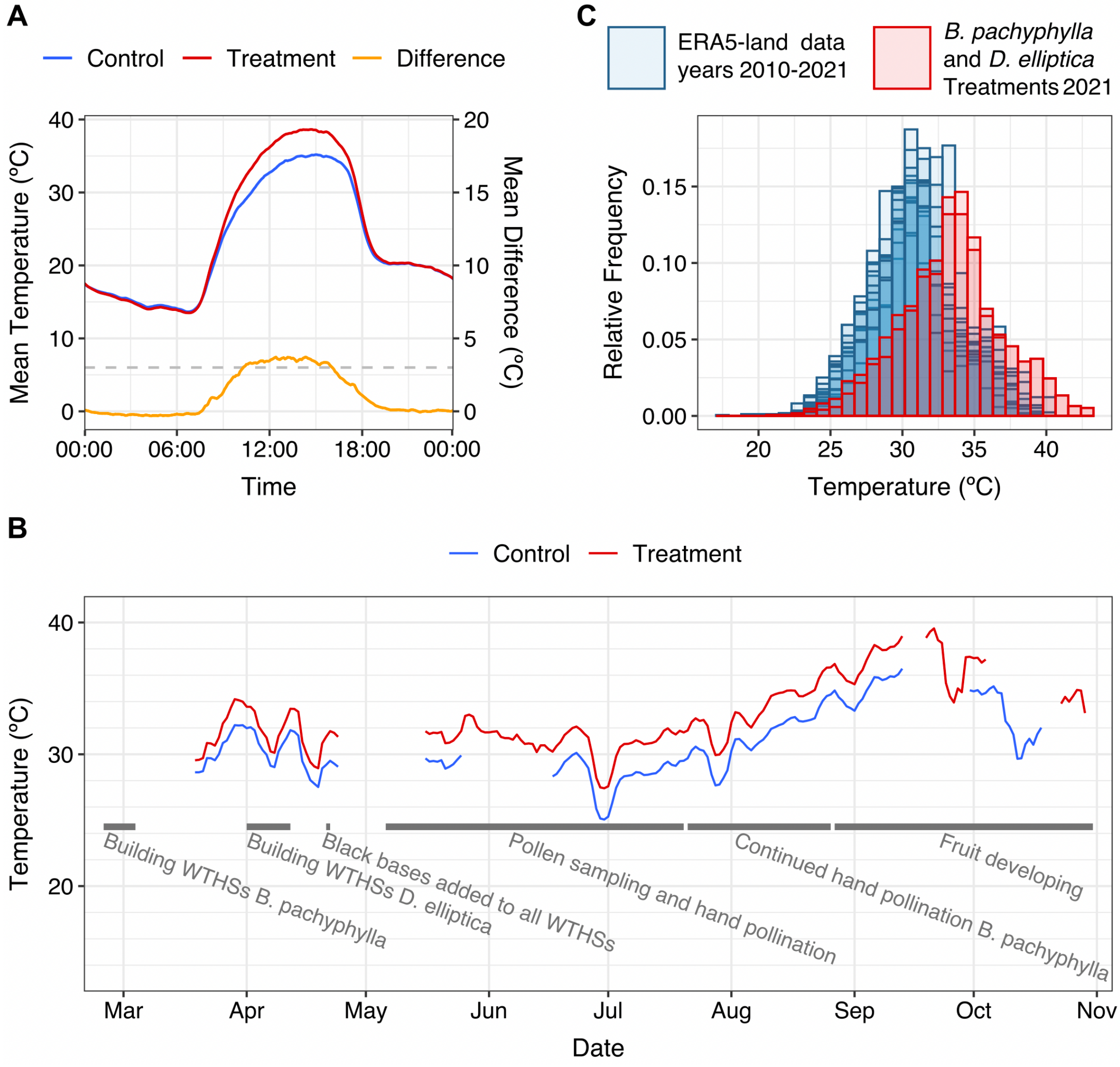

Figure 4.2 *Temperatures during the whole-tree heating experiment in 2021. (A) Mean diurnal pattern of 5-minute control and treatment temperatures for July 2021 (as example), showing the difference between control and treatment temperatures (heating effect) on the right-hand axis for clarity. Dashed line indicates target heating effect of 3°C. (B) 5-day moving averages of mean daytime (06:30 – 18:30) temperatures in 2021 recorded by our own sensors, showing marked periods of the experiment. Temperatures during each period are summarised in Table C1.5. (C) Peak hour (09:00 – 17:00) temperatures between March and November, comparing the estimated treatment temperatures for D. elliptica and B. pachyphylla in 2021 with the ERA5-land data for all years from 2010 to 2021 (inclusive).*

Comparing the ERA5-land data (taken as control temperature) with the complete dataset of treatment temperatures estimated for the entire experimental period of 2021, the treatment individuals of both *B. pachyphylla* and *D. elliptica* clearly and consistently experienced daytime temperatures significantly higher than the controls (Figure C2.5). Furthermore, comparing the estimated treatment temperatures for 2021 to the ERA5-land data since 2010 (for example looking specifically at the peak hours; Figure 4.2C), the treatment individuals of both species were exposed to high temperatures more frequently and to a greater degree in 2021 than in any previous year. Therefore, our heating treatment produced unprecedented thermal conditions for local individuals of the study species. Control temperatures in 2020 and 2021 were not markedly different from one another (Figure C2.6), or the previous years. However, brief unusually cold spells occurred during flowering in each year (Figure C2.6), but only one during pollen sampling (of *B. pachyphylla* in 2021). Control temperatures during sampling were very similar for *D. elliptica* individuals in both years (Figure C2.7). Whereas, *B. pachyphylla* individuals experienced much lower temperatures in 2021 than 2020 (Figure C2.7), and during this cold spell our sensors recorded minimum treatment and control temperatures of approximately 8°C (around dawn on 30th June 2021); low for the Cerrado and the coldest temperatures recorded during the entire study period.

4.4.2.3 Pollen viability

For both species in 2021 – following the development of treatment individuals under long-term daytime heating in WTHSs – the viability of pollen produced by the treatment individuals was significantly higher than that produced by the controls (p values < 0.05; Figure 4.3A; Table C1.4). The difference in mean pollen viability (between treatments and controls) was 12 ± 3% (*SE*) in *B. pachyphylla*. This difference was larger in *D. elliptica* at 24 ± 9%.

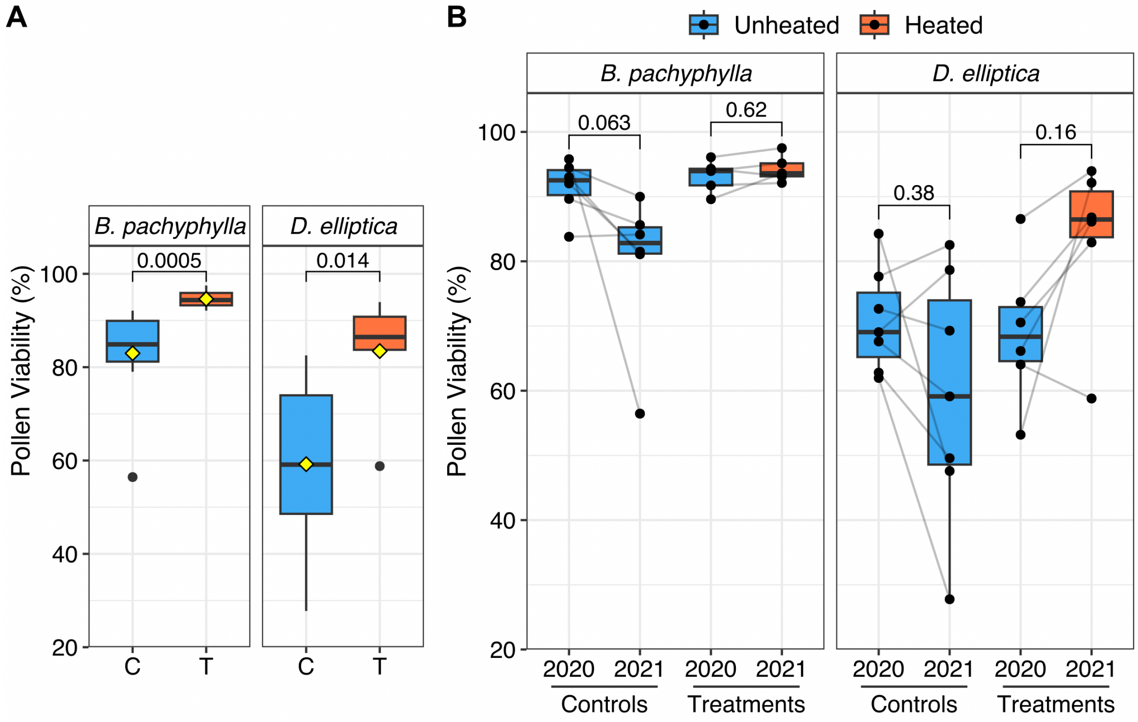

***Figure 4.3** Pollen viability of (A) all individuals sampled in 2021, grouped into controls (C) and treatments (T); and (B) individuals sampled in both 2020 and 2021, comparing individuals (grey lines) and groups between years. Yellow diamonds denote group means. Numbers in (A) denote p values of unpaired Wilcoxon rank-sum tests; and in (B) denote p values of paired Wilcoxon signed-rank tests.*

Of the individuals successfully sampled in both 2020 and 2021, several control individuals showed a reduction in pollen viability in 2021 (Figure 4.3B). Pollen viability of the treatment individuals generally remained very similar (in *B. pachyphylla*) or increased (in *D. elliptica*) in 2021, the year they were heated. However, these changes were not significant when compared using paired Wilcoxon signed-rank tests (all p values > 0.05; Figure 4.3B, Table C1.6), although the sample sizes were small (n = 5–7 depending on group).

More *B. pachyphylla* control individuals were sampled after the cold spell in June 2021 than before, including individual B16 (which showed the lowest pollen viability of all *B. pachyphylla*; Figure C2.8). As pollen viability varies with the individual, and few *B. pachyphylla* individuals were sampled both before and after this period, a direct impact of the cold temperatures on pollen viability could not be tested for. However, treatment pollen viability was already significantly higher than control before the cold spell (p = 0.056; Figure C2.9; Table C1.7) – although to a lesser degree than after (p = 0.018; Figure

C2.9) – and group results were not significantly different before and after the cold (*p* values > 0.1; Figure C2.9; Table C1.7).

4.4.2.4 Fruit set

Fruit set results for *D. elliptica* were unobtainable due to storm damage. Fruit set in *B. pachyphylla* showed high variability, both within and between individuals and groups (Figure C2.10). *CV*s in early fruit set results for example were 40% and 45% for controls and treatments respectively (Table C1.8). Early fruit set was significantly lower in the treatment individuals than in the controls, by 28 ± 9% (*SE*) on average ($R = 0.65$, $p = 0.0075$; Figure 4.4; Table C1.8). Final fruit set was similarly significantly lower in the treatment individuals, by 25 ± 9% ($R = 0.60$, $p = 0.016$; Figure 4.4; Table C1.8). Final fruit set was lower than early fruit set in almost all individuals (Figure C2.10). Fruit retention was around 85% for both controls and treatments (85 ± 4% and 84 ± 6% respectively; Table C1.8).

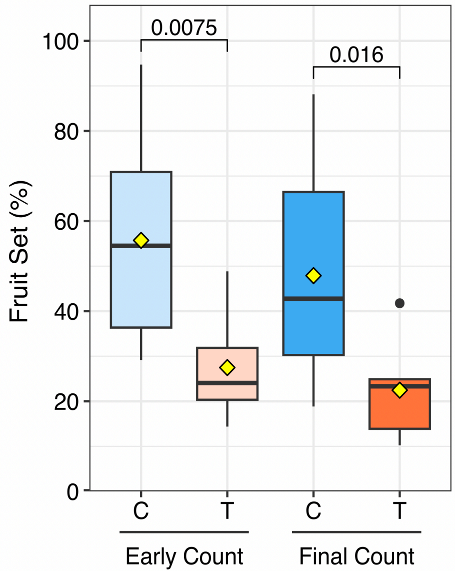

Figure 4.4. *Early and final fruit set in B. pachyphylla individuals in 2021, grouped into controls (C) and treatments (T). Yellow diamonds denote group means; numbers denote p values of Wilcoxon rank-sum tests.*

4.5 Discussion

To the best of our knowledge, this experiment was the first to employ long-term heating of entire mature individuals to investigate the high temperature responses of wild tree species *in situ* anywhere in the tropics, and one of few to assess the impacts of any form of direct heating on their reproductive success (others being Slot et al., 2023; and Werkmeister et al., in review; chapter two). Temperatures in the study area have been markedly increasing over recent decades (Marimon et al., 2020; Araújo et al., 2021; Tiwari et al., 2021), implying that local individuals may already be growing at or beyond their thermal optima (Sentinella et al., 2020; Araújo et al., 2021; Tiwari et al., 2021; Doughty et al., 2023). Our experiment added substantial further daytime heating, increasing the potential for temporary exceedance of physiological thresholds. However, contrary to our first hypothesis, heated individuals of both *B. pachyphylla* and *D. elliptica* displayed significantly higher pollen viability at anthesis than those experiencing ambient temperatures. Conversely, fruit set was significantly lower in *B. pachyphylla* individuals exposed to higher temperatures, agreeing with our second hypothesis, and suggesting a higher sensitivity of female (as opposed to male) reproductive development in the study species.

4.5.1 Pollen viability

Our whole-tree heating treatment – which was initiated prior to bud development – unexpectedly elicited significantly higher proportions of viable pollen grains (percent pollen viability) in the pollen produced by heated individuals of both *B. pachyphylla* and *D. elliptica* (compared to unheated controls; Figure 4.3A). This contrasts with the majority of available research on the susceptibility of plant sexual reproduction to temperature stress (focussed on crops) in which male reproductive development and viable pollen production is considered highly sensitive (Sage et al., 2015; Lohani et al., 2020; Goel et al., 2023), primarily due to the high temperature sensitivity of its initial stages (namely microsporogenesis; Pacini and Dolferus, 2016; Raja et al., 2019). High growth temperatures (above 25–30°C) negatively impact pollen viability in numerous crop species (Luo, 2011; Zhu et al., 2021) including several tropical and sub-tropical tree crops (for example clementine, peach and custard apple; Higuchi et al., 1998; Kozai et al., 2004; Nava et al., 2009; Lora et al., 2012; Distefano et al., 2018; Bennici et al., 2019). However, crop species are bred for high yield in optimum environments making them more susceptible to stresses (Kapazoglou et al., 2023), and experiments are often carried out in controlled environments (with constant day/night temperatures), which can elicit different responses to field experiments (Mesihovic et al., 2016).

Nevertheless, while there has been limited direct investigation of temperature impacts on reproduction in non-crop tropical species, a recent study by Slot et al. (2023) found a reduction in pollen viability in widespread tropical tree *M. calabura* (cut branches) under passive daytime warming (in greenhouses). This provided the first indication that increasing temperatures may directly impact pollen development in wild tropical species (as we hypothesised). Conversely however, Werkmeister et al. (in review; chapter two) previously found no impact of higher daytime temperatures on *B. pachyphylla* pollen when heating developing inflorescences *in situ* (in the Bacaba Park) from an early bud stage. While this suggested a high temperature threshold for pollen development in local individuals of *B. pachyphylla*, Werkmeister et al. (in review) also noted that the delayed initiation (possibly after microsporogenesis) and targeted nature of their heating treatment may have lessened its potential impacts, implying that heating entire individuals might still elicit a negative response. Nonetheless, we found the opposite (positive) response in both *B. pachyphylla* and *D. elliptica*, perhaps reflecting a greater resilience of Cerrado species to high temperatures than those native to less extreme environments, such as *M. calabura*.

While pollen viability varied between individuals in our study, there were no significant differences between treatment and control groups in 2020 (the pre-treatment year), providing confidence that higher pollen viability in the treatment individuals in 2021 was a result of the heating treatment. Interestingly, paired comparisons of pollen viability for individuals sampled in both years (Figure 4.3B) suggested an additional decrease in viability in the controls in 2021 – more so in *B. pachyphylla* – indicating a negative impact of ambient environmental conditions. Temperatures appeared to vary little from year to year, although a short spell of unusually low temperature (down to 8°C) was observed during the pollen sampling period of *B. pachyphylla* in 2021 (Figure 4.2B; Figure C2.6), and sudden low temperatures can negatively impact pollen viability (Pacini and Dolferus, 2016). More control individuals were sampled after the cold spell than treatments, which could have led to the lower control group viability had the cold temperatures reduced pollen viability overall. Nevertheless, no detectable impact was found on group-level results (Figure C2.9). Therefore, we surmise that higher pollen viability in the treatment individuals was indeed a result of the higher temperatures to which we exposed them.

Continued production of viable pollen at the treatment temperatures demonstrates a resilience to very high maximum temperatures in both study species, agreeing with the previous investigation of local individuals of *B. pachyphylla* (Werkmeister et al., in review; chapter two). An organism's ability to withstand high temperatures results from a mixture of basal thermotolerance (gained through long-term adaptation; Hasanuzzaman et al., 2013) and a range of responses at the transcriptome, proteome, and metabolome levels (the heat stress response) that act to maintain physiological homeostasis under heat stress (acclimation), which can also lead to acquired thermotolerance (Rieu et al., 2017; Raja et al., 2019; Goel et al., 2023). Being common species across the Cerrado biome (Ratter et al., 2003; Bridgewater et al., 2004), *B. pachyphylla* and *D. elliptica* are likely to be well adapted to the Cerrado environment. Furthermore, both baseline temperatures and the strength and frequency of heatwaves have been steadily increasing in the study region (Marimon et al., 2020; Araújo et al., 2021; Tiwari et al., 2021). High pollen viability at surprisingly high temperatures could therefore be a result of local adaptation of reproductive development to the high temperature growth environment, as seen for example in wild accessions of tomato (Driedonks et al., 2018), although no evidence of such adaptation has so far been found in wild tree species (Flores-Rentería et al., 2018). Expanding our experiment to heat the same species in areas of Cerrado with different mean temperatures could provide important insights into the adaptability of reproduction in woody Cerrado species, and indeed other wild trees in general.

Interestingly, our study species displayed not only high temperature thresholds, but also positive responses in pollen viability to experimental heating, which would suggest that at ambient temperatures they are growing below their thermal optima. Slot and Winter (2017a) have suggested that optimum temperatures for photosynthesis in tropical forest trees relate closely to their mean maximum daytime temperatures. As our mean treatment temperatures during the peak hours (09:00 – 17:00) were comparable to maximum ambient temperatures (Table C1.5), our treatment individuals potentially spent more time at optimum temperatures than the controls. Male reproductive development and pollen production are energy demanding processes, relying heavily on foliar photosynthesis and translocation of carbohydrates to the developing anthers and pollen (Ferguson et al., 2021; Liu et al., 2021). Furthermore, the proportion of viable pollen produced at anthesis has been linked to asynchronous development and competition for nutrients between developing pollen grains (Carrizo García et al., 2017). The higher temperatures experienced by the treatment individuals could therefore have led to the production of higher levels of viable pollen by allowing higher rates of photosynthesis overall, providing more energy for reproductive development and greater provision of photosynthates to – and less competition between – developing pollen. This may also explain why Werkmeister et al. (in review; chapter two) previously found no impact of heating on pollen viability, as heating only inflorescences would have had minimal impact on photosynthesis and nutrient availability.

In contrast, one investigation of local tree species (occurring across the Amazonia-Cerrado transition) showed that those in the Cerrado were growing close to their thermal maxima (rather than optima) for photosynthesis (Araújo et al., 2021). This would suggest that our study species were already stressed at the onset of the dry season when their flowers were developing. Furthermore, optimum temperatures for reproductive development are thought to be lower than for photosynthesis (Zhu et al., 2021). However, Cerrado temperatures rise seasonally even further in August and September, above those experienced (by either control or treatment individuals) earlier in the dry season (during pollen development in our experiment). To maintain physiological homeostasis during this time, local species may be able to acclimate to this seasonal temperature rise, for example by raising the thermal optima for photosynthesis (Gunderson et al., 2010; Slot and Winter, 2017b) and downregulating respiration rates (Slot and Kitajima, 2015; Cox et al., 2023).

Initial indications of physiological acclimation to seasonally higher temperatures were previously noted in one local Cerrado species (*Erythroxylum suberosum* A.St.-Hil.; Werkmeister et al., 2022; chapter three), and this has been detected in other tropical tree species (Sastry and Barua, 2017) including those growing nearby (Tiwari et al., 2021). It is therefore highly plausible that our study species – which exist in a high temperature environment and are seasonally exposed to even higher temperatures – have developed an effective heat stress response that allows them to acclimate (their photosynthetic, respiratory, and possibly pollen developmental pathways) to long periods of high temperature (Gunderson et al., 2010; Slot and Kitajima, 2015; Müller and Rieu, 2016; Rieu et al., 2017; Slot and Winter, 2017b; Raja et al., 2019), which we induced in our treatment individuals through long-term warming. Furthermore, acclimation of individuals inside the WTHSs to the higher daytime temperatures could have altered the balance between photosynthesis and respiration, potentially resulting in provision of more photosynthates to the developing pollen, increasing pollen viability (as mentioned above; Carrizo García et al., 2017). Future experiments such as this would be greatly improved by parallel measures of photosynthetic and respiratory activity (or photosynthate availability) to reveal whether they were indeed altered at the higher treatment temperatures.

Overall, our results suggest that viable pollen production in our study species is likely to persist, and may even increase, with increasing daytime temperatures predicted for the future of the Cerrado. Nevertheless, the combined stresses of heatwaves (of increasing strength and frequency) and increasing daytime temperatures may elicit different responses in pollen viability (and physiology), as might the addition of higher night-time temperatures (Djanaguiraman et al., 2013), which are also increasing in the Cerrado (Hofmann et al., 2021). Furthermore, heat stress responses and the ability to acclimate to higher temperatures are species-specific (Hovenden et al., 2007; Gray and Brady, 2016). Whether our results would be replicated in other Cerrado species – especially monoecious species or those that flower at different times of year – remains unknown, although easily testable through the expansion of this methodology to other species.

Additionally, while many treatment pollen grains appeared viable, development at high temperatures can lead to other, less conspicuous variations in pollen grain fitness. While these can affect pollen germination and fertilisation success reducing the quantity of fruit and seed produced, they can also lead to lower quality of seed given successful fertilisation, and even affect seedling growth (Higuchi et al., 1998; Rosbakh et al., 2018). Such impacts could not be detected in our experiment as treatment pollen was not used in pollinations. However, this could be investigated in future replications of these experiments through hand-pollination of control and treatment flowers using high-temperature developed pollen. Nevertheless, although higher temperatures appeared to have a positive effect on pollen viability, treatment flowers (pollinated with control pollen) showed lower fruit set, which will likely have greater repercussions for reproductive success overall.

4.5.2 Fruit Set

As hypothesised, higher daytime temperatures had a negative impact on fruit set in *B. pachyphylla*, as found in several tropical crop species (such as passion fruit, pitaya, custard apple, and *Citrus* species; Higuchi et al., 1998; Matsuda and Higuchi, 2020; Chu and Chang, 2020; Shafqat et al., 2021), and previously found in local individuals of *B. pachyphylla* when heating inflorescences alone (Werkmeister et al., in review; chapter two). Similar to pollen development, fruit production is a strongly energy demanding process requiring the transport of photosynthates from vegetative tissues (Génard et al., 2008; Ferguson et al., 2021). As we have suggested that provision of photosynthates was increased in our treatment individuals (heated in their entirety), we might have seen a more positive impact of whole-tree – as opposed to inflorescence – heating on fruit production, as we did for pollen. Nevertheless, fruit set (both at the early and final count) was significantly reduced in the heated individuals of *B. pachyphylla* (Figure 4.4), corroborating the results of Werkmeister et al. (in review).

Interestingly, our results suggest that female reproductive development in *B. pachyphylla* is more sensitive to high temperature stress than male. Male development is usually considered the most sensitive, with heat induced reductions in fruit set often attributed to low pollen viability causing pollination failure (Zinn et al., 2010; Sage et al., 2015; Pacini and Dolferus, 2016; Rosbakh et al., 2018). Nevertheless, negative impacts of heat stress on female development have been reported in a number of species (Lohani et al., 2020; Wang et al., 2021) including tropical and sub-tropical tree crops such as apricot, peach, sweet-cherry, and olive (Rodrigo and Herrero, 2002; Hedhly et al., 2003; Hedhly et al., 2005; Nava et al., 2009; Benlloch-González et al., 2018). However, in only a few species – for example pearl millet and peach (Kozai et al., 2004; Gupta et al., 2015; Djanaguiraman, Perumal, Ciampitti, et al., 2018) – has it been specifically shown that female development is *more* sensitive than male. Indeed, we cannot rule out that higher temperatures within the WTHSs reduced the germination and tube growth of (control) pollen used to pollinate our treatment individuals leading to the reduced fruit set. Yet we found that male development in *B. pachyphylla* was resilient to (if not positively impacted by) the treatment temperatures, and pollen germination is generally more resilient to temperature stress than pollen development (Higuchi et al., 1998; Distefano et al., 2018; Chu and Chang, 2022). This would suggest that germination of *B. pachyphylla* pollen *in vivo* was unaffected in the treatments, and instead that the reduction in fruit set in fact resulted from impacts on female development.

Female reproductive development in *B. pachyphylla* appears to be much more sensitive to high temperature before or during fertilisation than after. As with male development, high temperatures can act at any stage to negatively impact female development, from the initiation of the floral organs (or pistil; namely the stigma, style and ovary) and gametophyte (embryo sac) development, to post-anthesis stages of pollen-pistil interaction, fertilisation, and embryo development (Sage et al., 2015; Wang et al., 2021). In crop species, high temperatures have been widely demonstrated to cause reduced function, deformation, or degeneration of the female gametophyte or reproductive organs; reduced stigma receptivity; imbalance of important hormones or ROS (reactive oxygen species); sugar starvation of the pollen tube or reduced levels of attractants in the style; failure of fertilisation; incorrect development of the embryo or endosperm; and early fruit drop (reviewed in Lohani et al., 2020; Wang et al., 2021). Such impacts can lead to lower fruit or seed set, size, or quality, overall reducing reproductive success, although the isolated effects of higher temperatures post-fertilisation (on embryo development and fruit retention) are much less well studied than pre-fertilisation (Hedhly et al., 2009).

Heated individuals of *B. pachyphylla* already showed a significantly lower level of fruit set than controls at the early count after pollination (Figure 4.4), and fruit retention to maturity was the same in both groups (Table C1.8). Therefore, higher temperatures in the WTHSs acted before the early fruit count (before, during, or possibly immediately after fertilisation) to reduce fruit set. Furthermore, fruit development and maturation in *B. pachyphylla* – at least in local individuals – appears resistant to extremely high temperatures (reached during the peak of seasonal heating in September). However, pinpointing the most sensitive stages more precisely would require further investigation, for example, through replication of the experiment and microscopic – or potentially biochemical – analysis of floral tissues and fruits (of both heated and unheated individuals) throughout development.

The high-temperature induced reductions in fruit set detected here and by Werkmeister et al. (in review; chapter two) could have serious repercussions for *B. pachyphylla* persistence in this area (and similar areas) of the Cerrado – and impact its ability to disperse and migrate to areas with more favourable environmental conditions (Clark et al., 2021; McNichol and Russo, 2023) – given a mid-range climate warming scenario of ~3°C. Not to mention that fruit set was already low in the control individuals (at ambient temperatures) of both studies, even given hand-pollination. Future phenological changes in response to changing environmental cues could cause further reductions in fruit set resulting from altered relationships with pollinators and pests (Vilela et al., 2018; Gérard et al., 2020). Furthermore, continued fragmentation of the Cerrado biome will likely lead to further reductions in fecundity due to pollinator limitation, genetic drift, and additional changes in flowering phenology (Athayde and Morellato, 2014; Melo et al., 2014). Overall, this could have knock-on effects for pollinators and other fauna (Butt et al., 2015; Rabeling et al., 2019), and people given *B. pachyphylla*'s socioeconomic and ethnopharmacological uses (Bonacorsi et al., 2011; Guilhon-Simplicio and Pereira, 2011; Passos, 2023).

Due to storm damage before collection, we were unable to accurately study seed size and weight (as an indicator of seed viability and vigour; Kandasamy et al., 2020), therefore we cannot comment on the impacts of heating on seed quality. However, development at higher temperatures has also been linked to reductions in seed vigour (Pagamas and Nawata, 2008; Kochanek et al., 2010; Rosbakh et al., 2018), which could exacerbate the reduction in fertility due to reduced fruit set. Storm damage also made it impossible to measure fruit set in *D. elliptica*, therefore it is still not possible to say whether the negative impact of higher daytime temperatures that we found in *B. pachyphylla* would be replicated in *D. elliptica*, or indeed other unstudied Cerrado species. Although, it is highly plausible that some additional Cerrado species – and perhaps also species in comparably extreme ecosystems such as other tropical savannahs – will show reduced fruit production similar to *B. pachyphylla* given continued climate warming. Nevertheless, this requires further investigation, for example through the expansion of our methodology to other Cerrado species and physiognomies, and indeed other tropical ecosystems.

It is important to note that successful recruitment of Cerrado species depends not only on production of viable seeds, but also on successful dispersal, seed germination, and seedling survival. A global study of seed germination temperatures suggested that tropical species are growing close to their upper thermal limits (Sentinella et al., 2020). Although seeds of many Cerrado species are very tolerant to heat shock – due to the frequency of fire in the Cerrado biome (Miranda et al., 2009) – and some even require it to break dormancy before germination (Ribeiro et al., 2013), optimum temperatures for seed germination tend to range from 20°C to 35°C (Brancalion et al., 2010; Borghetti et al., 2021; Correa et al., 2021). Increases in peak temperatures, along with changes in precipitation, could therefore limit germination in this and other areas of the Cerrado (Borghetti et al., 2021), while increased fire frequency and severity is likely to favour species with dormant over non-dormant seeds (Daibes et al., 2022). Indeed, higher temperatures are likely to elicit different reproductive responses in species with different vegetative, floral, and seed traits, that flower and fruit at different times of year, and that inhabit different Cerrado physiognomies. Interspecific variation will likely lead to changes in species composition and community structure (Grubb, 1977; Vilela et al., 2018; Daibes et al., 2022). Ecological modelling of future species/range distributions would therefore benefit from greater inclusion of regeneration niche data, gathered for example through testing of reproductive thermal thresholds and experimental manipulations such as ours (Borghetti et al., 2021; Ferreira et al., 2022).

In conclusion, this investigation, novel in its approach of studying tropical tree reproduction through whole-tree heating, provides a unique understanding of the effects of higher daytime temperatures on the study species, namely *B. pachyphylla* and *D. elliptica*. It represents an important, although only initial step in understanding how reproduction in Cerrado species – and perhaps other tropical savannah species – will respond to a changing climate. While we found resilience and even potential acclimation of pollen production to higher daytime temperatures in both study species, the contrasting negative effects on *B. pachyphylla* fruit set point to potential negative impacts on fruit and seed production overall. Clearly, whether our results represent widespread Cerrado species responses to heat requires further testing. However, for some species the combination of reductions in fruit and seed production, along with limited germination and seedling establishment, and further ecosystem fragmentation, could severely impact reproduction and recruitment into the future. Changes in species richness and community structure have repercussions for associated fauna and the provisioning of essential ecosystem services including carbon capture and climate regulation. This investigation therefore highlights the importance of expanding the literature on the impacts of increasing temperatures on tropical species reproduction, and demonstrates the potential for direct environmental manipulation experiments in understudied ecosystems.

4.6 References

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Chapter 5

Synthesis, discussion, and conclusions

Having reviewed the research on the high temperature sensitivity of plant sexual reproduction in chapter one, there is clearly a potential for steadily increasing temperatures in already hot regions to negatively impact reproduction in native species. This includes species native to the undervalued and understudied Cerrado biome of Brazil, although very little is currently known. Reductions in viable pollen, or fruit and seed production could have repercussions for the continued reproductive success of individuals, and eventually population – or even species – survival and recruitment. The overall aim of this thesis was therefore to shed light on the effects that increasing temperatures will have on the ability of tropical woody species to reproduce, specifically those in the Cerrado. To achieve this, two novel methodologies for heating developing flowers of woody Cerrado species *in situ* were designed and tested: one for heating individual inflorescences and one for heating entire mature trees. These two methodologies were each employed at a field site in the Cerrado to investigate the direct impacts of higher temperatures on native woody species reproduction, evaluating any effects on pollen development and fruit production by measuring pollen viability at anthesis (the proportion of counted pollen grains that appear viable) and fruit set of hand-pollinated flowers (the proportion of flowers that develop into fruit).

Chapters two, three, and four consist of manuscripts (under second review, published, and ready for submission for publication, respectively) documenting the work carried out. This chapter will summarise the key findings of the included manuscripts (below), attempt to draw them together, and relate them back to the original question of: how will increasing temperatures affect reproduction in tropical tree species? This will include a synthesis and discussion of the results of the work as a whole, particularly on the themes of pollen production and fruit production and the interactions between them; discussion of the implications in a wider context and potential consequences for Cerrado communities; critical analysis of the work undertaken; suggestions for future research; and some final conclusions.

5.1 Summation of works

Chapter 2: Impacts of higher daytime temperatures on viable pollen and fruit production in common Cerrado tree *Byrsonima pachyphylla* (Malpighiaceae). This study employed the novel targeted passive heating methodology (developed for this experiment) to warm the air around developing inflorescences *in situ* (starting at an early bud stage) on five individuals of *Byrsonima pachyphylla* (common and widespread tree of the Cerrado biome; also known by synonym *Byrsonima crassa*; Francener, 2023). The targeted methodology performed well, heating by 3–4°C during the daytime – 3°C on average, rising to 4°C during the peak hours of heating between 09:00 and 17:00 – simulating a potential future climate scenario. To account for variation between individuals, control inflorescences were monitored on the same five individuals. Pollen viability at anthesis was estimated through the complementary techniques of differential pollen staining and *in vitro* pollen germination. Flowers were hand pollinated and counted, and the resulting fruits collected and counted to quantify fruit set. In contrast to the hypothesis, targeted heating had no impact on pollen viability, suggesting a high thermal threshold for male reproductive development in the study species. However, as anticipated, fruit set was significantly reduced in the heated inflorescences, suggesting a higher sensitivity of female development, and pointing to an overall negative impact of future higher temperatures on *B. pachyphylla* reproductive success.

Chapter 3: A novel *in situ* passive heating method for evaluating whole-tree responses to daytime warming in remote environments. This chapter detailed the methodology which was developed for passively heating entire individuals (up to 2.5 m tall, but easily expandable) *in situ* in the Cerrado. Multiple whole-tree heating structures (WTHSs; one larger 6-sided prototype and four smaller 4-sided structures) were built around individuals of two common woody Cerrado species (namely *Davilla elliptica* and *Erythroxylum suberosum*) and evaluated for their impact on internal air temperature and humidity. A short (4-week) study of the responses of foliar photosynthesis and dark respiration to heating was also carried out on the enclosed individuals of *E. suberosum* (and external controls) in order to demonstrate the use of the WTHSs in an experimental set up in the field. The WTHSs produced a consistent and reproducible heating effect during the day of 2.5–3.5°C, again simulating a possible future climate scenario, with minimal impact on humidity. No physiological response to the short-term (21-day) heating treatment was observed in the enclosed individuals of *E. suberosum*. However, a downward trend in respiration rates (at set temperatures) and upward trend in optimum and maximum temperatures for photosynthesis were observed in both the treatment and control groups, suggesting a possible acclimation response to seasonal warming in August.

Chapter 4: Contrasting responses of pollen and fruit to whole-tree heating in tropical savannah species. This work summarised an investigation carried out over two years. In the first year (pre-treatment year) pollen was sampled from (up to) 16 individuals of both *B. pachyphylla* and *D. elliptica* to gain an understanding of the levels of pollen viability in local individuals of each species under natural conditions. In the second (treatment year), six of these individuals (per species) were enclosed in the previously described WTHSs prior to floral structure development and heated until fruit maturation in order to quantify the impact on pollen viability at anthesis (through pollen sampling and staining) and fruit set (of hand-pollinated flowers). Contrary to expectation, pollen viability was higher in the heated individuals than the controls for both species. This indicated not only a high thermal threshold for the production of viable pollen in Cerrado species locally but also a positive impact of higher temperatures on pollen production, potentially as a result of local adaptation to the extreme environment, or physiological acclimation (of reproductive development and perhaps also of photosynthesis and respiration) to higher temperatures. Nevertheless, as hypothesised – and agreeing with the results of the original inflorescence heating study (chapter two) – fruit set was reduced at higher temperatures in *B. pachyphylla*, and this was already apparent at the earliest stages of fruit development. Fruit set results were unobtainable for *D. elliptica* and so how widespread this response would be remains unknown.

5.2 Synthesis of high temperature impacts on Cerrado ecosystems

5.2.1 Discussion of findings

These chapters clearly show the progression of my doctoral research, from the design, testing, and utilisation of the targeted passive heating methodology in a small-scale study of reproduction in one Cerrado species; through the development and testing of the WTHSs; to the utilisation of these in a larger-scale whole-tree heating experiment comprising more individuals and species. In their most simple form, the key findings of these works together are that in the common and widespread Cerrado species studied (namely *D. elliptica* and *B. pachyphylla*), pollen viability may increase with increasing temperatures; however, fruit production – and therefore final reproductive output – will likely decrease (at least for *B. pachyphylla*), with consequences for species recruitment and persistence into the future.

5.2.1.1 Higher temperatures and pollen

Targeted inflorescence heating (chapter two) had no noticeable impact on the production of viable pollen in *B. pachyphylla*, although a resilience of male reproductive development and pollen production to high temperatures (daily maxima above 40°C) was apparent. This was unexpected as the available literature on the thermal sensitivity of plant sexual reproduction (predominantly studying crops) suggests that pollen viability (and particularly the initial stages of development, including microsporogenesis) can be negatively affected by growth temperatures above 25–30°C (Luo, 2011; Zhu et al., 2021), even in species native to the tropics (Higuchi et al., 1998; Lora et al., 2012). Additionally, the only study of thermal thresholds in Cerrado trees (although photosynthetic thresholds) suggests that they are already temperature stressed during the dry season (at the time of floral development in *B. pachyphylla* and *D. elliptica*) without additional heating (Araújo et al., 2021). Furthermore, a recent study of cut branches of *Muntingia calabura*, a tree species widespread in the tropics, showed reduced pollen viability when exposed to daytime warming similar to our treatments (both inflorescence and whole-tree heating treatments; Slot et al., 2023).

Unfortunately, the initiation of this first experiment was unavoidably delayed, and it is therefore possible that the most sensitive stages of pollen development (such as microsporogenesis) had occurred before heating began. However, many buds developed later during the experiment (and therefore entirely under higher temperatures), yet length of heating had no obvious impact on pollen viability, suggesting that delayed heating initiation did not affect the results. Alternatively, it is possible that targeted inflorescence heating reduced the potential impacts of the heating treatment on pollen production by excluding any additional effects of higher temperatures on whole-tree physiology and their interactions with reproductive development. Sensing and responding to environmental change involves complex signalling pathways and resource re-allocation, potentially acting over long distances (involving the entirety of an individual; Hasanuzzaman et al., 2013; Belhassine et al., 2019; Delker et al., 2022). Furthermore, pollen production is an energy demanding process that relies on foliar photosynthesis and metabolite transport – processes also potentially altered by high temperatures (Sage and Kubien, 2007) – from vegetative tissues to the developing floral tissues and pollen grains (Ferguson et al., 2021; Liu et al., 2021), yet in this experiment the vegetative tissues remained predominantly unheated. However, a targeted heating methodology was initially chosen to allow comparison of inflorescences under control and treatment conditions on the same individual due to the anticipated (and eventually detected) variation in pollen viability and fruit set at the individual level. Nevertheless, given the lack of impact of targeted heating on pollen development, the methodology was developed to heat entire individuals (chapter three) to provide a more realistic simulation of climate warming, and we hypothesised that whole-tree heating (from as early as possible before floral development) would elicit a more negative response in pollen development.

The results of the whole-tree heating experiment (chapter four) confirmed the high temperature resilience of pollen development in *B. pachyphylla*, and the same resilience was also found in *D. elliptica*. However, conflicting with our first hypothesis, whole-tree heating induced an increase in viable pollen production in both species. As discussed in chapter four, this may have been a result of long-term adaptation of Cerrado species (Sarmiento et al., 1985) – and perhaps even local individuals (Driedonks et al., 2018; Maher et al., 2019) – to their high temperature growth environment, although little evidence currently exists of local adaptation of reproductive development in trees (Flores-Rentería et al., 2018). It could have also resulted from the activation of a strong heat stress response (HSR; Giorno et al., 2013; Qu et al., 2013) that the study species have likely developed to tolerate significant seasonal warming (that occurs later in the year), and which we may have induced by heating entire individuals, providing protection from and promoting physiological acclimation to the higher treatment temperatures (Aspinwall et al., 2016; Müller and Rieu, 2016). In fact, some indication of physiological acclimation (of photosynthesis and dark respiration) to local seasonal warming was noted during our short-term study of *E. suberosum* (chapter three), and has been detected in other tropical tree species (Sastry and Barua, 2017; Tiwari et al., 2021). Although, in comparison to controls, *E. suberosum* individuals did not noticeably acclimate to experimental heating within the WTHSs. However, the enclosed individuals were only heated for a short time (three weeks in total), and the small number of individuals and leaves measured meant statistical significance of any differences between treatment and control groups would have been difficult to detect.

Nonetheless, it is possible that during the longer-term heating experiment (chapter four), acclimation of photosynthesis and respiration (increases in photosynthetic optima and downregulation of respiration rates) and changes in the balance between them led to higher levels of photosynthates being transported to the developing inflorescences. This could have supported floral development and possibly reduced competition for nutrients during pollen development (Carrizo García et al., 2017), leading to higher proportions of viable pollen grains at anthesis. The possibility of CO_2 built up inside the WTHSs was also considered (although not discussed in chapter four), as elevated CO_2 in combination with warming can lead to increased photosynthesis and photosynthate provision for reproduction (Alzate-Marin et al., 2021). Nevertheless, short-term testing of an additional WTHS (erected the following year) provided no evidence of an impact on internal CO_2 concentrations (Figure D1.1). Alternatively, the same effect may have simply resulted from treatment individuals spending longer at temperatures close to already high photosynthetic optima (which may correspond to mean maximum ambient temperatures; Slot and Winter, 2017). Perhaps it is most plausible that adaptation and acclimation acted together to provide physiological resilience and increased pollen viability in *D. elliptica* and *B. pachyphylla* at higher temperatures (Rieu et al., 2017; Wittemann et al., 2022). In any case, testing of these theories requires long-term measurements of photosynthesis and respiration of enclosed individuals, which could be carried out in the future given repetition or replication of these experiments in other areas and species.

Given the recently found reduction in *M. calabura* pollen viability under heating (Slot et al., 2023), our contrasting results from *B. pachyphylla* and *D. elliptica* might suggest a higher temperature tolerance of pollen development in Cerrado trees than tropical forest species. This is not surprising considering Cerrado species are likely well adapted to their more extreme growth environment (Franco et al., 2008; Simon and Pennington, 2012; Araújo et al., 2021). This resilience of pollen production may therefore be shared by other species in similarly extreme environments such as other tropical savannah ecosystems, which also remain understudied (Murphy et al., 2016). However, Slot et al. (2023) warmed only cut branches of *M. calabura*, and it could later be found that exposure of entire individuals to higher temperatures would give different results, although achieving this experimentally would be difficult given the larger-stature of this tree species, and indeed other forest species. Furthermore, both our experiments and the study by Slot et al. (2023) provide only a very initial investigation of the impacts of temperature on pollen development in wild tropical trees, and any conclusions drawn are therefore mostly theoretical until similar studies are carried out on other species and in other ecosystems.

5.2.1.2 Higher temperatures and fruit

Fruit set following hand-pollination was reduced in *B. pachyphylla* inflorescences exposed to higher temperatures during development (compared to ambient controls), regardless of whether only inflorescences (chapter two) or entire trees (chapter four) were heated. This confirmed our second hypothesis (in both cases), and was consistent with heat-induced reductions in yield documented in many species of tropical crops (Higuchi et al., 1998; Chu and Chang, 2020; Matsuda and Higuchi, 2020; Shafqat et al., 2021). Since both targeted inflorescence and whole-tree heating had the same negative impact on fruit set, we have confidence that naturally increasing temperatures will indeed cause reductions in fruit production in this species (in this area).

In our whole-tree heating experiment (chapter four), the significant difference between treatment and control fruit set was already apparent at the early fruit count, and the proportions of fruit retained were the same (approximately 85%) for treatments and controls. It is therefore likely that higher temperatures acted prior to or during fertilisation – as opposed to after – to cause the reduced fruit set (although impacts on initial embryo development immediately after pollination are also possible). Furthermore, given the high levels of fruit retention, later embryo development and fruit maturation in *B. pachyphylla* appear resilient to very high temperatures (that both controls and particularly treatments reached during the peak of seasonal warming in September), which was not apparent during the inflorescence heating experiment (chapter two) as an initial fruit count was not carried out.

In both reproduction experiments (chapter two and chapter four), high temperatures may have acted during floral development, causing malformation or malfunction of female floral organs or gametophytes with direct effects (such as gametophyte sterility), or causing disruption of the interactions between the pollen grains and female tissues during and after pollination (discussed more thoroughly in chapters one, two, and four; and reviewed in Herrero, 2003; Sage et al., 2015; Lohani et al., 2020; Zhu et al., 2021). Although, male-female interactions could have also been affected by further impacts of high temperatures – on either female or male reproductive tissues and gametophytes – between pollination and fertilisation (which interestingly may occur roughly 48 hours after pollination; Hughes, 2021). However, while we did not test the germinability of *B. pachyphylla* pollen at the treatment temperatures, we found a strong resilience of pollen development to high temperature, and pollen is thought to be less sensitive to temperature after dispersal than during development (Higuchi et al., 1998; Distefano et al., 2018; Chu and Chang, 2022). We therefore assume that it was indeed female tissues that were affected, although testing of the thermal thresholds for pollen germination in the study species would have provided more confidence in this. Which elements of female development or tissues were most affected to cause the reduction in fruit set we cannot say without further floral heating and investigation, perhaps through microscopic analysis (given the necessary expertise in floral morphology) of buds and flowers at all stages of development, including after anthesis and pollination.

High-temperature development of seeds can impact early embryo and endosperm development, leading to underdeveloped or abnormal seeds with limited germinability (viability) or vigour (Pagamas and Nawata, 2008; Hampton et al., 2013; Wang et al., 2021). Although mentioned little in the previous chapters, attempts were made during the experiments to quantify the effects of heating on the seeds produced. Following inflorescence heating (chapter two) we attempted to clean, weigh, and measure the collected seeds of *B. pachyphylla*, and then germinate them in incubation chambers (after scarification). Unfortunately, the measurements taken were inaccurate (due to the accidental use of imprecise equipment) and none of the seeds (treatments or controls) germinated successfully, likely due to the difficulties of breaking seed dormancy and mimicking optimal conditions for Cerrado species seed germination in a laboratory setting (Zaidan and Carreira, 2008; Brasil, 2009; Alberto et al., 2011; Colado et al., 2020). During the whole-tree heating experiment (chapter four) we attempted again to weigh and measure the seeds. However, the issues of storm damage and early degeneration of fruits meant variation in seed mass could have resulted from falling early in the storms, fruit rotting in the protective net bags, or the treatment temperatures themselves; reliable comparisons between treatments and controls were therefore not possible. Attempts were made to study seed viability through the widely used method of tetrazolium testing (which distinguishes between living and dead tissue by detecting respiration; França-Neto and Krzyzanowski, 2019), although they were unsuccessful. This method (involving application of 2,3,5-triphenyl tetrazolium chloride solutions to excised embryos) was challenging and required strong species-specific knowledge of the process and the seed anatomy (Maldonado-Peralta et al., 2016; Assis et al., 2017; Grzybowski et al., 2017), which we lacked, yet the reliability of the results would have been questionable regardless considering the aforementioned issues at seed collection. Nevertheless, this could be considered in the future given more research and training with seeds of the study species.

Interestingly, although male reproductive development is generally considered more sensitive than female (Zinn et al., 2010; Giorno et al., 2013; Sage et al., 2015; Lohani et al., 2020), the contrasting impacts of heating on pollen viability and fruit set in *B. pachyphylla* suggest that female development was negatively affected by high temperatures while male development was not. Female development has been demonstrated to be more sensitive in some species, for example peach, in which growth temperatures of ≥20°C have been found to cause underdevelopment and degeneration of female gametophytes (embryo sacs; Kozai et al., 2004) or to reduce stigma receptivity (limiting pollen grain adherence, germination, and pollen tube growth; Hedhly et al., 2005). Peach pollen germination *in vitro* was not affected at the same temperatures (Kozai et al., 2004; Hedhly et al., 2005), suggesting a lower sensitivity of the male gametophyte, although these temperature treatments were applied to pollen after anthesis (rather than during development). However, pollen viability in other genotypes of peach has been shown to be unaffected by pre-blooming temperatures of 30°C (Carpenedo et al., 2017). Indeed, studies directly comparing male and female development and their relative sensitivities are rare (Lohani et al., 2020). Nevertheless, Gupta et al. (2015) have shown that female development of pearl millet (*Pennisetum glaucum*) is more sensitive than male, which has since been explained by greater oxidative damage (potentially due to fewer antioxidant enzymes) in female tissues than pollen grains at high temperatures (Djanaguiraman et al., 2018).

Female reproductive development and fruit and seed production are also water, energy, and nutrient demanding processes (Ruan et al., 2012). Additional possible causes of low *B. pachyphylla* fruit set under whole-tree heating (chapter four) could therefore include resource limitation or altered resource allocation. Cerrado soils are nutrient poor (Haridasan, 2008) and water availability in the Cerrado decreases throughout the dry season (Oliveira et al., 2005), increasing the likelihood of nutrient deficiency and drought stress in the study individuals as the experiment went on. Although we have already suggested that an increase in photosynthate availability in the heated individuals led to higher viability of pollen samples (due to lower competition for resources; Carrizo García et al., 2017), hand-pollination of study flowers continued later into the dry season than pollen sampling and temperatures had begun to increase seasonally during this time. Furthermore, while *B. pachyphylla* is considered evergreen (Boas et al., 2013), increasing temperature and drought stress during the Cerrado dry season can lead to foliar degeneration and leaf dropping (Araújo et al., 2021). It is therefore possible that at the time of pollination and initial embryo development (particularly for flowers pollinated later), treatment individuals had begun to experience temperatures above their thermal maxima for photosynthesis (regardless of any previously suggested acclimation) or were experiencing higher levels of foliar degeneration and leaf loss than controls, limiting the provision of photosynthates from source to floral sink tissues and therefore the resources available to support fruit set. Alternatively, individuals under increased stress may have allocated more resources to growth and survival at a cost to reproduction (Bazzaz et al., 1987; Ashman et al., 2004), or even allocated more resources to male over female reproduction (as found in longleaf pine, *Pinus palustris,* under warming; Guo et al., 2017), which might also explain the higher pollen viability in heated individuals. As we did not monitor leaf health, measure photosynthesis, or track the fate of each pollinated flower, we cannot know whether photosynthate availability or the frequency of fruit set decreased over the pollination period. Nevertheless, such variables could be monitored in future given repetition of this experiment, and the dynamics of fruit set evaluated through repeated fruit counts throughout (from the start of pollination).

Under targeted heating (chapter two), heated inflorescences on unheated individuals showed reduced fruit set compared to controls on the same individual, which would suggest a more specific detrimental impact of heating on the target tissues themselves. However, this could have also resulted from individuals reallocating resources away from the stressed branches (Sprugel, 2002), rather than away from fruit production as a whole. This simply confirms that further investigation is needed to identify the specific cause of the reduced fruit set, potentially through anatomical investigation looking for abnormalities in the floral structures and gametophytes throughout the periods of both floral development and male-female interaction.

5.2.1.3 Interacting impacts on pollen and fruit

While it is necessary to consider the pollen viability and fruit set results separately to try to elucidate which mechanisms will be affected by increasing temperatures, and how, it is also important to consider how the two diverging responses would act together given natural climate warming. It has been suggested that there is a high prevalence of pollen limitation in natural populations of flowering plants (Knight et al., 2005), meaning that the number of fruits and seeds produced are limited by the amount of suitable viable pollen received by flowers. If pollen limitation is prevalent in the Cerrado, increased production of viable pollen in response to warming (as detected in *B. pachyphylla* and *D. elliptica* under whole-tree heating) may help to alleviate pollen limitation and improve the likelihood of viable pollen deposition (and therefore fertilisation) in the future. Increased pollen viability in *B. pachyphylla* could potentially also counteract (at least partially) the reduction in fruit set under warming suggested by our results. Although, that depends on which element of female development was most affected (and whether pollen germination, pollen tube growth, and fertilisation were still possible in the heated flowers). As we did not monitor fruit set under open pollination (natural conditions) – only under hand-pollination, which would have provided surplus viable pollen for successful fertilisation and fruit production – we cannot know whether species in the study area are generally experiencing pollen limitation. Nevertheless, increased pollen viability could be advantageous regardless, considering the potential negative impacts that climatic changes will likely have on pollinator prevalence and activity in the future (Scaven and Rafferty, 2013; Elias et al., 2017; Rabeling et al., 2019).

Higher temperatures have also been shown to reduce (Nerd et al., 2002; Albrigo and Galán Saúco, 2004; Petrie and Clingeleffer, 2005) – or sometimes increase (del Cacho et al., 2013) – flower production, which will also feedback on pollen availability and the probability of successful pollination and seed production in the future. However, the number of flowers hand-pollinated per inflorescence on control and treatment individuals of *B. pachyphylla* during whole-tree heating (25 ± 7 *SD* and 28 ± 5 respectively; data not included in chapter) does not suggest an impact of heating on flower production, while pollinated sections of branches of *D. elliptica* varied in length (therefore flowers were not counted in comparable units). Nevertheless, flower production might be more strongly affected by longer-term warming, which could be investigated in the future through multi-year experiments (or long-term observation and correlation with climatic variables).

It is also important to consider that while heating entire individuals during floral development led to higher viability of pollen samples, it may have had additional, less conspicuous effects on pollen grain fitness, which could still impact reproductive success overall. For example, Higuchi et al. (1998) found that unheated flowers of cherimoya (*Annona cherimola*) pollinated with high-temperature developed pollen produced abnormal fruits, while Iovane and Aronne (2022) found that development at high temperature shortened the lifespan of tomato (*Solanum lycopersicum*) pollen by accelerating the senescence of originally viable grains. Furthermore, high temperature impacts on viable pollen may even affect seed germination, seedling vigour, and progeny phenotype (Galloway, 2001; Rosbakh et al., 2018). On the other hand, development at high temperatures can also lead to thermal acclimation of the pollen grains themselves, making them more resistant to high temperatures after dispersal (increasing pollen thermotolerance; Mesihovic et al., 2016; Müller and Rieu, 2016). As we potentially detected thermal acclimation in the heated study individuals, they may have also produced pollen with improved thermotolerance. This in turn could improve *in vivo* pollen germination and pollen tube growth at higher temperatures, which could also partially relieve any possible reductions in fruit set (again, depending on the mechanism by which fruit set was affected). Any additional effects of heating on pollen grain fitness – whether positive or negative – could not be detected in our experiments as no flowers were pollinated with high-temperature developed pollen, yet these could significantly influence the overall impact that warming has on reproduction in the future (and should be tested for given experiment repetition).

5.2.1.4 Additional climate factors

In addition to daytime temperatures, night-time temperatures are increasing (Hofmann et al., 2021). Furthermore, the Cerrado is experiencing heatwaves of increasing strength and frequency (Marimon et al., 2020; Tiwari et al., 2021) and potentially a longer dry season (Fu et al., 2013; Marengo et al., 2018), which will likely compound the effects of temperature stress already experienced by individuals due to gradual warming. Increased drought stress alone can lead to reduced growth and productivity, abnormal development of anther tissues and pollen grains, increased flower abortions, and reduced pollen release; overall reducing viable pollen availability and fruit set (Descamps et al., 2021; Lamin-Samu et al., 2021). Additionally, heatwaves at the time of flowering are likely to cause further reductions in fruit production in species that show sensitivity of fruit set (such as *B. pachyphylla*) and could potentially take individuals above their thermal thresholds for pollen production (which logically must exist although we didn't reach them in our experiments) and other physiological processes.

Higher night-time temperatures (alone) can also limit pollen viability and reproductive success (Shi et al., 2013; Djanaguiraman et al., 2013; Echer et al., 2014; Sadok and Jagadish, 2020). Having chosen to use passive heating methodologies, we were unable to test the effects of night-time warming along with daytime, although warmer nights would likely exacerbate the demonstrated negative impacts of warmer days on fruit set (Lohani et al., 2020). However, it is difficult to comment on whether the positive impact of daytime warming on pollen viability that we detected would persist given increasing night-time temperatures. Although the thermal maxima for pollen development is clearly high in our studied species, in some crops (for example wheat), specific genotypes already considered to be more tolerant to high daytime temperatures are among the most susceptible to night-time warming (McAusland et al., 2023). Higher night-time temperatures have been suggested to lead to upregulation of dark respiration, reducing the quantity of photosynthates available to reproductive tissues; affect transport of said photosynthates to the reproductive tissues; increase night-time vapour pressure deficit (VPD) and stomatal conductance, affecting water use efficiency and increasing water stress; and increase leaf senescence, reducing daytime photosynthetic capacity (again reducing photosynthate supply; Sadok and Jagadish, 2020). Therefore, it is plausible that higher night-time temperatures might counteract the positive impact we found of higher daytime temperatures on pollen viability (which was potentially a result of increased photosynthate availability), yet this requires further testing (through active heating).

5.2.2 Implications in a wider context

Increasing temperatures and other aspects of climate change have the potential to impact not only pollen viability and fruit set, but many other aspects of reproduction (such as flowering phenology, flower production, seed longevity and germination, and seedling survival; some of which have already been discussed), and affect not only the focal species, but also those with which they interact. It is therefore important to consider the results of these experiments in the wider context.

5.2.2.1 Species interactions

Higher levels of pollen viability under warming (suggested by the whole-tree heating experiment) may increase the likelihood of a pollination event given visitation by a pollinator and transference of said pollen to another individual, and may also provide higher nutrition to pollinators and other pollen foraging insects (Yeamans et al., 2014; Walters et al., 2022). However, as previously mentioned, increasing temperatures can also impact the number of flowers produced, with direct consequences for the availability of pollen. They can also cause changes in flower morphology and nectar availability, possibly altering their attractiveness to – or their ability to deposit pollen onto – pollinators (Scaven and Rafferty, 2013), influencing the likelihood and efficacy of floral visitations.

Changes to flowering and fruiting phenology may also impact the likelihood of interactions with pollinators (and pests). Changing environmental cues can shift the time of year a species flowers (Sherry et al., 2007; Numata et al., 2022), or even the time of day (Scaven and Rafferty, 2013), which again may have positive (for example creating more overlap between flowering time and peak pollinator activity; Alzate-Marin et al., 2021) or negative (for example causing pollinator mismatch or increasing competition for pollinators; Gérard et al., 2020) impacts on pollinator interactions, altering the likelihood of reproductive success. Changes in climate can also affect pollinator health and alter their lifecycles (Walters et al., 2022), affecting their abundance at certain times of year, or their activity at certain times of day, further impacting plant-pollinator interactions (Scaven and Rafferty, 2013). Although, species with specialist pollinators (as opposed to generalist or wind pollinated species) and those that require outcrossing (are not self-compatible) are likely to be more strongly affected. For example, both *B. pachyphylla* and *D. elliptica* – although visited by a variety of species – are predominantly pollinated by bees (Gottsberger, 1977; Boas et al., 2013; Melo et al., 2014; Hughes, 2021), and therefore might suffer pollinator limitation if bee populations are severely affected by climate change impacts (Elias et al., 2017).

Interestingly, by studying the reproduction and species interactions of four species in the Malphigiaceae family (the family of *B. pachyphylla*) in the Cerrado over six years, Vilela et al. (2018) demonstrated changes in flowering and fruiting phenology, which for some species correlated with variation in local temperature and precipitation. These changes caused greater overlap of flowering, altered species interactions with pollinators and herbivores, and affected fruit production. The study also highlighted that phenological responses to environmental change, pollinator and herbivore responses and interactions, and the impacts on fruit production can vary greatly with species (even those closely related). Study of reproductive phenology and pollinator interaction was outside the scope of this thesis. However, long-term use of the WTHSs could provide some indication of the impacts of higher temperatures on phenology of our study species, and the impacts this might have on reproductive success, if maintained over multiple years.

In addition to rising temperatures, the Cerrado faces very high rates of land use change (Klink and Machado, 2005; Garcia and Ballester, 2016; Strassburg et al., 2017) and this has left its vegetation highly fragmented (Carvalho et al., 2009). Fragmentation can lead to localised warming and other environmental changes (Camargo et al., 2011), which can potentially impact plant species fertility (Athayde and Morellato, 2014). In fact, Melo et al. (2014) demonstrated reduced fruit set of *B. pachyphylla* in fragments, decreasing further with decreasing size. This may be due particularly to the impacts of fragments on pollinator prevalence, activity, and interactions with plant species (Cunningham, 2000; Melo et al., 2014; Franceschinelli et al., 2015), potentially also altered by fragment impacts on flowering phenology (Camargo et al., 2011; Vogado et al., 2016). Furthermore, having fewer individuals within a population has been suggested to lead to reduced genetic variability and genetic drift within fragments, with deleterious effects on species fecundity (Bittencourt and Sebbenn, 2009; de Almeida et al., 2012). Not only will this likely reduce future recruitment within fragments, but it will also limit the ability of species to disperse seeds to other suitable areas of vegetation, potentially leading to the extirpation of fragmented populations and reductions in species' ranges (Aitken et al., 2008).

5.2.2.2 Regeneration from seed

Even though all of our study flowers were hand-pollinated, control inflorescences and individuals of *B. pachyphylla* (under ambient conditions) demonstrated low levels of fruit set in both reproduction experiments (chapters two and four), perhaps due to the already stressful environment and low levels of water and nutrient availability in the Cerrado. Hand-pollination of *B. pachyphylla* has been shown to be at least as effective as – if not more than – pollination by natural pollinators (given visitation; Boas et al., 2013; Hughes, 2021); therefore, fruit set under open pollination is likely to be even lower than in our controls. Further reductions in fruit set due to warming (suggested by our experiments) could severely limit species recruitment, with consequences for local species persistence over time (Walck et al., 2011; Daibes et al., 2022). Although, this would depend on reproductive strategy and the level of seed production and recruitment necessary to maintain a population. Vegetative reproduction (sprouting new shoots from roots or stem) is a common strategy for woody Cerrado species as it aids in the resilience to – and recovery of communities after – fire (Hoffmann, 1998). However, recruitment from seed is also common (Daibes et al., 2022) and is important for maintaining genetic variation within a population (de Andrade and Miranda, 2014).

When seeds fall, they often do not germinate immediately and instead become part of the soil seed bank, which might help to buffer the impacts of environmental variation on seed availability. Many woody Cerrado species (including many *Byrsonima* species; Zaidan and Carreira, 2008; Alberto et al., 2011; Grzybowski et al., 2017) produce seeds with some sort of dormancy, which require time or environmental cues (such as high or low temperature, water availability, or presence of smoke and other chemical cues) to break the dormancy and begin germination (Zaidan and Carreira, 2008; Fernandes et al., 2021), and which may be able to persist for a long time in the seed bank. However, the seed bank of Cerrado species is still considered transient (seeds remain for one year or less) rather than persistent (seeds remain viable for over a year; Salazar et al., 2011; de Andrade and Miranda, 2014). Furthermore, as a result of generally low seed production and dispersal, and high levels of seed predation and removal, many woody Cerrado species have been found to be seed-limited, including *B. pachyphylla* and *D. elliptica* (Salazar et al., 2012). This means that the negative impacts of a warming climate on fruit set (which we detected in *B. pachyphylla*) and possible further impacts of additional extreme heat events (discussed earlier) could have a large impact on seed availability and seedling recruitment, even if only affected in one year. This would have even more serious consequences for rarer species whose seed bank is contributed to by few individuals.

For the seeds that *are* produced and dispersed, increasing fire frequency and severity (Silva et al., 2019) – along with the aforementioned increases in temperature, heatwaves, and dry season length (Marengo et al., 2018; Hofmann et al., 2021; Tiwari et al., 2021) – will likely intensify degeneration of seeds in the soil seed bank (Miranda et al., 2009; Correa et al., 2021). Dormancy mechanisms can provide resistance to fire and heat shocks (Ribeiro et al., 2013) or help to control the timing of germination to occur when conditions are most favourable (such as when more water is available for seedling growth; Salazar et al., 2011). However, altered environmental cues may cause release of seeds, release of dormancy, or seed germination at inopportune times; for example before seeds have matured, or at times when – or in locations where – conditions are not suitable for seedling growth and survival (Correa et al., 2021; Daibes et al., 2022; Fontenele and Miranda, 2023). Additionally, although seeds of many Cerrado species are resistant to very high temperatures, optimum germination temperatures are thought to be more moderate (ranging between 20 and 35°C; Brancalion et al., 2010; Borghetti et al., 2021; Correa et al., 2021), and increasing average temperatures are reducing the extent of suitable areas and suitable periods of time available for seed germination and seedling establishment (Ribeiro et al., 2019; Correa et al., 2021; Daibes et al., 2022; Ferreira et al., 2022). Furthermore, warming may impact seedling growth and vigour, potentially affecting the success of recruitment given seed germination. However, such impacts could potentially be positive (Stevens et al., 2014) or negative (Ribeiro et al., 2019; Nottingham et al., 2023) and are not yet well understood. Nevertheless, each of these factors have the potential to limit the success of seed germination and continued recruitment of Cerrado species into the future, which will be of even greater consequence if the production of seeds is also limited by increasing temperatures, as suggested by our results.

5.2.2.3 Range edges

As the climate of the Cerrado changes, the suitability of areas currently occupied by many species will likely decline (Siqueira and Peterson, 2003; Angulo et al., 2021; Alves and Loeuille, 2022). To persist, species will have to acclimate and adapt, or migrate to more suitable areas (Aitken et al., 2008; Feeley et al., 2012). Migration relies on the production and dispersal of seeds to areas suitable for germination and recruitment, particularly by individuals at the leading edge of a species range (Rehm et al., 2015). For those species that respond similarly to *B. pachyphylla* – whether through the same mechanisms or others (some of which have been discussed) – possible reductions in reproductive output under warming will therefore limit their ability to disperse and migrate to new areas. Furthermore, exposure to increasing temperatures and extreme climate events is more intense at species range edges (Rehm et al., 2015; McNichol and

Russo, 2023), which could potentially further limit seed production and recruitment in these areas, making migration more challenging. This may reduce the likelihood of range shifts for Cerrado species, and lead to range contractions as ageing individuals – particularly at range edges – are not replaced, potentially over time leading to local extirpations and even species extinctions (Corlett and Westcott, 2013; McNichol and Russo, 2023).

5.2.2.4 Cerrado community structure and function

As *B. pachyphylla* (and *Byrsonima* as a genus) is common and widespread across the Cerrado (Ratter et al., 2003; Bridgewater et al., 2004), higher temperatures (and the resulting reduction in fecundity suggested by our results) could potentially have a widespread impact on *B. pachyphylla* recruitment and persistence, and Cerrado community composition. Reduced *B. pachyphylla* abundance and species range loss – in addition to increased fragmentation of Cerrado vegetation – could have cascading impacts for interacting species such as pollinating insects (predominantly bees; Boas et al., 2013; Melo et al., 2014; Hughes, 2021), herbivores, and seed predators (particularly birds; Purificação et al., 2014). This also includes human populations, as the fruits of *B. pachyphylla* (and other Byrsonima species) are eaten fresh or used to make sweets and alcohols (Passos, 2023), while the leaves and bark are used in medicine (Sannomiya et al., 2005; Bonacorsi et al., 2011; Guilhon-Simplicio and Pereira, 2011). Furthermore, many native and endemic Cerrado species (and their fruits and seeds) provide nutrition and have medicinal properties (de Oliveira et al., 2015; Valli and Bolzani, 2019; Arruda et al., 2022). Potential reductions in fruiting would therefore limit the socio-economic potential of – and possibility of sourcing new medicines from – the Cerrado.

As we were unable to collect fruit set results from *D. elliptica*, we cannot say with any certainty whether fruit set in other Cerrado species would be similarly negatively affected by increasing temperatures, but the extent of species in which high temperatures have been previously shown to reduce yield (although mostly crops; Zhao et al., 2017; Lohani et al., 2020; Zi et al., 2023) would suggest that the response of *B. pachyphylla* will not be unique. The predominance of *B. pachyphylla* may help to buffer the impacts of reduced recruitment of individuals, whereas high temperatures may have much larger repercussions for rarer species should they respond similarly (of which there are many; Martinelli et al., 2014). Furthermore, species with different reproduction strategies – such as those that flower or fruit at different times of year – may be differently affected. Some species have developed year-round flowering (Borges and de Assis Prado, 2014), which, although requiring continuous investment in reproductive development, reduces the likelihood of negative impacts of phenological shifts, or short-term inclement weather (such as heatwaves). On the other hand, species that flower for a short time, or perhaps only once in their lifetime (as in tropical forest tree *Tachigali versicolor*; Foster, 1977), will be at greater risk. For some species that have developed mast seeding (such as species of bamboo; Guala, 2003; Oliveira et al., 2004; Ojeda and Chazarreta, 2018) – a strategy to starve and then satiate seed predators through years of low and then high seed production, so that excess seeds remain for recruitment (Zwolak et al., 2022) – fewer seeds in mast years could lead to consumption of all seeds and low (or no) recruitment (Bogdziewicz et al., 2021; Hacket-Pain and Bogdziewicz, 2021). Additionally, there will be differing impacts for species that rely on wind as opposed to animals and insects for pollination and seed dispersal, although many Cerrado species are insect pollinated (Oliveira and Gibbs, 2000) and produce zoochorous (animal dispersed) seeds (Silvério and Lenza, 2010; de Souza et al., 2022).

Overall, the impacts that climate change will have on individual species will depend on their species-specific mechanisms of flowering, fruiting, seed production, dispersal, and dormancy; their own responses to changing environmental conditions and cues; their interactions with – and the responses of – interconnected species (including pollinators, herbivores, seed predators, seed dispersers and more); and the capacity of all these species to acclimate and adapt to said changes or migrate to more hospitable areas, especially at a speed that matches that of environmental change. Increasing temperatures – along with increased fragmentation and additional environmental perturbations – will undoubtedly lead to changes in the prevalence of some species and potential losses of flora and fauna, with consequences for Cerrado species assemblage and community structure, species interactions and ecosystem function, and potentially the extent of Cerrado vegetation. Furthermore, this will have repercussions for ecosystem services such as the provision of non-timber forest products for economy, nutrition, and medicine; local (and global) climate regulation; carbon capture and climate change mitigation; and water provisioning to (and hydroelectric stability of) much of South America.

5.2.2.5 Relevance to other ecosystems

What our results might also mean for species more prevalent in other related vegetation types such as the Cerradão (more closed canopy than the Cerrado *sensu stricto* in which our experiments were based) or for forest species is difficult to say given the species-specific nature of temperature responses and the lack of direct investigation of temperature impacts on reproduction in tropical species. Nevertheless, considering the divergent impacts of heating on pollen viability in our study species (*D. elliptica* and *B. pachyphylla*) and *M. calabura* studied by Slot et al. (2023; discussed earlier), and the fact that Cerrado species are generally better adapted for extreme conditions (Simon and Pennington, 2012; Araújo et al., 2021), it is highly plausible that reproduction of forest species will be more susceptible to the impacts of climate change and increasing temperatures. The varying responses of both Cerrado and forest species clearly require further investigation, although this is more difficult in the generally larger stature forest species. However, while our larger whole-tree heating methodology may not be suitable, it could be possible to employ our inflorescence heating chambers in forest canopies, given enough solar irradiance.

Nevertheless, our results might point to similar responses in species from other tropical savanna ecosystems exposed to similar environmental conditions (such as those in Africa, Asia, and northern Australia; Murphy et al., 2016), and the WTHSs could be easily utilised in the future to study similar shorter-stature woody species in said savannahs. Furthermore, they could be employed to study other significant non-forest ecosystems, such as tapia woodlands in Madagascar, where one tree species (tapia; *Uapaca bojeri*) – of significant ecological and socio-economic importance – dominates (Kull, 2002), and reductions in recruitment and persistence of this species under higher temperatures would have huge repercussions for Madagascar's ecology, culture, and economy.

5.3 Methodological limitations

Overall, each of the studies carried out in the preceding chapters are small, studying only a few species or individuals, and this is a clear limitation of the work. However, *in situ* direct heating experiments require investments of time, money, and physical effort (regardless of the method chosen for heating), and the size of each experiment was predominantly limited by the number of people and time available. Nevertheless, the study species *B. pachyphylla* and *D. elliptica* are both common and widespread in the Cerrado (Ratter et al., 2003; Bridgewater et al., 2004), as are the genera *Byrsonima* and *Davilla* (and families Malpighiaceae and Dilleniaceae; Ratter et al., 2003; Pereira et al., 2016), therefore the responses we detected in them locally may be relevant across the Cerrado. The number of branches studied per individual and samples taken per inflorescence per day were maximised in each study while allowing enough time for same day laboratory analysis of all samples (as the viability of released pollen declines over time; Brunet et al., 2019). Furthermore, during the photosynthesis and respiration study of chapter three, few individuals and leaves were studied at each time point, making it difficult to detect any significant differences between treatment and control groups. However, the sample size was again determined by the amount of time, people, and equipment available for both the building of the WTHSs, and the physiological testing of leaves. Furthermore, the study was primarily designed to test and demonstrate the usefulness of the WTHSs, and to that end it was successful.

5.3.1 Heating methodologies

The advantages and disadvantages of active and passive heating were already discussed in the thesis introduction (chapter one). The design and performance of the targeted and whole-tree heating methodologies developed for this project have also already been individually discussed in chapter two (and further in Appendix A1) and chapter three respectively. Both passive methodologies performed well and were much lower cost and simple to construct and maintain than active methods would likely have been. Furthermore, inclusion of a black base in each style of chamber removed the need for a closed system to reach the target heating effect of ~3°C, which reduced the unwanted effects of closed or semi-closed passive systems (Marion et al., 1997; Lewin et al., 2017). The only clear disadvantage of the passive methods was the inability to raise night-time temperatures in addition to daytime. However, passive heating methods were chosen predominantly due to the difficulties of providing a reliable supply of electrical power to potential active technologies in the Cerrado field site.

It is clear from the whole-tree heating experiment that targeted heating did not provide a complete picture of how individuals might respond to climate warming. Nevertheless, targeted heating led to the same response in fruit production as whole-tree heating, suggesting that for this stage of the reproductive cycle (perhaps the most important to overall reproductive success) it could still be very informative, and had we heated the inflorescences to above the thermal limit for pollen production it would have likely also been detectable using this method. Furthermore, it should be possible to employ these smaller chambers to heat target inflorescences on larger – for example forest – trees, whereas scaling up the passive WTHS design for very tall trees would perhaps not produce a relevant heating effect.

It is possible that the WTHSs altered additional aspects of the growth environment (possibly making it more favourable), such as soil humidity and temperature, and possibly ultraviolet-B (UV-B) radiation. What impact the black plastic would have had on soil temperature is not certain; possibly cooling as it shaded the soil from direct solar radiation, or possibly warming if it radiated infrared wavelengths downwards as well as into the structures. Since it was permeable to water, it was unlikely to have affected the movement of rain (of which there was little), although it may have limited evaporative water loss from the soil as the experiment was ongoing, possibly reducing water stress in the treatment individuals. However, Cerrado trees tend to have deep roots allowing them to access deep water (Oyedeji, 2023), so would have perhaps been unaffected potential alterations of surface conditions by the WTHSs.

The plastic used to construct the WTHSs was purchased from agricultural retailers in Brazil and little information was provided on the specific transmittance properties. Being sold for greenhouses, it may have had properties that provided some protection from UV radiation and dispersion of photosynthetically active wavelengths of radiation within the chambers (Balocco et al., 2018), perhaps creating a more favourable growth environment. This could have had a positive impact on the treatment individuals within the WTHSs, particularly as UV-B radiation has been shown to be harmful to pollen development (Koti et al., 2005). However, the WTHSs only encircled treatment individuals (not entirely containing them) so any impacts on solar transmittance should have been minimised. Unfortunately, UV transmittance (and soil temperature and humidity) could not be tested for in the additional WTHS erected the following year (to test CO_2 concentrations) due to lack of sensors.

Other variables not measured during the reproduction experiments include bud, and flower tissue temperatures (as opposed to air temperatures), which would have provided more information on the impacts of the increased air temperatures on plant tissues. Although attempts were made to measure bud temperature (using an infra-red camera and later thermocouples), they were unsuccessful. However, as we were interested in the impact of climate warming, air temperature was the most important variable to control and monitor.

5.3.2 Pollen analysis methodologies

No one method for analysing pollen viability is completely reliable (Dafni and Firmage, 2000). During the inflorescence heating experiment (chapter two), two complementary methods were therefore utilised (pollen staining and *in vitro* germination), whereas during whole-tree heating (chapter four) only staining was used to simplify the laboratory procedures and maximise the number of samples that could be analysed per day. While we found a strong correlation between the results of the two analysis methods for *B. pachyphylla* pollen (in chapter two) providing confidence in the reliability of staining alone, this relationship may not have held true for *D. elliptica* pollen (which was analysed in addition to *B. pachyphylla* in chapter four). Nevertheless, the response of pollen development to heating in the two species was very similar, providing confidence in the results.

Additional testing of the germinability of pollen grains (developed at ambient and high temperatures) at a greater range of incubation temperatures (to attempt to determine threshold temperatures for germination in our study species) would have improved our experiments and increased the confidence with which we suggest that female development was more affected than male (in *B. pachyphylla*). However, variation in pollen viability – between individuals, days of sampling, and even between flowers on one inflorescence – was high, and the viability of dehisced pollen grains tends to reduce over time, therefore one sample should be split and incubated simultaneously at different temperatures for comparative testing of pollen germination. This was not possible at the time of the inflorescence heating experiment (in which we tested pollen germination) as we had access to only one incubator.

Finally, pollen image processing by hand was incredibly time consuming for the number of samples that we collected. Given replication of these experiments, improved staining and imaging, along with more expertise in computer programming, could allow automation of image processing (Ascari et al., 2020; Zhang et al., 2023), vastly expediting the process. Furthermore, access to more specialised equipment (such as an impedance flow cytometer) would also expedite sample analysis allowing a greater number of samples to be taken and analysed, and might also give more information about pollen grain fitness as opposed to purely classification as viable or non-viable (Heidmann and Di Berardino, 2017; Langedijk et al., 2023).

5.4 Future perspectives

Having considered all three chapters and discussed the work and the results there are a number of ways in which we could improve or expand upon these experiments in the future, and a number of pertinent questions remain. Primarily, would *D. elliptica* also show reduced fruit set at higher temperatures? To answer this, it would be beneficial to repeat the whole-tree heating experiment on both *D. elliptica* and *B. pachyphylla*, monitoring the maturation of the seed capsules and fruits more closely and collecting them sooner, or perhaps finding an alternative method to allow the fruits to mature without losses from frugivory and herbivory (rather than using the net fruit bags). Through this repetition we should be able to detect the response of fruit set in *D. elliptica* to warming, and at the same time hopefully collect seeds of both species in a healthy, mature state. The seeds could then also be tested for viability – whether through accurate weighing and measuring (the simplest option), or improved seed germination or tetrazolium testing methodologies – providing insight into the impacts of higher temperatures on seed development of Cerrado species. Furthermore, given successful germination, the impact of heating during seed development on seedling growth could also be studied.

Given this repetition, it should be possible to determine whether the reduction in fruit set in *B. pachyphylla* indeed resulted from the impacts of temperature on female development (and if so, at which stage of development); or whether pollen was affected during pollination and fertilisation; or possibly whether resources were allocated away from fruit and seed production in favour of growth and survival. The previously suggested testing of thermal thresholds for pollen germination would provide some confidence in whether pollen was impacted by the treatment temperatures post-anthesis. Additionally, provided floral abnormalities were identifiable through microscopic analysis, flowers should be analysed throughout all stages of development – particularly post-anthesis during pollen germination and pollen tube growth – to detect any abnormalities and differences between control and heated flowers and the fate of germinating pollen grains in each. Furthermore, initial fruit set should be monitored throughout the pollination period to provide information on fruit set dynamics and the possibility of reduced allocation of resources to fruit production as the flowering period goes on.

The existence of pollen limitation in the study site could also be tested for by monitoring the levels of fruit set in additional control inflorescences under natural pollination and comparing this with fruit set under hand-pollination, which would be higher if natural pollen deposition is a limiting factor. Although monitoring the number of flowers that open on even more branches may be difficult, this could potentially be done by counting floral scars (as in Melo et al., 2014). Additional pollination of both control and treatment inflorescences with a mixture of high-temperature-developed pollen (which would need to be collected from multiple individuals each day and mixed together) would allow the detection of possible inconspicuous impacts of heating on the development of the pollen grains. Negative impacts on pollen development may result in further reductions in fruit set, or abnormal fruits and seeds, while possible acclimation of pollen to higher temperatures may result in higher fruit set or higher seed/seedling vigour at high temperatures (should seed germination experiments be successful).

As already discussed, possible impacts of the WTHSs (on soil temperature, soil humidity, or transmission of certain light wavelengths such as UV-B radiation) may have resulted in a more favourable growth environment inside the chambers. Given suggested repetition of the final experiment, these variables should be tested using the necessary sensors repeatedly over the course of the experiment, to evaluate any effects, and any changes in these effects over time. Furthermore, the additional testing of photosynthesis and respiration in control and treatment trees – or perhaps merely measurement of photosynthate availability within the reproductive structures – throughout the experiments would clarify the potential impacts of heating on photosynthate availability and the possibility of physiological acclimation to warming.

Although in position for most of a year, the WTHSs eventually became fragile and were disassembled after fruit collection. However, given strengthening of the structures, longer-term multi-year experiments could provide unique insights into the direct impacts of warming on flowering and fruiting phenology, and the quantity of flowers produced in Cerrado species. Multi-year warming may also impact resource investment in reproduction or the ability of species to acclimate, altering the effects of the heating treatment. Furthermore, monitoring of control individuals over multiple years and better long-term measurement of local climate parameters could allow for correlation analysis of reproductive phenology and output with natural environmental conditions.

Finally, although we chose to employ passive heating methodologies (for reasons already discussed; chapter one), the addition of night-time heating (requiring an active method) would provide a more realistic simulation of climate warming. To this end, the WTHSs could potentially be modified to include an active heating element purely for the night-time, requiring less electrical power than entirely active methods to produce a constant heating effect. Alternatively, and suggested previously (chapter three), the inclusion of a thermal mass (such as water filled black pipes; Godfree et al., 2011) could be trialled to passively reduce nocturnal cooling in the future.

Following repetition and improvement of these experiments, replication and expansion to other Cerrado species (locally and in different areas and vegetation types) and indeed other suitable ecosystems would provide further important insights into the – currently understudied – possible impacts of increasing temperatures on tropical tree reproduction. Furthermore, the data gathered through this expansion (such as thresholds temperatures for the reproductive niche of numerous wild species) could be integrated into species distribution models and utilised to better predict the potential impacts of climate change on Cerrado (and other tested) vegetation (as in Ribeiro et al., 2019; Ferreira et al., 2022).

5.5 Final conclusions

The work presented in this thesis has provided a unique, albeit initial, understanding of the potential impacts that climate change and increasing temperatures will have on reproduction in tropical woody species, more specifically those in the large and biodiverse tropical savannah region of Brazil known as the Cerrado. Furthermore, two novel methodologies for direct passive heating of reproductive structures (one heating entire individuals) of woody species *in situ* in remote environments were developed, tested, shown to be effective in simulating mid-range climate change scenarios, and utilised in the study of multiple Cerrado species. While the results may indicate a resilience of male reproductive development and physiological acclimation to very high temperatures, potential reductions found in fruit and seed production could point to an overall negative impact of climate warming on reproduction and recruitment, which may prove detrimental to species persistence and alter Cerrado community structure and function. However, the overall impact of changing environmental conditions will depend on species-specific responses and complex species interactions and feedbacks, and therefore requires much further investigation. Hopefully, the publication of the works within this thesis will initiate a greater interest in – and provide some guidance towards – further research of this subject and biome.

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Appendix A

Supporting information for Chapter two

A1. Details of heating methodology

A1.1 Description

In order to passively heat whole inflorescences (and developing flower buds) *in situ*, we developed a novel, elevated, open-topped chamber, designed to raise the internal air temperature (around inflorescences) to at least 3°C above ambient during sunny periods. It was comprised of two parts: a 30 × 30 cm square base (part A; Figure A3.1A) formed from modelling wire (1.6 mm galvanised mild steel) and 75% covered with black polythene sheeting (as used in construction), designed to absorb incident solar radiation (shortwave) and emit it as thermal radiation (longwave); and an upper section (part B; Figure A3.1A) made from a single rectangular piece of semi-rigid transparent acetate (25 × 100 cm, 0.3 mm thick) folded to produce the sides of the chamber and held in shape by wire, designed to allow transmission of solar radiation into the chamber (Orelma et al., 2020) but reflect thermal radiation internally, warming the air (and inflorescence) within. Part A was left partially (25%) uncovered (Figure A3.1A) and the top of part B was left completely uncovered to allow mixing of internal and external air in order to prevent overheating, while also reducing the impact of the chamber on light transmission, gas exchange and humidity. A hole was cut in one side of each part B (Figure A3.1A) to allow for positioning over an inflorescence-bearing branch, which was closed using a cable tie once each chamber was in place.

To allow installation of the chambers around inflorescences at varying heights we also developed a telescopic supporting structure (Figure A3.1B) consisting of two 1.5 m rebar (reinforcing steel bar) ‘feet’ hammered 0.5 m into the ground, with hollow polyvinyl chloride (PVC) pipe (of lengths relative to the height of each inflorescence) placed over each. Cable glands attached at the bottom of the PVC pipes could be loosened and tightened at different heights on the rebar, creating a flexible telescopic system for installing the chambers. Each supporting structure was stabilised by another 1.5 m piece of rebar hammered into the ground at an angle and attached to the vertical structure using a 0.5 m horizontal piece of rebar and strong cable ties (Figure A3.1B).

A1.2 Analysis

Sensors measured temperature at two of the three treatment inflorescences (T1, T2) and at their corresponding controls (C1, C2; pairs 1 and 2) on each tree (A–E); and measured temperature and relative humidity (RH) at the third treatment and control inflorescences (T3, C3; pair 3) on each tree. To determine whether chambers produced the desired heating effect, temperature and relative humidity data (recorded every minute) from the initiation of heating to the end of flowering (approximately eight weeks) were averaged for every 5 minutes and mean diurnal patterns calculated for each inflorescence, and all inflorescences together. The strongest and most consistent levels of heating were noted to occur between 09:00 and 17:00 (local time; BRT = UTC - 3) each day, referred to as the 'peak heating' period. Mean temperatures were calculated for the daytime (06:30 – 18:30), night-time (18:30 – 06:30) and peak heating periods (09:00 – 17:00) for each inflorescence each day, and used to compare air temperatures at treatment inflorescences and their corresponding controls.

To compare the levels of heating produced by the chambers with interannual variation in temperature, data assimilation based long-term ERA5-Land data (Muñoz Sabater, 2019) was downloaded from the Copernicus Climate Change Service (C3S; 2023) for the closest grid reference to the Bacaba field site (14°43'12.0" S, 52°21'36.0" W; 9 km resolution). Hourly, 2 m surface air temperatures from 2010 to end-2019 were used to estimate monthly mean daytime temperatures for each year, and the ten years together.

A1.3 Results

Treatment temperatures in our novel passive heating chambers correlated closely with controls, rising higher in the daytime as solar irradiance increased (Figure 2a in main article). From the initiation of heating to the end of flowering the chambers successfully raised air temperatures around the enclosed treatment inflorescences by 2.7 ± 1.0°C (*SD*) during the daytime compared to their corresponding ambient controls, and this rose to 4.0 ± 1.4°C during the peak heating period each day (Table A2.1). There was natural variation in heating patterns between chambers, with some producing a stronger heating effect (Figures S2a, S3), and treatment air temperature could reach as high as 15.0°C above ambient (occasionally in treatment chamber 3 tree C; Figure A3.3). However, on average the maximum difference between treatment and control temperatures each day was lower (7.5 ± 2.0°C; Table A2.1). The heating effect also fell earlier in the day in some chambers (for example on trees C and D; Figure A3.2A) due to localised shading by larger trees. As expected of passive heating devices, the chambers did not raise air temperature at night (Figure 2a). Night-time air temperatures inside the chambers were on average 0.7 ± 0.3°C below ambient, although this difference could sometimes be larger (mean maximum difference -1.6 ± 0.7°C, Table A2.1). From the end of flowering in August until fruits were collected and counted in November the treatment chambers continued to produce a strong heating effect (Table A2.3), in spite of being disrupted by heavier cloud cover towards the onset of the rainy season in late-September (Figure A3.4A).

RH was measured at one control and one treatment inflorescence per tree (pair 3). Between the initiation of heating and the end of flowering, daytime and night-time RH at the control inflorescences was 50 ± 6% (*SD*) and 83 ± 11% respectively (Table A2.2), with a large difference between the minimum (daytime) and maximum (night-time) RH experienced within a 24 hr period (31 ± 5% and 97 ± 4% on average respectively; Table A2.2). RH inside the treatment chambers correlated closely with ambient RH but fell on average 8 ± 3% lower inside the chambers during the peak heating period each day (Figure 2b, Table A2.2). This pattern reflected that of temperature (Figure 2). The difference between treatment and control RH at night was negligible (Table A2.2).

Long-term local temperature data from 2010–2020 (ERA5-Land data) showed a consistent annual pattern of seasonal temperature fluctuation, with peak temperatures occurring in September/October (Figure A3.5). Monthly mean daytime temperatures in 2019 were above the 10-year averages estimated for all months apart from June and July (during experiment initiation and flowering), which exhibited average temperatures. The standard deviation (interannual variation) around monthly daytime means ranged from ± 0.3 to 1.3°C (± 0.9°C on average). June, July, August, and September (months over which much of the experiment was carried out) displayed low levels of interannual variation compared to other months (*SD*s < ± 0.7°C; Figure A3.5).

A1.4 Discussion

The novel, *in situ* passive heating chambers that we developed and installed around developing inflorescences of *B. pachyphylla* were successful in heating the air around the inflorescences by 2.7°C on average during the daytime (close to our target of 3°C) and this heating effect was closer to 4°C on average during much of the day (between 09:00 – 17:00). The passive heating chambers therefore produced a strong heating effect within the range of temperature increases predicted for the future of the Cerrado (IPCC, 2013). Furthermore, considering the degree of interannual variation in monthly mean daytime temperatures, the chambers likely exposed the treatment inflorescences to temperatures above those that they would have experienced over the last 10 years, especially considering that 2019 was one of the warmest years since 2010 (according to the ERA5-Land dataset; Figure A3.5).

There was some variation between the heating effects produced by each chamber. However, while active heating methods can provide more control over treatment temperatures, large variations in temperature can still occur between different heated targets (for example different leaves on one tree; Carter and Cavaleri, 2018). The differences in temperature between inside our chambers and their corresponding ambient controls were at times much larger that 4°C. However, this could in some cases be attributed to a delay between the increase in air temperature inside the chambers and the increase in ambient air temperature between dawn and midday, when internal temperatures rose faster (Figure A3.2A). Furthermore, overheating is also possible using active heating methods, due to delays between the sensing of target temperatures and the curtailment of heating devices, sometimes with detrimental effects (such as leaf scorching; Carter and Cavaleri, 2018).

As expected, the chambers did not produce a heating effect at night. Internal air temperatures were in fact <1°C lower than ambient on average, and the maximum difference between chamber and ambient air could be closer to -2°C, although these larger differences most likely occurred just after dusk or just after dawn, when the air inside some chambers seemed to cool down faster or warm up slower than the air outside (Figure A3.2A), probably dependent on the angle of the chamber in relation to the sun. Night-time cooling can occur in passive chambers due to a lack of mixing of air between the inside and outside of the chambers (Marion et al., 1997), however we did not expect to see this in such open chambers. While these are common disadvantages of passive heating methods, we decided to employ passive heating because active heating equipment is often expensive and can be difficult to set up and unreliable in harsh and remote field situations. Conversely, our methodology was low-cost and flexible in design – fitting with the non-uniform environment of the Cerrado field site – and could be easily expanded to more inflorescences and more trees. However, we have to take into account when interpreting our results that we are only seeing a response to higher daytime temperatures, and not to a complete simulation of future climate conditions.

As mentioned previously, the passive heating chambers were designed to be very open to encourage mixing of internal and external air in order to reduce their impact on variables other than temperature (such as gas concentrations and humidity). While RH inside the chambers was very close to ambient at night, it repeatedly fell lower than ambient in the daytime (8% lower on average) during the peak period of heating. However, RH is temperature dependent, and as the diurnal patterns of RH mirrored those of temperature (Figure 4), the reduced RH within the chambers during the hottest part of the day may have been an unavoidable consequence of increasing temperatures. In addition, there is evidence that in recent years RH has decreased over much of the Cerrado biome and will continue to fall into the future (Hofmann et al., 2021) therefore our chambers may more accurately simulate future daytime climate conditions.

A1.5 References

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A2. Additional Tables

Table A2.1 *Air temperatures (°C) from the initiation of heating to the end of flowering, measured at the control and treatment inflorescences, and the differences generated between the treatment inflorescences and their corresponding controls, averaged across all days and inflorescences. Standard deviations are given in brackets, hours are in local time (BRT = UTC -3).*

	Peak heating period 09:00 – 17:00		**Daytime 06:30 – 18:30**	**Night-time 18:30 – 06:30**		
	Mean	**Max**	**Mean**	**Mean**	**Min**	**Max**
Control	32.9 (1.9)	36.7 (2.1)	29.4 (1.5)	18.7 (2.1)	14.4 (2.3)	-
Treatment	36.8 (2.5)	42.3 (2.9)	32.1 (2.0)	18.0 (2.0)	13.8 (2.4)	-
Difference	4.0 (1.4)	7.5 (2.0)	2.7 (1.0)	-0.7 (0.3)	-	-1.6 (0.7)

Table A2.2 *Relative humidity (%) from the initiation of heating to the end of flowering, measured at the control and treatment inflorescences, and the difference between the treatment inflorescences and their corresponding controls, averaged across all days and inflorescences measured. Standard deviations are given in brackets, hours are in local time (BRT = UTC -3).*

	Peak heating period 09:00 – 17:00			**Daytime 06:30 – 18:30**	**Night-time 18:30 – 06:30**	
	Mean	**Min**	**Max**	**Mean**	**Mean**	**Max**
Control	39 (5)	31 (4)	-	50 (6)	83 (11)	97 (4)
Treatment	31 (5)	23 (4)	-	43 (6)	83 (11)	97 (6)
Difference	-8 (3)	-	-15 (5)	-6 (3)	0 (3)	-

Table A2.3 *Mean monthly temperatures (°C) for the control and treatment inflorescences and the differences between them (treatment – control) from experiment initiation to end (fruit collection). Standard deviations are given in brackets, hours are in local time (BRT = UTC -3).*

		Month					
		Jun[1]	**Jul**	**Aug**	**Sep**	**Oct**	**Nov[1]**
Peak heating **09:00 – 17:00**	**Controls**	32.6 (0.6)	32.9 (1.9)	35.1 (2.1)	38.2 (2.6)	34.4 (3.4)	34.7 (3.2)
	Treatments	36.3 (0.6)	36.9 (2.0)	39.0 (2.3)	42.3 (3.1)	37.5 (4.3)	37.9 (4.1)
	Differences	*3.8 (0.2)*	*4.0 (0.2)*	*3.8 (0.5)*	*3.9 (0.6)*	*3.0 (1.0)*	*3.2 (0.9)*
Daytime **06:30 – 18:30**	**Controls**	29.2 (0.6)	29.3 (1.5)	32.1 (1.9)	35.1 (2.2)	31.9 (2.8)	32.1 (2.6)
	Treatments	31.7 (0.6)	32.0 (1.6)	34.8 (2.0)	38.1 (2.5)	34.2 (3.5)	34.4 (3.2)
	Differences	*2.6 (0.2)*	*2.8 (0.2)*	*2.7 (0.4)*	*2.8 (0.4)*	*2.2 (0.8)*	*2.3 (0.6)*
Night-time **18:30 – 06:30**	**Controls**	18.4 (1.8)	18.6 (1.9)	22.0 (2.4)	24.2 (1.7)	23.4 (0.9)	23.0 (0.8)
	Treatments	17.8 (1.7)	17.8 (1.9)	21.7 (2.4)	23.5 (1.8)	23.1 (0.9)	22.8 (0.8)
	Differences	*-0.7 (0.1)*	*-0.7 (0.1)*	*-0.7 (0.1)*	*-0.8 (0.2)*	*-0.3 (0.1)*	*-0.2 (0.0)*

[1] *Averages for June and November were calculated from data covering only the last and first half of the months respectively, so not reliable as full monthly averages.*

Table A2.4 *Size of each individual of Byrsonima pachyphylla in the experiment.*

Tree	**Height (m)**	**Diameter (cm) at soil height (30 cm)**	**Diameter (cm) at breast height (130 cm)**
A	3.90	10.7	7.0
B	3.39	11.9	8.3
C	2.45	8.2	5.1
D	2.56	8.3	5.9
E	3.00	9.5	4.1

Table A2.5 *In vitro pollen germination medium optimised through pre-experimental testing for pollen of B. pachyphylla; mixed in this order in up to 100ml of distilled water.*

Chemical	Amount per 100 ml
Boric acid	30 mg
Potassium nitrate	10 mg
Magnesium sulfate	20 mg
Calcium nitrate	30 mg
Sucrose	20 g
PEG 6000	5 g

Table A2.6 *Modified version of simplified Alexander's staining solution, optimised through pre-experimental testing for pollen of B. pachyphylla.*

Chemical	Amount per 100 ml
95% ethanol	10 ml
Malachite green (1% solution in 95% ethanol)	2 ml
Distilled water	53 ml
Glycerol	25 ml
Acid fuchsin (0.5% solution in water)	5 ml
Orange G (1% solution in water)	1 ml
Glacial acetic acid	4 ml

A3. Additional Figures

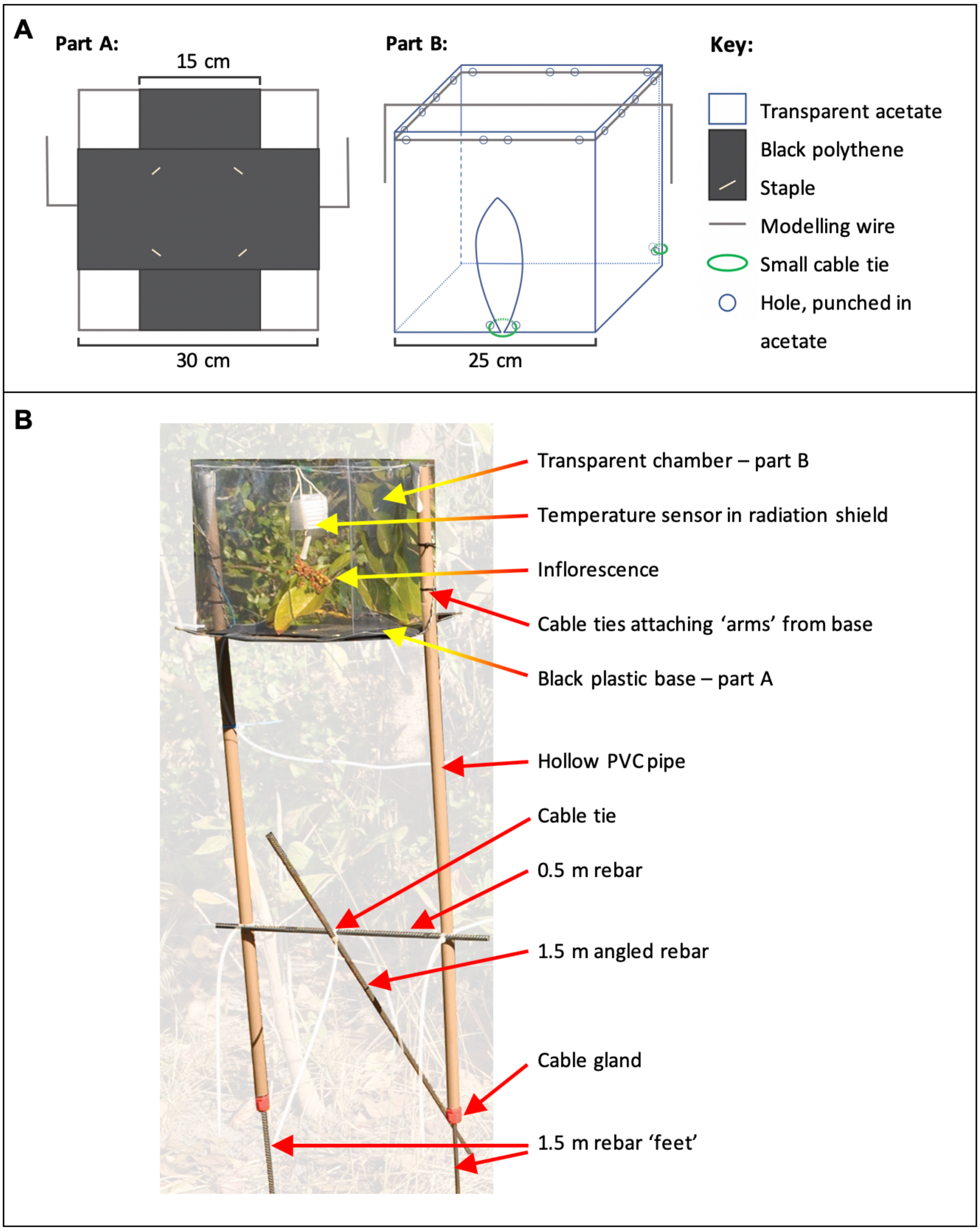

Figure A3.1 *The novel open-topped chamber developed for passively heating inflorescences in situ, showing (A) a diagram of the constituent parts; and (B) a labelled image of one chamber and supporting structure, erected in situ in the Cerrado, enclosing a single inflorescence of B. pachyphylla.*

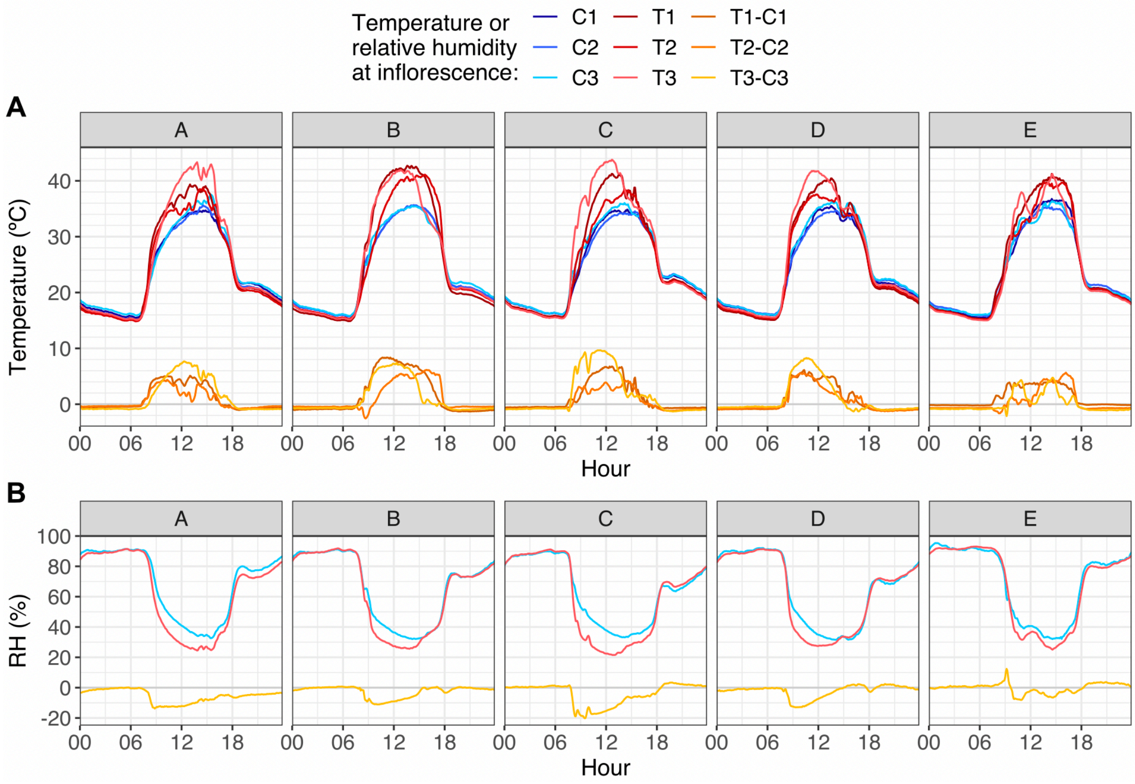

Figure A3.2 *Mean diurnal patterns of (A) temperature and (B) relative humidity (RH) measured at each control inflorescence (C1, C2 and C3) and each treatment inflorescence (T1, T2 and T3) per tree (A–E), including the differences in temperature and RH calculated between the treatment inflorescences and their corresponding controls. Values were averaged across every 5-minute interval of each day of recorded data between the initiation of heating and the end of flowering.*

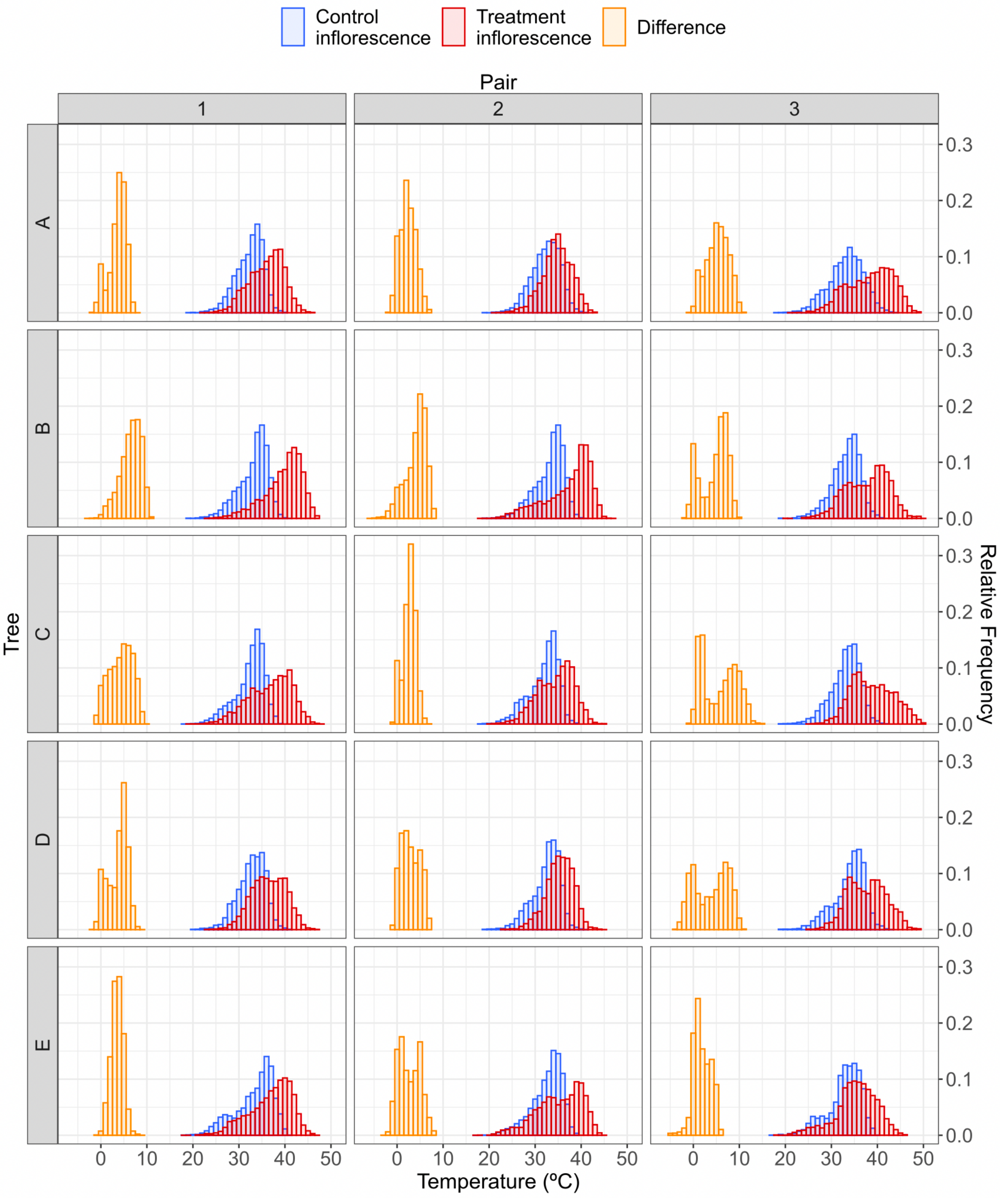

Figure A3.3 *Temperatures experienced every five minutes during peak heating (09:00 – 17:00) from the initiation of heating until the end of flowering at each of the control and treatment inflorescences (C1–C3 and T1–T3) on each tree (A–E), including the differences between T and C temperatures for each pair.*

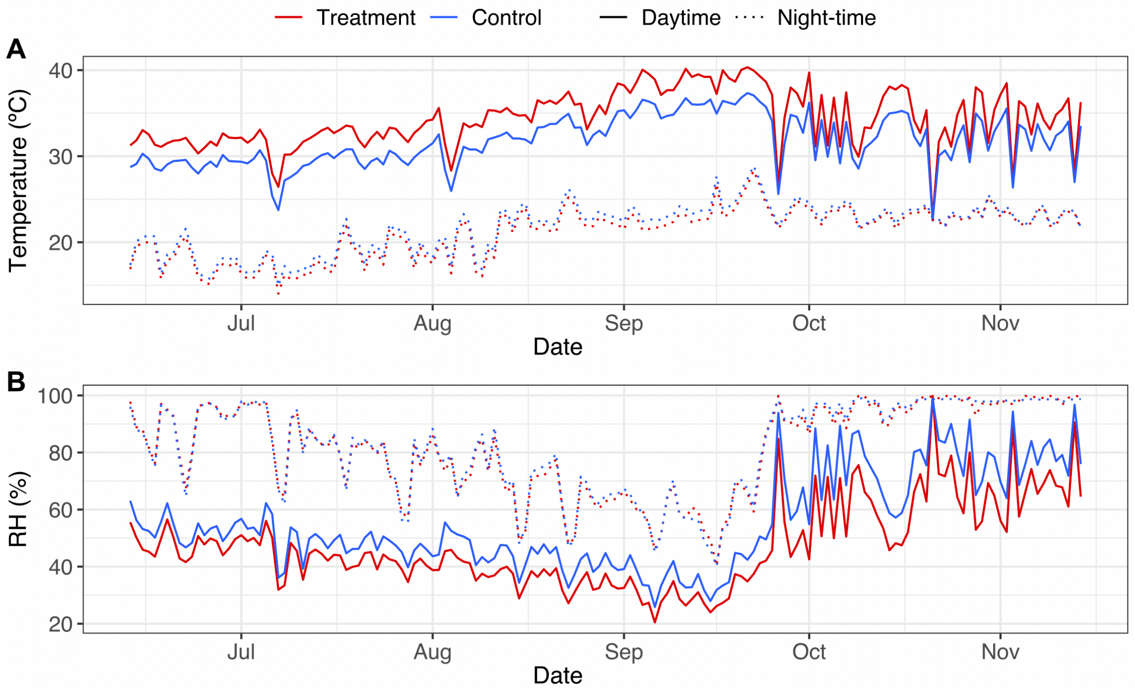

***Figure A3.4** Daily mean daytime and night-time values for treatment and control (A) temperature and (B) relative humidity (RH), from the initiation of heating in June to the end of the experiment and collection of fruits in November 2019.*

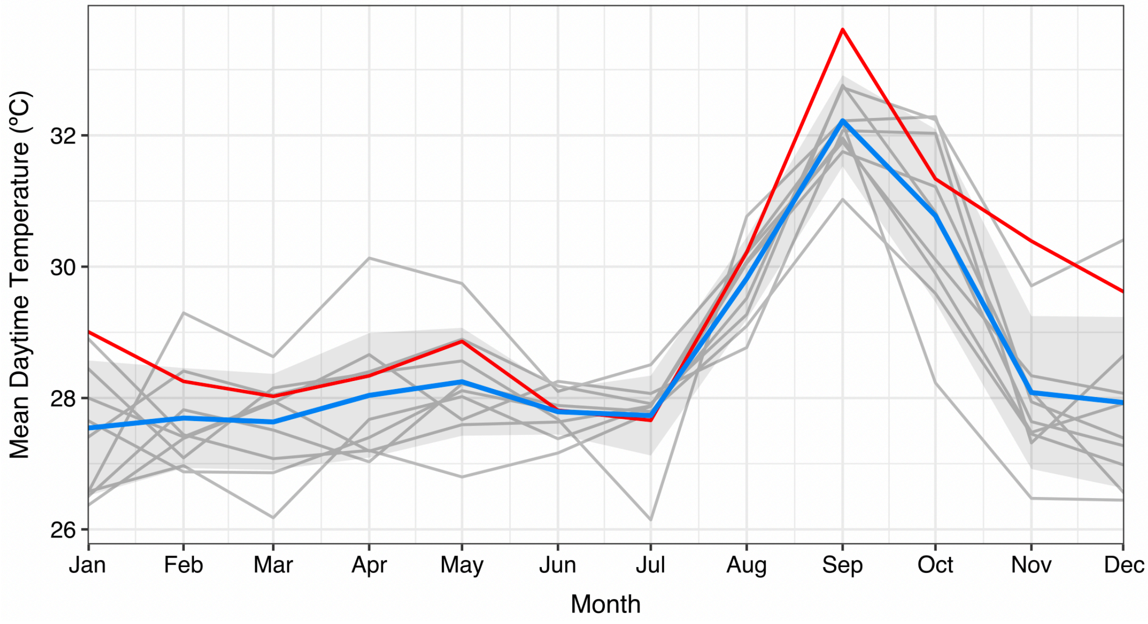

***Figure A3.5** Monthly mean daytime temperatures for each year (grey lines) from 2010 to 2019, with 2019 highlighted (red line), and including the mean values across all ten years (blue line). The shaded area denotes the standard deviation around the 10-year mean for each month. Values estimated from the ERA5-Land dataset (Muñoz Sabater, 2019) hourly 2m surface air temperatures, downloaded from the Copernicus Climate Change Service (C3S; 2023).*

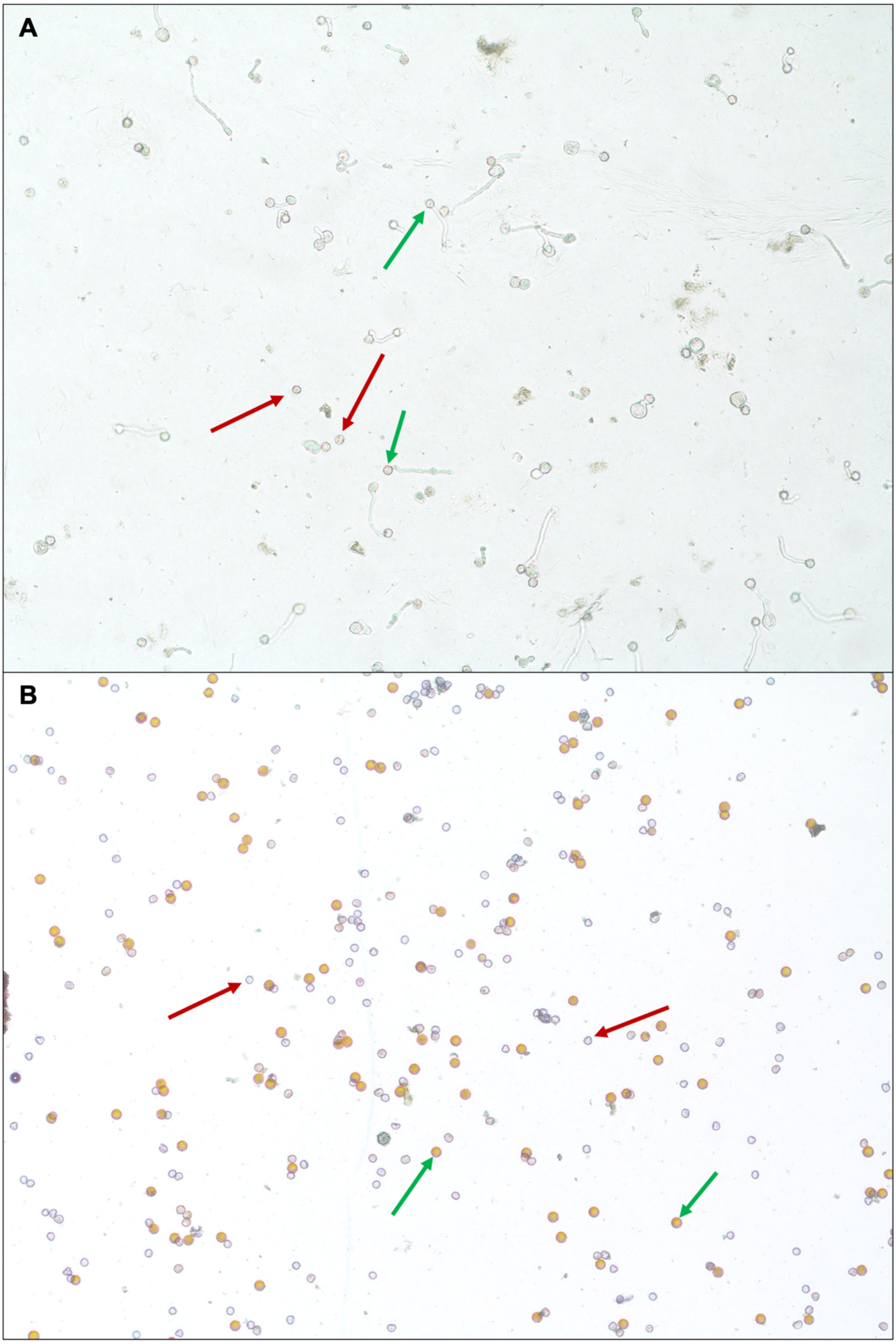

Figure A3.6 *Example images of pollen samples taken for (A) in vitro germination analysis and (B) pollen staining analysis, at 100 × magnification. Arrows point to examples of pollen grains classified as viable (green) and non-viable (red).*

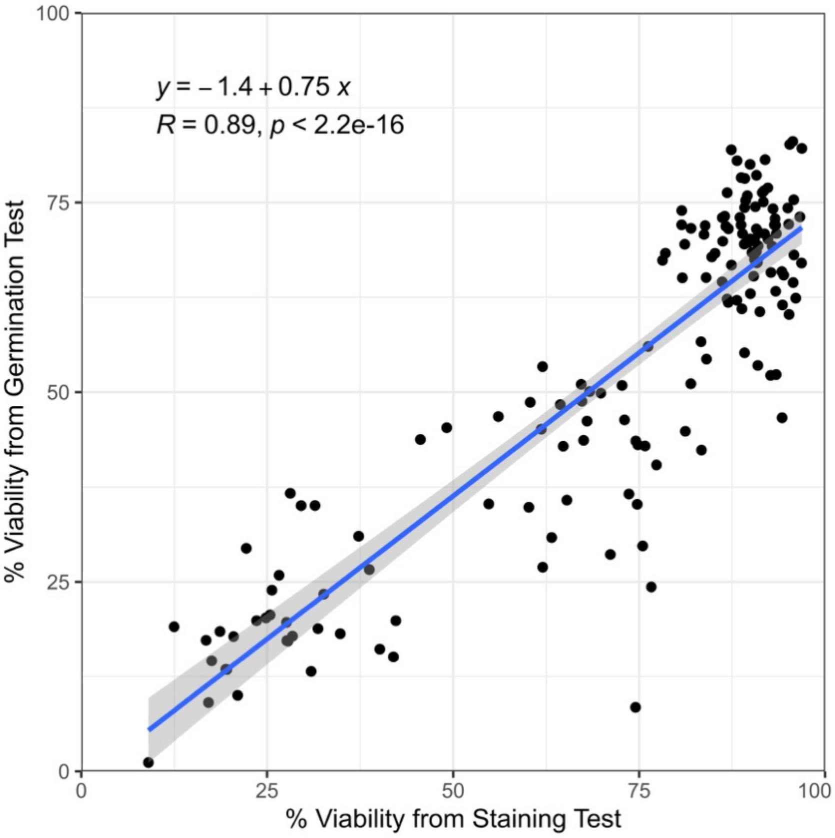

Figure A3.7 *Comparison of the pollen viability estimated for each sample analysed by differential pollen staining and in vitro pollen germination. Shaded area depicts the 95% confidence interval.*

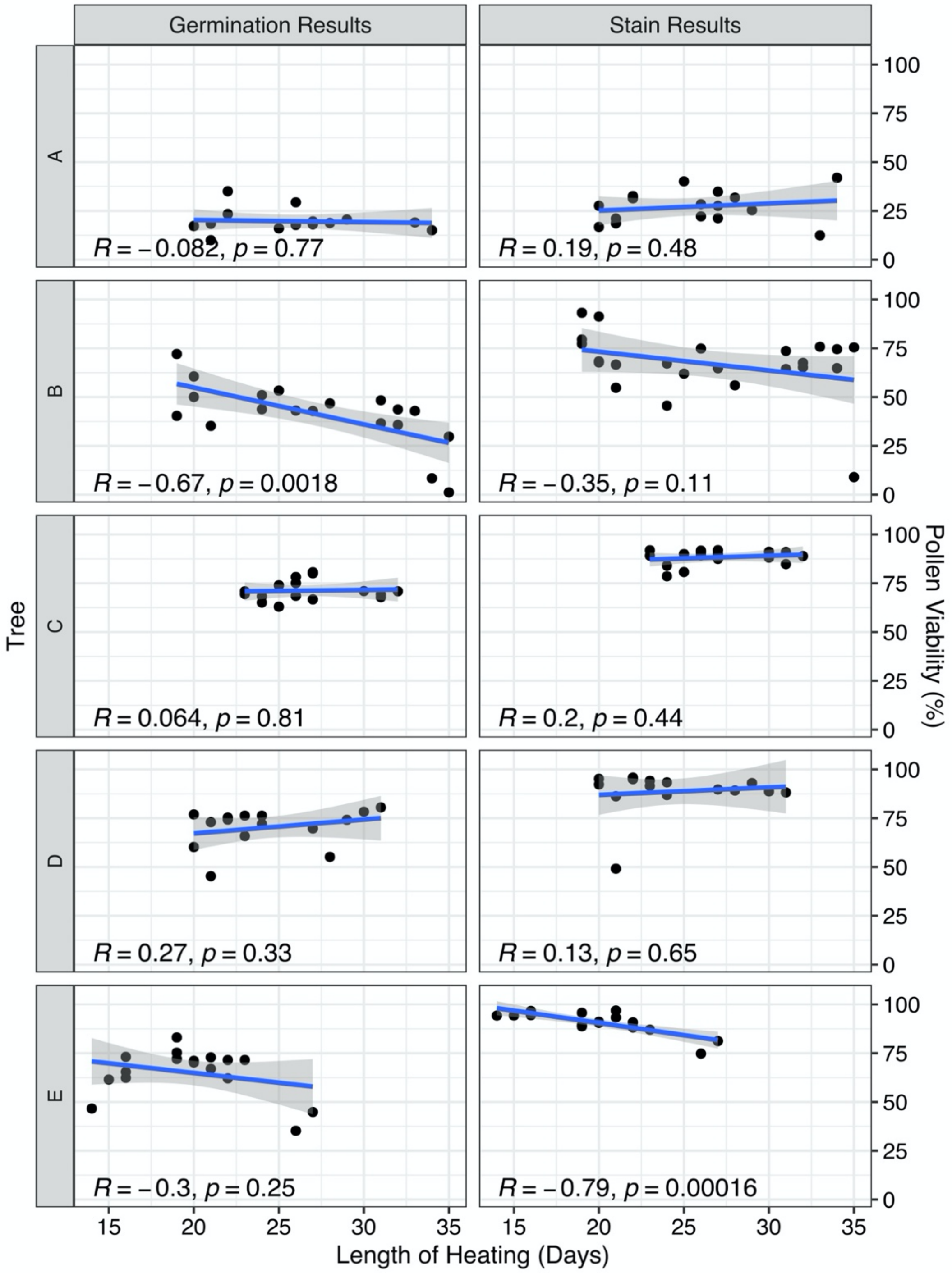

Figure A3.8 *Viability of pollen samples taken from the treatment buds of each tree compared against the length of time each bud was heated for before sampling. Shaded areas depict 95% confidence interval.*

Appendix B

Supporting information for Chapter three

B1. Investigating plant responses to short-term *in situ* heating, additional methods

B1.1 Physiological measurements

Photosynthesis and respiration temperature response curves were measured between 08:00 and 11:30 each day using two LI-6400XT portable photosynthesis systems (LI-COR Biosciences Inc., Nebraska, USA), one with a fluorometer (6400-40) chamber head and one with an LED (6400-02B) chamber head (for photosynthesis and respiration measurements respectively). One leaf was analysed with each LI-6400XT (two leaves per individual per day). Leaves were chosen that were healthy, fully expanded and equally mature, avoiding damage as much as possible. Leaves chosen for respiration analysis were covered at dawn to prevent sun exposure and kept in darkness for at least 40 minutes before testing began, while those for photosynthesis analysis were exposed to direct sunlight for at least 40 minutes prior to testing. Once the LI-6400XT chamber heads were clamped onto the still attached leaves (Figure B1.1), leaf temperature and either net photosynthesis or respiration rates were measured at 10 incremental steps in air temperature between 20 and 50°C, after full equilibration at each temperature. Temperatures above 40°C were achieved using temperature expansion water jackets (LI-COR Biosciences Inc.). Throughout measurement, CO_2 concentration was maintained at 400 ppm and relative humidity (RH) at 50% for all leaves, while light levels were maintained at 1100 $\mu mol\ m^{-2}\ s^{-1}$ for photosynthesis leaves and at 0 $\mu mol\ m^{-2}\ s^{-1}$ for respiration leaves.

B1.2 Analysis of physiological response to temperature

Net photosynthesis rates (A_{net}) were plotted against leaf temperature for each individual on each day of sampling, and a standard quadratic curve was fit to each one (Figure B1.2A). As the data fit to this equation was good for most leaves ($R^2 > 0.67$, mean $R^2 = 0.88$) no further models were tested. From the curves the optimum photosynthesis rate (A_{opt}), optimum temperature for photosynthesis (T_{opt}), maximum temperature for photosynthesis (T_{max}), and temperature range at which photosynthesis is at least 80% of A_{opt} (T_{span}) were extracted.

Figure B1.1 *Image of both LI-6400XT chamber heads clamped onto leaves of a treatment individual of E. suberosum while taking photosynthesis and respiration measurements inside one of the WTHSs (whole tree heating structures). The chamber heads were mounted on tripods to enable them to reach the leaves whilst still attached, and shaded by umbrellas to control temperature.*

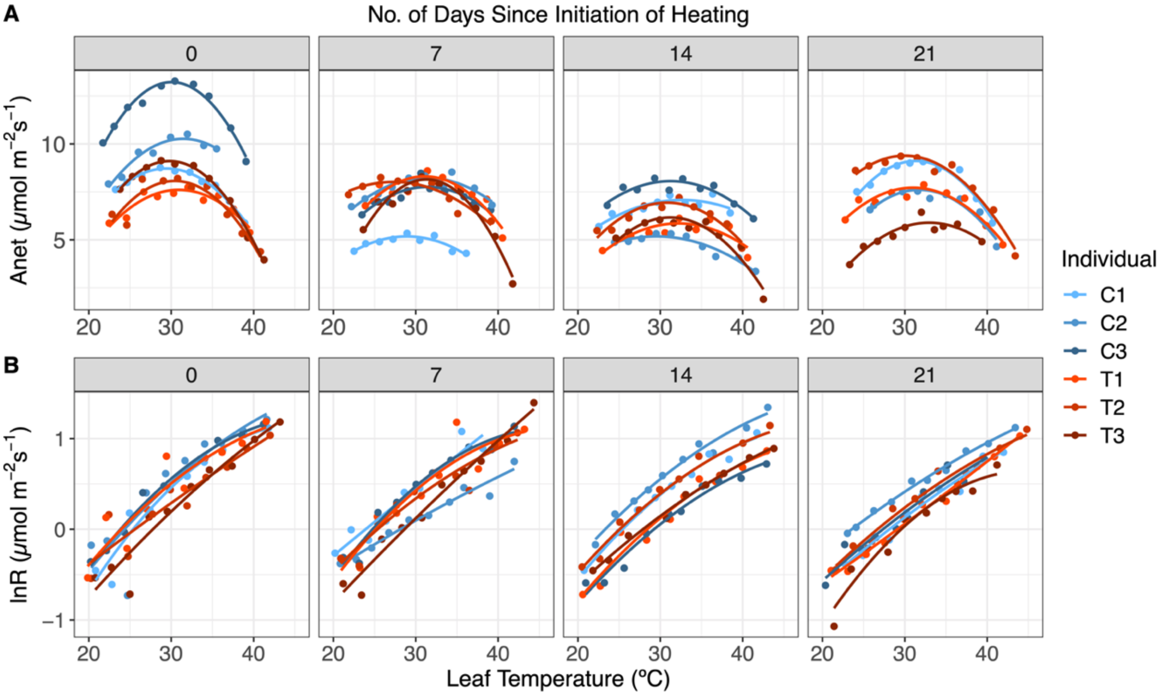

Figure B1.2 *Measured values and fitted temperature response curves of (A) net photosynthesis rate and (B) respiration rate (natural-log-transformed) for each leaf on each day of measurement.*

Respiration rates (R) were natural-log-transformed (*ln*) and plotted against leaf temperature for each individual on each day of sampling, and a quadratic curve was fit to each one (similar to Heskel et al., 2016; Figure B1.2B). The data fit to this equation was strong for most leaves ($R^2 > 0.80$, mean $R^2 = 0.94$) so no further models were tested. From these curves, values were then extracted for respiration rates at low (25°C; R_{25}), moderate (35°C; R_{35}), and high temperatures (45°C; R_{45}) for each individual and each day of measurement. Respiration rates R_{20} and R_{40} (at 20 and 40°C; a range of temperatures often experienced by *E. suberosum* individuals in the area) were also taken from the curves and used to calculate a Q_{10} value for the respiration rate of each individual on each day of measurement (using eq. 1 in Atkin and Tjoelker, 2003).

To evaluate whether the higher daytime temperatures experienced by the treatment individuals of *E. suberosum* had an impact on the temperature sensitivity of photosynthesis and respiration, all the parameters (namely T_{max}, T_{opt}, T_{span}, A_{opt}, R_{25}, R_{35}, R_{45} and Q_{10}; described above) calculated from measurements taken in the 3 weeks after the initiation of heating were compared between the treatment and control groups using 2-sided t-tests. The first two weeks of results post-heating were also tested separately in case a deterioration in general leaf health (which had been noticed in the field) was having an impact on the results by the third week of analysis. T-tests were also used to compare all the parameter results between the groups prior to heating to check for significant differences between the treatment and control groups that may have existed before the experiment began.

To further explore whether air temperature affected the temperature sensitivity of photosynthesis and respiration regardless of grouping, mean daytime and night-time temperatures were calculated for every 24 hours and every *E. suberosum* individual, and Pearson correlation analysis carried out between all photosynthesis and respiration parameters and the mean daytime and night-time temperatures of the day before leaf measurements were taken. Missing temperature data was estimated from temperature data recorded by the local weather station, and the previously calculated diurnal patterns of temperature differentials (see main article methods). The temperatures recorded on the days of testing were not used because leaf analysis started so early in the day that the leaves were not exposed to these temperatures before manipulation by the LI-COR systems. All data analysis was performed in R version 3.5.3 (R Core Team, 2021).

B1.3 References

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R Core Team 2021. R: A language and environment for statistical computing. Available from: https://www.R-project.org/.

B2. Additional figures

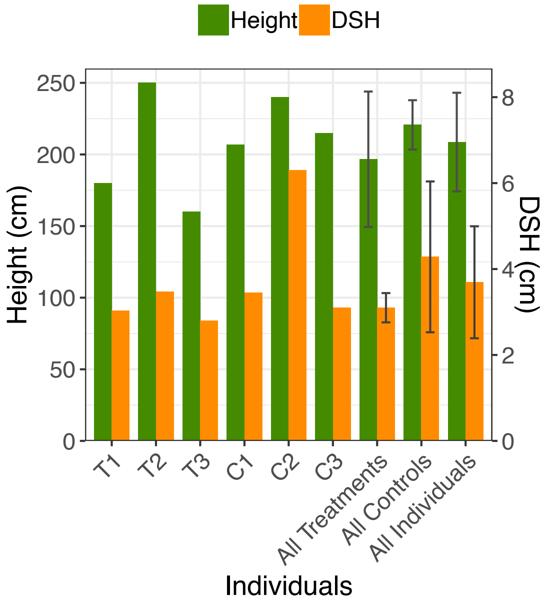

Figure B2.1 *Height and diameter at stump height (DSH; 30cm) of the three treatment individuals of E. suberosum (T1, T2, and T3) enclosed in the four-sided WTHSs (S1, S2 and S3 respectively); the individuals marked as controls (C1, C2 and C3); and the mean values for each group and all individuals together. Error bars denote standard deviation.*

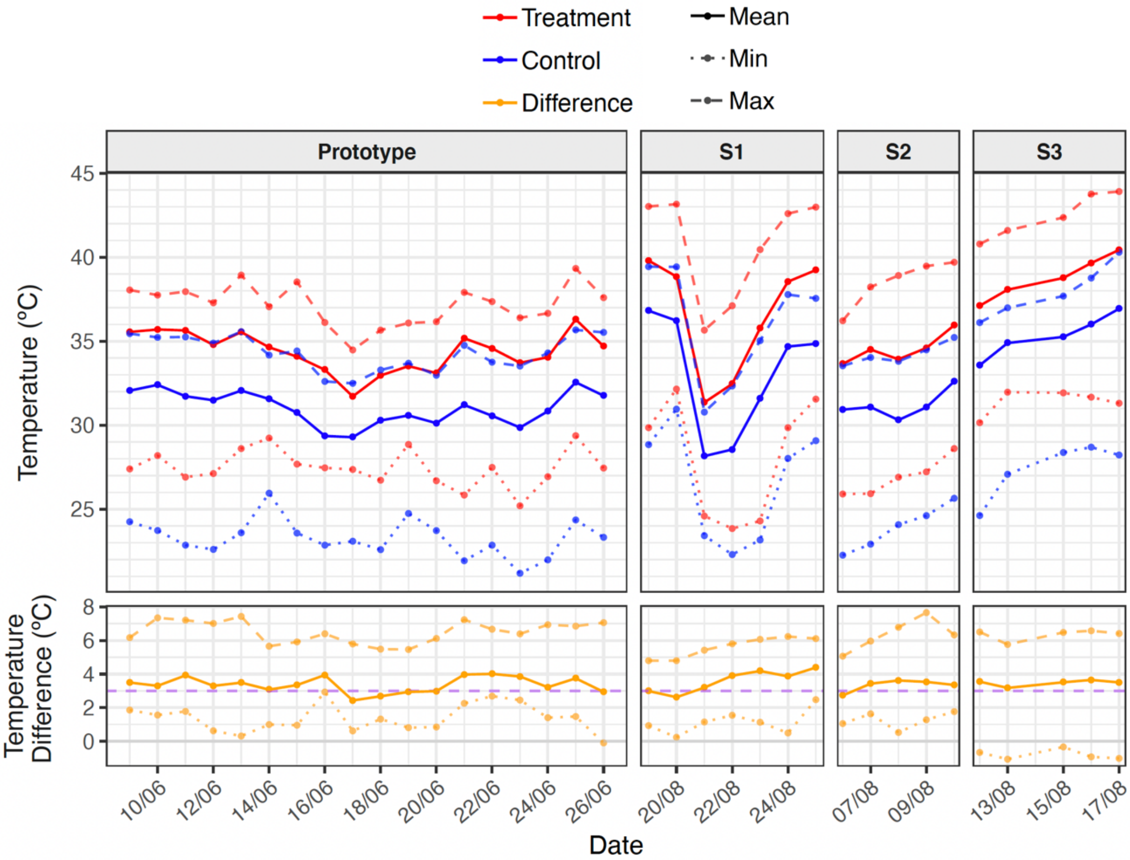

Figure B2.2 *Mean, minimum and maximum temperatures experienced at the treatment individuals in each of the WTHSs, and at their respective control individuals during the period of strongest heating (between 09:00 and 17:00) on each day of temperature recording that WTHSs were left closed throughout; with the mean, minimum and maximum differences in temperature between inside and outside the WTHSs (during this period of each day) given in the lower panel. The purple dashed line indicates the target temperature difference of 3°C.*

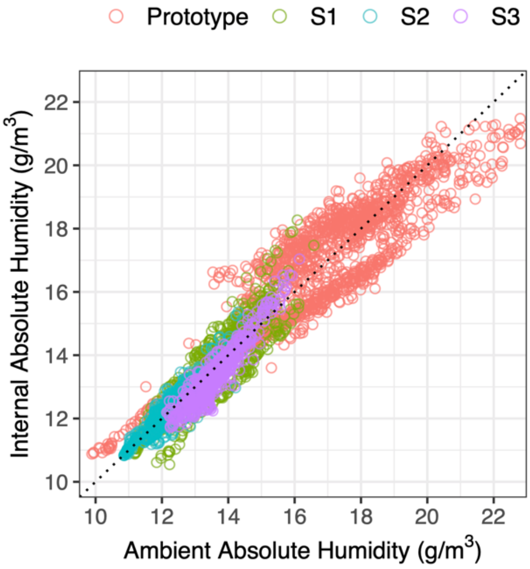

Figure B2.3 *Absolute humidity compared between the air inside each of the WTHSs and the ambient air outside, at each of their corresponding control individuals. The dotted line represents a 1:1 relationship.*

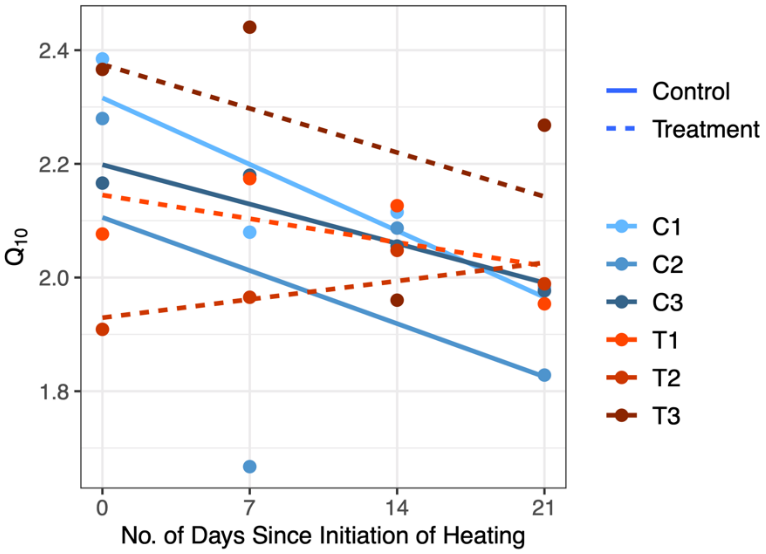

Figure B2.4 *Q_{10} results calculated between 20 and 40°C from measurements taken from each tree on each day of sampling. The trendline for each tree is shown for clarity, but there were no significant trends found within a 95% confidence limit.*

Appendix C

Supporting information for Chapter four

C1. Additional Tables

Table C1.1 *Modified version of simplified Alexander's staining solution, optimised for pollen of study species through pre-experimental testing.*

Chemical	**Amount per 100 ml**
95% ethanol	10 ml
Malachite green (1% solution in 95% ethanol)	2 ml
Distilled water	53 ml
Glycerol	25 ml
Acid fuchsin (0.5% solution in water)	5 ml
Orange G (1% solution in water)	1 ml
Glacial acetic acid	4 ml

Table C1.2 *Summary of temperature sensors being deployed in the field*

	Byrsonima pachyphylla				***Davilla elliptica***						
	Controls		**Treatments**		**Controls**		**Treatments**				
Day/month 2021	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Total C Individuals**	**Total T Individuals**	**Notes**
17/03	0	NA	1	1	0	NA	0	NA	*	1	*
18/03	0	NA	1	1	0	NA	0	NA	*	1	*
19/03	0	NA	1	1	0	NA	0	NA	*	1	*
20/03	0	NA	1	1	0	NA	0	NA	*	1	*
21/03	0	NA	1	1	0	NA	0	NA	*	1	*
22/03	0	NA	1	1	0	NA	0	NA	*	1	*
23/03	0	NA	1	1	0	NA	0	NA	*	1	*
24/03	0	NA	1	1	0	NA	0	NA	*	1	*
25/03	0	NA	1	1	0	NA	0	NA	*	1	*
26/03	0	NA	1	1	0	NA	0	NA	*	1	*
27/03	0	NA	1	1	0	NA	0	NA	*	1	*
28/03	0	NA	1	1	0	NA	0	NA	*	1	*
29/03	0	NA	1	1	0	NA	0	NA	*	1	*
30/03	0	NA	1	1	0	NA	0	NA	*	1	*
31/03	0	NA	1	1	0	NA	0	NA	*	1	*
01/04	0	NA	1	1	0	NA	0	NA	*	1	*
02/04	0	NA	1	1	0	NA	0	NA	*	1	*
03/04	0	NA	1	1	0	NA	0	NA	*	1	*
04/04	0	NA	1	1	0	NA	0	NA	*	1	*
05/04	0	NA	1	1	0	NA	0	NA	*	1	*
06/04	0	NA	1	1	0	NA	0	NA	*	1	*
07/04	0	NA	1	1	0	NA	0	NA	*	1	*
08/04	0	NA	1	1	0	NA	0	NA	*	1	*
09/04	0	NA	1	1	0	NA	0	NA	*	1	*
10/04	0	NA	1	1	0	NA	0	NA	*	1	*
11/04	0	NA	1	1	0	NA	0	NA	*	1	*
12/04	0	NA	1	1	0	NA	0	NA	*	1	*
13/04	0	NA	1	1	0	NA	0	NA	**	1	**
14/04	0	NA	1	1	0	NA	0	NA	**	1	**
15/04	0	NA	1	1	0	NA	0	NA	**	1	**
16/04	0	NA	1	1	0	NA	0	NA	**	1	**
17/04	0	NA	1	1	0	NA	0	NA	**	1	**
18/04	0	NA	1	1	0	NA	0	NA	**	1	**
19/04	0	NA	1	1	0	NA	0	NA	**	1	**
20/04	0	NA	1	1	0	NA	0	NA	**	1	**
21/04	0	NA	1	1	0	NA	0	NA	**	1	**
22/04	0	NA	1	1	0	NA	0	NA	**	1	**
23/04	0	NA	1	1	0	NA	0	NA	**	1	**
24/04	0	NA	1	1	0	NA	0	NA	**	1	**
25/04	0	NA	1	1	0	NA	0	NA	**	1	**
26/04	0	NA	1	1	0	NA	0	NA	**	1	**
27/04	0	NA	1	1	0	NA	0	NA	**	1	**
28/04	0	NA	0	NA	0	NA	0	NA	0	0	
29/04	0	NA	0	NA	0	NA	0	NA	0	0	
30/04	0	NA	0	NA	0	NA	0	NA	0	0	
01/05	0	NA	0	NA	0	NA	0	NA	0	0	
02/05	0	NA	0	NA	0	NA	0	NA	0	0	
03/05	0	NA	0	NA	0	NA	0	NA	0	0	

* On this day there is 1 sensor inside the structure (in the centre) measuring the Treatment temperature and 1 sensor outside as the Control.

** On this day there are 2 sensors inside the structure and 2 sensors outside as the Control (placed to the North and South sides of the tree and structure).

Table continues on next page…

	B. pachyphylla				*D. elliptica*						
	Controls		**Treatments**		**Controls**		**Treatments**				
Day/month 2021	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Total C Individuals**	**Total T Individuals**	**Notes**
04/05	0	NA	0	NA	0	NA	0	NA	0	0	
05/05	0	NA	0	NA	0	NA	0	NA	0	0	
06/05	0	NA	0	NA	0	NA	0	NA	0	0	
07/05	0	NA	0	NA	0	NA	0	NA	0	0	
08/05	0	NA	0	NA	0	NA	0	NA	0	0	
09/05	0	NA	0	NA	0	NA	0	NA	0	0	
10/05	0	NA	0	NA	0	NA	0	NA	0	0	
11/05	0	NA	0	NA	0	NA	0	NA	0	0	
12/05	0	NA	0	NA	0	NA	0	NA	0	0	
13/05	0	NA	0	NA	0	NA	0	NA	0	0	
14/05	0	NA	1	1	0	NA	0	NA	**	1	**
15/05	0	NA	1	1	0	NA	0	NA	**	1	**
16/05	0	NA	1	1	0	NA	0	NA	**	1	**
17/05	0	NA	1	1	0	NA	0	NA	**	1	**
18/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
19/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
20/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
21/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
22/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
23/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
24/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
25/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
26/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
27/05	0	NA	1	1	1	12	3	1, 3, 13	1	4	***
28/05	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
29/05	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
30/05	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
31/05	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
01/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
02/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
03/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
04/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
05/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
06/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
07/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
08/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
09/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
10/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
11/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
12/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
13/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
14/06	0	NA	0	NA	0	NA	3	1, 3, 13	0	3	
15/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
16/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
17/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
18/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
19/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
20/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
21/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	

** On this day there are 2 sensors inside the structure and 2 sensors outside as the Control (placed to the North and South sides of the tree and structure).

*** On this day the 2 sensors inside and 2 sensors outside the Byrsonima individual 1 remain, but full sets of 4 sensors are set up around each Davilla individual (12 being the Control; 1, 3, and 13 being Treatments).

Table continues on next page…

	B. pachyphylla				***D. elliptica***						
	Controls		**Treatments**		**Controls**		**Treatments**				
Day/month 2021	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Total C Individuals**	**Total T Individuals**	**Notes**
22/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
23/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
24/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
25/06	0	NA	0	NA	1	12	3	1, 3, 13	1	3	
26/06	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	****
27/06	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
28/06	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
29/06	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
30/06	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
01/07	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
02/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
03/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
04/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
05/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
06/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
07/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
08/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
09/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
10/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
11/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
12/07	2	12, 13	2	4, 11	1	12	3	1, 3, 13	3	5	
13/07	2	12, 13	1	4	1	12	3	1, 3, 13	3	4	
14/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
15/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
16/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
17/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
18/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
19/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
20/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
21/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
22/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
23/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
24/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
25/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
26/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
27/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
28/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
29/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
30/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
31/07	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
01/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
02/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
03/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
04/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
05/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
06/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
07/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
08/08	2	12, 13	2	1, 4	1	12	3	1, 3, 13	3	5	
09/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
10/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
11/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	

**** From this day, full sets of 4 sensors are set up around each *Byrsonima* individual (12 and 13 being Controls; 1, 4, and 11 being Treatments) in addition to the full sets of sensors at *Davilla* individuals.

Table continues on next page…

	B. pachyphylla				*D. elliptica*						
	Controls		**Treatments**		**Controls**		**Treatments**				
Day/month 2021	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Total C Individuals**	**Total T Individuals**	**Notes**
12/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
13/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
14/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
15/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
16/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
17/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
18/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
19/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
20/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
21/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
22/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
23/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
24/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
25/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
26/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
27/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
28/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
29/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
30/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
31/08	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
01/09	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
02/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
03/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
04/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
05/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
06/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
07/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
08/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
09/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
10/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
11/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
12/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
13/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
14/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
15/09	2	12, 13	3	1, 4, 11	1	12	2	3, 13	3	5	
16/09	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
17/09	0	NA	0	NA	0	NA	1	1	0	1	
18/09	0	NA	0	NA	0	NA	1	1	0	1	
19/09	0	NA	0	NA	0	NA	1	1	0	1	
20/09	0	NA	0	NA	0	NA	1	1	0	1	
21/09	0	NA	0	NA	0	NA	1	1	0	1	
22/09	0	NA	0	NA	0	NA	1	1	0	1	
23/09	0	NA	0	NA	0	NA	1	1	0	1	
24/09	0	NA	0	NA	0	NA	1	1	0	1	
25/09	0	NA	0	NA	0	NA	1	1	0	1	
26/09	0	NA	0	NA	0	NA	1	1	0	1	
27/09	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
28/09	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
29/09	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
30/09	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
01/10	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
02/10	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
03/10	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
04/10	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	

Table continues on next page…

	B. pachyphylla				***D. elliptica***						
	Controls		**Treatments**		**Controls**		**Treatments**				
Day/month 2021	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Number of individuals**	**Individual/s**	**Total C Individuals**	**Total T Individuals**	**Notes**
05/10	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
06/10	2	12, 13	3	1, 4, 11	1	12	3	1, 3, 13	3	6	
07/10	1	12	0	NA	0	NA	0	NA	1	0	
08/10	1	12	0	NA	0	NA	0	NA	1	0	
09/10	1	12	0	NA	0	NA	0	NA	1	0	
10/10	1	12	0	NA	0	NA	0	NA	1	0	
11/10	1	12	0	NA	0	NA	0	NA	1	0	
12/10	1	12	0	NA	0	NA	0	NA	1	0	
13/10	1	12	0	NA	0	NA	0	NA	1	0	
14/10	1	12	0	NA	0	NA	0	NA	1	0	
15/10	1	12	0	NA	0	NA	0	NA	1	0	
16/10	1	12	0	NA	0	NA	0	NA	1	0	
17/10	1	12	0	NA	0	NA	0	NA	1	0	
18/10	1	12	0	NA	0	NA	0	NA	1	0	
19/10	1	12	0	NA	0	NA	0	NA	1	0	
20/10	1	12	2	4, 11	0	NA	1	13	1	3	
21/10	0	NA	2	4, 11	0	NA	1	13	0	3	
22/10	0	NA	2	4, 11	0	NA	1	13	0	3	
23/10	0	NA	2	4, 11	0	NA	1	13	0	3	
24/10	0	NA	2	4, 11	0	NA	1	13	0	3	
25/10	0	NA	2	4, 11	0	NA	1	13	0	3	
26/10	0	NA	2	4, 11	0	NA	1	13	0	3	
27/10	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
28/10	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
29/10	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
30/10	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
31/10	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
01/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
02/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
03/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
04/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
05/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
06/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
07/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
08/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
09/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
10/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
11/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
12/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
13/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
14/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
15/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
16/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
17/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
18/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
19/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
20/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
21/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
22/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	
23/11	0	NA	3	1, 4, 11	0	NA	1	13	0	4	

Table C1.3 *Mean pollen viabilities for all individuals of each species in each year studied, and mean values for the treatment and control groups separately.*

Year	**Species**	**Group**	**Number of individuals**	**Mean pollen viability (%)**	***SE* (%)**	***CV* (%)**
2020	***B. pachyphylla***	All individuals	11	92	1	4
		Control	6	91	2	5
		Treatment	5	93	1	3
	D. elliptica	All individuals	16	70	2	13
		Control	10	70	3	12
		Treatment	6	69	5	16
2021	***B. pachyphylla***	All individuals	16	87	2	11
		Control	10	83	3	12
		Treatment	6	95	1	2
	D. elliptica	All individuals	13	70	6	29
		Control	7	59	7	33
		Treatment	6	83	5	15

Table C1.4 *Comparisons of pollen viability between control and treatment groups of each species in each year sampled, including results of unpaired Wilcoxon rank-sum tests (W, R and p values).*

Year	**Species**	**Difference in mean pollen viability Treatment – Control (%)**	***SE* (%)**	***W***	***R***	**Effect size**	***p***
2020	*B. pachyphylla*	2	2	13	0.11	Small	0.79
	D. elliptica	-1	5	31	0.03	Small	0.96
2021	*B. pachyphylla*	12	3	1	0.79	Large	0.0005**
	D. elliptica	24	9	4	0.67	Large	0.014 **

*** denotes strongly significant result (p < 0.05)*

Table C1.5 *Summary of temperatures (recorded by our sensors) experienced by the control and treatment individuals (and the differences between them, or heating effect) throughout different periods of the whole-tree heating experiment. Showing mean values for the daily mean and maximum temperatures during the peak heating hours (Peak H; 09:00 – 17:00), daytime means (06:30 – 18:30 local time), and daily mean and minimum temperatures at night (18:30 – 06:30).*

		Pre-bases, from first sensors to base insertion		**Post-bases pre-sampling**		**Pollen sampling and hand-pollinations**		**Continued hand-pollinations of *B. pachyphylla***		**Fruit development**	
		17/03/21 – 21/04/21		22/04/21 – 05/05/21		06/05/21 – 20/07/21		21/07/21 – 26/08/21		27/08/21 – 31/10/21	
	Individuals	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**
Peak H	Control	32.7 (3.0)	36.7 (3.1)	32.5 (0.7)	35.6 (0.1)	32.5 (2.2)	35.7 (2.3)	34.8 (2.6)	38.0 (2.4)	36.6 (3.4)	40.2 (3.0)
	Treatment	34.5 (3.6)	39.0 (3.4)	35.7 (1.0)	40.3 (0.4)	35.4 (2.2)	39.2 (2.2)	37.7 (2.7)	41.2 (2.5)	40.1 (3.7)	44.4 (3.0)
	Difference	**1.8 (0.7)**	**4.1 (1.2)**	**3.2 (0.3)**	**5.6 (0.4)**	**3.0 (0.5)**	**4.6 (0.6)**	**2.9 (0.4)**	**4.3 (0.5)**	**3.1 (0.4)**	**4.8 (0.6)**
Daytime	Control	30.1 (2.2)		29.1 (0.6)		28.7 (1.8)		31.3 (2.2)		34.0 (2.6)	
	Treatment	31.5 (2.6)		31.3 (0.8)		31.0 (1.7)		33.2 (2.2)		36.5 (3.0)	
	Difference	**1.4 (0.5)**	**Min**	**2.3 (0.2)**	**Min**	**2.2 (0.4)**	**Min**	**2.2 (0.3)**	**Min**	**2.3 (0.3)**	**Min**
Night	Control	21.7 (1.3)	19.7 (1.7)	18.8 (1.6)	15.8 (1.6)	17.3 (2.1)	13.7 (2.8)	19.3 (1.8)	14.5 (1.9)	24.1 (1.4)	21.0 (1.8)
	Treatment	22.0 (1.3)	19.9 (1.6)	18.9 (1.6)	15.8 (1.6)	17.8 (2.1)	14.3 (2.9)	19.0 (1.8)	14.2 (1.9)	23.3 (1.6)	20.0 (2.1)

Table C1.6 *Paired Wilcoxon signed-rank test results (V, R and p values) comparing pollen viability values between 2020 and 2021 for individuals sampled in both years.*

Species	Group	Number of individuals	*V*	*R*	Effect size	*p*
B. pachyphylla	Controls	6	20	0.81	Large	0.063 *
	Treatments	5	5	0.30	Moderate	0.62
D. elliptica	Controls	7	20	0.38	Moderate	0.38
	Treatments	6	3	0.64	Large	0.16

** denotes marginally significant result (0.05 < p < 0.1)*

Table C1.7 *Unpaired Wilcoxon rank-sum test results (W, R and p values) comparing pollen viability of groups calculated from samples taken before and after the unusually cold spell of June 30th 2021.*

Group 1	Number of individuals	Group 2	Number of individuals	*W*	*R*	Effect size	*p*
Controls Before	5	Treatments Before	5	3	0.63	Large	0.056 *
Controls After	9	Treatments After	3	1	0.67	Large	0.018 **
Controls Before	5	Controls After	9	25	0.09	Small	0.80
Treatments Before	5	Treatments After	3	4	0.37	Moderate	0.39

** and ** denote marginally and strongly significant results respectively (0.05 < p < 0.1 and p < 0.05)*

Table C1.8 *Fruit set and fruit retention of control (C) and treatment (T) individuals of B. pachyphylla in 2021, showing the difference between controls and treatments (D) and results of unpaired Wilcoxon rank-sum tests (W, R and p values).*

		C	T	D	*W*	*R*	Effect size	*p*	
Early fruit set (%)	Mean	56	27	-28	54	0.651	Large	0.00749	**
	SE	7	5	9					
	CV	40	45						
Final fruit set (%)	Mean	48	22	-25	52	0.597	Large	0.016	**
	SE	7	5	9					
	CV	48	51						
Fruit retention (%)	Mean	85	84	-1	29.5	0.0136	Small	1	
	SE	4	6	7					
	CV	16	18						
Number of individuals		10	6						

*** denotes strongly significant result (p < 0.05)*

C2. Additional Figures

Figure C2.1 *The first whole-tree heating structure erected in the Cerrado field site around an individual of Byrsonima pachyphylla.*

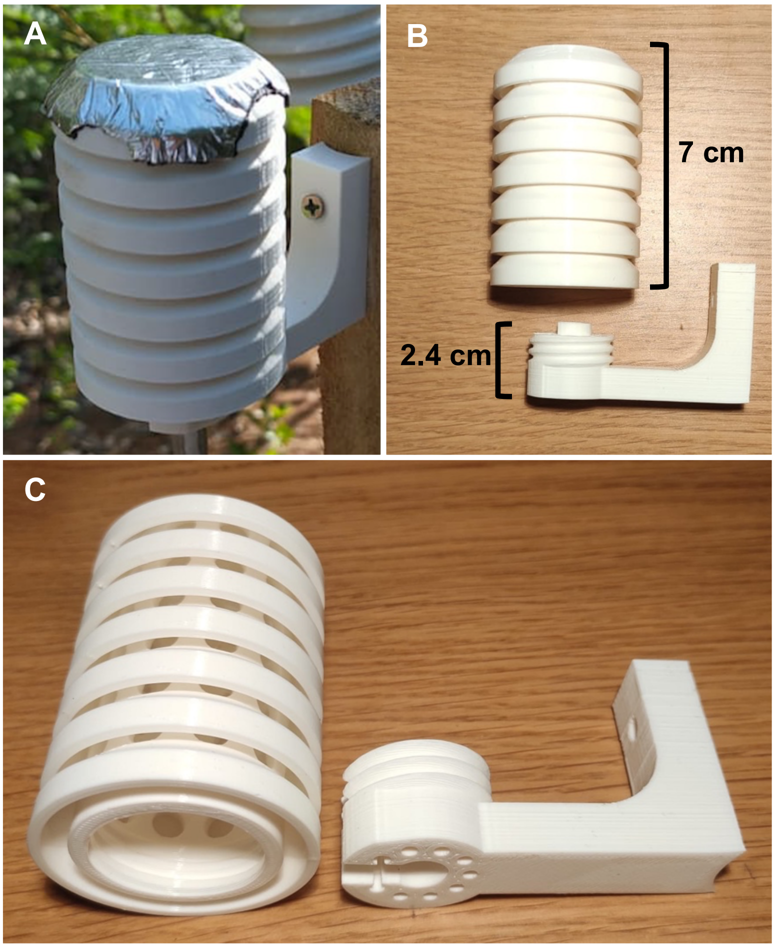

Figure C2.2 *3D printed radiation shield, made in two pieces. (A) A complete shield in the field, covered with thin, self-adhesive insulation to increase reflectance of solar radiation. (B) The two constituent pieces, showing size. (C) The two pieces in more detail. Shields were printed with 80% infill using 200µm white ASA (acrylonitrile styrene acrylate) filament for its UV resistant qualities.*

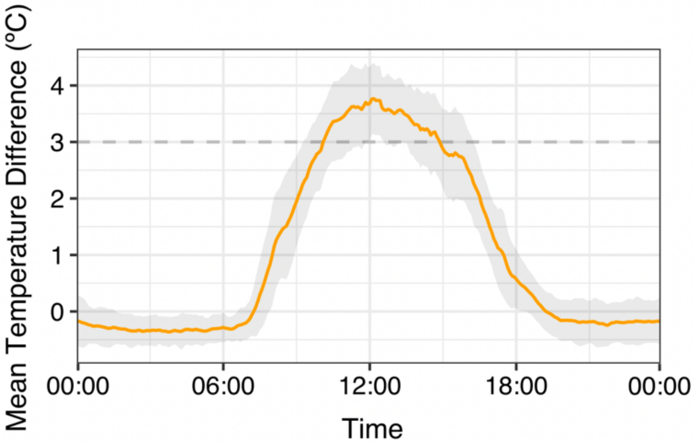

Figure C2.3 *Mean diurnal pattern of temperature differences (treatments – controls) after black base insertion, calculated from all own sensor data after the 26th June 2021 (once full sets of temperature sensors were set up on individuals of both species). Shaded area denotes SD around the mean values (for every five-minute record).*

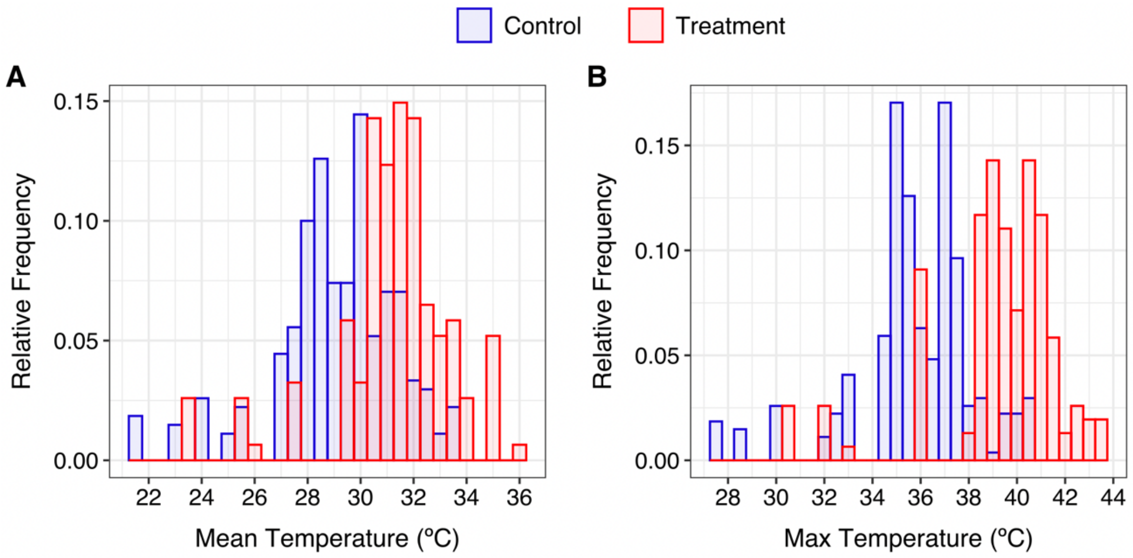

Figure C2.4 *(A) Mean and (B) maximum daytime air temperatures (measured by own sensors) experienced by control and treatment individuals of B. pachyphylla on days that flowers were pollinated.*

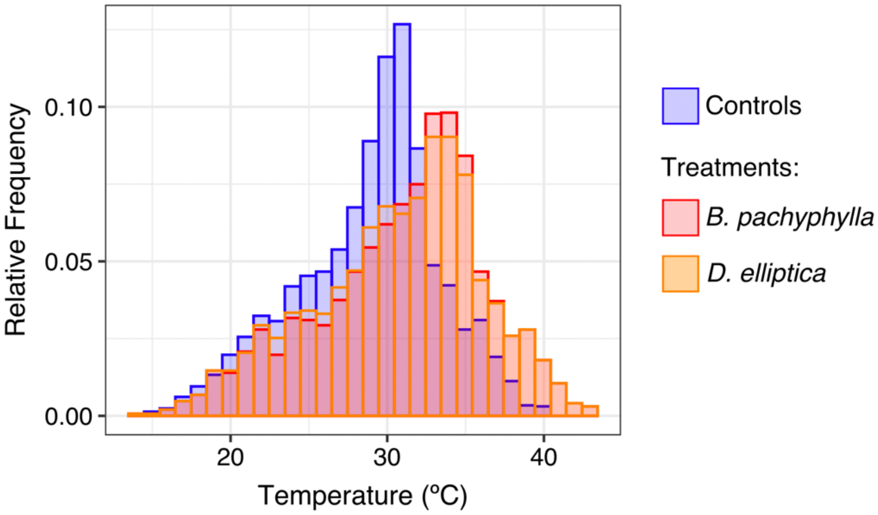

Figure C2.5 *Hourly daytime (06:30 – 18:30) temperatures experienced by the control and treatment individuals of both study species between March and November 2021, estimated using the ERA5-land data and mean diurnal patterns of the heating effect produced by the WTHSs.*

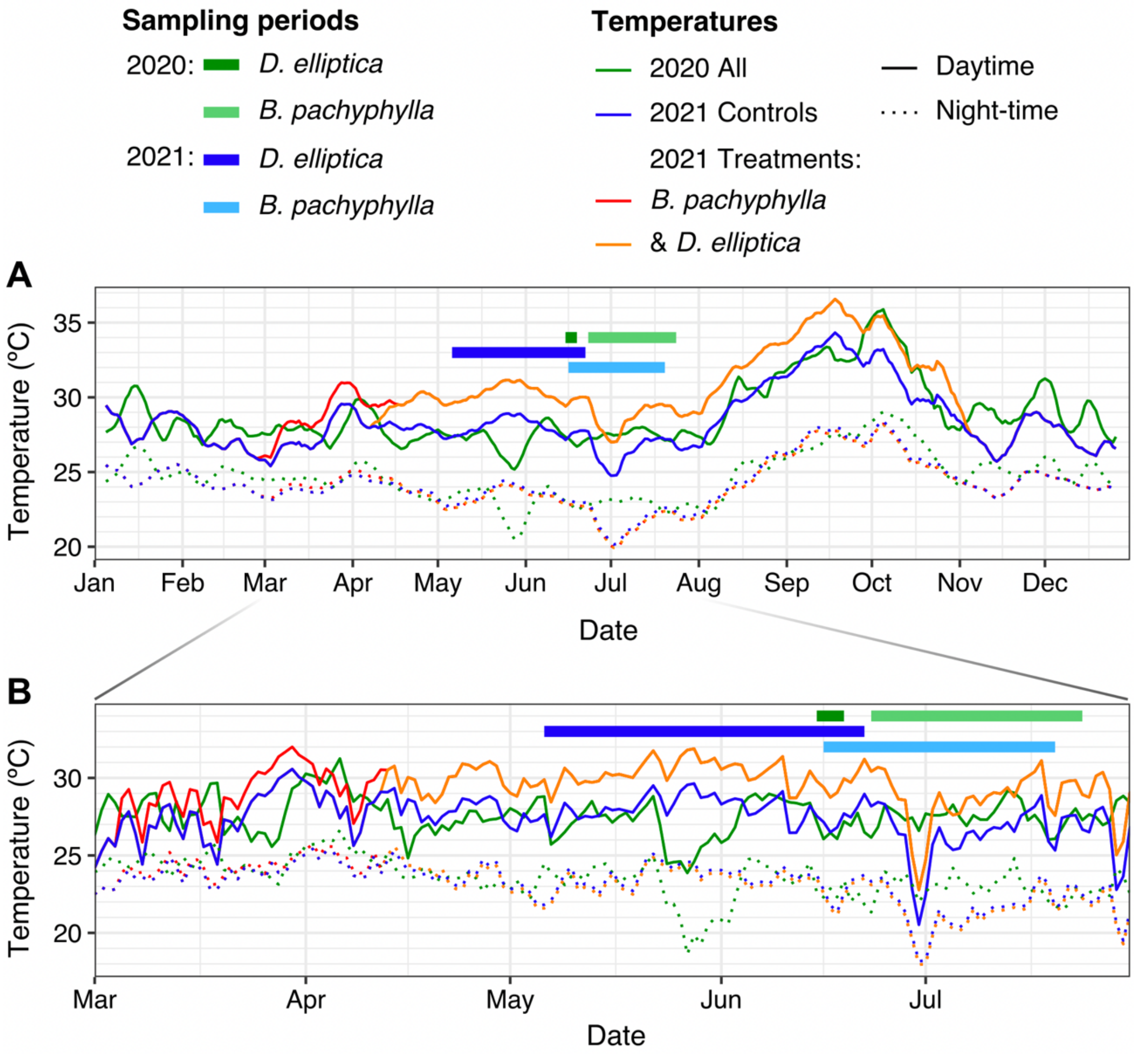

Figure C2.6 *Mean daytime and night-time temperatures (based on the ERA5-land data and estimated temperatures for the treatment individuals) for the years in which pollen samples were collected (2020 and 2021), with horizontal bars indicating the periods over which each species was sampled each year. Showing (A) 10-day moving average day and night temperatures for the entire year; and (B) mean temperatures for each day and night from March to August (the period in which Treatment individuals were heated and sampled in 2021) for more detail.*

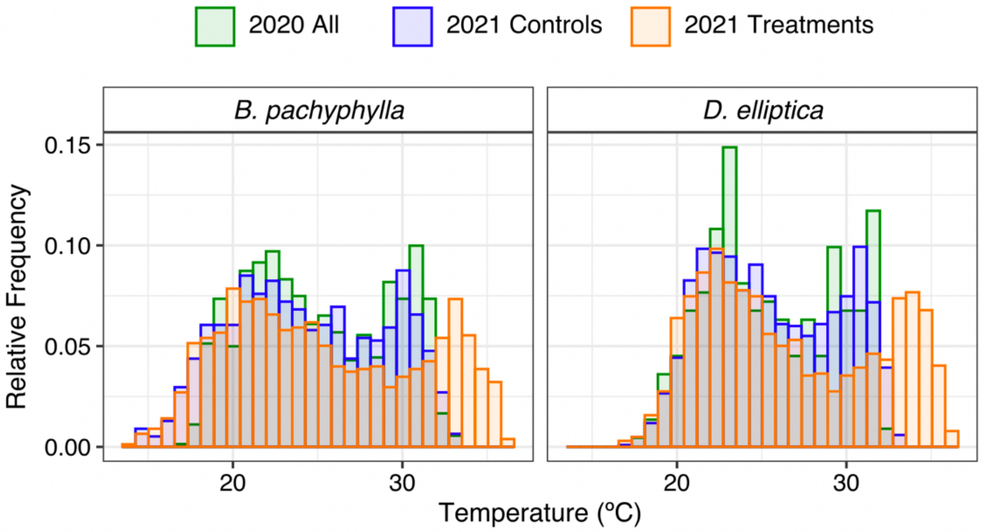

Figure C2.7 *Temperatures (estimated from the ERA5-land data) experienced by the control and treatment individuals during the pollen sampling periods of each species in 2020 and 2021; including the week before each sampling period, which may have affected the first flowers to open.*

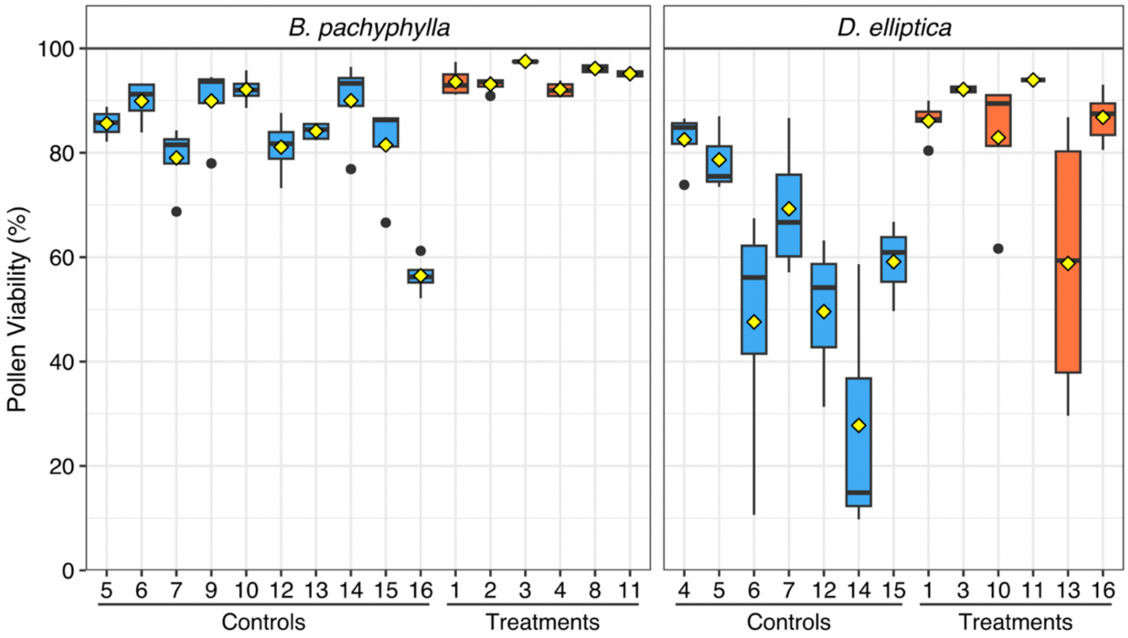

Figure C2.8 *Daily mean pollen viabilities for all individuals (1–16) of each species sampled in 2021 after treatment individuals were heated. Some samples/individuals were removed from analysis due to poor quality samples. Yellow diamonds denote individual means.*

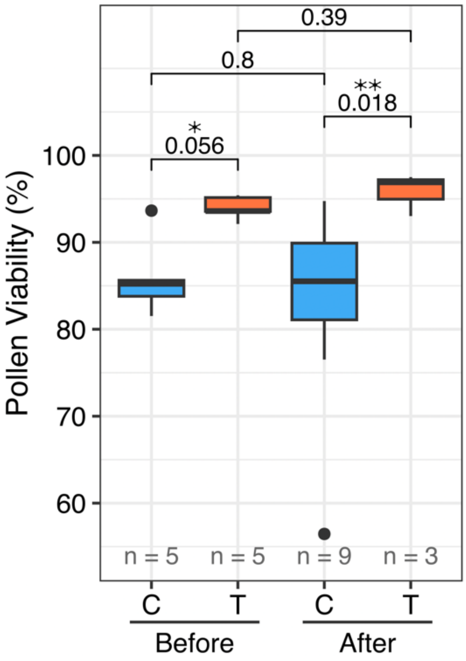

Figure C2.9 *Pollen viability values of B. pachyphylla individuals calculated from samples taken before and after the unusually cold spell of June 30th 2021. Horizontal lines indicate group comparisons and numbers denote p values of unpaired Wilcoxon rank-sum tests. Numbers in grey indicate how many individuals were sampled in that group and period.*

** and ** denote marginally and strongly significant differences between groups*

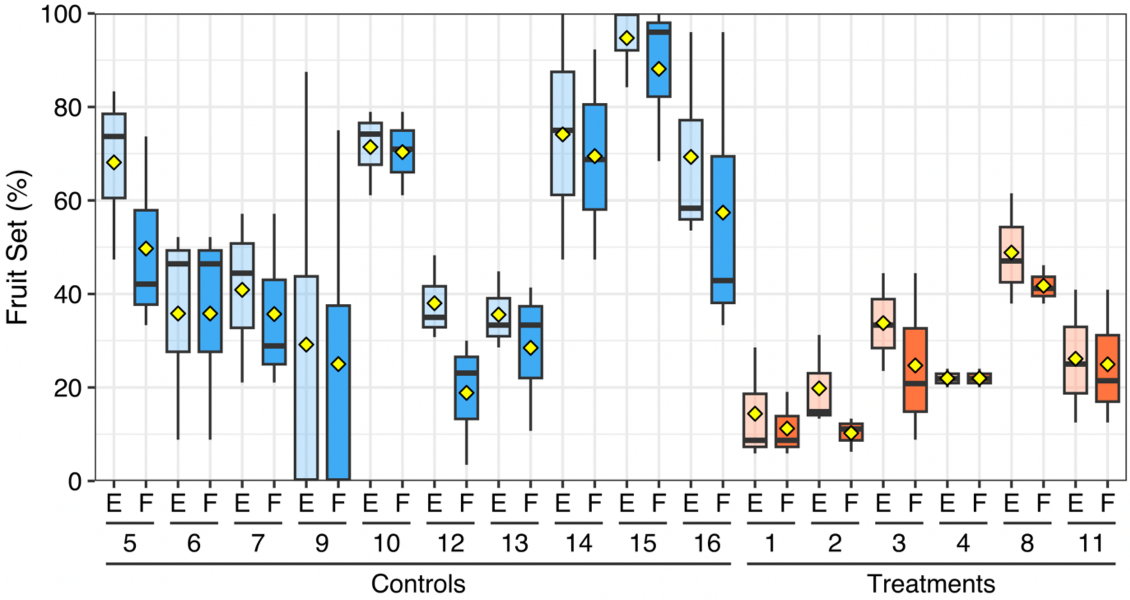

Figure C2.10 *Early (E) and final (F) percentage fruit set for the three inflorescences studied on each B. pachyphylla individual (1–16) in 2021.*

Appendix D

Supporting information for Chapter five

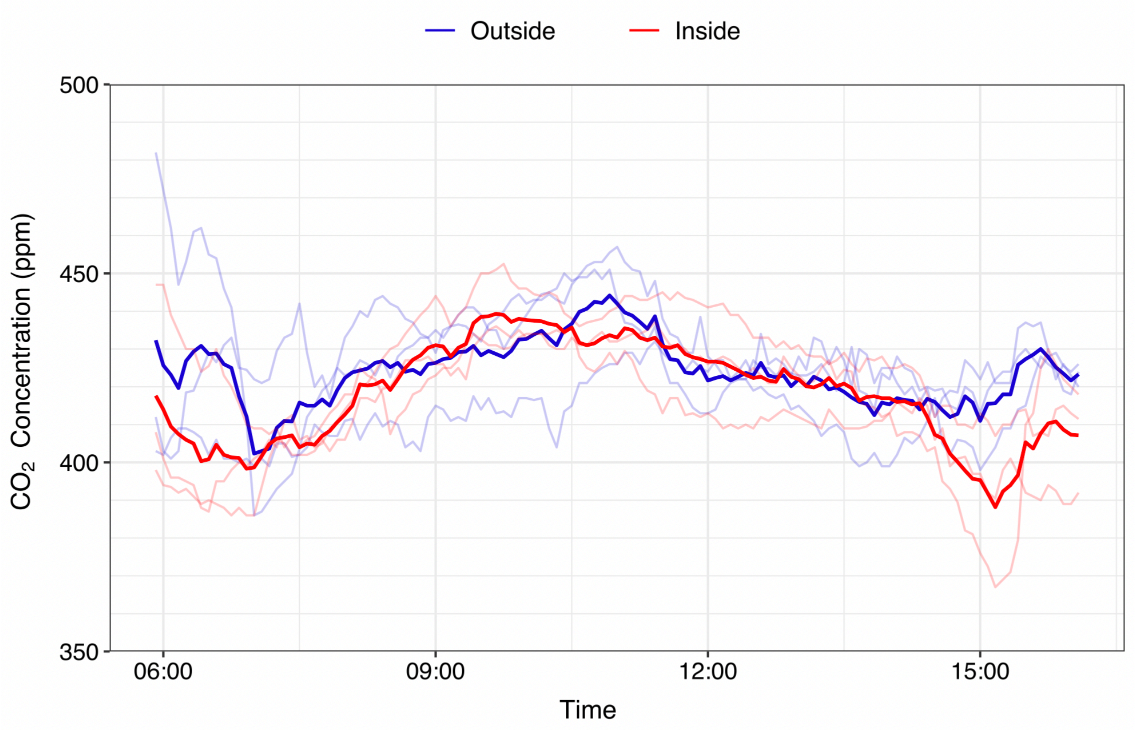

Figure D1.1 *CO_2 concentration measured inside and outside an additional whole-tree heating structure (WTHS) temporarily erected in 2022. Measurements taken using one Extech EA80 Air Quality Meter/Datalogger (accurate to ± 3% of reading; Extech Instruments Corporation) positioned 1.5 m from the ground near the centre of two individuals of Byrsonima pachyphylla (one inside and one outside the WTHS) on alternating days (six days in total; three days inside and three outside). Faded lines indicate CO_2 measurements on individual days; bold lines indicate mean values for the three days.*