The physiological effects of heat stress on anthesis and pollination in domesticated sunflowers (*Helianthus annuus L.*)

by

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FABI Forestry and Agricultural Biotechnology Institute

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List of abbreviations

ZT – Zeitgeber time

FABI – Forestry and Agricultural Biotechnology Institute

SANSOR – South African National Seed Organization

UV – Ultraviolet

ROS – Reactive oxygen species

IPCC – Intergovernmental Panel on Climate Change

SAWS – South African Weather Service

SAM – Shoot Apical Meristem

IM – Inflorescence Meristem

FM – Floral Meristem

DNA – Deoxyribonucleic Acid

D – Dark time

WL – White Light

VOS – Volatile Organic Compounds

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DECLARATION

I, Uyabongeka Memela, hereby declare that this dissertation, submitted to the University of Pretoria for the degree MSc Plant Sciences, contains my own work, and that the content that is contained within this dissertation has not been submitted to any other university or institution. Where use has been made of the work of others, it is indeed rightfully acknowledged in the text.

The experimental work was carried out under the Plant and Soil Sciences Department and Forestry and Agricultural Biotechnology Institute from January 2020 to February 2022. The study was done under the supervision of Dr. Nicky Creux and Dr. Gerda Fourie.

Signed: ________________

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PREFACE

Sunflower is cultivated worldwide for its ability to produce oil. Sunflowers are valuable from both an economic and an ornamental standpoint. Yellow dye is extracted from the flowers, and the seeds contain oil that can be consumed. By compressing the seeds, a sweet golden oil is obtained that can be used as a table oil. Stock and poultry are fed sunflower oil cake. The ongoing global warming is a serious problem affecting the sustainability of sunflower plant fitness and devepment, among many other biotic and abiotic factors. To maintain minimal impacts due to rising temperatures, they therefore require attention and extensive research.

Chapter 1 of this dissertation provides reports on global warming and how it causes extreme weather events like heatwaves. It talks about how plants and their reproductive organs get affected by heat stress during anthesis and pollination. The chapter also discusses effects of high temperatures on different plant species and on sunflowers specifically. It talks about how temperatures influence pollinator foraging patterns. There is also a detailed discussion on how anthesis, pollination and fertilization occur in flowering plants. It also provides a background of the importance of sunflower in agriculture worldwide.

Chapter 2 reports on the heat stress effects on sunflower reproductive organs in a field experiment. This chapter deals in particular with the analysis of sunflower reproductive organs, style, stigma and pollen under artificial heating and natural heatwave conditions. It also analyzes the timing of pollen emergence and the dynamics of pollinator visitors when plants are artificially heated and under a natural heatwave conditions. The chapter discusses detailed materials and methods used for the experiments. The finding was that pollen is the most sensitive reproductive part to high temperatures. High temperatures were found to advance anthesis process: style elongation and pollen release. Stigma receptivity was enhanced during heat stress conditions. Detailed results and the discussion of the results is supplied at the end if this chapter.

Chapter 3 provides a conclusion of this dissertation. We conclude this chapter by identifying the drawbacks and challenges of this study, highlighting what was discovered and learned through the experiments performed. There is also a discussion on how this information might be used in the future.

Chapter 1: Literature Review

The effect of high temperatures on the dynamics of anthesis

ABSTRACT

Global warming is defined as a gradual increase in average global temperatures. One of the many impacts of global warming includes negatively affecting crop yields and adding to the many other sources of yield variation. The continuous rise of average temperatures can cause an increase in the frequency and severity of extreme weather events such as heatwaves. Increases of a minimum of 5°C above maximum local average temperature are regarded as a heatwave. The elevating global temperatures threaten the sustainability of plant yields and their resources. In the second half of the 21st century, climate change predictions and model simulations indicate that heatwaves should become more sever, more frequent, and longer lasting. Plants that are undergoing anthesis are known to be extremely sensitive high temperature conditions, which directly impacts pollination, fertilization and yield. The ongoing rise in temperatures could therefore threaten food security and food chain systems. The mechanism of heat resistance has been studied in a large number of different plant species, but few direct efforts have been made to understand and assess heat tolerance in sunflowers during anthesis. This review explores the literature on climate change, heat waves and how they impact plants, especially during their reproductive stage. It outlines the different effects of high temperatures on the individual reproductive organs of sunflowers and other species during anthesis, pollination and fertilization processes. It also discusses the early history and production of sunflower in Southern Africa. For a sustainable yield under high temperatures, it is important to understand flower responses to heat stress to better develop thermo-tolerant plant cultivars or hybrids to ensure food security.

1.1 Introduction

Agriculture is severely affected by climate change due to increased emissions of greenhouse gases that cause an average global temperature increase. Crop productivity and yields have been profoundly compromised by extreme weather events such as drought, floods and heatwaves (Awais et al., 2018). High temperatures due to climate change were predicted to be cause significant yield reductions in sunflowers and maize (Abd-Elmabod et al., 2020, Debaeke et al., 2017, Moriondo et al., 2011). A growing number of studies demonstrate the impacts of climate change on biodiversity each year, with a growing number of reports demonstrating its effects on species fitness and persistence (Nunez et al., 2019, Gérard et al., 2020, Sintayehu, 2018). Plant pollination by animals, with insects occupying a predominant position in this interaction representing 9.5% of global food production. This mutualism has a risk of being disrupted by global warming, resulting in likely mismatches (a lack of successful interactions) putting plants and their pollinators at risk of extinction (Gérard et al., 2020). Therefore, there are a number of factors that can affect crop yield when they occur concurrently with anthesis, especially because it is a reproductive stage that is most sensitive to environmental stressors such as heat stress, which can be harmful to reproductive organs (Kalyar et al., 2014).

The process of pollination is highly sensitive to extreme temperatures and exposure to heat stress during this period can severely impact crop yields. Pollen dehiscence of the anther releases mature pollen grains from the locules of the anther and enables pollination to occur whereas indehiscence of anthers is the failure to release pollen, thus preventing pollination (Wang et al., 2021). Anthers in both indehiscent and dehiscent stages have a reduced ability to shed pollen under heat stress, with indehiscence anthers trapping pollen inside the anthers and dehiscence anthers fail to hydrate the pollen in locules (Wu et al., 2020, Wang et al., 2019, Wang et al., 2021a). Heat stressed anthers often fail to dehisce resulting fewer pollen grains contacting the stigma, thus impacting pollination. Anther dehiscence is driven mainly by the swelling of the pollen grains inside the anthers, therefore if anthers are exposed to high temperatures the diameter of pollen grains does not become big enough the result in anther dehiscence (Matsui et al., 2000). Heat stress also impacts the growth of the pollen tube during flowering and coupled with reduced pollen grains on the stigma it ultimately hinders ovule development through poor pollination and fertilization (Wu et al., 2020).

Domesticated sunflower, *Helianthus annuus L.,* is a charismatic, economically important oil crop grown worldwide. The genus name *Helianthus* originates from the Greek *helios,* for the sun, and *Anthos,* which means flower (Ram et al., 2018, Atamian et al., 2016, Seiler and Gulya, 2016). Only juvenile sunflowers follow the sun throughout the day; upon reaching the end of the vegetative process, they maintain a permanent eastward direction (Atamian et al., 2016). Sunflower is a member of the *Asteraceae* family, which is one of the largest plant families, hosting almost 1600 genera and 23 000 species (Fernández-Luqueño et al., 2014, Radanović et al., 2018). The *Helianthus* genus consists of 52 species within the Heliantheae tribe as part of the Asteraceae family. (Seiler and Gulya, 2016). Members of this family are characterized by a compressed inflorescence that contains up to 2000 tiny flowers/florets arranged in a cluster within a common receptacle called the capitulum or flower head (Katinas et al., 2007).

Sunflower is recognized as a significant source of high-quality edible oil that is widely used in culinary applications and provides dietary fiber that contributes greatly to human health. As a unique food source that contains essential amino acids, vitamin B, and minerals, sunflower meal is also an excellent additive to composite feeds for livestock (Adeleke and Babalola, 2020, Fernández-Luqueño et al., 2014). With sunflower being grown around the world primarily for its oil therefore there has been numerous studies that focuses on how heat stress affects sunflower plants by significantly reducing yields, oil and fatty acid content (Hernández et al., 2018, Debaeke et al., 2017, Moriondo et al., 2011). Sunflower cultivation has been reported to be susceptible to heat and high temperatures of 45 ºC, which result in severe yield reductions and alteration of fatty acids oil content and quality. Such effects pose a threat to the food industry. (Akladious, 2014, Angeloni et al., 2021). However, research on heat stress and its effects on sunflower reproductive organs and anthesis, however, is limited.

Sunflowers are considered hardy species due to how they are able to produce a stable yield even under unfavorable conditions. One of the many advantages of sunflower is its ability to adapt to different soil and climatic conditions, which has led to its wide cultivation and use as an oilseed plant throughout the world (Debaeke et al., 2017, Hernández et al., 2020). Farmers consider the crop to be flexible, inexpensive and easy to manage. Additionally, despite a number of environmental stresses that sunflowers are subjected to, they have a comparatively stable yield (Debaeke et al., 2021). Not much work has been done to assess its response to heat stress and studying how its floral organs respond to heat stress could provide an understanding of why it is more tolerant than other species. These kinds of studies will also assist with the identification of potential floral sensitivities in this species that might not have been previously known.

This review aims to examine how temperature increases influences flowering process, such as pollination and fertilization. We will explore the state of the literature on the impact of climate change influenced heatwaves on anthesis and what is known about the sunflower-specific response. Topics to be discussed also include sunflower production in Africa and worldwide. It is known that sunflowers can use their heads and leaves orientation as a mechanism to avoid heat stress during the process of anthesis (Kalyar et al., 2014). Although not much is known about sunflower anthesis or how its reproductive organs are affected by temperature, the literature available in this review will cover what is known.

1.2 Climate Change and Heat waves

1.2.1 Climate change

The term climate change describes a change in climate patterns primarily caused by the release of greenhouse gas from natural systems and human activities (Leisner, 2020, Fawzy et al., 2020, Wheeler and Von Braun, 2013). Anthropogenic activities have caused about 1.0°C of global warming so far, and if the current emissions rates continue, that warming will reach 1.5°C between the years 2030 and 2052 (Fawzy et al., 2020, Arora, 2019, Engelbrecht and Monteiro, 2021). Climate change may become irreversible if levels of global warming reach these thresholds. A sustained global warming of 1.5 – 2°C may trigger irreversible collapse of both the Greenland and West Antarctic ice sheets, as well as total loss of these ice sheets. An increase in global temperatures of 2 °C is estimated to result in sea level rise of six meters over a time period of 2000 years, indicating that if climate change is not urgently addressed, the next generation will have a completely different coastal landscape (Engelbrecht and Monteiro, 2021). In the event that greenhouse gas emissions continue at this rate, the CO_2 concentration in the atmosphere could double or triple by the end of the century, which would lead to higher surface temperatures (Del Buono, 2021, Wang et al., 2018). Due to their tendency to trap heat in the atmosphere, greenhouse gases cause a variety of effects, such as rising sea levels, extreme weather events, heatwaves, droughts, wildfires, heavy rainfall and frost (Nui et al., 2020).

Worldwide, agriculture is the main use of land, furthermore, it is the largest economic, social, and cultural activity practiced (Raza et al., 2019, Howden et al., 2007). Unpredictable and increasingly negative impacts on current agricultural practices are still anticipated and seen today due to increasing global temperatures as a results of climate change. It is expected that climate change might significantly modify plant distribution as well as cause extinctions and loss of biodiversity, which will negatively impact the ecosystems they are apart of (Aryal et al., 2020, Raza et al., 2019, Liu et al., 2018, Bakkenes et al., 2002). Climate change events can drastically decrease the production of important food crops (Ortiz-Bobea et al., 2021). This was observed in Russia around the year 2010, when more than 20% of their agricultural lands were severely affected by extreme high temperatures causing a decline in the overall crop production (Teixeira et al., 2013, Leisner, 2020). Another study by Ray et al. (2019) found that increases in temperature and climate changes have caused a loss in agricultural and crop yields in Europe, Africa and Australia. More of these disturbances due to climate changes are expected to occur as the global mean temperatures continue to rise (Wheeler and Von Braun, 2013, Engelbrecht and Monteiro, 2021).

The effect of these climate events on agricultural crop production, diversity and distribution of natural organisms, and other ecosystem services such as flowering time, pollination and fertilization has been well documented (Jagadish et al., 2016, Chandio et al., 2020, Fuglie, 2021, Kumar et al., 2018). Drastic climate changes interfere with ecosystem services, which ultimately cripple the normal development and growth of a plant. A study conducted by Craufurd and Wheeler (2009) showed that the process of crop production tends to be very sensitive towards inconsistencies in climate therefore affecting crop yield. For example, a rise of 1ºC in mean seasonal temperature can cause a decrease in yield of wheat crops by approximately 5-8 %.

About 85 percent of all higher plant species are C3 species, while about 5 percent are C4 species. Photosynthesis in C4 plants occurs when the stomata are closed, as opposed to C3 plants that require open stomata for photosynthesis. C4 plants do not undergo photorespiration, whereas C3 plants do. Therefore, C3 plants have a lower photosynthetic efficiency than C4 plants under normal environmental conditions (Boretti and Florentine, 2019 and Guidi et al., 2019). There are many environmental factors that influence the efficiency of photosynthetic processes, including energy, CO_2, and H_2O inputs. C3 plants tend to prefer cooler temperatures (optimum range of 18–24 °C) and moist environments, while C4 plants prefer warm (optimum range of 32-55 °C) and dry environments, however, both can be severely affected by extremely high temperatures.

Tito et al. (2018) found that growing maize (C4) and potatoes (C3) at warmer temperatures resulted in > 87 % less production, which clearly shows that climate change is a huge threat to agriculture and food security (Ehleringer, 2005, Obermeier et al., 2017). For plants that have evolved in drier or warmer environments, C4 photosynthesis is slightly better suited to maintaining their photosynthesis and productivity. Under temperate conditions, C3 photosynthesis is an excellent compromise between photosynthetic efficiency and some photorespiration (Kumar, et al, 2017). However, the increase in the CO_2 levels in the atmosphere could have beneficial impacts on species that are C3 plants (Ehleringer, 2005, Obermeier et al., 2017). As atmospheric CO_2 concentrations increase, C3 crops may yield more and their net primary productivity will increase through the CO_2 fertilization effect by stimulating photosynthesis, however, high temperatures will remain a barrier limiting these positive effect*s* (Jin et al., 2018, Obermeier et al., 2017). The report by Franke et al. (2020) concluded, however, that the increase in heat stress and water evaporation reduced the benefits gained by increasing CO_2 levels. It is therefore important for sessile organisms like plants to constantly find ways to deal with climate changes and environmental stressors such as high temperatures and droughts in order to survive (Tito et al., 2018, Ahuja et al., 2010, Zhu, 2016). Improving resistance to plant stress is crucial for both agricultural productivity and environmental sustainability by developing effective management strategies, as crops with low stress tolerance might not be able to endure the changes in climate leading to reductions in yield (Zhu, 2016).

1.2.2 Heat Waves

According to the South African Weather Services (SAWS), temperatures for a particular place that are at least 5°C or more above the average temperatures of the hottest month, for three or more consecutive days, is generally considered to be a heat wave (Meehl and Tebaldi, 2004, Lhotka et al., 2018). This being a meteorological definition of a heat wave, which can be described by their temperature magnitude, length and spatial extent (Lhotka et al., 2018). Heatwave simulations and future projections in Central African Republic and South Africa have been projected to increase by 3 to 6°C above the local average temperature, their length varies with a lower limit of three days for a standard heatwave (Dosio, 2017). A heatwave is generally characterized by median temperatures, taking into account the maximum temperatures, duration and frequency. Accordingly, a heat wave is then termed ‘severe’ if all of its characteristics are equal or greater than average medians (Lhotka et al., 2018). Regular, severe and longer lasting heat waves are expected to continue to occur as mean temperatures keep increasing as a result of global warming.

The botanical definition of a heat wave describes it as a period where the temperature rises beyond the threshold level for a long enough period to inflict significant harm to the plant’s growth and development (Wahid et al., 2007). Heat waves induce heat stress responses that can adversely affect practically every aspect of plant development, growth and reproduction (Mittler et al., 2012, Wahid et al., 2007). To some degree, heat stress can be tolerated by plants by changing their physical characteristics in the plant and often by generating signals to alter metabolism (Hasanuzzaman et al., 2013). In order to cope with heat stress, plants modify their metabolism and cell function in order to reduce the negative effects of extreme temperatures. (Mittler et al., 2012). The plants can only adapt slowly to changes in temperature, and if there is a breach of temperature thresholds, the plants will be negatively impacted.

1.2.3 Crop threshold temperatures

The threshold temperature is defined as the temperature under which the reproduction and growth of a species are negatively impacted as well as the temperature that exceeds a certain amount during which disturbance of physiological processes takes place. In 2007, Serbia experienced the most server heatwaves of approximately 44.9°C, which was 12.4°C above the regions threshold temperature of 32.5°C (Unkašević and Tošić, 2011, Profile, 2010). These temperatures were high enough to cause detrimental effects on plant species and most living species in the area. The threshold temperature varies significantly among plant species, developmental stages and reproductive growth processes. The average temperature threshold for most plant species ranges between 32 – 35°C. Heat stress has diminished the world's wheat and maize yields by 5.5 % and 3.8 %, respectively, during 1980 – 2008 (Mittler et al., 2012). In this sense, plant yield is heavily dependent on the environment in which the plant grows.

Sunflower is a well-known heat tolerant plant during seed germination, along with cotton, which has a temperature tolerance of >40ºC during reproduction and photosynthesis (Table 1.1). Sunflower is most sensitive during the anthesis stage and wheat during post-anthesis. Rice and canola have the same temperature threshold of 35ºC during anthesis. Temperatures above 35ºC are deemed detrimental to most plants at most developmental stages (Table 1.1). Climate change predictions have showed that the number of days above 35ºC are forecasted to increase in the near future (Coffel et al., 2017). Climate change could therefore present a threat to plant species by exceeding their threshold temperatures, which could in turn result to a decrease in their yield and production.

Table 1: Temperature threshold for important crop species and specific growth stages

Crop plants	Threshold temperature (°C)	Growth stage	References
Sunflower	26-29	Anthesis	(Kalyar et al., 2014)
	40	Seed germination	(Wen, 2015)
Safflower	34	Flowering	(Houshmand et al., 2021)
Canola	35	Reproductive seed yield	(Chen et al., 2021b)
Maize	38	Germination Grain filling	(Yang et al., 2017)
		Reproductive	(Siebers et al., 2017)
Cotton	>40	Reproductive Photosynthesis	(Sarwar et al., 2019)
Wheat	26	Post-anthesis	(Thomason et al., 2018)
Rice	35	Anthesis	(Kumar et al., 2015)
	37	Grain-filling	(Cheabu et al., 2018, Arshad et al., 2017)
Tomato	37	Anthesis	(Xu et al., 2017)

1.3 The process of Anthesis

The anthesis stage (flowering period of flower bud opening) in angiosperms is usually marked by the emergence of the first anther (Cummings et al., 1989, Beard, 1981). The process of flowers undergoing anthesis (or functional maturity) is associated with the start of pollen shedding from the open flower buds and opening of the male reproductive organs. The male reproductive organ is considered to be in anthesis when one anther has dehisced and pollen starts to emerge. The female reproductive organ is said to be in anthesis when styles and receptive stigmas first appear (Borrás et al., 2007). In most cases the flower bud opens as a result of cell expansion. In order for flowers to open, a minimum period of darkness and light are required, which is governed by the circadian clock (van Doorn and van Meeteren, 2003). There are thus a variety of developmental, hormonal, and environmental factors that control anthesis.

As flowers mature, pollen grains develop through two main stages, microsporogenesis and microgametogenesis in the locules of the anthers that contain pollen grains. Microsporogenesis involves division of a diploid microsporocyte during the meiotic cell cycle to produce a tetrad of haploid microspores. At this point, the tetrad is attached to a tapetum cell. Microsporogenesis is completed by the release of tetrad microspores into the locular fluid. Each microspore then undergoes microgametogenesis. A bicellular pollen develops from the vacuolated microspores after they undergo mitosis, resulting in a vegetative nuclear nucleus and a generative cell. During mitotic division, the bicellular pollen becomes a tricellular pollen with one vegetative nucleus and two sperm cells (Santiago and Sharkey, 2019).

1.3.1 Plant-pollinator interactions

Pollination is one of the primary ecosystem services for agriculture and natural productivity, with animals and plants engaging in mutual interactions 70 % of the time to make it a possibility (Kevan, 1990, Wojtaszek and Maier, 2014, Scaven and Rafferty, 2013). More than 200 000 vertebrate and insect species and a minimum of 300 000 plant species are engaged in mutualistic plant-pollinator interactions worldwide (Burkle and Alarcón, 2011). Since animal pollination is the most common form of pollination, it often changes when pollinators are scarce to ensure reproduction. A plant's floral morphology and inflorescence architecture largely reflect the pollination requirements needed for capture of pollen by stigmas after successful release from anthers (Friedman and Barrett, 2009).

The majority of plant pollinators are bees, flies and butterflies, facilitate successful cross pollination in many crop species (Dar et al., 2016). Bees use pollen and nectar for energy, metabolism as well as a source of proteins, vitamins, minerals, lipids etc., and in return they assist the pollination process (Terzić et al., 2017, Wojtaszek and Maier, 2014, Nicholls and Hempel de Ibarra, 2017, Jadhav et al., 2011). Therefore, the mutualistic relationship between plants and pollinators is equally important to ensure successful pollination in plants. Bees can detect temperature differences in flowers, which impacts the way they select flowers and their pollination behavior. Bees have been found to prefer warm nectar as opposed to cool nectar on flowers. Studies by Atamian et al. (2016) and Creux et al. (2021) found that sunflowers that have fixed flower orientation towards the easterly direction (direction of the sun rises) received more insect pollinators in the mornings due to the warmer flower microclimate than the west facing plants. A study by Harrap et al. (2017) showed that bumblebees had a preference for warmer flowers and they were able to differentiate between warm rewarding and non-rewarding artificial flowers. This finding shows that a floral cue can be derived from floral temperature patterns (Harrap et al., 2020).

The ideal flower temperature facilitates a lot of plant processes, such as embryo implantation, abortion, and development of the pollen tube, as well as the synthesis of components of flower scent. It is often imperative for successful plant reproduction that many plants develop adaptations to absorb exogenous heat in order to maintain their flower temperatures at optimal levels (Corbet, 1990, van der Kooi et al., 2019). Flowers are able to maintain stable temperature balance because many factors are involved, including the flowers size and shape, which determine how much light will be captured by the perianth and reproductive organs, as well as the orientation of the flower, which determines how much heat energy it absorbs from the sun (van der Kooi et al., 2019).

Plants that are animal-pollinated can often self-pollinate in the absence of pollinators or when there is limited pollinators present. The plant switch from cross-pollination to self-pollination in the long run causes shifts and mismatches causing inbreeding depression and genetic losses (Brown et al., 2017). However, another study by Bishop et al. (2017) showed different results. They indicated that faba bean (*Vicia faba*), which is a self-crossing species, went from self-pollination to cross-pollination after being stressed for 5 days (simulating a heatwave) during flowering, which caused a significant increase in the percentage of cross-pollinated seeds. Pollination failure as a result of no pollinators, can cause plants to evolve and depend on other pollen dispersal methods and abiotic factors, which includes wind and water (Besnard et al., 2021). In wind pollination, the distance of the anthers from the ground, its shape and size and its relation to the position of the stigma is very important. *Pennisetum clandestinum*, for example, displays extremely long filaments that extend anthers away from the plant, whereas stigmas are close to the ground. Pollen dispersal is more effective when male flowers are higher on the plant due to higher wind speeds that increase with distance from the ground, which also reduces interference by vegetative structure (Friedman and Barrett, 2009). In water pollinated species, pollen is carried by water currents to prospective stigmas. In some water plants, both anthers and stigma form a thread-like structure and they both meet one another halfway for pollen delivery (Cox, 1993). Therefore, in all plants that are to be pollinated, all reproductive organs involved during pollination should equally ready for successful pollination.

1.3.2 Pollination

All flowering plants undergo pollination, which is a very important evolutionary process in that it facilitates the relocation of pollen from the anther to the stigma and it ensures genetic dispersal within and between individuals (Besnard et al., 2021). The *Asteraceae* family possesses a semi-dry stigma, which means that the basal regions of stigmatic papillae are covered by a cuticle with a low level of lipid-rich secretions. The function of a cuticle is to protect the stigma from pathogens, which might make it difficult for pollen tube penetration (Hiscock et al., 2002, Allen and Hiscock, 2008, Edlund et al., 2004, Allen et al., 2011). When the pollen grain makes contact with the stigma, the secretion of the lipid-rich material gets enhanced (Allen et al., 2011).

The contact of pollen grains and stigma causes the pollen to adhere to the stigma, hydrate and germinate (Lord, 2000, Adhikari et al., 2020, Boavida et al., 2005). Pollen grain capture by a stigma causes a rapid release of a pollen coat from the exine on top of stigma surface. This leads to a fast-growing formation of cytoplasmic extension called attachment foot from an aperture or thin area in the wall of each pollen grain (Ito and Gorb, 2019, Allen et al., 2011, Lord and Russell, 2002, Boavida et al., 2005). During pollen hydration, water channels in the plasma membrane of the stigma cell helps re-organize the plasma membrane and cytoplasm of the stigma cell, re-restoring metabolic activity. (Boavida et al., 2005, Lord, 2000). The water permeates across the attachment foot resulting in pollen hydration (Ito and Gorb, 2019). In *Asteraceae* species such as *Senecio squalidus* L., this happens within 15-30 mins of landing on stigma surface during a compatible pollination (Hiscock et al., 2002). Pollen grains germinate after they are hydrated, producing a pollen tube that enters into the papillary secretory extracellular matrix of the stigmas by penetrating the stigma's cuticle (Allen et al., 2011, Hiscock et al., 2002, Lord, 2000).

Anthers and stigmas are the male and female reproductive organs, respectively. In self-pollinating plants and cross-pollinating plants, pollen is transferred from the anther to the stigma where it germinates (Lord and Russell, 2002, Kevan, 1990, Broz and Bedinger, 2021 and Wilcock and

Neiland, 2002). Pollen tubes are formed after pollen grains are germinated, and by doing so they penetrate the cuticle and enter the style (Lord, 2000, Lord and Russell, 2002). Even though a plant is fully self-compatible, pollen cannot germinate until the cuticle is ruptured (Zulkarnain et al., 2019). Pollen tube germination occurs in an extracellular matrix, which is normally a mixture of both pollen coat secretion and stigmatic exudates, which the pollen tube facilitates growth through the stigmatic tissue (Lord and Russell, 2002, Ito and Gorb, 2019). A special extracellular matrix forms around the pistil and forms the pollen tubes that are directed towards the ovary once pollen tubes break open the transmitting tract epidermis (Lord and Russell, 2002, Johnson and Preuss, 2002). The action of cell-wall degrading enzymes that are present on pollen grains or pollen tube walls aids the piercing of stigmatic tissues (Boavida et al., 2005).

1.3.3 Fertilization

A flowering plant's successful reproduction occurs when the plants sperm cell housed inside the pollen grains comes into contact with the female gametophyte called an embryo sac contained within the ovule, to create a diploid embryo and ultimately a viable seed (Adhikari et al., 2020, Ao, 2021). During a process of pollen germination, the pollen tube grows until it reaches and enters the opening of the ovule though the style (Lord and Russell, 2002). The pollen tube will eventually transport sperm cells to the embryo sac (Lord and Russell, 2002, Hiscock et al., 2002). Two sperm cells are released when the pollen tube tip bursts synergid. Each of the two will fuse with the egg cell to form a diploid embryo, and the other will fuse with the polar nucleus to form a triploid endosperm (Santiago and Sharkey, 2019). All angiosperms share this characteristic of double fertilization where sperm cells merge with egg and polar nucleus within the ovary (Johnson et al., 2019). Multiple ovaries exist in most flowers, and they contain egg cells that receive fertilization from sperm released from the pollen tube. When the egg cells are fertilized, seeds are formed (Johnson et al., 2019, Santiago and Sharkey, 2019, Lord and Russell, 2002).

Fertilization requires 2 main factors; 1) pollen grains should be viable and 2) the stigma should be in a receptive state (Zulkarnain et al., 2019). When the pollen tube enters the ovary, it grows along the placental tissue to an individual ovule and it also reaches the embryo sac through the mycropyle (Boavida et al., 2005). Steps leading to fertilization comprises an entry of pollen tube carrying two sperm cells inside via degenerating embryo sac. Upon entry the pollen tube ruptures releasing the two sperm cells where one sperm cell fuses with cells in the ovary to produce a zygote and an embryo and the other fuses with the central cell to produce the endosperm (Lord and Russell, 2002, Johnson and Preuss, 2002, Faure et al., 1996, Ao, 2021), therefore the development of sibling embryo is ensured (Boavida et al., 2005, Gehring, 2019).

1.4 Effects of high temperatures on anthesis

1.4.1 High temperatures interfere with plant-pollinator interactions

The frequent occurrence of heat waves can directly threaten the anthesis process success of plants and indirectly by disturbing plant-pollinator interactions (Descamps et al., 2018, Bishop et al., 2017, Scaven and Rafferty, 2013, Gérard et al., 2020). Distribution shifts in insect pollinators was observed, which were to the occurrence of warmer than normal temperatures, changing a plant preference of abundant pollinators due to an increased demand to pollinate because they were unable to self-pollinate (Bishop et al., 2017). Therefore, disruptions in plant-pollinator interactions is expected, which could lead to mismatch in time and space of plants and their pollinators as a result of their responses to warmer temperatures (Bishop et al., 2017, Swaminathan, 2019). Studies done by Burkle and Alarcón (2011) and Hoover et al. (2012) revealed that slight changes in precipitation, temperature, CO_2 concentration and nitrogen decomposition can change the phenology of the plant, floral quality and flower abundance. These changes therefore alters flower nutritional rewards and attractiveness to pollinators thus affecting the plant-pollinator interactions. Therefore the timing of these reproductive processes may be adjusted upon exposure to high temperatures (Gray and Brady, 2016, Ohnishi et al., 2010). For instance, the abundance and diversity of pollinators is directly enhanced at elevated temperatures as they can detect the increase in floral nectar sugar concentrations (Descamps et al., 2018, Mu et al., 2015).

Climate warming may lead to temporal mismatches between plant and insect mutualistic partnerships. Variations in temperature can shift flowering phenology, with temperature being the one of the driving forces and triggers for flowering (Herrera, 1995, Ohler et al., 2020). The process of reproduction in plants therefore depends on achieving the right flowering temperature (Ohler et al., 2020, Corbet, 1990). During the daytime, the temperature of the plant can increase due to direct solar radiation being absorbed by its structures and thermal radiation being emitted from structures surrounding the plant (van der Kooi, 2016, van der Kooi et al., 2019). Heat stress can affect quite a number of flower traits such as the pollen, nectar and volatiles release (Figure 1.1).

The results of the study by Descamps et al. (2018) on *Borago officinalis* found that stressed flowers were less frequently visited by bees. However, the visitation rate was positively correlated with nectar production in flowers without stress where they produced high nectar sugar quantity (3.9 mg/flower) and had more frequent visitors. Stressed flowers received fewer visitors due to lower nectar sugar quantity (1.3 mg/flower). They also found that the amount and quality of pollen produced also disturbed the pollinator foraging behavior, where plants that produced higher pollen quantity received more bee visitors than the ones with lower quantity pollen. A study conducted by Chamer et al. (2015) on sunflowers, revealed that the average number of pollinators that visited the plant and their attractiveness to bees was influenced and dependent on the amount of pollen produced and its availability. This affects flower-pollinator interactions because as much as flowering plants need bees for pollination and seed set, bees also need the flowering plants for nectar and pollen. An experiment done by Norgate et al. (2010) showed that bees prefer warm nectar, where they were attracted to plants with higher ambient temperatures. Even though, there is a great possibility that the nectar produced will have a decreased composition and concentration after being exposed to higher temperatures. An interesting study in Australia that is contradictory to Norgate et al. (2010) showed that stingless bees (*Trigona carbonaria*) preferred warmer nectar at higher ambient air temperatures (23 to 30°C), but when the air temperature reached 34°C, their behavior changed and they chose ambient temperature nectar for warmer nectar (Gérard et al., 2020).

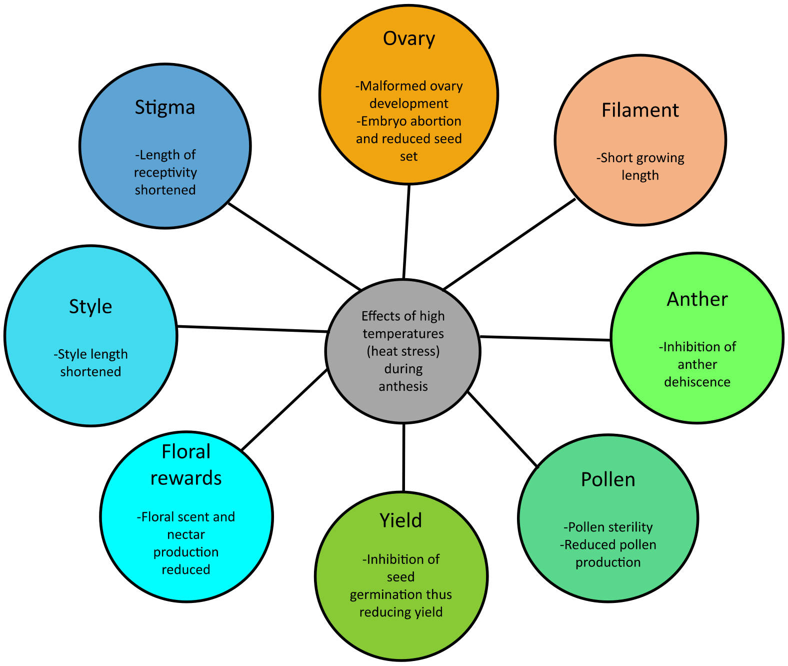


Figure 1.1. Major effects of heat stress temperatures on flowers during anthesis. The diagram shows the most common effects of high temperatures in angiosperms. Both the male and female parts of the plants were included represented by different colours. Female parts are found on the left side of the diagram, whereas the male parts are found on the right side of the diagram. Lastly, it outlines the main effects on plant yield (Chen et al., 2021a, Wen, 2015, Gray and Brady, 2016, Hedhly, 2011, Hasanuzzaman et al., 2013, Wang et al., 2021b, Scaven and Rafferty, 2013)**.**

Research has also revealed that warmer temperatures increases the release of volatility of organic compounds produced by flowers (Scaven and Rafferty, 2013). However, the study done by Sagae et al. (2008) provided evidence that increasing temperatures up to 30ºC increased the emission of volatiles while temperatures of 35ºC decreased floral scent production. Similarly, a study by Hu et al. (2013) that found that as temperature increased from 10 to 30ºC so did volatile emissions increase, however temperatures of 40ºC significantly decreased volatile emissions. These changes in rewards and floral odour could affect insect visits to some flowers and the benefits they gather. Pollinating insects, such as moths, that rely on long-range cues to identify floral resources could be affected by altered odor emissions or volatilization of floral scents at higher temperatures and being unable to detect rewarding flowers (Scaven and Rafferty, 2013).

1.4.2 High temperature negatively affects pollination

A number of studies have suggested that high temperatures affect male and female organ development in different ways (Pan et al., 2019, Nguyen et al., 2019, Wang et al., 2021b).

Specifically, Pan et al. (2017) determined that high temperatures reduced the lengths of both stamens and pistils. Stamens being reduced in length causes stigmas to protrude and therefore they limit self-pollination (Wang et al., 2021a, Pan et al., 2019). Under heat stress, a distorted pistil including ovule, stigma, and style was observed in species such as maize, chickpea, canola, tomatoes, apricot, wheat, rice and sorghum. In addition to protruded stigmas causing pollen capture to be compromised, shorter styles with shrunken ovaries negatively impacted pollination (Wang et al., 2021a, Giorno et al., 2013). However, a study by Nguyen et al (2019) showed that pistil development was unaffected by the heat stress because their growth occurred normally, whereas stamen filament length was reduced and anther sac size shrank. Their study was on Arabidopsis species, which found that heat treated stigmas still germinated healthy pollen grains and formed pollen tubes after being loaded onto these stigmas (Nguyen et al., 2019).

Heat stress effects in tomato during anthesis period showed significant alterations, which were presented by abnormal anthers and style elongation, and these changes were observed in tomato species subjected to high temperatures of about 36°C for long periods of time (Giorno et al, 2013). Stigma exertion during elevated temperatures in domesticated tomato species tempered with pollination process thus causing failure of fruit set (Pan et al., 2019, Wahid et al., 2007). Apart from excessive stigma exertion in high temperatures, it can also cause stigmas to lose their receptivity. High temperatures ≥30°C shortens the period of stigma receptivity, which then might compromise pollination (Hedhly et al., 2008), by disturbing pollen germination on the stigma, pollen tube formation in the stigma and style, as well as ovule fertilization thus reducing yield in canola, tomato, rice and cotton species (Sage et al., 2015).

Heat stress in maize resulted in blasted tassels, reduced pollen shedding, and poor pollen viability. Indehiscence of pollen anthers and a smaller tassel were responsible for the dramatic decrease in pollen shed weight (Wang et al., 2021b). In rice and cereal species, limitation of anther dehiscence under heat stress causes inhibition of pollination reducing the spikelet fertility caused by low pollen production as a result of anthers not breaking to release sufficient pollen therefore decreasing the number of germinating pollen grains on stigma (Oosterhuis and Snider, 2011, Barnabás et al., 2008). At anthesis, exposure to high temperatures resulted in decreased pollen production and pollen shed. It was hypothesized that no pollen grains swelled, anthers were not dehisced, and pollen grains were poorly released, resulting in a low number of germinating pollen grains on the stigma (Kumar et al., 2015). In plants that have separate male and female reproductive organs, there is a coordinated effort to ensure that anthers are positioned above the stigmas so that they can easily capture pollen after anther dehiscence (Sage et al., 2015, Nguyen et al., 2019, Wang et al., 2021a). Changes in these processes result in an altered fertilization process as they prevent pollen deposition on stigmas (Sita et al., 2017, Shi et al., 2018, Thakur et al., 2010).

Pollen viability in Sorghum cultivars was reduced by 61 % after being exposed to high temperature stress (Djanaguiraman et al., 2018). Pollen exposed to high temperatures displays a deeply pitted and non-smooth surface exine. A change in exine ornamentation caused by high temperature stress indicates disruption of tapetal cells and changes in exine thickness and roughness. Thus, damaged tapetal cells could affect the transport of nutrients to developing pollen, resulting in pollen sterility (Djanaguiraman et al., 2018). Exposure of male reproductive cells during development is believed to contribute to weak capacity pollen grains that influence pollen abortion and sterility (Giorno et al, 2013). The viability of pollen is an important factor in reproductive success, and its genetic variability under heat stress exposure has been documented in rice, sorghum, peanuts, and soybeans (Bheemanahalli et al., 2019). In fact, poor seed set cannot necessarily be attributed to poor pollen viability alone, as other parameters such as the viability of female reproductive organs (ovaries), pollen tube growth rates, and ovule-pollen tube interactions, which are not directly determined by pollen viability or pollen germination, can also contribute (Bheemanahalli et al., 2019).

An interesting study assessing pollen grain viability under high temperatures found the majority of the grains to be viable by manual observations (Endo et al., 2009). Even though, those pollen grains stained well with Alexander's solution despite their seemingly normal appearance, after exposure to high temperatures those pollen grains did not adhere to the stigma or germinate on it. The study revealed that pollen grains appeared viable but there were problems with stigma recognition, and pollen coats were showing evidence of sterility (Endo et al., 2009). Other changes due to higher temperatures include a reduced number of pollen grains produced, abnormal development of pollen grains, which causes reduced viability and germination capacity (Descamps et al., 2018). Elevated temperatures were also found to cause a reduced pollen transfer due to anthers abnormal morphology, leading to reduced fruit and seed set (Descamps et al., 2018, Deng et al., 2021). Flowering occurs at a specific time of day, and this time of day has a major influence on the pollen observations because pollen viability is declining at that time of day when the flower opens under heat stress (Kumar et al., 2015).

Development of anthers is resource-intensive, and heat stress affects the formation of tapetum cells and microspores that produce DNA, carbohydrates, proteins, and lipids. The development of microspores in anthers under heat stress is affected by degeneration of the tapetum, which results in a reduction in dehiscence, followed by the closure of the locules, and a decrease in pollen dispersal (Sita et al., 2017, Nguyen et al., 2019). Similar findings were seen in Arabidopsis species where by pollen sterility was greatly reduced, and plants displayed abnormal stamens, like short filaments, low pollen production, and anther dehiscence defects (Nguyen et al., 2019). The impaired sucrose metabolism in anthers causes reproductive failure by reduced anther dehiscence in chickpeas exposed to high temperatures. As a result, pollen is prevented from being deposited on the stigma, resulting in altered fertilization (Sita et al., 2017). Physiological events such as pollen grain development in anthers (gametogenesis), pollen viability, pollen tube growth on the stigma, and fertilization are negatively affected by heat stress during the reproductive stage.

The cytoskeleton and mechanical properties of the stigma or style are important in pollen germination and regulating the growth of the pollen tube (Wang et al., 2021a). In dry edible beans, high temperatures of 37ºC reduced the rate of pollen tube germination and pollen tube growth, which ultimately compromised fertilization (da Silva et al., 2020). In wheat species, heat stress caused a reduction in the mean number of pollen tubes going into the ovary and even if those ovaries managed to attract pollen tubes, they were still not fertilized effectively (Saini et al., 1983). Temperatures of 34°C, daytime temperature, reduced the pollen germination rate of wheat by 93% (Bheemanahalli et al., 2019). Studies on cherimoya (*Annona Cherimola*) found that temperatures of 30ºC and 35ºC decreased pollen tube growth and germination (Rosell et al., 1999). Stress, such as heat stress, disrupts the timing and synchronization of these events during pollen function (Raja et al., 2019). Higher temperatures of 30ºC promote faster growth of the pollen tube, resulting in less time required to reach the ovary as observed in sweet cherry and peach plants (Hedhly et al., 2005, Hedhly et al., 2004).

Higher temperatures in *Cherimoya* species resulted in elongating pollen tubes that extended along the ovarian wall and further to the lower end of the ovary. At temperatures of 27℃ or just below, pollen tube growth was found to be faster at higher temperatures. The pollen tube growth started rapidly at the first the start of exposure to temperatures of 30°C, the growth rate slowed down after it the pollen tube had just passed the ovary. A large number of pollen tubes stopped growing before they could reach the middle part of the ovary at 32°C (Matsuda et al., 2011). Higher temperatures of 30ºC promote faster growth of the pollen tube, resulting in less time required to reach the ovary as observed in sweet cherry plants. Another explanation could be that there are different temperature thresholds for different plant processes and plant species, which above the ambient temperature, it could have negative effects leading to inhibitions and timing mismatches (Hedhly et al., 2005).

The viability of male and female reproductive organs determines whether fertilization and seed formation occur successfully. Fertilization process is highly sensitive to high temperatures. In crop plants, reduced pollen germination and lowered pollen tube growth after pollination are severe restrictions for fertilization (Wang et al., 2021a, Sato et al., 2000). During an exposure to extreme heat stress of 38 / 20°C, cotton plants recorded a reduction in soluble carbohydrates (especially sucrose) and triphosphate (ATP) content in the pistil, which led to a decrease in the number of ovules and efficiency of fertilization. Rice plants exposed to heat stress during flowering failed to fertilize due to sugar starvation in pollinated pistils (Wang et al., 2021a). Their research provided proof of the significance of sugars in the growth of pollen tubes under heat stress by manipulating enzymes active in sugar hydrolysis and release. Low carbohydrate metabolism and low invertase activity during daytime heat stress limit the amount of sugar/energy that pistils provide to the pollen tube, thus limiting pollen tube growth and therefore preventing fertilization (Wang et al., 2021a).

1.5 Sunflower cultivation and production

Sunflower belongs to the Asteraceae family, which is a family that makes up approximately 10% of all the plant species. The first record of sunflower cultivation and domestication was from 4000 years ago in eastern North America by native Americans (Lentz et al., 2008, Seiler and Gulya, 2016, Radanović et al., 2018, Fernández-Luqueño et al., 2014). Early in the 1500s, sunflower was introduced to Europe through Spain, and they began growing it as an ornamental plant. After which it became widely distributed in Russia by the end of the 1800s. Through the Russian farmers, sunflower breeding improved oil content by approximately 50 % (Castro and Leite, 2018, Seiler and Gulya, 2016, Fernández-Luqueño et al., 2014). Russian varieties with higher oil levels renewed United States of America's (U.S) interest in the crop and there was a spike in U.S sunflower oil production (Seiler and Gulya, 2016). During the 1970s, sunflower production in the United States grew tenfold, averaging 7.3 billion pounds in 1979, but fell to only a third of that level by 1990. In response to growing demands for sunflower seed oil, U.S. production increased in the late 1970s. Since then, interest and production have increased, partly because there are new domestic farm policies in the U.S. that make it more attractive to grow sunflowers (Gupta, 2014).

Among the world's most important oil crops, sunflower is one of the most widely cultivated (Castro and Leite, 2018, Unakıtan and Aydın, 2018) among vegetable oils selected for quality, sunflower oil ranks first (Suyunovna, 2021). Sunflower ranks fourth in the world's oil production after soybeans, rapeseed, and safflower (Adeleke and Babalola, 2020). In India and South Africa, sunflowers may be planted and used as a substitute crop in low producing years (Adeleke and Babalola, 2020). Ukraine and the Russian Federation currently produce nearly 80 % of the world's sunflower oil, which means Ukraine and the Russian Federation combined produce more than half the world's sunflower seeds (FAO, 2022). Over 10% of world's sunflower seeds came from Eastern European countries and Argentina (Adeleke and Babalola, 2020). A total of 2.7 million tons of sunflowers are produced in China each year, making it the world's fifth largest sunflower producer. Between 2013 and 2017 the USA, Brazil, Argentina and china emerged as major seed producing nations (Adeleke and Babalola, 2020).

Enriched in high linoleic content is the sunflower oil and it is normally used as frying oil, salad oil and it can also be used in making margarines. The oil from sunflower can be a very useful alternative or supplement to diesel fuel to make biodiesel. Crushed sunflower seeds are usually used in livestock and poultry feeds. The sunflower seeds can be used for human consumption, they are normally roasted, salted and consumed as a food snack (Seiler and Gulya, 2016, Nhemachena and Muchara, 2020). They can also be mixed with rye flour to make bread (Nhemachena and Muchara, 2020). Made from roasted sunflower is a SunButter, which is a fairly new product in the market, it is an alternative for peanut butter made for people with nut allergies. Sunflower kernels are also eaten as a snack after being coated with chocolate (Nhemachena and Muchara, 2020, Seiler and Gulya, 2016).

1.5.1 Sunflower Production in Africa

In the years from 1947 to 1975, the Food and Agricultural Organization (FOA) reported that over 400 000 ha of sunflower were grown in Africa. South Africa was the major producer with 32 000 ha in 1946, most of which was exported to England and other countries as oil (Putt, 1997). Another report of sunflower production was in Zimbabwe during the years 1924 to 1925. The area covered a little over 2400 ha, at which the first official grades of sunflower seeds were recorded. Aside from exports to England, sunflower seeds were used locally in South Africa and Zimbabwe as feed for poultry and other livestock (Putt, 1997). Early in the 20th century, Europeans introduced sunflower to Kenya, and the seeds were primarily used for bird feed. Early in 1906, there was also evidence of sunflower cultivation in the Belgian Congo, however it was not as extensive as it is today. In North Africa, the annual production of sunflower was just under 1000 tonnes by 1972, and it was used exclusively for oil production (Putt, 1997).

Sunflowers, which are an important oil crop in South Africa, contribute about 82 % of all edible oil (Chigeza et al., 2012). A recent increase of approximately 40 % in the demand for oil cakes and vegetable oils in South Africa, along with the lack of sufficient crushing capacity to meet the increasing demand, has led to challenges and shortfall for sunflower production in the country over the last 5 years (Meyer and Van der Burgh, 2015). Due to the rising demand, South Africa has since increased the production of sunflower and yield through factors such as seed prices and introduction of new hybrid cultivars (Chigeza et al., 2012). Over the last two decades, annual sunflower seed production in South Africa has increased. Fluctuations in production levels are primarily due to uncertainty about price expectations, high input costs and high stock levels (Loubser, 2002). Therefore, if the production of sunflower continues at this rate it could meet or exceed the rising demand of vegetable oil in South Africa. It is more cost-effective to import crude oil than sunflower seed during years of reduced sunflower production, since crushing plants are less active. During the period 2019/20 to 2020/21, the average price increased by 25, 5 %, from R5 359 to R7 201/ton. The sunflower price moved from R6 029 to R8 804/ton between July 2020 and July 2021, an increase of 31, 5 %, between June and July 2021 prices grew by 5 % on a monthly basis (Maluleke, 2021).

1.6 Sunflower capitulum structure, anthesis and pollination

The sunflower buds are initiated soon after the leaves have completed their vegetative phase (Sharma and Bhatla, 2013a) at approximately 38 days after sowing, with swelling that covers the entire apical meristem as a first visible sign of transition to reproductive phase (Marc and Palmer, 1978). An involucral bract or phyllaries that functions as a sepal surrounds and protects the developing capitulum by being ovate to ovate-laceolate in shape. (Sharma and Bhatla, 2013a). As the bud grows and increase in diameter, it forms a clear dome-shape with visible ray florets that are folded inwards (Sharma and Bhatla, 2013b). A continued expansion occurs until there is a formation of a flat disc that has a rim elevated at the periphery (Zhao et al., 2016, Marc and Palmer, 1978). Each of the florets within the bud form a single true flower with the initiation starting at the center of the disc and these are completely separated at the time of bud development. This activity of early bud differentiation should be complete before anthesis starts (Sharma and Bhatla, 2013b, Steele, 2000).

Ray and disc florets are generated at the flat surface where the floret primodia appears. Fibonacci sequence is the arrangement of disc florets, which in turn gives rise to the emergence of a spiral pattern as florets arise in rows of bumps that are composed of a bract and a floret (Dosio et al., 2006). Before the beginning of the anthesis process, the ray florets, with bright yellow elongated corollas that form the petals that surround the head will then start to unfold (Sharma and Bhatla, 2013b). Anthesis commences only once the ray petals are fully open (Sharma and Bhatla, 2013b), at approximately 60 days after sowing (Marc and Palmer, 1978). Anthesis starts as the disc florets in the capitulum gradually open in pseudo-whorls towards the center of the head and continues to the middle of the head (Sharma and Bhatla, 2013a, Fernández-Luqueño et al., 2014). Such developmental pattern increases the capitulum's anthesis time thus attracting pollinator visitors for pollination (Sharma and Bhatla, 2013b).

Secondary pollen presentation is a phenomenon that refers to a process of pollen movement from anthers to another reproductive organ, which will be the one to act as a pollen presenter for pollination. Sixteen angiosperm families have been identified to use secondary floral organs for their pollen presentation including the Asteraceae family, which uses a style (Howell et al., 1993). Pollen discharged inside the anthers gets pushed out by the style as it elongates, which makes it available to the pollinators. This type of pollen presentation is different from the pollen dispersal where by the male organ is the primary and competent disperser of the pollen. A study by Budumajji and Solomon Raju, (2018) was not specifically a temperature experiment, but it was performed during their hot season where the maximum temperatures were 42ºC and they found that this process advanced pollen exportation which improved the fitness of both the male and female organs. In sunflowers, during the mature bud stage, with stamens and stigmas appearing in different positions on the disc florets, their anthers dehisce inwardly and release pollen grains into the anther tube. The style located below the anthers and has aligned stylar arms, elongates inside the anther tube and brushes pollen out, primarily through external stylar hairs known as "sweeping hairs", which present pollen only on the outside of the style and stylar arms immediately after anthesis. In such a pollen presentation manner, the pollen is presented through a brush mechanism, which is also referred to as secondary pollen presentation. (Budumajji and Solomon Raju, 2018, Howell et al., 1993, Rao, 2017).

The centrally located disc flowers in sunflowers are dichogamous and contains both male (stamen) and female (pistil) reproductive organs (Wojtaszek and Maier, 2014, Putt, 1940). Pollen-producing anthers and filaments together represent the male reproductive organ called a stamen and pistil is the female reproductive organ including the stigma, style and ovary (Bawa and Beach, 1981). The anthers and filaments, both are housed inside the corolla tube. There are five anthers arranged in a tube-like form around the style, and the independent filaments are attached to the base of the corolla tube (Lobello et al., 2000, Wojtaszek and Maier, 2014). Five anthers are joined together to create a cylinder around the style, whereas the individual filaments are joined to the base of the corolla tubes and the bottom and the anther column at the top, as shown in Figure 1.2 below (Putt, 1940, Lobello et al., 2000). During the bud stage, the style is housed within the anther tube, with the stigma that is not receptive as the two lobes are held together (Lobello et al., 2000, Dedio and Putt, 1980). During anthesis, the elongating corolla cracks open the bud exposing the anther. After the dark period, the anther filaments begin to elongate for six hours. Approximately, two to three hours after the light period begins, 80% of the elongation is complete. After that, the pollen is discharged inside the anther tube.

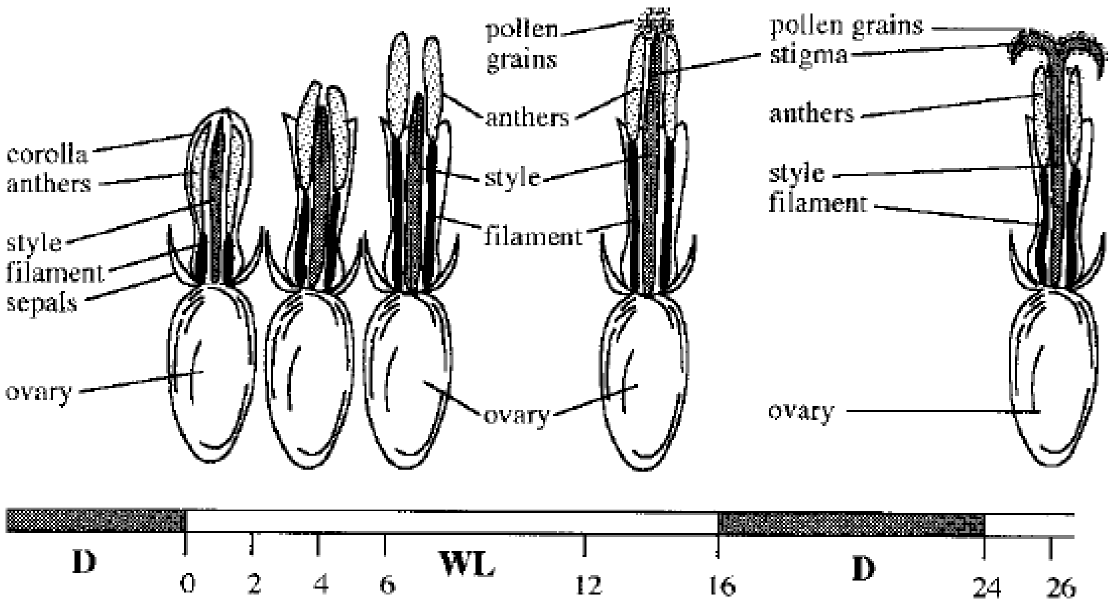


Figure 1.2. Graphical representation of sunflowers (*Helianthus annuus L.*) disc florets during anthesis process under 16 hours of while light and darkness of 8 hours. Each label is pointed at each floral organ. Bottom bars represents D= dark period (night-time), WL= represents light period (day-time). Figure adapted from Lobello et al., 2000.

As the filament elongates, so do the styles, but at a slower rate for 16-20 hours. As the style elongates, it pushes the pollen out of the anted cylinder, making it readily available to pollinators (Lobello et al., 2000). The only time that nectar and pollen is produced is during disc floret development is at the staminate stage (Wojtaszek and Maier, 2014). The anther column gets drawn back into the corolla tube during the pistillate stage of the disc floret development and subsequently style and stigma emergence starts on the interior of the anther cylinder elongating upwards, allowing stigma receptivity by opening into two lobes once elongation in complete. When the stigmatic lobes first extend upwards it is short and stuck together but each lobe coils downwards upon maturation (Love et al., 2016). The two-lobed receptive stigma is masked with hairs called papillae, which indicated that stigma is ready for pollen attachment (Sharma and Bhatla, 2013b). It is only the following day after anthesis that the stigma is fully receptive to receive pollen grains (Lobello et al., 2000). Assurance of cross pollination in sunflowers then happens when the honeybees go through the outer part of the disc florets heading to staminate florets to collect pollen, alien grains of pollen gets attached on the receptive stigmas of the pistillate disc florets (Wojtaszek and Maier, 2014).

Anther filament and style elongation are regulated by light and temperature. Anther filaments elongation was found to be regulated by light. Whereas style elongation is regulated by temperature (Lobello et al., 2000, Creux et al., 2021, Atamian et al., 2016). In a study by Lobello et al. (2000), they found that under 16 hours of white light and 8 hours of dark (WL16/D8) period, anther filament started their elongation just after the dark period and elongated well enough to extrude the corolla tube (Figure 1.2). Whereas they found that under continuous light, anther filament growth was inhibited. The style only elongates faster after exposure to increasing temperatures of the east-facing plants that are in direct sunlight and heated sunflower heads (Creux et al., 2021). These studies suggest that anther filament and style elongation are regulated by different environmental cues.

In sunflowers, when the style elongates, it pushes the pollen out from the anthers, making it available to the pollinators. Pollinators visited the east-facing (in direct sunlight early in the morning) plants earlier and five times more than the west-facing (shade, away from the sun in the morning) plants. The difference was only seen because east-facing flowers were warmer than west-facing flowers (Creux et al 2021, Atamian et al 2016). Therefore, these results show that eastward facing flowers microclimate has an increase in temperature due to the sun rays quickly warming them earlier in the day. Insect visits pattern may be dependent on temperature increases, release of flower volatiles and the availability of floral rewards like pollen and nectar. Because they found that both insect visits and pollen release exhibited 30-minute phase advantage on east facing flowers compared with the west facing plants. Therefore, the earlier the release of floral rewards and increasing temperature on flowers, the earlier the insects visits.

Through evolution, sunflower, species acquired adaptations that assist in optimizing the pollination process and seed set formation successfully by cross-pollination via insects. The pollination is predominantly performed by bees increasing plants seed set and quality (de Oliveira et al., 2019, Wojtaszek and Maier, 2014, Terzić et al., 2017). A review study by Wojtaszek and Maier (2014) discussed the mutualistic relationship that exist between honeybee and sunflowers. Ray florets attract honeybees towards the flower with its bright colours and by honey guides. Sunflower florets mature in a concentric manner, which serves as a cue for honeybees to pollinate. Inflorescences of sunflowers form a target pattern that is visible in UV light, which honeybees can detect since their compound eyes specialize in detecting UV light (Avarguès-Weber et al., 2012). In sunflowers, the conical epidermal cells on the abaxial side of the ray florets reflect UV light, thus providing a landing site to bees (Avarguès-Weber et al., 2012, Wojtaszek and Maier, 2014). The bees are then lured into the sunflower inflorescence by the basal UV-absorbing cells. As soon as these honeybees sits on the ray florets, they get drawn into the center of the inflorescence called the disk florets for collection of their rewards (pollen and nectar). Discharge of pollen grains happens from inside the fused anthers and as they gather inside the anther column they appear as a star on each anther tip on the disc flowers (Wojtaszek and Maier, 2014). Emission of floral scent, which is a complex composition of volatile organic compounds (VOCs) indicate that nectar and pollen is ready for collection (Bloch et al., 2017). These signs and indicators are processed by sunflower pollinators therefore identifying nectar and pollen to be harvested from the florets (Wojtaszek and Maier, 2014).

The process by which the juvenile sunflowers track the sunlight from east to west on a daily basis is called heliotropism. The solar tracking of the sun is guided by the plant circadian clock (Atamian et al., 2016). When florets are fully developed and sunflower transitions from vegetative stage into reproductive stage heliotropism stops and the sunflower heads remain facing an eastwardly direction (Atamian et al., 2016). Atamian et al. (2016) showed, that under ambient temperature conditions, floral temperatures increased in eastward facing sunflowers in the morning ultimately increased their attractiveness to pollinators, resulting in more insect visits.

1.6.1. Effects of high temperature during sunflower anthesis

Sunflower's anthesis is defined by the florets that are open and flowering. Sunflower tolerates a threshold temperature between 26 and 29°C (Table 1.1); temperatures above the threshold cause heat stress. Research performed by Chimenti and Hall (2001) illustrated that temperatures of about 25°C increased the rate of anthesis process and embryo growth in sunflower species. Whereas with temperatures above 25°C, there was a significant decrease in embryo weight and duration of anthesis was shortened. Research done by Chimenti and Hall (2001) revealed that during the grain-filling period, temperatures of 25°C stimulates the growth speed in the embryo, whereas there was a reduced growth speed and embryo growth in sunflowers at temperatures higher than 27°C. Temperatures of ≥ 31°C at anthesis can be very harmful to the sunflower yield as they compromise pollination and leading to floret sterility (Debeake et al, 2017). A short period of heat stress at temperatures of 37°C negatively affects grain filling duration and development of the embryo (Van der Merwe et al., 2015). This shows a lower tolerance for high temperatures in sunflowers during these reproductive processes (Kalyar et al., 2014).

In sunflower species, pollen emergence occurs at the top of the anthers as the style elongate. When temperatures elevate, this process of pollen emergence then accelerates. Sunflowers exposed to heat stress temperatures daytime of 47ºC during anthesis showed a great decrease in pollen viability. Also known from the sunflower genotypes studies by Razzaq et al. (2019) was that genotypes with downward sunflower head positions at anthesis had a high pollen fertility. In contrast to the upward head position, the downward head position protected sunflower pollen from high solar radiation. A heat experiment performed under heat tents to evaluate the effects of high temperatures in pollen viability found that it decreased significantly during the reproductive phase. The loss of pollen viability resulted due to a low number of yield components such as seed weight and number, which means not all ovules were fertilized (Hernández et al., 2018).

1.7. Conclusion

In conclusion, high temperature increases are detrimental to plants floral reproductive organs for various plant species. Different floral organs and processes have different temperature thresholds. Heat stress is known to affect pollen viability and stigma receptivity in plants undergoing anthesis. They cause ovule sterility and ovule abortion at temperatures that are above certain temperatures that stress the plant. Temperatures increases however affects different plant species differently. The style of a sunflower will lengthen early due to increased morning temperatures, causing pollinator visitors to arrive earlier, causing the pollen to release earlier. Heat stress, however, could harm the reproductive organs of the plant. Sometimes the effects of heat stress on reproductive organs vary between species and between male and female reproductive organs. Many studies have highlighted the negative effects of increased temperatures during pollination and fertilization such as reduced stigma receptivity, reduced pollen viability, and abortion of embryos in ovules, but little is known about the anthesis processes in sunflowers and how they are impacted by increased temperatures. There are also gaps in the literature regarding the effects of heat stress and high temperatures on the release of volatiles in sunflowers. Observations that demonstrate how plants undergoing anthesis respond during heat stress may indicate that sunflowers may also be affected in a similar manner. Therefore, there should be more research that suggests mitigation strategies with the temperatures continuing to rise in order to ensure food security.

1.8 References

ABD-ELMABOD, S. K., MUÑOZ-ROJAS, M., JORDÁN, A., ANAYA-ROMERO, M., PHILLIPS, J. D., JONES, L., ZHANG, Z., PEREIRA, P., FLESKENS, L. & VAN DER PLOEG, M. 2020. Climate change impacts on agricultural suitability and yield reduction in a Mediterranean region. *Geoderma,* 374**,** 114453.

ADELEKE, B. S. & BABALOLA, O. O. 2020. Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and health benefits. *Food Science & Nutrition,* 8**,** 4666-4684.

ADHIKARI, P. B., LIU, X., WU, X., ZHU, S. & KASAHARA, R. D. 2020. Fertilization in flowering plants: an odyssey of sperm cell delivery. *Plant Molecular Biology,* 103**,** 9-32.

AHUJA, I., DE VOS, R. C., BONES, A. M. & HALL, R. D. 2010. Plant molecular stress responses face climate change. *Trends in plant science,* 15**,** 664-674.

AKLADIOUS, S. A. 2014. Influence of thiourea application on some physiological and molecular criteria of sunflower (*Helianthus annuus L.*) plants under conditions of heat stress. *Protoplasma,* 251**,** 625-638.

ALLEN, A. & HISCOCK, S. 2008. Evolution and phylogeny of self-incompatibility systems in angiosperms. *Self-incompatibility in flowering plants.* Springer, 73 – 101.

ALLEN, A. M., THOROGOOD, C. J., HEGARTY, M. J., LEXER, C. & HISCOCK, S. J. 2011. Pollen–pistil interactions and self-incompatibility in the Asteraceae: new insights from studies of Senecio squalidus (*Oxford ragwort*). *Annals of Botany,* 108**,** 687-698.

ANGELONI, P., AGUIRREZÁBAL, L. & ECHARTE, M. M. 2021. Assessing the mechanisms underlying sunflower grain weight and oil content responses to temperature during grain filling. *Field Crops Research,* 262**,** 108040.

AO, C. 2021. The origin of double fertilization in flowering plants: looking into the history of plant reproduction evolution. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology,* 155**,** 994-1000.

ARORA, N. K. 2019. Impact of climate change on agriculture production and its sustainable solutions. Springer, 2523 – 8922.

ARSHAD, M. S., FAROOQ, M., ASCH, F., KRISHNA, J. S. V., PRASAD, P. V. V. & SIDDIQUE, K. H. M. 2017. Thermal stress impacts reproductive development and grain yield in rice. *Plant Physiology and Biochemistry,* 115**,** 57-72.

ARYAL, J. P., SAPKOTA, T. B., KHURANA, R., KHATRI-CHHETRI, A., RAHUT, D. B. & JAT, M. L. 2020. Climate change and agriculture in South Asia: Adaptation options in smallholder production systems. *Environment, Development and Sustainability,* 22**,** 5045-5075.

ATAMIAN, H. S., CREUX, N. M., BROWN, E. A., GARNER, A. G., BLACKMAN, B. K. & HARMER, S. L. 2016. Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits. *Science,* 353**,** 587-590.

AVARGUÈS-WEBER, A., MOTA, T. & GIURFA, M. 2012. New vistas on honey bee vision. *Apidologie,* 43**,** 244-268.

AWAIS, M., WAJID, A., SALEEM, M. F., NASIM, W., AHMAD, A., RAZA, M. A. S., BASHIR, M. U., MUBEEN, M., HAMMAD, H. M., HABIB UR RAHMAN, M., SAEED, U., ARSHAD, M. N. & HUSSAIN, J. 2018. Potential impacts of climate change and adaptation strategies for sunflower in Pakistan. *Environmental Science and Pollution Research,* 25**,** 13719-13730.

BAKKENES, M., ALKEMADE, J., IHLE, F., LEEMANS, R. & LATOUR, J. 2002. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. *Global change biology,* 8**,** 390-407.

BARNABÁS, B., JÄGER, K. & FEHÉR, A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant, cell & environment,* 31**,** 11-38.

BAWA, K. S. & BEACH, J. H. 1981. Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden***,** 254-274.

BEARD, B. H. 1981. The sunflower crop. *Scientific American,* 244**,** 150-161.

BESNARD, G., DUFAY, M. & SCHATZ, B. 2021. Investigating pollination strategies in a changing world. Taylor & Francis, 168, 311-315.

BHEEMANAHALLI, R., SUNOJ, V. J., SARIPALLI, G., PRASAD, P. V., BALYAN, H., GUPTA, P., GRANT, N., GILL, K. S. & JAGADISH, S. K. 2019. Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. *Crop Science,* 59**,** 684-696.

BISHOP, J., JONES, H. E., O'SULLIVAN, D. M. & POTTS, S. G. 2017. Elevated temperature drives a shift from selfing to outcrossing in the insect-pollinated legume, faba bean (Vicia faba). *Journal of Experimental Botany,* 68**,** 2055-2063.

BLOCH, G., BAR-SHAI, N., CYTTER, Y. & GREEN, R. 2017. Time is honey: circadian clocks of bees and flowers and how their interactions may influence ecological communities. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 372**,** 20160256.

BOAVIDA, L. C., VIEIRA, A. M., BECKER, J. D. & FEIJO, J. A. 2005. Gametophyte interaction and sexual reproduction: how plants make a zygote. *International Journal of Developmental Biology***,** 615-632.

BORRÁS, L., WESTGATE, M. E., ASTINI, J. P. & ECHARTE, L. 2007. Coupling time to silking with plant growth rate in maize. *Field Crops Research,* 102**,** 73-85.

BORETTI, A. & FLORENTINE, S. 2019. Atmospheric CO_2 concentration and other limiting factors in the growth of C3 and C4 plants. Plants, 8, 92.

BROWN, J., YORK, A., CHRISTIE, F. & MCCARTHY, M. 2017. Effects of fire on pollinators and pollination. *Journal of Applied Ecology,* 54**,** 313-322.

BROZ, A. K. & BEDINGER, P. A. 2021. Pollen-Pistil interactions as reproductive barriers. *Annual review of plant biology,* 72**,** 615-639.

BUDUMAJJI, U. & SOLOMON RAJU, A. J. 2018. Pollination ecology of Bidens *p*ilosa L.(Asteraceae). Taiwania, 63.

BURKLE, L. A. & ALARCÓN, R. 2011. The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *American journal of botany,* 98**,** 528-538.

CASTRO, C. D. & LEITE, R. D. C. 2018. Main aspects of sunflower production in Brazil. *Embrapa Soja-Artigo em periódico indexado (ALICE)*.

CHAMER, A. M., MEDAN, D., MANTESE, A. I. & BARTOLONI, N. J. 2015. Impact of pollination on sunflower yield: is pollen amount or pollen quality what matters? *Field Crops Research,* 176**,** 61-70.

CHANDIO, A. A., JIANG, Y., REHMAN, A. & RAUF, A. 2020. Short and long-run impacts of climate change on agriculture: an empirical evidence from China. *International Journal of Climate Change Strategies and Management*.

CHEABU, S., MOUNG-NGAM, P., ARIKIT, S., VANAVICHIT, A. & MALUMPONG, C. 2018. Effects of heat stress at vegetative and reproductive stages on spikelet fertility. *Rice Science,* 25**,** 218-226.

CHEN, J., MIAO, W., FEI, K., SHEN, H., ZHOU, Y., SHEN, Y., LI, C., HE, J., ZHU, K. & WANG, Z. 2021a. Jasmonates alleviate the harm of high-temperature stress during anthesis to stigma vitality of Photothermosensitive genetic male sterile rice lines. *Frontiers in Plant Science,* 12**,** 412.

CHEN, S., STEFANOVA, K., SIDDIQUE, K. H. & COWLING, W. A. 2021b. Transient daily heat stress during the early reproductive phase disrupts pod and seed development in *Brassica napus L. Food and Energy Security,* 10**,** e262.

CHIGEZA, G., MASHINGAIDZE, K. & SHANAHAN, P. 2012. Seed yield and associated trait improvements in sunflower cultivars over four decades of breeding in South Africa. *Field Crops Research,* 130**,** 46-56.

CHIMENTI, C. & HALL, A. 2001. Grain number responses to temperature during floret differentiation in sunflower. *Field Crops Research,* 72**,** 177-184.

COFFEL, E. D., HORTON, R. M. & DE SHERBININ, A. 2017. Temperature and humidity based projections of a rapid rise in global heat stress exposure during the 21st century. *Environmental Research Letters,* 13**,** 014001.

CORBET, S. A. 1990. POLLINATION AND THE WEATHER. *Israel Journal of Plant Sciences,* 39**,** 13-30.

COX, P. A. 1993. Water-pollinated plants. *Scientific American,* 269**,** 68-74.

CRAUFURD, P. Q. & WHEELER, T. R. 2009. Climate change and the flowering time of annual crops. *Journal of Experimental botany,* 60**,** 2529-2539.

CREUX, N. M., BROWN, E. A., GARNER, A. G., SAEED, S., SCHER, C. L., HOLALU, S. V., YANG, D., MALOOF, J. N., BLACKMAN, B. K. & HARMER, S. L. 2021. Flower orientation influences floral temperature, pollinator visits and plant fitness. *New Phytologist,* 232**,** 868-879.

CUMMINGS, J. L., GUARINO, J. L. & KNITTLE, C. E. 1989. Chronology of blackbird damage to sunflowers. *Wildlife Society Bulletin (1973-2006),* 17**,** 50-52.

DA SILVA, D. A., PINTO-MAGLIO, C. A. F., DE OLIVEIRA, É. C., DOS REIS, R. L. D. M., CARBONELL, S. A. M. & CHIORATO, A. F. 2020. Influence of high temperature on the reproductive biology of dry edible bean (*Phaseolus vulgaris L.*). *Scientia Agricola,* 77**,** e20180233-e20180233.

DAR, S. A., MIR, G., PARRY, M., AHMAD, S., GANIE, M., RAJA, T., YAQOB, M., KHRUSHEED, R. & PARRY, S. 2016. Diversity and richness indices and the Whittaker plot value of insect pollinators of Peach *Prunus persica* in landscapes of temperate India. *Academic Journal of Entomology,* 9**,** 62-73.

DE OLIVEIRA, A. C., JUNQUEIRA, C. N. & AUGUSTO, S. C. 2019. Pesticides affect pollinator abundance and productivity of sunflower (*Helianthus annuus L.*). *Journal of Apicultural Research,* 58**,** 2-8.

DEBAEKE, P., CASADEBAIG, P., FLENET, F. & LANGLADE, N. 2017. Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe. *OCL Oilseeds and fats crops and lipids,* 24**,** 15 p.

DEBAEKE, P., CASADEBAIG, P. & LANGLADE, N. 2021. New challenges for sunflower ideotyping in changing environments and more ecological cropping systems. *OCL Oilseeds and fats crops and lipids,* 28.

DEDIO, W. & PUTT, E. D. 1980. Sunflower. *Hybridization of crop plants***,** 631-644.

DEL BUONO, D. 2021. Can biostimulants be used to mitigate the effect of anthropogenic climate change on agriculture? It is time to respond. *Science of The Total Environment,* 751**,** 141763.

DENG, F., ZENG, Y., LI, Q., HE, C., LI, B., ZHU, Y., ZHOU, X., YANG, F., ZHONG, X. & WANG, L. 2021. Decreased anther dehiscence contributes to a lower fertilization rate of rice subjected to shading stress. *Field Crops Research,* 273**,** 108291.

DESCAMPS, C., QUINET, M., BAIJOT, A. & JACQUEMART, A. L. 2018. Temperature and water stress affect plant–pollinator interactions in *Borago officinalis* (*Boraginaceae*). *Ecology and Evolution,* 8**,** 3443-3456.

DJANAGUIRAMAN, M., PERUMAL, R., JAGADISH, S., CIAMPITTI, I., WELTI, R. & PRASAD, P. 2018. Sensitivity of sorghum pollen and pistil to high-temperature stress. *Plant, cell & environment,* 41**,** 1065-1082.

DOSIO, A. 2017. Projection of temperature and heat waves for Africa with an ensemble of CORDEX regional climate models. *Climate Dynamics,* 49**,** 493-519.

DOSIO, G. A., TARDIEU, F. & TURC, O. 2006. How does the meristem of sunflower capitulum cope with tissue expansion and floret initiation? A quantitative analysis. *New phytologist,* 170**,** 711-722.

EDLUND, A. F., SWANSON, R. & PREUSS, D. 2004. Pollen and stigma structure and function: the role of diversity in pollination. *The Plant Cell,* 16**,** S84-S97.

EHLERINGER, J. R. 2005. The influence of atmospheric CO_2, temperature, and water on the abundance of C 3/C 4 taxa. *A history of atmospheric CO_2 and its effects on plants, animals, and ecosystems.* Springer, 241-231.

ENDO, M., TSUCHIYA, T., HAMADA, K., KAWAMURA, S., YANO, K., OHSHIMA, M., HIGASHITANI, A., WATANABE, M. & KAWAGISHI-KOBAYASHI, M. 2009. High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology,* 50**,** 1911-1922.

ENGELBRECHT, F. A. & MONTEIRO, P. M. 2021. The IPCC Assessment Report Six Working Group 1 report and southern Africa: Reasons to take action. *South African Journal of Science,* 117.

FAURE, J.-E., ALDON, D., ROUGIER, M. & DUMAS, C. 1996. Emerging data on pollen tube growth and fertilization in flowering plants, 1990–1995. *Protoplasma,* 193**,** 132-143.

FAWZY, S., OSMAN, A. I., DORAN, J. & ROONEY, D. W. 2020. Strategies for mitigation of climate change: a review. *Environmental Chemistry Letters***,** 1-26.

FERNÁNDEZ-LUQUEÑO, F., LÓPEZ-VALDEZ, F., MIRANDA-ARÁMBULA, M., ROSAS-MORALES, M., PARIONA, N. & ESPINOZA-ZAPATA, R. 2014. An introduction to the sunflower crop. *Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases. Valladolid, Spain: Nova Science Publishers***,** 1-18.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2022. Oilseeds, oils & meals monthly update, No. 152.

FRANKE, A., MUELELWA, L. & STEYN, J. M. 2020. Impact of climate change on yield and water use efficiencies of potato in different production regions of South Africa. *South African Journal of Plant and Soil,* 37**,** 244-253.

FRIEDMAN, J. & BARRETT, S. C. 2009. Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of botany,* 103**,** 1515-1527.

FUGLIE, K. 2021. Climate change upsets agriculture. *Nature Climate Change,* 11**,** 294-295.

GEHRING, M. 2019. Epigenetic dynamics during flowering plant reproduction: evidence for reprogramming? *New Phytologist,* 224**,** 91-96.

GÉRARD, M., VANDERPLANCK, M., WOOD, T. & MICHEZ, D. 2020. Global warming and plant–pollinator mismatches. *Emerging Topics in Life Sciences,* 4**,** 77-86.

GIORNO, F., WOLTERS-ARTS, M., MARIANI, C. & RIEU, I. 2013. Ensuring reproduction at high temperatures: the heat stress response during anther and pollen development. *Plants,* 2**,** 489-506.

GRAY, S. B. & BRADY, S. M. 2016. Plant developmental responses to climate change. *Developmental biology,* 419**,** 64-77.

GUIDI, L., LO PICCOLO, E. & LANDI, M. 2019. Chlorophyll fluorescence, photoinhibition and abiotic stress: Does it make any difference the fact to be a C3 or C4 species? Frontiers in Plant Science, 10.

GUPTA, M. K. 2014. Sunflower oil and its applications. *Lipid Technology,* 26**,** 260-263.

HARRAP, M. J., DE IBARRA, N. H., WHITNEY, H. M. & RANDS, S. A. 2020. Floral temperature patterns can function as floral guides. *Arthropod-plant interactions***,** 1-14.

HARRAP, M. J., RANDS, S. A., DE IBARRA, N. H. & WHITNEY, H. M. 2017. The diversity of floral temperature patterns, and their use by pollinators. *Elife,* 6**,** e31262.

HASANUZZAMAN, M., NAHAR, K., ALAM, M., ROYCHOWDHURY, R. & FUJITA, M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International journal of molecular sciences,* 14**,** 9643-9684.

HEDHLY, A. 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environmental and Experimental Botany,* 74**,** 9-16.

HEDHLY, A., HORMAZA, J. & HERRERO, M. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). American journal of botany, 91, 558-564.

HEDHLY, A., HORMAZA, J. & HERRERO, M. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology,* 7**,** 476-483.

HERNÁNDEZ, F., POVERENE, M., MERCER, K. L. & PRESOTTO, A. 2020. Genetic variation for tolerance to extreme temperatures in wild and cultivated sunflower (*Helianthus annuus*) during early vegetative phases. *Crop and Pasture Science,* 71**,** 578-591.

HERNÁNDEZ, F., POVERENE, M. & PRESOTTO, A. 2018. Heat stress effects on reproductive traits in cultivated and wild sunflower (*Helianthus annuus L.*): evidence for local adaptation within the wild germplasm. *Euphytica,* 214**,** 146.

HERRERA, C. M. 1995. Microclimate and individual variation in pollinators: Flowering plants are more than their flowers. *Ecology,* 76**,** 1516.

HISCOCK, S. J., HOEDEMAEKERS, K., FRIEDMAN, W. E. & DICKINSON, H. G. 2002. The stigma surface and pollen-stigma interactions in *Senecio squalidus L.*(Asteraceae) following cross (compatible) and self (incompatible) pollinations. *International Journal of Plant Sciences,* 163**,** 1-16.

HOOVER, S. E., LADLEY, J. J., SHCHEPETKINA, A. A., TISCH, M., GIESEG, S. P. & TYLIANAKIS, J. M. 2012. Warming, CO_2, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecology letters,* 15**,** 227-234.

HOUSHMAND, P., SHIRANI, M. & EHSANZADEH, P. 2021. Insights into temperature and soil moisture-induced alterations in safflower physiological, seed filling, quality, and yield attributes. *International Journal of Plant Production*, 1735-8043.

HOWDEN, S. M., SOUSSANA, J.-F., TUBIELLO, F. N., CHHETRI, N., DUNLOP, M. & MEINKE, H. 2007. Adapting agriculture to climate change. *Proceedings of the national academy of sciences,* 104**,** 19691-19696.

HU, Z., ZHANG, H., LENG, P., ZHAO, J., WANG, W. & WANG, S. 2013. The emission of floral scent from Lilium 'siberia'in response to light intensity and temperature. *Acta Physiologiae Plantarum,* 35**,** 1691-1700.

ITO, S. & GORB, S. N. 2019. Attachment-based mechanisms underlying capture and release of pollen grains. *Journal of the Royal Society Interface,* 16**,** 20190269.

JADHAV, J. A., SREEDEVI, K. & PRASAD, P. R. 2011. Insect pollinator diversity and abundance in sunflower ecosystem. *Current Biotica,* 5**,** 344-350.

JAGADISH, S., BAHUGUNA, R. N., DJANAGUIRAMAN, M., GAMUYAO, R., PRASAD, P. & CRAUFURD, P. Q. 2016. Implications of high temperature and elevated CO_2 on flowering time in plants. *Frontiers in plant science,* 7**,** 913.

JIN, Z., AINSWORTH, E. A., LEAKEY, A. D. & LOBELL, D. B. 2018. Increasing drought and diminishing benefits of elevated carbon dioxide for soybean yields across the US Midwest. *Global change biology,* 24**,** e522-e533.

JOHNSON, M. A., HARPER, J. F. & PALANIVELU, R. 2019. A fruitful journey: pollen tube navigation from germination to fertilization. *Annual review of plant biology,* 70**,** 809-837.

JOHNSON, M. A. & PREUSS, D. 2002. Plotting a course: multiple signals guide pollen tubes to their targets. *Developmental cell,* 2**,** 273-281.

KALYAR, T., RAUF, S., TEIXEIRA DA SILVA, J. A. & SHAHZAD, M. 2014. Handling sunflower (*Helianthus annuus L.*) populations under heat stress. *Archives of Agronomy and Soil Science,* 60**,** 655-672.

KATINAS, L., CRISCI, J. V., TELLERÍA, M. C., BARREDA, V. & PALAZZESI, L. 2007. Early history of Asteraceae in Patagonia: evidence from fossil pollen grains. *New Zealand Journal of Botany,* 45**,** 605-610.

KEVAN, P. Pollination: keystone process in sustainable global productivity. *VI International Symposium on Pollination,* 288, 1990. 103-110.

KUMAR, N., KUMAR, N., SHUKLA, A., SHANKHDHAR, S. C. & SHANKHDHAR, D. 2015. Impact of terminal heat stress on pollen viability and yield attributes of rice (*Oryza sativa L.*). *Cereal Research Communications,* 43**,** 616-626.

KUMAR, P., TOKAS, J., KUMAR, N., LAL, M. & SINGAL, H. 2018. Climate change consequences and its impact on agriculture and food security. *International Journal of chemical studies,* 6**,** 124-133.

LEISNER, C. P. 2020. Climate change impacts on food security-focus on perennial cropping systems and nutritional value. *Plant Science,* 293**,** 110412.

LENTZ, D. L., POHL, M. D., ALVARADO, J. L., TARIGHAT, S. & BYE, R. 2008. Sunflower (*Helianthus annuus L.*) as a pre-Columbian domesticate in Mexico. *Proceedings of the National Academy of Sciences,* 105**,** 6232-6237.

LHOTKA, O., KYSELÝ, J. & FARDA, A. 2018. Climate change scenarios of heat waves in Central Europe and their uncertainties. *Theoretical and applied climatology,* 131**,** 1043-1054.

LIU, H., MI, Z., LIN, L., WANG, Y., ZHANG, Z., ZHANG, F., WANG, H., LIU, L., ZHU, B., CAO, G., ZHAO, X., SANDERS, N. J., CLASSEN, A. T., REICH, P. B. & HE, J.-S. 2018. Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proceedings of the National Academy of Sciences,* 115**,** 4051-4056.

LOBELLO, G., FAMBRINI, M., BARALDI, R., LERCARI, B. & PUGLIESI, C. 2000. Hormonal influence on photocontrol of the protandry in the genus *Helianthus*. *Journal of experimental botany,* 51**,** 1403-1412.

LORD, E. 2000. Adhesion and cell movement during pollination: cherchez la femme. *Trends in plant science,* 5**,** 368-373.

LORD, E. M. & RUSSELL, S. D. 2002. The mechanisms of pollination and fertilization in plants. *Annual review of cell and developmental biology,* 18**,** 81-105.

LOUBSER, H. 2002. Sunflower production and important production strategies in South Africa. *SA Grain (South Africa)*.

LOVE, J., GRAHAM, S. W., IRWIN, J. A., ASHTON, P. A., BRETAGNOLLE, F. & ABBOTT, R. J. 2016. Self-pollination, style length development and seed set in self-compatible Asteraceae: evidence from *Senecio vulgaris L. Plant Ecology & Diversity,* 9**,** 371-379.

MALULEKE, I., (2021). Sunflower makes giant strides internationally. SA Grain (South Africa).

MARC, J. & PALMER, J. 1978. Determination of the length of the vegetative and pre-floral stages in the day-neutral plant *Helianthus annuus* by chilling pulses. *Journal of Experimental Botany,* 29**,** 367-373.

MATSUDA, H., HIGUCHI, H., KOZAI, N. & OGATA, T. 2011. Effect of temperature on the time requirement of pollen tubes to penetrate into the embryo sac after pollination in cherimoya (*Annona cherimola Mill.*). *Tropical Agriculture and Development,* 55**,** 157-161.

MATSUI, T., OMASA, K. & HORIE, T. 2000. High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in Rice(*Oryza sativa L.*). Plant production science, 3, 430-434.

MEEHL, G. A. & TEBALDI, C. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. *Science,* 305**,** 994-997.

MEYER, F. & VAN DER BURGH, G. 2015. The competitiveness of the South African sunflower value chain. *Oilseeds Focus,* 1**,** 28-31.

MITTLER, R., FINKA, A. & GOLOUBINOFF, P. 2012. How do plants feel the heat? *Trends in biochemical sciences,* 37**,** 118-125.

MORIONDO, M., GIANNAKOPOULOS, C. & BINDI, M. 2011. Climate change impact assessment: the role of climate extremes in crop yield simulation. *Climatic change,* 104**,** 679-701.

MU, J., PENG, Y., XI, X., WU, X., LI, G., NIKLAS, K. J. & SUN, S. 2015. Artificial asymmetric warming reduces nectar yield in a Tibetan alpine species of Asteraceae. *Annals of botany,* 116**,** 899-906.

NGUYEN, T. D., JANG, S., SOH, M.-S., LEE, J., YUN, S. D., OH, S. A. & PARK, S. K. 2019. High daytime temperature induces male sterility with developmental defects in male reproductive organs of Arabidopsis. *Plant Biotechnology Reports,* 13**,** 635-643.

NHEMACHENA, C. R. & MUCHARA, B. 2020. Structure of the sunflower plant breeders' rights landscape in South Africa. *South African Journal of Science,* 116**,** 1-6.

NICHOLLS, E. & HEMPEL DE IBARRA, N. 2017. Assessment of pollen rewards by foraging bees. *Functional Ecology,* 31**,** 76-87.

NORGATE, M., BOYD-GERNY, S., SIMONOV, V., ROSA, M. G., HEARD, T. A. & DYER, A. G. 2010. Ambient temperature influences Australian native stingless bee (*Trigona carbonaria*) preference for warm nectar. *PLoS One,* 5**,** e12000.

NUI, N. H., BIEN, V. T., TRUNG, N. D., LUA, T. H., CUONG, N. T., SPILLANE, C., BRYCHKOVA, G., MCKEOWN, P. & LE VINH, B. 2020. Cassava farmers' perception on climate change: A case study in van Yen district, Yen Bai Province. *Vietnam Journal of Agricultural Sciences,* 3**,** 700-711.

NUNEZ, S., ARETS, E., ALKEMADE, R., VERWER, C. & LEEMANS, R. 2019. Assessing the impacts of climate change on biodiversity: is below 2°C enough? *Climatic Change,* 154**,** 351-365.

OBERMEIER, W. A., LEHNERT, L. W., KAMMANN, C. I., MÜLLER, C., GRÜNHAGE, L., LUTERBACHER, J., ERBS, M., MOSER, G., SEIBERT, R., YUAN, N. & BENDIX, J. 2017. Reduced CO_2 fertilization effect in temperate C3 grasslands under more extreme weather conditions. *Nature Climate Change,* 7**,** 137-141.

OHLER, L.-M., LECHLEITNER, M. & JUNKER, R. R. 2020. Microclimatic effects on alpine plant communities and flower-visitor interactions. *Scientific Reports,* 10**,** 1-9.

OHNISHI, S., MIYOSHI, T. & SHIRAI, S. 2010. Low temperature stress at different flower developmental stages affects pollen development, pollination, and pod set in soybean. *Environmental and Experimental Botany,* 69**,** 56-62.

OOSTERHUIS, D. M. & SNIDER, J. L. 2011. High temperature stress on floral development and yield of cotton. *Stress physiology in cotton, edited by Oosterhuis DM The Cotton Foundation, Cordova, Tennessee***,** 1-24.

ORTIZ-BOBEA, A., AULT, T. R., CARRILLO, C. M., CHAMBERS, R. G. & LOBELL, D. B. 2021. Anthropogenic climate change has slowed global agricultural productivity growth. *Nature Climate Change,* 11**,** 306-312.

PAN, C., YANG, D., ZHAO, X., JIAO, C., YAN, Y., LAMIN-SAMU, A. T., WANG, Q., XU, X., FEI, Z. & LU, G. 2019. Tomato stigma exsertion induced by high temperature is associated with the jasmonate signalling pathway. *Plant, cell & environment,* 42**,** 1205-1221.

PAN, C., YE, L., ZHENG, Y., WANG, Y., YANG, D., LIU, X., CHEN, L., ZHANG, Y., FEI, Z. & LU, G. 2017. Identification and expression profiling of microRNAs involved in the stigma exsertion under high-temperature stress in tomato. *BMC genomics,* 18**,** 1-16.

PROFILE, C. 2010. Serbia. *Global Burden of Diseases, Injuries, and Risk Factors Study*.

PUTT, E. 1940. Observations on morphological characters and flowering processes in the sunflower (*Helianthus annuus L.*). *Scientific Agriculture,* 21**,** 167-179.

PUTT, E. D. 1997. Early history of sunflower. *Sunflower technology and production,* 35**,** 1-19.

RADANOVIĆ, A., MILADINOVIĆ, D., CVEJIĆ, S., JOCKOVIĆ, M. & JOCIĆ, S. 2018. Sunflower genetics from ancestors to modern hybrids—A review. *Genes,* 9**,** 528.

RAJA, M. M., VIJAYALAKSHMI, G., NAIK, M. L., BASHA, P. O., SERGEANT, K., HAUSMAN, J. F. & KHAN, P. S. S. V. 2019. Pollen development and function under heat stress: from effects to responses. *Acta Physiologiae Plantarum,* 41**,** 1-20.

RAM, J. J., SINGH, U., SINGH, S. & KRISHNA, B. 2018. Study of genetic diversity in Sunflower (Helianthus annuus L.). *Int. J. Curr. Microbiol. App. Sci,* 7**,** 2266-2272.

RAO, M. M. 2017. SECONDARY POLLEN PRESENTATION, PSYCHOPHILY AND ANEMOCHORY IN SOME ASTERACEAE SPECIES. Journal of Palynology Vol, 53, 25-82.

RAY, D. K., WEST, P. C., CLARK, M., GERBER, J. S., PRISHCHEPOV, A. V. & CHATTERJEE, S. 2019. Climate change has likely already affected global food production. *PloS one,* 14**,** e0217148.

RAZA, A., RAZZAQ, A., MEHMOOD, S. S., ZOU, X., ZHANG, X., LV, Y. & XU, J. 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants,* 8**,** 34.

RAZZAQ, M. K., RAUF, S., KHURSHID, M., IQBAL, S., BHAT, J. A., FARZAND, A., RIAZ, A., XING, G. & GAI, J. 2019. Pollen viability an index of abiotic stresses tolerance and methods for the improved pollen viability. *Pakistan Journal of Agricultural Research,* 32.

ROSELL, P., HERRERO, M. & GALÁN SAÚCO, V. 1999. Pollen germination of cherimoya (*Annona cherimola Mill.*).: In vivo characterization and optimization of in vitro germination. *Scientia Horticulturae,* 81**,** 251-265.

SAGAE, M., OYAMA-OKUBO, N., ANDO, T., MARCHESI, E. & NAKAYAMA, M. 2008. Effect of temperature on the floral scent emission and endogenous volatile profile of Petunia axillaris. *Bioscience, biotechnology, and biochemistry***,** 0711300686-0711300686.

SAGE, T. L., BAGHA, S., LUNDSGAARD-NIELSEN, V., BRANCH, H. A., SULTMANIS, S. & SAGE, R. F. 2015. The effect of high temperature stress on male and female reproduction in plants. *Field Crops Research,* 182**,** 30-42.

SAINI, H., SEDGLEY, M. & ASPINALL, D. 1983. Effect of heat stress during floral development on pollen tube growth and ovary anatomy in wheat (*Triticum aestivum L.*). *Functional Plant Biology,* 10**,** 137-144.

SANTIAGO, J. P. & SHARKEY, T. D. 2019. Pollen development at high temperature and role of carbon and nitrogen metabolites. *Plant, cell & environment,* 42**,** 2759-2775.

SARWAR, M., SALEEM, M. F., ULLAH, N., ALI, S., RIZWAN, M., SHAHID, M. R., ALYEMENI, M. N., ALAMRI, S. A. & AHMAD, P. 2019. Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. *Scientific Reports,* 9**,** 13022.

SATO, S., PEET, M. & THOMAS, J. 2000. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum Mill.*) under chronic, mild heat stress. *Plant, cell & environment,* 23**,** 719-726.

SCAVEN, V. L. & RAFFERTY, N. E. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current zoology,* 59**,** 418-426.

SEILER, G. J. & GULYA, T. 2016. Sunflower: Overview, 10.1016/B978-0-08-100396-5.00027-5.

SHARMA, B. & BHATLA, S. 2013a. Accumulation and scavenging of reactive oxygen species and nitric oxide correlate with stigma maturation and pollen–stigma interaction in sunflower. *Acta physiologiae plantarum,* 35**,** 2777-2787.

SHARMA, B. & BHATLA, S. C. 2013b. Structural analysis of stigma development in relation with pollen–stigma interaction in sunflower. *Flora-Morphology, Distribution, Functional Ecology of Plants,* 208**,** 420-429.

SHI, W., LI, X., SCHMIDT, R. C., STRUIK, P. C., YIN, X. & JAGADISH, S. K. 2018. Pollen germination and in vivo fertilization in response to high-temperature during flowering in hybrid and inbred rice. *Plant, cell & environment,* 41**,** 1287-1297.

SIEBERS, M. H., SLATTERY, R. A., YENDREK, C. R., LOCKE, A. M., DRAG, D., AINSWORTH, E. A., BERNACCHI, C. J. & ORT, D. R. 2017. Simulated heat waves during maize reproductive stages alter reproductive growth but have no lasting effect when applied during vegetative stages. *Agriculture, Ecosystems & Environment,* 240**,** 162-170.

SINTAYEHU, D. W. 2018. Impact of climate change on biodiversity and associated key ecosystem services in Africa: a systematic review. *Ecosystem Health and Sustainability,* 4**,** 225-239.

SITA, K., SEHGAL, A., HANUMANTHARAO, B., NAIR, R. M., VARA PRASAD, P. V., KUMAR, S., GAUR, P. M., FAROOQ, M., SIDDIQUE, K. H. M., VARSHNEY, R. K. & NAYYAR, H. 2017. Food legumes and rising temperatures: Effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. *Frontiers in Plant Science,* 8.

STEELE, C. R. 2000. Shell stability related to pattern formation in plants. *J. Appl. Mech.,* 67**,** 237-247.

SUYUNOVNA, T. A. S. 2021. THE EFFECT OF SOWING SCHEME ON THE YIELD PARAMETRES OF SUNFLOWER VARIETIES.

SWAMINATHAN, S. 2019. Impact of climate change on insect pollination. *Management Research,* 1**,** 1-12.

TEIXEIRA, E. I., FISCHER, G., VAN VELTHUIZEN, H., WALTER, C. & EWERT, F. 2013. Global hot-spots of heat stress on agricultural crops due to climate change. *Agricultural and Forest Meteorology,* 170**,** 206-215.

TERZIĆ, S., MIKLIČ, V. & ČANAK, P. 2017. Review of 40 years of research carried out in Serbia on sunflower pollination. *OCL-Oilseeds & Fats Crops and Lipids,* 24.

THAKUR, P., KUMAR, S., MALIK, J. A., BERGER, J. D. & NAYYAR, H. 2010. Cold stress effects on reproductive development in grain crops: an overview. *Environmental and Experimental Botany,* 67**,** 429-443.

THOMASON, K., BABAR, M. A., ERICKSON, J. E., MULVANEY, M., BEECHER, C. & MACDONALD, G. 2018. Comparative physiological and metabolomics analysis of wheat (*Triticum aestivum L.*) following post-anthesis heat stress. *PLoS One,* 13**,** e0197919.

TITO, R., VASCONCELOS, H. L. & FEELEY, K. J. 2018. Global climate change increases risk of crop yield losses and food insecurity in the tropical Andes. *Global Change Biology,* 24**,** e592-e602.

UNAKıTAN, G. & AYDıN, B. 2018. A comparison of energy use efficiency and economic analysis of wheat and sunflower production in Turkey: A case study in Thrace Region. *Energy,* 149**,** 279-285.

UNKAŠEVIĆ, M. & TOŠIĆ, I. 2011. The maximum temperatures and heat waves in Serbia during the summer of 2007. *Climatic Change,* 108**,** 207-223.

VAN DER KOOI, C. J. 2016. Plant biology: flower orientation, temperature regulation and pollinator attraction. *Current Biology,* 26**,** R1143-R1145.

VAN DER KOOI, C. J., KEVAN, P. G. & KOSKI, M. H. 2019. The thermal ecology of flowers. *Annals of Botany,* 124**,** 343-353.

VAN DER MERWE, R., LABUSCHAGNE, M. T., HERSELMAN, L. & HUGO, A. 2015. Effect of heat stress on seed yield components and oil composition in high-and mid-oleic sunflower hybrids. *South African Journal of Plant and Soil,* 32**,** 121-128.

VAN DOORN, W. G. & VAN MEETEREN, U. 2003. Flower opening and closure: a review. *Journal of Experimental Botany,* 54**,** 1801-1812.

WAHID, A., GELANI, S., ASHRAF, M. & FOOLAD, M. R. 2007. Heat tolerance in plants: an overview. *Environmental and experimental botany,* 61**,** 199-223.

WANG, J., VANGA, S. K., SAXENA, R., ORSAT, V. & RAGHAVAN, V. 2018. Effect of climate change on the yield of cereal crops: a review. *Climate,* 6**,** 41.

WANG, Y., IMPA, S., SUNKAR, R. & JAGADISH, S. K. 2021a. The neglected other half-role of the pistil in plant heat stress responses. *Plant, Cell & Environment,* 0140-7791.

WANG, Y., LIU, X., HOU, X., SHENG, D., DONG, X., GAO, Y., WANG, P. & HUANG, S. 2021b. Maximum lethal temperature for flowering and seed set in maize with contrasting male and female flower sensitivities. *Journal of Agronomy and Crop Science*, 158, 80-88.

WANG, Y., TAO, H., TIAN, B., SHENG, D., XU, C., ZHOU, H., HUANG, S. & WANG, P. 2019. Flowering dynamics, pollen, and pistil contribution to grain yield in response to high temperature during maize flowering. *Environmental and Experimental Botany,* 158**,** 80-88.

WANG, Z., YUAN, C., ZHANG, S., TIAN, S., TANG, Q., WEI, D. & NIU, Y. 2021. Screening and interaction analysis identify genes related to anther dehiscence in *Solanum melongena L.* Frontiers in Plant Science, 12.

WEN, B. 2015. Effects of high temperature and water stress on seed germination of the invasive species Mexican sunflower. *PLoS One,* 10**,** e0141567.

WHEELER, T. & VON BRAUN, J. 2013. Climate change impacts on global food security. *Science,* 341**,** 508-513.

WILCOCK, C. & NEILAND, R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in plant science,* 7**,** 270-277.

WOJTASZEK, J. & MAIER, C. 2014. A microscopic review of the sunflower and honeybee mutualistic relationship. *International Journal of AgriScience,* 4**,** 272-282.

WU, C., CUI, K., TANG, S., LI, G., WANG, S., FAHAD, S., NIE, L., HUANG, J., PENG, S. & DING, Y. 2020. Intensified pollination and fertilization ameliorate heat injury in rice (*Oryza sativa L.*) during the flowering stage. *Field Crops Research,* 252**,** 107795.

XU, J., WOLTERS-ARTS, M., MARIANI, C., HUBER, H. & RIEU, I. 2017. Heat stress affects vegetative and reproductive performance and trait correlations in tomato (*Solanum lycopersicum*). *Euphytica,* 213**,** 1-12.

YADEGARI, R. & DREWS, G. N. 2004. Female gametophyte development. The Plant Cell, 16, S133-S141.

YANG, H., HUANG, T., DING, M., LU, D. & LU, W. 2017. High temperature during grain filling impacts on leaf senescence in waxy maize. *Agronomy Journal,* 109**,** 906-916.

ZHANG, T. & ELOMAA, P. 2021. Don’t be fooled: false flowers in Asteraceae. *Current Opinion in Plant Biology,* 59**,** 101972.

ZHAO, Y., ZHANG, T., BROHOLM, S. K., TÄHTIHARJU, S., MOUHU, K., ALBERT, V. A., TEERI, T. H. & ELOMAA, P. 2016. Evolutionary co-option of floral meristem identity genes for patterning of the flower-like Asteraceae inflorescence. *Plant Physiology,* 172**,** 284-296.

ZHU, J.-K. 2016. Abiotic stress signaling and responses in plants. *Cell,* 167**,** 313-324.

ZULKARNAIN, Z., ELIYANTI, E. & SWARI, E. I. 2019. Pollen viability and stigma receptivity in *Swainsona formosa* (G. Don) J. Thompson (*Fabaceae*), an ornamental legume native to Australia. *Ornamental Horticulture,* 25**,** 158-167.

Chapter 2

Time of anthesis: Pollination in sunflowers during heat stress

ABSTRACT

Anthesis and pollination are precisely timed events, which change with the surrounding environment depending on light, temperature, and humidity. Plants and pollinators may exhibit mismatched behaviors as a result of temperature changes, and relatively little information exists about how heat stress and heat waves affect anthesis and pollination in sunflowers (*Helianthus Annuus* L.). This study investigates the effect of heat stress on timing of pollen emergence, style elongation rate, pollinator visits, pollen viability and stigma receptivity. We sampled during the anthesis period in a field setting under different summer conditions, including sunny and overcast days each with artificially heated and unheated plants; and a natural heatwave. Sunflower insect visits were video imaged in all test conditions, and individual insects were counted. A variety of staining and imaging techniques were used to determine pollen fertility, style elongation, and stigma receptivity. Heating sunflower capitula to approximately 10°C above the natural temperature resulted in a faster rate of style elongation, earlier pollen emergence and reduced stigma receptivity. In both the sunny and overcast days, time-lapse images showed that pollen emergence was 15 minutes earlier on heated plants than unheated ones. Heatwaves, however, bring in significantly more early morning visitors than unheated sunny, which was not observed by artificial heating. At temperatures of 40°C on sunny days, stigma receptivity and pollen fertility appears to significantly decrease over time. Sunflowers are considered a robust and temperature hardy plant, which maintains yields even when subjected to harsh conditions. This is one of the first studies to assess the effect of heat stress on sunflower anthesis. Our findings suggest that even though sunflower anthesis and pollination can be negatively affected by the high temperatures associated with heatwave events, it is able to adapt and manage stress by changing timing of pollen emergence and insects visits to earlier in the day before the high temperatures can have their detrimental effect.

2.1 Introduction

While the challenges associated with climate change are significant and far-reaching, there are increasing indications and predictions that climate change is having a substantial impact on humanity and agricultural practices (Pittock, 2017, Ozturk et al., 2015). In recent years, high temperatures have become more likely to occur (Engelbrecht and Monteiro, 2021b). In the Fifth and sixth Assessment Report from the Intergovernmental Panel on Climate Change, there is a high probability that global temperatures will be higher by the end of the 21st century, and heat waves will be more frequent and longer in duration (Allen et al., 2014, Engelbrecht and Monteiro, 2021a). Since crop production and agriculture are directly affected by climate and weather conditions, studying potential adverse impacts is a priority (Bisbis et al., 2018, Taraz, 2018). In the last couple of decades, high temperatures especially during reproductive periods have become a major concern as they negatively affect seed production and result in yield losses (Tiwari and Yadav, 2019).

Heat stress during the developmental phase of the male reproductive organs may result in sterility, impaired fertility, and inviable pollen grains. In plant species exposed to high temperatures during their reproductive stages, pollen production was lower because the microspore mother cells did not divide properly, which led to poor pollen germination and slow pollen tube growth (Kumar et al., 2015, Wang et al., 2021). In another study examining heat stress' effects on pollen and male reproductive organs, researchers found that excessive heat induces premature or untimely degeneration of anther wall layers, especially of the tapetum. Increased temperatures also inhibit pollination in flowering plants by reducing the number of pollen grains produced by the anther (Raja et al., 2019, Bheemanahalli et al., 2019).

It is generally known that male reproductive organs are more sensitive to heat stress than the female reproductive organs. However, according to recent studies, female reproductive organs in some plant species are more sensitive than male reproductive organs (Chen et al., 2021, Wang et al., 2021, Lohani et al., 2020). Pistils are the female reproductive organs of angiosperm flowers. They are composed of stigma, style, and ovary, where the gametophyte develops. Heat stress has been shown to partly cause the female organ sterility to contribute to a decrease in seed set percentage (Hassan et al., 2021). A high temperature impairs pistil development, contributing to asynchronous development of male and female reproductive organs, resulting in poor pollination (Wang et al., 2021). Some species such as apricot, sorghum and tomatoes are found to have abnormal pistils, including stigmas, styles and ovules, when stressed by heat. Physiological changes such as protruding stigmas interfered with pollen capture, shorter styles and shrunken ovaries with desiccated, flaccid stigmas and styles negatively affects pollination (Wang et al., 2021)

There are multiple drivers of global change that threaten plant-pollinator interactions, such as climate warming and land-use intensification. A significant change in climate is modifying plant-pollinator interactions by decreasing the attractiveness of plants to pollinators and consequently reducing their abundance and diversity (Duque et al., 2021). Pollinators find flowers less appealing when nectar volume decreases due to high temperatures (Descamps et al., 2018, Haverkamp et al., 2019). Flower orientation affects the amount of time and energy pollinators must spend to access nectar and pollen in addition to flower detectability. In complex environments, bees have also been shown to use secondary cues like flower colour or spatial location to choose warmer flowers that are more rewarding (Norgate et al., 2010). As a result, the reward model of warmth and nutrition could have a significant impact on pollinator decision-making, even in complex natural environments. Moreover, bumblebees are able to process flower temperature and perceived sucrose sweetness independently, showing that hotter flowers serve as distinct rewards for pollinators (Norgate et al., 2010, Gérard et al., 2020). As much as pollinators do prefer warm nectar in order to feed off that energy, there could be limited visualization due to altered composition on the nectar and pollen.

Sunflower (*Helianthus annuus L.*) is an important oil seed plant grown throughout the world. It belongs to the Asteraceae family. Some of the most commercially important plants in this family include edible leaf, stem and seed oil crops (lettuce, artichoke, endive, sunflower, safflower), herbs and medicinal plants (Artemisia, Calendula, Echinacea), as well as ornamental cut flowers (gerbera, chrysanthemum). The inflorescences of these plants resemble dense flowers, creating pseudanthia flowers, which combine thousands of flowers into a single structure (Zhang and Elomaa, 2021). Sunflower is considered to be tolerant and hardy plant of extreme weather conditions, as it is known to produce stable yields even under conditions such as heat waves. Its anthesis and pollination process is highly susceptible to elevated temperatures whereby temperatures above 26 - 29°C can pose a threat to sunflowers reproductive organs and yield. Beyond these temperature thresholds, heat stress causes various disturbances, indicating a reduced tolerance of reproductive organs to heat stress (Kalyar et al., 2013). Heat stress can also widely affect pollen viability and stigma receptivity in sunflowers thus impacting on seed yield. Sunflowers are mainly grown for their oil, which is extracted from the seeds therefore high temperature exposure can impact on the oil quality and contents produced from the seeds (Van der Merwe et al., 2015).

The studies by Atamian et al, 2016 and Creux et al, 2021 focused on the natural, non-heat stressed temperatures and our study is a follow up from these, focusing specifically on the effects of heat stress on sunflower anthesis. Atamian et al, 2016 discovered that the east-facing flower heads were getting warm faster than the west-facing heads in the mornings. When they warmed the west-facing plants with an aim of matching the morning temperatures with those of the east-facing heads, they found that the number of insect visitors significantly increased. Creux et al, 2021 found that heating the west-facing heads to temperatures matching the east-facing heads also resulted to more and earlier insect visitors, earlier style elongation and pollen presentation. Creux et al, 2021 also showed that temperature directly regulates style elongation and the timing of pollen presentation. The main research question of our study follows this work to further understand how heat waves and heat stress affect the timing of late-stage floral organ development, pollinator visits and pollination of sunflowers. The aim and objectives of this study are to investigate the effects of heatwaves and heat stress on sunflower reproductive organs by harvesting them at different time points to analyze them after exposure to high temperatures. In this study we investigated timing of pollen emergence, style elongation rate, insect visits, stigma receptivity and pollen viability through imaging and direct measurements under different summer conditions. The results of the heat stress in the study was found to have different effects on different reproductive organs. Styles elongate faster sunflowers that are exposed to temperatures that are higher than ambient temperatures releasing earlier pollen and attracting earlier pollinator visitors. It also shows that pollen losses its fertility and some stigma lose their receptivity. Therefore, sunflowers still need an increased amount of attention to mitigate and well understand the reproductive processes.

2.2 Materials and methods

2.2.1 Plant materials

Sunflower (*Helianthus annus* L.) seeds from the AGSUN 8251 cultivar, used in this study, were provided by the South African National Seed Organization (SANSOR) in Pretoria, Gauteng Province, South Africa and kindly donated for this research by Syngenta. The seeds were germinated in the Forestry and Agricultural Biotechnology Institute (FABI) phytotrons, set at a constant daytime temperature of 23ºC - 27ºC and humidity ranging from 60% to 99% with 16 hours light and 8 hours dark phases. Seeds were sown with potting soil (Gromor (pty) Ltd, Durban, South Africa) in plastic seeding trays of 60 cm^3 with 128 loose inserts.

Seedlings were transplanted in the field at Innovation Africa@UP, Hatfield campus, when they had at least two true leaves. In total, 616 plants were planted in the field from the 30th September 2020 to the 9th of February 2021. Individual seedlings were each transplanted with an all-purpose slow-release fertilizer (Bio Ganic All-purpose Atlantic fertilizers, Bellville, South Africa). Approximately 40 seedlings were planted weekly over two rows, with ±20 seedlings planted per row. The rows were 25 m long, with a distance of 1m between each row and seedlings were planted about 1 m apart from each other. There were 34 rows planted in total for a field size of 25 m x 45 m. Over a period of 5 months, plants were irrigated via button drippers every second night for four hours using a Hunter NODE SDMX50 50 Ohm multiplexer meter (Campbell Scientific Inc, Logan, Utah, United States of America). Drippers were inserted directly opposite each plant.

2.2.2 Heating field grown plants

2.2.1.1 Artificial heating

Plants were heated with a gas heater (Alva, Johannesburg, South Africa), to mimic morning heatwave temperatures. Four plants were used each day of the experiment, two were unheated (control) and two were heated. Plants were heated for about five hours from 05:30 to 10:30. This was done to make sure that the temperature is at least 10°C higher than the normal summer temperature. Heated and unheated plants per day were monitored and temperature data was collected with digital food thermometers (PT-101 and Prepworks Digital), which provided the exact temperatures of the sunflower head at a specific time. The artificial heating data was divided into two groups: sunny weather conditions and overcast weather conditions. The data collection for sunny conditions took place over 17 randomly selected days (presented by blue circles in supplementary figure S2.1), while the data collection for overcast weather took place over seven randomly selected days (presented by orange circles supplementary figure 2.1).

2.2.1.2 Natural heat wave

According to the SAWS, a heatwave occurs when the maximum temperature in a particular town or city meets or exceeds 5°C above the average maximum temperature of "the hottest month" for that place, and continues in that mode for three days or more. A natural heat wave took place over three days, from 19 to 21 February 2021. The heat wave days are represented on a graph figure S2.1c (supplementary data by red circles), where temperatures were ≥33℃ for three consecutive days in Pretoria, Gauteng, South Africa. Temperature readings were taken by digital food thermometers on all the sampling time points. The experiments conducted and measurements taken under heatwave days were similar to those taken for the heated and unheated plants except no artificial heating was performed. Control for the experiment was the unheated plants under a sunny weather condition.

2.2.3 Style elongation rate

Styles from each sunflower under all conditions, were collected every 30 minutes to determine style elongation rates. Forceps were used to detach five-disc florets per capitulum and remove their intact styles over five hours. Due to lockdown restrictions and curfew, there were two collection batches, at different times. Time designation used is Zeitgeber time (ZT), which is a convention for indicating the phase in a 24-hour cycle. ZT 0 represents the start of the day, or light phase. ZT was chosen to keep the start times aligned as the season progressed. The first collection time points for styles from the heated and unheated plants were ZT 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5. The second collection time points were ZT 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0. Harvesting ended either at ZT 4.5 or at 4.0, to ensure sufficient style elongation.

Photographs of harvested styles were taken with the Canon SX430 Powershot digital camera over a period of five hours. The styles were aligned on a clean white background with lines serving as a scale. The image analysis used these lines as a standard measure and length of styles could be compared against this. Images were analyzed with the FIJI, ImageJ (Rueden et al, 2017) application to measure style length. The software had to be calibrated by measuring the distance between the page lines. The segmented line tool was to accurately measure style length. Images were processed by tracing a free segmented line over them. Measurements were recorded in an Excel spreadsheet, followed by an analysis of a two-way ANOVA with multiple comparisons on GraphPad Prism 9 software (founded by Dr. Harvey Motulsky, University of California, San Diego).

2.2.4 Timing of pollen emergence

The sunflowers pollen emergence was observed by time lapse imaging using Wingscapes TimelapseCam Pro (Moultie, Birmingham, Alabama, U.S) cameras. Images were captured every 15 minutes from the time of its mounting on Mivision 5858D tripods (Audiolens, Cape Town, South Africa) at ZT 0.5 and ZT 0.25. Cameras were positioned to face each sunflower head. Two sunflower plants were used each day for time-lapse photography, one heated and one unheated, and camera was started as soon as it was set up. Each day, two different sunflowers were sampled under all conditions, which ensured that no plant was exposed to daily heating. The cutoff times for daily sampling were between ZT 4.5 and ZT 5.0 since insect activity had stabilized.

Images were downloaded and the memory cards formatted daily. All images were time and date stamped and used for further analysis. Images taken in the normal summer temperatures and overcast conditions (control unheated plants) were subsequently compared to those taken under heat waves conditions that included both the natural heat wave as well as the artificially heated plants. Images were analyzed using Fiji, as a virtual stack, in order to make sure the same area of florets were analyzed in each image for one condition on a particular day. Analysis involved counting the number of florets with pollen on the same area over the time series until all 100 florets displayed pollen. Analysis done was using a two-way ANOVA with multiple comparison on GraphPad Prism 9 software.

2.2.5 Insect visitor counts

GoPro Hero 7 White video cameras (GoPro Inc., California, USA) were used to monitor the insect visits for all conditions. Every morning of the sampling, the video cameras were mounted on Mivision 5858D tripods (Audiolens, Cape Town, South Africa) to film two sunflower plants per day. The first collection time points were ZT 1.5, 2.5, 3.5 and 4.5 and the second collection time points were ZT 0.5, 1.5, 2.5, 3.5 and 4.5. Videos were taken from ZT 0.5 for five hours. Each hour, a 20-minute video was recorded to monitor the insect visits to each plant at a later stage. The difference in the number of pollinator visits between heated and unheated plants was assessed across the time points. At the end of each day, there was a total of ten videos per day five from two sunflowers. The number of pollinator visitors from each video and each time points were counted and recorded on an excel spreadsheet together with regular temperature readings that were taken and analyzed using a two-way ANOVA with multiple comparison on GraphPad Prism 9 software.

2.2.6 Stigma receptivity test

Stigma receptivity procedure was done as described by Dafni and Maués (1998). Hydrogen peroxide 6% bubbles as a positive indication of receptivity on the stigma. Stigmas were collected from florets that opened the previous day with stigmas protruding from plants under all conditions. Stigmas were collected at five time points, namely ZT 0.5, 1.5, 2.5, 3.5 and 4.5. From each plant, two stigmas were collected, using forceps, at each time point. Collected stigmas were placed on microscope slides in closed petri dishes with two drops of hydrogen peroxide 6% (Dischem, Mondoer, Johannesburg, South Africa) for three minutes. Thereafter pictures of the stigmas were taken with a Canon SX430 digital camera (Canon Inc, Tokyo, Japan) that is set on macro setting for a clear imaging and analyzed on FIJI, ImageJ to count the number of bubbles in each image.

2.2.7 Pollen fertility test

Pollen fertility test was conducted as described by de Jesus Vieira et al. (2015) and Melloni et al. (2013). Lactophenol stain test for pollen tests had to be a total of 100mL, adding 1-5 mL of 1% aqueous solution of aniline blue. Our stain was prepared by mixing the following quantities of ingredients: 5 mL of phenol (Sigma aldrich, Co, St Louis, Missouri, USA), 10 mL of glycerin (Merck (pty) Ltd, Modderfontein, Gauteng), 5 mL of tap water, 5 mL of lactic acid (Merck (pty) Ltd, Wadeville, Gauteng) and 0.25% of methyl blue stain (Sigma aldrich. Co, St Louis, Missouri, USA). Pollen collection was done every hour at five time points ZT 0.5, ZT 1.5, ZT 2.5, ZT 3.5 and ZT 4.5, from all conditions. One drop of the lactophenol stain was placed on the microscope slide (Lasec, Lauda-koningshofen, Germany). Pollen was collected by brushing it off from the sunflower florets and placing the pollen grains on the drop of the staining solution. A cover slip (Lasec, Lauda-koningshofen, Germany) was subsequently placed over the pollen and staining solution. Clear nail vanish (PEP, China) was used around the edges of the coverslips to seal and preserve the sample. For each slide, a minimum of 100 pollen grains were counted for the natural heat wave, heated and unheated plants under the 10x objective of an Axioskop 2 plus stereomicroscope (Microscope central, Feasterville, Panama). Photos of the pollen slides were taken using a Zeiss camera (Carl Zeiss, Jena, Germany) linked to a microscope. A minimum of 15 pictures per slide were taken in order to count up to 100 grains per slide. The number of stained pollen grains were counted against the pollen grains that were not stained for a percentage of stained pollen grains out of 100. Analysis done was using a two-way ANOVA with multiple comparison on GraphPad Prism 9 software.

2.3 Results

Results are divided into three sections, the sunny, overcast and heatwave days. Sunny and overcast days mean that the experiment was done by artificial heating to imitate a heatwave. Whereas, heatwave data shows data collected on days with a natural heatwave.

2.3.1 High temperatures promote faster style elongation and earlier pollen emergence

The artificially heated styles harvested from sunny summer conditions exhibit a faster elongation rate than the unheated styles and were already significantly longer than unheated styles as early as ZT 0.5. Between ZT1.0 and ZT 1.5, unheated styles start elongating but grow considerably slower than artificially heated styles (Figure 2.1a). Artificial heating under overcast conditions increased the rate of style elongation significantly from ZT 0.5 - 3.0 in comparison to the unheated plants under the same condition at the same time points. At ZT 4.0, the length appears to be similar in both the artificially heated styles and the unheated styles during overcast conditions (Figure 2.1b). Under natural heatwaves, there were no significant differences possibly due to the limited number of replicates, however, there seems to be a trend with styles exposed to heat waves elongating a little more than the control. At ZT 2.5 and 3.0. At ZT 4.0, the control grows longer than the natural heatwave treatment, while styles under natural heatwaves appear to shrink (Figure 2.1c). Both the sunny (Figure 2.1a) and overcast (Figure 2.1b) show an increased style length at ZT 0.5 with the heated styles at 10.8 mm long. Data collection under the natural heatwave (Figure 2.1c) started later at ZT 1.5 as opposed to the sunny and overcast, which started at ZT 0.0. However, the similarities observed at time point ZT 1.5 is that heated and natural heatwave styles are all above the length of 11 mm (Figure 2.1a, b and c). Figure 2.1d shows that the heat-treated sunflower heads have temperatures that are 10°C higher than the ones that are not heated suggesting that temperature is regulating the style elongation, as observed in previous studies (Creux et al 2021). The higher the temperatures, the longer the styles elongated (figure 2.1b and d)

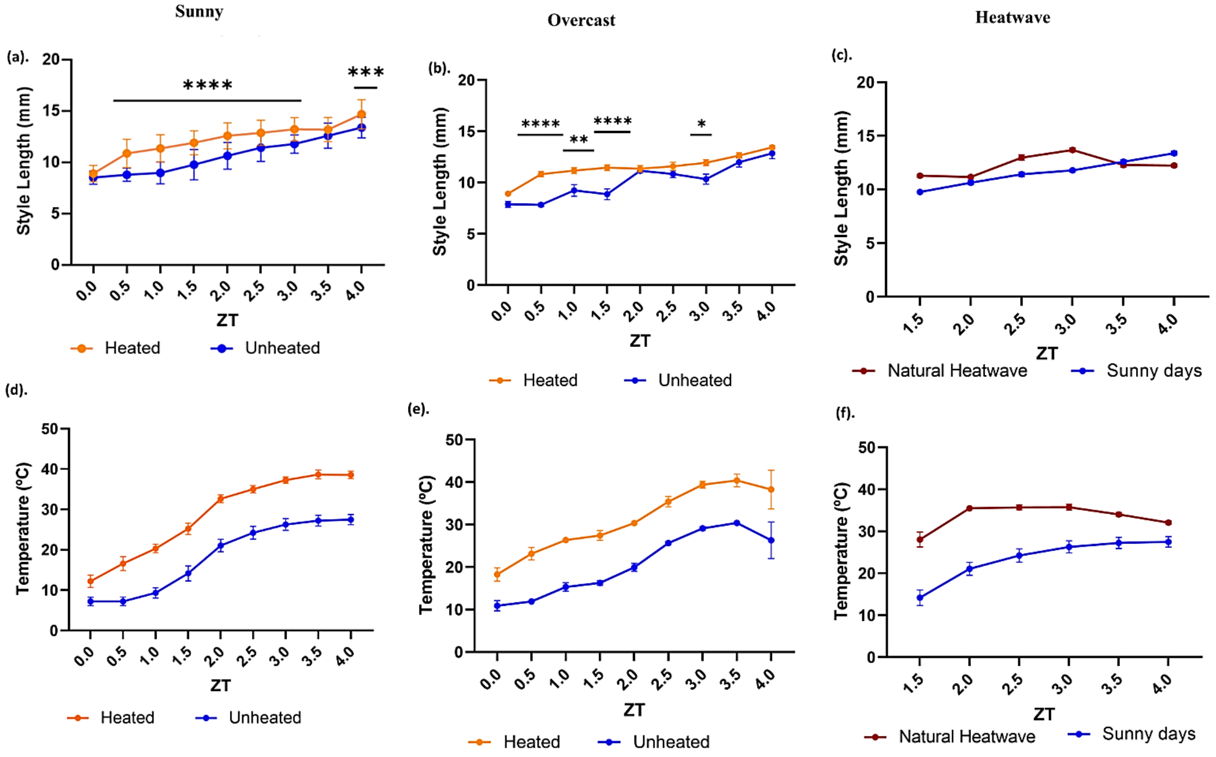


Figure 2.1. Sunflower style elongation rate under heated (orange) and unheated (blue) sunny conditions (a) and overcast conditions (b) at different temperatures. Style elongation rate on control (blue) and heat wave (red) conditions (c). Average temperature readings of heated and unheated plants over the collection time for heated and unheated plants under sunny conditions (d) and overcast conditions (e). Temperature reading on control (blue) and natural heatwave days (red) (f) Error bars indicate SE. ZT (Zeitgeber Time) 0 represents sunrise. One-way ANOVA analysis followed by Tukey's multiple comparison test indicated significant differences. * $P < 0.05$, *** $P < 0.0001$ and ****$P < 0.0001$.

Time-lapse imaging of florets in sunny conditions show the pollen presentation at the same time point at ZT 0.0 for both artificially heated and unheated florets but for the artificially heated florets, the pollen presentation occurs at a faster pace than the unheated sunflower florets (Figure 2.2a). On both the artificially heated sunflower florets and unheated, there is pollen emergence at ZT 0.0 however as time progresses there is fewer florets with pollen on unheated florets than heated florets (Figure 2.3a). The artificially heated florets has more florets with pollen presentation at all time points from ZT 0.25 onwards with significant differences (* $P < 0.05$) at ZT 0.5 and 1.25. The heated sunflower florets showed earlier pollen presentation then the unheated florets (Figure 2.3b). Under overcast conditions, pollen emergence on heated florets occurs 30 minutes earlier than on unheated florets, however both appear to have the same number of florets displaying pollen emergence at ZT 1.0 with no significant differences between them (Figure 2.3b). The first pollen presentation is observed at ZT 0.25 with the artificially heated heads. Time point ZT 0.75 is the earliest time point with pollen presentation on unheated sunflower florets. Artificially heated florets get earlier pollen presentation but from ZT 1.25 the unheated florets seem to be catching up and all florets show pollen presentation at ZT2.25 under both conditions (Figure 2.3b). Earlier pollen presentation on the sunflower florets is observed in the control sunny days, but there was a faster pollen emergence under the heatwave days with all the sunflower florets having emerged pollen on all florets by ZT 1.25. The main difference between the sunny conditions and overcast conditions is the timing in the emergence of the pollen. Sunny showed few differences in the time to start pollen emergence, but heated plants displayed a faster rate of pollen. Whereas under overcast conditions there is a clear difference in the timing of first pollen emergence and in the heated plants, pollen emerges earlier (Figure 2.3 a and b).

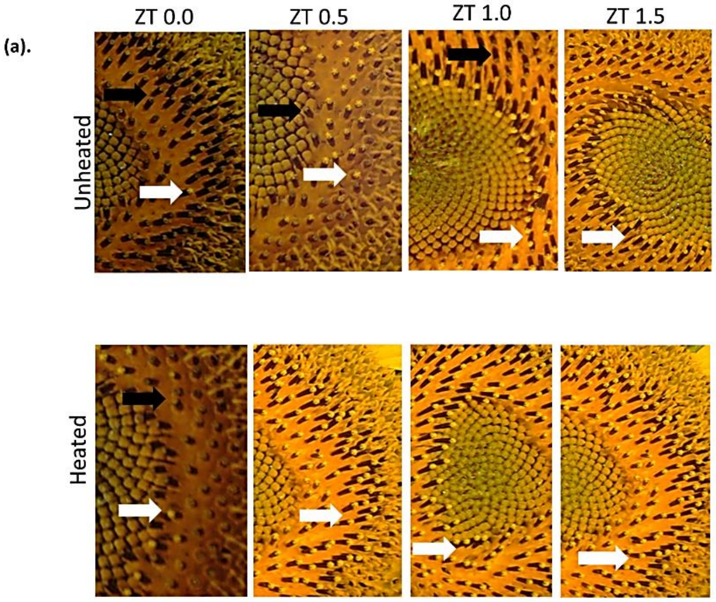


Figure 2.2. Comparison of time-lapse images taken on the heated and the unheated sunflower heads showing the timing of first pollen emergence, with white arrows showing appearance of pollen on anthers and black arrows show anthers without pollen (a).

Under natural heat wave conditions, a large shift towards earlier pollen presentation is observed at ZT 1.0 when compared to the control none heat wave sunny days ($P < 0.0001$, Figure 2.3e). Figure

2.3b shows that under the overcast weather, there are always more florets with pollen under the artificially heated than the unheated sunflower florets. At higher temperatures with artificially heated sunflower heads having much more florets with pollen emergence at ZT 2.0 and unheated florets only catching up at ZT 3.25, which is also at the same time with sunny control (figure 2.3b and e). Temperature differences between the sunflower heads in the control and heatwave showed that heatwave temperatures start higher than the control, however, the control sunflower heads eventually got warmer than the heatwave day at ZT 2.5. Essentially, the plants in a heat wave have hotter heads early in the morning while in the normal sunny day, the flower heads can get hotter later in the day than the flower heads during a heat wave. (Figure 2.3g).

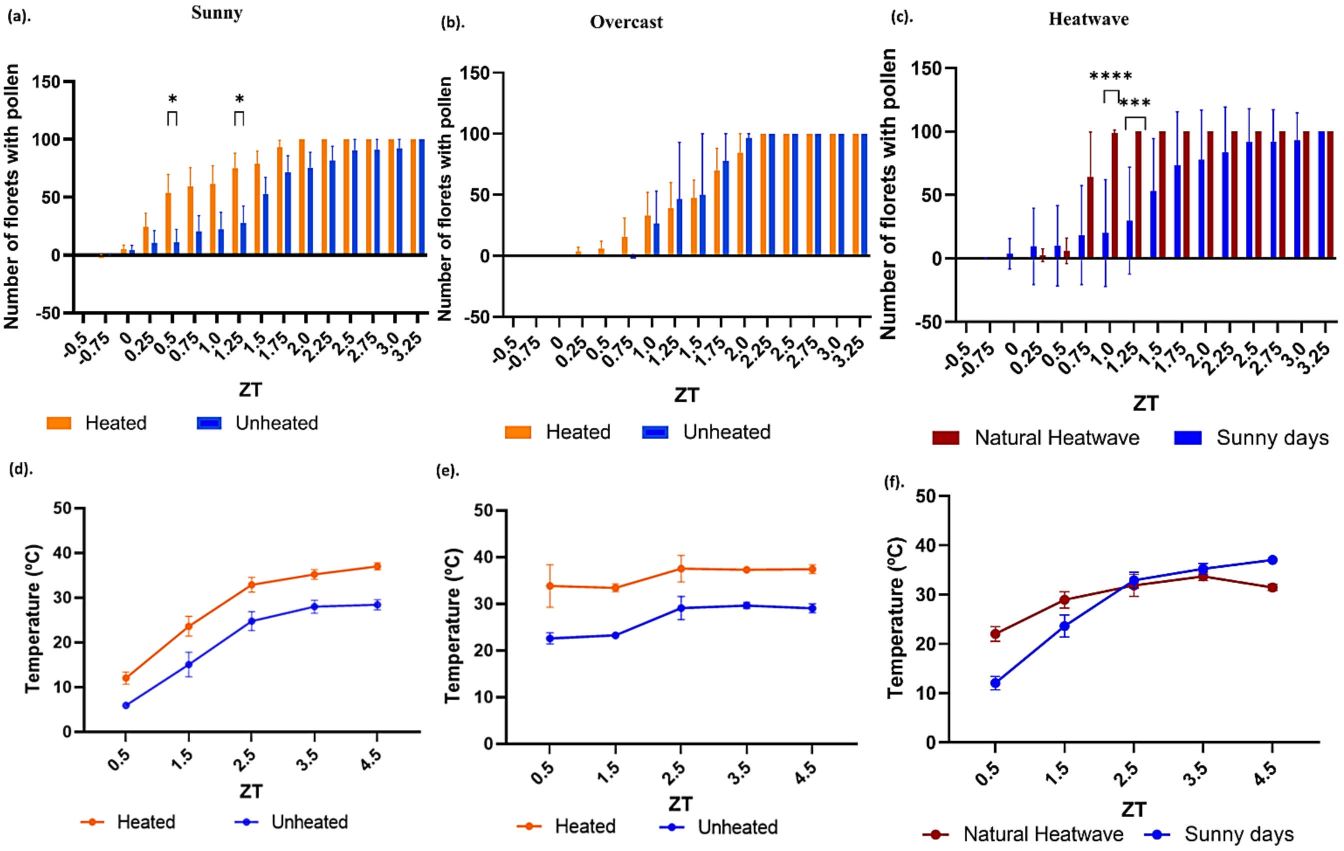


Figure2.3. Sunflower timing of pollen emergence under heated (orange) and unheated (blue) during summer (a) and overcast summer conditions (b). Pollen emergence on heat wave (red) conditions (c). Temperature readings on sunny days (d) overcast days (e). Temperature reading on control (blue) and natural heatwave days (red) (f). Error bars indicate SE. ZT (Zeitgeber Time) 0 represents sunrise. One-way ANOVA analysis followed Tukey's multiple comparison Test indicated significant differences. *P <0.05, ** P < 0.01, ***P < 0.001 and ****P < 0.0001.

2.3.2 Environmental factors affecting the timing of pollinator visits

The timing of insect visits did not show a significant difference at between heated and unheated plants under sunny conditions. However, trends suggest that time point ZT 1.5 had more insect visits with a higher median for the artificially heated than the unheated plants. ZT 3.5 and 4.5 do not show the difference in the number of visitors between the artificially heated and unheated (Figure 3a) even with clear differences in temperature readings between the artificially heated and unheated plants of 9.5 °C (Figure 2.4d). Under overcast conditions, there again seems to be no significant differences, however, trends again indicate that ZT 1.5 and ZT 2.5 show a higher average number of visitors on the artificially heated plants (insects visits over 40) then the unheated plants (insects visits below 20). Artificially heated shows a higher median value of visitors on all time points than the unheated ones (Figure 2.4b). Under the heatwave conditions, time point ZT 1.5 and 3.5 show a significant number of insect visits compared to normal sunny days (Figure 2.4c). The high insect's visits especially at time point ZT 1.5 and ZT 3.5 correlates with high temperatures of 27.9°C and 33.5°C respectively. Therefore, the significant differences in the amount of insect visits to the sunflower heads coincides with the high difference in temperature between the two flower heads, control and under a natural heatwave (Figure 2.4f). In all the three summer conditions, at time point ZT 1.5 there appeared to be higher insect's visits as compared to the controls.

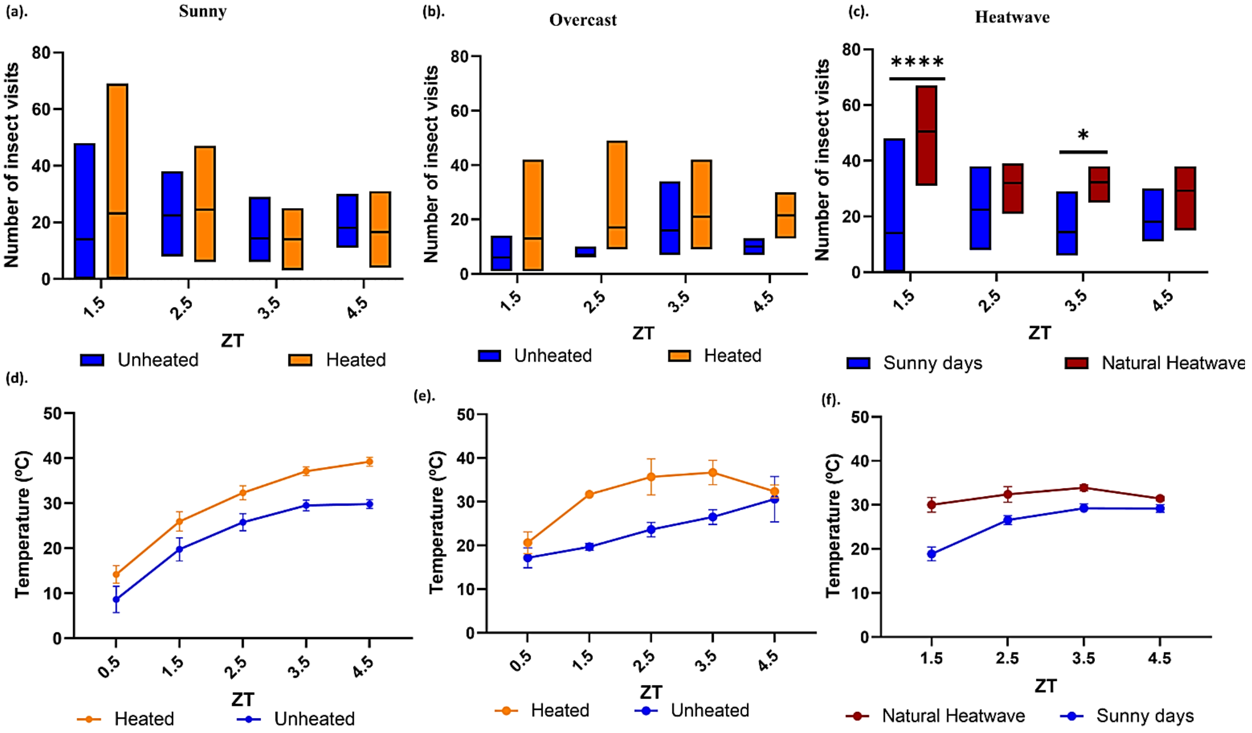


Figure 2.4. Sunflower insect visitors under heated (orange) and unheated (blue) conditions on sunny days (a), overcast days (b) and a comparison of sunny days unheated (blue) and heatwaves (red) (c). Average temperature readings on sunny days (d) overcast days (e) natural heatwave days (f). Error bars indicate SE. ZT (Zeitgeber Time) 0.0 represents sunrise. The top line represents the upper quartile, middle line represents the median and the lower line representing the lower quartile. One-way ANOVA analysis followed Tukey's multiple comparison test indicated significant differences. *P < 0.05 and **** P < 0.0001.

2.3.3 Stigma receptivity changes at high temperatures

The representative images showing the difference in the stigmatic receptivity between the artificially heated plants and unheated florets (Figure 2.5a), were assessed by bubble count. The bubbles observed represent receptivity of the stigma. The bubbles occur when the hydrogen peroxide reacts with the peroxidase enzymes that get released by the stigma when it is receptive and ready to receive pollen (Dafni and Maués, 1998). There is a significant difference at time point ZT 4.5 at $**P < 0.01$ with heated stigmas at an average of 27 bubbles and 38 bubbles with the unheated stigmas (Figure 2.5b). The last time point ZT 4.5 shows the lowest number of bubbles across all time points with artificially heated at the average number of 10 bubbles and unheated at the average number of 22 bubbles (Figure 2.5c). At elevated temperatures in sunny conditions (Figure 2.5b and e), stigma receptivity is slightly, non-significantly reduced in artificially heated plants when compared to unheated plants. Under the overcast weather, there seem to be no significant differences, but it seems that the stigmas are similar to sunny conditions with lower trend in receptivity when heated. The higher the temperatures in overcast weather (heated), the lower the receptivity of heat exposed stigmas (Figure 2.5f). Under the natural heatwave, there are no significant differences at all collection time points, however there seem to be a trend towards increased stigma receptivity under natural heatwave when compared to the control. Time point ZT 4.5 has the highest receptivity across all time points with an average number of 63 average number of bubbles (Figure 2.5d). Natural heatwave stigmas were observed to have a slightly higher stigma receptivity at all time points whereas the sunny and overcast weather conditions show a decrease in the stigma receptivity with the artificial heating.

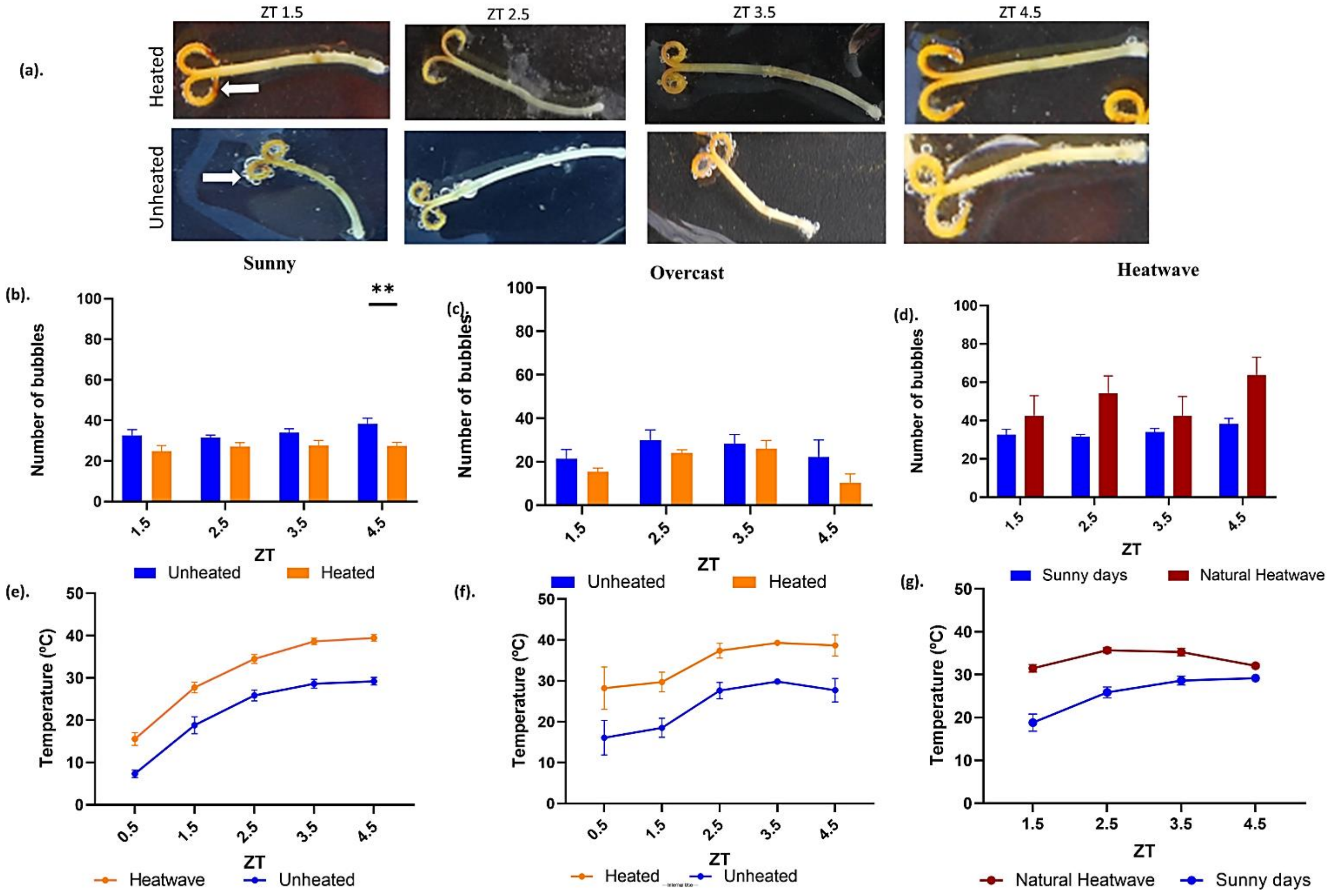


Figure 2.5. Stigmas in hydrogen peroxide pictures compared under the heated and unheated conditions, with white arrows showing the bubbles counted in the analysis (a). Stigma receptivity of the stigmas under heated (orange) and unheated (blue) conditions, collected on sunny days (b), Stigma receptivity of the stigmas under heated (orange) and unheated (blue) conditions collected on overcast (c) days and stigmas collected under natural heatwave days (red) and control (d). Control was taken from the sunny unheated weather days as a comparison. Average temperatures readings of the stigmas collected on sunny days (c) overcast days (f) natural heatwave days (g) were noted down at different time points. Error bars indicate SE. ZT (Zeitgeber Time) 0 represents sunrise. One-way ANOVA analysis followed Tukey's multiple comparison test indicated significant differences. **P < 0.01.

2.3.4 The viability of pollen changes at high temperatures

Figure 2.6 represents the differences between the viable (blue) and non-viable (white) pollen grains between the artificially heated pollen (a) and the unheated (control) pollen (b). Under the sunny weather conditions, the amount of non-viable pollen grains increases with increasing temperatures at the later collection time points (Figure 2.7a and d). There are significant differences in the amount of non-viable pollen grains at time point ZT 3.5 (**$P < 0.01$) and ZT 4.5 (**** $P < 0.0001$) between the heated and the unheated florets. Across all the time points in the sunny condition, the artificially heated florets show a higher proportion of non-viable pollen grains than the unheated florets. The artificially heated pollen grains show 16.9 % more non-viable pollen grains at time point ZT 3.5 (Figure 2.7a). At the time point ZT 4.5, the artificially heated pollen grains showed 36.8 % more non-viable pollen grains than the unheated grains (Figure 2.7a). In GraphPad Prism,the mean points on these graphs were used to calculate the difference in percentage between the control and artificially heated pollen.

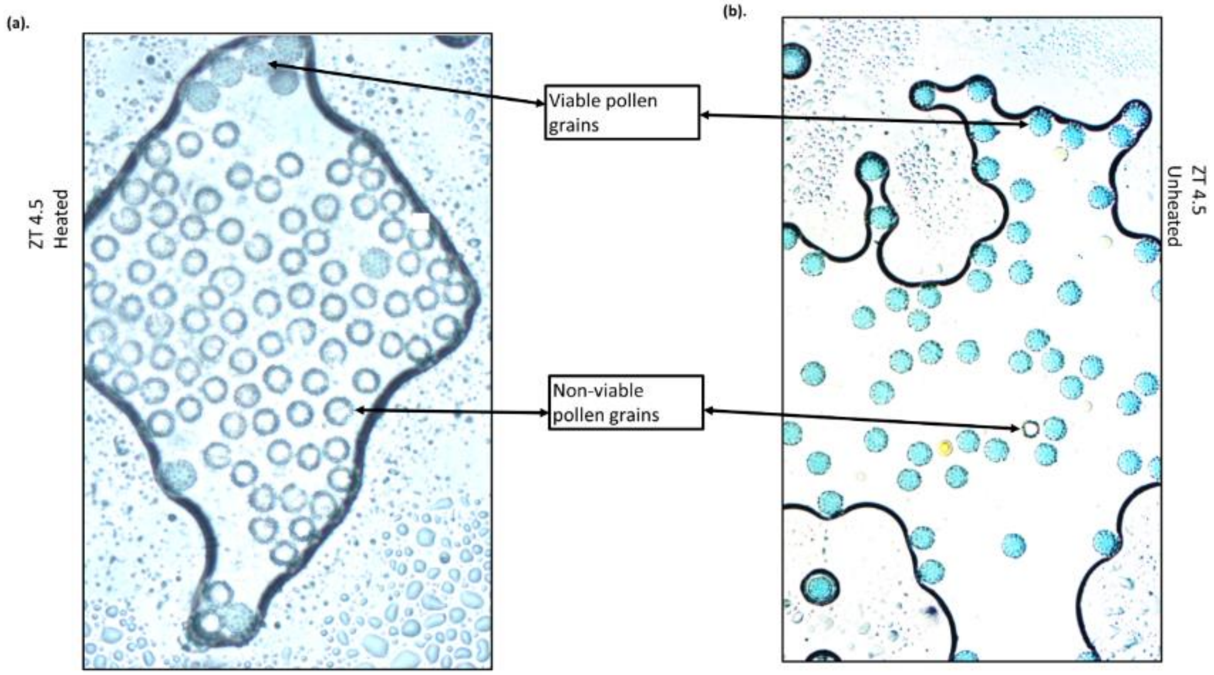


Figure 2.6. Representative images of pollen grains viewed under the microscope collected under the heated (a) and unheated (b) conditions, with black arrows showing the viable (blue) and non-viable (white) pollen grains.

Under the overcast conditions, there are no significant differences in all the four time points. At time points ZT 1.5 and ZT 2.5, the two lines representing the artificially heated pollen and unheated ones seem to be at the same number even though the temperature differences between the two points is about 10°C (Figure 2.7b and e). There is a slight difference of about 10% in the number of non-viable pollen grains at time points ZT 3.5 and ZT 4.5 (Figure 2.7b). Natural heatwave showed an overall higher percentage of non-viable pollen grains then control throughout all the time points. It is the time point ZT 4.5 only that shows a significant difference (**P < 0.01) in the number of viable pollen grains by 14 % (Figure 2.7c). Across all the weather conditions, only the sunny and the natural heatwave conditions show a clear difference between heated and controls (with the two lines not touching throughout the time points), though not always significant. Whereas the overcast only starts showing distinction between the two lines at ZT 3.5 and ZT 4.5 (figure 2.7b).

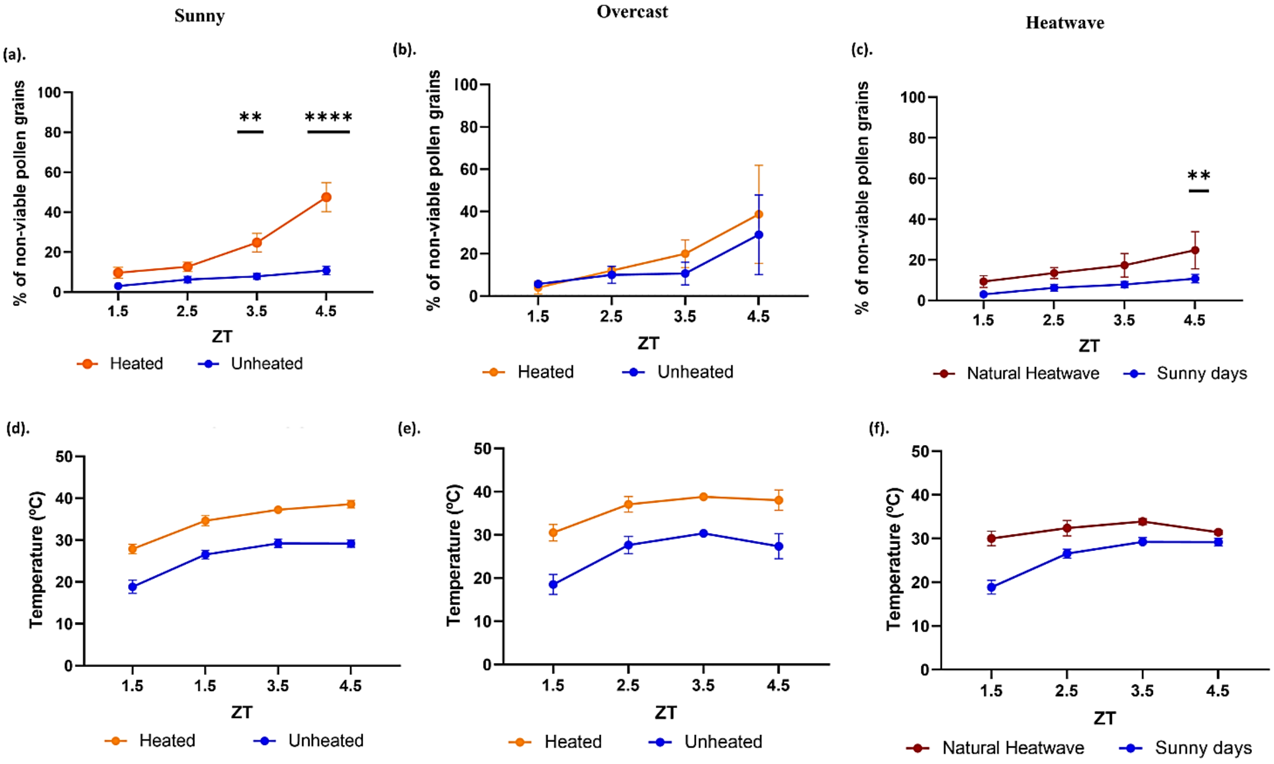


Figure 2.7. Percentage of non-viable pollen grains under heated (orange) and unheated (blue) conditions, collected on sunny days (a), Percentage of non-viable pollen grains under heated and unheated conditions, collected on overcast days (b) and Percentage of non-viable pollen grains collected under natural heatwave days (red) and control (c). Control was taken from the sunny unheated weather days as a comparison. Average temperatures readings of the stigmas collected on sunny days (d) overcast days (e) natural heatwave days (f) were noted down at different time points. Error bars indicate SE. ZT (Zeitgeber Time) 0 represents sunrise. One-way ANOVA analysis followed Tukey's multiple comparison test indicated significant differences. **P < 0.01 and **** P < 0.0001.

2.4 Discussion

The main objective of this study was to investigate the effects of heatwaves and initial heating of sunflower reproductive organs to determine the effect on the timing of sunflower anthesis and pollination. The major findings in this study are that even though sunflowers are considered a temperature hardy crops, we present evidence of decreased stigma receptivity and pollen fertility (Figure 2.5 and 2.7) respectively, which could affect pollination and ultimately seed production. Additionally, we noted the timing of pollen emergence and insect visits shifted earlier under both artificial heating and natural heat waves. This study also proved that artificial heating and heat stress during a natural heatwave have opposite responses when it comes to stigma receptivity. Pollen viability was also seen to decrease with increasing temperatures in both artificially heated and natural heat wave conditions. A study by (Jagadish, 2020) on cereal crops shows that pollen is very sensitive to heat stress. It causes pollen to lose its viability which results in poor pollen loading onto the stigma. A heat stressed stigma showed great sensitivity towards heat stress, lowering its receptivity. However, they outlines how rice species keep their stigmas enclosed during heat stress as a mechanism to preserve stigma viability (Jagadish, 2020).

In this study, pollen viability was decreased and stigma receptivity of artificially heated florets (Figure 2.7, 2.5b, and c). Similar findings were seen in this study when stigma receptivity was increased during a heat wave (Figure 2.5d). The rapid elongation of the styles at elevated temperatures resulted in earlier pollen presentation and much more insect visits (Creux et al., 2021, Atamian et al., 2016). Figure 2.1 showed that there was a faster elongation of the artificially heated styles than the unheated styles. The faster elongation in the early morning was in coincidence with earlier pollen release by plants (Figure 2.3). The earlier pollen release was also showed to attract more pollinator visitors to the plant (Figure 2.4). The process of rapid style elongation and earlier pollen emergence could have been a mechanism that sunflowers developed to avoid emergence of pollen during the hottest part of the day. This suggests an adaptation and may confer tolerance to higher temperatures in sunflower.

Despite the challenging nature of field studies difficulties, an advantage is that the results generated in this study are representative of what occurs under the natural environmental conditions and allowed for assessment of pollinator interactions. One disadvantage of this study was the unpredictable weather, limiting collection days. Appropriate and good weather condition was essential to also make sure it coincided with flowering plants in the field. This was ensured through weekly plantings, so that our plants were all a week apart from anthesis. Another surprising result from this study was the higher stigma receptivity during the heatwave days. This could be an intriguing future study to assess exactly if this could be another way sunflowers compensate during high temperatures in order to protect their reproduction and seed development.

2.4.1 Sunflower pollen and stigma display temperature sensitivity

Stigma receptivity to pollen grains is defined by its ability to hold and germinate those grains (Kumar et al., 2013). Dafni and Maués (1998) used hydrogen peroxide (H_2O_2) to estimate stigma receptivity by assessing peroxidase activity and a similar approach was used for this study. When mature and most receptive to pollen, stigmas exhibit high levels of peroxidase activity. Therefore, peroxidase might be a regulating agent in pollen-stigma interactions as well as an aid to fertilization (Archana and Pandey, 2021). These peroxidase enzymes lead to the oxidation of a variety of substrates by breaking down hydrogen peroxide into highly oxidizing intermediates. A wide range of substrate preferences and a lack of tissue specificity have for a long time made it difficult to understand how peroxidases function in plants. There have been many theories about the function of peroxidases since they were identified as an essential component of stigmas as this enzyme is present when they are in receptive. In *Nicotiana* species, early isozyme studies suggested that they might be involved in pollination processes (McInnis et al., 2006b).

In chicory root plants, peroxidase and esterases activity were on average 82 % lower in high temperature exposed stigmas than in controls, according to a study by Mathieu et al, 2020. Different ways that heat can negatively affect the pistil include altering the stigmas ability to attach and germinate pollen. For instance, an elevated temperature exposure in the sweet cherry plant reduced the length of stigmatic receptivity (Hedhly et al., 2003). Temperature clearly affected the stigma receptivity especially in our current study under the sunny and overcast conditions where fewer bubbles were counted indicating lower receptivity in stigmas from artificially heated flowers (Figure 2.5b and c). Similarly in Sorghum, heat stress decreased the antioxidant enzyme activity and reactive oxygen species in stigmatic tissue, which suggests that there is a decrease in stigma receptivity (Djanaguiraman et al., 2018). In lentils exposed to temperatures of 35/25°C, significantly lower stigma receptivity was observed with a reduction of about 44 – 50 % (Bhandari et al., 2020). Pollen-pistil interaction success lies in the coincidence of stigma receptivity and pollen viability, heat stress effects could interfere with this interaction leading to pollination failure (Mathieu et al., 2020, He et al., 2017, Lohani et al., 2020).

Contradictory to the above findings, stigma receptivity during heat wave conditions in this study was higher than unheated controls (Figure 2.5d). Heat stress in plants or exposure to high temperatures causes a production of reactive oxygen species (ROS). ROS can harm and damage plants components and photosynthetic capacity which results in reduced plant yield. Peroxidases are among the antioxidant enzymes generated by plants in order to combat ROS production (Kothari and Lachowiec, 2021, Raja et al., 2019, Singh et al., 2019, Jovanović et al., 2018). When Sunflowers are under stress and attack, they produce excessive amounts of peroxidases in the whole plant (Nandeeshkumar et al., 2008). It is likely that ROS/ H_2O_2 are playing a functional role in stigmatic papillae due to their high constitutive levels. In receptive stigmas, high levels of ROS/ H_2O_2 and high levels of peroxidase activity appear to be related, suggesting a regulated turnover of H2O2 in this tissue. In this way, stigmatic peroxidases may contribute to controlling H_2O_2 levels through regulated degradation, or they may even drive its generation.

The possibility also exists that stigma peroxidases use H_2O_2 to undergo peroxidation reactions within the cell wall. Stigmatic ROS/ H_2O_2 could therefore be involved in signaling networks that promote pollen germination and/or pollen tube growth on the stigma (McInnis et al., 2006a). The elevated amounts in peroxidases on the stigmas may be the possible explanation of the increased stigma receptivity under heatwave conditions as seen in Figure 2.5d. The abundance of peroxidases increased amount of bubbles when tested with hydrogen peroxide, made these stigmas more receptive. In heat stressed rice species, they also found the increased production of peroxidases enzymes. Furthermore, heat stress during pre- and post-flowering stage resulted in an increased peroxidase activity (Kothari and Lachowiec, 2021). Another study by Raja et al. (2019) also showed that ROS levels were elevated in wheat and sorghum exposed to high temperatures. These studies support the possibility of elevated peroxidase activity in stigmas during a heat wave.

As mentioned above, in order for the pollen-pistil interaction to work, stigma receptivity and pollen viability need to be in accordance. For successful pollination and seed set in plants, pollen viability and stigma receptivity are the fundamental pre-requisites (He et al., 2017). In order for pollen to interact successfully with the stigma for successful fertilization, they must be in a viable state. As the temperature increases, so do the counts of infertile pollen grains (Figure 2.6 and 2.7). The results of this study agree with the results from previous studies because the amount of non-viable pollen grains increased as temperature increased with artificial heating. Commonly, pollen development is considered the most sensitive stage in plant growth to heat. A decrease in pollen viability and function is commonly associated with heat stress (Lohani et al., 2020). Feldeverd et al. (2020) found that elevated temperatures resulted in loss of pollen cells, male and female sterility, and a decreased attachment to the stigma in Arabidopsis species.

In a study of wheat species under controlled environments, such as greenhouses that simulate warming temperatures, heat stress and high CO_2 reduced the number and viability of mature pollen grains (Bokshi et al., 2020). Furthermore, Dwivedi et al. (2017) examined wheat plants and found that high temperatures impaired the development of pollen, viability, and fertilization. Similar results were obtained in Magnoliopsid species, tomato species and grain sorghum species where there was significant decrease of pollen viability at elevated temperatures (Zhuikova et al., 2020, Xu et al., 2017, Prasad et al., 2006). This was due to elevated CO_2 levels in their treatments and could be related to degeneration of tapetum layer and decreased carbohydrate metabolism (Prasad et al., 2006). These results therefore show that while sunflower is considered a temperature resilient plant, its flowers are still sensitive to high temperatures.

2.4.2 Coordinated timing of pollen emergence and pollinator visits as an adaptation mechanism under heat stress

Asteraceae species such as sunflowers are referred to as protandry species with hermaphroditic disc flowers with a phenomena called secondary pollen presentation. Secondary pollen presentation process involves pollen transfer from anthers to another floral organ that functions as a pollen presenting organ (Howell et al., 1993, Pacini and Dolferus, 2019). The style acts as a pollen presenter, with pollen accumulation in the anthers, it then pushes pollen out using a pump mechanism as it elongates (Hong et al., 2008, Sharma and Bhatla, 2013). In this study, it was observed that artificially heated flowers or flowers exposed to a natural heatwave condition possessed styles that elongated more rapidly than the unheated styles, thus exerting pollen earlier (Figure 2.1 and 2.3). Under high temperatures, florets had earlier and quicker pollen emergence than the unheated control. This verified that as styles elongated faster, they then pushed the pollen out earlier from the anthers. It was also observed from the field experiments that temperature increases were able to advance these processes, style elongation and pollen emergence (Figure 2.1

and 2.3). Creux et al. (2021) found that when heating the sunflowers to ambient temperatures, there was earlier pollen presentation due to the faster elongating styles which act as a pollen presenter. A similar mechanism was observed in domesticated tomatoes. When domesticated tomatoes are grown at moderately elevated temperatures, they show high stigma exertion during anthesis, which significantly impedes self-pollination and reduces fruit yields and quality (Pan et al., 2017, Pan et al., 2019). Giorno et al. (2013) found that even on young tomato buds and flowers heat stress produced abnormal anthers and style elongation.

The pollen released by many flowering plants serves as both a carrier for the male gametophyte and as reward for the attraction to pollinators (De Luca et al., 2013). It is often insects, such as bees, that transport pollen to flowering plants, which ensures reproductive success. Nectar and pollen are offered to pollinators as rewards, providing them with protein, lipids, vitamins, minerals, carbohydrates, and water. Therefore, in order for pollinators to locate and identify flowers with food resources such as nectar and pollen, they rely on both visual and scent cues provided by flowers. When given a choice between several possible types of artificial flowers, bumblebees are more likely to develop floral constancy when the varieties differ by scent cues rather than visual cues. (Junker and Blüthgen, 2010, Wojtaszek and Maier, 2014).

An increase in ambient temperatures has been found to influence and increase pollinator visitors to flowers. Their study found that bumblebees shifted their foraging behavior towards increasing flower temperatures (Norgate et al., 2010). Our study also showed an increase in the number of visitors when morning floral temperatures were increasing (Figure 2.4). Thermal radiation from structures surrounding the plant coupled with absorption of direct solar radiation can cause flower temperatures to rise (van der Kooi, 2016, Harrap et al., 2017). As a result of capturing solar radiation, flowers often warm quickly, creating a series of floral displays that attract pollinators. In addition to providing signals that allow pollinators to find and locate flowers, floral temperature also enables them to recognize and learn about them (Basari et al., 2018, Harrap et al., 2017, Walker, 2007). According to a study performed by Walker, 2007, it was observed that various insect species prefer basking in warm, sunlit leaves or flowers. Atamian et al. (2016) and Creux et al. (2021) also found that east-facing plants (in direct sunlight) received more visitors than the west-facing plants. They also found that heating west-facing plants increased the number of visiting insects as compared to west-facing unheated plants.

In this current study, the impact of sunlight was evident in the insect visits in sunny conditions (Figure 2.4a and c), where both artificially heated and unheated plants received almost the same number of visitors since both were in full and direct sunlight. Greater light availability usually leads to large floral displays. Larger displays are proven to draw pollinators and increase the number of visitors (Kilkenny and Galloway, 2008). Pollinators are attracted by a number of factors, like the availability of food resources such as pollen and nectar. They are drawn to the plants by UV markings that they follow as a cue when pollen has emerged or when there is an availability of resources. A study by Creux et al., (2021) showed that east and west facing plants displayed very differently in the morning when pollinators are most active due to the sun rising in the east. This resulted in fewer insects visiting the west and more visitors coming to the east-facing plants. All the plants in our study were oriented in an easterly direction and therefore likely appeared more similar to the insect visitors. The fact that there was no difference seen between heated and unheated plants is suggesting that visual clues are a very important driver of pollinator attraction and heat alone in some instances in not sufficient to drive a change in pollinator visits (Figure 2.4 a and b). The study by Kilkenny and Galloway, (2008) also confirmed that the visitation of bumblebee pollinators was influenced by light conditions in String lily angiosperm species. Those plants placed in the sun received seven times as many visitors as those placed in the shade, and the number of visitors increased with the size of the display. This supports the findings of our study where the control plants in the overcast conditions received much fewer visitors then the heated ones (Figure 2.4b).

Another factor that plays a huge role in the pollinator visitation patterns is the UV markings or floral displays. These are the UV absorbing pigments or nectar guides that are visible to pollinators but humans. UV markings help improve the flowers attractiveness to pollinators and lead them to the most rewarding part of the flower (Moyers et al., 2017, Harrap et al., 2020, Brock et al., 2016, Wojtaszek and Maier, 2014, Todesco et al., 2021, Creux et al., 2021). These findings can conclude that apart from visual cues and floral scent, other pollination factors that are likely influencing and facilitating visitation rates is the temperature, UV markings and irradiance levels especially when plants are in full sunlight than those in the shade.

Many plant species, including sunflower, have genetic mechanisms to stop pollen of the same plant germinating on its own stigma in a mechanisms known as self-incompatibility Wild sunflowers are self-incompatible, which means that they are heavily reliant on pollinators from other plants, therefore outcrossing is highly enforced (Wojtaszek and Maier, 2014). Domesticated sunflowers are self-compatible meaning that they have less reliance in pollinators because they can self-pollinate. Even though domesticated sunflowers are not entirely dependent on pollinators it has been found that an abundance of pollinators will increase seed set and yield (Degrandi-Hoffman and Chambers, 2006). This is a benefit to a certain extent because if the increased number of pollinators visiting the plant, which safeguards the variability of the genes (Wojtaszek and Maier, 2014). A difference between the wild and the domesticated sunflowers in the attractiveness and pollinator visits was not observed by Creux et al, 2021. They showed similar increases in the insect visits and timing of visits. These finding suggest that while domesticated sunflowers are self-fertile they appear to have similar anthesis dynamics to wild species, similar pollinator activities and gain at least some advantage in cross pollination.

Foraging activities of bees are not only influenced by temperatures (Kilkenny and Galloway, 2008) but food source availability and colony condition (Basari et al., 2021, Mallinger and Prasifka, 2017). According to a study conducted by Basari et al, 2021, the number of stingless pollinating bees increased in the morning up until noon and then gradually declined thereafter in the ten different flower species they studied. They also found that early morning nectar has high sugar concentration, which is a better reward to pollinator visitors (Basari et al., 2021). Similarly, in the current study, we found that even though there were no significant differences but there were much earlier visitors to the artificially heated and natural heatwave flowers than the unheated (Figure 2.4). The study also shows that there are fewer pollinator visitors later on the last collection time of the day points as compared to earlier time points (Figure 2.4). This was due to the availability of floral rewards, warm nectar and pollen earlier in the day to the plant pollinators (Basari et al., 2021, Shrestha et al., 2018). The decreased number of visitors on the last time point may be due to the lower availability of floral rewards. Apart from the floral rewards being less available, foraging activity also decreased due to temperatures exceeding 30°C later in the day. These temperatures may be too high for foragers, as they would require even more energy therefore causing dehydration (Basari et al., 2021).

2.5 Conclusion

In conclusion, the results of this study show how sunflowers reproductive organs respond to heat stress. Artificially heating sunflower florets showed that it can cause pollen to emerge earlier, attracting more and earlier visitors. The earlier emergence of pollen is due to the rapid elongation of the style under elevated temperatures which pushes out the pollen. However, pollen viability seems to be affected by high temperatures on both artificially heated and natural heatwave pollen. Sunflowers exposed to high temperatures exhibit an earlier than normal emergence of pollen. The earlier availability of pollen tends to attract earlier and more pollinator visitors to the flowers. The strategy of sunflowers to release pollen earlier when exposed to high temperatures could be a mechanism that avoids a decrease in pollen grain viability. The release of pollen grains just when temperatures are starting to spike still ensures pollen grains are still fertile to fertilize the ovule before temperatures get too high. Especially because pollen has been found to have a negative response to elevated temperatures, which is also seen by significant differences in the sunny and heatwave weather conditions. What was interesting is that only artificially heated stigmas showed a decrease in receptivity whereas in a heat wave it is increased. The possible explanation to this possibility is still unknown and it is a huge gap in the sunflower literature. This is something that could be done in future to answer this question.

2.6 References

ALLEN, S. K., PLATTNER, G.-K., NAUELS, A., XIA, Y. & STOCKER, T. F. Climate Change 2013: The physical science basis. An overview of the working group 1 contribution to the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC). *EGU General Assembly Conference Abstracts*, 2014. 3544.

ARCHANA & PANDEY, N. 2021. Reproductive development and pollen-stigma interaction in sunflower plants receiving boron deficient and toxic supply. *Journal of Plant Nutrition*, 1-10.

ATAMIAN, H. S., CREUX, N. M., BROWN, E. A., GARNER, A. G., BLACKMAN, B. K. & HARMER, S. L. 2016. Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits. *Science,* 353**,** 587-590.

AWAIS, M., WAJID, A., SALEEM, M. F., NASIM, W., AHMAD, A., RAZA, M. A. S., BASHIR, M. U., MUBEEN, M., HAMMAD, H. M., HABIB UR RAHMAN, M., SAEED, U., ARSHAD, M. N. & HUSSAIN, J. 2018. Potential impacts of climate change and adaptation strategies for sunflower in Pakistan. *Environmental Science and Pollution Research,* 25**,** 13719-13730.

BASARI, N., RAMLI, S. N., ABDUL-MUTALID, N. A., SHAIPULAH, N. F. M. & HASHIM, N. A. 2021. Flowers morphology and nectar concentration determine the preferred food source of stingless bee, *Heterotrigona itama*. *Journal of Asia-Pacific Entomology,* 24**,** 232-236.

BASARI, N., RAMLI, S. N. & KHAIRI, M. 2018. Food reward and distance influence the foraging pattern of stingless bee, Heterotrigona itama. *Insects,* 9**,** 138.

BHANDARI, K., SITA, K., SEHGAL, A., BHARDWAJ, A., GAUR, P., KUMAR, S., SINGH, S., SIDDIQUE, K. H., PRASAD, P. V. & JHA, U. 2020. Differential heat sensitivity of two cool-season legumes, chickpea and lentil, at the reproductive stage, is associated with responses in pollen function, photosynthetic ability and oxidative damage. *Journal of Agronomy and Crop Science,* 206**,** 734-758.

BHEEMANAHALLI, R., SUNOJ, V. J., SARIPALLI, G., PRASAD, P. V., BALYAN, H., GUPTA, P., GRANT, N., GILL, K. S. & JAGADISH, S. K. 2019. Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. *Crop Science,* 59**,** 684-696.

BISBIS, M. B., GRUDA, N. & BLANKE, M. 2018. Potential impacts of climate change on vegetable production and product quality – A review. *Journal of Cleaner Production,* 170**,** 1602-1620.

BROCK, M. T., LUCAS, L. K., ANDERSON, N. A., RUBIN, M. J., CODY MARKELZ, R., COVINGTON, M. F., DEVISETTY, U. K., CHAPPLE, C., MALOOF, J. N. & WEINIG, C. 2016. Genetic architecture, biochemical underpinnings and ecological impact of floral UV patterning. *Molecular Ecology,* 25**,** 1122-1140.

CHEN, S., SARADADEVI, R., VIDOTTI, M. S., FRITSCHE-NETO, R., CROSSA, J., SIDDIQUE, K. H. M. & COWLING, W. A. 2021. Female reproductive organs of Brassica napus are more sensitive than male to transient heat stress. *Euphytica,* 217**,** 117.

CREUX, N. M., BROWN, E. A., GARNER, A. G., SAEED, S., SCHER, C. L., HOLALU, S. V., YANG, D., MALOOF, J. N., BLACKMAN, B. K. & HARMER, S. L. 2021. Flower orientation influences floral temperature, pollinator visits and plant fitness. *New Phytologist,* 232**,** 868-879.

DAFNI, A. & MAUÉS, M. M. 1998. A rapid and simple procedure to determine stigma receptivity. *Sexual plant reproduction,* 11**,** 177-180.

DEGRANDI-HOFFMAN, G. & CHAMBERS, M. 2006. Effects of honey bee (*Hymenoptera: Apidae*) foraging on seed set in self-fertile sunflowers (*Helianthus annuus L.*). Environmental Entomology, 35, 1103-1108.

DE JESUS VIEIRA, L., DE SANTANA, J. R. F., ALVES, A. A. C., DA SILVA LEDO, C. A. & SOUZA, F. V. D. 2015. Use of aniline blue stain to observing pollen tubes development in different Manihot Mill. species. *African Journal of Agricultural Research,* 10**,** 1805-1809.

DE LUCA, P. A., BUSSIERE, L. F., SOUTO-VILAROS, D., GOULSON, D., MASON, A. C. & VALLEJO-MARÍN, M. 2013. Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. *Oecologia,* 172**,** 805-816.

DESCAMPS, C., QUINET, M., BAIJOT, A. & JACQUEMART, A. L. 2018. Temperature and water stress affect plant–pollinator interactions in *Borago officinalis* (*Boraginaceae*). *Ecology and Evolution,* 8**,** 3443-3456.

DJANAGUIRAMAN, M., PERUMAL, R., JAGADISH, S., CIAMPITTI, I., WELTI, R. & PRASAD, P. 2018. Sensitivity of sorghum pollen and pistil to high-temperature stress. *Plant, cell & environment,* 41**,** 1065-1082.

DUQUE, L., POELMAN, E. H. & STEFFAN-DEWENTER, I. 2021. Effects of ozone stress on flowering phenology, plant-pollinator interactions and plant reproductive success. *Environmental Pollution,* 272**,** 115953.

DWIVEDI, S., BASU, S., KUMAR, S., KUMAR, G., PRAKASH, V., KUMAR, S., MISHRA, J., BHATT, B., MALVIYA, N. & SINGH, G. 2017. Heat stress induced impairment of starch mobilisation regulates pollen viability and grain yield in wheat: Study in Eastern Indo-Gangetic Plains. *Field Crops Research,* 206**,** 106-114.

ENGELBRECHT, F. A. & MONTEIRO, P. 2021a. The IPCC Assessment Report Six Working Group 1 report and southern Africa: Reasons to take action. *South African Journal of Science,* 117**,** 1-7.

ENGELBRECHT, F. A. & MONTEIRO, P. M. 2021b. The IPCC Assessment Report Six Working Group 1 report and southern Africa: Reasons to take action. *South African Journal of Science,* 117.

FELDEVERD, E., PORTER, B. W., YUEN, C. Y., IWAI, K., CARRILLO, R., SMITH, T., BARELA, C., WONG, K., WANG, P. & KANG, B.-H. 2020. The Arabidopsis protein disulfide isomerase subfamily M isoform, PDI9, localizes to the endoplasmic reticulum and influences pollen viability and proper formation of the pollen exine during heat stress. *Frontiers in plant science,* 11**,** 2035.

GÉRARD, M., VANDERPLANCK, M., WOOD, T. & MICHEZ, D. 2020. Global warming and plant–pollinator mismatches. *Emerging Topics in Life Sciences,* 4**,** 77-86.

GIORNO, F., WOLTERS-ARTS, M., MARIANI, C. & RIEU, I. 2013. Ensuring reproduction at high temperatures: the heat stress response during anther and pollen development. *Plants,* 2**,** 489-506.

HARRAP, M. J., DE IBARRA, N. H., WHITNEY, H. M. & RANDS, S. A. 2020. Floral temperature patterns can function as floral guides. *Arthropod-plant interactions***,** 1-14.

HARRAP, M. J., RANDS, S. A., DE IBARRA, N. H. & WHITNEY, H. M. 2017. The diversity of floral temperature patterns, and their use by pollinators. *Elife,* 6**,** e31262.

HASSAN, M. U., CHATTHA, M. U., KHAN, I., CHATTHA, M. B., BARBANTI, L., AAMER, M., IQBAL, M. M., NAWAZ, M., MAHMOOD, A. & ALI, A. 2021. Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—A review. *Plant*

Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 155**,** 211-234.

HAVERKAMP, A., LI, X., HANSSON, B. S., BALDWIN, I. T., KNADEN, M. & YON, F. 2019. Flower movement balances pollinator needs and pollen protection. *Ecology,* 100**,** e02553.

HE, G., HU, F., MING, J., LIU, C. & YUAN, S. 2017. Pollen viability and stigma receptivity in Lilium during anthesis. *Euphytica,* 213**,** 1-10.

HEDHLY, A., HORMAZA, J. & HERRERO, M. 2003. The effect of temperature on stigmatic receptivity in sweet cherry (*Prunus avium L.*). *Plant, Cell & Environment,* 26**,** 1673-1680.

HONG, L., SHEN, H., YE, W., CAO, H. & WANG, Z. 2008. Secondary pollen presentation and style morphology in the invasive weed Mikania micrantha in South China. *Botanical Studies,* 49**,** 253-260.

HOWELL, G., SLATER, A. & KNOX, R. 1993. Secondary pollen presentation in angiosperms and its biological significance. *Australian Journal of Botany,* 41**,** 417-438.

JAGADISH, S. K. 2020. Heat stress during flowering in cereals–effects and adaptation strategies. *New Phytologist*, 226**,** 1567-1572.

JOVANOVIĆ, S. V., KUKAVICA, B., VIDOVIĆ, M., MORINA, F. & MENCKHOFF, L. 2018. Class III peroxidases: functions, localization and redox regulation of isoenzymes. *Antioxidants and antioxidant enzymes in higher plants.* Springer.

JUNKER, R. R. & BLÜTHGEN, N. 2010. Floral scents repel facultative flower visitors, but attract obligate ones. *Annals of Botany,* 105**,** 777-782.

KALYAR, T., RAUF, S., TEIXEIRA DA SILVA, J. & SHAHZAD, M. 2013. Handling Sunflower (*Helianthus annuus L.*) populations under heat stress. *Archives of Agronomy and Soil Science,* 60.

KILKENNY, F. F. & GALLOWAY, L. F. 2008. Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia,* 155**,** 247-255.

KOTHARI, A. & LACHOWIEC, J. 2021. Roles of Brassinosteroids in Mitigating Heat Stress Damage in Cereal Crops. *International Journal of Molecular Sciences,* 22**,** 2706.

KUMAR, N., KUMAR, N., SHUKLA, A., SHANKHDHAR, S. C. & SHANKHDHAR, D. 2015. Impact of Terminal Heat Stress on Pollen Viability and Yield Attributes of Rice (*Oryza sativa L.*). *Cereal Research Communications,* 43**,** 616-626.

KUMAR, S., THAKUR, P., KAUSHAL, N., MALIK, J. A., GAUR, P. & NAYYAR, H. 2013. Effect of varying high temperatures during reproductive growth on reproductive function, oxidative stress and seed yield in chickpea genotypes differing in heat sensitivity. *Archives of Agronomy and Soil Science,* 59**,** 823-843.

LOHANI, N., SINGH, M. B. & BHALLA, P. L. 2020. High temperature susceptibility of sexual reproduction in crop plants. *Journal of experimental botany,* 71**,** 555-568.

MALLINGER, R. & PRASIFKA, J. 2017. Bee visitation rates to cultivated sunflowers increase with the amount and accessibility of nectar sugars. *Journal of applied entomology,* 141**,** 561-573.

MATHIEU, A.-S., PÉRILLEUX, C., JACQUEMIN, G., RENARD, M.-E., LUTTS, S. & QUINET, M. 2020. Impact of vernalization and heat on flowering induction, development and fertility in root chicory (*Cichorium intybus L. var. sativum*). *Journal of Plant Physiology,* 254**,** 153272.

MCINNIS, S. M., DESIKAN, R., HANCOCK, J. T. & HISCOCK, S. J. 2006a. Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? *New Phytologist,* 172**,** 221-228.

MCINNIS, S. M., EMERY, D. C., PORTER, R., DESIKAN, R., HANCOCK, J. T. & HISCOCK, S. J. 2006b. The role of stigma peroxidases in flowering plants: insights from further characterization of a stigma-specific peroxidase (SSP) from *Senecio squalidus* (Asteraceae). *Journal of Experimental Botany,* 57**,** 1835-1846.

MELLONI, M. L. G., SCARPARI, M. S., DE MENDONÇA, J. R., PERECIN, D., DE ANDRADE LANDELL, M. G. & PINTO, L. R. 2013. Comparison of two staining methods for pollen viability studies in sugarcane. *Sugar Tech,* 15**,** 103-107.

MOYERS, B. T., OWENS, G. L., BAUTE, G. J. & RIESEBERG, L. H. 2017. The genetic architecture of UV floral patterning in sunflower. *Annals of botany,* 120**,** 39-50.

NANDEESHKUMAR, P., RAMACHANDRAKINI, K., PRAKASH, H., NIRANJANA, S. & SHEKAR SHETTY, H. 2008. Induction of resistance against downy mildew on sunflower by rhizobacteria. *Journal of Plant Interactions,* 3**,** 255-262.

NORGATE, M., BOYD-GERNY, S., SIMONOV, V., ROSA, M. G., HEARD, T. A. & DYER, A. G. 2010. Ambient temperature influences Australian native stingless bee (*Trigona carbonaria*) preference for warm nectar. *PLoS One,* 5**,** e12000.

OZTURK, T., CEBER, Z. P., TÜRKEŞ, M. & KURNAZ, M. L. 2015. Projections of climate change in the Mediterranean Basin by using downscaled global climate model outputs. *International Journal of Climatology,* 35**,** 4276-4292.

PACINI, E. & DOLFERUS, R. 2019. Pollen Developmental Arrest: Maintaining Pollen Fertility in a World With a Changing Climate. *Frontiers in Plant Science,* 10.

PAN, C., YANG, D., ZHAO, X., JIAO, C., YAN, Y., LAMIN-SAMU, A. T., WANG, Q., XU, X., FEI, Z. & LU, G. 2019. Tomato stigma exsertion induced by high temperature is associated with the jasmonate signalling pathway. *Plant, cell & environment,* 42**,** 1205-1221.

PAN, C., YE, L., ZHENG, Y., WANG, Y., YANG, D., LIU, X., CHEN, L., ZHANG, Y., FEI, Z. & LU, G. 2017. Identification and expression profiling of microRNAs involved in the stigma exsertion under high-temperature stress in tomato. *BMC genomics,* 18**,** 1-16.

PITTOCK, A. B. 2017. *Climate change: turning up the heat,* Routledge, 1315065843.

PRASAD, P. V., BOOTE, K. J. & ALLEN JR, L. H. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor (L.*) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agricultural and forest meteorology,* 139**,** 237-251.

RAJA, M. M., VIJAYALAKSHMI, G., NAIK, M. L., BASHA, P. O., SERGEANT, K., HAUSMAN, J. F. & KHAN, P. S. S. V. 2019. Pollen development and function under heat stress: from effects to responses. *Acta Physiologiae Plantarum,* 41**,** 1-20.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW in BMC Bioinformatics, 2017; 18(1):529 ImageJ2: ImageJ for the next generation of scientific image data. doi: 10.1186/s12859-017-1934-z

SHARMA, B. & BHATLA, S. 2013. Accumulation and scavenging of reactive oxygen species and nitric oxide correlate with stigma maturation and pollen–stigma interaction in sunflower. *Acta physiologiae plantarum,* 35**,** 2777-2787.

SHRESTHA, M., GARCIA, J. E., BUKOVAC, Z., DORIN, A. & DYER, A. G. 2018. Pollination in a new climate: assessing the potential influence of flower temperature variation on insect pollinator behaviour. *PLoS One,* 13**,** e0200549.

SINGH, D., SINGH, C. K., TAUNK, J., JADON, V., PAL, M. & GAIKWAD, K. 2019. Genome wide transcriptome analysis reveals vital role of heat responsive genes in regulatory mechanisms of lentil (*Lens culinaris Medikus*). *Scientific reports,* 9**,** 1-19.

TARAZ, V. 2018. Can farmers adapt to higher temperatures? Evidence from India. *World Development*, 112**,** 205-219.

TIWARI, Y. K. & YADAV, S. K. 2019. High temperature stress tolerance in maize (*Zea mays L.*): Physiological and molecular mechanisms. *Journal of Plant Biology,* 62**,** 93-102.

TODESCO, M., BERCOVICH, N., KIM, A., IMEROVSKI, I., OWENS, G. L., RUIZ, Ó. D., HOLALU, S. V., MADILAO, L. L., JAHANI, M. & LÉGARÉ, J.-S. 2021. Genetic basis and dual adaptive role of floral pigmentation in sunflowers. *BioRxiv*.

VAN DER MERWE, R., LABUSCHAGNE, M. T., HERSELMAN, L. & HUGO, A. 2015. Effect of heat stress on seed yield components and oil composition in high-and mid-oleic sunflower hybrids. *South African Journal of Plant and Soil,* 32**,** 121-128.

VAN DER KOOI, CASPER J. 2016. Plant Biology: Flower Orientation, Temperature Regulation and Pollinator Attraction. *Current Biology,* 26**,** R1143-R1145.

WALKER, J. 2007. Warm flowers, happy pollinators. *Biologist,* 54**,** 154.

WANG, Y., IMPA, S., SUNKAR, R. & JAGADISH, S. K. 2021. The neglected other half-role of the pistil in plant heat stress responses. *Plant, Cell & Environment*, 0140-7791.

WOJTASZEK, J. & MAIER, C. 2014. A microscopic review of the sunflower and honeybee mutualistic relationship. *International Journal of AgriScience,* 4**,** 272-282.

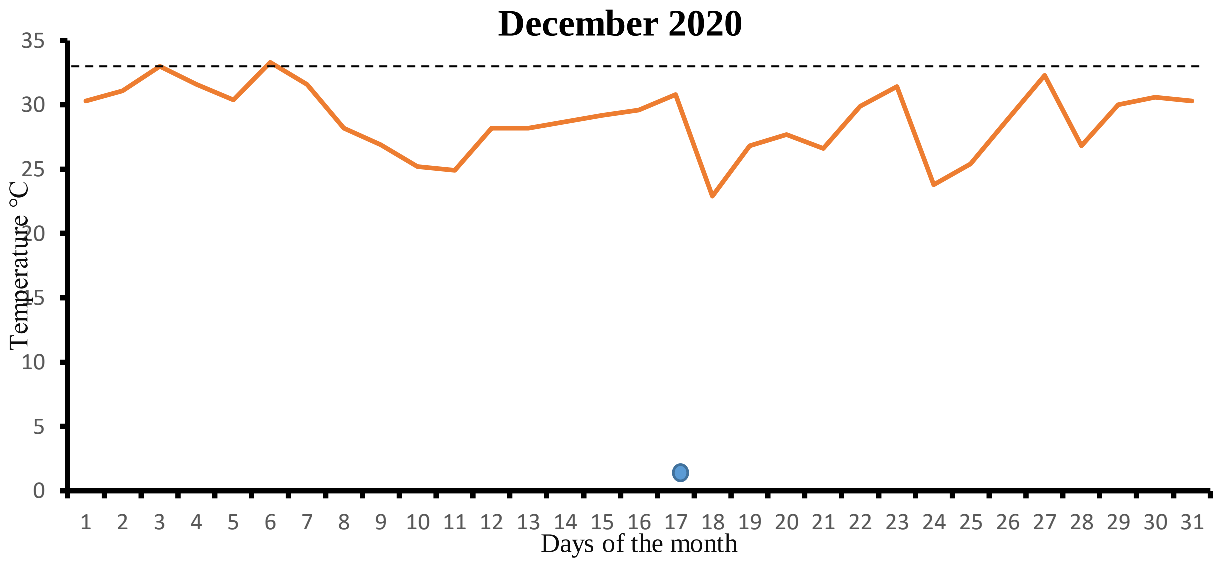
XU, J., WOLTERS-ARTS, M., MARIANI, C., HUBER, H. & RIEU, I. 2017. Heat stress affects vegetative and reproductive performance and trait correlations in tomato (*Solanum lycopersicum)*. *Euphytica,* 213**,** 1-12.

ZHUIKOVA, T., BEZEL, V., BERGMAN, I., MELING, E. & KRIVOSHEEVA, A. 2020. Fertility and viability of pollen grains of *Taraxacum officinale* Wigg. sl (Asteraceae, Magnoliopsida) in a Gradient of an Anthropogenically Transformed Environment. *Biology Bulletin,* 47**,** 1351-1358.

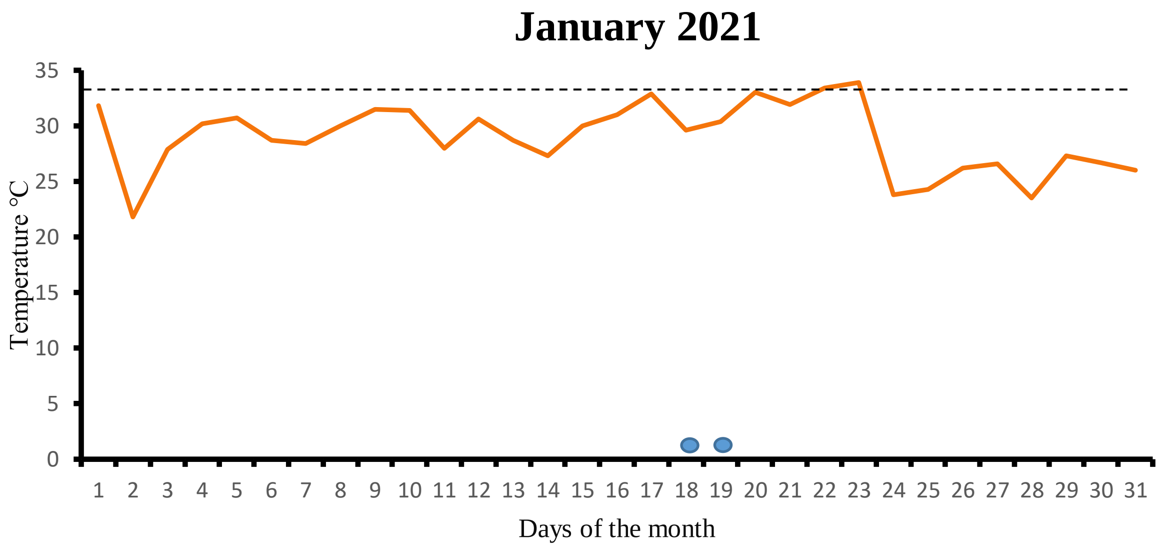
Supplementary data

Supplementary figure 2.1. Graphical representation of the daily temperatures on sampling months 2020/2021. The data collection started in December 2020 (a), maximum daily temperatures on the month of January 2021 (b). The heatwave took place in February 2021 (c). The maximum temperatures during March (d), April (e), May (f) and June (g) 2021 are also plotted. Red circles represents the heatwave sampling days. The orange circles represent the overcast sampling days and the blue circles represent the normal summer sunny day's samplings.

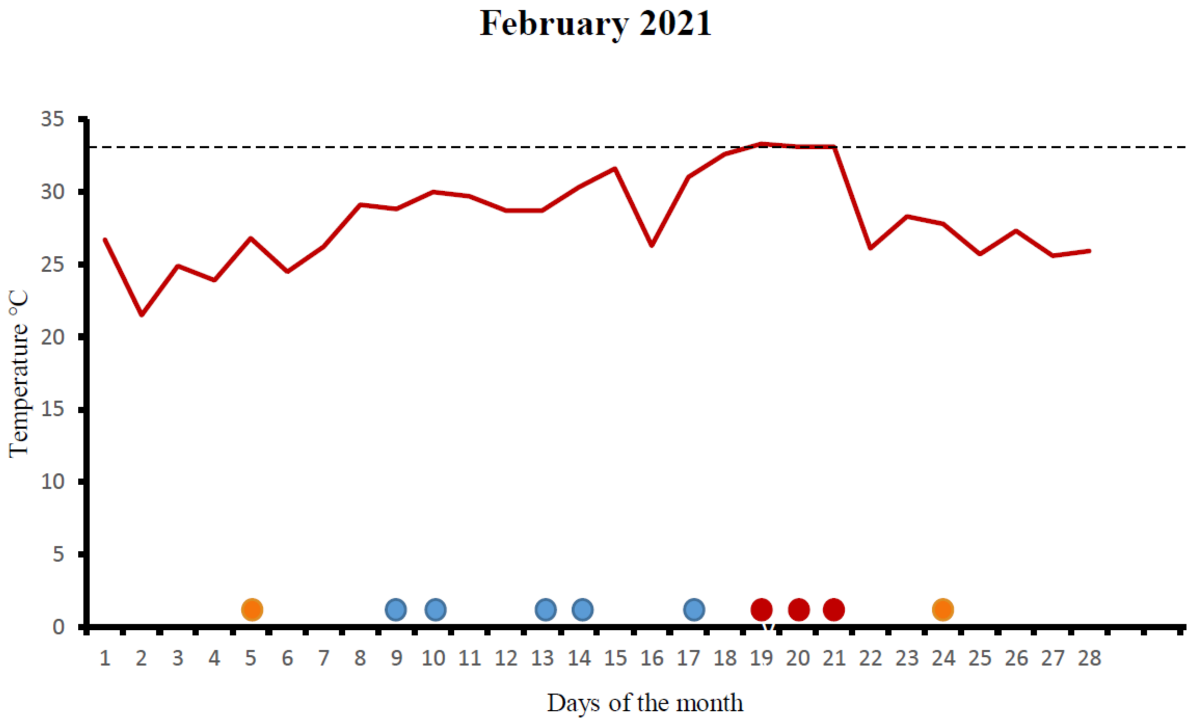
a)



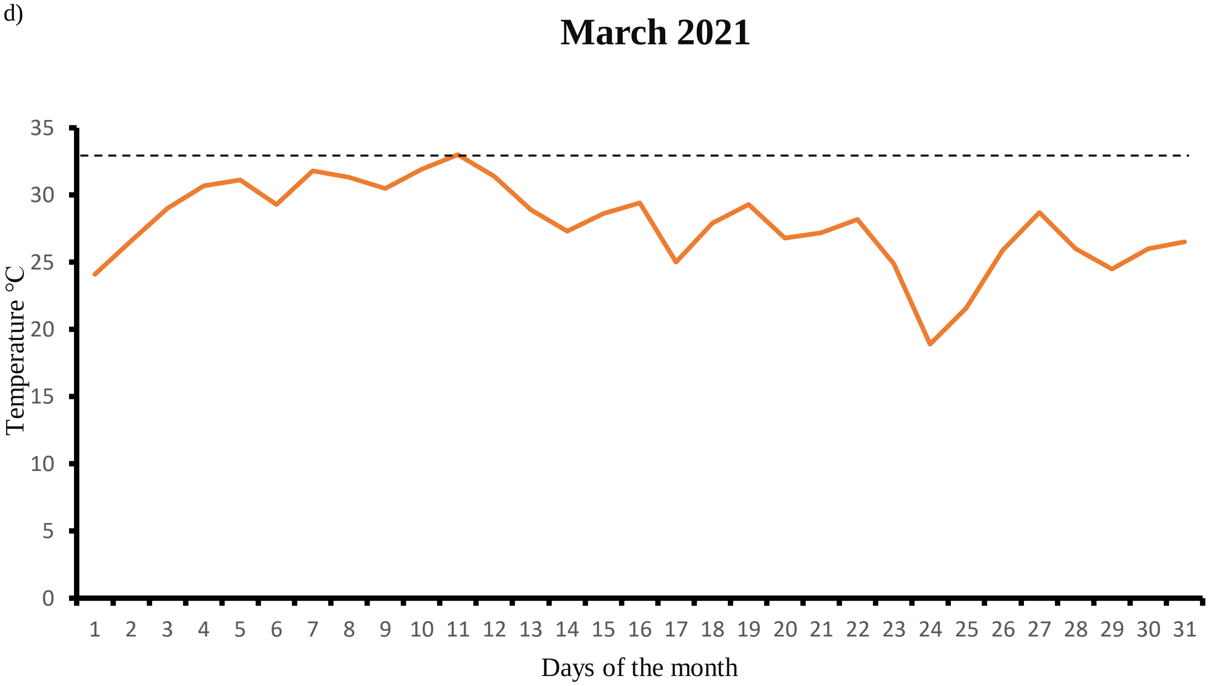
b)


January 2021

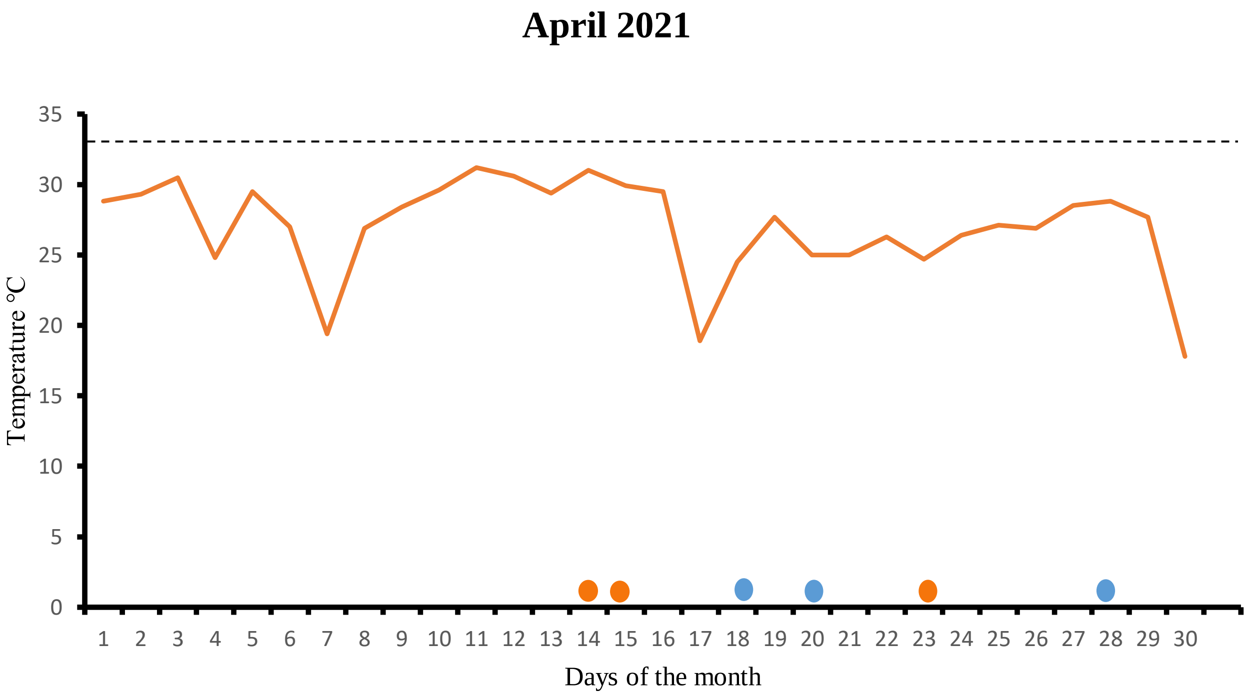
c)


February 2021

d)

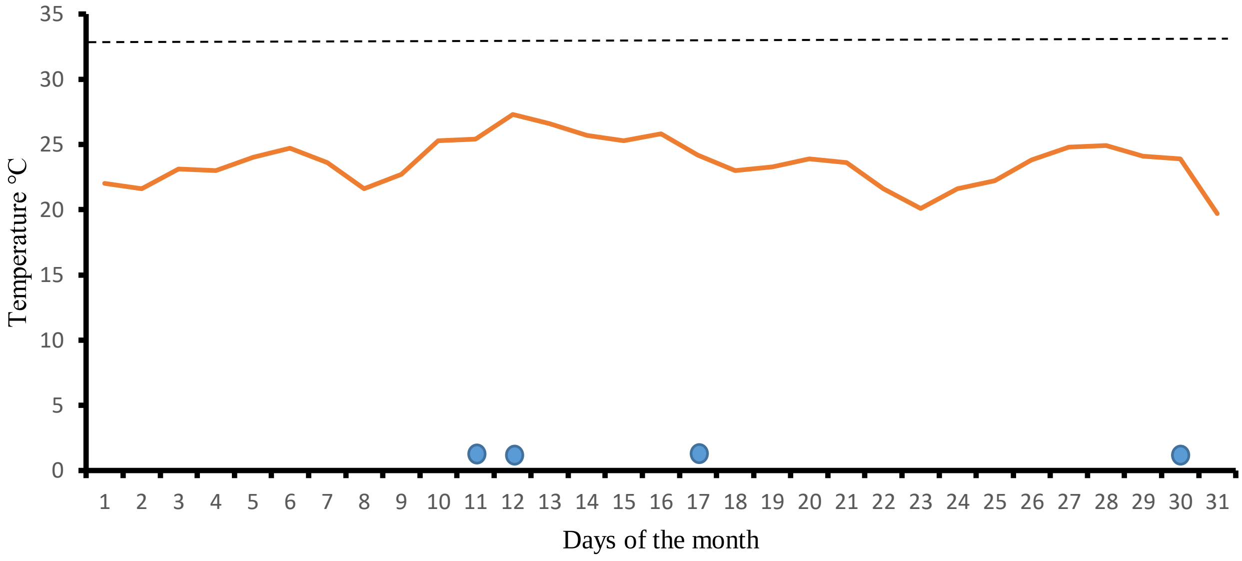

March 2021
Temperature °C
0
5
10
15
20
25
30
35
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Days of the month

e)


April 2021
Temperature °C
0
5
10
15
20
25
30
35
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Days of the month

f)

May 2021



g)

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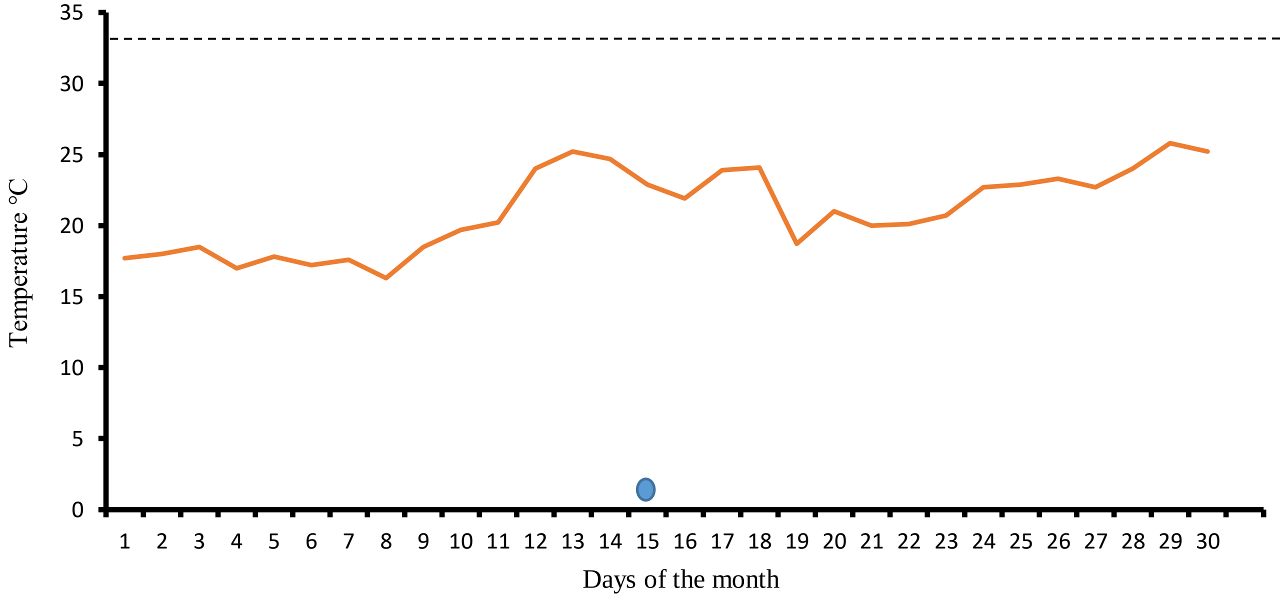


Chapter 3

Concluding Remarks

3.1 Introduction

Sunflowers (*Helianthus annuus* L.) are members of the Asteraceae family. There are 65 species in the *Helianthus* genus. Sunflowers are grown mainly for oil, ornamental purposes and bird feed (Fernández-Luqueño et al., 2014). The sunflower plant is grown worldwide, contributing approximately 8 % to the world oilseed market. During the summer months, high temperatures significantly limit growth in sunflowers. Anthesis is the period when sunflowers are most susceptible to heat stress, resulting in a loss of yield, decreased oil content, and altered fatty acid composition of sunflower seeds sown in spring (Debaeke et al., 2017). Climate change simulations and predictions show that temperatures are going to keep increasing causing an increase in the overall average temperatures, Due to these reasons, extreme weather events such as heat waves will become much more common in the future. Despite a sunflower being viewed as a tolerant plant, temperatures that increase due to climate change will negatively impact sunflower yields unless appropriate adaptation measures are taken (Debaeke et al., 2017).

Through breeding or biotechnology, improved management practices and heat-tolerant varieties can be developed by understanding the mechanisms of plant tolerance to high temperatures (Abdel Razik et al., 2021). Several studies looked at the effects of heat stress on sunflowers before and after anthesis (Khan et al., 2017), but not much specifically focuses on how the timing of reproductive organ development is directly impacted by heat stress ad heat waves. Our study is very important in a sense that it is one of the very first studies that look at heatwave and heat stress effects on the timing of sunflower anthesis and pollination. The main aim of this study was to investigate the different impacts of heats stress and heatwaves on the floral organs in sunflowers during anthesis and pollination.

3.2 Style elongation, pollen emergence and insect visits under high temperatures

The experiments in ***Chapter 2*** were performed under field conditions to ensure pollinator access to flowers. After the styles were harvested and measured at several time points under heat stress conditions, their behavior was compared to that of control styles. At elevated temperatures, styles were found to be elongating at a faster rate than the unheated controls. This was seen in our study for both the artificially heated and natural heatwave styles (Figure 2.1). The faster elongation of the styles caused an earlier release of pollen on anthers. The release of pollen was monitored using timelapse photography taking images every fifteen minutes and it was noted that heated florets had earlier pollen emergence than the unheated control florets. During a natural heatwave there was also a much earlier pollen presentation on florets.

The availability of floral rewards such as pollen may have been the main attractors of pollinators to the flowers. The artificially heated plants in the sunny condition had a few more visitors than the control, even though there were no significant differences (Figure 2.4a). This was due to the fact that both the heated and the unheated were in direct sunlight which has been found to also influence floral temperatures and insects visits (Creux et al., 2021). During overcast conditions, there were noticeable differences of more and earlier insect visitors on the heated than the unheated (Figure 2.4b). Therefore, there was earlier and more insect's visits to the heated and under a natural heatwave plants (Figure 2.4b and c). Insect visits were monitored and counted using a video recorder at different time points of each sampling morning. Sunflowers might have evolved this mechanism to bypass adverse possible effects due to heat stress. The earlier release of pollen earlier in the day could avoid impacts high temperatures have on pollen and other reproductive organs.

3.3 Pollen infertility and stigma receptivity under high temperatures

In our study, there were different results indicating reproductive organs responses to heat stress and high temperatures in the field. The pollen produced was harvested and observed in slides with stains to test for fertility. Pollen grains were found to be significantly affected by artificial heating and heatwave. If pollen grains lose their viability, they are unable to fertilize the ovule, suggesting that fertilization and seed formation do not take place. Male reproductive organs were found to be more sensitive to high temperatures including studies done by Razzaq et al. (2019) and Kalyar et al. (2014). Our study also showed a decrease in pollen fertility with increasing temperatures for both artificial heating and a natural heatwave (Figure 2.7). This is an indication that while sunflowers are considered hardy crops that can tolerate high temperatures our results show that the pollen is sensitive to high temperatures like other plant species. However, instead of having a more temperature resistant pollen grain, it is likely due to their earlier pollen release that accounts for their tolerance to temperature.

Stigmas were harvested and submerged in hydrogen peroxide that bubbled as a measure of stigma receptivity. The analysis of the pictures showed the stigma receptivity to be reduced during artificial heating and interestingly increased during a natural heatwave. The reason could be the increased amounts of ROS and peroxidases that get produced when the plant is under attack (Nandeeshkumar et al., 2008), therefore increasing the overall receptivity of the stigmas as a counter attack strategy for heat stress. Additionally, increased receptivity could be related to the chemical reaction between stigma and hydrogen peroxide, which might be making a faster reaction when the test was conducted with high ambient temperatures. It will be possible to confirm these possibilities by running further experiments in growth chambers with elevated temperatures and drawing concrete conclusions.

3.4 Experimental advantages and limitations

The insect visit results showed no significant differences in the sunny and overcast weather conditions even though there a trend showing an increase in the heated than the unheated. , More insect visits data would real assist in identifying clear differences. If the trend seen in the overcast (heated and control) is significant or not significant. It was important for all of these experiments to take place in the field in order for the results to reflect true effects of high temperatures. The field setting also allowed for the assessment of the heatwave and pollinator which would not have been possible in a lab or growth chamber. Some time points were done a bit later than they should have been due to lockdown regulations, therefore they could not be used because sunflower anthesis begins just before dawn and curfews prohibited in the inclusion of these time points. For this experiment, there was an initial plan to record the timing of bud opening. Due to lockdown/curfew time constraints it could not be done. Therefore, instead of starting time-lapse cameras at ZT 0.0 (dawn), the experiment would be run from ZT -0.5 (before dawn). This should show the whole process of bud opening from a bud stage.

As mentioned above, the plant to use time-lapse photography to capture the timing of bud opening and anther emergence did not work because every time we got to the field we found the buds were already open and anthers had already started emerging. This observation was very unconventional due to the study done by Creux et al. (2021) showed that they observed bud and anther elongation staring at dawn not before that. Our speculation is that the spot light in a distance from the field may be affecting the timing of these events, bud opening and anther emergence. A study by Lobello et al. (2000) found that light regulates the anther filament elongation. Therefore, the speculation is that the spot light in the field is adjusting the timing of these events. An experimental field that would be ideal is the one that is completely free from any light pollution. And then start time-lapse collection from the time when the sunflower buds have just swelled up and have not opened yet. This will give a nice visual representation from when the buds are closed to when they actually open up taking note of the differences between the control and heated.

Increasing the number of replicates in the stigma receptivity experiment would help validate the stigma receptivity increase during a heatwave and the decrease during artificial heating. Redoing this experiment in a greenhouse or chamber experiment under heatwave simulated conditions could shed some light on those validations needed because stigma and hydrogen peroxide together form a chemical reaction. It could be that the outside environment enhances and advances this biological chemical reaction, hence the increased bubbling of the stigmas. In this study, stigmas were collected and put in petri dishes with slides containing hydrogen peroxide. It is possible that the wind might have some sort of a disadvantage or interference when it comes into contact with hydrogen peroxide. Therefore, doing this experiment in a closed setting, controlled growth conditions where wind and external environmental factors have no influence.

As a general impression, the field experiment went well. Being able to experience and assess a natural heatwave in Pretoria during a month of February 2021 really ensured nice and comparable data to artificial heating experiment. The occurrence of a natural heatwave is a huge success of the study because it showed that artificial heating can give us an indication of how plants respond to a natural heatwave due to similarities in our data set. Figure 2.1, 2.3 and 2.7 in ***Chapter 2*** show similar trends between artificially heated and a natural heatwave. Our data sets also allowed for situations that were not comparable, where by artificial heating and a natural heat wave gave different results. This was seen in Figure 2.5 where stigma behaved differently between the artificially heated and a natural heatwave. It is also an important consideration when performing artificial heating experiments because it shows that there are differences between natural and artificial heat. An artificial heating experiment will not exhibit all the changes a plant may undergo during a natural heat wave. Additionally, the study was done under field conditions which allowed for the observation of plant-pollinator interactions. This would not be possible in a controlled growth condition such as a greenhouse or a chamber.

Figure 2.6 shows how pollen viability changes when exposed to elevated temperatures. Pollen might be even less viable under heat stress than what the pollen grains show when observed especially if one test is done to test viability. A study by Endo et al. (2009) suggests that if pollen viability is only tested by one staining technique, it might not be a true reflection of the viability of pollen grains. More tests need to be done to support a staining test so that there is conclusive results. They discovered this because there were pollen grains that seemed viable but failed to germinate on the stigmas.

3.5 Future studies

For future work, it would be good to do this study this under two or more natural heatwave seasons prolonged natural heatwave conditions to actually see how this affects the sunflowers maybe over three planting trials. There is also not enough literature on the effects of high temperatures on reproductive structures of sunflowers during anthesis and pollination. Sunflower reproductive processes might be advancing earlier in the morning so that they avoid pollen exposure to high temperatures. It would therefore be beneficial to understand and explore this change in timing that occurs as an adaptive measure to the plant. Testing heat stressed pollen on normal florets to see if the ovules do get fertilized would help for greater understanding. It would also be good to check if heat stressed pollen does germinate well and to find out if pollinated florets do produce viable seeds at the end.

In light of all of that, exploring the genes and molecular mechanisms underlying the changes in this study by RNA sequencing is a nice project that Miss Roder, an MSc student, has been working on based on this study. She is specifically investigating the light effects on bud opening and anther emergence. It would also interesting to look at a combination of different stresses in sunflowers anthesis and pollination. Such as drought and heat stress together to compare the impacts together and also individually. It would also be useful to assess high osmotic pressure or water logging and see how that affects plant-pollinator interactions.

Another thing would be to collect of volatiles from sunflowers and access the differences between the control, artificially heated and the natural heatwave conditions. Volatiles and scents are some of the environmental cues that pollinators look out for when plants are ready for pollination. A study on volatile emission did show that temperature increases up to 30°C increase volatile release, however temperatures of 35°C and over decreases the emissions and change the volatile compositions thus reducing the activity of floral pigments (Sagae et al., 2008). Thermal desorption gas chromatography time-of-flight mass spectrometry can be used for sampling and analysis of field volatiles (Cheung et al., 2015). How these volatiles changes and how they affect interactions of the pollinators with the flowers during heat stress is important to explore in sunflowers.

There could be similarities or differences in the results and the processes during reproductive stage across different cultivars. The commercial cultivars that were used and tested for both this study and the Creux et al., 2021 study appeared to have similar trends in terms of the observations recorded. In Creux et al, 2021 the cultivar used showed similar observations to our study with a different cultivar, especially in terms of insect visits patterns and the style elongation rate between the heated and unheated ones. The same trend was observed in our study even at higher temperatures with a different cultivar. Oleic and linoleic sunflower cultivars were also found to be very similar in their behavior and response to other environmental conditions due to the same characteristics and traits of both the female and male reproductive organs (Chabert et al., 2020).

However, there are cultivars that could show differences in their trends like cytoplasmic male sterile lines that are used for breeding purposes, but they have no male reproductive organs such as anthers or pollen. The differences in the genetic variation also means that there is no pollen emergence and secondary pollen presentation that is commonly found in Asteraceae plant species (Yamgar et al., 2018). Different sunflower lines such as dwarf sunflowers (sunspot) were found to be unresponsive to the Gibberellic acid, which is a phytohormone that promotes and regulate plant height and flowering time in plants, hence their dwarfism. This means that there is a huge genetic variation, which could also yield different results from tall cultivars of sunflower (Best et al., 2016). Another study by Lobello et al, 2000 showed the important role that Gibberellic acid have in the elongation of the anther filament. This study showed that high levels of GA3 inhibited the anther filament elongation therefore different results could also be expected due to different genetic backgrounds. It would be interesting to further explore how these genetic differences might impact the timing of pollen emergence and pollinator visits.

3.6 Conclusion

Sunflowers are used as catch crops because they are able to be grown under very adverse environmental conditions and yet still produce stable yields even under these adverse environmentally stressing conditions (Shah et al., 2013). Despite this, it produces a lower yield than cash crops like soybeans and maize under good growing conditions, but these crops perform more poorly during adverse weather conditions. The main findings from this study suggest that even a hardy crop such as sunflower still has pollen that is sensitive to heat stress. However, the rapid elongation of the style, earlier release of pollen and earlier pollinators to pollinate the plant are seen as adaptation measures, to avoid the effects of heat stress conditions. The increase of the stigma receptivity during a heatwave weather condition could be another adaptation strategy to deal with elevated temperatures. Therefore, these findings in sunflower could be used in future to help breed pollen that is thermo-tolerant or sensitive. These breeding techniques will be used during climate change as temperatures continue rising to protect yields, therefore securing food systems.

5.7 References

ABDEL RAZIK, E. S., ALHARBI, B. M., PIRZADAH, T. B., ALNUSAIRI, G. S., SOLIMAN, M. H. & HAKEEM, K. R. 2021. γ-Aminobutyric acid (GABA) mitigates drought and heat stress in sunflower (*Helianthus annuus L.*) by regulating its physiological, biochemical and molecular pathways. *Physiologia Plantarum,* 172**,** 505-527.

BEST, N. B., WANG, X., BRITTSAN, S., DEAN, E., HELFERS, S. J., HOMBURG, R., MOBLEY, M. L., SPINDLER, T. L., XIE, B. & ZHANG, M. 2016. Sunflower 'Sunspot'is hyposensitive to GA_3 and has a missense mutation in the DELLA motif of HaDella1. *Journal of the American Society for Horticultural Science*, 141, 389-394.

CHABERT, S., SÉNÉCHAL, C., FOUGEROUX, A., POUSSE, J., RICHARD, F., NOZIÈRES, E., GEIST, O., GUILLEMARD, V., LEYLAVERGNE, S. & MALARD, C. 2020. Effect of environmental conditions and genotype on nectar secretion in sunflower (Helianthus annuus L.). *OCL Oilseeds and fats crops and lipids*, 27, 51.

CHEUNG, W. H., PASAMONTES, A., PEIRANO, D. J., ZHAO, W., GRAFTON-CARDWELL, E. E., KAPAUN, T., YOKOMI, R., SIMMONS, J., DOLL, M. & FIEHN, O. 2015. Volatile organic compound (VOC) profiling of *Citrus tristeza* virus infection in sweet orange citrus varietals using thermal desorption gas chromatography time of flight mass spectrometry (TD-GC/TOF-MS). *Metabolomics,* 11**,** 1514-1525.

CREUX, N. M., BROWN, E. A., GARNER, A. G., SAEED, S., SCHER, C. L., HOLALU, S. V., YANG, D., MALOOF, J. N., BLACKMAN, B. K. & HARMER, S. L. 2021. Flower orientation influences floral temperature, pollinator visits and plant fitness. *New Phytologist,* 232**,** 868-879.

DEBAEKE, P., CASADEBAIG, P., FLENET, F. & LANGLADE, N. 2017. Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe. *OCL Oilseeds and fats crops and lipids,* 24**,** 15 p.

ENDO, M., TSUCHIYA, T., HAMADA, K., KAWAMURA, S., YANO, K., OHSHIMA, M., HIGASHITANI, A., WATANABE, M. & KAWAGISHI-KOBAYASHI, M. 2009. High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology,* 50**,** 1911-1922.

FERNÁNDEZ-LUQUEÑO, F., LÓPEZ-VALDEZ, F., MIRANDA-ARÁMBULA, M., ROSAS-MORALES, M., PARIONA, N. & ESPINOZA-ZAPATA, R. 2014. An introduction to the sunflower crop. *Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases. Valladolid, Spain: Nova Science Publishers***,** 1-18.

KALYAR, T., RAUF, S., TEIXEIRA DA SILVA, J. A. & SHAHZAD, M. 2014. Handling sunflower (*Helianthus annuus L.*) populations under heat stress. *Archives of Agronomy and Soil Science,* 60**,** 655-672.

KHAN, M., RAUF, S., MUNIR, H., KAUSAR, M., HUSSAIN, M. M. & ASHRAF, E. 2017. Evaluation of sunflower (*Helianthus annuus L.*) single cross hybrids under heat stress condition. *Archives of Agronomy and Soil Science,* 63**,** 525-535.

LOBELLO, G., FAMBRINI, M., BARALDI, R., LERCARI, B. & PUGLIESI, C. 2000. Hormonal influence on photocontrol of the protandry in the genus *Helianthus*. *Journal of experimental botany,* 51**,** 1403-1412.

NANDEESHKUMAR, P., RAMACHANDRAKINI, K., PRAKASH, H., NIRANJANA, S. & SHEKAR SHETTY, H. 2008. Induction of resistance against downy mildew on sunflower by rhizobacteria. *Journal of Plant Interactions,* 3**,** 255-262.

RAZZAQ, M. K., RAUF, S., KHURSHID, M., IQBAL, S., BHAT, J. A., FARZAND, A., RIAZ, A., XING, G. & GAI, J. 2019. Pollen viability an index of abiotic stresses tolerance and methods for the improved pollen viability. *Pakistan Journal of Agricultural Research,* 32.

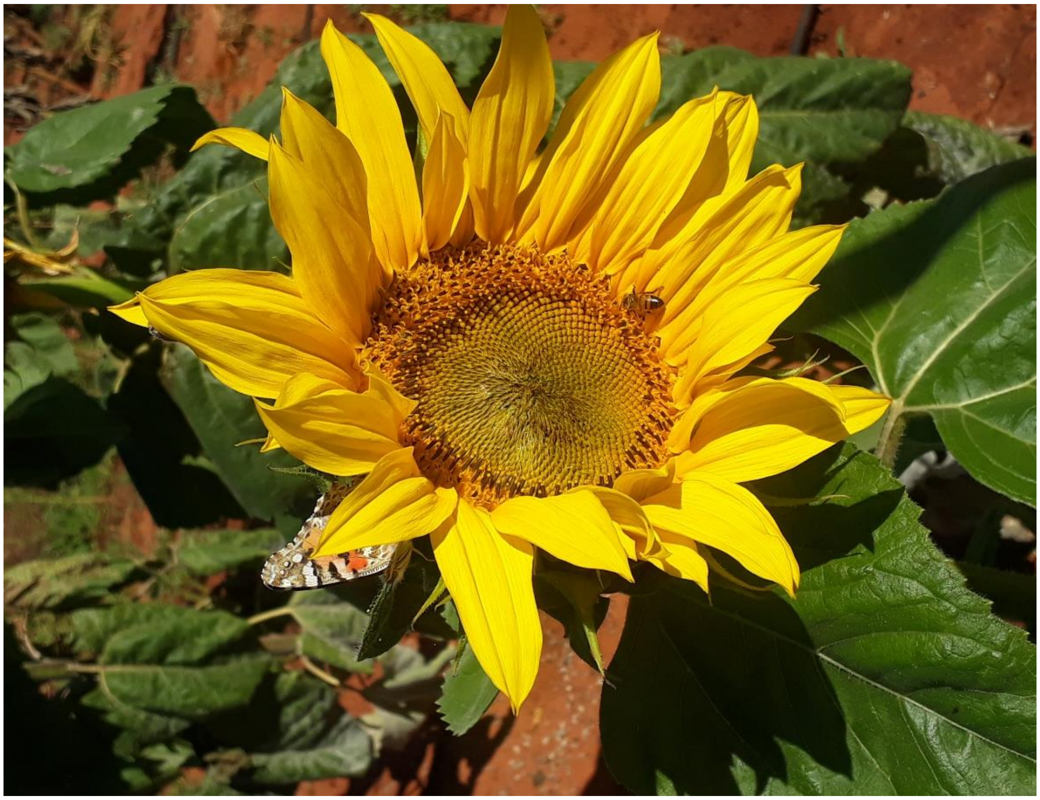
SAGAE, M., OYAMA-OKUBO, N., ANDO, T., MARCHESI, E. & NAKAYAMA, M. 2008. Effect of temperature on the floral scent emission and endogenous volatile profile of Petunia axillaris. *Bioscience, biotechnology, and biochemistry***,** 0711300686-0711300686.

SHAH, N., AUJLA, K., ISHAQ, M. & FAROOQ, A. 2013. Trends in sunflower production and its potential in increasing domestic edible oil production in Punjab, Pakistan. *Sarhad J. Agric,* 29**,** 7-13.

YAMGAR, S., DHONE, P. U., PATHARE, S. M. & KARANDE, G. R. 2018. Cytoplasmic diversity studies in sunflower (*Helianthus annuus L.*): A review. International Journal of Current Microbiology and Applied Sciences, 7, 36-41.

“IT ALWAYS SEEM IMPOSSIBLE UNTIL ITS DONE”

(Nelson Mandela)



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