# EFFECTS OF EXTREME HEAT ON NORTHERN HIGHBUSH BLUEBERRY POLLEN AND THE NATIVE POLLINATOR, *OSMIA LIGNARIA*

By

Jenna Marie Walters

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology – Doctor of Philosophy
Ecology, Evolution, and Behavior – Dual Major

2024

## ABSTRACT

Extreme heat poses a major threat to flowering plants and bee pollinators, yet the direct and indirect consequences of heat stress are not well understood, particularly for spring-blooming crops and native solitary bees. Pollen germination and tube growth are highly sensitive to extreme heat, yet few studies have determined the effects of extreme heat on pollen performance of spring-blooming perennial crop plants. To understand how northern highbush blueberry (*Vaccinium corymbosum* L.) pollen responds to different temperatures, pollen germination and tube growth were measured *in vitro* using four cultivars ('Bluecrop', 'Elliott', 'Jersey', and 'Liberty') at temperatures between 10-40°C and 30-40°C. Recovery from high heat was also tested in 'Bluecrop' pollen to determine whether pollen tubes can still germinate and grow after short bursts of extreme heat. Across all tested cultivars, the greatest germination success and longest pollen tubes occurred at 20 and 30°C and the lowest at 10 and 40°C, with nearly complete inhibition at 40°C. Significant reduction in pollen germination and tube growth occurred at temperatures at and above 35°C across all cultivars and assessment times. Exposure to 37.5°C for only 4 hours resulted in substantial reductions in pollen germination and tube growth, even after pollen was moved to optimal conditions of 25°C. These results demonstrate that exposure to extreme heat, even for a short duration, can significantly inhibit pollen germination and tube growth and may have cascading effects on fruit set and crop yields. To determine whether extreme heat affects blueberry fruit set and berry parameters, 'Bluecrop' bushes at several floral bud development stages (tight bud, bud swell, bud break, early pink bud, late pink bud) were exposed to heat stress (37.5°C) or normal (25°C) conditions for 4 hours and flowers were hand-pollinated at anthesis. Fruit set was significantly lower when heat treatment was applied at bud swell, but there were no clear patterns for pre-bloom heat stress affecting fruit quality. Given the negative consequences of extreme heat on blueberry pollen, I investigated whether extreme heat exposure to host plants also affects the behavior, fecundity, development, or survival of *Osmia lignaria*, a native solitary bee pollinator of blueberry. A no-choice semi-field cage experiment was conducted to provide female *O. lignaria* bees with host plants (blueberry, lacy phacelia, white clover) previously exposed to extreme heat (37.5°C) or normal temperatures (25°C) for 4 hours at 25% bloom. Despite a similar number of open flowers and floral visitation between the two temperature treatments, female bees provided heat stressed plants laid significantly fewer eggs. Progeny were provided similar quantities of pollen provisions between the two temperature treatments, yet larvae consuming pollen from heat

stressed plants had significantly lower survival as larvae and adults. Delayed emergence and reduced adult longevity were observed when larvae consumed heat stressed pollen. This study is the first to document how short, field-realistic bursts of extreme heat exposure to flowering host plants can indirectly affect bee pollinators and their offspring, with important implications for crop pollination and native, solitary bee populations. Given the consequences of extreme heat on northern highbush blueberry pollen performance and *O. lignaria* bee fecundity and survival, poor pollen nutrition is a potential mechanism driving these responses. To determine whether extreme heat affects the nutritional content of blueberry pollen, 'Bluecrop' pollen was collected from bushes exposed to extreme heat (37.5°C) or normal temperatures (25°C) for 4 hours at five development stages. Exposure to extreme heat had variable consequences for pollen nutrients across floral development stages. Pollen starch content was significantly reduced following heat stress at the tight bud stage. No significant differences were observed for glucose or sucrose. These results suggest that exposure to heat had stronger effects on pollen starch content and may have disrupted sugar metabolism, utilization, or transport, with potential negative consequences for pollen performance and bee nutrition. Pollen protein was significantly reduced at bud swell following heat exposure, but no significant effects were observed at other development stages. Total amino acid content was significantly lower at bud swell following heat stress, driven by reductions in aspartate, glutamate, methionine, proline, and lysine, potentially explaining the observed negative consequences for pollen performance, fertilization, bee fecundity, and brood development. When comparing essential and non-essential amino acids, exposure to extreme heat had no significant effects on essential amino acids yet it significantly reduced non-essential amino acids at bud swell. Some essential amino acids were increased in response to heat stress, with potentially conflicting effects on plant production and bee nutrition. Overall, these findings suggest the greatest heat sensitivity in blueberries occurs at bud swell, with negative consequences of brief extreme heat stress on pollen performance, fruit production, and bee health. This research provides novel insight into the effects of extreme heat in blueberry pollination systems and suggests that studies evaluating the direct effects of heat stress on bees or plants may have underestimated the consequences for plants, bees, and their interactions.

*This dissertation is dedicated to my mom and dad, for loving me fiercely, believing in me, and instilling the importance of integrity, passion, and empathy in everything I do.*

## ACKNOWLEDGEMENTS

Thank you to my partner Ryan, who has celebrated and supported me at every stage of my graduate studies. Thank you for reminding me to rest, explore, and laugh (even when I didn't want to), for making sure there were always fresh flowers in our home, and for embracing the uncertainty of life in wherever it may take us. Thank you to my mom, who loves deeper than anyone I've ever known, for believing in me always. For teaching me that respect is earned, not given, and to proudly use my voice to stand up for what I believe in. Thank you to my dad, for showing me unwavering love, support, and pride for who I am. For being my rock and always giving me a safe space to land. Thank you to my sibling Wes, for teaching me to do everything honestly and earnestly, even when it's hard. For being my lifelong best friend and making me laugh harder than anyone I know. Thank you to my best friends, Julia, Jade, Caitlin, and Hunter for helping mold me into who I am today. For knowing when to make me laugh and when to let me cry, and to happily be there regardless.

Thank you to Rufus for exceeding all expectations at an advisor and mentor. For encouraging me to explore grandiose ideas and fueling my excitement for science. For answering panicked phone calls in the middle of the night when freezers break or greenhouses overheat, meeting my frustration but always providing solutions. For amplifying my voice and the voices of so many, celebrating disruption and change for the better. For showing me the importance of doing science to support community, whether it be farmers, neighbors, or bees. For pushing me to live life to the fullest even when there's deadlines, because there are always deadlines. Following your lead, I will always do science with kindness, passion, curiosity, and joy.

This research would not have been possible without the excellent mentorship, support, and technical assistance of so many people over the past 6 years. Thank you to my wonderful committee members, Drs. Zsofia Szendrei, Greg Lang, and Pat Edger for your guidance and enthusiasm over the past several years. For encouraging me and refining my skills, preparing me to be the best scientist I can be. Thank you to Ronnie Miller and Lauren Goldstein for growing with me during our graduate studies and becoming my lifelong friends. The unexpected, unconditional love, support, and laughter over the past several years in the Isaacs lab is something I will always cherish. Thank you to Jackie Perkins, Phil Fanning, Kelsey Graham, and James Santiago for your mentorship, guidance, and friendship- I would not have been able to navigate academic research without you. Thank you to my previous academic mentors, Zsofia Szendrei and

Lars Brudvig, for believing in me and fostering my love for ecology as a young student, preparing me for a future in research. Thank you to Thomas Sharkey, James Santiago, and Sean Weise who were excellent collaborators, uplifting my interest in biochemistry and sharing their time, expertise, and resources that made the pollen nutrition analyses in Chapter 5 possible. Thank you to McKenna Barlass and Robin Fisher who provided dedicated, passionate assistance over several years- this research would not have been possible without you, and having the opportunity to be your mentor is one of my greatest accomplishments of my Ph.D.

Thank you to Ronnie Miller, Henry Pointon, Andie Veach, Alexandrea Peake, Michael Killewald, Madeline Henrickson, Steve Van Timmeren, Joy Johnson, Andrew Jones, and Mia Bianchi for technical assistance across various projects. Thank you to the many Michigan State University facility managers, including Chrislyn Particka, Anthony Schilmiller, Tony Boughton, and Mitchell Fox for your dedicated care and assistance enabling my research. Thank you to Sichao Wang for your patient, dedicated advice on statistical analyses. Thank you to Lisa DeVetter for your support and quick responses to any and all horticultural questions. Thank you to Timothy Miles for providing facilities and expertise to make agar plates, and Douglas Landis for access to environmental chambers. Thank you to the Entomology business office staff, especially Heather Lenartson-Kluge and Linda Gallagher, and IT specialist, Lee Duynslager, for always answering questions and making sure things run smoothly in the background. Thank you to the many custodial staff for maintaining our building, allowing me to focus on research.

I would also like to acknowledge the Specialty Crop Research Initiative of the National Institute of Food and Agriculture within the United States Department of Agriculture (USDA SCRI) for financial support of this research within the project titled Optimizing Blueberry Pollination To Ensure Future Yields (Grant 2020-51181-32155). I also thank Michigan State University for funding, including Project GREEEN, the Roger and Barbara Hoopingarner Endowed Graduate Fellowship, and the Dissertation Completion Fellowship from the College of Agriculture and Natural Resources for their generous support of this research.

Lastly, thank you to all those who did not believe in me. You've taught me the importance of perseverance and self-worth and have shown me that I am capable of so much more than I ever could have imagined.

## TABLE OF CONTENTS

# CHAPTER 1. DIRECT AND INDIRECT EFFECTS OF EXTREME HEAT ON POLLINATION SYSTEMS

## 1.1 INTRODUCTION

Climate change is increasing the intensity and frequency of extreme heat events with negative implications for plants, pollinators, and their interactions (Zinn et al., 2010; Hatfield and Prueger, 2015; Mesihovic et al., 2016; Müller and Rieu, 2016; Settele et al., 2016; Vanderplanck et al., 2019; IPCC 2018; Walters et al., 2022; IPCC 2023). Exposure to extreme heat, or temperatures 5-10°C above an organism's critical development threshold for a sufficient amount of time, results in direct heat stress (Wahid et al., 2007) with adverse consequences for bee and plant physiology (Bordier et al., 2017; Hamblin et al., 2017; Chaturvedi et al., 2021; Zhao et al., 2021; Zhu et al., 2021), capacity for acclimation (Martinet et al., 2015; Mesihovic et al., 2016; Oyen and Dillon, 2018; Martinet et al., 2021a; Gonzalez et al., 2022a, b; Hernández-Fuentes et al., 2023; Sepúlveda and Goulson, 2023), and reproductive potential (Vanderplanck et al., 2019; Amuji et al., 2020; Lohani et al., 2020; Zhao et al., 2021; Campion et al., 2023). When extreme heat events occur, both bees and plants can endure heat stress that results in compounding, interactive ramifications for these organisms. Studies on direct and indirect effects of extreme heat on bees, plants, and their interactions are currently limited (but see Greenop et al., 2020; Descamps et al., 2021; Hemberger et al., 2023), resulting in an incomplete understanding of extreme heat repercussions for pollination systems. There is a need for more research on the direct and indirect consequences for bees and crop plants that are particularly at risk from high temperatures, including native, solitary bee species and spring-blooming perennial crops.

### 1.1.1 Direct effects of extreme heat on plants, flowers, and pollen

In contrast to gradual temperature increases (>1.5-3°C) that are the focus of most climate change research and policies, extreme heat is increasingly recognized as a major driver of stress in natural and managed systems experiencing the effects of climate change. These extreme events leave little time to acclimate and can adversely affect plant functioning and survival across all development stages. For example, severe and sometimes irreversible reductions in photosynthesis can occur following exposure to extreme heat, even for just a few hours (Hüve et al., 2011; Feller and Vaseva, 2014; Moore et al., 2021). Reduced photosynthetic activity due to heat stress has been attributed to the deactivation of Rubsico, the key enzyme for $CO_2$ assimilation during photosynthesis, limiting electron transport activity and ATP synthesis in plants (Salvucci and

Crafts-Brandner, 2004; Perdomo et al., 2017; Waheeda et al., 2023). Rubisco requires Rubisco activase for its activation, but this is highly sensitive to heat and can be negatively affected by temperatures above 30°C (Salvucci and Crafts-Brandner, 2004; Sharkey, 2005; Waheeda et al., 2023). Such reductions in photosynthesis have negative consequences for entire plants, limiting the metabolism and transport of nutrients required for plant growth, reproduction, and survival (Moore et al., 2021).

All developmental stages in plants are potentially affected by heat stress, but reproductive development (floral development, pollination and fertilization) is more sensitive to heat than vegetative development (Zinn et al., 2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Raja et al., 2019; Lohani et al., 2020; Soroye et al., 2020; Chaturvedi et al., 2021; Moore et al., 2021). Furthermore, certain stages of plant sexual reproduction are more sensitive to heat than others. Reproductive development occurs in three general stages: gametophyte development (from meiosis to pollination), progamic phase (from pollination to zygote formation), and embryo development (from zygote to seed) (Snider and Oosterhuis, 2011). During progamic development, a series of coordinated processes must occur for successful fertilization, where anthers release mature pollen grains to be transferred to a receptive stigma surface. These adhered pollen grains germinate and penetrate the stigma surface, and pollen tubes grow up the style of the flower towards the ovules (Snider and Oosterhuis, 2011). Thus, viable pollen and a receptive stigma are required for fertilization, yet these processes can be disrupted following brief bouts of heat stress. While female floral organs (stigma, style, and ovary) are considered less sensitive to heat stress than male floral organs (Lohani et al., 2020), under natural conditions heat stress will simultaneously affect both reproductive tissues and can have additive negative repercussions on fertilization success. When extreme heat occurs during floral development, maturation, and/or dehiscence, it can have compounding adverse consequences. For example, extreme heat exposure during flower development can result in fewer or morphologically abnormal flowers with altered or atypical arrangement of petals (Descamps et al., 2018; Chen et al., 2019b; Amuji et al., 2020; Lohani et al., 2020; Alsamir et al., 2021; Abdellatif et al., 2022) as well as abnormalities in the size, length, and number of stamens and pistils (Descamps et al., 2018; Lohani et al., 2020; Matsuda and Higuchi, 2020; Alsamir et al., 2021).

Among the gametophyte and progamic phases, greater sensitivity to heat extremes have been observed during pollen development, pollen germination, pollen tube elongation, and

fertilization, where even a few hours of extreme heat exposure can be detrimental (Zinn et al., 2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021). Pollen development occurs in two sequential stages, microsporogenesis and microgametogenesis (Fadón et al., 2019). During microsporogenesis, meiotic division occurs forming the tetrads of haploid microspores and during microgametogenesis, microspores enlarge and differentiate as pollen grains following haploid mitotic divisions (Carrizo García et al., 2017; Fadón et al., 2019). While these processes occur within a few days in annual plants, woody perennial plants require several months to complete this development as they undergo a dormancy period in the winter (Koltunow et al., 1990; Smyth et al., 1990; El-Ghazaly and Grafström, 1995; Julian et al., 2011; Mirgorodskaya et al., 2015; Fadón et al., 2019). Heat stress during floral development can also result in the degradation of the tapetum (i.e., innermost cell layer lining the anther locule), failure to release microspores, altered metabolism and transport of nutrients in pollen, reduced pollen viability, poor anther dehiscence and failure to release pollen (Snider and Oosterhuis, 2011; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021; Kumar et al., 2022). When heat inhibits nutrient sequestration in developing pollen grains, concentrations of carbohydrates, proteins, lipids, and amino acids can be reduced or altered (Borghi and Fernie, 2017; Borghi et al., 2019; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021). Many of these nutrients drive reproductive processes including pollen germination and tube growth, which are necessary for fertilization, so this depletion of nutrients can reduce pollen quality, performance, and subsequent reproduction (Borghi and Fernie, 2017; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021; Kumar et al., 2022).

Synergistic effects can occur when female floral organs are also exposed to heat stress, reducing soluble carbohydrates and ATP content in pistils, further inhibiting pollen tube growth and fertilization (Snider et al., 2009). Initially, pollen grains rely on pre-existing carbohydrate reserves to fuel pollen tube growth, but eventually utilize carbohydrates provided by the transmitting tract of the style (Herrero and Arbeloa, 1989; Zinn et al., 2010), emphasizing the potential for additive effects of heat stress experienced by male and female floral tissues. Rapid reductions in stigmatic receptivity are also reported following heat stress, inhibiting support for pollen penetration, germination, and adhesion (Hedhly et al., 2003, 2005). Heat stress during female gametophyte development can also decrease and malform ovules, desiccate the stigma and style, and lead to ovule abortion (Snider et al., 2009; Lohani et al., 2020), further perpetuating

fertilization failure in addition to poor pollen performance. Understanding the adverse effects of extreme heat on pollen performance (i.e., pollen germination and pollen tube growth) and stigmatic receptivity is important given their sensitivity to high temperatures and essential role in fertilization and crop yields (Snider and Oosterhuis, 2011; Mesihovic et al., 2016).

A recent focus of research in this field is evaluating the consequences of heat stress at different floral and male gametophyte (pollen) development stages, including specific stages during microsporogenesis and microgametogenesis (Lohani et al., 2020). During microsporogenesis, meiotic division forms the tetrads of haploid microspores, followed by microgametogenesis when the microspores enlarge and differentiate as pollen grains following haploid mitotic divisions (Carrizo García et al., 2017; Fadón et al., 2019). The consequences for mature pollen varies greatly depending on when heat is applied during these developmental processes (Lohani et al., 2020) but is relatively unknown for many different crop species. In barley florets, exposure to heat stress during the pre-meiotic stage of development resulted in stunted anther development, failure to produce pollens grains, and altered progression of microspore mother cell (MMC) meiosis, while heat stress during meiosis limited starch accumulation in pollen grains (Sakata et al., 2000; Draeger and Moore, 2017). Other studies have found that barley fertility is most sensitive to heat stress during the MMC stage (early pollen development) and the pollen mitosis stage (late pollen development) (Callens et al., 2023). In *Arabidopsis* flowers exposed to 42°C for 4 h, greater sensitivity was reported during the MMC stage (early pollen development) and during anther dehiscence (late pollen development), both of which had significant reductions in fruit set (Kim et al., 2001). These reports, and the preceding studies discussed above, highlight that the effects of brief extreme heat are variable for different flower development stages, while also showing that greater research effort has been focused on annual than perennial crops.

Most studies of how extreme heat affects crop pollination have focused on high acreage annual crops (Hatfield and Prueger, 2015; Mesihovic et al., 2016; Raja et al., 2019). High temperatures (35-40°C) inhibit pollen function in several crops, including rice (*Oryza* spp.) (Satake and Yoshida, 1978; Zhang et al., 2018), cotton (*Gossypium hirsutum*) (Masoomi-Aladizgeh et al., 2021), and tobacco (*Nicotiana tabacum*) (Parrotta et al., 2016). The timing of heat exposure, as well as the reproductive organs enduring heat stress, can also have substantial influence on fertilization success and yield. In one study, tomato (*Solanum lycopersicum*) seed and fruit production were completely inhibited when pollinated using pollen developed under heat stress

conditions despite the plant being grown in optimal conditions (Peet et al., 1998; Snider and Oosterhuis, 2011). In the same study, when tomato plants were exposed to heat stress conditions and pollinated with pollen developed under optimal conditions, fruit set and seed production were similar to control plants (Peet et al., 1998; Snider and Oosterhuis, 2011). Similar findings have been reported in maize (*Zea mays*), where exposing pollen to 40°C for 4 h prior to pollination resulted in complete inhibition of fertilization, even though spikelets were maintained under optimal conditions for the remainder of the experiment. When heat stressed spikelets were pollinated with non-stressed pollen, only 43% fertilization was observed (Dupuis and Dumas, 1990; Snider and Oosterhuis, 2011). For these species, and several other annual crops, it is well established that pollen performance and fertilization are particularly sensitive to high heat applied in short bursts (Zinn et al., 2010; Mesihovic et al., 2016; Raja et al., 2019). Far less is known about the tolerance of perennial fruit crops to heat extremes, perhaps because most are grown in climates where hot weather conditions during bloom were very rare. As extreme heat becomes more common during the spring season (IPCC 2021), when many perennial crops are blooming, it will be important to understand the thermal limits of the pollination processes in these crop species.

Studies of plants that flower earlier in the season indicate greater sensitivity to heat stress than summer flowering species (Hedhly et al., 2009). For spring-blooming crops including strawberry (*Fragaria* x *ananassa*), sweet cherry (*Prunus avium*), peach (*Prunus persica*), and apricot (*Prunus armeniaca*), optimal temperatures for pollen growth and performance are similar to temperatures historically experienced during the spring, typically between 20 and 30°C (Cerovlć and Ružić, 1992; Austin et al., 1998; Hedhly et al., 2004, 2005; Kozai et al., 2004; Ledesma and Sugiyama, 2005). At temperatures 5-10°C above this optimum range, pollen viability and performance can be negatively affected. For example, in several genotypes of *Pistacia* spp., pollen germination rates decreased rapidly as temperatures increased from 25°C to 35°C and no pollen germination was observed when exposed to 40°C for 24 h (Acar and Kakani, 2010). In strawberries, pollen germination was significantly reduced when exposed to 30°C for just 4 h compared to a normal temperature of 23°C (Ledesma and Sugiyama, 2005). Ledesma and Kawabata (2016) exposed strawberry flowers to 42°C for 4 h at various flower ages, from 12 days before anthesis to anthesis (at 3-day increments), and found that the degree of damage to fruit set and fruit quality varied by flower age, flower location, and cultivar (Ledesma and Kawabata, 2016). In the ‘Nyoho’ strawberry cultivar, total fruit set was significantly reduced when heat was

applied 9, 3, and 0 days before anthesis and at 12, 9, 3, and 0 days before anthesis for the 'Toyonoka' cultivar, indicating greater sensitivity to heat stress at earlier and later floral development stages (Ledesma and Kawabata, 2016). At earlier development stages, strawberry flowers were at the MMC development stage and at later development stages, flower anthers were undergoing dehiscence (Ledesma and Kawabata, 2016). They also evaluated fruit weight and size following heat exposure and found that 'Toyonoka' flowers were most sensitive at earlier and later floral development stages with reduced fruit weight and size (Ledesma and Kawabata, 2016). Interestingly, while the 'Nyoho' cultivar showed heat sensitivity in fruit set at early floral development stages, it had no significant effect on the size and weight of berries (Ledesma and Kawabata, 2016). The results from this study highlight the importance of evaluating various floral development stages to heat stress to capture the full effects on flowering crop plants, particularly those most at risk, including spring-blooming perennial crops. The temperatures that negatively affect plant reproduction vary among crop type (e.g., annual vs. perennial), by species, and even genotype within species (Hedhly et al., 2005; Hamidou et al., 2013; Lohani et al., 2020) so there is a need to understand how high heat affects development of many modern crops and their cultivars, particularly those most at risk, including spring-blooming perennial crops.

### 1.1.2 Northern highbush blueberry (*Vaccinium corymbosum* L.)

Northern highbush blueberry, *Vaccinium corymbosum* L. (Ericales: Ericaceae), is a spring-blooming, woody perennial crop native to eastern North America, historically grown in regions with cold winters and mild summers (Retamales and Hancock, 2012; Lobos and Hancock, 2015). As with most *Vaccinium* species, blueberries are highly dependent on wild pollinators for fertilization, seed set, and maximizing yields (Tuell et al., 2009; Gibbs et al., 2016; Pinilla-Gallego and Isaacs, 2018; DeVetter et al., 2022). While native to eastern North America, northern highbush blueberries are now cultivated globally in regions that experience mild, moist summers and cold winters, including Australia, Africa, China, Europe, South America, and across North America (Retamales and Hancock, 2012; Retamales and Hancock, 2018). In the US, northern highbush blueberry production primarily occurs in the Pacific Northwest, the Southeast, Midwest, and in New Jersey (Retamales and Hancock, 2018). In Michigan, common and commercially important cultivars include 'Aurora', 'Bluecrop', 'Draper', 'Jersey', 'Liberty', 'Elliott', 'Legacy', and 'Nelson' (Retamales and Hancock, 2012; Retamales and Hancock, 2015; Vander Weide et al., 2024). The ever-growing international production of highbush blueberry yields over 650 million

tons annually (Retamales and Hancock, 2018). Northern highbush blueberry cultivars are well-adapted for cold conditions, requiring 800 to 1000 hours of winter chilling (temperatures below 2°C) for proper floral bud development, and they can withstand mid-winter temperatures below -20ºC (Retamales and Hancock, 2018). Blueberries also require well-drained, acidic soil with ample moisture for optimal growth and production (Retamales and Hancock, 2018). Northern highbush blueberry plants are composed of several canes emerging from the crown of the plant, which become woody during the second season of growth, and can be as tall as 1.8-4.0 m (Retamales and Hancock, 2018). Shoots emerge from these canes and, after at least one year of growth, floral and vegetative buds begin to develop on these shoots (Retamales and Hancock, 2018). Cane productivity (i.e., number of flower buds per cane) increases with cane diameter and cane age (Palma et al., 2023).

The inflorescence of blueberry is a raceme, where the corolla of fused petals is inverted and urn-shaped, typically white to pinkish-white when fully open (Retamales and Hancock, 2018). The pistil, containing the stigma and style of the flower (i.e., female floral organs) can be longer or shorter than the corolla, and varies depending on the cultivar (Courcelles et al., 2013; Retamales and Hancock, 2018). The ovary of the blueberry flower is inferior with four to five locules and many ovules per locule (Retamales and Hancock, 2018). At the base of the corolla, surrounding the style, are eight to ten stamens, each of which contain an anther and filament (i.e., male floral organs). Blueberry anthers are poricidal, releasing pollen when disturbed via sonication or vibration, typically induced by a bee pollinator. Blueberry pollen tetrads are developed and stored in these poricidal anthers until flower opening (i.e., anthesis), after which pollen is mature and dehydrated, ready for pollination. Depending on temperature, individual blueberry flowers remain open for about a week. However, high spring temperatures can accelerate blueberry bloom timing and hasten petal drop, limiting the window for pollination under elevated temperature conditions (Chabert and Mallinger, in prep.). Blueberry floral morphology can significantly affect pollinator attraction and activity and, in turn, affect fruit set and berry weight (Sampson et al., 2013; DeVetter et al., 2022). Depending on temperature conditions, blueberry pollen is most viable 24 hours after flower opening, or within the first five days of flower opening, and stigmas are receptive to pollen for up to 5-8 days following anthesis (Retamales and Hancock, 2018). However, fruit set is considerably reduced if pollination is delayed by 3 to 4 days (Retamales and Hancock, 2018). When viable blueberry pollen is deposited on a receptive stigma by bee pollinators, pollen tubes

will germinate and grow toward the ovary for fertilization, typically reaching floral ovules 24-72 h following pollen deposition (Dogterom et al., 2000).

Fewer outcrossed pollen tetrads are needed for stigmatic saturation than selfed ones in blueberry, but once germinated, both grow in the style at the same rate (Retamales and Hancock, 2018). Fertilized ovaries develop seeds which affect blueberry fruit set and size, and thus, affect yields (Dogterom and Winston, 1999; Dogterom et al., 2000; Gan et al., 2020). Blueberry fruit grow in three stages: Stage I berries undergo rapid cell division and dry weight gain (Birkhold et al., 1992; Cano-Medrano and Darnell, 1997a; Cano-Medrano and Darnell, 1997b) lasting from 25-35 days, Stage II berries go through and active period of seed development, but little fruit growth occurs, typically between 30-40 days (Edwards et al., 1972), Stage III berries go through rapid fruit growth for about 30-60 days, where sugars accumulate and the berry turns from green to blue as anthocyanins accumulate (Eck and Stretch, 1986; Birkhold et al., 1992; Cano-Medrano and Darnell, 1997b). In 'Jersey', the percentage of total soluble sugars (TSS) increased for 6 days following color change then leveled off while titratable acidity (TA) decreased continually during berry ripening, resulting in a steady increase in the ratio of sugar:acid during berry ripening (Retamales and Hancock, 2018). In northern highbush blueberry, fruit development ranges from 42-90 days (Darnell, 2000). Several berry quality metrics are assessed when determining the success of pollination, marketability of fruit, and other berry traits that affect yield, and vary by cultivar, region, pollen donor (i.e., selfed or crossed) and other abiotic conditions experienced during bloom (i.e., temperature, humidity). Common parameters determining fruit quality include fruit set, berry weight, berry diameter, % TSS, % TA, the number of fertilized seeds, and the % fertilized seeds. In common northern highbush blueberry cultivars, including 'Aurora', 'Bluecrop', 'Elliott', 'Draper', 'Legacy', 'Nelson', percent fruit set can vary from 64 to 94% (Krebs and Hancock, 1988; Ehlenfeldt and Prior, 2001; Kim et al., 2013; Gündüz et al., 2015; Strik et al., 2017; Retamales and Hancock, 2018; Strik and Vance, 2019), berry weight (g) can vary from 0.92 to 2.75 g (Krebs and Hancock, 1988; Ehlenfeldt and Prior, 2001; Strik et al., 2017; Retamales and Hancock, 2018), berry diameter (mm) can vary from 14.97 to 17.75 mm (Jorquera-Fontena et al., 2017; Lin et al., 2020), % TSS can vary from 9.8 to 15.6 (Yang et al., 2009; Kim et al., 2013; Gündüz et al., 2015; Strik et al., 2017; Retamales and Hancock, 2018), % TA can vary from 0.46 to 2.7 (Kim et al., 2013; Gündüz et al., 2015; Retamales and Hancock, 2018), and the number of fertilized seeds per fruit can vary from 4.7 to 65 (Krebs and Hancock, 1988; Ehlenfeldt and Prior,

2001; Retamales and Hancock, 2018; Strik and Vance, 2019). Fertilized seeds are typically plump and dark brown while those that were not fertilized are small, lighter brown or white, and collapsed (Desjardins and Oliveira, 2006). Blueberry fruit are true berries with many seeds and typically ripen 2-3 months after pollination (Darrow, 1958; Retamales and Hancock, 2018). Under optimal conditions, including good weather and ample pollination services by bees, highbush blueberry has the potential to set nearly 100% of its flowers into fruits (Ehlenfeldt and Prior, 2001; Kumarihami et al., 2021; DeVetter et al., 2022).

Blueberry floral bud initiation begins in late summer and development continues into the fall. Gough et al. (1978) described floral organ development in the 'Bluecrop' cultivar and provides a limited description of gametophyte development over time. In the fall (early November), microspore mother cell formation begins with some separation of sporogenous tissue but pollen development ceases in the first part of December (Gough et al., 1978). At this stage, development is arrested over the duration of winter. By mid-March, blueberry bud development continues where microsporogenous tissue in the anthers is further separated and tapetal disintegration had begun and continues through bud swell (Gough et al., 1978). By mid-April, pollen grains appear to be fully formed and loosely packed in the antherine locules and remnants of tapetal tissue are apparent (Gough et al., 1978). In recent years, northern highbush blueberry floral bud development has been characterized using these terms to indicate the progression of growth: tight bud, bud swell, bud break, tight cluster, early pink bud, late pink bud, and anthesis (blueberries.msu.edu), developing over the span of several weeks as photoperiods get longer, and temperatures rise. However, Gough et al. (1978) did not describe floral bud development stages occurring at the same time as pollen development (with the exception of bud swell at microsporogenesis), so the relative timing of pollen and floral bud development is still largely unclear for northern highbush blueberry. Research on willow (*Salix* spp.), provides a framework for blueberry floral development as this woody perennial grows in similar regions as blueberry and some species flower during the same part of the spring. Willow floral bud initiation and floral organ development occur in summer, and by the fall, anthers are at the MMC stage and undergo winter dormancy (Zhang and Fernando, 2005). At this stage, meiosis has not yet occurred and thus pollen is still immature. In the spring, after MMC meiosis and subsequent mitosis occurs within the developing floral bud, mature pollen is produced causing floral buds to expand and elongate. The shedding of bud scales in willow indicates fully developed anthers, and once the filaments

elongate, the flower opens (Zhang and Fernando, 2005). In the spring when blueberry floral buds continue meiosis (microsporogenesis) and mitosis (microgametogenesis) in developing pollen grains, exposure to extreme heat could result in negative, compounding effects on the quality of pollen produced as observed in other flowering plants as discussed above. However, given the limited research done to identify when male gametophyte development (i.e., microsporogenesis-microgametogenesis) occurs during blueberry floral bud development (tight bud, bud swell, bud break, tight cluster, early pink bud, late pink bud, and anthesis), our understanding of when their flowers are most sensitive to extreme heat is extremely lacking. As discussed in the preceding sections, the consequences of heat stress for developing pollen includes (but is not limited to) premature degradation of tapetal cells, degraded tapetum, failure to release microspores, altered sugar-starch utilization, metabolism and transport, male sterility, altered pollen exine formation, loss of cell membrane integrity, cell death, reduced pollen viability, poor anther dehiscence and failure to release pollen grains (Lohani et al., 2020), all of which could affect blueberry flower and pollen development with potential negative consequences for fruit set, fruit production, and fruit quality.

Despite the importance for crop yield, the sensitivity of pollen to heat stress is not well characterized in northern highbush blueberry (Gan et al., 2020; Yang Q et al., 2019) particularly for commercially important cultivars (Gan et al., 2020). Eaton (1966) found that *in vitro* tetrad germination in highbush blueberry differed among cultivars, ranging from 5.5% to 70.6%. Brewer and Dobson (1969) reported greater rates of germination in 'Rubel' (45%) compared to 'Jersey' (23%). Lang and Parrie (1992) observed significant differences in pollen tube growth and germination rates across blueberry cultivars, with lengths ranging from 26 to 40 $\mu m \cdot h^{-1}$ and germination ranging from 79.5% to 96.3%. The above studies were conducted at 25°C, so the effect of temperature on pollen performance was not determined. Gan et al. (2020) explored pollen performance of four blueberry cultivars exposed to 2, 7, 13, 18, or 24°C and found increases in germination and tube growth as temperatures increased. Yang Q et al. (2019) assessed pollen germination and tube growth of the rabbiteye blueberry (*Vaccinium virgatum)* cultivar 'Gardenblue' in 'Brightwell' pistils *in situ* and found that after 24 h the percentage of styles with germinated pollen was over 90% at 20 and 25°C, while at 30 and 35°C, the percentage of styles with germinating pollen was reduced to 63 and 57%, respectively. Yang Q et al. (2019) also reported that at 35°C, no pollen tubes reached the ovules. Such reductions in blueberry pollen

performance under field realistic high temperature regimes raise concerns for agricultural productivity, the supply of fresh blueberries, and food security for consumers.

Indeed, in 2018, Michigan blueberry growing regions endured temperatures exceeding 35°C for several hours during bloom where these daily maximum temperatures were >20°C hotter than historical daily maximums for that region and time of year (Global Historical Climatology Network). This was the hottest May on record for the region in the past 92 years. As with many other spring-blooming plants, the optimal temperature for northern highbush blueberry is similar to the temperatures historically endured in the spring, or between 20-25°C (Lobos and Hancock 2015). According to bloom predictive models (www.enviroweather.msu.edu), blueberry bushes experienced this heat event at approximately 25% bloom, where floral bud development stages ranged from bud swell, bud break, early and late pink bud, and anthesis. Following this extreme heat event in May, Michigan blueberry producers reported a 30-50% reduction in yield for the berries harvested in July and August, with 30 million pounds less than 2017. Studies have reported optimal temperatures for photosynthesis at 14-22°C in 'Bluecrop' cultivars, and 30°C has been shown to reduce photosynthesis by 22-51% in northern highbush cultivars (Hancock et al., 1992; Lobos and Hancock, 2015), emphasizing the potential damage during this 2018 heatwave. Another study found that 'Aurora', 'Brigitta', and 'Duke' blueberry cultivars grown under high tunnels sometimes experienced temperatures between 40-50°C, and these plants produced significantly less fruit, yet the fruit grown under the high tunnel had higher total and individual sugar content compared to plants grown under hail nets (Smrke et al., 2021). Other blueberry growing regions are also experiencing the negative effects of extreme heat on berry production (Yang Q et al., 2019), but little research has been done to understand how heat affects blueberry blooms and the subsequent implications for berry production, as most studies have focused on the impact of heat on $CO_2$ assimilation (i.e., photosynthesis) and on berry ripening (Chen et al., 2012; Lobos and Hancock, 2015). However, as discussed in previous sections, photosynthesis and embryo development are considered less sensitive to heat stress than pollen development and performance (Snider and Oosterhuis, 2011).

Because most blueberry cultivars are not completely self-fertile, they benefit from cross-pollination provided by bees to maximize fruit production and fruit quality (Retamales and Hancock, 2018). In northern highbush blueberry, selfing reduced fruit set by up to 133%, inhibited the number of seeds per fruit between 36 and 1469%, and reduced fruit weight by up to 35%

(Retamales and Hancock, 2018), clearly demonstrating the importance of bees and other insects moving pollen for blueberry quantity and quality. One study found that 112 different bee species were present and active in blueberry fields during bloom, most of which were solitary bees (Tuell et al., 2009). The native, solitary bee *Osmia lignaria* and other native *Osmia* species have been identified as potential targets for management in blueberry fields as they emerge around the time of blueberry bloom and they forage on blueberry flowers and nest in habitat adjacent to fields (Tuell et al., 2009; Pinilla-Gallego and Isaacs, 2018; Fortuin et al., 2021; DeVetter et al., 2022). Compared to honey bees, some *Osmia* species and other native bees are more efficient pollinators of blueberry given their capacity to tolerate cooler temperatures (Bosch and Kemp, 2000, 2002; Tuell and Isaacs, 2010) and different physiological and behavioral traits that allow them to readily collect and move blueberry pollen between bushes (Bosch and Kemp, 2002; Graham et al., 2023), and their greater fidelity to blueberry flowers (Graham et al., 2021, 2023). Despite honey bees collecting little blueberry pollen and mainly visiting flowers for nectar (Graham et al., 2023), they are still the primary bees used for blueberry pollination services given the ease of renting and placing mass quantities of bees in farms. In Michigan, blueberry producers also receive pollination from a diversity of wild bee species with various physiologies and behaviors to aid in cross-pollination in order to maximize the yield and quality of their crop. However, if blueberry pollen quality is disrupted due to stressors like heat, it may have indirect effects on bee foraging or bee health, yet no studies have explored this.

Most northern highbush blueberry breeding has focused on improving flavor, longer storing fruit, expanded harvest dates, disease and pest resistance, machine harvestability, increasing the size of berries, and ensuring a sweet, crunchy fruit with a trace of acidity (Lobos and Hancock, 2015; Gallardo et al., 2018; Retamales and Hancock, 2018; Edger et al., 2022; Krishna et al., 2023). Some breeding interest and efforts have also been done to promote late blooming blueberry varieties, as these cultivars suffer less frost damage than those flowering earlier in the season (Retamales and Hancock, 2018; Edger et al., 2022). However, blueberry growing regions are experiencing more early season extreme heat events rather than late season frosts, yet little research or breeding efforts have been focused on high heat stress concerns. Furthermore, blueberry breeding programs have also not focused on pollination attractiveness, even though blueberries are highly dependent on bee pollinators for fertilization and maximizing yield potential (Egan et al., 2018). Compared to wild blueberry pollen, domesticated blueberry

pollen and nectar are less attractive and potentially less nutritious for some bee pollinators (Egan et al., 2018). While mostly unexplored, extreme heat has potential to further exacerbate lower blueberry pollen attractiveness and the quality of pollen provided to pollinators.

1.1.3 Direct effects of extreme heat on social and solitary bees

Temperature is one of the most important factors affecting development, emergence, and survival of insects within a range of optimal temperatures (González-Tokman et al., 2020; Buckley, 2022; Johnson et al., 2023). Temperatures above and below the optimal range can disrupt normal development, affecting the distribution of insects at continental and local scales (Bosch et al., 2010; Sgolastra et al., 2016; González-Tokman et al., 2020; Buckley, 2022; Walters et al., 2022; Johnson et al., 2023). Extreme high heat can negatively affect bee physiology, phenology, and behavior (Bosch et al., 2010; Sgolastra et al., 2016; Zhao et al., 2021; Walters et al., 2022; Johnson et al., 2023), but our understanding of this is informed primarily by studies on social bees, particularly honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.). These studies provide insights into how extreme heat directly affects bee physiology (Zhao et al., 2021; Bordier et al., 2017), foraging behavior (Descamps et al., 2018; Hemberger et al., 2023; Naumchik and Youngsteadt, 2023), capacity for acclimation (Gonzalez et al., 2022a,b; Sepúlveda and Goulson, 2023), and fecundity (Zhao et al., 2021; Campion et al., 2023). Heat stress in honey bee larvae can result in altered or malformed proboscis, wing, and leg structures in adult bees (Medina et al., 2018) and can also hinder neural development and memory (Groh et al., 2004), inhibiting foraging. Extreme heat can also alter gene expression in bees, changing task-related behaviors like redirecting honey bee foragers to perform thermoregulation in order to maintain optimal hive temperatures (Bordier et al., 2017). Traits related to honey bee and bumble bee reproduction can also be affected, including male fertility, sperm count, and sperm DNA integrity (McAfee et al., 2020; Martinet et al., 2021a,b) which can inhibit brood production. However, there is evidence for greater sensitivity to extreme heat in solitary bees than social bees (Hamblin et al., 2017), so results derived from social bees may not reflect the full consequences for the majority of bee species that are solitary (Danforth et al., 2019). For example, wing fanning and water collection allows social bees and their brood greater resilience to heat stress compared to solitary bees, who do not perform such behaviors (Gardner et al., 2007; Hamblin et al., 2017; Maebe et al., 2021; Johnson et al., 2023). Indeed, female solitary bees do not perform brood-care behaviors following egg-laying, so

progeny confined within the nest during development must endure bouts of extreme heat without any mitigation.

For some solitary bee species, direct exposure to high temperature during development can cause significant delays in emergence and reductions in survival, body mass, and fat content (CaraDonna et al., 2018; Song et al., 2023). High heat conditions can also affect solitary bee mating and nesting behaviors, limiting their reproductive potential (Pitts-Singer and James, 2009; Conrad et al., 2017; Kierat et al., 2017; Wilson et al., 2020; Ostap-Chec et al., 2021). Some studies report that male bees may be more sensitive to heat than female bees, with just one hour of heat shock delaying *Megachile rotundata* male bee emergence, but not female emergence (Hayes and López-Martínez, 2021). Different bee development stages can vary in their sensitivity to extreme heat, where pupae and adults are considered more resilient to extreme heat than larvae (Bordier et al., 2017; Hayes and López-Martínez, 2021; Song et al., 2023). For example, *Osmia lignaria* larvae exposed to 37°C increased mortality by 130% and slowed the timing of larval development (Melone et al., 2024). It is clear that exposure to direct heat stress has negative consequences for bee survival and functioning, but greater attention is needed for bees most at risk of decline, including the spring-emerging, native, solitary bees that provide crop pollination services.

### 1.1.4 The Blue Orchard Mason bee (*Osmia lignaria* Say)

*Osmia lignaria* Say (Hymenoptera: Megachilidae) is a solitary, stem nesting bee native to North America. Male *O. lignaria* bees emerge first from their cocoons in the spring (typically April-June) and patrol nearby nesting sites waiting for females to emerge, typically 1-3 days after male emergence (Bosch and Kemp, 2002). Mating occurs soon after female emergence, and females will typically wait 1-2 days before nest initiation to complete ovary maturation. During this time females consume pollen and nectar and investigate potential nest sites (Bosch and Kemp, 2002). Once an adequate nest site has been selected, female bees will start gathering mud using their mandibles and carry a small clump back to the nest, where it is deposited to form the initial mud partition at the deepest end of the nest cavity (Bosch and Kemp, 2002). Once the initial mud partition is completed, females forage for pollen and nectar to provision brood cells, with each brood cell provisioned with a mass of pollen and nectar. Once a sufficient size of provision is collected, female *Osmia* bees lay a single egg on top of this provision and seal this brood cell with another layer of mud (Bosch and Kemp, 2002). The average pollen provision requires approximately 2,000 flower visits for this species (Bosch and Kemp, 2002). *Osmia lignaria* nests

consist of a linear series of adjacent cells within a cavity, each containing a single provision and egg, separated from one another via mud partitions. Once a female bee has filled a cavity, she builds a thicker mud partition to act as a plug, sealing off the cavity entrance. Given ample floral resources, *O. lignaria* bees can complete 1-2 provisioned brood cells per day or approximately 20-60 brood cells throughout their lifetime, which is typically 20-30 days (Bosch and Kemp, 2002; Spendal and Cane, 2022). Because mated female *O. lignaria* bees store sperm in their spermatheca, they can selectively fertilize eggs before oviposition, where fertilized eggs produce female brood and unfertilized eggs produce male brood (Bosch and Kemp, 2002). Fertilized eggs (i.e., female brood) are typically laid in the back, innermost cells of the nest and are provided larger pollen provisions than unfertilized (male) eggs, accounting for the larger body size and energy demands of female offspring.

*Osmia lignaria* bees are strictly univoltine, meaning they only produce one generation per year (Bosch and Kemp, 2000, 2002). Unlike honey bees and bumble bees, *O. lignaria* offspring take several months to complete development from egg to adulthood, so mother bees and her brood have no overlap in activity as adults. Once an egg is laid, it takes about a week for it to hatch into the first larval instar (Bosch and Kemp, 2002). The first instar larva remains inside the egg's chorion, feeding on egg fluids, before actively hatching and molting into the second larval instar a few days later. At the second and third larval instars, *O. lignaria* brood is small, translucent-white, slow-moving, and grub-like and begins feeding on the pollen provision provided by the mother bee. At the third and fourth larval instars, these brood have grown considerably, consuming pollen faster and in larger quantities and appear white-cream colored with pollen and frass visible in their digestive tract. By the fifth and final larval instar, the brood are large, consuming pollen rapidly, and appear white-cream colored. The fifth larval instar is characterized by the presence of frass as these brood have now consumed a large portion of their provision. Extrinsic cues regulated via starvation and hormone signaling (Helm et al., 2017) promote fifth instar larvae to begin spinning silk using their salivary glands, forming a dark brown cocoon around themselves, designated as the cocoon spinning stage. By late spring these larvae have fully enclosed themselves in their cocoon, reaching the prepupal stage, and undergo a summer dormant period (Bosch and Kemp, 2004). By late summer, the *O. lignaria* prepupa molts into a pupa and begins to darken and develop into an adult bee. These brood remain dormant in their cocoons over the fall and winter and require exposure to cold temperatures (3-5ºC), emerging the following spring as adults. During the

prepupal, pupal, and adult overwintering stages, these *O. lignaria* brood have no access to food and thus rely on the metabolic reserves accumulated from larval provisions to sustain them until their spring emergence (Bosch et al., 2010).

*Osmia lignaria* bees are polylectic, meaning they visit a variety of different plant species (Bosch and Kemp, 2002). Both wild and managed *O. lignaria* populations are important pollinators of spring-blooming plants, including those in the Rosaceae, Ericaceae, and Salicaceae families, among others (Bosch and Kemp, 2002). This bee species has been increasingly considered and managed for blueberry production (DeVetter et al., 2022). The foraging behavior of *O. lignaria* bees, moving from plant to plant across a crop field or natural area, helps facilitate cross-pollination, providing crucial pollination services that other bees may be lacking (Bosch and Kemp, 2002). Additionally, these spring bees are well adapted to fly in cool conditions, as low as 12ºC, and are active early in the morning and later in the evening providing important pollination services when other bees, like honey bees, are inactive (Bosch and Kemp, 2002). However, recent studies suggest *O. lignaria* populations are in decline in the US, driven by increased competition for resources, disease prevalence, and pesticide exposure (Artz and Pitts-Singer, 2015; Eeraerts et al., 2020; LeCroy et al., 2020; Russo et al., 2021; Kopit et al., 2022; Gutierrez et al., 2023). Direct nutritional and heat stress can also negatively affect *Osmia* bees (Conrad et al., 2017; Kierat et al., 2017; CaraDonna et al., 2018; Lee et al., 2018; Filipiak and Filipiak, 2020; Stuligross and Williams, 2020; Knauer et al., 2022; Song et al., 2023; Melone et al., 2024), and recent climate change predictive models have shown that this trend in *Osmia* populations is projected to continue in the coming decades (Kazenel et al., 2024). Given the evidence for declining populations and the importance of their pollination services, it is imperative to identify and mitigate stressors affecting wild bees, including extreme heat.

1.1.5 The indirect effects of extreme heat on bees and plants

The majority of studies investigating the effects of climate change or extreme heat on bees have focused on the direct consequences of heat stress, but few studies have explored the indirect effects of extreme heat on bee pollinators. In plant-pollinator networks, synchrony is critical for their success and survival, and extreme heat can disrupt the timing of these interactions (Scaven and Rafferty, 2013; CaraDonna et al., 2018; Slominski and Burkle, 2021; de Manincor et al., 2023). While phenological synchrony is important to understand in the context of climate change, few studies have explored whether heat stress also affects the synergy between bee pollinators and their

host plants. For example, there is little information on whether heat stressed plants adequately support the dietary needs of their bee pollinators. As discussed above, when plants endure extreme heat during floral development, maturation, and dehiscence, it can inhibit nutrient sequestration in developing pollen grains, limiting or altering concentrations of carbohydrates, proteins, lipids, and amino acids in pollen and furthermore, and it can hinder the production and release of pollen grains (Borghi and Fernie, 2017; Borghi et al., 2019; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021). Many of these nutrients that support reproductive processes in plants are also fundamental nutrients in bee diets (Roulston and Cane, 2000; Vaudo et al., 2020). This has led some researchers to hypothesize that such heat-induced nutrient reductions in pollen could also negatively affect bees (Borghi and Fernie, 2017; Borghi et al., 2019; Descamps et al., 2021). However, no studies have confirmed the connection between heat stressed pollen and bee nutrition, requiring further research.

Pollen provides carbohydrates, proteins, lipids, amino acids, and other micronutrients to bees (Roulston and Cane, 2000; Vaudo et al., 2020). When bees are fed a high-quality diet during larval development, they tend to have greater survival, longevity, and resilience to stress as adults (Vaudo et al., 2015, 2020; Vanderplanck et al., 2019; Woodard et al., 2019; Knauer et al., 2022). In contrast, bees fed low-quality diets can experience various adversities regarding their development, sex ratios, body size, survival, and longevity (Bosch, 2008; Bukovinszky et al., 2017; Filipiak and Filipiak, 2020; Stuligross and Williams, 2020). For example, in *O. lignaria* larvae fed honey bee collected pollen, only the highest protein diets supported development to adulthood (Levin and Haydak, 1957; Roulston and Cane, 2000). Other studies have found that carbohydrates and lipids, rather than protein, mediate *Osmia* larval growth and survival to pupation (Austin and Gilbert, 2021; Westreich and Tobin, 2024). *Osmia bicornis* and *O. cornuta* larvae failed to develop on *Tanacetum* pollen, which authors suggest is due to insufficient quantity or quality of nutrients (Sedivy et al., 2011). *Osmia cornifrons* larvae failed to develop when fed multifloral and single-source pollen diets, even when these diets had similar protein:lipid ratios as surveyed provisions, suggesting certain micronutrients were lacking and must be present for proper development (Crone et al., 2023). *Osmia* cocoons are an important sink for nutrients assimilated and used during larval development, and underdeveloped cocoons may indicate the scarcity of specific elements present in pollen (Filipiak et al., 2021). *Osmia bicornis* larvae fed a single-source pollen diet failed to enclose their cocoon and had high larval mortality, suggesting chronic nutrient

deficiency (Bukovinszky et al., 2017). When fed high quantities of rapeseed pollen, the same species exhibited hindered cocoon development and high male mortality, but when supplemented with additional nutrients, these negative effects were absent (Filipiak et al., 2022). These studies indicate the importance of meeting nutritional needs of *Osmia* bees for proper development and survival.

Extreme heat may also reduce the production and release of pollen (Raja et al., 2019; Amuji et al., 2020; Hedhly et al., 2020; Lohani et al., 2020), further limiting access to floral rewards for adult bees and their offspring. In low-resource conditions, *Osmia* bees produced 26% fewer offspring and 48% fewer daughters (Stuligross and Williams, 2020). Other studies have shown that when *Osmia* females are denied access to pollen they fail to mature oocytes or lay eggs (Cane, 2016). *Osmia cornuta* females can produce 40-50 oocytes but rarely lay more than 10-20 eggs, suggesting limitations on fecundity may be attributed to constraints on brood cell provisioning (like reduced resource availability) rather than egg production potential (Bosch and Vicens, 2006). When brood provisioning is impeded, adjustments in parental investment allocation can occur, where females may limit the number of brood produced but maintain the provision size provided to offspring (Bosch and Vicens, 2006). Over time, this could affect bee populations on a local scale. While egg production and oviposition are energetically costly for all bees, it is particularly costly for solitary bees like *Osmia* who lay large eggs relative to their body size and require pollen for oocyte maturation (Cane, 2016), emphasizing the importance of abundant, nutritionally rewarding diets for solitary bees. Additionally, foraging choices are influenced by the quality and quantity of floral resources available where bees preferentially visit patches of flowers where floral resources are both abundant and nutritionally rewarding, potentially limiting bee pollinator abundances in crop fields experiencing extreme heat (Ruedenauer et al., 2015, 2019, 2021; Somme et al., 2015; Ogilvie and Forrest, 2017; Greenop et al., 2020; Russell and McFrederick, 2021; Hemberger et al., 2023). Consequently, extreme heat may indirectly limit crop reproduction due to reduced visitation and efficiency of pollination services in response to diminished quantities and quality of floral resources. There is a clear need for more research on the direct and indirect consequences of extreme heat for bees and crops that are particularly at risk, including solitary bee species and spring-blooming perennial crops, and their interactions with one another.

## 1.2 RESEARCH OBJECTIVES

As extreme heat events become more common and intense, and bee populations continue to decline, there is an increasing need to understand how native solitary bees such as *Osmia lignaria*, and spring-blooming crop plants such as northern highbush blueberry are affected by extreme heat. To address this, my first objective (Chapter 2) aimed to understand how temperature affects northern highbush pollen germination and pollen tube growth, and to determine an upper temperature threshold for pollen. I also explored whether or not blueberry pollen could recover after short bursts (4 h) of extreme heat. I hypothesized that blueberry pollen would perform best at temperatures historically experienced during it spring-bloom period, and that pollen performance would decline as temperatures increased. I also hypothesized that pollen would be unable to recover from brief bouts of extreme heat.

Using the upper threshold temperature determined from the previous study, my second objective (Chapter 3) explored the effects of extreme heat exposure on blueberry fruit set and some berry quality parameters. Specifically, I evaluated how different blueberry floral development stages, including tight bud, bud swell, bud break, early pink bud, and late pink bud, varied in their sensitivity to extreme heat by exposing bushes at these development stages to heat stress, or normal conditions, for 4 h and hand-pollinating flowers once they opened. Once berries developed, I assessed fruit set, fruit ripening duration, and several fruit quality measurements including berry weight, size, the number and percent fertilized seeds, % total soluble sugar content, and % titratable acidity content. For this chapter, I hypothesized that plants exposed to heat stress would have lower fruit set, reduced berry quality, and altered berry ripening duration. I also hypothesized that the severity of damage following heat stress on fruit set and berry quality would vary by development stage.

In my third objective (Chapter 4), I aimed to understand whether heat stressed host plants would have indirect effects on a native, solitary bee pollinator, *Osmia lignaria.* I provided mated, female *O. lignaria* bees access to blueberry, phacelia, and clover plants previously exposed to extreme heat conditions (or normal conditions) for 4 h and monitored their foraging and egg laying behaviors in a no-choice semi-field cage study. I collected the eggs laid by these female bees and assessed their development and survival as they consumed pollen provisions from either heat-stressed or non-stressed plants as larvae. For offspring that successfully pupated, I monitored their emergence success and timing, survival and longevity as adults. I hypothesized that female *O.*

*lignaria* bees provided heat stressed plants would have similar foraging rates but reduced fecundity compared to female bees provided non-stressed host plants. I also hypothesized that *Osmia* offspring consuming pollen from heat stressed plants would have altered development and lower survival. For the adult offspring who consumed heat stressed diets as larvae, I hypothesized they would exhibit reduced emergence success and survival, smaller body size, altered timing of emergence and longevity, and male-dominated sex ratios.

Finally, in my last objective (Chapter 5) I investigated whether the nutrient content of blueberry pollen is altered following heat stress exposure at various floral development stages. This objective was aimed to explore the mechanisms that link the negative effects of extreme heat exposure for blueberry plants (Chapters 2 and 3) to negative effects observed in bees (Chapter 4). Specifically, I measured the pollen protein, carbohydrate (sucrose, glucose, and starch), and amino acid profiles in blueberry pollen exposed to extreme heat or normal conditions for 4 h at tight bud, bud swell, bud break, early/late pink bud, and anthesis development stages. I hypothesized that the nutrient content of blueberry pollen would be reduced or altered when exposed to extreme heat, but the severity of these changes would vary depending on the development stage exposed to heat stress.

# CHAPTER 2. POLLEN GERMINATION AND TUBE GROWTH IN NORTHERN HIGHBUSH BLUEBERRY ARE INHIBITED BY EXTREME HEAT

This chapter was published as Walters, J. and Isaacs, R. (2023). Pollen germination and tube growth in northern highbush blueberry are inhibited by extreme heat. *HortScience* 58(6): 635-642. https://doi.org/10.21273/HORTSCI17075-23

## 2.1 INTRODUCTION

The intensity and frequency of extreme heat events are increasing in every region of the globe (IPCC, 2021), with negative consequences for agricultural production (Mesihovic et al., 2016; Hatfield et al., 2020; Lohani et al., 2020; van Es, 2020; IPCC, 2021). Global yields are expected to decline by 2.5-10% across various crops in the twenty-first century due to extreme heat stress and other effects of climate change, threatening food security for a growing human population (Hatfield et al. 2011; IPCC, 2018). Much of the research on the effects of climate change on crop production has focused on long-term gradual temperature increases (e.g., 1.5-3°C), yet short-lived extreme heat events are also expected to increase and, depending on their timing and intensity, may be more detrimental to crop production (Zinn et al., 2010; Hatfield and Prueger, 2015; Mesihovic et al., 2016; Walters et al., 2022). While plants have evolved strategies to endure a wide range of temperatures, adequate acclimation time is needed for plants to adjust to new conditions (Larkindale and Vierling, 2008; Müller and Rieu, 2016), highlighting the potential for injury following short bursts of extreme heat compared to gradual temperature increases. As extreme heat events intensify, it will be critical to determine the effects of these conditions on crop performance and productivity (Hatfield et al., 2020; Lohani et al., 2020), and to develop mitigation measures.

Extreme heat triggers stress in flowering crops, and the resulting physiological and ecological responses can have profound repercussions on reproduction, development, and productivity (Zinn et al., 2010; Müller and Rieu, 2016; Hatfield et al., 2020; Lohani et al., 2020). During the gametophyte and progamic phases of reproductive development, greater sensitivity to heat extremes has been observed during pollen development, pollen germination, and pollen tube elongation (Zinn et al., 2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Lohani et al., 2020). Several adverse effects may occur when heat is endured during these developmental stages,

including reduced rates of pollen germination and pollen tube growth and reduced seed set and yield (Hedhly, 2011; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Lohani et al., 2020). Understanding the adverse effects of extreme heat on pollen performance (i.e., pollen germination and pollen tube growth) is important given its sensitivity to high temperatures and essential role in fertilization (Snider and Oosterhuis, 2011; Mesihovic et al., 2016).

Most studies of how extreme heat affects crop pollination have been limited to high acreage annual crops (Hatfield and Prueger, 2015; Mesihovic et al., 2016; Raja et al., 2019). High temperatures (36-40°C) have been shown to inhibit pollen function in several crops, including rice (*Oryza* spp.) (Satake and Yoshida, 1978; Zhang et al., 2018), cotton (*Gossypium hirsutum*) (Masoomi-Aladizgeh et al., 2021), and tobacco (*Nicotiana tabacum*) (Parrotta et al., 2016). For these species, and several other annual crops not discussed here, it is well established that pollen performance and fertilization are particularly sensitive to high heat applied in short bursts (Zinn et al., 2010; Mesihovic et al., 2016; Raja et al., 2019). Far less is known about the tolerance of perennial fruit crops to heat extremes, perhaps because most are grown in climates where hot weather conditions during bloom were very rare. As extreme heat becomes more common during the spring season (IPCC 2021), when many perennial crops are blooming, it will be important to understand the thermal limits of the pollination processes in these crop species.

Studies of plants that flower earlier in the season, including various perennial crops, indicate potential for greater sensitivity to heat stress than summer flowering species (Hedhly et al., 2009). In several genotypes of *Pistacia* sp. plants, pollen germination rates decreased rapidly as temperatures increased from 25°C to 35°C and no pollen germination was observed when exposed to 40°C for 24 h (Acar and Kakani, 2010). In strawberries (*Fragaria* x *ananassa*), pollen germination was significantly reduced when exposed to 30°C for just 4 h compared to a normal temperature of 23°C (Ledesma and Sugiyama, 2005). The temperatures that negatively affect plant reproduction vary among crop type (e.g., annual vs. perennial), by species, and even genotype within species (Hedhly et al., 2005; Hamidou et al., 2013; Lohani et al., 2020) so there is a need to understand how high heat affects development of many modern crops and their cultivars.

Northern highbush blueberry (*Vaccinium corymbosum*) is a woody perennial crop native to eastern North America, historically grown in regions with cold winters and mild summers (Retamales and Hancock 2012). When viable blueberry pollen is deposited on a receptive stigma, pollen tubes will germinate and grow toward the ovary for fertilization (Dogterom et al., 2000).

Fertilized ovaries develop seeds which affect blueberry fruit size, and thus, affect yields (Dogterom and Winston, 1999; Dogterom et al., 2000; Gan et al., 2020). However, pollen performance in northern highbush blueberry is not well characterized (Gan et al. 2020; Yang Q et al. 2019) and few studies have evaluated northern highbush blueberry pollen performance in current commercially important cultivars (Gan et al., 2020). Eaton (1966) found that *in vitro* tetrad germination in highbush blueberry differed across cultivars, ranging from 5.5% to 70.6%. Brewer and Dobson (1969) reported greater rates of germination in 'Rubel' (45%) compared to 'Jersey' (23%). Lang and Parrie (1992) observed significant differences in pollen tube growth and germination rates across blueberry cultivars, with lengths ranging from 26 to 40 $\mu m \cdot h^{-1}$ and germination ranging from 79.5% to 96.3%. The above studies were conducted at 25°C, so the effect of temperature on pollen performance for those described blueberry cultivars was not determined. Gan et al. (2020) explored pollen performance of four blueberry cultivars exposed to 2, 7, 13, 18, or 24°C and found increases in germination and tube growth as temperatures increased. Yang Q et al. (2019) assessed pollen germination and tube growth of the rabbiteye blueberry (*Vaccinium virgatum)* cultivar 'Gardenblue' in 'Brightwell' pistils *in situ* and found that after 24 h the percentage of styles with germinated pollen was over 90% at 20 and 25°C, while at 30 and 35°C, the percentage of styles with germinating pollen was reduced to 63 and 57%, respectively. Yang Q et al. (2019) also reported that at 35°C, no pollen tubes reached the ovules. No studies, to our knowledge, have assessed the impact of extreme heat on northern highbush blueberry cultivars.

Future agricultural production will be increasingly threatened as climate change intensifies (Motha and Baier, 2005; Malikov et al., 2020), but the negative impacts of extreme heat on crop production are already evident. In May 2018, the main blueberry production region in Michigan experienced mid-day conditions of >32°C for over 4 hours during bloom. These temperature conditions are uncommonly hot for the region and time of year. Despite high bloom density and strong crop potential for Michigan in 2018, blueberry yields were 30-50% lower than normal. Reductions in blueberry production due to extreme heat have also been observed globally, with Yang Q et al. (2019) listing high temperature stress as the biggest hindrance to rabbiteye blueberry flowering and fruit set in the Guizhou province, China. Despite the losses in global blueberry production under extreme heat conditions, our understanding of critical threshold temperatures in blueberry pollen performance is not well characterized. This study was conducted to explore the effects of extreme heat on northern highbush blueberry pollen performance by asking the following

questions: (1) how does blueberry pollen germination and tube growth respond to a broad range of temperatures? (2) which high temperatures inhibit blueberry pollen germination and tube growth? and (3) can blueberry pollen germination and tube growth recover after a brief exposure to extreme heat? Determining blueberry pollen performance under various heat conditions, both in severity and duration, will be critical for our understanding of this crop and for developing mitigation measures as climate change intensifies.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Plant material and maintenance

Dormant 2-year-old northern highbush blueberry bushes were purchased in late winter (Hartmann Nursery, Grand Junction, Michigan and DeGrandchamp Farms, South Haven, Michigan). Four commercially relevant cultivars were selected for these studies, including 'Bluecrop', 'Elliott', 'Jersey', and 'Liberty'. Dormant bushes were immediately placed in dark cold storage (MSU Horticultural Teaching and Research Center, Holt, Michigan) at 2°C until at least 1200 chilling hours had been accumulated. Thereafter, bushes were moved from cold storage as needed for experiments. In 2019 and 2021, all bushes were moved from cold storage to a greenhouse (22 ± 5°C 16:8 L:D) for the duration of the experiments. In 2020, bushes had to be moved to lab conditions (20 ± 3°C, constant light) due to the COVID-19 pandemic. Bushes were treated with 1% v:v Superior Oil (petroleum distillates, Loveland Products, Greeley, Colorado) after removal from cold storage to reduce pest infestation. Plants were watered regularly to maintain moist soil and soil pH was monitored every 3-4 weeks to ensure it was <6.0. When necessary, bushes were treated with Jobe's Organics soil acidifier (calcium sulfate (80%), Sulphur (18%), and bentonite clay (2%), Easy Gardener Products, Inc., Waco, Texas) according to the manufacturer's label. Osmocote Smart-Release Plant Food Flower and Vegetable (nitrogen (14%), available phosphate (14%), soluble potash (14%), The Scotts Company, Marysville, Ohio) granules were also added to potted blueberry bushes following the manufacturer's label.

### 2.2.2 Pollen collection setup for experiments

Pollen was collected from the most productive and healthy bushes (those with a higher density of flowers and minimal damage from pests or pathogens), from flowers located in the mid- to upper-canopy. To ensure that the pollen for experiments was fresh (within 24 h of anthesis), we marked every open flower on each bush by lightly dotting the corolla with a permanent marker (Kearns and Inouye, 1993). Thus, the following day, any open flower without a mark had been

open for less than 24 h. Pollen from a single flower was released from the anthers by touching a vibrating sonication tool (AeroGarden, Boulder, Colorado) onto the outside of the corolla. The pollen was collected on the surface of a 16 x 60 mm Petri dish (Fisher Scientific, Hampton, New Hampshire) containing a blueberry specific nutrient medium (Lang and Parrie, 1992) held directly under the flower. Petri dishes were immediately covered with their lids, then placed in an environmental growth chamber set at 16L:8D and 60 ± 5% relative humidity. These *in vitro* experiments were performed in climate chambers to allow precise temperature control, which is ideal for experiments where extreme temperature is applied for a short time (Mesihovic et al., 2016). Indeed, much of the research on this topic has used climate chambers to identify the temperature sensitivity of fully mature male gametophytes for several crop plants (Snider and Oosterhuis, 2011). Details of the specific treatments, temperatures, and experimental designs are discussed below for each experiment. To record temperatures in growth chambers, HOBO data loggers (Onset Computer Corporation, HOBO Pendant Temperature/Light Data Logger, Bourne, Massachusetts) were placed in each chamber and these recorded temperature every 30 min.

2.2.3 Pollen germination and pollen tube growth evaluations

To quantify pollen germination and tube growth, Petri dishes were removed from the growth chambers at the appropriate timing and assessed immediately under a 100x light microscope. For each sample, a randomized sample of 10 pollen tetrads was observed per Petri dish using an eyepiece graticule (10mm/100 Division Reticle eyepiece with crossline, Accu-scope, Commack, New York). For each tetrad, the number of pollen tubes produced and the length of each germinated pollen tube on the same tetrad was recorded. Pollen tetrads were considered successfully germinated when the pollen tube length was equal to or greater than the pollen tetrad diameter (Lang and Parrie, 1992; Jumrani et al., 2018). When no germinated pollen tubes were observed, no lengths were recorded and thus they could not be analyzed. Pollen tube length was measured by aligning the graticule eye piece along the length of the pollen tube, which was first recorded in graticule units and later converted to millimeters (mm).

In Experiment 1, I investigated how pollen germination and growth responded to a range of temperatures between 10 and 40°C. For each cultivar described above, pollen was collected from six bushes. Once a week, one bush per cultivar was randomly selected and moved from cold storage to the greenhouse. Placement of bushes within the greenhouse benches was also randomized. Pollen from open flowers was collected onto a Petri dish as described above, and the

dishes were exposed to 10, 20, 30, or 40°C in environmental growth chambers. These temperatures broadly encompass the conditions that northern highbush blueberries may be exposed to during bloom. After 4 and 24 h of temperature exposure, 10 pollen tetrads were randomly assessed. There was a total of six Petri dishes per cultivar for each temperature, resulting in 60 pollen tetrad observations for each cultivar, temperature, and assessment time.

Based on the results of Experiment 1, I conducted Experiment 2 to determine the temperature threshold inhibiting pollen germination and tube growth. I exposed pollen from cultivars described above to 30, 32.5, 35, 37.5, or 40°C. This experiment followed similar methods described in Experiment 1, but due to the COVID-19 pandemic, some bushes for this experiment were moved from cold storage to our greenhouse (prior to March 2020 shutdown) and some bushes were moved into our laboratory space (in late April 2020) and grown under grow lights (California Lightworks, SolarFlare 220 LED Grow Light, Canoga Park, California). No significant differences were found among pollen samples taken in the two conditions, and thus were analyzed together. In this experiment, we used the same experimental design and replication as described above.

I conducted Experiment 3 to determine the potential for blueberry pollen germination and tube growth to recover after brief exposure to extreme heat. The 'Bluecrop' cultivar pollen was exposed to either a control temperature (CT) treatment (25°C for 24 h), a high temperature (HT) treatment (37.5°C for 24 h), or a relieving temperature (RT) treatment which combined brief HT followed by CT conditions (37.5°C for 4 h then 25°C for 20 h). For each treatment, I assessed the proportion of successfully germinated pollen grains and the length of pollen tubes as described above. After 4, 10, and 24 h of exposure, Petri dishes were removed from growth chambers and evaluated as described above.

2.2.4 Data analysis

All data were analyzed in RStudio (Version 4.2.2). The proportion of germinated pollen tetrads and the pollen tube length data were analyzed separately for each cultivar and assessment time. The proportion of germinated pollen tetrads was calculated for each observation within a replicate group (N=6) for each cultivar, temperature, and assessment time, based on the number of tetrads observed with at least one successfully germinated pollen tube. A generalized linear model (GLM) with a Gaussian distribution was used to compare pollen germination across temperatures (glm(mean germination success ~ temperature, family= gaussian)). Mean pollen tube lengths were determined for each observation within a replicate group (N=6) for each cultivar, temperature, and

assessment time. A GLM with a gamma distribution and log link function was used to transform these right-skewed data and to determine the effects of temperature on mean pollen tube length (glm(mean length ~ temperature, family = gamma(link = "log")). Models were tested for normality by comparing deviance residual values and using the Shapiro-Wilk test. For normally distributed data, a one-way analysis of variance (ANOVA) was used to determine if temperature had a significant effect on pollen germination or pollen tube length for each cultivar and assessment time. For data that were not normally distributed, a Kruskal Wallis test of significance was used. If the ANOVA or Kruskal Wallis test was significant ($P < 0.05$), a Sidak post-hoc test was used for pairwise comparisons among means. Statistically different means are represented by different letters in tables and figures ($P < 0.05$).

## 2.3 RESULTS

### 2.3.1 Experiment 1: Pollen response to 10-40°C

#### 2.3.1.1 Pollen germination

Across all cultivars and sampling times, temperature significantly affected pollen germination (Table 2.1). The highest proportion of germinated pollen tetrads was observed at 20 and 30°C while significantly lower germination was found at 40°C for all cultivars and sampling times. After 4 h of incubation, germination rates were similar between 10 and 40°C. However, after 24 h, pollen germination was significantly lower in 40°C conditions compared to 10°C. Between 4 and 24 h assessments at 10°C, the proportion of germinated pollen tetrads increased by 0.63, 0.53, 0.48, and 0.38 for 'Bluecrop', 'Elliott', 'Jersey', and 'Liberty', respectively. For all cultivars (except 'Elliott'), no significant differences were found among germination proportions after 4 h of exposure to 20 and 30°C. After 24 h of exposure, a high proportion of pollen tetrads germinated at 20 and 30°C, ranging between 0.63 and 0.98 across all cultivars. Within a cultivar, no significant differences were found among germination proportions at 20 and 30°C at 24 h. Germination observed at 40°C was very limited and significantly lower than at 20 or 30°C across all cultivars and sampling times (Table 2.1). Across all cultivars and sampling times, no pollen tetrads exposed to 40°C produced more than one pollen tube (Supplemental Table 2.4).

#### 2.3.1.2 Pollen tube length

Pollen tube length was significantly affected by temperature for most cultivars and sampling times (Figure 2.1). After 4 and 24 h incubation, pollen tube length increased as temperatures increased from 10 to 30°C, but at 40°C it was severely or completely inhibited. For

'Bluecrop' and 'Elliott' at 10°C, tube lengths increased approximately 7-8 fold (respectively) from 4 to 24 h (Supplemental Table 2.1). For 'Jersey' and 'Liberty' at 10°C, pollen tube lengths were observed at 24 h, but not at 4 h. Pollen tube lengths were not significantly different at 10-30°C (except for 'Bluecrop') at 24 h. Comparing 20 and 30°C at 4 h, significantly longer pollen tubes were recorded at 30°C for 'Jersey' and 'Liberty'. After 24 h, pollen tube lengths for 20 and 30°C were not significantly different for any cultivar. After 4 h exposure to 40°C, only 'Jersey' and 'Liberty' had measurable pollen tube growth, yet was significantly shorter than lengths observed at 20 or 30°C. After 24 h exposure to 40°C, only 'Bluecrop' pollen tubes germinated, and these were significantly shorter than those observed at all other temperatures.

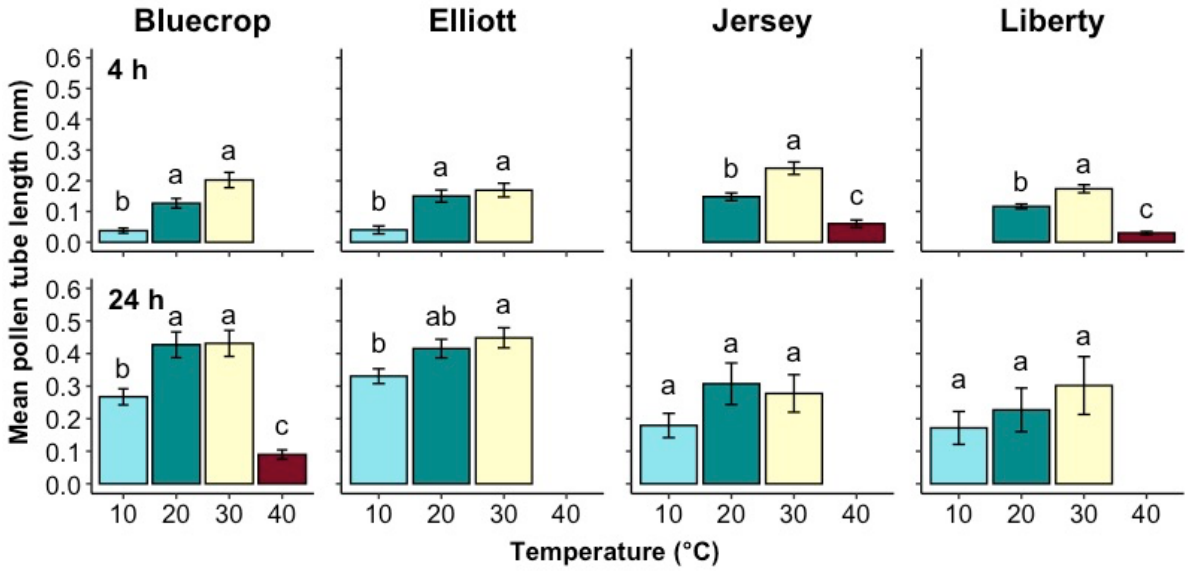


**Figure 2.1** Mean pollen tube length of four northern highbush blueberry cultivars after 4 h and 24 h of exposure to 10, 20, 30 or 40°C. Each bar represents the mean value of six independent replicates shown with standard error bars. Bars with a common letter, based on a Sidak post-hoc test, are not statistically different at $P < 0.05$ within each graph.

2.3.2 Experiment 2: Pollen response to 30-40 °C

2.3.2.1 Pollen germination

Across all cultivars and sampling times, temperature conditions between 30 and 40°C had a significant effect on blueberry pollen germination (Table 2.1). For each of the tested cultivars and assessment times, the highest germination proportions were observed at 30-35°C, and these values did not significantly differ from one another. For all cultivars, germination rates were significantly lower at 37.5°C compared to 30-35°C at both 4 and 24 h. For example, across all

cultivars and incubation times, proportion successfully germinated pollen tetrads ranged from 0.42 to 0.95 for 30-35°C, while at 37.5°C, germination ranged from 0.20 to 0.73 (Table 2.1). At 40°C, pollen germination was completely inhibited for all cultivars and sampling times, except for 'Elliott' at 24 h where a single germination observation was recorded. For this sample, exposure to 40°C reduced pollen germination by 98% compared to rates observed at 30°C. The proportion of pollen tetrads with two or more pollen tubes was also substantially reduced at 37.5°C, and no tetrads produced more than one pollen tube at 40°C (Supplemental Table 2.5).

2.3.2.2 Pollen tube length

Temperature had a significant effect on pollen tube length for all cultivars and sampling times except for 'Liberty' at 24 h (Figure 2.2). For all cultivars at 4 and 24 h, pollen tube length decreased as temperature increased from 30 to 37.5°C. For all cultivars and sampling times (except 'Jersey' at 24 h), the longest pollen tubes were observed at 30°C. For 'Jersey' at 24 h, the longest pollen tubes were observed at 32.5°C, although it did not significantly differ from lengths observed at 30°C. Compared 30°C at 4 h, significant reductions in tube length were observed at 35°C for 'Bluecrop' and 'Jersey' and at 37.5°C for 'Elliott' and 'Liberty'. At 24 h, lengths were significantly reduced at 37.5°C for 'Bluecrop', 'Elliott', and 'Jersey' compared lengths observed at 30°C (no significant differences among temperatures for 'Liberty' at 24 h). Although not statistically different, pollen tube lengths were shorter by 40%, 18%, 39%, and 14% at 35°C compared to 30°C at 24 h for 'Bluecrop', 'Elliott', 'Jersey', and 'Liberty', respectively (Supplemental Table 2.2). For 'Liberty', after 24 h, pollen tube length did not significantly differ among temperatures, although there was a trend of shorter pollen tubes as temperature increased. Exposure to 40°C completely inhibited pollen tube growth for all cultivars at 4 h and for 'Bluecrop', 'Jersey', and 'Liberty' at 24 h. After 24 h of exposure to 40°C conditions, 'Elliott' pollen tubes were significantly inhibited compared to all other temperatures sampled (Supplemental Table 2.2).

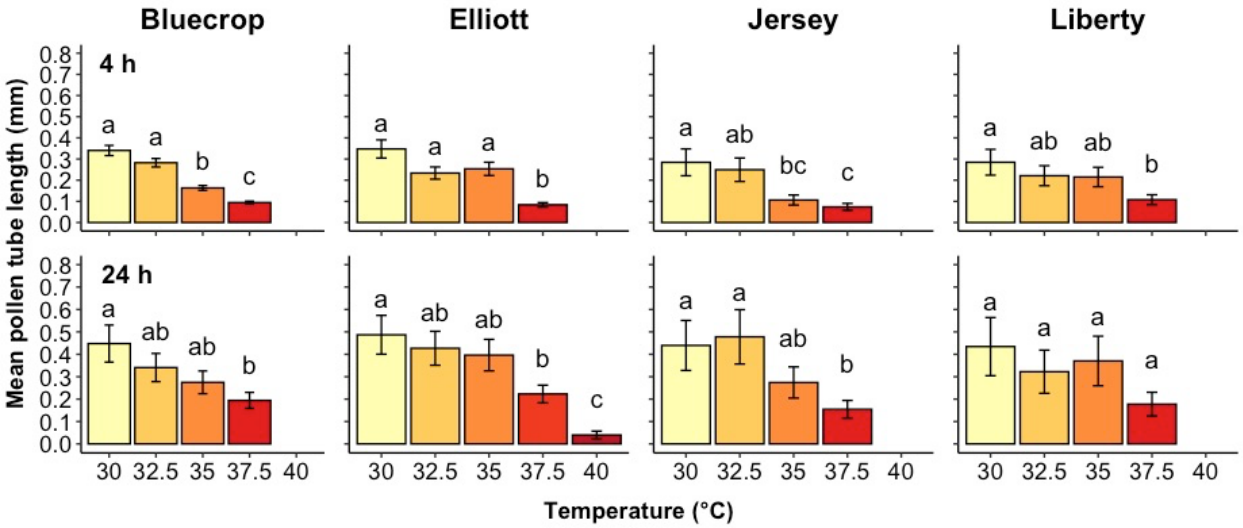


**Figure 2.2** Mean pollen tube length of four northern highbush blueberry cultivars after 4 h and 24 h of exposure to 30, 32.5, 35, 37.5, or 40°C. Each bar represents the mean value of six independent replicates shown with standard error bars. Bars with a common letter, based on a Sidak post-hoc test, are not statistically different at $P < 0.05$ within each graph.

2.3.3 Experiment 3: Recovery from extreme heat

2.3.3.1 Pollen germination

Germination rates of 'Bluecrop' pollen were significantly affected by CT, HT, or RT temperature regimes at 4 and 24 h, but not at 10 h (Table 2.3). The greatest germination levels were observed in the CT regime, where 89%, 91%, and 91% of observed pollen tetrads germinated at least one pollen tube at 4, 10, and 24 h, respectively. In comparison, pollen germination for the HT group was reduced by 26%, 14%, and 27% at 4, 10, and 24 h, respectively, but only significantly reduced at 24 h. Germination rates between HT and RT groups did not differ significantly from one another across all sampling times. Compared to CT conditions, the proportion of pollen tetrads with more than one pollen tube was also reduced for HT and RT groups by approximately 30-40% across all sampling times (Supplemental Table 2.6).

2.3.3.2 Pollen tube length

Temperature regime had a significant effect on pollen tube length at all assessment times (Figure 2.3). Pollen tube lengths were significantly shorter in HT and RT groups compared to the CT group across all sampling times. Pollen tube lengths increased from 4 to 10 h across all temperature regimes but changed variably between 10 and 24 h (Supplemental Table 2.3). At 4 h, pollen tube lengths for HT and RT groups were 74% and 77% shorter (respectively) compared to

the CT group. This trend continued at 10 and 24 h, where pollen tube lengths were shorter in the HT and RT groups compared to the CT group. At 4 and 10 h, pollen tube lengths did not statistically differ between the HT and RT groups. However, after 24 h, pollen exposed to the RT regime had significantly longer pollen tubes than those exposed to the HT regime.

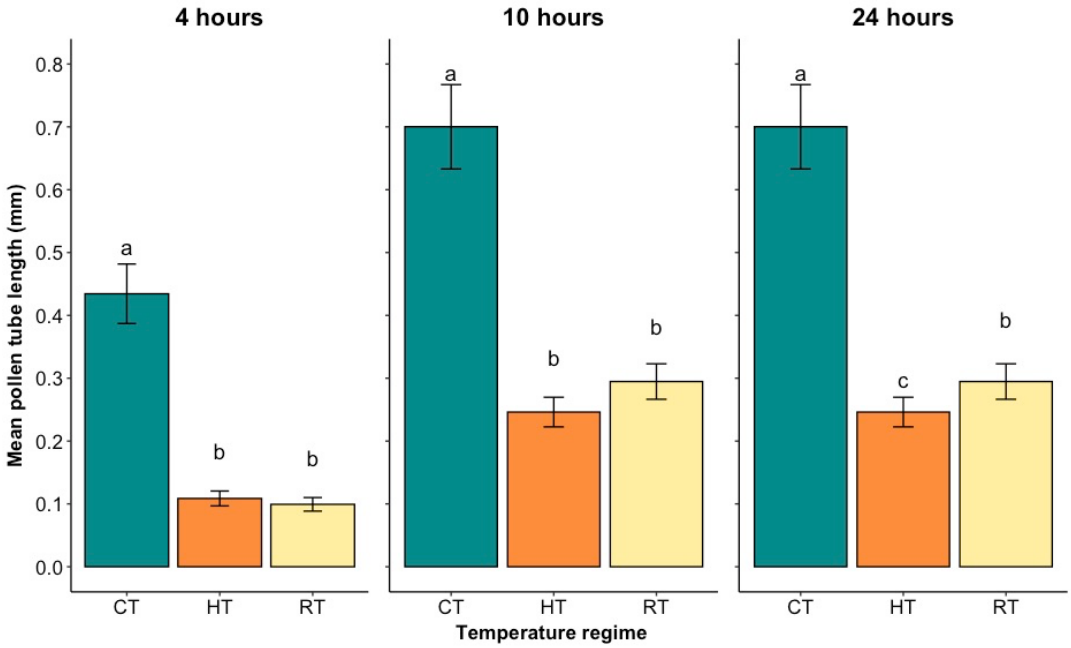


**Figure 2.3** Mean pollen tube length of the 'Bluecrop' blueberry cultivar after 4, 10, and 24 h of exposure to three different temperature regimes (CT = 25°C, HT = 37.5°C, RT = 37.5°C for 4 h and 25°C for 20 h). Each bar represents the mean value of six independent replicates ± SE. Bars with a common letter, based on a Sidak post-hoc test, are not statistically different at $P < 0.05$ within each graph.

## 2.4 DISCUSSION

Temperature is the most important factor controlling plant growth and development, particularly for reproductive stages of development including pollen germination and pollen tube growth (Zinn et al. 2010; Snider and Oosterhuis 2011; Yang Q et al. 2019). In this first study to document upper thermal limits of northern highbush blueberry, we found that pollen germination and tube growth in northern highbush blueberry had high performance at 20 and 30°C but became inhibited between 30 and 40°C. I determined the upper threshold for pollen performance to be 35°C, as higher temperatures cause significant and irreversible damage to northern highbush blueberry pollen performance. Furthermore, I found that short bursts of extreme heat, only 4 hours of exposure to 37.5°C, is enough to cause significant and irreversible damage in blueberry pollen

performance. These new findings highlight the sensitivity of blueberry reproduction, specifically pollen germination and tube growth, to extreme temperatures experienced recently in major production regions of this crop.

Our first experiment revealed that the greatest pollen germination rates and tube growth of northern highbush blueberry cultivars occurred at 20 and 30°C, whereas exposure to 40°C either significantly or completely inhibited pollen germination and tube growth. Given that northern highbush blueberry is commonly cultivated in temperate regions and blooms in the spring, this indicates that pollen tube growth is optimized for temperatures typically encountered during spring bloom, similar to strawberry, sweet cherry (*Prunus avium*), peach, and apricot (*Prunus armeniaca*) (Cerovlć and Ružić, 1992; Austin et al., 1998; Hedhly et al., 2004, 2005; Kozai et al., 2004; Ledesma and Sugiyama, 2005).

The optimal temperature range for pollen performance found in this study deviated slightly from reports in rabbiteye blueberry, where Yang Q et al. (2019) determined 21.4 and 18.4°C as the ideal temperatures for pollen germination and tube growth, respectively. They also reported that at 30°C, pollen tubes grew quickly but mostly stopped growing in the middle of the style (Yang Q et al. 2019). It is possible that the *in situ* methods used by Yang Q et al. (2019) using 'Gardenblue' pollen measured in 'Brightwell' pistils contributed to a greater sensitivity to heat than in our study. As Yang Q et al. (2019) suggest, higher temperatures inhibit stigmatic mucus secretion, which may contribute to poor stigmatic receptivity or a decrease in pistil nutrient supply which can reduce pollen germination and inhibit tube growth (Herrero and Hormaza, 1996; Hedhly et al., 2003, 2005; Mesihovic et al., 2016), and thus these pollen-stigma interactions could explain some of the differences in our findings.

Differences in pollen response to cooler and hotter temperatures across time were also observed in our study. For example, pollen germination and tube lengths were similar at 10 and 40°C across cultivars after 4 h of exposure. However, after 24 h, germination and tube length for pollen exposed to 10°C increased amongst all cultivars. Similarly, Yang Q et al. (2019) reported that the percentage of rabbiteye styles with germinated pollen at 10°C was around 12% at 4 h but increased to 43% at 24 h. At 40°C, we found little to no increases in pollen germination and tube growth after 24 h, and for some cultivars, we observed decreases in pollen germination and tube growth after 24 h. These findings were further confirmed by assessing the proportion of tetrads that produced two or more pollen tubes, a measure of pollen viability in blueberry (Lang and Parrie,

1992). At 24 h across all cultivars (excluding 'Liberty'), several pollen tetrads were observed with two or more tubes at 10°C, but no tetrads exposed to 40°C produced more than one tube. These findings suggest that exposure to cold temperatures may temporarily delay development in pollen germination in tube growth, but exposure to high temperatures results in rapid and potentially irreversible inhibition of pollen performance.

Our second experiment revealed that pollen germination and tube growth decreased as temperature increased, with almost complete inhibition at the highest temperature across all sampling times and cultivars. While germination was greatest between 30-35°C, the longest pollen tubes were observed primarily at 30°C ('Bluecrop', 'Elliott', 'Liberty') or 32.5°C ('Jersey'). Interestingly for 'Bluecrop' and 'Jersey' cultivars, pollen germination rates were similar between 30-35°C yet pollen tube lengths were reduced at 35°C, potentially indicating different temperature optima for pollen germination and pollen tube growth processes. For all sampling times and cultivars, pollen germination and pollen tube lengths were substantially reduced at 37.5°C compared to cooler temperatures. While pollen germination is a necessary pre-requisite for fertilization, it is critical that pollen tubes from germinating grains grow long enough to reach the ovules for successful fertilization to occur. Thus, we consider the upper temperature threshold for northern highbush blueberry pollen to be 35°C, as temperatures above 35°C result in significant and often irreversible consequences in pollen performance. These results align with observations from Yang Q et al. (2019) who reported significantly reduced pollen germination and no pollen tubes fully traversing the style at 35°C in rabbiteye blueberries.

Exploration of how 'Bluecrop' pollen responds to a brief exposure of extreme heat followed up by a cooler, relieving temperature indicated very little recovery. In this third experiment, pollen tube lengths did not differ between high temperature (HT) and relieving temperature (RT) groups for the first two sampling times and increased very little (albeit significantly) for the RT group at the last sampling period. Given this meager increase in length, and that pollen tetrads and their associated pollen tubes were randomly assessed at each sampling time (i.e., not the same tetrads and tubes were tracked across sampling times), it is possible that this growth for the RT group after 24 h is not biologically significant. Further evidence for this can be seen in the germination data, as pollen germination rates did not significantly differ between HT and RT groups across all sampling times. Compared to pollen tube lengths observed at the CT group, lengths for the RT group are consistently much shorter at all sampling times, suggesting

that any recovery observed is still insufficient for a full recovery in pollen performance. Our findings suggest that that even a short duration of extreme heat can have long-term effects on pollen performance that we expect to limit blueberry pollination and yield. Similar results have been observed by Dupuis and Dumas (1990) who reported that exposing maize (*Zea mays*) pollen to 40°C for 4 h prior to pollination completely inhibited *in vitro* fertilization, despite the fact that pollination occurred on spikelets maintained at 28°C throughout the experiment. Furthermore, Iwahori (1966) found that exposing tomato (*Solanum lycopersicum*) pollen to 40°C for 3 h following pollination completely prevented fertilization and resulted in ovule abortion. Our results, in tandem with findings in other well-studied crops, emphasize the permanent impacts of short-term extreme heat exposure on plant reproductive processes.

Our findings suggest that extreme heat contributed to blueberry yield deficits when fields in bloom are exposed to temperatures greater than 35°C for several hours and highlights the need to develop production systems that can maintain yields during extreme weather. Coupled sensor and cooling systems, for example, could be employed to mitigate risk of heat-associated injury in blueberry fields. For example, recent research in Oregon has highlighted that intermittent misting in blueberry farms using microsprinklers can reduce air temperatures at the blueberry canopy level by approximately 10°C (Yang F-H et al., 2019; Yang et al., 2020). While this strategy was originally used for preventing heat damage in ripening blueberries, it could be adapted for use during blueberry bloom to help mitigate heat stress during pollination. Our findings emphasize the negative consequences of brief extreme heat on blueberry pollen, and the impact a few degrees can make on pollen performance. Thus, strategies that reduce blueberry field temperatures during this developmentally sensitive period could help prevent losses in yields. However, field trials are needed to investigate the effectiveness and potential pitfalls of using irrigation systems to cool during blueberry bloom. In addition to mitigation strategies for currently established blueberry fields, our observations of cultivar-specific responses to heat also highlights the potential to explore breeding for heat tolerant blueberry cultivars. Marker-assisted breeding can integrate traits for climatic adaptation into new blueberry cultivars faster than conventional breeding strategies (Rowland et al., 2012; Lobos and Hancock, 2015; Wang et al., 2019). However, using marker-assisted breeding to develop heat-tolerant blueberry cultivars will require further evaluation of genotypes with a range of tolerance (Lobos and Hancock, 2015; Driedonks et al., 2016). Using *in vitro* techniques, our research provides novel information on the thermotolerance of four northern

highbush blueberry cultivars which should be considered when identifying heat-tolerant germplasm for future breeding efforts (Zinn et al., 2010).

This first report of pollen performance for northern highbush blueberry cultivars under extreme heat conditions provides novel insight into the thermal thresholds of commercial cultivars. Our results describe the negative consequences of brief periods of extreme heat, mirroring conditions experienced in the field, on northern highbush blueberry pollen performance. New regions of blueberry expansion and existing regions growing blueberry such as south China (Yang Q et al., 2019) and Michigan (Lobos and Hancock, 2015) have already experienced the negative effects of extreme heat exposure on berry yields, so understanding the tolerance and sensitivity of this crop to high heat is critical as incidences of extreme heat increase in intensity, frequency, and duration (Lobos and Hancock, 2015; Yang Q et al., 2019; van Es, 2020; IPCC, 2021; Walters et al., 2022). Our results from these experiments should inform future *in vivo* research in northern highbush blueberry and advise prospective breeding and mitigation strategies to reduce heat stress in blueberry fields and maintain yields as the climate continues to change.

## TABLES

**Table 2.1** Mean proportion germinated pollen tetrads (± SE) of four northern highbush blueberry cultivars after two durations of exposure to 10, 20, 30, or 40°C.

| | | Mean ± SE proportion germination | |
|---|---|---|---|
| Cultivar | Temperature (°C) | 4 h | 24 h |
| 'Bluecrop' | 10 | 0.05 ± 0.09 b | 0.68 ± 0.08 a |
| | 20 | 0.52 ± 0.09 a | 0.93 ± 0.08 a |
| | 30 | 0.75 ± 0.09 a | 0.98 ± 0.08 a |
| | 40 | 0.00 ± 0.00 b | 0.03 ± 0.08 b |
| | | $\chi^2 = 52.08$, df = 3, $P < 0.001$ | $\chi^2 = 16.74$, df = 3, $P < 0.001$ |
| 'Elliott' | 10 | 0.02 ± 0.04 c | 0.55 ± 0.07 b |
| | 20 | 0.23 ± 0.04 b | 0.78 ± 0.07 ab |
| | 30 | 0.57 ± 0.04 a | 0.95 ± 0.07 a |
| | 40 | 0.00 ± 0.00 c | 0.00 ± 0.00 c |
| | | $\chi^2 = 161.88$, df = 3, $P < 0.001$ | $\chi^2 = 103.33$, df = 3, $P < 0.001$ |
| 'Jersey' | 10 | 0.00 ± 0.00 b | 0.48 ± 0.05 b |
| | 20 | 0.63 ± 0.04 a | 0.82 ± 0.05 a |
| | 30 | 0.63 ± 0.04 a | 0.88 ± 0.05 a |
| | 40 | 0.02 ± 0.04 b | 0.00 ± 0.00 c |
| | | $\chi^2 = 19.33$, df = 3, $P < 0.001$ | $\chi^2 = 180.59$, df = 3, $P < 0.001$ |
| 'Liberty' | 10 | 0.00 ± 0.00 b | 0.38 ± 0.08 a |
| | 20 | 0.37 ± 0.06 a | 0.70 ± 0.08 a |
| | 30 | 0.42 ± 0.06 a | 0.63 ± 0.08 a |
| | 40 | 0.02 ± 0.06 b | 0.00 ± 0.00 b |
| | | $\chi^2 = 16.52$, df = 3, $P < 0.001$ | $\chi^2 = 16.62$, df = 3, $P < 0.001$ |

Values within each cultivar and sampling time with a common letter are not statistically different at $P < 0.05$, based on a Sidak post-hoc test. Statistical comparisons of the proportions were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data.

**Table 2.2** Mean proportion germinated pollen tetrads (± SE) of four northern highbush blueberry cultivars after two durations of exposure to 30, 32.5, 35, 37.5, or 40°C.

| | | Mean ± SE proportion germination | |
|---|---|---|---|
| Cultivar | Temperature (°C) | 4 h | 24 h |
| 'Bluecrop' | 30 | 0.95 ± 0.05 a | 0.95 ± 0.04 a |
| | 32.5 | 0.95 ± 0.05 a | 0.92 ± 0.04 a |
| | 35 | 0.92 ± 0.05 a | 0.95 ± 0.04 a |
| | 37.5 | 0.58 ± 0.05 b | 0.73 ± 0.04 b |
| | 40 | 0.00 ± 0.00 c | 0.00 ± 0.00 c |
| | | $\chi^2 = 20.38$, df = 4, $P < 0.001$ | $\chi^2 = 19.03$, df = 4, $P < 0.001$ |
| 'Elliott' | 30 | 0.77 ± 0.03 a | 0.88 ± 0.04 a |
| | 32.5 | 0.75 ± 0.03 a | 0.83 ± 0.04 a |
| | 35 | 0.82 ± 0.03 a | 0.87 ± 0.04 a |
| | 37.5 | 0.37 ± 0.03 b | 0.45 ± 0.04 b |
| | 40 | 0.00 ± 0.00 c | 0.02 ± 0.04 c |
| | | $\chi^2 = 435.59$, df = 4, $P < 0.001$ | $\chi^2 = 357.55$, df = 4, $P < 0.001$ |
| 'Jersey' | 30 | 0.48 ± 0.07 ab | 0.60 ± 0.06 a |
| | 32.5 | 0.55 ± 0.07 a | 0.68 ± 0.06 a |
| | 35 | 0.42 ± 0.07 ab | 0.58 ± 0.06 a |
| | 37.5 | 0.20 ± 0.07 bc | 0.30 ± 0.06 b |
| | 40 | 0.00 ± 0.00 c | 0.00 ± 0.00 c |
| | | $\chi^2 = 42.07$, df = 4, $P < 0.001$ | $\chi^2 = 21.36$, df = 4, $P < 0.001$ |
| 'Liberty' | 30 | 0.73 ± 0.04 a | 0.93 ± 0.03 a |
| | 32.5 | 0.82 ± 0.04 a | 0.87 ± 0.03 a |
| | 35 | 0.87 ± 0.04 a | 0.85 ± 0.03 a |
| | 37.5 | 0.38 ± 0.04 b | 0.50 ± 0.03 b |
| | 40 | 0.00 ± 0.00 c | 0.00 ± 0.00 c |
| | | $\chi^2 = 359.18$, df = 4, $P < 0.001$ | $\chi^2 = 503.85$, df = 4, $P < 0.001$ |

Values within each cultivar and sampling time with a common letter are not statistically different at $P < 0.05$, based on a Sidak post-hoc test. Statistical comparisons of the proportions were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data.

**Table 2.3** Mean proportion germinated pollen tetrads (± SE) of the northern highbush blueberry cultivar 'Bluecrop' under three temperature regimes[1].

| Sampling time (h) | Temperature regime | Mean ± SE proportion germination |
|---|---|---|
| 4 | CT | 0.89 ± 0.08 a |
| | HT | 0.66 ± 0.08 ab |
| | RT | 0.59 ± 0.08 b |
| | | $\chi^2 = 7.63$, df = 2, $P < 0.05$ |
| 10 | CT | 0.91 ± 0.06 a |
| | HT | 0.78 ± 0.06 a |
| | RT | 0.77 ± 0.06 a |
| | | $\chi^2 = 3.28$, df = 2, NS |
| 24 | CT | 0.91 ± 0.06 a |
| | HT | 0.66 ± 0.06 b |
| | RT | 0.75 ± 0.06 ab |
| | | $\chi^2 = 9.59$, df = 2, $P < 0.01$ |

[1]CT = 25°C, HT = 37.5°C, RT = 37.5°C for 4 h and 25°C for 20 h. Mean values with a common letter are not statistically different at $P < 0.05$ within sampling time, based on a Sidak post-hoc test. Non-significant comparisons ($P > 0.05$) are indicated by NS.

# CHAPTER 3. EFFECTS OF PRE-BLOOM EXTREME HEAT EXPOSURE ON FRUIT SET AND QUALITY IN NORTHERN HIGHBUSH BLUEBERRY

## 3.1 INTRODUCTION

Extreme heat poses an increasing risk for agricultural production (Hatfield et al., 2020; Lohani et al., 2020; Parker et al., 2020; Brás et al., 2021; Kopecká et al., 2023), with potential to cause significant changes in crop yield. For example, abnormally warm temperatures in the winter and spring in California in 2015 resulted in more than $240 million in combined crop indemnity payments for several woody perennial crops, including almond, cherry, grape, pistachio, peach, and walnut (Parker et al., 2020). Such reductions in crop yields threaten food security, food quality, and the cost of fresh food for a growing human population (Hatfield et al., 2011; IPCC, 2023). While most research has focused on understanding the effects of gradual long-term temperature increases (e.g., 1.5-3°C), short-lived extreme heat events are also expected to increase and, depending on their timing and intensity, may be more detrimental to crop production (Zinn et al., 2010; Hatfield and Prueger, 2015; Mesihovic et al., 2016; Walters et al., 2022). Plants have evolved strategies to function within a wide range of temperatures and abiotic conditions, but adequate time is needed for plants to acclimate (Larkindale and Vierling, 2008; Müller and Rieu, 2016), resulting in greater potential for injury following short bursts of extreme heat compared to gradual temperature increases. As extreme heat events intensify, it will be critical to determine the effects of brief heat stress on crop performance and productivity (Hatfield et al., 2020; Lohani et al., 2020), and to develop mitigation measures.

Extreme heat can have negative repercussions for flowering plants at all development stages, but particular sensitivity has been recorded for reproductive development in angiosperms (Zinn et al., 2010; Müller and Rieu, 2016; Hatfield et al., 2020; Lohani et al., 2020). During reproductive development, a series of coordinated processes must occur for successful fertilization. First, pollen microspores must undergo meiosis and mitosis to form mature pollen grains, stored in floral anthers until anthesis (Lohani et al., 2020). Once a flower opens, anthers release mature pollen to be transferred to a receptive stigma surface, where adhered pollen grains must germinate and penetrate the stigma surface, growing pollen tubes that traverse the style towards the ovules, finally resulting in fertilization (Snider and Oosterhuis, 2011). Thus, viable pollen and a receptive stigma are required for fertilization, yet these processes can be disrupted following short bouts of heat stress, particularly during pollen development, germination, and tube elongation (Zinn et al.,

2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021). While female floral organs (stigma, style, ovary) are considered less sensitive to heat stress than male floral organs (Lohani et al., 2020), under natural conditions heat stress will simultaneously affect both reproductive tissues and can have additive negative effects on fertilization. Evaluating how male and female floral organs differ, or align, in their sensitivity to extreme heat will be important for assessing field-realistic consequences for crop production.

During male gametophyte development, heat stress can result in the degradation of the tapetum (the specialized layer of nutritive cells supporting pollen development), failure to release microspores (i.e., immature pollen grains), altered metabolism and transport of nutrients in developing pollen, reduced pollen viability, poor anther dehiscence, and failure to release mature pollen (Snider and Oosterhuis, 2011; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021; Kumar et al., 2022). Together, heat stress can disrupt the physiological, morphological, and biochemical processes necessary for pollen development and functioning, potentially limiting fertilization. Synergistic effects can occur when female floral organs are also exposed to heat stress, reducing soluble carbohydrates and ATP content in pistils, further inhibiting pollen tube growth and fertilization (Snider et al., 2009). Initially, pollen grains rely on pre-existing carbohydrate reserves to fuel pollen tube growth, but eventually utilize carbohydrates provided by the transmitting tract of the style (Herrero and Arbeloa, 1989; Zinn et al., 2010), resulting in potential for additive effects of heat stress experienced by male and female floral tissues. Rapid reductions in stigmatic receptivity are also reported following heat stress, inhibiting support for pollen penetration, germination, and adhesion (Hedhly et al., 2003, 2005). Heat stress during female gametophyte development can also cause malformed ovules, desiccated stigmas, and can lead to ovule abortion (Snider et al., 2009; Lohani et al., 2020), further perpetuating fertilization failure in addition to poor pollen performance.

The timing of heat exposure, as well as the reproductive organs enduring heat stress, can also have substantial influence on fertilization and yield. In one study, tomato fruit and seed production were completely inhibited when pollinated using pollen developed under heat stress conditions despite the plant being grown in optimal conditions (Peet et al., 1998; Snider and Oosterhuis, 2011). In the same study, when tomato plants were exposed to heat stress conditions and pollinated with pollen developed under optimal conditions, fruit set and seed production were similar to control plants (Peet et al., 1998; Snider and Oosterhuis, 2011). Similar results have been

reported in maize (Dupuis and Dumas, 1990). The available literature suggests that male gametophyte development and performance are more sensitive to heat stress than other developmental stages. A recent focus of research in this field has evaluated the consequences of heat stress at different floral and male gametophyte (pollen) development stages, including specific stages during microsporogenesis and microgametogenesis (Lohani et al., 2020). The consequences for mature pollen vary greatly depending on when heat is applied during these developmental processes (Lohani et al., 2020) but is relatively unknown for many different crop species. In barley florets, exposure to heat stress during the pre-meiotic stage of development resulted in stunted anther development, failure to produce pollens grains, and altered progression of the pollen Microspore Mother Cell (MMC) meiosis, while heat stress during meiosis limited starch accumulation in pollen grains with negative consequences for pollen viability (Sakata et al., 2000; Draeger and Moore, 2017; Callens et al., 2023). Studies in barley and *Arabidopsis* indicate that brief extreme heat effects are variable across different flower development stages (Kim et al., 2001; Callens et al., 2023). There is also a strong bias to studies on annual crop responses to extreme heat, with much less known about perennial crops.

Far less is known about the tolerance of perennial fruit crops to heat extremes, perhaps because many are grown in climates where hot weather conditions during bloom were very rare. However, some studies suggest greater sensitivity in spring-blooming perennial crops than summer flowering species (Hedhly et al., 2009). Of the studies done in perennial crops, greater efforts have been made in evaluating pollen performance *in vitro,* primarily at anthesis, including strawberry, sweet cherry, peach, apricot, and blueberry (Austin et al., 1998; Cerovlć and Ružić, 1992; Hedhly et al., 2004; Hedhly et al., 2005; Kozai et al., 2004; Ledesma and Sugiyama, 2005; Yang et al., 2019; Walters and Isaacs, 2023). While *in vitro* studies provide valuable and easily reproducible information on pollen thermotolerance, these studies do not report the effects of heat stress on fertilization success, fruit set, or fruit quality. Thus, *in vivo* approaches, such as hand-pollination studies, provide insight into how heat can affect plant reproductive success under natural conditions by exposing both male and female floral organs. These studies can take into account the potential additive effects of heat stress on stigmatic receptivity, pistil support for pollen tube growth, and ovule development. For example, Ledesma and Kawabata (2016) exposed strawberry flowers to 42°C for 4 h and found that the degree of damage to fruit set and fruit quality varied by flower age and cultivar. In the ‘Nyoho’ cultivar, total fruit set was significantly reduced

when heat was applied 0-9 days before anthesis and at 0-12 days before anthesis for the 'Toyonoka' cultivar, indicating greater sensitivity to heat stress at earlier and later floral development stages. At earlier development stages, strawberry flowers were at the MMC development stage and at later development stages, flower anthers were undergoing dehiscence (Ledesma and Kawabata, 2016). Authors also found that 'Nyoho' had no significant change in the size or weight of berries following heat stress, despite a reduction in fruit set (Ledesma and Kawabata, 2016). The results from this study highlight the importance of evaluating the sensitivity of various floral development stages to heat stress to capture the full potential effects on flowering crop plants, particularly those most at risk, including spring-blooming perennial crops.

Northern highbush blueberry, *Vaccinium corymbosum,* is a spring-blooming woody perennial native to eastern North America, and historically grown in regions with cold winters and mild summers (Retamales and Hancock, 2012; Lobos and Hancock, 2015). In Michigan, the most widely grown fresh blueberry cultivar is 'Bluecrop', comprising 25% of the state's blueberry acreage for its cold-hardiness, vigor, yield, flavor, and low need for cross-pollination (Vander Weide et al., 2024). As with most *Vaccinium* species, blueberries are highly dependent on wild pollinators for fertilization, seed set, and maximizing yields (Tuell et al., 2009; Gibbs et al., 2016; Pinilla-Gallego and Isaacs, 2018; DeVetter et al., 2022). Under optimal conditions, including good weather and ample pollination services by bees, highbush blueberry has the potential to set nearly 100% of its flowers into fruits (Kumarihami et al., 2021; DeVetter et al., 2022). Gough et al. (1978) described floral organ development in 'Bluecrop' and provided a limited description of blueberry gametophyte development over time. In early November, MMC formation begins but development ceases in the first part of December as conditions become cooler and photoperiods are shorter (Gough et al., 1978). By mid-March, development is again initiated when microsporogenous tissue in the anthers is separated and tapetal disintegration begins, continuing through bud swell (Gough et al. 1978). By mid-April, pollen grains are fully formed and loosely packed in the antherine locules and remnants of tapetal tissue are apparent (Gough et al. 1978). Northern highbush blueberry floral bud development is typically characterized using the following terms, indicating the progression of growth as photoperiods get longer and temperatures rise: tight bud, bud swell, bud break, tight cluster, early pink bud, late pink bud, and anthesis (blueberries.msu.edu). However, Gough et al. (1978) did not describe floral bud development stages occurring at the same time as pollen development (with the exception of bud swell at microsporogenesis), so the relative

timings of pollen and floral bud development are still largely unclear for northern highbush blueberry. In the spring when blueberry floral buds continue meiosis (microsporogenesis) and mitosis (microgametogenesis) in developing pollen grains, exposure to extreme heat could result in negative, compounding effects on the quality of pollen produced as observed in other flowering plants as discussed in other flowering crops above. However, given the limited research identifying when male gametophyte development (i.e., microsporogenesis-microgametogenesis) occurs during blueberry floral bud development, our understanding of when blueberry flowers are most sensitive to extreme heat is lacking.

Recent pollen performance studies in northern highbush blueberry have shown that exposure to brief periods (4 h) of extreme heat (37.5°C) can significantly, and irreversibly, reduce *in vitro* pollen germination and tube growth, which may have negative implications for blueberry fruit set and fruit quality (Walters and Isaacs, 2023). Indeed, In May 2018, the main blueberry production region in Michigan experienced mid-day conditions of 35°C for over 4 hours during bloom, where these daily maximum temperatures were >20°C hotter than historical daily maximums for that region and time of year (Global Historical Climatology Network). This was the hottest May on record for the region in the past 121 years. Despite high bloom density and strong crop potential 2018, Michigan blueberry yields were 30-50% lower than normal. Pollen performance studies can provide insights into pollen thermotolerance (see Chapter 2), but additional research is required to determine the effects of heat on blueberry fruit set and fruit quality. To my knowledge, no studies have assessed the consequences of pre-bloom heat stress on blueberry fruit production or berry quality. To address this, I evaluated the consequences of brief, extreme heat exposure on 'Bluecrop' northern highbush blueberry fruit set and fruit quality at various floral development stages, including tight bud, bud swell, bud break, early pink bud, and late pink bud. By exposing 'Bluecrop' bushes to normal (25°C) or extreme heat conditions (37.5°C) for 4 h at the development stages described above, and self-pollinating open flowers with pollen exposed to the same conditions, the effects pre-bloom heat stress on blueberry pollination could be quantified. Bushes exposed to heat stress or non-stress conditions were compared for fruit set, fruit ripening, and fruit quality to test the hypothesis that heat sensitivity varies by development stage, and thus, fruit set and berry quality vary depending on when extreme heat exposure occurs relative to floral development stage.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Plant material and maintenance

Dormant 2-year-old northern highbush blueberry 'Bluecrop' bushes were purchased in late winter in 2021 (Hartmann Nursery, Grand Junction, Michigan and DeGrandchamp Farms, South Haven, Michigan). The bushes were immediately placed in dark cold storage (MSU Horticultural Teaching and Research Center, Holt, Michigan) at 2°C until at least 1200 chilling hours had accumulated. Thereafter, bushes were moved from cold storage into the MSU greenhouses (East Lansing, MI) as needed for experiments, and maintained at 22 ± 5°C and 16:8 L:D photoperiod for the duration of the experiments. Plants were watered regularly to maintain moist soil, and soil pH was monitored every 3-4 weeks to ensure it was < 6.0 using a soil pH meter (Bluelab Soil pH pen, Bluelab Corporation, New Zealand). When necessary, bushes were treated with Jobe's Organics soil acidifier (calcium sulfate (80%), sulphur (18%), and bentonite clay (2%), Easy Gardener Products, Inc., Waco, Texas) according to the manufacturer's recommendations. Osmocote Smart-Release Plant Food Flower and Vegetable granules (nitrogen (14%), available phosphate (14%), soluble potash (14%), The Scotts Company, Marysville, Ohio) were also added to potted blueberry bushes following the manufacturer's directions.

### 3.2.2 Blueberry temperature treatments

Heat treatments were randomly assigned to either a control temperature (CT) treatment (25°C for 4 h) or high temperature (HT) treatment (37.5°C for 4 h). The temperature for the HT regime was selected to mimic recently experienced extreme heat events where daily maximum temperatures exceeded 35°C for 4 h during blueberry bloom (Global Historical Climatology Network) which can negatively affect pollen performance in blueberry (Walters and Isaacs, 2023, Chapter 2). Bushes were exposed to temperature treatments using environmental growth chambers (Darwin Chambers, St. Louis, MO) set to CT and HT conditions (60 ± 5% relative humidity), and temperatures were monitored every 30 min using HOBO temperature loggers (Onset Computer Corporation, Bourne, MA). Plants were assigned in pairs such that multiple plants received the HT and CT treatment conditions at the same time in each respective chamber. Following temperature exposure, bushes were immediately brought back to the greenhouse and maintained under the conditions described above for the remainder of the experiment.

3.2.3 Experimental design

'Bluecrop' bushes were exposed at several floral bud development stages, including tight bud, bud swell, bud break, early pink bud, or late pink bud growth stages to CT or HT conditions for 4 h. Following placement in the greenhouse, bushes were randomly assigned to a development stage and temperature treatment. Blueberry bushes were monitored daily to assess floral bud development, ensuring plants were exposed at the correct time. For each development stage, we used 12 bushes, with six bushes exposed to CT conditions and six exposed to HT conditions. Using colored twist ties, floral bud development stages were identified and marked on shoots prior to exposure, where twist ties were loosely (but securely) wrapped around the shoot just below floral buds assessed in the experiment. From the floral buds selected for treatment, colored twist ties were again used to randomly assign floral buds to be hand pollinated or to be a pollen donor. At least six floral bud locations were selected per bush, where three floral buds were randomly assigned to be pollinated or to be a pollen donor. Across all bushes and development stages, we selected apical buds for treatments as the number of flowers found in inflorescence buds is negatively correlated with distance from shoot tip (Retamales and Hancock, 2018). From each selected floral bud, several flowers developed, but only 2-3 flowers were kept per location for pollination treatments, typically resulting in six flowers to be pollinated and six flowers used to collect pollen for donor pollen (i.e., typically 12 total flowers per plant). However, depending on flower health and availability, as few as four and as many as 13 flowers were used per plant for each pollination treatment. Flowers considered unhealthy or showing signs of disease were excluded from the experiment. In some cases, entire bushes were excluded from analysis if they showed significant signs of disease.

3.2.4 Pollen collection and hand-pollination

Flowers assigned to be pollen donors were used to collect pollen from flowers on the same plant as those to be pollinated. Thus, the donor and recipient flowers experienced the same temperature treatment at the same development stage, and all flowers in this study were self-pollinated. Pollen was collected from at least the same number of flowers being hand-pollinated, ensuring sufficient pollen available to saturate floral stigmas for optimal fertilization. To ensure that the pollen and flowers used for experiments were fresh (within 24 h of anthesis), every open flower was marked on each bush by lightly dotting the corolla with a permanent marker (Kearns and Inouye, 1993). Thus, the following day, any open flower without a mark had been open for

less than 24 h. Donor pollen was released from the blueberry flower anthers by touching a vibrating sonication tool (AeroGarden, Boulder, Colorado) onto the outside of the corolla and collecting into a 1.5 mL microcentrifuge tube, allowing pollen to accumulate at the conical end of the centrifuge tube. Flowers were self-pollinated by dipping the end of a fine, clean paint brush in the microcentrifuge tube containing the recently collected donor pollen and lightly touching it to the surface of the flower stigma until there was an even layer of pollen saturating the stigma. A small, white paper tag on a thin thread was loosely wrapped around the pedicel of hand-pollinated flowers to record relevant information on berry ripening timing, described in more detail in the subsequent section. Hand-pollination flowers were bagged using small, white mesh jewelry bags which remained until fruit collection. Bagging flowers helped ensure that no cross contamination or additional pollination could occur while flowers and berries developed in the greenhouse. Once all flowers were treated, identified, and bagged, all other flowers and flower buds were removed from the whole bush. Flowers not used for treatments were removed to provide maximum resource allocation for the experimental flowers and subsequent berries.

3.2.5 Fruit set assessments and berry collection

Blueberry bushes were maintained in the greenhouse throughout berry development and ripening under optimal conditions (22 ± 5°C, 16:8 L:D). Treated flowers were checked daily to monitor ovary swelling (indicating fruit growth or flower abortion), green fruit growth, and timing of berry color change (from pink to blue). Paper tags attached to flowers and developing fruit included relevant information such as the date a berry starts to change color (changing from green to pink), the date it turned completely blue, and the date it was collected. Berries were collected five days after turning completely blue to ensure enough time to ripen and accumulate sugar and were immediately placed in a -20°C freezer until ready for analysis. Green berries, aborted flowers, and small, dried blue berries with no juice or flesh were excluded from fruit set and fruit quality analyses, as these are not considered marketable fruit. Flowers were considered to have successfully set fruit if the berries turned 100% blue and had enough juice for fruit quality assays.

3.2.6 Fruit quality assays

Berries were removed from the -20°C freezer and kept in room temperature conditions for 4 h until they thawed completely (Yang et al., 2009). Each individual berry was assessed for berry weight (g), berry diameter (mm), the number of fertilized seeds, the percent (%) fertilized seeds, total soluble solids (TSS), titratable acidity (TA). The weight of each berry was determined using

a precision balance (Mettler Toledo, Columbus, OH), accurate to 1 mg. Berry diameter was measured using a digital caliper, where the caliper was placed at the cross section of the berry (i.e., calyx facing up) at the widest part of the berry. The number of fertilized seeds and percent fertilized seeds was determined by squishing each berry separately in a small, clear, clean plastic bag and counting the number of fertilized or unfertilized seeds (together representing the total number of seeds) using a dissection microscope. Seeds were considered fertilized if they were large, brown, and round/full while unfertilized seeds were small, white or light tan, and deflated or malformed. Following seed assessments, the juice from the squished berry was set aside to assess the % TSS and % TA using the Pocket Brix-Acidity Meter (CPAL-BXIACID7, Cat. No 7107, Atago USA Inc, Bellevue, WA) following manufacturer instructions. Briefly, to measure % TSS, at least 0.3 mL of undiluted berry juice was added to the Pocket Brix-Acidity Meter sensor, where each berry was assessed separately. Berry juice was extracted by squeezing a single berry onto the sensor. After each assessment, the sensor was cleaned with purified water and dried with a Kimwipe (Kimberly-Clark Professional, Rosewell, GA). To measure % TA, juice (from a single berry) was diluted at a 1:50 ratio using purified water, mixing juice and water in a small beaker for about 30 sec, then placing at least 0.6 mL of diluted juice onto the Pocket Brix-Acidity Meter sensor. Again, the sensor was cleaned with purified water and dried with a Kimwipe after each assessment.

3.2.7 Data analysis

All statistical analyses were conducted in R (R version 4.2.3) (R Core Team, 2023), using generalized linear mixed-models (GLMM) and linear mixed-models (LMM) ('lme4' package) (Bates et al., 2015) for all analyses. Final models were selected based on the type of data (i.e., count vs continuous), meeting the assumptions of the model distribution, assessing the lowest AIC model scores ('bbmle' and 'stats' packages) (Bolker et al., 2023; R Core Team, 2023), the model deviance residuals ('base' package) (R Core Team, 2023), and other model performance metrics ('performance' package) (Lüdecke et al., 2024). Model assumptions were checked by assessing scaled residuals using the 'performance' and 'car' packages (Fox et al., 2023; Lüdecke et al., 2024). All models described below adequately met model distribution assumptions. Test statistics were calculated using Likelihood Ratio Tests (LRT) ('stats' package) (R Core Team, 2023). The 'stats', 'emmeans' and 'multcomp' packages (Hothorn et al., 2023; R Core Team, 2023; Lenth et al., 2024) were used for pairwise comparisons between temperature treatments for each development stage and to derive means and standard errors of response variables from models.

Figures were made using the 'ggplot2' and 'ggsignif' packages (Ahlmann-Eltze and Patil, 2021; Wickham et al., 2024).

A generalized linear mixed model (GLMM) with a binomial distribution and logit link function was used to test the effects of temperature exposure on blueberry fruit set, where the response variable was a binary indicator for fruit set success or failure. Floral development stage (tight bud, bud swell, bud break, early pink bud, late pink bud) and temperature treatment (CT or HT) were included as fixed effects, and replicate and bush code were included as random effects. A GLMM with a negative binomial distribution was used to test the effects of temperature exposure on berry ripening time, where the response variable was the number of days until berries turned fully blue after they started to change color. Berry ripening time was only assessed for berries that were considered to have successfully set fruit. Development stage and temperature treatment were included as fixed effects, and bush code and replicate were included as random effects.

To test whether temperature exposure affected blueberry fruit quality, GLMM and LMM models were used depending on the fruit quality measurement, representing the response variable (i.e., berry weight, berry diameter, number of fertilized seeds, % fertilized seeds, % TSS, % TA). Floral development stage and temperature treatment were included as fixed effects, and replicate and bush code were included as random effects in all models. For berry weight (g) a GLMM model with gamma distribution was used with an inverse link function. For berry diameter (mm), the number of fertilized seeds, % fertilized seeds, % TSS, and % TA, a LMM model with a Gaussian distribution was used. Berry quality measurements were only assessed for berries that were considered to have successfully set fruit.

## 3.3 RESULTS

### 3.3.1 Fruit set and berry ripening

Temperature treatment had a significant effect on fruit set for only one development stage, but otherwise there was no significant difference between treatments (Figure 3.1: $\chi^2$=12.02, df=2, $p$=0.21). In the bud swell stage, exposure to HT conditions significantly reduced fruit set (z=2.32, $p$=0.02), where the proportion fruit set for CT plants was 0.95 ± 0.05 while the proportion fruit set for the HT plants was 0.58 ± 13, a 39% reduction compared to non-stressed conditions. No significant effect of temperature treatment on berry ripening time was found across all development stages (Figure 3.2: $\chi^2$=4.91, df=2, $p$=0.30).

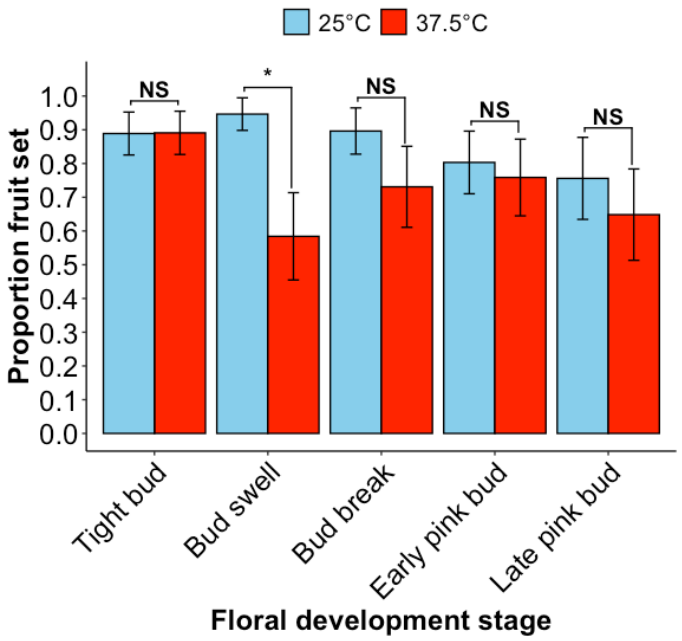


**Figure 3.1** The mean (± SE) proportion fruit set for 'Bluecrop' bushes exposed to control (CT: 25°C) or high (HT: 37.5°C) temperature treatments for 4 h at different floral bud development stages. Significant difference between means is indicated by the asterisk above error bars ('*' = $p \leqq 0.05$).

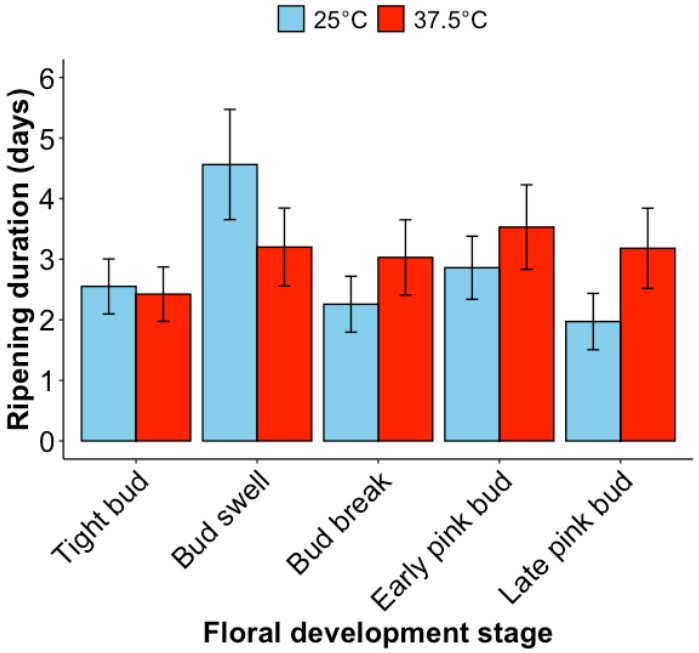


**Figure 3.2** The mean (± SE) berry ripening duration (days) for 'Bluecrop' bushes exposed to control (CT: 25°C) or high (HT: 37.5°C) temperature treatments for 4 h at different bud development stages. No significant differences were found between means for any of the development stages ($p > 0.05$).

3.3.2 Berry quality

Across all development stages (Table 3.1), temperature treatment had no significant effect on berry weight ($\chi^2$=3.17, df=2, $p$=0.53), berry diameter (F=0.83, df=2, $p$=0.51), the number of fertilized seeds (F=0.35, df=2, $p$=0.84), or % TSS (F=0.75, df=2, $p$=0.57). Temperature treatment had a significant effect on the percent fertilized seeds in one development stage (early pink bud) but was not statistically significant between treatments overall (Table 3.1: F=1.23, df=2, $p$=0.31). Berries collected from bushes exposed to HT conditions at the early pink bud stage had approximately 78% fertilized seeds while berries collected from bushes exposed to CT conditions had approximately 65% fertilized seeds (t=-2.46, $p$=0.02). Temperature treatment had a significant effect on the % TA in one development stage but no other stages (Table 3.1: F=2.05, df=2 $p$=0.10). At the bud break stage (t=-2.45, $p$=0.02), % TA was significantly higher (69%) in HT-treated plants (0.66 ± 0.08) than CT-treated plants (0.39 ± 0.08).

## 3.4 DISCUSSION

Heat stress during flower and pollen development threatens reproduction in flowering crops by reducing pollen viability and performance and inhibiting pollen-pistil interactions necessary for fruit set and development. Previous studies have shown the negative effects of extreme heat stress in northern highbush blueberry pollen performance *in vitro* (Walters and Isaacs, 2023, Chapter 2), however, no studies to my knowledge have assessed which blueberry floral development stages are most sensitive to heat stress, or the cascading effects brief heat stress exposure may have on fruit set, fruit ripening, and berry quality. This study provides evidence for development-dependent responses in floral buds exposed brief extreme heat exposure (37.5°C, 4 h) on fruit set in northern highbush blueberry. At the bud swell floral development stage, a significant negative response to heat stress was observed with a 39% reduction in fruit set, while other development stages (bud break, early and late pink bud) were not significantly affected. Results in this chapter suggest a stronger effect of heat stress on fruit set than fruit ripening and berry quality, particularly during the bud swell development stage. The results also highlight resilience to heat stress during the tight bud development stage, immediately following dormancy, compared with later development stages. Identifying which floral development stages are most at risk of heat stress is important for targeting mitigation efforts and timing in blueberry fields to help promote an abundant, high-quality crop despite seasonal heat waves.

At tight bud, the earliest floral development stage in blueberry, fruit set and fruit quality were nearly the same between heat stressed and control temperature treatments. Based on Gough et al. (1978) and other studies on woody perennial spring-blooming plants (Zhang and Fernando, 2005; Fadón et al., 2019), it is likely that northern highbush blueberry pollen is at the microspore mother cell stage (MMC) during tight bud and has not yet undergone meiosis. During dormancy in temperate-zone woody perennial plants, floral buds are typically more resistant to unfavorable conditions (Campoy et al., 2011). While abiotic resilience in dormant perennial plants has mostly been studied through the lens of cold tolerance (Anderson et al., 2010), dormancy may also enable resiliency to short bouts of extreme heat, but additional studies are needed to confirm this in blueberry. Future studies should consider testing the effects of repeated heat stress events at the tight bud blueberry floral stage on fruit set and quality to more realistically depict the consequences of heatwaves. However, this development stage is fleeting and develops into bud swell rather quickly in the spring, so the likelihood of this floral stage experiencing spring-time heat stress is quite low. Conversely, late summer and fall time heat waves could affect floral bud initiation and early stages of pollen microspore development and deserves greater exploration.

As plants become active after winter dormancy, blueberry floral buds transition into the bud swell stage where previously dormant MMC are likely now undergoing meiosis (Gough et al., 1978). This is a critical stage for developing pollen grains to accumulate nutrients needed to fuel pollen germination and tube growth (Lohani et al., 2020). During microsporogenesis, but particularly during MMC meiosis, exposure to heat stress can degrade the tapetum, inhibit auxin biosynthesis, and alter amino acid and carbohydrate metabolism, limiting the nutrient supply and hormone production necessary for pollen development, germination, and tube growth. This often results in pollen sterility (Zinn et al., 2010; Giorno et al., 2013; Lohani et al., 2020). The lower fruit set of self-pollinated ‘Bluecrop’ flowers following extreme heat exposure at bud swell indicates substantial disruption to pollen development and subsequent pollen performance, including pollen germination and tube growth. These findings agree with previous reports that just four hours of extreme heat (37.5°C) can significantly and irreversibly inhibit blueberry pollen germination and tube growth *in vitro* (Walters and Isaacs, 2023). Sensitivity to extreme heat during MMC meiosis and microsporogenesis have also been reported in several other crops, including barley (Callens et al., 2023), sorghum (Jain et al., 2010), wheat (Sakata et al., 2000; Draeger and Moore, 2017), tomato (Iwahori, 1966; Peet et al., 1998; Pressman et al., 2002; Firon et al., 2006;

Sato et al., 2006), *Arabidopsis* (Kim et al., 2001), apricot (Rodrigo and Herrero, 2002), groundnut (Vara Prasad et al., 2000) and strawberry (Ledesma and Kawabata, 2016), with notable reductions in fruit set following heat stress in these crops.

These results suggest a mechanism by which blueberry yields were reduced by 30-50% across Michigan in 2018, as this heat wave likely reduced pollen performance and limited blueberry fertilization. According to bloom prediction models (www.enviroweather.msu.edu), blueberry bushes experienced this 2018 heatwave at approximately 25% bloom, where floral bud development stages ranged from bud swell, bud break, early pink bud, late pink bud, and anthesis. However, fruit set was only significantly reduced at the bud swell stage following heat stress in the present study. It is possible that anthesis is also highly sensitive to heat stress in blueberry, as indicated by previous *in vitro* studies (Walters and Isaacs, 2023), but this floral stage was not evaluated due to limitations in healthy available bushes. Thus, future studies should assess the consequences of heat stress at anthesis for blueberry fruit set to better understand how extreme heat affects pollination. Additionally, heat stress was only applied in a single 4-hour event in this study, but additive heat stress events over several days (as experienced in May 2018) would likely amplify these negative consequences for fruit set. However, additional studies are required to confirm this. Overall, fruit set reported in the present study is similar to previous reports for self-pollinated 'Bluecrop' bushes, between 56-94% (MacKenzie, 1997; Strik et al., 2017).

Berry ripening time, or the number of days it took fruit to turn fully blue once they started changing color (from green to pink), was similar between temperature treatments. Similar to fruit set results discussed above, berry ripening was nearly the same at the tight bud development stage regardless of temperature treatment, further suggesting resilience to heat stress at this stage. Overall, berry ripening timing recorded in the current study is similar to other reports in blueberry, between 2-6 days (Forney et al., 2012; Lin et al., 2020; Watanabe et al., 2021). It is possible that such marginal differences in berry ripening time across temperature treatments could be attributed to blueberry bushes experiencing the same, optimal conditions immediately before and after heat stress exposure. Given that vegetative development is more resilient to heat stress than reproductive development (Zinn et al., 2010; Chaturvedi et al., 2021), the negative effects of a single 4-hour heat stress event during floral development may have been insufficient to disrupt photosynthesis functioning and subsequent berry development in these blueberry plants. Future

studies should be conducted that expose blueberry floral buds to repeated, brief (4 h) extreme heat events to evaluate the consequences of multi-day heat waves on subsequent berry ripening time.

Hao et al (2019) assessed optimal vegetative growth conditions in northern highbush blueberry, exposing 'Bluecrop' bushes to varying temperature conditions and found that net photosynthetic activity was highest at 35/30°C (day/night) and lowest at 40/35°C. In contrast, 'Bluecrop' pollen exposed to temperatures at or above 35°C *in vitro* for just 4 h showed significant declines in performance, with complete inhibition of pollen germination and tube growth at 40°C after 4 and 24 h of exposure (Chapter 2). This highlights resilience to heat during vegetative growth and the sensitivity to heat during floral development and pollen performance in northern highbush blueberry. The present study echoes these reports, as pre-bloom extreme heat exposure had a stronger effect on fruit set, implying greater limitation on fertilization, than on berry ripening. Thus, once blueberry flowers set fruit, berries may have been supplied with similar resources during development and ripening, regardless of temperature exposure during floral development. To confirm this, comparative studies should be performed to document blueberry photosynthetic activity, fertilization success, berry production, and berry quality when brief extreme heat is applied at various vegetative and reproductive development stages.

Among the blueberry flowers that set fruit, berry weight and berry diameter were similar between temperature treatments across all floral development stages and were comparable to other reports in blueberry (MacKenzie, 1997; Kim et al., 2013; Jorquera-Fontena et al., 2017; Strik et al., 2017; Retamales and Hancock, 2018; Strik and Vance, 2019; Lin et al., 2020). Similar findings have been reported in strawberry, where 'Nyoho' was exposed to a single-day heat stress event during early floral and pollen development, inhibiting fruit set but no measurable changes in fruit weight or size of those fruits that did set (Ledesma and Kawabata, 2016). In apricot, fruit set was reduced following a 6-7°C increase in maximum temperature during floral bud development, yet the fruit produced under the warming treatment was slightly heavier (as could be expected from a lower crop load) (Rodrigo and Herrero, 2002). Nonetheless, the overall crop yield was still reduced under the warming treatment in apricot, despite the slight increase in individual fruit weight (Rodrigo and Herrero, 2002). Observations in the present study and related studies indicate that heat stress during floral development has a stronger effect on fertilization and fruit set than on the quality of fruit that develops after fertilization.

The number of fertilized seeds per berry was comparable between temperature treatments within development stages, and overall was similar to previous studies in 'Bluecrop' (Strik and Vance, 2019). The percent fertilized seeds were also mostly unaffected by temperature treatment, although heat significantly increased the percent fertilized seeds (~19%) in the early pink bud stage compared to the control. It is unclear why heat exposure increased the percent fertilized seeds at this stage, but nonetheless no other berry quality measurements were significantly affected at early pink bud, so overall berry quality was not improved following heat exposure. Across all tested development stages, % TSS and TA values were mostly similar between temperature treatments. However, at the bud break stage, exposure to heat stress significantly increased berry acidity by 69%. While such an increase in TA would result in a more acidic taste (Guiné et al., 2016), the cause of such an increase in acidity is unclear given that fruit set, ripening time, and other berry quality measurements were not significantly affected at bud break. Overall, these results indicate no consistent pattern of pre-bloom heat stress exposure on the tested berry quality parameters.

In this study, I found that brief heat stress exposure to developing blueberry floral buds had greater limitations on fruit set than fruit quality, comparable to other studies in strawberry (Ledesma and Kawabata, 2016) and apricot (Rodrigo and Herrero, 2002). Among sufficiently fertilized blueberry blooms, heat stress may not have substantial consequences for the quality of berries produced. This suggests that the yield declines reported in Michigan in 2018 were from poor fruit set, but berry quality metrics influencing yield (weight, size) may have been mostly retained. Given that fruit quality measurements were conducted on berries that successfully set fruit, it is likely that short bursts of heat stress had a greater effect on pollen performance and fertilization, in agreement with previous *in vitro* studies in blueberry (Chapter 2). Significant reductions in fruit set following heat stress occurred at the bud swell stage, but the other floral development stages showed some resilience to temperature stress. This aligns with my prediction that bud swell is occurring during MMC meiosis, as this pollen development stage has shown high sensitivity to heat for many different crop plants and is an important time for pollen and flowers to accumulate resources required for proper development and performance. However, studies should be done following the methods of Gough et al. (1978) to validate pollen and floral organ development timing with floral bud morphology in northern highbush blueberry. While additional research is required, these findings indicate greater attention is needed to protect the pollination phase of blueberry production (from pollen development to fertilization), particularly during bud

swell, to ensure high fruit set rates, as fruit quality is less affected when heat waves occur during floral bud development.

My results also show the importance of blueberry growers and field managers to have strategies to maintain fields at optimal maximum temperatures (20-30°C) during floral development and pollination, just as they do for maintaining optimal minimum temperatures to prevent frost damage. Strategies to cool fields during bouts of extreme heat include intermittent misting using overhead irrigation systems, which have been shown to reduce air temperatures in Oregon blueberry fields by approximately 10°C during berry ripening (Yang et al., 2019a, 2020a) and by 5-10°C in Michigan blueberry farms during bloom (Walters, Van Timmeren, and Isaacs, unpublished).

## TABLE

**Table 3.1** Mean ± SE values of berry quality measurements for blueberry fruit from 'Bluecrop' bushes exposed to control (CT: 25°C) or high (HT: 37.5°C) temperature treatments for 4 h at five different floral development stages. Comparisons between treatments that were significantly different are presented in bold text. Test statistics ($\chi^2$, F, df, *p*) were derived from a likelihood ratio test (LRT) of full GLMM or LMM models. Pairwise comparisons (z or t, *p*) between temperature treatments within a given development stage were also performed, derived from models.

| **Berry quality parameter** | **Development stage and temperature treatment** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Tight bud** | | **Bud swell** | | **Bud break** | | **Early pink bud** | | **Late pink bud** | |
| | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** |
| **Weight (g)** | 1.42 ± 0.16 | 1.95 ± 0.34 | 1.71 ± 0.27 | 1.49 ± 0.19 | 1.32 ± 0.14 | 1.59 ± 0.20 | 1.45 ± 0.15 | 1.87 ± 0.28 | 1.51 ± 0.17 | 1.72 ± 0.21 |
| $\chi^2$=3.17, df=4, *p*=0.53 | z=1.63, *p*=0.10 | | z=-0.70, *p*=0.48 | | z=1.14, *p*=0.26 | | z=1.42, *p*=0.16 | | z=0.78, *p*=0.44 | |
| **Diameter (mm)** | 9.39 ± 0.53 | 10.50 ± 0.57 | 13.05 ± 0.65 | 12.59 ± 0.60 | 11.50 ± 0.59 | 12.99 ± 0.61 | 12.55 ± 0.54 | 13.69 ± 0.61 | 12.44 ± 0.60 | 12.81 ± 0.60 |
| F=0.83, df=4, *p*=0.51 | t=-1.44, *p*=0.16 | | t=0.52, *p*=0.61 | | t=-1.77, *p*=0.09 | | t=-1.40, *p*=0.17 | | t=-0.43, *p*=0.67 | |
| **Fertilized seeds** | 39.64 ± 3.64 | 47.76 ± 4.35 | 58.34 ± 6.24 | 53.95 ± 5.52 | 48.28 ± 4.84 | 48.69 ± 5.13 | 45.19 ± 4.20 | 49.47 ± 5.15 | 49.10 ± 5.37 | 54.20 ± 5.85 |
| F=0.35, df=4, *p*=0.84 | z=-1.44, *p*=0.15 | | z=0.53, *p*=0.60 | | z=-0.06, *p*=0.95 | | z=-0.65, *p*=0.52 | | z=-0.64, *p*=0.52 | |
| **% fertilized seeds** | 58.47 ± 3.21 | 65.79 ± 3.40 | 81.65 ± 3.97 | 78.52 ± 3.69 | 80.76 ± 3.58 | 83.32 ± 3.73 | 65.42 ± 3.29 | 77.62 ± 3.71 | 74.31 ± 3.78 | 79.62 ± 3.74 |
| F=1.23, df=4, *p*=0.31 | t=-1.57, *p*=0.13 | | t=0.58, *p*=0.57 | | t=-0.50, *p*=0.62 | | **t=-2.46, *p*=0.02** | | t=-1.00, *p*=0.32 | |
| **TSS (%)** | 12.48 ± 0.64 | 13.60 ± 0.68 | 13.15 ± 0.78 | 14.07 ± 0.73 | 14.02 ± 0.71 | 13.28 ± 0.74 | 12.97 ± 0.65 | 14.41 ± 0.73 | 13.36 ± 0.74 | 14.11 ± 0.73 |
| F=0.75, df=4, *p*=0.57 | t=-1.23, *p*=0.23 | | t=-0.88, *p*=0.38 | | t=0.75, *p*=0.46 | | t=-1.50, *p*=0.14 | | t=-0.73, *p*=0.47 | |
| **TA (%)** | 0.54 ± 0.07 | 0.65 ± 0.07 | 0.68 ± 0.08 | 0.50 ± 0.08 | 0.39 ± 0.08 | 0.66 ± 0.08 | 0.52 ± 0.07 | 0.61 ± 0.08 | 0.49 ± 0.08 | 0.57 ± 0.08 |
| F=2.05, df=4, *p*=0.10 | t=-1.10, *p*=0.28 | | t=1.55, *p*=0.13 | | **t=-2.45, *p*=0.02** | | t=-0.93, *p*=0.36 | | t=-0.71, *p*=0.48 | |

# CHAPTER 4. EXTREME HEAT EXPOSURE OF HOST PLANTS INDIRECTLY REDUCES SOLITARY BEE FECUNDITY AND SURVIVAL

This chapter was published as Walters, J., Barlass, M., Fisher, R., Isaacs, R. 2024. Extreme heat exposure of host plants indirectly reduces solitary bee fecundity and survival. *Proceedings of the Royal Society B.* 291: 20240714. https://doi-org.proxy1.cl.msu.edu/10.1098/rspb.2024.0714

## 4.1 INTRODUCTION

The warming climate is a key driver of insect population declines (Soroye et al., 2020; Wagner, 2020; Raven and Wagner, 2021; Janousek et al., 2023), yet the various ways in which these changes affect insects are still being elucidated. Acute bouts of extreme heat are becoming more frequent and intense (IPCC, 2023), negatively affecting plants and the insects that they depend on, including bees and their pollination services (Memmott et al., 2007; Hatfield et al., 2020; Nicholson and Egan, 2020; Kammerer et al., 2021; Walters et al., 2022). Studies investigating the direct effects of extreme heat on bees or plants offer important insights on the consequences of heat for their physiology (Bordier et al., 2017; Hamblin et al., 2017; Chaturvedi et al., 2021; Zhao et al., 2021; Zhu et al., 2021), capacity for acclimation (Martinet et al., 2015; Mesihovic et al., 2016; Oyen and Dillon, 2018; Martinet et al., 2021a; Gonzalez et al., 2022b, a; Hernández-Fuentes et al., 2023; Sepúlveda and Goulson, 2023) and reproductive potential (Vanderplanck et al., 2019; Amuji et al., 2020; Lohani et al., 2020; Zhao et al., 2021; Campion et al., 2023). However, when extreme heat events occur in the environment, bees and their host plant both endure heat stress, potentially resulting in compounding, interactive ramifications for these organisms. Despite this, investigations intersecting the effects of extreme heat on bee-plant interactions are limited (Greenop et al., 2020; Descamps et al., 2021; Hemberger et al., 2023), fragmenting our understanding of heat stress repercussions on pollination systems. In plant-pollinator networks, synchrony is critical for their success and survival, and extreme heat can disrupt the timing of these interactions (Scaven and Rafferty, 2013; CaraDonna et al., 2018; Slominski and Burkle, 2021; de Manincor et al., 2023). While phenological synchrony is important to understand in the context of climate change, few studies have explored whether heat stress also affects the synergy between bee pollinators and their host plants. For example, there is little information on whether heat stressed plants adequately support the dietary needs of their bee

pollinators. As bouts of extreme heat continue to intensify, it is critical to broaden our understanding of how indirect heat stress affects bee-plant interactions.

Plant reproductive development, particularly gametophyte (i.e., pollen) development and performance are the most sensitive development stages to heat stress (Zinn et al., 2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021). When extreme heat occurs during floral development, maturation, and/or dehiscence, it can have compounding adverse consequences, including degradation of the tapetum, failure to release microspores, altered metabolism and transport of nutrients in pollen, reduced pollen viability, poor anther dehiscence and failure to release pollen (Snider and Oosterhuis, 2011; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021; Kumar et al., 2022). When heat inhibits nutrient sequestration in developing pollen, concentrations of carbohydrates, proteins, lipids, and amino acids can be reduced or altered (Borghi and Fernie, 2017; Borghi et al., 2019; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021). Many of these nutrients drive reproductive processes including pollen germination and tube growth, which are necessary for fertilisation, so this depletion of nutrients can reduce pollen quality, performance, and subsequent reproduction (Borghi and Fernie, 2017; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021; Kumar et al., 2022). Some researchers have recently hypothesized that these heat-induced pollen nutrient reductions could also negatively affect bees, as these insects rely on the nutrients present in pollen for their diets (Borghi and Fernie, 2017; Borghi et al., 2019; Descamps et al., 2021; Walters et al., 2022). However, no studies have confirmed the connection between heat stressed pollen and bee nutrition, requiring further research. Extreme heat may also reduce the production and release of pollen (Raja et al., 2019; Amuji et al., 2020; Hedhly et al., 2020; Lohani et al., 2020), limiting bee access to floral rewards. These changes could lead to nutritional stress with important consequences for bee fecundity, behavior, and development (Vaudo et al., 2015, 2020; Woodard et al., 2019; Knauer et al., 2022). Subsequent offspring can also be affected as reduced quantity and quality of pollen provisions may lead to altered developmental timing, shifts in sex ratios, reduced body size, and higher rates of mortality (Bosch, 2008; Bukovinszky et al., 2017; Filipiak and Filipiak, 2020; Stuligross and Williams, 2020). These adverse effects on bees could limit pollination services, affecting reproduction of wild plants (de Manincor et al., 2023) and crops (Slominski and Burkle, 2021; Walters et al., 2022). Despite the potential ramifications,

there is limited cross-disciplinary understanding of the effects of extreme heat on bees, plants, and their interactions.

While most research exploring the impacts of extreme heat on bee pollinators has focused on social bees, studies suggest greater sensitivity to extreme heat in solitary bees (Hamblin et al., 2017), such as *Osmia* bees. *Osmia lignaria* Say (Hymenoptera: Megachilidae) is a solitary, polylectic, stem-nesting mason bee native to North America. Wild and managed *O. lignaria* are important pollinators of spring-blooming wildflowers and crops (Kraemer et al., 2014; Sheffield, 2014; Pitts-Singer et al., 2018; Boyle et al., 2020). However, recent studies suggest *O. lignaria* populations are in decline in the US, driven by increased competition and disease prevalence (LeCroy et al., 2020; Russo et al., 2021; Gutierrez et al., 2023) as well as pesticide exposure (Artz and Pitts-Singer, 2015; Eeraerts et al., 2020; Kopit et al., 2022). Direct nutritional and heat stress can also negatively affect these bees (Conrad et al., 2017; Kierat et al., 2017; CaraDonna et al., 2018; Lee et al., 2018; Filipiak and Filipiak, 2020; Knauer et al., 2022; Song et al., 2023; Melone et al., n.d.), but there is limited understanding of the indirect effects of extreme heat on *Osmia* bees and their offspring. Given the evidence for declining populations and the importance of their pollination services, it is imperative to identify and mitigate stressors affecting wild bees.

Northern highbush blueberry (*Vaccinium corymbosum*) is a spring-blooming perennial fruit crop native to North America that is highly dependent on wild pollinators and is visited by *O. lignaria* (Pinilla-Gallego and Isaacs, 2018; Fortuin et al., 2021). In temperate regions where this crop grows, spring temperatures are typically moderate, but extreme heat events have become more common (Lobos and Hancock, 2015; IPCC, 2023). In 2018, Michigan blueberry growing regions endured temperatures exceeding 35°C for several hours during bloom, associated with 30-50% yield reductions (Walters and Isaacs, 2023). Blueberry pollen exposed to extreme heat, even for 4 hours, can drastically and irreversibly inhibit performance, potentially limiting fertilization and yields (Walters and Isaacs, 2023). Lacy phacelia (*Phacelia tanacetifolia*) is a herbaceous flowering plant native to North America, visited by *O. lignaria* in the wild and semi-field experiments (Williams, 2003; Ladurner et al., 2008; Stuligross and Williams, 2020). While no studies to our knowledge have assessed the effects of heat stress on blooming phacelia, optimum temperatures are reported to be between 23-30°C (Owayss et al., 2020; Hernández-Fuentes et al., 2023) with high seedling mortality following acute heat stress (Thomson et al., 2017, 2018). White clover (*Trifolium repens*) is an herbaceous flowering plant native to Europe and Central Asia,

introduced and widely distributed across North America. It is commonly found blooming near blueberry fields and frequently visited by *O. lignaria* (Pinilla-Gallego and Isaacs, 2018; Graham et al., 2023). Brief heat exposure can cause abiotic stress in white clover (Iqbal et al., 2022), resulting in fewer inflorescences (Zaleski, 1964) or vegetative tissue loss and mortality (Wright et al., 2022). While crop plants have received greater attention, many plants enduring high temperatures (>35°C) during floral development, even for a few hours, experience adverse repercussions on pollen quality, performance, and subsequent plant functioning (Zinn et al., 2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Raja et al., 2019; Lohani et al., 2020).

As extreme heat events become more common and intense, and bee declines continue to escalate, further research is required to provide a broader understanding of how native solitary bees are affected by extreme heat. To address this, I investigated the indirect effects of extreme heat on *O. lignaria* and their offspring by releasing females in field cages to forage on blueberry, phacelia, and white clover exposed to extreme heat (37.5°C for 4h) or control conditions (25°C for 4h) during bloom. Bees were observed during foraging and egg-laying, and their offspring were monitored *in vitro* from eggs to adults. The study was designed to determine whether: 1) Heat stressed host plants affect *O. lignaria* maternal foraging and fecundity; 2) Development and survival of larvae are affected by consuming pollen from heat stressed plants; and 3) Emergence, survival, body size and sex ratio of adult progeny are affected by heat stressed larval diets. I expected female *O. lignaria* bees provided with heat stressed plants to have similar foraging rates but reduced fecundity. I also predicted altered larval development and reduced survival for offspring fed pollen from heat-treated plants. For adult offspring who consumed heat stressed diets as larvae, I hypothesized reduced emergence success, survival, and body size, altered timing of emergence and longevity, and male-dominated sex ratios.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Biological material

This study used three different host plants: blueberry, lacy phacelia, and white clover. Dormant 2-year-old ‘Bluecrop’ blueberry bushes were purchased in winter (Hartmann Nursery, Grand Junction, MI and DeGrandchamp Farms, South Haven, MI) and immediately placed in dark cold storage (2°C) until 1200 chilling hours had accumulated. When needed, bushes were moved to a greenhouse at 22 ± 5°C and 16:8 Light:Dark (L:D) cycle. Plants were watered regularly, and soil pH was monitored every 3-4 weeks to ensure it was <6.0 (Yang et al., 2022). When necessary,

bushes were treated with acidifier (Jobe's Organics, Easy Gardener Products, Inc., Waco, TX) and fertilizer (Osmocote, The Scotts Company, Marysville, OH) following the manufacturer label. Phacelia and white clover seeds were purchased from L.A. Hearn Company (King City, CA), and sown in 1-liter plastic pots with a mixture of potting soil (Michigan Grower Products, Inc., Galesburg, MI) and field soil at a 50:50 ratio, ensuring optimal soil moisture for growth (Seker et al., 2003; Kilian, 2016; Mitropolova et al., 2023) and kept in a greenhouse (22 ± 5°C, 16:8 L:D). Approximately 20 pots of phacelia and clover were sown each week (January-April 2022) to ensure sufficient blooming plants for experiments.

4.2.2 Exposing host plants

At approximately 25% bloom, plants were randomly assigned to control (25°C for 4h - CT) or high temperature (37.5°C for 4h - HT) conditions. Plants were exposed at 25% bloom because: this was the approximate blueberry development stage in 2018 during the extreme heat event described above, it allowed for a wider breadth of floral development stages exposed to heat (from developing buds to open blooms), and this maximized the duration of bloom (and thus floral resources) available to bees. Phacelia and white clover were also exposed at 25% bloom to ensure consistency of heat stress exposure. The temperature for the HT regime was selected to mimic recently experienced acute extreme heat events where daily maximum temperatures were > 20°C hotter than historical daily maximums in 2018, exceeding 35°C for 4 hours during blueberry bloom (Global Historical Climatology Network). In general, heat stress occurs when temperatures are 10-15°C above ambient (Wahid et al., 2007). Previous studies have shown that short exposure (<5h) to >35°C can negatively affect pollen performance in blueberry (Walters and Isaacs, 2023) and other plant species (Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Chaturvedi et al., 2021). While several studies discussed above have found a relationship between altered pollen nutrition and poor pollen viability following heat stress, no studies have reported a connection to nutrition availability for bees. However, myself and others hypothesize that such reductions in pollen viability driven by nutritional deficits may also have negative consequences for bee nutrition (Borghi et al., 2019; Descamps et al., 2021; Walters et al., 2022).

Environmental growth chambers were set to CT and HT conditions and temperatures were monitored every 30 min using HOBO temperature loggers (Onset Computer Corporation, Bourne, MA). Plants were assigned in pairs such that multiple plants received the HT and CT treatment

conditions at the same time in each respective chamber. After exposure to the appropriate temperature regime, plants were immediately moved to the field cages as described below.

4.2.3 *Osmia lignaria* bees for experiments

Male and female *Osmia lignaria* cocoons were purchased from Meyer Bees (Meyer Bees, Minooka, IL) in March 2022. Cocoons were kept dormant (no light, 4°C, 60% RH) until needed for experiments (Bosch and Kemp, 2004; Eeraerts et al., 2020). In June 2022, I began moving cocoons to emergence conditions. I placed 20 male and 10 female cocoons in 0.3 x 0.3 m plastic mesh cages (BioQuip, Rancho Dominguez, CA) next to a window for natural light, maintained at 20-22°C in three cohorts on June 10, 15, and 20, 2022. Bees took 3-5 days to emerge and were provided sugar water (50% sucrose) *ad libitum* via a dental wick in a glass flask which was replaced every week. Once emerged, observations were made daily to ensure successful mating. Bees typically mated 2-3 days following emergence. Mated females were marked on their thorax using a uniquely colored non-toxic paint pen (Mitsubishi Pencil Co., Ltd., Tokyo, Japan), then moved to a separate cage maintained under the same conditions until release in field cages. Bees were marked to allow for individual behavior assessments in field cages. Once sufficient floral resources were available, three mated females were released in each cage. Each bee was similar in size and age (3-4 days old). After all cages received their initial three bees, some cages received replacement bees if one was found dead or missing within 1 day of its release or if bees died and failed to produce eggs. Replacement bees were released in two cages under these criteria to maintain activity and egg laying.

4.2.4 Cage experimental design

Using a no-choice design, I measured foraging and egg laying of *O. lignaria* in field cages provided HT or CT host plants. At the Entomology Research Farm (East Lansing, MI), eight 3.7 x 1.8 m mesh field cages (BioQuip, Rancho Dominguez, CA) were constructed, spaced 2 m apart. Field cages were randomly assigned to temperature treatment, where four cages received HT-treated plants and four cages received CT-treated plants. Ambient air temperature averaged 25°C throughout the study at a nearby weather station (enviroweather.msu.edu), ranging from 18 to 31°C.

Within each field cage, a nest box was placed on the opposite end from the opening, facing east. Each nest box, adapted from (Knauer et al., 2022), consisted of ten 170 x 180 mm pieces of wood stacked inside a plywood box with an open face and slanted roof, mounted on two metal

poles, at 1.2 m height. In each nesting plank, 10 cavities were routered (Ryobi, Anderson, SC), each 10 mm wide and deep, 160 mm long, and spaced 7 mm apart, providing 100 blind-ended cavities in each cage. Clear acetate sheets cut to the same dimensions were placed on top of each plank, allowing observation and extraction of eggs and provisions with minimal disturbance. In front of each nest box, a 0.3 x 0.6 m bare patch of moist soil was created to allow bees access to mud for their nest construction.

Cages were randomly paired in four replicate blocks, with one cage from both temperature groups in each block. Cages were paired to match timing of plant and bee placement while also accounting for the capacity of growth chambers to expose plants to treatments. This allowed for a consistent distribution of newly exposed, blooming plants, ensuring sufficient resources for female bees and their offspring. Following heat exposure, plants were immediately moved to field cages (~5 km away). The exposure and initial placement of all host plants occurred on the same day within paired blocks, between June 14 and 29, 2022. Bees were released in cages within 2-3 days of initial blueberry plant placement, between June 16 and 27, 2022. Phacelia and clover plants were added 0-5 and 2-13 days, respectively, after bee releases and bees were able to forage on these plants the same day of plant exposure and placement. To maintain floral densities, subsequent additions of newly exposed plants (and removal of non-blooming plants) occurred from June 22 to July 26, 2022, with 5, 3, and 3 days between replacements for blueberry, phacelia, and clover, respectively. All replacement plants were moved to field cages immediately after their temperature treatment. In each cage, 26-30 blueberry, 10-15 phacelia, and 6-13 clover plants were provided throughout the experiment. The number of open flowers on each plant was counted, typically the same day as behavior assessments, or within 2-3 days, throughout the experiment. Plants were placed in the cages in rows, with blueberry placed on the outer rows (closest to the cage walls) and phacelia and clover placed on inner rows, between the blueberries. This ensured visibility of the nest box and floral visitation observations.

4.2.5 Behavior assessments

Bees were released the same day within blocked pairs, between June 16 and 27, 2022. To allow time to adjust to new conditions, foraging observations began the day following release. Bee foraging observations occurred from June 17 to July 29, 2022. Observations were 30 min per cage, conducted 3-5 days/week, typically between 9am and 3pm when weather conditions were suitable. The order in which field cages were assessed was randomized daily. For each individual bee, I

recorded the plant visited and the number of floral visits per plant. Floral visits were recorded when bees were actively collecting nectar or pollen. All bees (in each cage) were assessed by a single observer sitting inside the cage by the entrance, opposite the nest box. These observations also allowed identification of nest cavities being provisioned by *Osmia* bees. Field cage observations concluded in early August once all bees died, and the flowers were depleted.

4.2.6 *Osmia* development assays

Eggs and pollen provisions were collected from nest boxes typically the same day the eggs were laid, or within two days. Egg collection occurred at night, minimizing disturbance and potential damage to mother bees and eggs. I used sterilized featherweight forceps (BioQuip, Rancho Dominguez, CA) to place each egg and associated pollen provision in a 1.5 mL centrifuge tube with a piece of sterilized tin foil placed inside, holding them upright. These were transferred to the lab and each pollen provision was weighed on a precision balance (Mettler Toledo, Columbus, OH), accurate to 1 mg. Pollen provisions and eggs were transferred to sterilized 48-well cell culture plates with 10 mm diameter cells, adapted from (Dharampal et al., 2018; Eeraerts et al., 2020). Each well plate received 3-7 eggs, with at least one egg from both temperature treatments. Plates were kept in a dark environmental growth chamber (22°C + 60% RH) and were removed for brief periods every 1-2 days to assess larval survival and growth stage using a dissection microscope. Development stages were recorded as egg/1$^{st}$ instar, 2$^{nd}$/3$^{rd}$ instar, 3$^{rd}$/4$^{th}$ instar, 5$^{th}$ instar, cocoon spinning, or fully spun cocoon (Claus et al., 2021). Eggs were considered alive if they appeared undamaged (no holes, not deflated), and larvae were considered alive if they were moving or if spiracles on the side of the body were dilating, indicating breathing. Developing offspring were maintained at 22°C + 60% RH for 120 days, then moved to pre-wintering conditions (21 days: 14°C + 60% RH), wintering conditions (120 days: 4°C + 60% RH), and finally emergence conditions (22°C + 60% RH). The pre-winter weight of cocoons was recorded using a precision balance immediately before moving them to winter conditions.

4.2.6 Adult emergence and survival assessments

The day cocoons were moved to emergence conditions, the post-winter weight was recorded to determine the change in weight following overwintering. Daily checks were conducted to determine the timing of emergence and survival of adult bees. Emerged bees were kept in well plates without food provisions to determine their longevity. Upon the first day of adult emergence, bees were weighed and placed back in well plates. Once bees died, they were removed, sexed, and

the intertegular distance was recorded as a proxy of body size and expected flight capacity (Cane, 1987). All emerged progeny in this study were identified as males.

4.2.7 Data analysis

All statistical analyzes were conducted in R (R version 4.2.3) (R Core Team, 2023). Generalized linear mixed-models (GLMM) ('lme4' package) (Bates et al., 2015) and mixed effects Cox models ('coxme' and 'survival' packages) (Therneau, 2024; Therneau et al., 2024) were used. Decisions on final models was based on the nature of the data taken, meeting the assumptions of the model distribution, assessing the lowest AIC model scores ('bbmle' and 'stats' packages) (Bolker et al., 2023; R Core Team, 2023), the model deviance residuals ('base' package) (R Core Team, 2023), and other model performance metrics ('performance' package) (Lüdecke et al., 2024). GLMM model assumptions were checked by assessing scaled residuals using 'performance' and 'car' packages (Fox et al., 2023; Lüdecke et al., 2024). All GLMM models met model distribution assumptions. To test the assumptions of mixed effect Cox models, the relationship between scaled Schoenfeld residuals and time was quantified ('finalfit' package) (Harrison et al., 2023). The proportional hazard assumptions were met for all Cox models. Test statistics were calculated using Likelihood Ratio Tests ('stats' package) (R Core Team, 2023) for GLMM models and Cox models ('performance' package) (Lüdecke et al., 2024). The residual degree of freedom for GLMM models and hazard ratios for Cox models were derived from model summaries ('base' package) (R Core Team, 2023). The 'emmeans' and 'multcomp' packages (Hothorn et al., 2023; Lenth et al., 2024) were used to derive means and standard errors of response variables from GLMM models. Figures were made using 'ggplot2', 'ggsignif', 'ggsurvfit', and 'survival' packages (Ahlmann-Eltze and Patil 2021; Sjoberg et al., 2023; Therneau et al., 2024; Wickham et al., 2024).

Detailed descriptions of models, error distributions, and R syntax can be found in supplemental materials and Table S4.1, with brief descriptions provided below. GLMM models were used to test the effect of host plant temperature exposure on: number of open flowers (blueberry, phacelia, clover), number of flowers visited (blueberry, phacelia, clover), number of eggs laid, pollen provision weights, larval development duration, pre-winter cocoon weights, post-winter cocoon weights, pupal weight lost after overwintering, adult emergence timing, adult longevity, adult intertegular distance (ITD), and adult body weight. Cox models were used to test the effect of host plant temperature exposure on the proportion larval survival, proportion adult

emergence, and proportion adult survival. Random effects were included in all models (see Table S4.1 for details). When appropriate, a unique identifier for mother bee identity was created using the 'dplyr' package (Wickham et al., 2023) and used as a random effect. Mother bee identity was created considering the thorax paint color associated with each bee, the field cage it was placed in, and the date when it was released into a field cage, ensuring each mother bee was associated with its offspring. For analysis of the number of eggs laid, I ran two separate models. The first model excluded all bees who failed to produce eggs, testing whether temperature treatment affected fecundity of individuals successfully initiating egg laying. The second model excluded all bees who lived < 6 days, as these bees did not live long enough to begin egg laying, but otherwise included all bees.

## 4.3 RESULTS

### 4.3.1 Bee behavior

For each host plant type (blueberry, phacelia, clover), no significant effect of temperature treatment (CT vs. HT) was found for the number of open flowers, or the number of flowers visited by females (Table 4.1). No significant effect of host plant temperature exposure was observed for the amount of pollen provisions collected by female bees ($\chi^2$=0.94, df=1, $p$=0.33). The mean (± SE) pollen provision collected by females provided CT plants was 0.12 ± 0.09g compared to 0.06 ± 0.02g by females provided HT plants. Host plant temperature treatment had a significant effect on female fecundity, both for females that successfully initiated egg laying (Figure 4.1; $\chi^2$=4.20, df=1, $p$=0.04) and for female bees who lived in cages for more than six days ($\chi^2$=7.02, df=1, $p$=0.008). For bees who successfully initiated egg laying, the mean number of eggs laid was significantly lower (68%) in HT cages (1.57 ± 0.65) compared to CT cages (4.95 ± 1.51). For female bees who lived in cages for >6 days, significantly fewer eggs laid (78%) in HT cages (0.68 ± 0.30) compared to CT cages (3.08 ± 1.11) was observed. I released 16 bees in HT cages, and 10 bees (62.5%) failed to produce eggs. For CT cages, 12 bees were released, and 4 bees (33.3%) failed to produce eggs. In the HT cages, 5 bees who lived >6 days failed to lay eggs (31.3%) and 1 bee in CT cages lived >6 days and failed to lay eggs (8.3%). In total, females in cages with CT plants laid 33 eggs while those in cages with HT plants laid 10 eggs, a 70% decrease in total egg production.

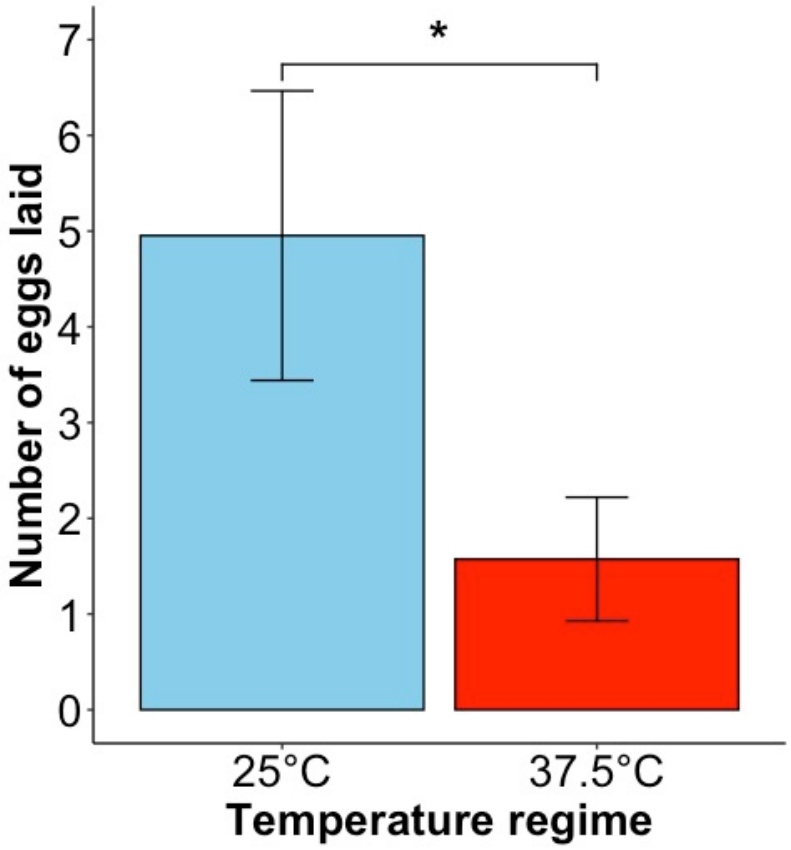


**Figure 4.1** Mean (±SE) number of eggs laid by *Osmia lignaria* provided host plants (blueberry, phacelia, and clover) exposed to CT (25°C for 4h) or HT (37.5°C for 4h) treatments. Significant difference between means is indicated by the asterisk above error bars ('*' = $p \leqq 0.05$). *p*-value indicating significance derived from a likelihood ratio test (LRT) of GLMM model.

4.3.2 Larval development and survival

Host plant temperature exposure had no significant effect on the duration of larval development across all development stages (Figure 4.2: $\chi^2$=1.33, df=2, $p$=0.93). In contrast, larval survival was significantly affected by host plant temperature exposure (Figure 4.3: $\chi^2$=17.83, df=1, $p$=0.002, n.events=42). Larvae consuming pollen from HT plants were 8.3 times more likely to die compared to larvae consuming pollen from CT plants (coef=2.11, exp(coef)=8.26, se(coef)=0.92). In CT cages, 29 of the 33 eggs laid survived to pupation, resulting in 12% larval mortality, whereas in HT cages, 4 of the 10 eggs laid survived to pupation, resulting in 60% larval mortality. Host plant temperature treatment had no significant effect on pre-winter weight of pupae ($\chi^2$=0.06, df=1, $p$=0.80; CT: 0.053 ± 0.009g; HT: 0.055 ± 0.012g), post-winter weight of pupae ($\chi^2$=0.31, df=1, $p$=0.58; CT: 0.045 ± 0.005g; HT: 0.048 ± 0.010g) or weight lost during overwintering ($\chi^2$=0.28, df=1, $p$=0.60; CT: 0.016 ± 0.003g; HT: 0.014 ± 0.003g).

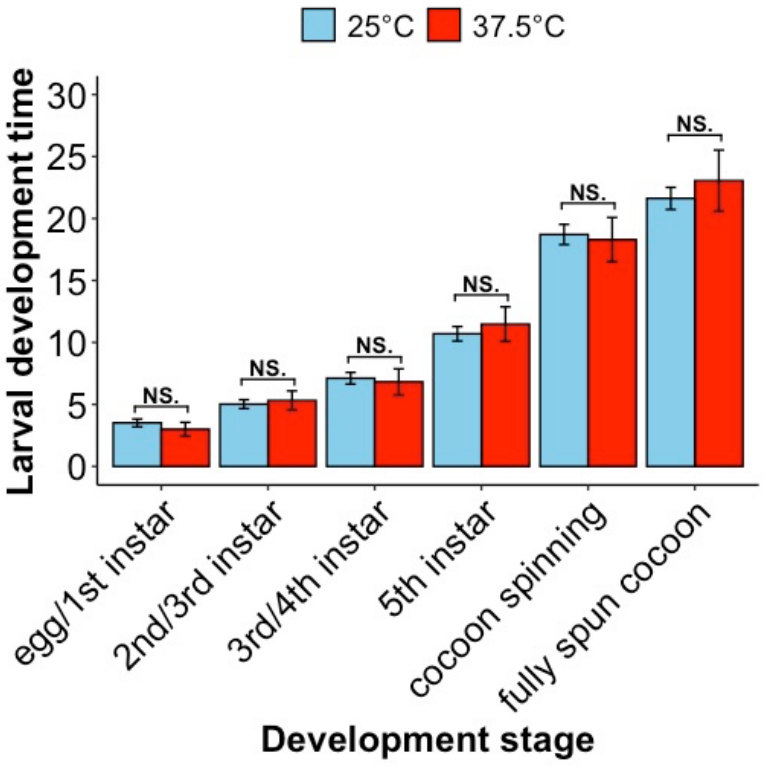


**Figure 4.2** Mean (±SE) larval development timing (days) of *Osmia lignaria* larvae fed pollen from host plants exposed to CT (25°C for 4h) or HT (37.5°C for 4h) treatments. *p*-values derived from likelihood ratio test (LRT) of GLMM model showed no significant difference of development timing between temperature treatments, for each development stage, indicated by 'NS' above error bars.

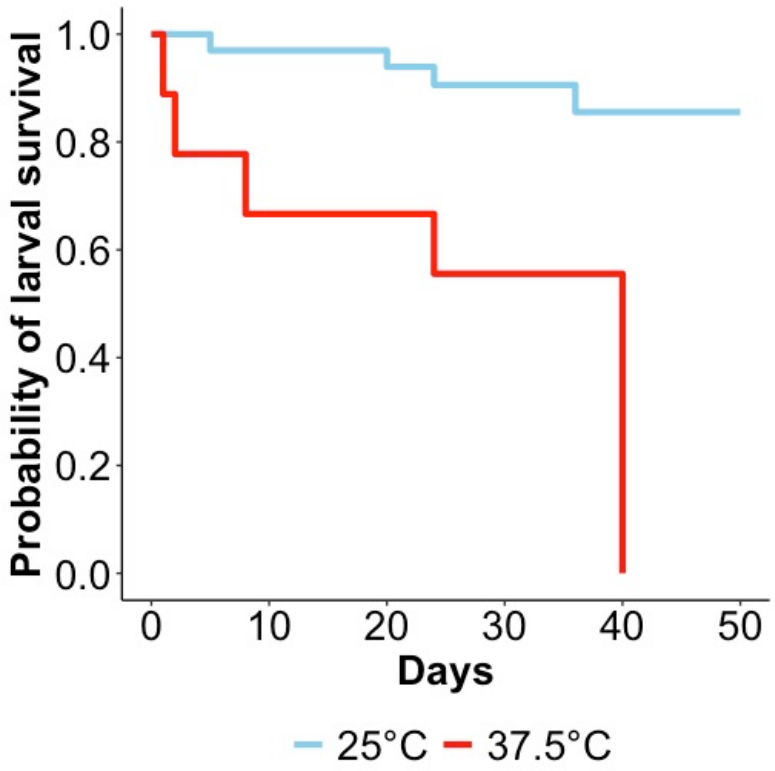


**Figure 4.3** Kaplan-Meier survival probability curve during larval development of *Osmia lignaria* larvae fed pollen from host plants exposed to CT (25°C for 4h) or HT (37.5°C for 4h) treatments.

4.3.3 Adult emergence, body size, and survival

Adult bee emergence was significantly affected by host plant temperature exposure ($\chi^2$=10.59, df=1, $p$=0.035, n.events=33). Progeny fed HT pollen as larvae were 50% less likely to emerge as adults compared to progeny fed CT pollen (coef=-0.69, exp(coef)=0.50, se(coef)=0.78). The timing of adult emergence was not significantly affected by host plant temperature exposure ($\chi^2$=1.02, df=1, $p$=0.31). The mean (±SE) days until emergence was 10.64 ± 2.36 for bees consuming HT pollen and 8.45 ± 0.65 for bees consuming CT pollen. Adult body weight ($\chi^2$=0.09, df=1, $p$=0.77) and ITD ($\chi^2$=0.25, df=1, $p$=0.61) were not significantly affected by host plant temperature exposure. The mean (±SE) adult body weight and ITD (respectively) for progeny fed HT pollen as larvae was 0.037 ± 0.009g and 2.71 ± 0.22mm, compared with 0.035 ± 0.005g and 2.58 ± 0.16mm for those who consumed CT pollen as larvae. Adult bee survival was significantly affected by host plant treatment (Figure 4.4: $\chi^2$=21.39, df=1, $p$=0.001, n.events=31). Progeny provided HT pollen provisions as larvae were 3.5 times more likely to die as adults compared to progeny provided CT pollen (coef=1.26, exp(coef)=3.54, se(coef)=0.77). The longevity of adult

survival was not significantly affected by host plant temperature ($\chi^2$=1.37, df=1, p=0.24). Bees fed HT pollen as larvae lived 5.39 ± 1.56 days as adults and bees fed CT pollen lived 7.66 ± 0.74 days as adults. Far fewer progeny survived into adulthood when provided pollen from HT plants. Of the 10 eggs laid by females provided HT plants, only 3 emerged as adults (70% mortality from egg to adulthood) compared to the bees provided CT plants where 28 of the 33 eggs laid emerged as adults (15% mortality).

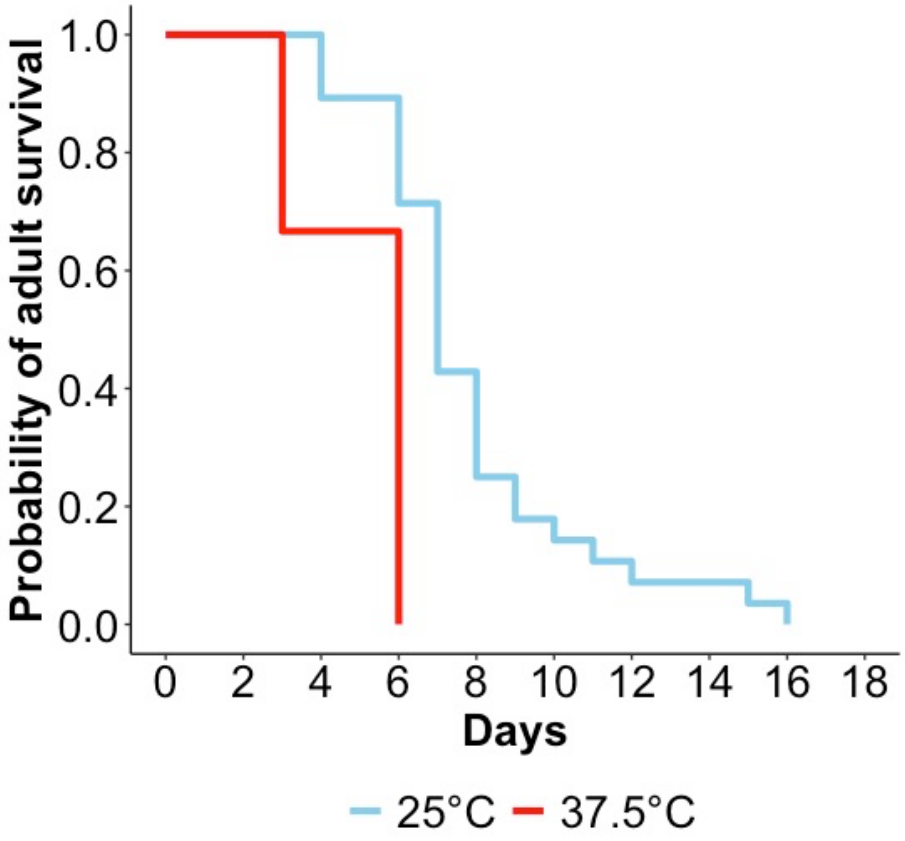


**Figure 4.4** Kaplan-Meier survival probability curve of *Osmia lignaria* adults fed pollen from host plants exposed to CT (25°C for 4h) or HT (37.5°C for 4h) treatments during larval development.

## 4.4 DISCUSSION

Anthropogenic climate change has caused substantial damage to ecosystems across the globe, and extreme heat events are a main driver for these changes (IPCC, 2023). Despite the importance of insect pollinators for ecosystem health and global agriculture, we know relatively little about the current and long-term effects of extreme heat on their health, productivity,

functioning, and interactions with other organisms. Increasingly, researchers are considering the indirect impacts of climate warming on bees by evaluating changes in floral rewards and bee behavior (Descamps et al., 2018, 2021; Borghi et al., 2019; Hemberger et al., 2023), but no studies to our knowledge have evaluated how field-realistic acute extreme heat exposure to host plants affects mother bees and their offspring. Our investigation into the indirect effects of extreme heat provides the first evidence of adverse consequences for solitary bees and their brood, mediated through their host plants. Female *O. lignaria* foraging on plants exposed to only four hours of extreme heat during bloom laid significantly fewer eggs, and the majority of larvae consuming heat stressed pollen died before pupation. The offspring who consumed heat stressed pollen as larvae and pupated had lower emergence and greater risk of mortality as adults. Trends for delayed adult emergence and shorter adult lifespan compared to those provided pollen from non-stressed plants were also observed. These results highlight that even brief periods of extreme heat on host plants can have detrimental repercussions for foraging bees and their offspring, expanding our understanding of the implications of climate change for plant-pollinator interactions beyond the direct negative effects (Memmott et al., 2007; Hegland et al., 2009).

The number of open flowers and flower visitation rates were similar between the two temperature groups in this study, indicating consistency of resources and resource use across the treatments. Despite this, I observed considerably lower egg laying by bees foraging on heat stressed plants. This suggests that floral rewards from heat stressed plants were of lower quantity and/or quality compared to non-stressed plants. In a related study, exposing host plants to heat wave conditions (35/22°C) for three days resulted in 70% lower nectar production compared to plants developing at 25°C, yet the number of flowers visited by bumble bees was the same between treatments (Hemberger et al., 2023). Other studies have found lower pollen production and release following heat exposure (Ozga et al., 2017; Hedhly et al., 2020; Lohani et al., 2020; Descamps et al., 2021). Our finding of similar-sized pollen provisions between treatments, yet fewer eggs laid in the heat stressed treatments, may reflect reduced availability of floral resources from individual flowers. Thus, bees provided heat stressed plants may need greater foraging effort to collect enough pollen for provisions. However, other studies have reported similar amounts of pollen produced from stressed- and non-stressed plants, yet the viability of pollen is lower, which may affect the quality of diet provided to bees (Descamps et al., 2018; Walters et al., 2022). Additional

studies are needed to measure changes in pollen production and composition following heat stress, and subsequent effects on pollinator visitation and plant reproduction.

Reduced egg laying by bees provided with heat stressed plants may also be attributed to an insufficient diet, adversely affecting their reproduction. Egg production and oviposition are energetically costly for all bees, but particularly for solitary bees who lay large eggs relative to their body size and require pollen for oocyte maturation (Cane, 2016). Other *Osmia* studies have shown that females foraging in low-resource conditions produced fewer brood cells per day (Stuligross and Williams, 2020), and when denied access to pollen, females failed to mature oocytes or lay eggs (Cane, 2016). *Osmia cornuta* females can produce 40-50 oocytes but rarely lay more than 10-20 eggs, suggesting limitations on fecundity may be attributed to constraints on brood cell provisioning (like reduced resource availability) rather than egg production potential (Bosch and Vicens, 2005). When brood provisioning is impeded, adjustments in parental investment allocation can occur, where females may limit the number of brood produced but maintain the provision size provided to offspring (Bosch and Vicens, 2005). This offers additional insight into why females provided heat stressed plants produced less brood but retained similar amounts of provisions for those offspring. Under extreme heat conditions, pollen may not only be in short supply, but the nutrients present in pollen may also be altered if heat disrupts the sequestration and metabolic transport of nutrients into developing pollen grains (Borghi and Fernie, 2017; Carrizo García et al., 2017; Borghi et al., 2019; Santiago and Sharkey, 2019; Descamps et al., 2021). Alterations or deficiencies in pollen nutrition can have devastating consequences for bee reproduction. In honey bees, low-protein diets can inhibit ovarian and egg development (Hoover et al., 2006; Human et al., 2007) and diets lacking essential amino acids can prevent brood production and development (Herbert et al., 1970; Roulston and Cane, 2000; Barraud et al., 2022). *Bombus terrestris* provided diets deficient in essential amino acids delayed nest initiation and inhibited brood production (Ryder et al., 2021). Future studies should compare oocyte and brood production in female bees provided optimal, low-quantity, low-quality, or heat stressed pollen diets.

The consequences of heat-altered nutrient availability on pollen performance and fertilisation is well studied (Carrizo García et al., 2017; Raja et al., 2019; Santiago and Sharkey, 2019; Lohani et al., 2020; Chaturvedi et al., 2021), but there is far less research on the subsequent consequences for bee nutrition (but see Borghi et al., 2019; Descamps et al., 2021; Hemberger et

al., 2023). In the present study, bees consuming pollen from heat-exposed plants had much higher mortality during larval development, despite similar provision sizes compared to those in control cages. While enough pollen may have been provided for larval development, it is possible that heat stressed provisions lacked certain essential nutrients, inhibiting their survival. Previous research on inadequate pollen nutrition emphasizes the consequences of a poor diet on bee development and survival. For *O. lignaria* larvae fed honey bee-collected pollen, only the highest protein diets supported development to adulthood (Levin and Haydak, 1957; Roulston and Cane, 2000). Other studies have found that carbohydrates, not protein, mediate *Osmia* larval growth and survival to pupation (Austin and Gilbert, 2021). *Osmia bicornis* and *O. cornuta* larvae failed to develop on *Tanacetum* pollen, which authors suggest is due to insufficient quantity or quality of nutrients (Sedivy et al., 2011). *Osmia cornifrons* larvae failed to develop when fed multifloral and single-source pollen diets, even when these diets had similar protein:lipid ratios as surveyed provisions, suggesting certain micronutrients were lacking and must be present for proper development (Crone et al., 2023). Abnormal development was also observed in our study for larvae that consumed heat stressed pollen, where a third of these larvae spun unusually light-colored silk and failed to enclose themselves for pupation. *Osmia* cocoons are an important sink for nutrients assimilated and used during larval development, and underdeveloped cocoons may indicate the scarcity of specific elements present in pollen (Filipiak et al., 2021). *Osmia bicornis* larvae fed a single-source pollen diet failed to enclose their cocoon and had high larval mortality, suggesting chronic nutrient deficiency (Bukovinszky et al., 2017). When fed high quantities of rapeseed pollen, the same species exhibited hindered cocoon development and high male mortality, but when supplemented with additional nutrients, these negative effects were absent (Filipiak et al., 2022). In the context of these other studies, our results suggest a nutritional mechanism for the adverse effects of indirect heat stress on larval development and survival. However, additional research is required to quantify nutrient composition of heat stressed pollen to better understand how it affects nutrition and silk production in *Osmia* bees.

I found variable effects of heat stressed diets on bee development, with higher larval mortality and abnormal cocoon spinning, but no effect on larval development duration. This finding is surprising, but understanding the physiological processes of *Osmia* development may provide insight. Solitary bee larvae provided pesticide-contaminated diets experience slower development compared to untreated diets, and authors attribute this to detoxification processes that

divert time, energy, and nutrients away from development (Anderson and Harmon-Threatt, 2019; Claus et al., 2021). In the present study, no detoxification was required as brood were not exposed to pesticides, and the pollen provided came from known host plants, protected from pesticide exposure. Other studies have shown extrinsic cues for *Osmia* development timing and pupation, regulated via starvation and hormone signaling rather than meeting a critical mass as previously assumed (Helm et al., 2017), possibly providing insight on the similar development timing observed between treatments in our study. There are strong positive correlations between pollen provision mass and weight of *Osmia* cocoons and adults (Zdzisław et al., 2004; Claus et al., 2021), and no differences in the weights of pollen provisions, cocoons, or adults between treatments was observed. The results indicate similar fat body depletion and respiration rates among surviving brood (Bosch and Kemp, 2003, 2004; Bosch et al., 2010; Sgolastra et al., 2016) and suggest that the indirect effects of extreme heat are primarily mediated through pollen quality affecting progeny survival as larvae and adults.

In spring-emerging bees, like *Osmia*, emergence timing is critical for maximizing resource allocation and fecundity (Bosch and Kemp, 2003; Bosch and Vicens, 2006; CaraDonna et al., 2018). When emergence phenologies are altered, individuals may experience lower mating opportunities, foraging potential, and fecundity (CaraDonna et al., 2018; Farzan and Yang, 2018; Kehrberger and Holzschuh, 2019; Pelletier and Forrest, 2023). In the present study, 50% of bee pupae fed heat stressed pollen as larvae failed to emerge as adults and took three additional days to emerge compared to the control diet group. Ovary maturation in *O. lignaria* takes 2-3 days, after which females initiate nesting (Bosch, 2008). In another study, *O. lignaria* females who successfully established nests took ~3 days to emerge, while females who failed to establish nests took ~6 days (Sgolastra et al., 2016). So, a three-day delay in emergence could have repercussions for *Osmia* egg laying potential. For males, delayed emergence could impede mating opportunities due to shorter copulation events and heightened reproduction failure, as observed in *O. cornuta* (Felicioli et al., 2023). Delayed emergence is also relevant for crop pollination, where mismatches in bee emergence and bloom could inhibit pollination services (Bosch and Blas, 1994; Bosch and Kemp, 2000; Pitts-Singer et al., 2018; Boyle et al., 2020).

All emerged progeny in this study were males. This could be because the nutrients present in pollen provisions were adequate for male development and survival, but not for females (Filipiak et al., 2021). Higher concentrations of certain nutrients are found in female *Osmia*

cocoons, potentially reflecting higher production costs and nutrient demands for female bees than male bees (Filipiak et al., 2021). Alternatively, female bees may preferentially produce progeny of the smaller sex (males) when floral resources are limited (Bosch and Vicens, 2005). Given the limited sample size in this study, future research should consider scaling up this design in the field or exploring these mechanisms in laboratory settings. Nonetheless, our study is amongst the first to provide strong evidence that brief periods of extreme heat can indirectly reduce female fecundity and subsequent offspring survival.

The longevity of *Osmia lignaria* adults in the absence of food reflects the quality of diet received as larvae and survival under natural conditions when food sources are limited (Bosch and Kemp, 2000; Eeraerts et al., 2020). Bees fed heat stressed pollen as larvae had a greater risk of mortality and shorter lifespans (~3 days) as adults compared to those fed non-stressed pollen. Considering emergence delays and reduced lifespans, bees fed heat stressed pollen would be active for ~6 fewer days compared to offspring fed non-stressed pollen. *Osmia lignaria* are typically active for 20-30 days during the spring (Bosch, 2008), so this could result in a 22-35% reduction in adult lifespan. Female *O. lignaria* can complete 1-2 provisioned brood cells per day (Spendal and Cane, 2022) or 20-60 brood cells throughout their lifetime, so 6 fewer days of activity could limit brood production, considering the strong relationship between fecundity and longevity in *Osmia* (Bosch and Vicens, 2006). These bees are already in decline in certain regions of the US (LeCroy et al., 2020), so increasing constraints on their fecundity and longevity may further perpetuate these patterns. It is also important to highlight that the bees in this study were reared and maintained under optimal conditions, so negative consequences are likely to be amplified if adult bees and their offspring are directly exposed to heat stress or other stressors including resource limitation, pesticide exposure, and pathogens.

This study highlights that indirect effects of climate change and extreme heat can have cumulative impacts to bees, their offspring, and their host plants. Furthermore, the results suggest that studies evaluating direct heat stress on bees may underestimate the consequences of climate change on bee-plant interactions. There is an urgent need for mitigation strategies to help protect host plants, and in return, help protect bees such as overhead irrigation to cool fields during bouts of extreme heat (Yang et al., 2019a, 2020a) which may protect pollen and its nutritional quality. Combatting the effects of climate change-induced heat extremes will require creative solutions on both short- and long-term scales. To effectively address the implications of heat stress on bees and

their interactions, we must broaden our understanding from direct, isolated stressors to indirect and interactive stressors. These results demonstrate that the indirect effects of extreme heat events for bee physiology and development should be further explored to better understand the implications for bee populations, wild plants, and agricultural production.

## TABLE

**Table 4.1** Mean (±SE) number of flowers open and number of flowers visited (per 30-min) for three host plants (blueberry, phacelia, clover) exposed to CT (25°C for 4h) or HT (37.5°C for 4h) treatments. Effect sizes ($\chi^2$), degrees of freedom (df), and *p*-values reported from likelihood ratio tests (LRT) of GLMM models.

| | | **Blueberry** | | **Phacelia** | | **Clover** | |
|---|---|---|---|---|---|---|---|
| | | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** |
| **# flowers open** | Mean ± SE | 8.6 ± 6.6 | 8.3 ± 6.3 | 16.1 ± 3.3 | 14.1 ± 3.0 | 2.0 ± 0.2 | 2.4 ± 0.2 |
| | # plants (N) | 110 | 112 | 52 | 45 | 36 | 33 |
| | | $\chi^2$=0.09, df=1, *p*=0.77 | | $\chi^2$=1.19, df=1, *p*=0.28 | | $\chi^2$=1.80, df=1, *p*=0.18 | |
| **# flowers visited** | Mean ± SE | 4.6 ± 0.6 | 3.8 ± 0.5 | 13.2 ± 2.1 | 10.0 ± 1.6 | 2.2 ± 0.4 | 2.8 ± 0.5 |
| | # plants (N) | 77 | 86 | 43 | 42 | 27 | 24 |
| | | $\chi^2$=3.33, df=1, *p*=0.07 | | $\chi^2$=1.87, df=1, *p*=0.17 | | $\chi^2$=1.88, df=1, *p*=0.17 | |

# CHAPTER 5. NUTRITIONAL CONTENT OF NORTHERN HIGHBUSH BLUEBERRY POLLEN EXPOSED TO EXTREME HEAT

## 5.1 INTRODUCTION

Climate change is intensifying, perpetuated by the anthropogenic emission of greenhouse gases, and this is causing environmental change across the globe (IPCC, 2023). One consequence of climate change is increasingly common and intense extreme heat events, where air temperatures are 5-10°C higher than historically normal conditions for a certain time of year in a geographic region (Wahid et al., 2007; IPCC, 2023). When these extreme heat conditions exceed an organism's critical threshold for a sufficient duration, it can result in direct heat stress (Wahid et al., 2007) that causes compounding effects on organism functioning, survival, and interaction with other organisms. Plant-pollinator systems are at particular risk, as extreme heat events threaten to disrupt the functioning of plants and insects, as well as their interactions (Walters et al., 2022). Studies investigating the direct effects of extreme heat on bees or plants provide insights into the consequences for their physiology (Bordier et al., 2017; Hamblin et al., 2017; Chaturvedi et al., 2021; Zhao et al., 2021; Zhu et al., 2021), capacity for acclimation (Martinet et al., 2015; Mesihovic et al., 2016; Oyen and Dillon, 2018; Martinet et al., 2021a; Gonzalez et al., 2022a, b; Hernández-Fuentes et al., 2023; Sepúlveda and Goulson, 2023), and reproductive potential (Vanderplanck et al., 2019; Amuji et al., 2020; Lohani et al., 2020; Zhao et al., 2021; Campion et al., 2023). However, few studies have explored the direct and indirect effects of extreme heat on bees, plants, and their interactions (but see Hemberger et al., 2023; Descamps et al., 2021; Greenop et al., 2020), limiting our ability to predict how extreme heat will affect pollination systems. Pollen is the common denominator driving the relationship between flowering plants and bees, as many flowering plants require insect-mediated pollination for their reproduction, and many bees rely on pollen to fulfill their and their offspring's nutritional needs. Thus, exploring the ways in which extreme heat affects pollen will provide important insights into the direct and indirect consequences of extreme heat for food production, natural ecosystems, and bee declines.

During plant reproductive development, a series of coordinated processes must occur for successful fertilization. First, pollen microspores must undergo microsporogenesis and microgametogenesis development, eventually forming mature pollen grains (Lohani et al., 2020). Once a flower opens, anthers release mature pollen to be transferred to a receptive stigma surface, where adhered pollen grains must germinate and penetrate the stigma surface, growing pollen

tubes that traverse the style towards the ovules, finally resulting in fertilization (Snider and Oosterhuis, 2011). Thus, viable pollen and a receptive stigma are required for fertilization, yet these processes can be disrupted following short bouts of heat stress, particularly during pollen development, germination, and tube elongation (Zinn et al., 2010; Snider and Oosterhuis, 2011; Mesihovic et al., 2016; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021). Heat stress can inhibit pollen development and performance due to the altered metabolism, availability, and utilization of nutrients in developing microspores that fuel pollen development and functioning (Lohani et al., 2020), and this is also expected to affect bees that consume this pollen.

Pollen development is energetically costly in plants, with photosynthetic tissues delivering sucrose to the anthers (via the filament) where sucrose is hydrolyzed to glucose and fructose then imported into pollen grains where it is converted and stored as starch and/or lipids (Taurino et al., 2018; Borghi et al., 2019). Sugar transporter proteins facilitate the movement of carbohydrates to the tapetum, which is the layer of nutritive cells between the anther wall and sporogenous tissue. The tapetum then transfers nutrients to the locular fluid and pollen microspores (Borghi et al. 2019). During male gametophyte (i.e., pollen) development, heat stress can cause degradation of tapetum cells, altering nutrient sequestration in developing anthers and pollen grains, including carbohydrates, proteins, lipids, and amino acids (Borghi and Fernie, 2017; Borghi et al., 2019; Santiago and Sharkey, 2019; Lohani et al., 2020; Santiago et al., 2021). These nutrients are essential for pollen development and functioning following anther dehiscence and pollen release, so altered or reduced concentrations of nutrients can result in male sterility (Lohani et al., 2020).

While male gametophyte development is broadly considered the most heat sensitive process in plants, the timing of heat exposure across various male gametophyte development stages (i.e., microsporogenesis and microgametogenesis) can have a strong influence on pollen nutrient concentrations, pollen performance, and fertilization (Lohani et al., 2020). For example, in barley florets, exposure to heat stress during the pre-meiotic stage of development resulted in stunted anther development, failure to produce pollen grains, and altered progression of meiosis in the microspore mother cells (MMC). When heat stress occurs during meiosis in barley, starch accumulation in pollen grains was limited (Sakata et al. 2000, Draeger and Moore (2017). MMC meiosis is an important stage for nutrient metabolism and partitioning in developing pollen microspores, and exposure to heat stress at this stage can lead to premature degeneration of the tapetum, altering amino acid and carbohydrate metabolisms which, in turn, can limit the nutritive

supply necessary for pollen development, germination, and tube growth (Zinn et al., 2010; Giorno et al., 2013; De Storme and Geelen, 2014; Borghi and Fernie, 2017; Borghi et al., 2019; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021; Kumar et al., 2022). Sensitivity to extreme heat during MMC meiosis and microsporogenesis has been reported in several crops including (but not limited to) barley (Callens et al., 2023), sorghum (Jain et al., 2010), wheat (Sakata et al., 2000; Draeger and Moore, 2017), tomato (Iwahori, 1966; Peet et al., 1998; Pressman et al., 2002; Firon et al., 2006; Sato et al., 2006), *Arabidopsis* (Kim et al., 2001), apricot (Rodrigo and Herrero, 2002), groundnut (Vara Prasad et al., 2000), and strawberry (Ledesma and Kawabata, 2016), with notable reductions in fruit or seed set following heat stress in these crops. Later pollen development stages (i.e., microgametogenesis) also require ample accumulation of carbohydrates and certain amino acids, like proline, to help fuel pollen germination and tube growth, and exposure to extreme heat can result in pollen sterility (Polowick and Sawhney, 1993; Giorno et al., 2013).

From these studies, it is clear that heat stress has various consequences for developing floral buds and is dependent on the development stage at which heat is applied, with negative repercussions for pollen germination, pollen tube growth, fertilization, and overall crop production (Borghi and Fernie, 2017; Raja et al., 2019; Lohani et al., 2020; Chaturvedi et al., 2021; Kumar et al., 2022). However, the diversity of crops investigated in the literature is limited, with a greater focus on annual crops despite the potential for greater heat sensitivity in spring-blooming perennial crops (Hedhly et al., 2009). In northern highbush blueberry, exposure to extreme heat (37.5°C, 4 h) during bud swell (likely when MMC meiosis occurs) reduced fruit set by 39%, suggesting heat stress at this development stage inhibited pollen development and germination, pollen tube growth, and fertilization (Chapter 3). Walters and Isaacs (2023) (Chapter 2) also reported how brief extreme heat (37.5°C for 4 h) inhibited blueberry pollen germination and tube growth *in vitro*, demonstrating the potential for extreme heat to limit reproductive success in blueberries. However, no studies have evaluated the consequences of heat stress for blueberry pollen nutrition. Such information may provide important insight into the drivers inhibiting pollen performance and fertilization in heat stressed blueberry plants. Additionally, evaluating differences in blueberry pollen nutrient concentrations at various floral development stages is entirely unexplored.

Even less attention has been given to determine if heat stressed pollen affects bee health, despite growing concerns for bee declines and the potential loss of pollination services (but see Chapter 4). The depletion or alteration of pollen nutrients following heat stress, as discussed above,

could adversely affect bee pollinators as pollen provides protein, carbohydrates, lipids, and amino acids necessary for bee development and survival as larvae and adults (Borghi and Fernie, 2017; Borghi et al., 2019; Vaudo et al., 2020; Descamps et al., 2021; Walters et al., 2022). Despite this, no studies to my knowledge have quantified how heat-stressed pollen alters the nutritional profile for bees and the consequences for their development or survival. Drawing from bee nutritional studies, unbalanced, insufficient pollen diets can alter bee fecundity, foraging behaviors, development, and survival (Vaudo et al., 2015, 2020; Woodard et al., 2019; Knauer et al., 2022). Subsequent offspring may also be affected as reduced quantity and/or quality of pollen provisions could lead to altered developmental timing, shifted sex ratios, reduced body size, and higher mortality (Bosch, 2008; Bukovinszky et al., 2017; Filipiak and Filipiak, 2020; Stuligross and Williams, 2020). Low-protein diets can inhibit ovarian and egg development in bees (Hoover et al., 2006; Human et al., 2007) and diets lacking essential amino acids can prevent brood production and development (Herbert et al., 1970; Roulston and Cane, 2000; Ryder et al., 2021). In a recent study on *Bombus terrestris*, bees provided with diets deficient in essential amino acids had delayed nest initiation and inhibited brood production (Barraud et al., 2022). While it is clear that altered pollen nutrition can have profound consequences for bees, most studies have focused on social species, like honey bees and bumble bees. However, 75% of the world's bee species are solitary (Danforth et al., 2019), and many solitary bee taxa like *Osmia* are in decline (LeCroy et al., 2020; Kazenel et al., 2024), so greater research on the nutritional requirements among these taxa is necessary. Furthermore, understanding the role of heat stress altering bee nutrition is entirely unexplored, but critical, on an ever-warming planet (IPCC, 2023).

In Chapter 4, I demonstrated that native solitary *Osmia lignaria* female bees provided host plants (blueberry, phacelia, white clover) exposed to extreme heat (37.5°C) for 4 h at 25% bloom and had significantly reduced egg laying. Other studies with *Osmia* bees have shown that females foraging in low-resource conditions produced fewer brood cells per day (Stuligross and Williams, 2020), and when denied access to pollen, females failed to mature oocytes or lay eggs (Cane, 2016). Among the *O. lignaria* offspring that consumed pollen diets from heat stressed plants, there were significantly higher mortality rates as larvae and as adults (Chapter 4). Some studies have found that only the highest protein diets supported *O. lignaria* development to adulthood (Levin and Haydak, 1957; Roulston and Cane, 2000) while others have found that carbohydrates, not protein, mediate *Osmia* larval growth and survival to pupation (Austin and Gilbert, 2021).

Importantly, I did not expose female *O. lignaria* bees or offspring to heat stress conditions and the quantity of pollen diets was similar between heat stressed and control diets, so negative repercussions observed in female fecundity and offspring survival were likely due to nutritionally altered diets following heat stress in host plants (Chapter 4). These findings highlight the potential adverse effects of heat-altered pollen nutrition on bee reproduction, development, and survival and could also limit pollination services in wild plants and crops (Scaven and Rafferty, 2013; Borghi and Fernie, 2017; Borghi et al., 2019; Slominski and Burkle, 2021; Walters et al., 2022; de Manincor et al., 2023). Despite the potential ramifications, there is limited cross-disciplinary understanding of the effects of extreme heat on bees, plants, and their interactions.

Pollen is a common denominator underlying crop yields and bee health, yet no studies to my knowledge have explored how extreme heat may alter pollen nutrition with consequences for plants, bees, and their interactions. In Chapter 2, I demonstrated that extreme heat (37.5°C for 4 h) inhibited pollen germination and tube growth *in vitro* (Walters and Isaacs, 2023) and in Chapter 3 I found that extreme heat exposure at various floral development stages limited blueberry fruit set. I also found that extreme heat exposure during floral bud development and early bloom inhibited *Osmia lignaria* female fecundity and offspring survival. Based on these findings, it is possible that heat-driven alterations in blueberry pollen nutrition are driving negative consequences for both plants and bees. To understand how short-term extreme heat affects pollen nutrition, I analyzed the composition of northern highbush blueberry pollen exposed to heat stressed (37.5°C) or non-stressed (25°C) conditions for 4 h at several floral bud development stages including tight bud, bud swell, bud break, early/late pink bud, and anthesis. Several pollen metabolites were assessed, such as carbohydrates, including soluble sugars (sucrose and glucose) and starch, total soluble proteins, and amino acids in northern highbush blueberry. These were analyzed given their important roles in pollen development and performance and bee nutrition. Given the results in the preceding chapters, I hypothesized that heat stress would alter pollen nutrition in northern highbush blueberries, and the severity of change in pollen nutrition would vary by the floral development stage exposed to extreme heat.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Plant material and maintenance

Dormant 2-year-old northern highbush blueberry ‘Bluecrop’ bushes were purchased in late winter in 2021 (Hartmann Nursery, Grand Junction, Michigan and DeGrandchamp Farms, South

Haven, Michigan). 'Bluecrop' was selected as it is the mostly widely grown fresh market blueberry cultivar grown in Michigan, comprising 25% of the state's blueberry acreage (Vander Weide et al., 2024) and was used in the preceding studies (Chapters 2-4). The bushes were immediately placed in dark cold storage (MSU Horticultural Teaching and Research Center, Holt, Michigan) at 2°C until at least 1200 chilling hours had been accumulated. Thereafter, bushes were moved from cold storage into the MSU greenhouses (East Lansing, MI) as needed for experiments, maintained at 22 ± 5°C and 16 h photoperiod for the duration of the experiments. Plants were watered regularly to maintain moist soil and soil pH was monitored every 3-4 weeks to ensure it was < 6.0. When necessary, bushes were treated with Jobe's Organics soil acidifier (calcium sulfate (80%), sulphur (18%), and bentonite clay (2%), Easy Gardener Products, Inc., Waco, Texas) according to the manufacturer's label. Osmocote Smart-Release Plant Food Flower and Vegetable (nitrogen (14%), available phosphate (14%), soluble potash (14%), The Scotts Company, Marysville, Ohio) granules were also added to potted blueberry bushes following the manufacturer's label.

5.2.2 Blueberry temperature treatments

Following placement in the greenhouse, blueberry bushes were randomly assigned to either a control (CT: 25°C for 4 h) or high (HT: 37.5°C for 4 h) temperature treatment. The temperature for the HT regime was selected to be similar to recently experienced extreme heat events where daily maximum temperatures exceeded 35°C for 4 h during blueberry bloom (Global Historical Climatology Network). Bushes were exposed to temperature treatments using environmental growth chambers (Darwin Chambers, St. Louis, MO) set to CT and HT conditions (60 ± 5% relative humidity), and temperatures were monitored every 30 min using HOBO temperature loggers (Onset Computer Corporation, Bourne, MA). Plants were assigned in pairs such that multiple plants received the HT and CT treatment conditions at the same time in each respective chamber. Following temperature exposure, bushes were immediately brought back to the greenhouse and maintained under the conditions described above for the remainder of the experiment.

5.2.3 Experimental design

'Bluecrop' bushes at several floral bud development stages, including tight bud, bud swell, bud break, early pink bud, late pink bud, or anthesis growth stages were exposed to CT or HT conditions for 4 h in the growth chambers. Following placement in the greenhouse, bushes were

randomly assigned to a development stage and temperature treatment. The bushes were monitored daily to assess floral bud development, ensuring the correct flower developmental stage was exposed. Using colored twist ties, individual floral buds were identified and marked prior to exposure, where twist ties were loosely (but securely) wrapped around the shoot just below a given floral bud targeted for treatment. Following temperature exposure, plants were maintained under optimal conditions (22 ± 5°C and 16:8 L:D photoperiod) in the greenhouse until anthesis, at which time pollen was collected. To ensure that the pollen and flowers used for experiments were fresh (within 24 h of anthesis), open flowers on each bush were marked by lightly dotting the corolla with a permanent marker (Kearns and Inouye, 1993). Thus, the following day, any open flower without a mark had been open for less than 24 h. Pollen was released from the blueberry flower anthers by touching a vibrating sonication tool (AeroGarden, Boulder, Colorado) onto the outside of the corolla and collecting into a 1.5 mL microcentrifuge tube, allowing pollen to accumulate at the bottom of the tube. Each tube contained pollen from a single collection date, blueberry bush, and development stage. The fresh pollen weight of blueberry pollen was recorded using a precision balance by taking an 'empty' weight of 1.5 mL centrifuge tubes (prior to collection) and a 'filled' tube weight (after collection), with the difference providing the fresh pollen weight. The location on the bush canopy from which flowers were collected was also recorded (e.g., top, mid, low). After collection, tubes were stored in a -80°C freezer until ready for analysis. Across all development stages and temperature treatments, pollen was collected from 434 blueberry bushes to provide enough pollen for biochemical assays.

To prepare samples for biochemical assays, tubes with pollen were taken out of the -80°C freezer in small batches for short durations (10 min or less) to combine pollen from separate vials into new 2 mL conical flat bottom microcentrifuge tubes (Fisherbrand™ Premium Microcentrifuge 2.0 mL tubes, Waltham, MA, USA). For each biochemical assay, pollen was weighed and combined (within a given temperature treatment and development stage) to meet the pollen weight needed for each method. With the exception of the tight bud stage, six replicates were prepared for both temperature treatments (CT, HT) across blueberry floral bud developments stages where pollen amounts per sample ranged from 5-16 mg for the protein assay, 8-15 mg for the carbohydrate assay, and 5-12 mg for the amino acid assay. For all assays, pollen was limited at the tight bud development stage and thus received less pollen (1-7 mg) and fewer replicates (4) across all metabolite assays. Differences in pollen weights did not affect the quality of the assays, and

variation in weight per replicate was accounted for in the final measurement calculations across all assays performed. Immediately after pollen was combined into the tubes, samples were placed back in the -80°C freezer until they were ready for metabolite extraction.

5.2.4 Carbohydrate extraction and biochemical assay

Sucrose, glucose, and starch extractions and assays were adapted from Lu et al. (2006) by James Santiago in 2021 and 2022. To prepare for grinding frozen pollen samples, each centrifuge vial had a sanitized 2 mm stainless steel ball (Thermo Scientific, Waltham, MA, USA) added to the pre-weighed pollen sample. Sample vials were placed in a Retsch mill block and placed back in a -80°C freezer for 5 min. The block with tubes containing pollen samples was placed in a Retsch MM 301 vibratory mill (Verder Scientific Inc., Newton, PA) and milled twice, each for 1 min at 30 Hz (e.g., 30.0 1/s). Pollen samples were milled to crack open pollen exine walls to ensure pollen nutrient contents were available for bioassays. Immediately after grinding pollen samples, tubes were placed in ice. Then, 300 µL of chilled 3.5% (v/v) perchloric acid was added to each sample and vortexed for ~5 sec, then placed back on ice for 5 min to incubate. After incubation, samples were centrifuged in a pre-chilled centrifuge set at 4°C for 10 min at 14,000 RPM. In a new 1.5 mL microcentrifuge tube, 200 µL of supernatant was transferred while avoiding the pollen pellet at the bottom of the previous tube. These sample aliquots were used for glucose and sucrose assays. In the tubes containing pollen pellets, 850 µL of 80% ethanol was added to each tube then placed in the -80°C freezer. These were used the following day for starch extraction. For glucose and sucrose extractions, 70 µL of neutralizing buffer (2 M KOH, 150 mM HEPES, 10 mM KCl, 250 mL milli-Q water) was added to each sample vial, raising the sample pH close to 7.0, and vortexed for 5 sec. Samples were then placed in a pre-chilled centrifuge (4°C) for 2 min at 14,000 RPM. From these vials, 200 µL of supernatant was extracted and placed in a new 1.5 mL centrifuge tube, immediately placed in the -80°C freezer until ready for the soluble sugars (glucose, sucrose) assay.

For starch extraction, vials previously placed in -80°C freezer with 80% ethanol were removed from the freezer and vortexed for ~15 sec to break up the suspended pollen pellet. The vials were then centrifuged at 4°C and 20,817 RCF for 5 min. The ethanol supernatant was then removed by pipetting after centrifugation. This step was repeated (add 80% ethanol, vortex, centrifuge, discard ethanol) twice, then placed in a dri-bath set to 70°C for 1-2 min to dry out any remaining ethanol. Once all ethanol had been dried out, 250 µL of 0.2 M KOH was added to each

vial and vortexed for ~5 sec to resuspend the pellet in KOH solution. The samples were then placed in a preheated dri-block set to 95°C and incubated for 30 min. The samples were then allowed to cool for 2-5 min at room temperature, then 45 µL of 1 M acetic acid was dispensed to each tube and vortexed for 5 sec. An enzyme cocktail containing amyloglucosidase and α-amylase was mixed, then 52 µL of the enzyme solution was dispensed into each sample tubes and vortexed for ~5 sec. The vials containing starch extractions were then set in tube racks and placed on an orbital shaker (VWR, Radnor, PA) set to 20°C at 20 RPM for 48 h to allow enzymatic digestion of starch.

For the carbohydrate measurements, an NADPH-linked protocol described by Lu and Sharkey (2004) was used. Soluble sugars (glucose, sucrose) were measured by first moving the tubes containing extracted samples from the -80°C freezer and placed on ice to thaw. In a clear, 96-well acrylic plate, 5 µL of the sugar extracts were aliquoted to each well. Using a multi-channel pipette, 195 µL of enzyme assay solution (150 mM HEPES buffer (pH 7.2), 50 mM NADP, 50 mM ATP, 0.25 U/µL G6PDH) was added to each well containing soluble sugar extracts. The 96-well plate was then placed in a microplate reader (FilterMax F5 Multi-Mode microplate reader, Molecular Devices LLC, San Jose, CA). The plate containing samples was shaken in the plate reader to ensure thorough mixing of sugar extract and enzyme assay solution. Using the SoftMax® Pro plate reader software (Molecular Devices LLC, San Jose, CA), absorbance was set at 340 nm and a kinetic assay was performed to provide a stable absorbance baseline. Then, the well plate was removed and 5 µL of hexokinase (HXK) enzyme was added to each well using a homemade flat end 96-prong tool, allowing addition of HXK to each well and starting the reactions in each well all at the same time. The plate was then placed back in the plate reader, again running a kinetic assay (λ=340 nm) until it stabilized, providing absorbance readings for use in glucose calculations. The plate was then carefully removed from the plate reader, 10 µL of invertase (INV) was added to each well using the 96-prong tool, incubated at 37°C for 30 min, and then an end-point assay was started (λ=340 nm). The absorbance readings were then used for sucrose calculations. For the starch assay, 5 µL of starch extracts and 195 µL of assay solution (see above) were pipetted into a fresh 96-well acrylic plate and a kinetic assay was started until a stable baseline was achieved. Invertase (10 µL) was then added, and the samples incubated at 37°C for 1 h to break down any remaining sucrose before starting the kinetic assay at 340 nm. The absorbance data from these assays were then used to calculate the molar concentration using the following formula: Molar concentration = (ΔAbsorbance ÷ 6220) [change in absorbance from baseline divided by the

extinction coefficient of NADPH at 340 nm] ÷ (0.003 * volume of solution in the well in µl) [path length] * 240e-6 [volume of the well in Liters] ÷ 40e-6 [amount of sample added to the well in Liters].

Molar concentration was then converted to moles per sample. Accounting for the aliquot and the original sample extract volumes, and pollen weights, concentrations in nmol/mg (sugars) or mmol/mg (starch) was calculated. Finally, concentrations were converted to µg/mg (sugars) or g/mg (starch) for reporting.

5.2.5 Protein extraction and biochemical assay

Methods for protein extraction from pollen were developed by James Santiago at Michigan State University in 2021 and 2022, based on the approach of Santiago and Tegeder (2016). Breaking open pollen samples for protein extraction followed the same protocol described above. Chilled protein extraction buffer (50 mM HEPES, 5 mM $MgCl_2$, 1 mM EDTA, 10% [v/v] glycerol, 50 mL Milli-Q water, pH 7.5) was added to each pollen tube sample, with 200 µL extraction buffer added per sample. Milled pollen samples suspended in the extraction buffer were vortexed for 10 sec, then shaken for 10 sec, and immediately placed back on ice. These vials were then placed in a pre-chilled (4°C) Eppendorf 5417R Refrigerated Centrifuge (Eppendorf North America, Inc. Westbury, NY, USA) run at 14,000 RPM for 10 min. After samples were centrifuged, 150 µL of the supernatant was pipetted and placed in a new 2.0 mL microcentrifuge tube, careful to not disturb cellular debris at the bottom of the vial. If disturbed, pollen vials were run for another 10 min in the centrifuge, as described above. Vials with the pollen extract were placed back in the -80°C freezer until biochemical assays.

Total soluble protein quantification was conducted using a NanoOrange® Protein Quantitation Kit (Invitrogen, Ltd., Paisley PA4 9RF, UK), where bovine serum albumin (BSA) was used as the protein standard. This assay provides highly accurate detections of total soluble proteins with improved sensitivity compared to the more common absorption-based protein solution assays (Jones et al., 2018) and does not require large amounts of pollen for assessments. Minimizing the amount of pollen required for the assay is crucial given the time and labor limitations involved in pollen collection from blueberry and allowed for a more robust statistical analysis by increasing the number of replications. The protein assay was performed following the manufacturer's instructions. Briefly, the protein quantitation diluent was prepared along with the BSA standard solution (provided in the kit) based on the number of samples we assessed and then

kept on ice. Using the 2 µg/mL BSA stock solution provided in the kit, I prepared 0, 0.6, 0.8, 1, 3, 6, and 10 µg/mL diluted BSA solutions in 1.5 mL centrifuge tubes (kept in ice) to provide a protein standard curve for the assay. This reference standard curve was used to convert the fluorescence signal to µg/mL protein concentration after samples were run in the plate reader. Both protein quantitation diluent and protein standard curve solutions were made immediately before the assays. Protein extract samples were then taken out of the -80 °C freezer and thawed on ice. Once thawed, tubes were vortexed for ~30 sec then placed in a pre-chilled centrifuge (4°C, 20,817 RCF) for 5 min and placed on ice immediately afterwards. From each protein extraction sample, 10 µL of supernatant was moved into a new 2.0 mL centrifuge tube, after which 490 µL of chilled protein extraction buffer (described above) was added to new tubes, producing a 1:50 protein sample dilution.

For the assay reaction, 5 µL of the 1:50 diluted protein sample was dispensed into a new 2.0 mL microcentrifuge tube along with 205 µL of the NanoOrange working solution, then vortexed for ~5 sec (each). Tubes containing the standard curve and pollen samples were placed on an aluminum block heated to 95 °C, allowing samples to incubate at this temperature for 10 min while covered with aluminum foil. Another aluminum block was then set on top of the tubes to prevent the lids from opening and to protect samples from light. After incubation, tubes containing pollen samples and standard solutions were transferred to a tube rack and left to cool at room temperature for 20 min, with an aluminum sheet placed on top of tubes to protect samples from light. After the samples and standards cooled down, they were placed in a pre-chilled centrifuge (4°C, 13,000 RCF) for 1 min. After spinning, 200 µL of standard curve solutions and diluted pollen protein extracts were transferred to a black, flat-bottom 96-well plate, with care taken to ensure no light exposure of the samples. The black 96-well plate containing standard curve and protein extraction solutions was then loaded into a microplate reader (FilterMax F5 Multi-Mode microplate reader, Molecular Devices LLC, San Jose, CA) controlled by the SoftMax® Pro plate reader software (Molecular Devices LLC, San Jose, CA). The excitation wavelength was set at 485 nm and emission at 595 nm. Fluorescence measurements were then plotted against the prepared BSA standard concentrations (0-10µg/mL) to create a standard curve. Fluorescence readings from pollen samples were then calculated using the standard curve. Taking into account the dilution, sample volume used and the original extract volume, the final total soluble protein was then calculated and expressed in µg/mg pollen.

5.2.6 Amino acid extraction and biochemical assay

The protocol for free amino acid extraction and assay was provided by the Michigan State University Mass Spectrometry and Metabolomics Core (MSMC) (Protocol MSMC-002 version 1.1). The amino acid internal standards ($^{13}C$,$^{15}N$ stable isotope-labeled amino acid internal standards) were provided by the MSMC and served as the extraction solvent. To prepare the internal standards, the labeled amino acids in a lyophilized pellet form was resuspended in 1 mL sterile Milli-Q water for a 1000X strength. The internal standard also doubles as the extraction buffer and was prepared at 1X strength. Breaking open pollen samples for amino acid extraction followed the same protocol provided above for carbohydrate and protein extraction. After milling using a Retsch mill, 400 µL of extraction solvent was added to each sample, vortexed for ~3 sec, then incubated in a dri-bath at 90°C for 5 min. Samples were left to cool on ice for ~1 minute before placing samples in a pre-chilled centrifuge (13,000 RCF at 4°C) for 10 min. Immediately after, 100 µL of supernatant was removed and dispensed into a new 1.5 mL tube, careful to not extract the pellet at the bottom of the tube. This extracted supernatant was mixed with 10 mM PFHA (in equal volume), then vortexed for ~3 sec until homogenized. Samples were again centrifuged (13,000 RCF at 4°C) for 5 min, then placed on ice. In glass inserts placed inside a Liquid Chromatography (LC) autosampler vials, 200 µL of amino acid extract was dispensed and capped, then stored in the -80°C freezer until ready for amino acids quantification using Ultra Pressure LC (UPLC)-tandem Mass Spectrometry (MS-MS). Waters Xevo TQ-S Micro tandem MS (Mildford, MA) operated in a positive model with an electrospray ionization (ESI) interface in the MSU Mass Spectrometry and Metabolomics Core (MSMC) facility was used. Reagent blanks were also prepared in LC autosampler vials, including Milli-Q water which was placed in order on the sample carousel rack before the standard curve vials and between each developmental stage samples.

Standard curve vials were positioned on the carousel before and after the amino acid samples. For the standard curve, ½ serial dilutions of unlabeled amino acids prepared with 20 mM PFHA in water at 50 µM, 12.5 µM, 3.13 µM, 0.78 µM, 0.195 µM, 0.049 µM, and 0.012 µM concentrations were prepared (see MSMC extraction protocol). The protocol allowed for the assessment of 19 different amino acids in blueberry pollen samples, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparagine, aspartate, cysteine, glutamate, glutamine, proline, serine, and tyrosine. Data from LC-

MS assay was uploaded to the MassLynx Mass Spectrometry Software (Waters Corporation, Milford, MA), where peak concentrations were assessed by James Santiago and adjusted as needed. Using amino acid concentrations (μM) provided from MassLynx software, data was transformed to provide the concentration of each amino acid in each sample (μg/mg).

5.2.7 Data analysis

All statistical analyses were conducted in R (version 4.2.3, R Core Team, 2023). Generalized linear mixed-models (GLMM) and linear mixed-models (LMM) ('lme4' package) (Bates et al., 2015) were used for all analyses to compare between heat treatments and blueberry bush development stages for each metabolite assay. Final models were selected based on the nature of the data taken (i.e., count vs continuous), meeting the assumptions of the model distribution, assessing the lowest AIC model scores ('bbmle' and 'stats' packages) (Bolker et al., 2023; R Core Team, 2023), the model deviance residuals ('base' package) (R Core Team, 2023), and other model performance metrics ('performance' package) (Lüdecke et al., 2024). Model assumptions were checked by assessing scaled residuals using 'performance' and 'car' packages (Fox et al., 2023; Lüdecke et al., 2024). All models described below adequately met model distribution assumptions. Test statistics were calculated using Likelihood Ratio Tests (LRT) ('stats' package) (R Core Team, 2023). The 'stats', 'emmeans' and 'multcomp' packages (Hothorn et al., 2023, Lenth et al., 2024) were used for pairwise comparisons between temperature treatments within a development stage and to derive means and standard errors of response variables from models. Figures were made using the 'ggplot2' and 'ggsignif' packages (Ahlmann-Eltze and Patil, 2021; Wickham et al., 2024).

Models assessing pollen nutrient content (protein, carbohydrate, amino acids) across various floral development stages (tight bud, bud swell, bud break, early pink bud, late pink bud) for CT and HT temperature treatments included several random effects including replicate, bush identity, pollen collection date, and flower location. In these models, nutrient content was included as the response variable and development stage and temperature treatment were included as predictor variables. A linear mixed model (LMM) was used to test the effects of temperature exposure on several different nutrient measurements in blueberry pollen, including protein, sucrose, starch, and several individual amino acids (asparagine, aspartate, glutamate, proline, arginine, phenylalanine). A GLMM with a gamma distribution and inverse link function were used to test the effects of temperature exposure on several different nutrient measurements in blueberry

pollen, including glucose, total amino acids, essential and non-essential amino acids, and several individual amino acids (serine, threonine, methionine, histidine, lysine, tryptophan, tyrosine, valine, leucine, alanine, cysteine, isoleucine, glutamine). Additional models (using the same distribution and model type described previously) were made to assess the effects of temperature on protein, glucose, sucrose, starch, and total amino acids across all development stages, where pollen nutrient content was included as the response variable and temperature was included as the predictor variable. The random effects included in these models were replicate, bush identity, pollen collection date, flower location, and development stage.

## 5.3 RESULTS

### 5.3.1 Carbohydrate analysis

The starch content of blueberry pollen was significantly affected by temperature treatment at one floral bud development stage (Figure 5.1: $F=4.24$, $df=4$, $p=0.003$). Specifically, exposure to HT conditions at the tight bud stage ($t=4.56$, $p=0.0001$) significantly reduced pollen starch content by 56% compared to the CT conditions. At the bud swell stage, mean pollen starch content was 57% lower in the HT pollen samples compared to CT-treated pollen, but this difference was not statistically significant. Across all development stages pooled together, temperature had a significant effect on pollen starch content ($F=5.61$, $df=2$, $p=0.02$), where exposure to HT conditions ($0.30 \pm 0.17$ g/mg) reduced pollen starch content by 35% compared to plants exposed to CT conditions ($0.46 \pm 0.18$ g/mg).

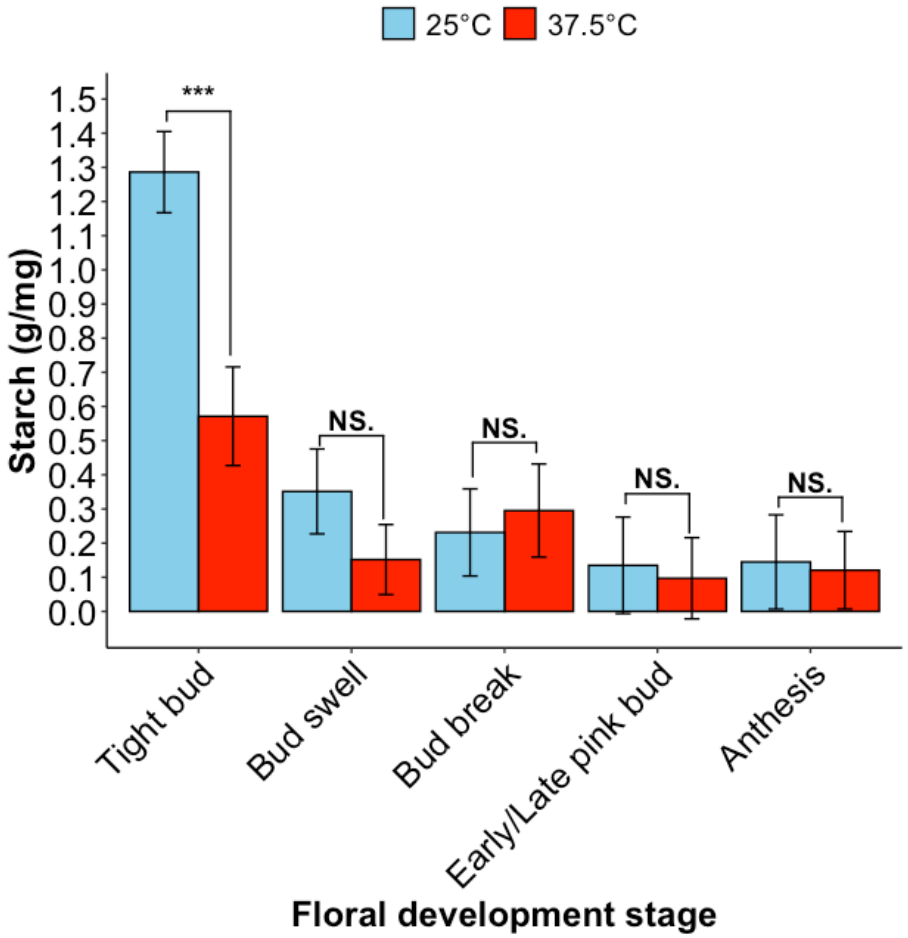


**Figure 5.1** Starch content (g/mg) of northern highbush blueberry pollen (cv. 'Bluecrop') collected at anthesis from flowers exposed to either CT (25°C, 4 h) or HT (37.5°C, 4 h) at tight bud, bud swell, bud break, early/late pink bud, or anthesis floral bud stages. Data is presented as mean ± SE, derived from a linear mixed model. Significant differences between means are indicated by the asterisks above error bars ('***' = $p \leqq 0.0001$). 'NS' indicates no significant difference between means ($\alpha > 0.05$).

The soluble sugar content of blueberry pollen was not significantly affected by temperature treatment at any floral bud development stage, both for glucose ($\chi^2$=7.28, df=4, p=0.12) and sucrose ($\chi^2$=0.97, df=4, p=0.43) (Table 5.1). Across all development stages pooled together, temperature treatment had no significant effect on pollen glucose content ($\chi^2$=0.62, df=2, $p$=0.43; CT: 0.28 ± 0.04 µg/mg, HT: 0.27 ± 0.04 µg/mg) or pollen sucrose content (F=0.21, df=2, $p$=0.65; CT: 19.02 ± 0.67 µg/mg, HT:18.84 ± 0.65 µg/mg).

5.3.2 Protein analysis

Total soluble protein content in blueberry pollen was significantly different between temperature treatments at one floral bud development stage, but otherwise there was no significant difference between treatments (Figure 5.2: F=1.89, df=4, $p$=0.11). At the bud swell stage, exposure to HT conditions significantly reduced the total soluble protein content in pollen grains (t=2.89, $p$=0.006), where the mean protein content in control-treated pollen was 160.3 ± 12.6 µg/mg while the mean protein content in heat-treated pollen was 124.3 ± 12.2 µg/mg (Figure 5.2). Thus, exposure to heat stress (37.5°C) for 4 h at the bud swell development stage reduced the total soluble protein content in blueberry pollen by 22%. Across all development stages, temperature had no significant effect on the total soluble protein content (F=3.44, df=2, $p$=0.07). Across all development stages and temperature treatments, the mean total soluble protein content of northern highbush blueberry pollen was 135.90 µg/mg and ranged from 64.76 µg/mg to 370.17 µg/mg.

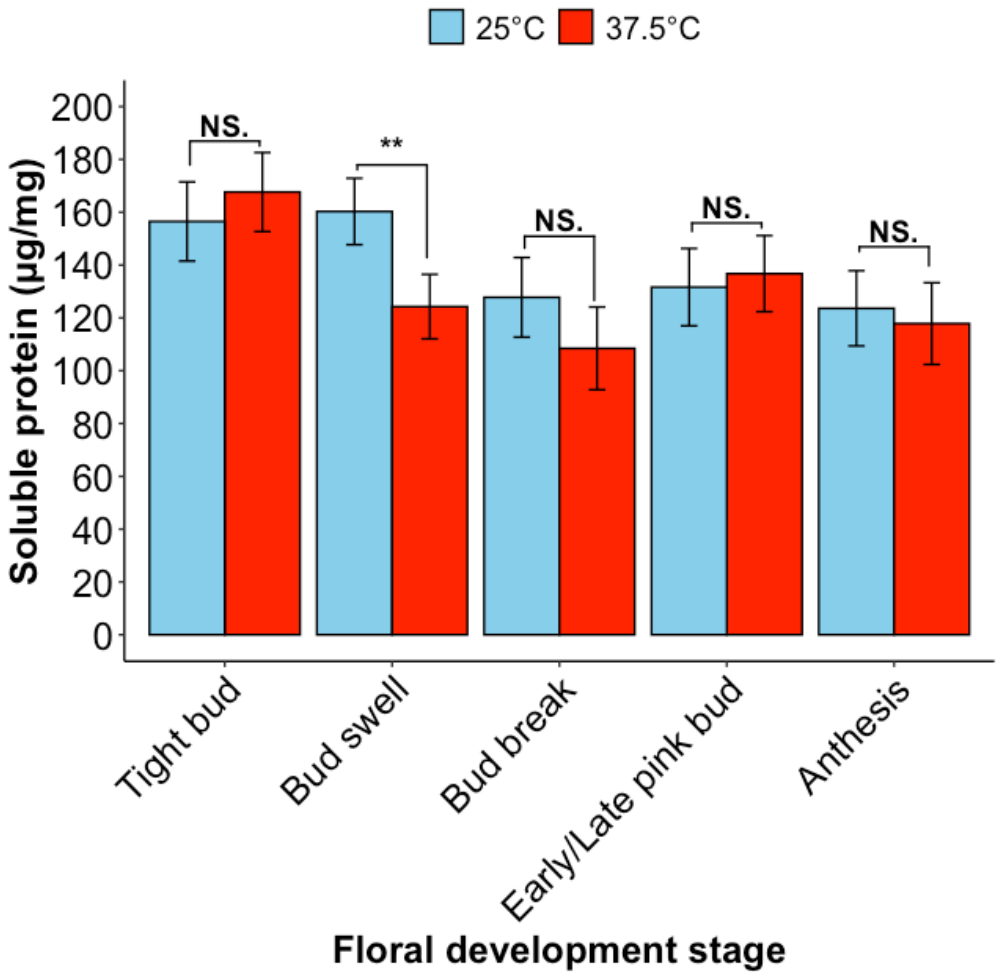


**Figure 5.2** Total soluble protein content (µg/mg) of pollen from northern highbush blueberry (cv. 'Bluecrop') collected at anthesis from flowers exposed to either CT (25°C, 4 h) or HT (37.5°C, 4 h) at tight bud, bud swell, bud break, early/late pink bud, or anthesis development stages. Data is presented as mean ± SE, derived from a linear mixed model. Significant differences between means are indicated by the asterisks above error bars ('**' = $p \leqq 0.01$). 'NS' indicates no significant difference between means ($\alpha > 0.05$).

5.3.3 Amino acid analysis

The mean total amino acid content of blueberry pollen was significantly different between heat treatments at one development stage (Figure 5.3: $\chi^2$=15.66, df=4, $p$=0.004). Exposure to HT conditions significantly reduced the total amino acid content of blueberry pollen at the bud swell stage (z=-4.20, $p < 0.0001$) by 35% compared to pollen exposed to CT conditions. Across all development stages, temperature treatment had no significant effect on the mean total amino acid content (F=1.63, df=2, $p$=0.20). The sum amino acid content across all development stages in HT-treated plants was 302.96 µg/mg and 322.60 µg/mg in CT-treated plants.

Temperature treatment had no significant effect on the mean total essential amino acids across all floral bud stages (Figure 5.4: $\chi^2$=5.19, df=4, $p$=0.27), including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Essential amino acids necessary for bee development and survival was determined by De Groot (1952). I observed trends for increases in essential amino acids following heat stress at bud break (25%), early/late pink bud (39%), and anthesis (24%) stages, but the differences between temperature treatments were not statistically significant. Temperature treatment had a significant effect on non-essential amino acids at only one development stage (Figure 5.4: $\chi^2$=9.54, df=4, $p$=0.05), where exposure to extreme heat at bud swell significantly reduced non-essential amino acids in pollen by 38% (z=-3.49, $p$=0.0005), but this was also influenced by a much higher level in the CT pollen.

Temperature treatment had variable effects on individual amino acid levels across development stages (Table 5.2). Of the 19 total amino acids quantified, the top five most abundant amino acids found in blueberry pollen, across all development stages and temperature treatments, were proline, aspartate, asparagine, glutamate, and arginine. At the tight bud development stage, there was no significant effect of temperature treatment on amino acid content across all 19 amino acids assessed. At the bud swell stage, exposure to HT significantly reduced levels of aspartate, glutamate, methionine, proline, and lysine. At the bud break stage, exposure to HT significantly increased concentrations of asparagine, glutamate, arginine, lysine, tryptophan, tyrosine, phenylalanine, leucine, and isoleucine. At the early/late pink bud stage, significant reductions in methionine and proline were observed after exposure to HT conditions. In contrast, asparagine, serine, threonine, arginine, lysine, tryptophan, tyrosine, valine, phenylalanine, leucine, isoleucine, and glutamine all were increased following HT exposure at the early/late pink bud stage. Exposure to HT at anthesis significantly reduced glutamine concentrations, but significantly increased glutamate, threonine, proline, arginine, lysine, tryptophan, tyrosine, valine, phenylalanine, cysteine, and isoleucine.

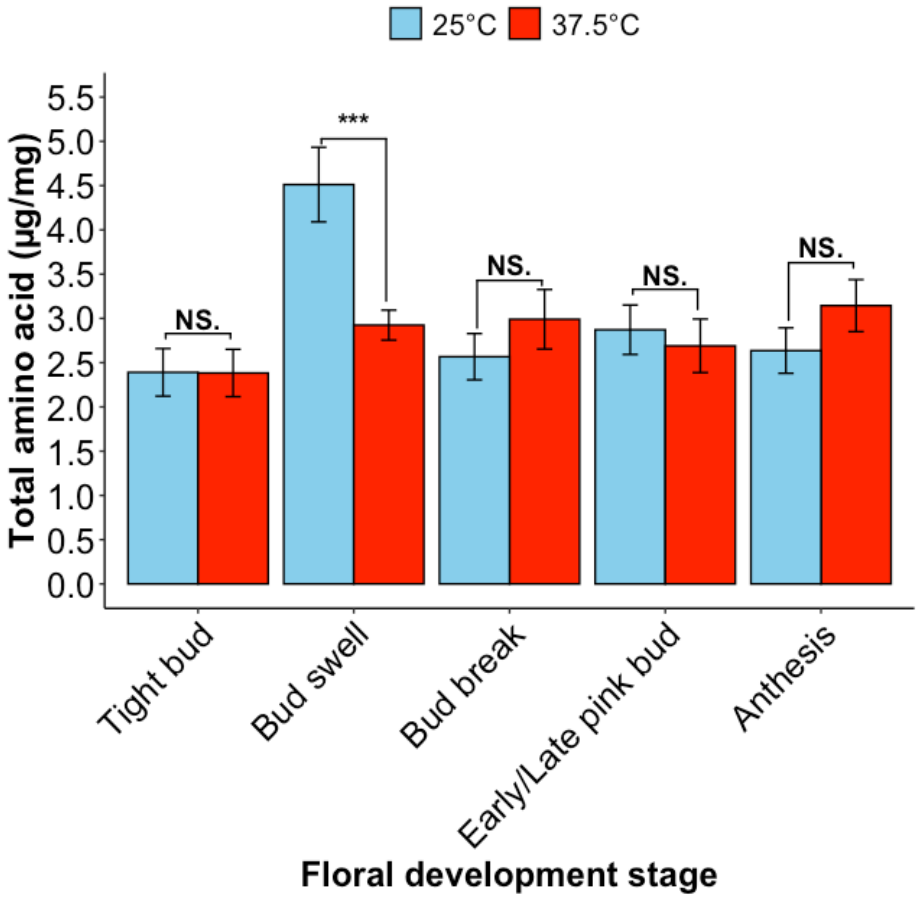


**Figure 5.3** Mean (± SE) total amino acid content (µg/mg) of northern highbush blueberry pollen (cv. 'Bluecrop') collected at anthesis from flowers exposed to either CT (25°C, 4h) or HT (37.5°C, 4h) at tight bud, bud swell, bud break, early/late pink bud, or anthesis development stages. Significant differences between means are indicated by the asterisks above error bars ('***' = $p \leqq 0.0001$). 'NS' indicates no significant difference between means ($\alpha > 0.05$).

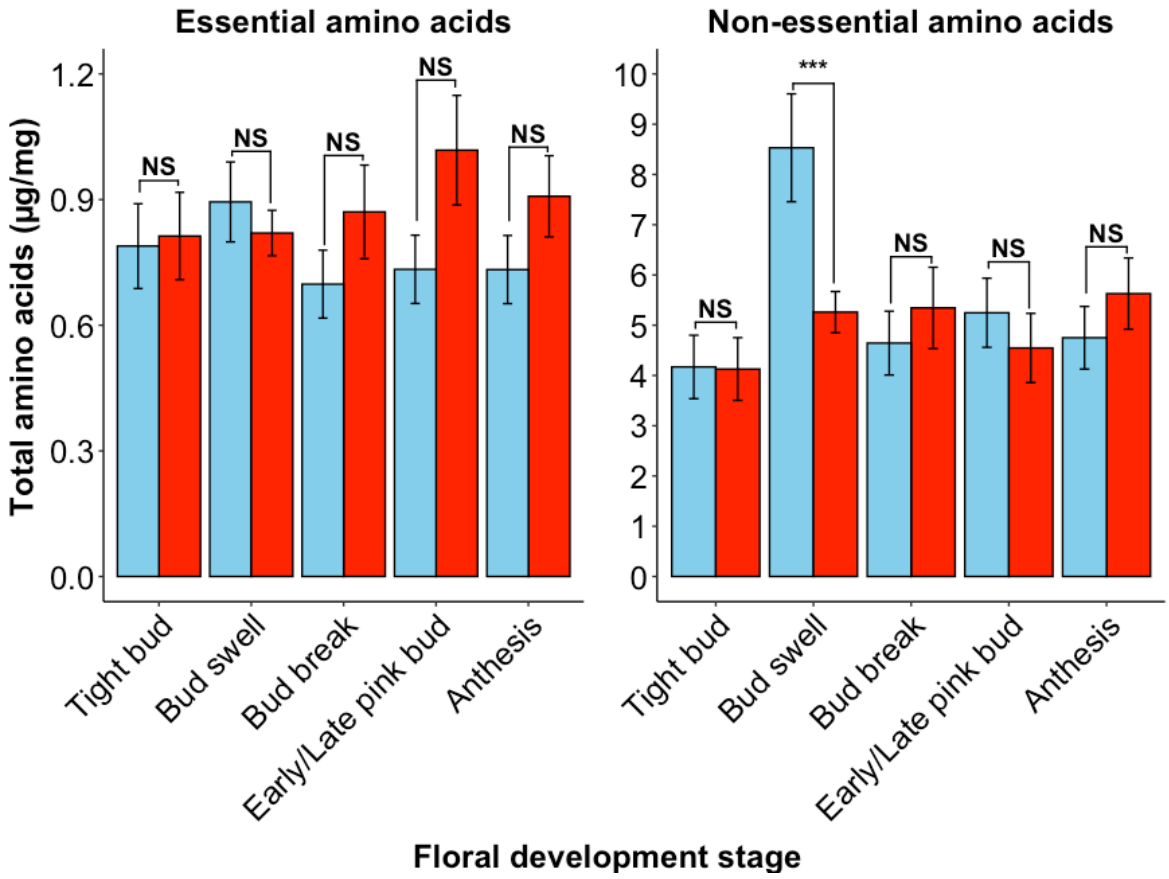


**Figure 5.4** Mean (± SE) essential (left) and non-essential (right) amino acid content (µg/mg) in pollen grains of northern highbush blueberry (cv. 'Bluecrop') collected at anthesis from flowers exposed to either CT (25°C, 4 h) or HT (37.5°C, 4 h) at tight bud, bud swell, bud break, early/late pink bud, or anthesis floral bud stages Significant differences between means are indicated by the asterisks above error bars ('***' = $p \leqq 0.0001$). 'NS' indicates no significant difference between means ($\alpha > 0.05$).

## 5.4 DISCUSSION

Nutrient rich pollen is required to support pollination and fertilization in plants, in addition to meeting the dietary needs of insect pollinators (Roulston and Cane, 2000; Borghi et al., 2019). Most studies have focused on the direct consequences of heat stress on plants or bees (reviewed by Walters et al., 2022), but few have explored the indirect consequences of extreme heat on pollination systems (but see Greenop et al., 2020; Descamps et al., 2021; Hemberger et al., 2023). Under extreme heat conditions, pollen nutrients including proteins, carbohydrates and amino acids may be reduced or altered with negative consequences for plant reproduction (Lohani et al., 2020; Chaturvedi et al., 2021), yet no studies to my knowledge have explored this in northern highbush blueberry. Furthermore, it is well known that insufficient pollen diets can have negative effects on

bee development, fecundity, and survival (Vanderplanck et al., 2014; Borghi and Fernie, 2017; Vanderplanck et al., 2019; Woodard et al., 2019; Filipiak and Filipiak, 2020; Stuligross and Williams, 2020; Knauer et al., 2022), yet no studies have investigated how extreme heat may alter the nutritional content of pollen with regard to bee nutrition. In this chapter, I determined the nutritional content (protein, carbohydrates, amino acids) of northern highbush blueberry pollen exposed to 4 h of heat stress (at 37.5°C) and found that the sensitivity or resilience to extreme heat was notably different across development stages. At bud swell, significant reductions in pollen protein, total amino acids, and several individual amino acids, as well as a trend for reduced pollen starch content, were observed following heat stress. Significant reductions in pollen starch (tight bud) and several individual amino acids (early/late pink bud and anthesis) were also observed following heat stress, which are expected to have consequences for both fertilization and diet quality for bees. Additionally, some amino acids were significantly increased following heat stress at bud break, early/late pink bud, and anthesis development stages, with variable effects on pollen functioning but potentially negative effects on bee development. Overall, this study highlights a mechanism through which heat stress can affect blueberry pollination, and the importance of interdisciplinary studies for uncovering the consequences of extreme heat in pollination systems.

5.4.1 Consequences of heat stress on pollen development, functioning, and fertilization

Immediately after winter dormancy, northern highbush blueberry floral buds are at the tight bud development stage (blueberries.msu.edu) and likely have not yet undergone MMC meiosis (Gough et al., 1978). Exposure to four hours of extreme heat at this early floral bud development stage revealed high sensitivity to extreme heat for pollen starch content, reducing starch levels by 56%, whereas soluble sugars, protein, total amino acid and individual amino acid content was unaffected by heat stress. For many angiosperms, MMC accumulate starch just before the first meiotic division (Fadón et al., 2018), likely aligning with the tight bud stage in blueberry. In sweet cherry, starch accumulation was highest during pre-meiotic development, and authors suggest these starch reserves support the process of pollen meiosis and early anther growth (Fadón et al., 2018). Other studies emphasize the importance of starch for flower development (Hedhly et al. 2016) and mitigating abiotic stress (Thalmann and Santelia, 2017). Heat-induced reductions in starch accumulation for developing microspores has been observed in multiple systems, associated with reduced pollen viability (Pressman et al., 2002; Firon et al., 2006; Pressman et al., 2006; Raja et al., 2019; Callens et al., 2023). In barley, reduced pollen starch content following heat stress

was attributed to reduced conversion of sucrose to starch (Callens et al., 2023). In the present study, the observations of reduced starch concentrations but unchanged sucrose concentrations in heat stressed blueberry pollen at tight bud could indicate disruptions in sugar utilization rather than sugar transport processes (Jin et al., 2013, Borghi et al., 2019). However, further detailed metabolomic studies are required to confirm this for blueberry.

In tomato and barley plants, altered pollen carbohydrate profiles under short or prolonged heat exposure were associated with fruit or seed set failure (Pressman et al., 2002; Sato et al., 2006; Firon et al., 2006), yet blueberry plants at tight bud exposed to the same heat stress regime as this study showed no reduction in blueberry fruit set (Chapter 3). Despite the significantly lower concentration of starch observed at tight bud following heat exposure compared to the control, this concentration was nonetheless higher compared with other flower development stages studied, regardless of temperature exposure. While it is unclear why starch content was higher at the tight bud stage compared to latter development stages, given that all pollen assessed in this study was collected at the anthesis stage, this suggests that starch concentrations were sufficient to support pollen development and subsequent performance, regardless of temperature treatment. Additionally, it is possible that carbohydrate reserves in pollen exposed to heat stress at tight bud may have had enough accumulated resources to fuel initial pollen tube growth, and subsequent pollen tube growth may have supported by the carbohydrates provided through the transmitting tract of the style (Herrero and Arbeloa, 1989; Zinn et al., 2010). While pollen sample weight was accounted in final nutrient conversions, pollen was limited for the tight bud stage resulting in smaller pollen sample quantities per replicate and fewer replicates overall compared to other development stages. Thus, repeated assessments should be performed to determine whether the pollen starch content measured in this study is biologically valid for the tight bud stage in northern highbush blueberry. Future studies should also confirm the pollen development stage occurring during tight bud in northern highbush blueberry and explore the consequences of pollen nutrition and performance under alternative heat stress regimes, such as prolonged mildly elevated temperatures or repeated short bursts of extreme heat over several days. Indeed, confirming the pollen development stage associated with tight bud will provide insight into which organs and regulatory systems may be altered or disrupted under heat stress conditions. It is also possible that a single 4 h heat stress event at this stage was insufficient to disrupt the subsequent functioning of pollen, possibly inferring tolerance to heat stress at this pre-meiotic floral bud stage in blueberry.

Overall, my results suggest that for the tight bud stage, carbohydrate metabolism and utilization are more strongly affected following heat stress compared to other pollen metabolites. However, additional assessments are required for pollen starch content at tight bud, given the variability among the controls.

Soon after the tight bud stage, northern highbush blueberry floral buds transition into the bud swell stage where previously dormant MMC are likely now undergoing meiosis (Gough et al., 1978). This stage is the first sign of growth for blueberries in the early spring and is considered tolerant to cold temperatures (-12 to -9°C; blueberries.msu.edu), yet no studies have investigated heat tolerance of this development stage. I found that this floral development stage had substantial sensitivity to heat stress, reflected by significant reductions in pollen protein, total amino acids, and several individual amino acids (aspartate, glutamate, methionine, proline, and lysine). When extreme heat occurs during MMC meiosis, the stage likely associated with bud swell in northern highbush blueberry, it can result in premature degradation of tapetal cells, disrupted cell division, altered auxin and amino acid biosynthesis, inhibited nitrogen assimilation, and reduced protein quality (Borghi et al., 2019; Lohani et al., 2020). At the transcriptome level, transcripts altered following microspore heat stress includes ROS-related genes, genes related to ethylene and abscisic acid signaling, heat shock factors and proteins, and genes related to carbohydrate metabolism (Lohani et al., 2020). Several crop plants have been studied when heat stress occurs at this MMC meiosis stage, such as wheat plants which experience early tapetum degradation and subsequent pollen sterility (Sakata et al., 2000). The metabolite reductions following heat stress observed at the bud swell stage is similar to findings for several other crops (Iwahori, 1966; Peet et al., 1998; Sakata et al., 2000; Vara Prasad et al., 2000; Kim et al., 2001; Pressman et al., 2002; Rodrigo and Herrero, 2002; Firon et al., 2006; Sato et al., 2006; Jain et al., 2010; Ledesma and Kawabata, 2016; Callens et al., 2023), with the overall recognition that MMC meiosis is a critical development stage for nutrient metabolism and partioning in developing microspores of angiosperms with important consequences for pollen development and performance (Lohani et al., 2020).

Like many of these studies that reported reduced fruit or seed set following heat stress at the MMC meiosis stage, my previous chapter exposing blueberry plants to the same heat stress regime (Chapter 3) revealed bud swell to be the most affected floral development stage with a 39% reduction in fruit set following heat stress. Reductions in total and several individual amino acids

following heat stress may have contributed to poor pollen performance and limited fruit set at the bud swell stage. For instance, proline was significantly reduced following heat stress at bud swell, and this is an important amino acid required for pollen development and fertility in angiosperms (Schwacke et al., 1999; Biancucci et al., 2015; Mattioli et al., 2018). During normal development, proline helps prevent protein denaturation, protects cellular structures, and provides energy for pollen tube growth (Chiang and Dandekar, 1995; Biancucci et al., 2015; Raja et al., 2019). In *Arabidopsis* mutants deficient in proline synthesis enzymes, pollen grains were abnormal and sterile, supporting the claim that proline is essential for pollen development and functioning (Mattioli et al., 2012; Raja et al., 2019). In tomato, gene expression involved in proline uptake was inhibited under heat stress conditions (Sato et al., 2006). In rice, pollen proline levels decreased following high temperature stress, affecting pollen development and performance (Tang et al., 2008). Glutamate, an amino acid that plays a central role in plant nitrogen metabolism (Forde and Lea, 2007), was also significantly reduced following heat stress at bud swell. Glutamate is a precursor for proline, arginine, asparagine, and GABA synthesis in plants, all of which play an important role in response to stressors, including heat shock (Forde and Lea, 2007; Biancucci et al., 2015). The glutamate derivative GABA has been reported to play an important role in pollen tube growth and guidance (Palanivelu et al., 2003; Biancucci et al., 2015), so reductions in glutamate following heat stress could have negative effects on pollen performance and fertilization. Significant reductions in methionine were also observed at the bud swell stage, and considering this amino acid is a precursor for ethylene production in plants (Chen et al., 2019a), significant reductions of this amino acid could have repercussions for plant heat tolerance, pollen quality, and pollen performance (i.e., germination and tube growth) (Jegadeesan et al., 2018; Raja et al., 2019; Chaturvedi et al., 2021).

Aspartate, another central amino acid driving plant nutrition, energy and stress responses (Han et al., 2021), was also significantly reduced following heat stress at bud swell. Aspartate is a precursor for many other amino acids, including glutamate, methionine, threonine, lysine, isoleucine, asparagine, arginine, tyrosine, and phenylalanine (Han et al., 2021). Aspartate is also a precursor for the synthesis of nicotinamide adenine dinucleotide ($NAD^+$), an essential coenzyme necessary for metabolic reactions in plants and an essential component in pollen development (Hashida et al., 2009; Lin et al., 2010; Han et al., 2021). When $NAD^+$ pathways are disrupted, it can have negative consequences for pollen maturation and inhibit pollen tube growth (Hashida et

al., 2007, 2009, 2013). Given the importance of aspartate in the biosynthesis of several fundamental metabolites, changes in aspartate levels can have serious consequences for downstream processes important to plant metabolism, including glycolysis and the TCA cycle (Han et al., 2021). Lysine, another amino acid significantly reduced following four hours of heat stress at the bud swell stage, also plays an important role in the TCA cycle, abiotic and biotic stress responses in plants and starch metabolism (Yang et al., 2020c; Trovato et al., 2021), and may have contributed to observed trends of reduced pollen starch content at this floral bud stage. In the present study, exposure to four hours of extreme heat at bud swell significantly reduced pollen proline (13%), glutamate (33%), methionine (33%), aspartate (75%), and lysine (18%), and given the roles of these amino acids in plant metabolism, pollen development and functioning, such reductions could affect pollen tube growth and fertilization. Indeed, exposure to the same extreme heat regime at bud swell reduced fruit set by 39% in my previous study (Chapter 3), and such declines in fertilization could have, in part, been driven by reduced pollen amino acid content. Overall, the findings in the present study indicate that the bud swell stage in northern highbush blueberry is highly sensitive to brief exposure of extreme heat stress with negative consequences on pollen quality, performance, and subsequent blueberry fruit production. Further studies should be done to confirm whether MMC meiosis is occurring at this bud swell stage in northern highbush blueberry, adding to the growing literature that MMC meiosis is particularly sensitive to extreme heat stress and the negative repercussions it can have on crop plants.

Bud break occurs after bud swell stage in northern highbush blueberry, where bud scales begin to open, revealing the tips of individual flowers. Given the lack of formal analysis on the stage of pollen development occurring at the bud break stage in northern highbush blueberry, it is unclear whether microspores are still undergoing meiosis or have completed meiosis. In the present study, the consequence of heat stress at bud break appears to show resilience, more than any other development stage tested. Starch, sucrose, and glucose levels were relatively unchanged by temperature treatment at this development stage, so it is unlikely that heat induced any substantial changes in sugar-starch utilization, metabolism, or transport at bud break (Lohani et al., 2020). Protein levels were also not significantly affected by heat stress at this development stage. Increases in certain individual amino acids following heat stress, such as asparagine and glutamate, also suggest pollen heat tolerance at bud break as these amino acids play an important role in heat shock response (Forde and Lea, 2007; Biancucci et al., 2015). It is possible that significant

increases in certain amino acids following heat stress could be due to the alteration of certain pathways, either up- or down-regulated, in an effort to reduce damage caused by heat stress (Zhang et al., 2022). In strawberry, exposure to a one-time extreme heat event during floral development revealed heat tolerance at intermediate floral development stages, but heat sensitivity at earlier and later stages (Ledesma and Kawabata, 2016). While the exact mechanisms are unknown, it is possible that this intermediate bud break stage is more heat tolerant than earlier and later blueberry floral development stages. Data in the present study supports this, as well as previous work showing heat stress at bud break had no significant effect on blueberry fruit set (Chapter 3). Further investigation should be done to evaluate the effect of repeated, brief extreme heat events at this development stage to more realistically assess the effects of heat waves on pollen quality and performance. Overall, it is clear that further research is required to understand what pollen development stage is occurring during bud break in northern highbush blueberry, providing insight into the relative heat tolerance at this floral bud stage.

At the early and late pink bud stage, blueberry anthers begin to elongate resulting in the presence of floral corollas and subsequent flower elongation. Drawing from studies in sweet cherry, a woody perennial crop that blooms at a similar time as northern highbush blueberry, it is possible that blueberry pollen is undergoing mitosis at the early and late pink bud floral bud stage (Fadón et al., 2018). While future studies are required to confirm the pollen development stage occurring at early and late pink bud in blueberry, it is broadly accepted that when extreme heat occurs during pollen mitosis it can alter sugar-starch utilization, metabolism, and transport (Jain et al., 2007; Raja et al., 2019; Lohani et al., 2020). In the present study, there were significant reductions in methionine and proline concentrations following heat stress exposure at early/late pink bud. As discussed previously, proline plays an important role in pollen development and pollen performance and has the potential to affect fertilization and yield (Chiang and Dandekar, 1995; Schwacke et al., 1999; Biancucci et al., 2015; Mattioli et al., 2018). Significant reductions in methionine at the early/late pink bud stage following heat stress could also have negative consequences for plant heat tolerance, pollen quality, and pollen germination (Porch and Jáhn, 2001; Jegadeesan et al., 2018; Raja et al., 2019; Chaturvedi et al., 2021). However, in my previous study (Chapter 3), exposure to the same heat regime (37.5°C, 4h) at early and late pink bud did not significantly affect fruit set in ‘Bluecrop’ bushes. Furthermore, several amino acids increased following heat stress at the early/late pink bud stage, potentially aiding in blueberry pollen

thermotolerance and mitigating substantial reductions in fertilization and fruit production. Overall, this early/late pink bud floral stage in blueberry appears to have greater heat tolerance than bud swell, yet greater heat sensitivity than tight bud and bud break. As discussed for the preceding floral development stages, future studies should explore repeated heat stress events on pollen quality and functioning, and imaging should be done to confirm whether mitosis is occurring at early/late pink bud in northern highbush blueberry.

At anthesis, or flower opening, blueberry pollen microspores have now matured into pollen grains and are prepared for anther dehiscence once sonicated or disturbed by a bee pollinator. When extreme heat occurs during anthesis, it can result in altered carbohydrate and protein metabolism, reduced membrane integrity, and the accumulation of reactive oxygen species (ROS) which ultimately can reduce pollen viability, inhibit anther dehiscence, and result in failure to release pollen grains (Lohani et al., 2020). In the current study, exposure to extreme heat for four hours at anthesis had no significant effect on blueberry pollen protein or carbohydrate content, and several amino acids such as glutamate and proline significantly increased. As discussed for bud break and early/late pink bud development stages, this could potentially indicate improved thermotolerance at later pollen development stages via upregulated metabolic pathways. However, this requires much greater exploration. One amino acid, glutamine was significantly reduced following heat stress at anthesis and may play an important role in pollen functioning. The conversion of glutamine from glutamate is considered to be a major pathway incorporating inorganic nitrogen into organic molecules, ultimately playing an important role in the nitrogen and carbon metabolism (Cren and Hirel, 1999; Lee et al., 2023b). Glutamine also plays an essential role in protein synthesis and serves as a signaling molecule regulating metabolism and stress responses in plants (Lee et al., 2023b). In my previous study (Chapter 2), 'Bluecrop' pollen exposed to the same heat stress regime (37.5°C, 4 h) *in vitro* significantly inhibited pollen tube growth (74%) and pollen germination (39%) compared to pollen exposed to 30°C. Given the discrepancies in these results for anthesis pollen, with most of the pollen nutrients assayed being unaffected by heat stress yet pollen viability being substantially reduced *in vitro*, it is clear that further research is needed for this development stage. Additional studies should be performed to assess the consequences of heat stressed blueberry pollen at the anthesis stage on fruit set, as disruptions in pollen-pistil interactions may not have been captured in this research but could have contributed to yield declines observed in Michigan fields in 2018.

5.4.2 Consequences of heat stress on pollen nutrient quality and bee nutrition

As discussed above, insufficient pollen nutrition, including proteins, carbohydrates, and amino acids, can have devastating consequences for pollen development, functioning (i.e., pollen germination and tube growth), and ultimately fertilization. These changes can also lead to nutritional stress in bees, as pollen provides the primary source of protein and amino acids for bees, as well an important resource of carbohydrates (Roulston and Cane, 2000; Vanderplanck et al., 2014; Vaudo et al., 2016, 2020). Unbalanced pollen diets can hinder bee development, fecundity, resilience to stress, and survival as adults and developing larvae (Roulston and Cane, 2000; Vaudo et al., 2015; Bukovinszky et al., 2017; Woodard et al., 2019; Filipiak and Filipiak, 2020; Crone et al., 2022; Knauer et al., 2022). Bees are particularly sensitive to altered pollen nutrition, either in excess or deficient amounts, given their high metabolic activity and low energy stores that ultimately requires a well-balanced diet for proper development (Ruedenauer et al., 2015; Vaudo et al., 2020; Crone et al., 2023). Despite this, no studies have quantified the consequences of heat-altered pollen diets for bee pollinators and their offspring. In my previous study (Chapter 4), *Osmia lignaria* females were provided blueberry plants exposed to extreme heat (37.5°C, 4h) or non-stressed (25°C, 4h) conditions at 25% bloom, exposing a range of floral buds from bud swell to anthesis. Female bees provided plants exposed to extreme heat laid 70% fewer eggs compared to females provided non-stressed host plants, and of the brood produced, 60% died before completing larval development. Considering female bees provided brood similar quantities of pollen provisions, regardless of temperature treatment, it is likely that heat altered pollen nutrition had important consequences for female fecundity and the development and survival of their brood. Furthermore, female *O. lignaria* bees were not exposed to heat stress and were reared under the same optimal conditions regardless of the host plants they were provided, emphasizing the potential role of host plant temperature treatment on female fecundity. Hereafter, I discuss the implications of heat-altered blueberry pollen nutrition on bee health, development, reproduction, and survival, particularly in light of previous studies in *O. lignaria* (Chapter 4).

While nectar is often considered the main source of carbohydrates for bees, the carbohydrates present in pollen also provide the energy needed for the biosynthesis of proteins, tissues, and enzymes necessary for development and survival (Nepi et al., 2005; Lau et al., 2022). Carbohydrate content in pollen is particularly important for the development and survival of solitary bees like *Osmia* (Austin and Gilbert, 2021). Pollen provisions in some solitary bee larvae,

including several *Osmia* species, are comprised of mostly pollen and small amounts of nectar, thus solitary bee larvae must acquire carbohydrates from pollen for proper development and survival (Austin and Gilbert, 2021). While minimum protein concentrations are needed in larval provisions to complete development (Levin and Haydak, 1957; Roulston and Cane, 2000), carbohydrates may be more limited in solitary bee provisions than protein given the large quantity of pollen provided in their provisions. Indeed, several studies have reported that carbohydrates may have a stronger effect on growth, development, and survival to pupation in solitary bees than protein (Wasielewski et al., 2013; Austin and Gilbert, 2021). Furthermore, studies evaluating the process of pollen digestion in bees highlight the importance of pollen carbohydrate levels for bee development and survival, both directly and indirectly. The outer exine layer of pollen is indigestible to bees (Roulston and Cane, 2000; Nepi et al., 2005), yet bees require the nutritive contents within pollen grains for their development and survival. As such, several processes must occur to break open the exine layer of pollen to allow the extraction of pollen nutrients to fulfill the nutrient demands of bees (Roulston and Cane, 2000). Several mechanisms have been described for pollen nutrient extraction (see Roulston and Cane, 2000), including the onset of pollen germination (or pseudo-germination) to release nutrients from pollen grains. Adult bees possess a crop where pollen and nectar mix, providing a sugar-rich environment that stimulates pollen germination (or pseudo-germination) similar to when pollen grains land on the surface of a floral stigma (Roulston and Cane, 2000). As pollen grains germinate and pollen tubes protrude, the proteins, carbohydrates, and amino acids are released from the interior of pollen grains (Stanley and Linskens, 1965; Linskens and Schrauwen, 1969; Roulston and Cane, 2000). As adult bees digest germinating pollen grains, the contents of pollen are steadily emptied, allowing bees access to these nutrients fueling their development and activity (Roulston and Cane, 2000). While bee larvae do not have a crop like adult bees, larval pollen diets are mixed with nectar to create a pollen provision, providing a sugar-rich environment stimulating pollen germination and subsequent access to pollen nutrients (Roulston and Cane, 2000; Nepi et al., 2005). This pollen germination mechanism for bee digestion is important in the context of the present study, as several nutrients such as starch, proteins, and certain amino acids like proline, are required to fuel pollen germination and subsequent tube growth. Blueberry plants exposed to extreme heat at various stages of floral development exhibited notable reductions in pollen starch, protein, and proline content, potentially inhibiting pollen germination and tube growth with cascading effects on bees' access to pollen nutrients.

Furthermore, observations in Chapter 2 revealed significant reductions in pollen germination and tube growth in 'Bluecrop' pollen exposed to extreme heat conditions for four hours, which has obvious implications for plant fertilization, but may also play an important role in bee digestion and their capacity to access pollen nutrition (Roulston and Cane, 2000; Nepi et al., 2005). Thus, reductions in certain pollen nutrients (including starch) may indirectly inhibit bees access to nutrients by impeding processes necessary for digestion and nutrient uptake.

In one study, Nepi et al. (2005) evaluated the process of pollen digestion in *Osmia cornuta* larvae by quantifying the starch content of pollen from anthers, pollen provisions, and the larval digestive tract and gut. Unlike other bee species, solitary bees like *Osmia* can effectively remove starch from pollen grains and have shown a preference for "starchy pollen" (Simpson and Neff, 1983; Nepi et al., 2005). Interestingly, authors observed reductions in pollen starch content before bee larvae even began consuming provisions, and authors hypothesized that once pollen came in contact with nectar in the provision, starch granules underwent hydrolysis, activating starch depolymerization to simple sugars, providing a digestible form of sugar for bees (Ladurner et al., 1999; Nepi et al., 2005). Starch content continued to decrease along the larval digestive tract, attributed to pseudo-germination via protoplasm extrusion occurring when pollen grains collapsed in the gut, continuing to provide a digestible sugar resource for solitary bee larvae (Nepi et al., 2005). Authors also observed some unmodified (i.e., no germination), starch-containing pollen grains in the provision and suggested that the lack of depolymerization or poor digestion could be due to degeneration during pollen development, rendering pollen "dead" and unable to respond to stimuli (i.e., germinate) after coming in contact with the nectar present in the provision and/or osmotic shock in the gut (Nepi et al., 2005). Ultimately, the study by Nepi et al. (2005) provides a few important insights for the current body of work. First, these findings highlight that heat-induced reductions in pollen germination and tube growth observed in blueberry (Chapter 2) likely play a role in bees' access to pollen nutrition, before and during digestion (Roulston and Cane, 2000; Nepi et al., 2005). Further studies should explore the role of pollen germination (or pseudo-germination) as possible mechanism limiting bees' access to pollen nutrients following heat stress, following a similar approach to Nepi et al. (2005). Second, Nepi et al. (2005) provide evidence for the importance of pollen starch content in solitary bee larvae diets to meet soluble sugar demands necessary for development. In the present study, a significant reduction in pollen starch content (35%) was observed following heat stress across all blueberry floral development stages, while

soluble sugars (sucrose and glucose) were not significantly affected by extreme heat exposure. However, the starch depolymerization process described by Nepi et al. (2005) suggests that such reductions in pollen starch content could also inhibit bees access to soluble sugars during digestion. Thus, while extreme heat exposure did not directly alter or reduce pollen soluble sugar content, the reductions in pollen starch may have downstream effects inhibiting the dietary needs of bees. Such inhibition is particularly concerning for *Osmia* bee species, given the important role carbohydrates play mediating their growth and survival, particularly as larvae (Austin and Gilbert, 2021). In my previous study (Chapter 4), *O. lignaria* larvae provided pollen from heat stressed plants experienced significantly higher larval mortality compared to larvae fed pollen from non-stressed plants, despite brood receiving similar quantities of pollen provisions regardless of host plant temperature treatment. Such substantial reductions in larval survival suggest heat-driven limitations in pollen nutrient availability during development, contributed by poor digestion via reduced pollen germination success and/or insufficient pollen nutrient concentrations via reduced starch content. Inhibitions in larval survival may have been further perpetuated by the reduced or altered levels of pollen protein and amino acids. In the context of current and previous studies, this suggests a positive feedback loop driven by heat stressed pollen sources with negative consequences for *O. lignaria* development and survival.

Pollen is the primary resource of protein for bees, providing the building blocks necessary for development (Vanderplanck et al., 2014; Vaudo et al., 2015; Quinlan and Grozinger, 2023; Westreich and Tobin, 2024). Pollen protein content is also important for bee reproduction and longevity (Roulston and Cane, 2000). Egg-laying female bees require a sufficient protein diet to properly develop their ovaries and eggs, so a low-protein diet can inhibit reproductive potential among female bees (Hoover et al., 2006; Human et al., 2007; Pirk et al., 2010; Cane, 2016). Egg production and oviposition are energetically costly for all bees, but particularly for solitary bees such as *O. lignaria* that lay large eggs relative to their body size and require pollen for oocyte maturation (Cane, 2016). The substantial reduction in egg laying for *O. lignaria* bees provided heat stressed host plants in my previous study (Chapter 4) may, in part, be in response to insufficient pollen protein content inhibiting ovary development and egg production. However, follow up studies should be done to assess differences in oocyte maturation and ovary size among bees provided pollen from heat stressed host plants to confirm this. Pollen protein content is also important for larval development, demonstrated by Levin and Haydak (1957) who transplanted *O.*

*lignaria* eggs onto honey bee-collected pollens and found that only the richest protein diets supported development to adulthood. Additionally, the adult body size of *O. lignaria* bees increased linearly with total protein consumed (Levin and Haydak, 1957; Roulston and Cane, 2000). While *O. lignaria* adult body size was not significantly affected by heat stressed pollen diets in my previous study (Chapter 4), substantial reductions in larval survival was observed among brood provided pollen from heat stressed host plants, indicating chronic nutrient deficiencies. Among other heat-mediated pollen nutrient deficiencies in pollen starch and amino acids, it is possible that reductions in pollen protein content may have contributed to nutritional stress and subsequent larval mortality in *O. lignaria* bees. While floral preference was not evaluated between heat stressed and non-stressed plants in the previous study (Chapter 4), other studies report a significant correlation between pollen protein content, bee abundance, and bee visitation rates (Russo et al., 2019), suggesting future behavioral or ecological studies should assess potential shifts in floral preference following heat stress. However, the capacity of bees, particularly solitary bees, to detect differences in pollen quality is still largely unknown.

Amino acids present in pollen diets are crucial for bee growth, somatic maintenance, reproduction, and longevity (Paoli et al., 2014; Vanderplanck et al., 2014; Barraud et al., 2022; Jeannerod et al., 2022). Barraud et al. (2022) provided honey bees (*Apis mellifera*), bumble bees (*Bombus terrestris*), and *Osmia* bees (*Osmia bicornis* and *Osmia cornuta*) diets of variable quality and found that bumble bees and *Osmia* bees struggled to develop on diets with low total amino acid content, while honey bees were relatively unaffected. Specifically, poor amino acid diets inhibited brood production in bumble bees and reduced cocoon and adult body weights in *Osmia* bees (Barraud et al., 2022), indicating the importance of a sufficient, well-balanced amino acid pollen diet for bee development, reproduction, and fitness. In the present study, extreme heat exposure at bud swell significantly reduced total amino acids, while other floral development stages were not significantly affected. It is possible that a reduction in total amino acids at the bud swell stage could have contributed to reduced *Osmia* egg laying and offspring survival in my previous study (Chapter 4), however, the total amino acid content exposed to control conditions at bud swell is notably higher compared to controls at other development stages. It is unclear why such differences in total amino acids were observed for the control group, given all pollen was collected at the anthesis stage. Repeated measurements of total amino acid content in blueberry pollen should be considered in order to validate the biological significance of heat stress at the bud

swell stage. Additionally, further pollen metabolite studies should assess the effect of heat stress on phacelia and white clover blooms, as these host plants were also provided to *O. lignaria* bees in addition to blueberry, likely contributing to declines in fecundity and survival.

Certain essential amino acids (arginine, methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, tryptophan) cannot be synthesized by bees and thus, they rely on dietary resources (i.e., pollen) to meet these nutrient demands (De Groot, 1952). Given this limitation, amino acid composition has been considered to have a greater influence on the amount of pollen required by bees than crude protein content (Nicolson, 2011; Vanderplanck et al., 2014), although this likely varies across bee genera. While not statistically significant, some essential amino acid levels were higher following heat exposure at bud break (25%), early/late pink bud (39%), and anthesis (24%). Several studies have reported deleterious effects of higher, unbalanced amino acid pollen content for bees, inhibiting bee flight, shortening bee life spans, and causing higher morality (Paoli et al., 2014; Stabler et al., 2015; Archer et al., 2021; Austad et al., 2024). However, these interpretations should be considered with caution given variations among means for essential amino acids following heat stress were not statistically different from the control in blueberry pollen.

Some researchers have hypothesized that the consequences of altered amino acid diets on bee fitness is contingent on the concentration and/or ratio of individual amino acids present in the diet, as certain amino acids (or interactions between amino acids) could result in toxic side effects or impair certain physiological processes (Grandison et al., 2009; Stabler et al., 2015; Archer et al., 2021; Austad et al., 2024). This aligns with my previous study (Chapter 4), where extreme heat exposure increased certain essential and non-essential amino acids, yet *Osmia lignaria* larvae fed pollen from heat stressed blueberry plants experienced significantly higher mortality rates. Given the overall high survival rate among *O. lignaria* larvae provided pollen from non-stressed plants, it is possible that the elevated concentrations of certain amino acids following heat stress, and reduced concentrations of some amino acids, contributed to nutrient imbalance, ultimately resulting in higher rates of mortality. Delineating the concentrations and combinations of individual amino acids that may impair (or improve) bee functioning and survival have received little attention, particularly for solitary bees, and requires further research. Thus, reports from other well-studied insect taxa such as *Drosophila* flies may provide important insights for the present study. For example, Grandison et al. (2009) assessed the lifespan and fecundity of *Drosophila*

under variable amino acid diets and found that methionine was crucially important for increasing fecundity and ensuring egg quality. Interestingly, under restricted diets devoid of all essential amino acids besides methionine, *Drosophila* fecundity increased, and was even comparable to unrestricted diets where all essential amino acids were present (Grandison et al., 2009). Thus, the authors suggest that methionine is a key limiting nutrient necessary for reproduction (Grandison et al., 2009). In the present study, methionine was significantly reduced in blueberry pollen following heat stress at bud swell (33%) and early/late pink bud (13%). Furthermore, methionine was one of the most limited amino acids present in blueberry pollen, across all temperature treatments and development stages. Another study also reported limited methionine concentrations in pollen across several plant genera (Jeannerod et al., 2022). While further exploration is needed to confirm the role of methionine in bee reproduction, these findings may provide important insight into the results of my previous study (Chapter 4), where *O. lignaria* bees provided heat stressed host plants produced 70% fewer eggs compared to females provided non-stressed host plants. For example, the role of heat stress exacerbating already low concentrations of methionine in blueberry pollen may have caused a nutrient imbalance in *O. lignaria* pollen diets, inhibiting their reproductive development and egg laying capacity (Cane, 2016).

Additionally, Grandison et al. (2009) found no changes in *Drosophila* reproduction or lifespan when provided diets that excluded tryptophan, despite this amino acid being essential and in low concentrations. Other reports have found that restricting levels of leucine, isoleucine, and valine in *Drosophila* diets can extend longevity and induce stress resistance (Juricic et al., 2020; Fulton et al., 2024). In the present study, blueberry plants exposed to heat stress at various development stages had significantly higher levels of tryptophan, isoleucine, leucine, and valine in pollen grains. While all these amino acids are considered essential for bees, it is possible that increases in these specific essential amino acids resulted in nutritional imbalance. This heat-mediated diet imbalance could have damaging effects for bees. Indeed, higher mortality rates as larvae and adults, as well as trends for reduced adult longevity, were observed for *O. lignaria* larvae provided diets from heat stressed blueberry plants (Chapter 4). However, further studies are required to determine whether similar patterns observed in *Drosophila* are similar for various bee taxa, including social and solitary bees. Nonetheless, these results suggest that heat-driven imbalances in amino acid concentrations can have varying consequences for insect reproduction and survival and emphasize the importance of investigating nutrient limitations caused by

individual amino acids. Development of an artificial diet system for *Osmia* larvae, similar to methods described by Grandison et al. (2009), could facilitate exploration of how specific amino acid nutrient imbalances may influence bee development and survival.

It is unclear whether bees, particularly solitary bees, are able to detect and regulate their intake of essential amino acids from pollen ensuring they consume a well-balanced diet. In one study, bumble bees did not regulate their pollen intake and over-consumed essential amino acids resulting in higher rates of mortality, and authors offered several potential explanations for this (Stabler et al., 2015). It is possible that bees were not able to easily taste differences in the concentrations of amino acids in pollen diets, resulting in a passive, over-ingestion of essential amino acids (Stabler et al., 2015). Unlike nectar, which several bee species can readily detect the quality of during foraging bouts (Carter et al., 2006; Howell and Alarcón, 2007; Nicolson and Thornburg, 2007; Bertazzini et al., 2010; Nicolson, 2011; Darvishzadeh et al., 2015; Ruedenauer et al., 2015; Tafi et al., 2023), pollen requires greater manipulation during digestion before nutrients are accessible (Roulston and Cane, 2000), potentially inhibiting sensory cues informing feeding decisions by bees. It is also possible that the post-ingestive mechanisms regulating essential amino acid intake may be tied to a specific concentration of amino acids, where bees may be unable to adjust or reduce their intake when such concentrations are in excess for a given pollen diet (Stabler et al., 2015). Other studies have also found that bumble bees are able to detect and discriminate between different types of pollen (i.e., floral source), but were unable to discriminate between different concentrations of pollen diets (Ruedenauer et al., 2015). In my previous work, *O. lignaria* females provided similar quantities of pollen provisions to brood regardless of host plant temperature exposure, yet findings in the present study make it clear that extreme heat exposure altered nutrient balance in blueberry pollen. It is possible that female *Osmia* bees were unable to detect insufficient nutrient concentrations in pollen, including amino acids, and therefore did not alter the quantity of provisions provided to brood to help ensure dietary balance. While little is known about the effects of non-essential amino acids on bee diets, they may play an important role in foraging decisions for bees. For example, Ruedenaur et al. (2021) has shown that honey bees are able to detect proline concentrations in pollen which may serve as a guide during foraging bouts (Ruedenauer et al., 2021). In the present study, proline was significantly reduced in blueberry pollen at bud swell and early/late pink bud stage following heat stress, but significantly increased at anthesis, so whether or not such alterations would influence *Osmia*

foraging decisions is unclear. Choice studies should be done to determine whether *O. lignaria* are able to discern nutritional differences in pollen following heat stress, and the subsequent effects of these foraging choices on female fecundity and offspring survival.

This research is among the first to examine how brief extreme heat exposure (37.5°C, 4 h) during floral development affects the nutritional content of pollen and the downstream consequences on an entire pollination system. Altered concentrations of pollen protein, starch, and several amino acids in response to heat stress in northern highbush blueberry may have inhibited various pollen development processes, contributing to reduced pollen germination and tube growth (Chapter 2) and reduced fruit set (Chapter 3). Additionally, such alterations in the nutritional profile of blueberry pollen likely resulted in dietary imbalances for the *Osmia lignaria* bees feeding on it, limiting the reproductive potential of adult female bees and contributing to high mortality rates among subsequent brood, both as larvae and adults (Chapter 4). My research suggests that studies evaluating the effects of direct heat stress, either for plants or bees, may underestimate the consequences of climate change on bee-plant interactions, with significant consequences for future crop production and bee declines. Broadening our understanding from direct, isolated stressors to indirect and interactive stressors is important for combatting climate change in pollination systems and emphasizes the important role pollen plays for mediating this. As such, there is an urgent need to develop mitigation strategies to help protect the nutritional quality of pollen under extreme heat conditions, for the protection of both crop yields and pollinators alike.

## TABLES

**Table 5.1** Mean (± SE) soluble sugars content (µg/mg), including glucose and sucrose, in northern highbush blueberry pollen (cv. 'Bluecrop') collected at anthesis from flowers exposed to either CT (25°C, 4h) or HT (37.5°C, 4h) at tight bud, bud swell, bud break, early/late pink bud, or anthesis floral bud stages. Across all development stages, no means were statistically different from one another between temperature treatments ($\alpha > 0.05$).

| Floral bud development stage | Mean (± SE) soluble sugar assay (µg/mg) | | | |
|---|---|---|---|---|
| | Glucose | | Sucrose | |
| | CT | HT | CT | HT |
| Tight bud | 0.43 ± 0.08 | 0.35 ± 0.08 | 51.30 ± 2.32 | 52.47 ± 2.87 |
| | z=-0.77, *p*=0.44 | | t=-0.41, *p*=0.69 | |
| Bud swell | 0.29 ± 0.04 | 0.23 ± 0.02 | 55.47 ± 2.42 | 56.90 ± 2.08 |
| | z=-1.84, *p*=0.07 | | t=-0.60, *p*=0.56 | |
| Bud break | 0.31 ± 0.04 | 0.39 ± 0.06 | 57.51 ± 2.53 | 57.58 ± 2.67 |
| | z=1.55, *p*=0.12 | | t=-0.02, *p*=0.98 | |
| Early/Late pink bud | 0.31 ± 0.04 | 0.28 ± 0.03 | 58.59 ± 2.77 | 55.39 ± 2.32 |
| | z=-0.68, *p*=0.49 | | t=1.17, *p*=0.25 | |
| Anthesis | 0.25 ± 0.03 | 0.26 ± 0.02 | 56.63 ± 2.75 | 52.77 ± 2.26 |
| | z=0.25, *p*=0.80 | | t=1.37, *p*=0.17 | |

**Table 5.2** Mean (± SE) amino acid content (µg/mg) of northern highbush blueberry pollen (cv. 'Bluecrop') collected at anthesis from flowers exposed to either CT (25°C, 4h) or HT (37.5°C, 4h) at tight bud, bud swell, bud break, early/late pink bud, or anthesis floral bud stages. Amino acids were ordered from most abundant (i.e., proline) to least abundant (i.e., cysteine) across all development stages and temperature treatments in this table. For all amino acids tested, the degrees of freedom = 4 and residual degrees of freedom = 116, derived from likelihood ratio tests of GLMM or LMM models and GLMM/LMM model summaries, respectively. Other test statistics ($\chi^2$, F, *p*) reported for each amino acid was derived likelihood ratio tests of GLMM or LMM models. Significant differences between means within a given development stage are indicated by the bolded test statistic ($\alpha \leqq 0.05$).

| **Amino acid (µg/mg)** | **Development stage and temperature treatment** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Tight bud** | | **Bud swell** | | **Bud break** | | **Early/Late pink bud** | | **Anthesis** | |
| | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** |
| **Proline** | 17.07±1.45 | 15.61±1.36 | 24.68±1.21 | 21.42±0.87 | 22.40±1.20 | 22.03±1.28 | 21.55±1.17 | 17.31±1.34 | 20.19±1.14 | 23.62±1.18 |
| F=4.70, *p*=0.003 | t=0.84, *p*=0.40 | | **t=2.62, *p*=0.01** | | t=0.23, *p*=0.82 | | **t=2.66, *p*=0.009** | | **t=-2.28, *p*=0.02** | |
| **Aspartate** | 5.05±2.43 | 6.35±2.31 | 31.51±1.99 | 7.80±1.36 | 4.01±2.03 | 6.42±2.19 | 9.78±1.99 | 5.16±2.28 | 5.90±1.93 | 7.26±1.98 |
| F=24.64, *p*<0.0001 | t=-0.43, *p*=0.67 | | **t=10.74, *p*<0.0001** | | t=-0.84, *p*=0.40 | | t=1.61, *p*=0.11 | | t=-0.51, *p*=0.61 | |
| **Asparagine** | 6.58±0.58 | 6.53±0.54 | 7.80±0.50 | 8.15±0.37 | 6.80±0.48 | 8.55±0.51 | 6.40±0.47 | 8.41±0.53 | 6.73±0.46 | 7.78±0.47 |
| F=2.18, *p*=0.08 | t=0.07, *p*=0.94 | | t=-0.67, *p*=0.51 | | **t=-2.86, *p*=0.005** | | **t=-3.25, *p*=0.002** | | t=-1.83, *p*=0.07 | |
| **Glutamate** | 4.56±0.54 | 4.90±0.51 | 7.08±0.47 | 4.73±0.36 | 4.05±0.45 | 5.91±0.48 | 5.32±0.45 | 5.01±0.49 | 5.56±0.43 | 6.69±0.44 |
| F=12.02, *p*<0.0001 | t=-0.55, *p*=0.58 | | **t=5.26, *p*<0.0001** | | **t=-3.36, *p*=0.001** | | t=0.57, *p*=0.57 | | **t=-2.20, *p* = 0.03** | |
| **Arginine** | 5.06±0.43 | 5.02±0.39 | 5.40±0.37 | 5.22±0.24 | 4.35±0.31 | 5.52±0.33 | 4.57±0.31 | 6.51±0.36 | 4.59±0.30 | 5.87±0.30 |
| F=4.02, *p*=0.005 | t=0.06, *p*=0.95 | | t=0.42, *p*=0.68 | | **t=-2.61, *p*=0.01** | | **t=-4.18, *p*=0.0001** | | **t=-3.02, *p*=0.003** | |
| **Alanine** | 2.19±0.19 | 2.30±0.17 | 2.75±0.27 | 2.67±0.18 | 2.49±0.17 | 2.68±0.21 | 2.51±0.17 | 2.74±0.21 | 2.75±0.20 | 3.12±0.27 |
| $\chi^2$=1.53, *p*=0.82 | z=0.54, *p*=0.59 | | z=-0.32, *p*=0.75 | | z=0.92, *p*=0.36 | | z=1.12, *p*=0.26 | | z=1.61, *p*=0.11 | |

**Table 5.2** (cont'd)

| Amino acid (µg/mg) | Development stage and temperature treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Tight bud** | | **Bud swell** | | **Bud break** | | **Early/Late pink bud** | | **Anthesis** | |
| | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** |
| **Serine** | 0.90±0.14 | 1.06±0.18 | 1.11±0.17 | 1.18±0.21 | 1.02±0.13 | 1.13±0.20 | 0.93±0.14 | 1.34±0.29 | 1.04±0.20 | 1.11±0.19 |
| $\chi^2$=10.60, $p$=0.03 | z=1.62, $p$=0.11 | | z=0.72, $p$=0.47 | | z=1.19, $p$=0.24 | | **z=4.27, $p$<0.0001** | | z=0.82, $p$=0.41 | |
| **Lysine** | 0.58±0.04 | 0.61±0.04 | 0.78±0.06 | 0.64±0.03 | 0.57±0.02 | 0.65±0.03 | 0.61±0.03 | 0.73±0.04 | 0.59±0.02 | 0.73±0.04 |
| $\chi^2$=22.31, $p$<0.001 | z=0.64, $p$=0.52 | | **z=-2.71, $p$=0.007** | | **z=2.61, $p$=0.009** | | **z=3.09, $p$=0.002** | | **z=4.16, $p$<0.0001** | |
| **Valine** | 0.43±0.05 | 0.45±0.05 | 0.64±0.08 | 0.59±0.07 | 0.56±0.05 | 0.59±0.07 | 0.56±0.06 | 0.83±0.12 | 0.51±0.06 | 0.64±0.08 |
| $\chi^2$=12.79, $p$=0.01 | z=0.54, $p$=0.59 | | z=-0.81, $p$=0.42 | | z=0.58, $p$=0.56 | | **z=3.87, $p$=0.0001** | | **z=2.76, $p$=0.006** | |
| **Threonine** | 0.44±0.03 | 0.49±0.03 | 0.59±0.05 | 0.55±0.03 | 0.52±0.03 | 0.58±0.04 | 0.53±0.03 | 0.67±0.05 | 0.50±0.02 | 0.57±0.03 |
| $\chi^2$=9.16, $p$=0.06 | z=1.31, $p$=0.19 | | z=-0.83, $p$=0.41 | | z=1.82, $p$=0.07 | | **z=3.40, $p$<0.001** | | **z=2.36, $p$=0.02** | |
| **Histidine** | 0.33±0.08 | 0.36±0.11 | 0.24±0.04 | 0.27±0.04 | 0.31±0.06 | 0.23±0.03 | 0.23±0.04 | 0.32±0.06 | 0.21±0.03 | 0.27±0.04 |
| $\chi^2$=8.51, $p$=0.07 | z=-0.23, $p$=0.82 | | z=0.54, $p$=0.59 | | z=-1.34, $p$=0.18 | | z=1.72, $p$=0.09 | | z=1.56, $p$=0.12 | |
| **Tyrosine** | 0.24±0.04 | 0.34±0.06 | 0.25±0.03 | 0.31±0.04 | 0.27±0.02 | 0.35±0.05 | 0.24±0.02 | 0.50±0.09 | 0.19±0.01 | 0.30±0.09 |
| $\chi^2$=16.32, $p$=0.003 | z=1.55, $p$=0.12 | | z=1.43, $p$=0.15 | | **z=2.26, $p$=0.02** | | **z=5.44, $p$<0.0001** | | **z=5.30, $p$<0.0001** | |
| **Isoleucine** | 0.19±0.02 | 0.21±0.02 | 0.27±0.03 | 0.23±0.02 | 0.21±0.01 | 0.28±0.03 | 0.22±0.02 | 0.25±0.04 | 0.22±0.02 | 0.27±0.02 |
| $\chi^2$=17.85, $p$=0.001 | z=1.19, $p$=0.23 | | z=-1.68, $p$=0.09 | | **z=3.19, $p$=0.001** | | **z=4.03, $p$=0.0001** | | **z=2.17, $p$=0.03** | |
| **Phenylalanine** | 0.24±0.02 | 0.26±0.02 | 0.29±0.02 | 0.26±0.02 | 0.24±0.02 | 0.30±0.02 | 0.23±0.02 | 0.31±0.02 | 0.21±0.02 | 0.27±0.02 |
| F=4.91, $p$=0.001 | t=-1.02, $p$=0.31 | | t=1.35, $p$=0.18 | | **t=-3.18, $p$=0.002** | | **t=-4.14, $p$=0.0001** | | **t=-3.26, $p$=0.002** | |

**Table 5.2** (cont'd)

| Amino acid (µg/mg) | Development stage and temperature treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Tight bud** | | **Bud swell** | | **Bud break** | | **Early/Late pink bud** | | **Anthesis** | |
| | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** | **CT** | **HT** |
| **Glutamine** | 0.10±0.01 | 0.08±0.01 | 0.12±0.02 | 0.16±0.03 | 0.11±0.01 | 0.11±0.02 | 0.11±0.01 | 0.20±0.05 | 0.09±0.01 | 0.07±0.01 |
| $\chi^2$=34.93, $p$<0.0001 | z=-1.02, $p$=0.31 | | z=1.74, $p$=0.08 | | z=0.51, $p$=0.61 | | **z=3.58, $p$=0.0003** | | **z=-3.49, $p$=0.0005** | |
| **Leucine** | 0.13±0.01 | 0.15±0.02 | 0.18±0.02 | 0.15±0.01 | 0.14±0.01 | 0.19±0.02 | 0.14±0.01 | 0.25±0.03 | 0.14±0.01 | 0.16±0.01 |
| $\chi^2$=21.98, $p$<0.001 | z=1.29, $p$=0.20 | | z=-1.51, $p$=0.13 | | **z=2.83, $p$=0.005** | | **z=4.85, $p$<0.0001** | | z=1.69, $p$=0.09 | |
| **Methionine** | 0.050 ± 0.005 | 0.051 ± 0.005 | 0.094 ± 0.020 | 0.063 ± 0.006 | 0.041 ± 0.003 | 0.046 ± 0.004 | 0.053 ± 0.005 | 0.046 ± 0.004 | 0.064 ± 0.007 | 0.065 ± 0.007 |
| $\chi^2$=9.23, $p$=0.06 | z=0.11, $p$=0.91 | | **z=-2.91, $p$=0.004** | | z=1.48, $p$=0.14 | | **z=-1.96, $p$=0.05** | | z=0.16, $p$=0.87 | |
| **Tryptophan** | 0.042 ± 0.006 | 0.048 ± 0.007 | 0.052 ± 0.009 | 0.046 ± 0.006 | 0.043 ± 0.005 | 0.057 ± 0.009 | 0.040 ± 0.004 | 0.090 ± 0.022 | 0.042 ± 0.004 | 0.055 ± 0.009 |
| $\chi^2$=29.06, $p$<0.0001 | z=0.86, $p$=0.39 | | z=-1.06, $p$=0.29 | | **z=2.61, $p$=0.009** | | **z=6.15, $p$<0.0001** | | **z=2.75, $p$=0.006** | |
| **Cysteine** | 0.038 ± 0.003 | 0.044 ± 0.003 | 0.052 ± 0.005 | 0.054 ± 0.003 | 0.050 ± 0.003 | 0.048 ± 0.003 | 0.052 ± 0.003 | 0.058 ± 0.004 | 0.047 ± 0.002 | 0.062 ± 0.004 |
| $\chi^2$=12.82, $p$=0.01 | z=1.73, $p$=0.08 | | z=0.43, $p$=0.66 | | z=-0.56, $p$=0.57 | | z=1.40, $p$=0.16 | | **z=3.76, $p$=0.0002** | |

# CHAPTER 6. HEAT-BEE-PLANT INTERACTIONS: LESSONS LEARNED AND FUTURE DIRECTIONS

In this research, I aimed to uncover the direct and indirect effects of extreme heat on blueberry pollination systems by investigating how realistic short bursts of extreme heat directly affect blueberry pollen performance (Chapter 2), blueberry fruit production and fruit quality (Chapter 3), and how the native solitary bee *Osmia lignaria* and subsequent offspring respond to host plant exposure to heat stress (Chapter 4). To understand the mechanisms affecting blueberry pollen, pollination, , and bee health, I assessed the nutritional content of blueberry pollen exposed to extreme heat (Chapter 5). The results provided in this dissertation add to our understanding of bee-plant-climate interactions and provide new insights into how extreme heat has detrimental repercussions for crop production and bee health, development, and survival. My research also suggests that studies evaluating the direct effects of extreme heat on flowering plants or bees underestimate the consequences of heat stress on pollination systems. Given the increasing incidence of seasonal climactic extremes globally, there is an urgent need to continue interdisciplinary research that blends entomology, plant science, horticulture, and climatology to better understand these interactions and to develop solutions.

In Chapter 2, I found that blueberry pollen performance (i.e., pollen germination and tube growth) was optimal between 20 and 30°C, similar to temperatures typically experienced during bloom. These findings deviate from reports in rabbiteye blueberry, where Yang Q et al. (2019) determined 21.4 and 18.4°C as the ideal temperatures for pollen germination and tube growth, respectively. They also reported that at 30°C, pollen tubes grew quickly but mostly stopped growing in the middle of the style (Yang Q et al. 2019). It is possible that the *in situ* methods described by Yang Q et al. (2019) contributed to greater heat sensitivity than the *in vitro* approaches I used in Chapter 2. As the authors suggest, higher temperatures can inhibit stigmatic mucus secretion, which may contribute to poor stigmatic receptivity or a decrease in pistil nutrient supply, reducing pollen germination and inhibiting tube growth (Herrero and Hormaza, 1996; Hedhly et al., 2003, 2005; Mesihovic et al., 2016). Thus, these altered pollen-stigma interactions observed in rabbiteye could explain some of the differences in our findings. Future studies should consider employing *in situ* methods for northern highbush blueberry and explore pollination when the pollen, stigma, or both experience extreme heat. Additionally, evaluating pollen germination and tube growth across various blueberry floral development stages using simple, reliable, and

easily replicable *in vitro* methods (Snider and Oosterhuis, 2011; Mesihovic et al., 2016) would provide important additional information on the consequences of heat stress at various stages of crop development during the spring.

The heat recovery assessment performed in Chapter 2 explored the capacity for blueberry pollen to rebound after just four hours of extreme heat exposure, yet little recovery was observed. The four-hour heat exposure was selected to reflect the duration of time Michigan blueberry growing regions experienced temperatures above 35°C during the day in 2018, resulting in a 30-50% yield loss for Michigan blueberry producers. However, additional heat stress durations should be explored for blueberry pollen using *in vitro* approaches. For instance, evaluating whether pollen is able to rebound after one, two, or three hour(s) of heat exposure would be important information for blueberry growers managing their fields during extreme heat events. Additionally, exposing blueberry pollen *in vitro* to a heat wave regime where temperatures ramp up in the morning, reach a maximum, then ramp down in the evening over the span of several days would better reflect blueberry pollen performance under field realistic conditions. Overall, the *in vitro* methods used in Chapter 2 proved to be a reliable and reproducible approach for assessing northern highbush blueberry pollen thermotolerance and should be utilized in future studies.

The results obtained in Chapter 2 were used to determine an upper threshold temperature for northern highbush pollen (37.5°C) and showed that just four hours of extreme heat is enough to disrupt performance. This brief extreme heat regime was used for all subsequent studies in this dissertation. In Chapter 3, 'Bluecrop' bushes at various floral bud development stages, from tight bud to late pink bud, were exposed to this heat stress regime then hand pollinated with pollen exposed to the same conditions, once flowers reached anthesis. Fruit set was significantly reduced following heat stress at bud swell and trends also showed reductions at bud break. Such reductions in fruit set supports the hypothesis that pollen germination and tube growth were disrupted due to damage from heat stress, resulting in inhibited fertilization. However, there is a clear difference in pollen performance reductions following heat stress in Chapters 2 and 3, suggesting that *in vitro* pollen performance assessments were not necessarily predictive of blueberry reproductive potential. It seemed that blueberry plants exposed to heat stress during pre-bloom development stages were more resilient than mature blueberry pollen exposed to heat stress on a nutrient medium. However, pollen collected at the anthesis stage was used exclusively for Chapter 2 assessments while no flowers or pollen were exposed to heat stress at the anthesis stage for Chapter

3 fruit set assessments due to shortages in healthy bushes, making comparisons between Chapter 2 and 3 results challenging. Future studies should fill this knowledge gap by assessing *in vitro* pollen performance following heat stress at pre-bloom development stages as well as assessing fruit set and quality measurements for plants exposed to heat stress at anthesis. Additionally, I recommend that entomologists working in this field should collaborate with blueberry horticulturalists who are experts at growing and maintaining healthy blueberry bushes in greenhouse and field conditions. This can avoid wasted years of research due to poorly growing plants, especially given the very unique soil conditions favored by highbush blueberry.

Compared to similar studies in sweet cherry (Hedhly et al., 2003), it is possible that heat stress at anthesis could have inhibited pollen performance and reduced stigmatic receptivity, further impeding support for pollen penetration, germination, and adhesion. It is unclear in the literature how developing female floral organs respond to heat stress during floral development, yet female gametophytes are generally considered less sensitive to heat stress than male gametophytes (Lohani et al., 2020). It is possible that four hours of heat stress was enough to inhibit pollen performance at several pre-bloom stages, yet the stigma, style and ovaries of flowers may have been less affected by heat stress and thus were able to contribute energy (i.e., carbohydrates, ATP) necessary to help fuel pollen germination and tube growth for improved fertilization (Snider and Oosterhuis, 2022; Lohani et al., 2020). Future work should assess the receptivity of northern highbush blueberry stigmas following heat stress at various floral development stages. To minimize time and labor constraints, hand pollination of blueberry stigmas was performed by visually applying an even, thin coating of pollen rather than counting out a standardized number of pollen grains per stigma. It is possible that, despite some blueberry pollen grains being inhibited by heat, enough were able to germinate, grow, and fertilize floral ovules at several floral bud stages, mitigating losses in fruit set and fruit quality. Future studies could standardize the number of pollen grains applied to floral stigmas when comparing fruit set and fruit quality under heat stress conditions to reduce variability across treatments.

As described in Chapter 3, the patterns of heat stress affecting strawberry floral bud development (Ledesma and Kawabata, 2016) are similar to those observed in northern highbush blueberry. However, no studies have confirmed which blueberry floral bud stage is associated with a given pollen development stage. The only study providing clues that bud swell occurs at MMC meiosis was done by Gough et al. (1978), and needs updating given that no morphological bud

stage (i.e., tight bud, bud swell, etc.) was associated with a pollen development stage (i.e., MMC meiosis). It will be important for future research to fill this knowledge gap, with relevant techniques such as scanning electron microscopy (SEM) (Gough et al. 1978, Fadón et al. 2018) to confirm the pollen development stage associated with each morphological floral bud stage. This will be crucial for providing targeted advice to blueberry growers related to the timing of applying heat mitigation measures in fields.

Despite fruit set reductions at certain development stages following heat stress, fruit ripening duration and fruit quality measurements seemed relatively unaffected by extreme heat conditions across all development stages. This reflects results observed in strawberry (Ledesma and Kawabata, 2016) and apricot (Rodrigo and Herrero, 2002), where fruit set was reduced following heat stress, but fruit quality was either unaffected or even improved following heat stress. Taken together, this suggests that fertilization is the greatest limitation for developing blueberry flowers exposed to heat stress. This is important for blueberry crop producers and extension educators, putting a greater emphasis on ensuring ample pollination services and keeping fields below the upper threshold temperature during floral development and bloom to promote fertilization, with the expectation that berries that are fertilized will still reach marketable size and quality. Heat stress mitigation measures, such as overhead sprinkler irrigation show promise for maintaining crop fields below threshold temperatures (Walters, Van Timmeren, and Isaacs, unpublished), with ongoing research to investigate the potential negative effects of extended overhead irrigation use on pollination and disease prevalence.

During this Ph.D. program, I also attempted to perform a study to expose various cultivars (‘Aurora’, ‘Bluecrop’, ‘Draper’, ‘Elliott’, ‘Legacy’, and ‘Nelson’) at anthesis to heat stress or typical ambient (control) conditions and evaluate fruit set and fruit quality. However, this study was omitted from this dissertation for various reasons. First, the number of bushes was extremely limited due to poor bush health in response to diseases (particularly Phomopsis), pest outbreaks (scales, thrips, aphids), insufficient nutrient uptake from pot-bound roots, and an early spring heat event causing greenhouse temperatures to exceed threshold temperatures (> 35°C), rendering over 100 blueberry bushes unusable for experiments. Additionally, bushes had to be moved several times across various greenhouse rooms due to various unexpected circumstances following hand pollination treatments, which may have additionally stressed plants and/or disrupted floral fertilization processes. Of the bushes deemed healthy enough for experimentation, fruit set was

uncharacteristically low regardless of experimental temperature treatment, resulting in very few berries to measure. Specifically, the total number of berries ranged from 2-19 across all cultivars for the control treatment (11-64% fruit set) while the number of berries ranged from 0-17 across all cultivars for the heat treatment (0-55% fruit set). With large variability within treatments, this sample size was insufficient to test the main hypothesis. A similar experiment should consider a comparison of heat tolerance across blueberry cultivars. Regardless, the results presented in Chapter 3 align with observations in Chapter 2 that extreme heat inhibits pollen germination and tube growth, providing additional key evidence explaining the 30-50% yield reductions reported in Michigan blueberry growing regions in 2018 following spring heat wave conditions.

The results in Chapters 2 and 3 show that northern highbush blueberry pollen is negatively affected by extreme heat exposure, with important implications for pollen functioning and crop production. However, the implications for bees was unknown. The research presented in Chapter 4 demonstrated that extreme heat exposure of plants can significantly affect bees through reduced egg laying, and inhibited brood development and survival. While pollen nutrition likely played a key role in inhibiting female fecundity (discussed more below), it is also possible that heat stress limited the quantity of floral rewards (i.e., pollen and nectar) available on a per-flower basis. Specifically, the amount of pollen produced and released per flower may have been reduced following heat stress compared to control bushes, as observed in other plant types (Ozga et al., 2017; Lohani et al., 2020; Descamps et al., 2021). Future studies should consider taking a subset of flowers exposed to heat (and non-heat) conditions and quantifying the average pollen produced per flower to determine any restraints of floral reward access. Nonetheless, a reduction in pollen quantity and/or quality can inhibit *Osmia* oocyte development (Cane, 2016), so given the stark reduction in *O. lignaria* egg laying observed in Chapter 5, this suggests extreme heat exposure to host plants disrupted pollen quantity or quality. Future studies should consider dissecting female bees provided heat stressed host plants to determine effects evident in the number or size of oocytes, potentially contributing to reduced fecundity. Using field trapped *Osmia* eggs and feeding them pollen collected from heat exposed and control pollen would be one approach to increase the chance of female bees developing through to the adult stage.

The amount of nectar produced and the quality of nectar (i.e., sugar concentration) was not assessed in this dissertation, but may have played an interactive role limiting the quantity and/or quality of nutrition available to *O. lignaria* bees. For instance, Hemberger et al. (2023) found a

70% reduction in nectar production in heat stressed host plants (*Brassica napus*, oilseed rape), yet the number of bumble bee visits to flowers was the same regardless of temperature treatment. Similarly, female bee *O. lignaria* activity did not differ between host plant temperature treatments in Chapter 4, but reductions in nectar quantity or quality could have negative effects on female nutrition, potentially inhibiting brood production. Reductions in nectar availability could have also negatively affected the quality of pollen provisions provided to *O. lignaria* brood. In cages provided with heat stressed host plants, pollen provisions may have been too dry due to insufficient nectar added, potentially limiting digestion processes in *Osmia* larvae (Nepi et al., 2005). Additionally, insufficient nectar contributions in larval provisions may have further inhibited carbohydrate concentrations necessary for *Osmia* larval development and survival (Austin and Gilbert, 2021). Future studies should consider quantifying the amount of nectar produced, as well as the concentration of sugar produced, on a per-flower basis following heat stress exposure during floral development in blueberry.

*Osmia lignaria* females were also provided three different host plants to forage on (blueberry, phacelia, clover), given these bees are polylectic, requiring a multifloral diet for development and survival. While blueberry was the primary floral source provided to bees in this study, phacelia flowers were also frequently visited by females and contributed greatly to pollen provisions. Clover flowers were also visited, but far less than blueberry and phacelia flowers. The composition of pollen provisions provided to larvae, or the relative abundance of the three different plant types within a provision, should be assessed in future studies as these different host plants likely vary in their sensitivity to heat stress, potentially exacerbating or buffering the nutritional stress in female *Osmia* bees and her brood. Future studies could assess the nutritional and defense chemical profiles of each host plant pollen following heat stress in addition to the assessment of blueberry pollen described in Chapter 5, as well as assessing entire pollen provisions provided by mother bees. Understanding the differences in pollen nutrition across host plant type and temperature treatment would provide important insights into which plant type is most sensitive to heat stress. This could inform decisions on planting heat-tolerant plants along crop margins, potentially buffering the nutritional stress of bees when extreme heat events occur to maintain healthy bee populations in future years.

Many bee species can detect differences in nectar quality during foraging bouts (Carter et al., 2006; Howell and Alarcón, 2007; Nicolson and Thornburg, 2007; Bertazzini et al., 2010;

Nicolson, 2011; Darvishzadeh et al., 2015; Ruedenauer et al., 2015; Tafi et al., 2023), but it is unclear whether solitary bees such as *Osmia* are able to detect differences in pollen nutritional quality following heat stress conditions. If there are differences among plant types in pollen and nectar nutritional quality following heat stress, then the ability of female bees to discriminate and make choices among floral resources would be extremely beneficial for ensuring a well-balanced diet necessary for development and survival, both for the mother bee and her brood. Alternatively, if unable to detect differences in pollen nutritional quality, then female bees may be investing time and energy into foraging on inadequate host plants with negative repercussions on her fecundity and brood survival. Future studies should conduct choice studies with *O. lignaria* female bees to evaluate their ability to make foraging decisions, such as avoiding heat stressed plants or visiting certain host plants more than others that may be more tolerant to heat stress conditions. Evaluating *O. lignaria* capacity to detect such differences among host plants and temperature treatments would provide important evidence on whether female bees are able to balance floral diets during bouts of extreme heat, with important implications for female development and fecundity as well as larval development and survival. Additionally, studies could be done to assess differences in pollination efficiency of *O. lignaria* bees (i.e., number of pollen grains deposited per stigma) and respective seed/fruit set among heat stressed and non-stressed plants. If bees are unable to detect differences between host plant temperature treatments, resulting in similar visitation frequencies, assessing fruit production could provide important insights into the role of heat stress impeding fruit production despite sufficient pollination services provided by bees. Alternatively, if bees avoid heat stressed plants thereby limiting pollination services, compounding effects of extreme heat on seed/fruit production may be found through the effects directly on pollen and on the behavior of pollinators delivering insufficient pollination services.

All emerged progeny in the experiments described in Chapter 4 were male bees, despite the female, mother bees being mated prior to release in field cages. It is possible that the diets provided to the larvae were sufficient for male, but not female, development. Indeed, other *Osmia* studies male and female broods differed in their nutritional requirements (Filipiak et al., 2021). It is also possible that, given the limited floral resources provided in field cages, mother *Osmia* bees preferentially produced male progeny as this is the smaller sex and requires a smaller provision (Bosch and Vicens, 2005). The number of host plants provided in field cages was limited given that potted host plants needed to be exposed to temperature treatments in growth chambers prior

to placement in field cages, and the number of growth chambers and space within growth chambers was limited. Additionally, host plants needed to be potted for heat treatments rather than directly sown into the ground as done in other field cage *Osmia* studies that produced female brood (Stuligross and Williams, 2020; Knauer et al., 2022), limiting floral abundance within the already limited area of the field cages. Furthermore, female *Osmia* foraging observations on a per flower basis were conducted to validate female bees were accessing a similar number of flowers across temperature treatments. Thus, plants had to be far enough apart from one another to ensure accurate visitation counts by a human observer. While observation techniques have been developed to measure floral visitation preference in bumble bees (Hemberger et al., 2023) and nesting behaviors in *Osmia* bees (Stuligross and Williams, 2020; Knauer et al., 2022), no affordable or reliable technology is available, to my knowledge, to assess *Osmia* foraging visitation across different plant types and at a floral density that supports ample brood development. Thus, I conducted visual, in-person 30-minute foraging observations for each field cage daily, limiting the number of replicate cages due to time and labor constraints. While sufficient brood was produced to make measurements in Chapter 4, future studies should nonetheless consider increasing the number and/or size of cages with additional personnel, providing a greater abundance of floral resources to increase total brood production and potentially promote female brood production. In-field artificial heating is another approach that could be developed with sufficient resources, but few in-field heat chambers are currently capable of reliable extreme heat exposure. Alternatively, laboratory assessments could be conducted with pollen collected from heat stressed or non-stressed host plants, providing a standardized provision to all brood. Laboratory *in vitro* bee rearing on an artificial diet would also remove any effects of the mother bee on larval survival, as eggs would be collected from trap nests with larvae provided the same floral resources and exposed to the same conditions, allowing direct assessment of how brood mortality is influenced by differences in pollen nutrition.

The study described in Chapter 4 was intended to assess the indirect effects of heat stress on solitary bees, however, when extreme heat events happen in nature, both the plant and bee endure heat stress conditions. Future studies should consider employing similar methods to Hemberger et al. (2023), who performed a fully crossed factorial experiment exposing (or not exposing) bees and host plants to heat stress conditions to evaluate the direct and indirect effects of heat exposure on bumble bees. Such a study would provide insights into how field realistic

extreme heat conditions affect solitary bees exposed to direct and indirect heat stress, which to my knowledge is missing entirely from the literature. Additionally, such a study would provide an interesting comparison with a social bee species (i.e., bumble bees) conducted by Hemberger et al. (2023), potentially shedding light on the differences in heat tolerance or resiliency across levels of bee sociality. The literature is also entirely lacking on how the second generation, or surviving brood provided pollen provisions from heat stressed host plants, would perform the following spring in terms of mating success, fecundity, foraging capacity, and adult longevity. Such a study would build our understanding of how bee populations perform in subsequent years following extreme heat events. With so many unknowns and potential studies to explore, it is clear that evaluating the direct, indirect, and interactive effects of extreme heat on bee behavior, development, and survival is an emerging field that deserves greater attention. This study conducted in Chapter 4 was the first to evaluate how indirect heat exposure affects the native, solitary bee *Osmia lignaria*, and suggests greater exploration across a wider diversity of bee taxa is needed.

After investigating the effects of extreme heat on blueberries and mason bees, I was interested in the underlying mechanisms driving these responses. Collaboration with plant biochemists who have studied pollen nutrition in other crops enabled the evaluation of how extreme heat affects pollen carbohydrates, protein, and amino acid concentrations. The pollen assays reported in Chapter 5 provide additional evidence that blueberry floral bud development stages vary in their sensitivity to extreme heat, with bud swell being significantly more affected by extreme heat than other development stages. Reductions in pollen protein, starch, and several amino acids following extreme heat exposure suggest disruption of MMC meiosis during bud swell. This is a first examination of this topic and additional studies are required to confirm pollen development stages associated with each morphological floral bud stage, as discussed above.

The greatest limitation for Chapter 5 was tied to the amount of pollen material required for metabolite assays and, in turn, the amount of floral resources and labor required to collect sufficient pollen quantities. The metabolite protocols developed in collaboration with Dr. James Santiago for this study were designed to conduct highly sensitive metabolite assays with reduced pollen quantities to improve replication for statistical analysis. Using this method was crucial, given that this approach still required yearlong, daily pollen collection across 434 different blueberry bushes, 6437 blueberry flowers, and several personnel. Despite this, the amount of pollen collected at the

tight bud developmental stage was still limited, reducing the number of replicate samples for this development stage to four while all other development stages received six replicate samples per temperature treatment. Future studies should take care to follow methods similar to those described in Chapter 5 to minimize the pollen material required for metabolite assays, ensuring highly accurate measurements with limited pollen samples. This also emphasizes the importance of interdisciplinary collaboration in pollination ecology research, given the expertise and resources provided from collaborators and their respective labs.

The reduction in pollen starch content detected for the tight bud and bud swell development stages following heat stress has potential for interactive, indirect heat stress effects on bees, potentially contributing to inhibited *Osmia* female fecundity and brood survival observed in Chapter 4. Blueberry floral buds exposed to heat stress not only inhibited pollen starch content, limiting a key nutrient for *Osmia* bees (Roulston and Cane, 2000; Nepi et al., 2005), but it may also have impeded pollen digestive processes in bees by inhibiting pollen germination (Nepi et al., 2005). Thus, even if pollen nutrient content was sufficient, heat stress inhibiting pollen germination may limit bees' access to pollen nutrients during digestion, contributing to nutrient deficiencies. Future studies should follow similar methods described by Nepi et al. (2005), assessing the nutrient content of heat stressed pollen from anthers, pollen provisions, in the bee gut (at various stages during bee digestion), and post-digestion (i.e., frass). Future studies should evaluate this for both adult and larval bees, as bee life stage may differ in their sensitivity to nutrient stress following host plant heat exposure.

The unexpected result showing extreme heat exposure at bud break, early/late pink bud, and anthesis increasing certain essential amino acids is the first recorded for blueberry and has potentially conflicting implications for blueberry pollination systems. Certain increases in individual amino acids following heat stress could be a sign of improved thermotolerance at these development stages, given the important role amino acids play in pollen development and performance and the statistically insignificant differences in fruit set following heat stress at bud break, early/late pink bud, and anthesis. However, such increases in these individual amino acids following heat stress could have resulted in unbalanced diets, causing deleterious effects for bee development and survival as observed in other bees and *Drosophila* (Grandison et al., 2009; Paoli et al., 2014; Stabler et al., 2015; Archer et al., 2021; Austad et al., 2024). These unbalanced pollen diets in response to heat stress may have contributed to the reduced *O. lignaria* female fecundity

and brood survival observed in Chapter 4. While many climate change studies discuss phenological mismatch between bees and flowering plants (reviewed by Walters et al., 2022), the results from this dissertation suggest an additional mechanism of heat affecting pollinators through nutritional mismatch following heat stress between bees and plants. However, the role of different individual amino acids driving (or impeding) certain developmental or physiological processes in bees, especially solitary bees, is unclear. Future studies should consider following the approach described by Grandison et al. (2009) in *Drosophila*, where each essential amino acid was added into diets in a stepwise manner to evaluate their respective role in bee development and functioning.

This research used various approaches to investigate the direct and indirect effects of brief extreme heat exposure on the blueberry pollination system, revealing negative effects for pollen, plants, and bees. Extreme heat can have compounding, additive consequences negatively affecting both plants and bees, but this is not always the case. Blueberry plants may have evolved certain strategies to handle extreme heat stress by upregulating the synthesis of certain amino acids, potentially mitigating damage to pollen performance and fruit production at certain development stages. However, this upregulation of pollen amino acid content following heat stress may have worsening, negative repercussions for bees consuming this pollen. It is clear that there remain many unknown aspects of direct and indirect effects of extreme heat on pollination systems. It will be imperative that future research shifts from direct, isolated studies towards indirect, interactive studies with interdisciplinary approaches to uncover the complexities of climate change on entire pollination systems.

## REFERENCES


Abdellatif, I. M. Y., S. Yuan, R. Na, S. Yoshihara, H. Hamada, T. Suzaki, H. Ezura, and K. Miura. 2022. Functional characterization of tomato phytochrome a and b1b2 mutants in response to heat stress. *International Journal of Molecular Sciences* 23: 1681.

Acar, I., and V. G. Kakani. 2010. The effects of temperature on in vitro pollen germination and pollen tube growth of *Pistacia* spp. *Scientia Horticulturae* 125: 569–572.

Ahlmann-Eltze, C., and I. Patil. 2021. ggsignif: R Package for Displaying Significance Brackets for 'ggplot2'. doi.org/10.31234/osf.io/7awm6

Alsamir, M., T. Mahmood, R. Trethowan, and N. Ahmad. 2021. An overview of heat stress in tomato (*Solanum lycopersicum* L.). *Saudi Journal of Biological Sciences* 28: 1654–1663.

Amuji, C. F., L. J. Beaumont, and B. J. Atwell. 2020. The effect of co-occurring heat and water stress on reproductive traits and yield of tomato (*Solanum lycopersicum*). *The Horticulture Journal* 89: 530–536.

Anderson, J. V., D. P. Horvath, W. S. Chao, and M. E. Foley. 2010. Bud dormancy in perennial plants: a mechanism for survival. *In* E. Lubzens, J. Cerda, and M. Clark [eds.], *Dormancy and Resistance in Harsh Environments*, 69–90. Springer, Berlin, Heidelberg.

Anderson, N. L., and A. N. Harmon-Threatt. 2019. Chronic contact with realistic soil concentrations of imidacloprid affects the mass, immature development speed, and adult longevity of solitary bees. *Scientific Reports* 9: 3724.

Archer, C. R., J. Fähnle, M. Pretzner, C. Üstüner, N. Weber, A. Sutter, V. Doublet, and L. Wilfert. 2021. Complex relationship between amino acids, fitness and food intake in *Bombus terrestris*. *Amino Acids* 53: 1545–1558.

Artz, D. R., and T. L. Pitts-Singer. 2015. Effects of fungicide and adjuvant sprays on nesting behavior in two managed solitary bees, *Osmia lignaria* and *Megachile rotundata*. *PLOS ONE* 10: e0135688.

Austad, S. N., J. R. Smith, and J. M. Hoffman. 2024. Amino acid restriction, aging, and longevity: an update. *Frontiers in Aging* 5.

Austin, A. J., and J. D. J. Gilbert. 2021. Solitary bee larvae prioritize carbohydrate over protein in parentally provided pollen. *Functional Ecology* 35: 1069–1080.

Austin, P. T., E. W. Hewett, D. Noiton, and J. A. Plummer. 1998. Self incompatibility and temperature affect pollen tube growth in 'Sundrop' apricot (*Prunus armeniaca* L.). *Journal of Horticultural Science and Biotechnology* 73: 375–386.

Barraud, A., L. Barascou, V. Lefebvre, D. Sene, Y. Le Conte, C. Alaux, F.-V. Grillenzoni, et al. 2022. Variations in nutritional requirements across bee species. *Frontiers in Sustainable Food Systems* 6.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67: 1–48.

Bertazzini, M., P. Medrzycki, L. Bortolotti, L. Maistrello, and G. Forlani. 2010. Amino acid content and nectar choice by forager honeybees (*Apis mellifera* L.). *Amino Acids* 39: 315–318.

Biancucci, M., R. Mattioli, G. Forlani, D. Funck, P. Costantino, and M. Trovato. 2015. Role of proline and GABA in sexual reproduction of angiosperms. *Frontiers in Plant Science* 6.

Birkhold, K. T., K. E. Koch, and R. L. Darnell. 1992. Carbon and nitrogen economy of developing rabbiteye blueberry fruit. *Journal of the American Society for Horticultural Science* 117: 139–145.

Bolker, B., R. D. C. Team, and I. Giné-Vázquez. 2023. bbmle: Tools for General Maximum Likelihood Estimation. https://cran.r-project.org/web/packages/bbmle/index.html

Bordier, C., S. Suchail, M. Pioz, J. M. Devaud, C. Collet, M. Charreton, Y. Le Conte, and C. Alaux. 2017. Stress response in honeybees is associated with changes in task-related physiology and energetic metabolism. *Journal of Insect Physiology* 98: 47–54.

Borghi, M., and A. R. Fernie. 2017. Floral metabolism of sugars and amino acids: Implications for pollinators' preferences and seed and fruit set. *Plant Physiology* 175: 1510–1524.

Borghi, M., L. Perez de Souza, T. Yoshida, and A. R. Fernie. 2019. Flowers and climate change: a metabolic perspective. *New Phytologist* 224: 1425–1441.

Bosch, J. 2008. Production of undersized offspring in a solitary bee. *Animal Behaviour* 75: 809–816.

Bosch, J., and M. Blas. 1994. Effect of over-wintering and incubation temperatures on adult emergence in *Osmia cornuta* Latr (Hymenoptera, Megachilidae). *Apidologie* 25: 265–277.

Bosch, J., and W. Kemp. 2002. How to manage the blue orchard bee as an orchard pollinator. *Sustainable Agriculture Network (SAN)* handbook series; bk. 5. ISBN 1-888626-06-2.

Bosch, J., and W. P. Kemp. 2000. Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology* 29: 8–13.

Bosch, J., and W. P. Kemp. 2004. Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Apidologie* 35: 469–479.

Bosch, J., and W. P. Kemp. 2003. Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology* 32: 711–716.

Bosch, J., F. Sgolastra, and W. P. Kemp. 2010. Timing of eclosion affects diapause development, fat body consumption and longevity in *Osmia lignaria*, a univoltine, adult-wintering solitary bee. *Journal of Insect Physiology* 56: 1949–1957.

Bosch, J., and N. Vicens. 2006. Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behavioral Ecology and Sociobiology* 60: 26–33.

Bosch, J., and N. Vicens. 2005. Sex allocation in the solitary bee *Osmia cornuta*: do females behave in agreement with Fisher's theory? *Behavioral Ecology and Sociobiology* 59: 124–132.

Boyle, N. K., D. R. Artz, O. Lundin, K. Ward, D. Picklum, G. I. Wardell, N. M. Williams, and T. L. Pitts-Singer. 2020. Wildflower plantings promote blue orchard bee, *Osmia lignaria* (Hymenoptera: Megachilidae), reproduction in California almond orchards. *Ecology and Evolution* 10: 3189–3199.

Brás, T. A., J. Seixas, N. Carvalhais, and J. Jägermeyr. 2021. Severity of drought and heatwave crop losses tripled over the last five decades in Europe. *Environmental Research Letters* 16: 065012.

Brewer, J. W., and R. C. Dobson. 1969. Seed count and berry size in relation to pollinator level and harvest date for the highbush blueberry, *Vaccinium corymbosum*. *Journal of Economic Entomology* 62: 1353–1356.

Buckley, L. B. 2022. Temperature-sensitive development shapes insect phenological responses to climate change. *Current Opinion in Insect Science* 52: 100897.

Bukovinszky, T., I. Rikken, S. Evers, F. L. Wäckers, J. C. Biesmeijer, H. H. T. Prins, and D. Kleijn. 2017. Effects of pollen species composition on the foraging behaviour and offspring performance of the mason bee *Osmia bicornis* (L.). *Basic and Applied Ecology* 18: 21–30.

Callens, C., J. Fernandez-Goméz, M. R. Tucker, D. Zhang, and Z. A. Wilson. 2023. Heat stress responses vary during floret development in European spring barley cultivars. *Frontiers in Plant Science* 13.

Campion, C., A. Rajamohan, and M. E. Dillon. 2023. Sperm can't take the heat: Short-term temperature exposures compromise fertility of male bumble bees (*Bombus impatiens*). *Journal of Insect Physiology* 146: 104491.

Campoy, J. A., D. Ruiz, and J. Egea. 2011. Dormancy in temperate fruit trees in a global warming context: A review. *Scientia Horticulturae* 130: 357–372.

Cane, J. H. 2016. Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. *Journal of Insect Physiology* 95: 105–109.

Cane, J. H. 1987. Estimation of bee size using intertegular span (Apoidea). *Journal of the Kansas Entomological Society* 60: 145–147.

Cano-Medrano, R., and R. L. Darnell. 1997a. Cell number and cell size in parthenocarpic vs . pollinated blueberry (*Vaccinium ashei*) fruits. *Annals of Botany* 80: 419–425.

Cano-Medrano, R., and R. L. Darnell. 1997b. Sucrose metabolism and fruit growth in parthenocarpic vs seeded blueberry (*Vaccinium ashei*) fruits. *Physiologia Plantarum* 99: 439–446.

CaraDonna, P. J., J. L. Cunningham, and A. M. Iler. 2018. Experimental warming in the field delays phenology and reduces body mass, fat content and survival: Implications for the persistence of a pollinator under climate change. *Functional Ecology* 32: 2345–2356.

Carrizo García, C., M. Nepi, and E. Pacini. 2017. It is a matter of timing: asynchrony during pollen development and its consequences on pollen performance in angiosperms—a review. *Protoplasma* 254: 57–73.

Carter, C., S. Shafir, L. Yehonatan, R. G. Palmer, and R. Thornburg. 2006. A novel role for proline in plant floral nectars. *Naturwissenschaften* 93: 72–79.

Cerovlć, R., and D. Ružić. 1992. Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. *Journal of Horticultural Science* 67: 333–340.

Chaturvedi, P., A. J. Wiese, A. Ghatak, L. Záveská Drábková, W. Weckwerth, and D. Honys. 2021. Heat stress response mechanisms in pollen development. *New Phytologist* 231: 571–585.

Chen, D., Q. Shao, L. Yin, A. Younis, and B. Zheng. 2019a. Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science* 9: 1945.

Chen, W., W. Cen, L. Chen, L. Di, Y. Li, and W. Guo. 2012. Differential sensitivity of four highbush blueberry (*Vaccinium corymbosum* L.) Cultivars to heat stress. *Pak. J. Bot* 44: 853–860.

Chen, X., L. Shi, Y. Chen, L. Zhu, D. Zhang, S. Xiao, A. Aharoni, et al. 2019b. Arabidopsis HSP70-16 is required for flower opening under normal or mild heat stress temperatures. *Plant, Cell & Environment* 42: 1190–1204.

Cheung, A. Y. 1996. Pollen—pistil interactions during pollen-tube growth. *Trends in Plant Science* 1: 45–51.

Chiang, H.-H., and A. M. Dandekar. 1995. Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh during development and in response to desiccation. *Plant, Cell & Environment* 18: 1280–1290.

Claus, G., M. Pisman, P. Spanoghe, G. Smagghe, and M. Eeraerts. 2021. Larval oral exposure to thiacloprid: Dose-response toxicity testing in solitary bees, *Osmia* spp. (Hymenoptera: Megachilidae). *Ecotoxicology and Environmental Safety* 215: 112143.

Conrad, T., C. Stöcker, and M. Ayasse. 2017. The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). *Ecology and Evolution*: 8966–8975.

Courcelles, D. M. M., L. Button, and E. Elle. 2013. Bee visit rates vary with floral morphology among highbush blueberry cultivars (*Vaccinium corymbosum* L.). *Journal of Applied Entomology* 137: 693–701.

Cren, M., and B. Hirel. 1999. Glutamine synthetase in higher plants regulation of gene and protein expression from the organ to the cell. *Plant and Cell Physiology* 40: 1187–1193.

Crone, M. K., D. J. Biddinger, and C. M. Grozinger. 2022. Wild bee nutritional ecology: integrative strategies to assess foraging preferences and nutritional requirements. *Frontiers in Sustainable Food Systems* 6.

Crone, M. K., N. K. Boyle, S. T. Bresnahan, D. J. Biddinger, R. T. Richardson, and C. M. Grozinger. 2023. More than mesolectic: Characterizing the nutritional niche of *Osmia cornifrons*. *Ecology and Evolution* 13: e10640.

Danforth, B. N., R. L. Minckley, and J. L. Neff. 2019. The solitary bees: biology, evolution, conservation. Princeton University Press.

Darnell, R. L. 2000. Blueberries. *In* A. Erez [ed.], Temperate Fruit Crops in Warm Climates, 429–444. Springer Netherlands, Dordrecht.

Darrow, G. 1958. Seed number in blueberry fruits. *Proceedings. American Society for Horticultural Science* 72: 212–215.

Darvishzadeh, A., V. Hosseininaveh, G. Nehzati, and J. Nowzari. 2015. Effect of proline as a nutrient on hypopharyngeal glands during development of *Apis mellifera* (Hymenoptera: Apidae). *ARTHROPODS* 4: 137–143.

De Storme, N., and D. Geelen. 2014. The impact of environmental stress on male reproductive development in plants: Biological processes and molecular mechanisms. *Plant, Cell and Environment* 37: 1–18.

Descamps, C., A. Jambrek, M. Quinet, and A. L. Jacquemart. 2021. Warm temperatures reduce flower attractiveness and bumblebee foraging. *Insects* 12: 1–13.

Descamps, C., M. Quinet, A. Baijot, and A.-L. Jacquemart. 2018. Temperature and water stress affect plant–pollinator interactions in *Borago officinalis* (Boraginaceae). *Ecology and Evolution* 8: 3443–3456.

Desjardins, E. V.-C., and D. D. Oliveira. 2006. Commercial bumble bee bombus impatiens (Hymenoptera: Apidae) as a pollinator in lowbush blueberry (Ericale: Ericaceae) fields. *Journal of Economic Entomology* 99.

DeVetter, L. W., S. Chabert, M. O. Milbrath, R. E. Mallinger, J. Walters, R. Isaacs, S. P. Galinato, et al. 2022. Toward evidence-based decision support systems to optimize pollination and yields in highbush blueberry. *Frontiers in Sustainable Food Systems* 6.

Dharampal, P. S., C. M. Carlson, L. Diaz-Garcia, and S. A. Steffan. 2018. In vitro rearing of solitary bees: A tool for assessing larval risk factors. *Journal of Visualized Experiments* 2018: 1–14.

Dogterom, M. H., and M. L. Winston. 1999. Pollen storage and foraging by honey bees (Hymenoptera: Apidae) in highbush blueberries (Ericaceae), cultivar Bluecrop. *The Canadian Entomologist* 131: 757–768.

Dogterom, M. H., M. L. Winston, and A. Mukai. 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. 'Bluecrop' (*Vaccinium corymbosum*; Ericaceae). *American Journal of Botany* 87: 1584–1591.

Draeger, T., and G. Moore. 2017. Short periods of high temperature during meiosis prevent normal meiotic progression and reduce grain number in hexaploid wheat (Triticum aestivum L.). *Theoretical and Applied Genetics* 130: 1785–1800.

Driedonks, N., I. Rieu, and W. H. Vriezen. 2016. Breeding for plant heat tolerance at vegetative and reproductive stages. *Plant Reproduction* 29: 67–79.

Dupuis, I., and C. Dumas. 1990. Influence of temperature stress on in vitro fertilization and heat shock protein synthesis in maize (*Zea mays* L.) reproductive tissues 1. *Plant Physiology* 94: 665–670.

Eaton, G. W. 1966. Production of highbush blueberry pollen and its germination in vitro as affected by pH and sucrose concentration. *Canadian Journal of Plant Science* 46: 207–209.

Eck, P., and A. W. Stretch. 1986. Nitrogen and plant spacing effects on growth and fruiting of potted highbush blueberry. *HortScience* 21: 249–250.

Edger, P. P., M. Iorizzo, N. V. Bassil, J. Benevenuto, L. F. V. Ferrão, L. Giongo, K. Hummer, et al. 2022. There and back again; historical perspective and future directions for *Vaccinium* breeding and research studies. *Horticulture Research* 9: uhac083.

Edwards, T. W., W. B. Sherman, and R. H. Sharpe. 1972. Seed development in certain florida tetraploid and hexaploid blueberries. *HortScience* 7: 127–128.

Eeraerts, M., M. Pisman, R. Vanderhaegen, I. Meeus, and G. Smagghe. 2020. Recommendations for standardized oral toxicity test protocols for larvae of solitary bees, *Osmia* spp. *Apidologie* 51: 48–60.

Egan, P. A., L. S. Adler, R. E. Irwin, I. W. Farrell, E. C. Palmer-Young, and P. C. Stevenson. 2018. Crop domestication alters floral reward chemistry with potential consequences for pollinator health. *Frontiers in Plant Science* 9: 1–14.

Ehlenfeldt, M. K., and R. L. Prior. 2001. Oxygen Radical Absorbance Capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural and Food Chemistry* 49: 2222–2227.

El-Ghazaly, G., and E. Grafström. 1995. Morphological and histochemical differentiation of the pollen wall of *Betula pendula* Roth, during dormancy up to anthesis. *Protoplasma* 187: 88–102.

Fadón, E., M. Herrero, and J. Rodrigo. 2019. Anther and pollen development in sweet cherry (*Prunus avium* L.) in relation to winter dormancy. *Protoplasma* 256: 733–744.

Farzan, S., and L. H. Yang. 2018. Experimental shifts in phenology affect fitness, foraging, and parasitism in a native solitary bee. *Ecology* 99: 2187–2195.

Felicioli, A., S. Sagona, F. Coppola, C. B. Boni, and M. Pinzauti. 2023. Effect of ageing in the mating behaviour sequence of *Osmia cornuta* Latr. (Hymenoptera: Megachilidae). *Insects* 14: 335.

Feller, U., and I. I. Vaseva. 2014. Extreme climatic events: impacts of drought and high temperature on physiological processes in agronomically important plants. *Frontiers in Environmental Science* 2.

Filipiak, M., M. Woyciechowski, and M. Czarnoleski. 2021. Stoichiometric niche, nutrient partitioning and resource allocation in a solitary bee are sex-specific and phosphorous is allocated mainly to the cocoon. *Scientific Reports* 11: 652.

Filipiak, Z. M., B. Denisow, E. Stawiarz, and M. Filipiak. 2022. Unravelling the dependence of a wild bee on floral diversity and composition using a feeding experiment. *Science of The Total Environment* 820: 153326.

Filipiak, Z. M., and M. Filipiak. 2020. The scarcity of specific nutrients in wild bee larval food negatively influences certain life history traits. *Biology* 9: 1–16.

Firon, N., E. Pressman, S. Meir, R. Khoury, and L. Altahan. 2012. Ethylene is involved in maintaining tomato (*Solanum lycopersicum*) pollen quality under heat-stress conditions. *AoB PLANTS* 2012: pls024.

Firon, N., R. Shaked, M. M. Peet, D. M. Pharr, E. Zamski, K. Rosenfeld, L. Althan, and E. Pressman. 2006. Pollen grains of heat tolerant tomato cultivars retain higher carbohydrate concentration under heat stress conditions. *Scientia Horticulturae* 109: 212–217.

Forde, B. G., and P. J. Lea. 2007. Glutamate in plants: metabolism, regulation, and signalling. *Journal of Experimental Botany* 58: 2339–2358.

Forney, C. F., W. Kalt, M. A. Jordan, M. R. Vinqvist-Tymchuk, and S. A. E. Fillmore. 2012. Blueberry and cranberry fruit composition during development. *Journal of Berry Research* 2: 169–177.

Fortuin, C. C., E. McCarty, and K. JK. Gandhi. 2021. Acute contact with imidacloprid in soil affects the nesting and survival success of a solitary wild bee, *Osmia lignaria* (Hymenoptera: Megachilidae). *Chemosphere* 264: 128572.

Fox, J., S. Weisberg, B. Price, D. Adler, D. Bates, G. Baud-Bovy, B. Bolker, et al. 2023. car: Companion to Applied Regression. https://cran.r-project.org/web/packages/car/index.html

Fulton, T. L., M. R. Wansbrough, C. K. Mirth, and M. D. W. Piper. 2024. Short-term fasting of a single amino acid extends lifespan. *GeroScience*.

Gallardo, R. K., Q. Zhang, M. Dossett, J. J. Polashock, C. Rodriguez-Saona, N. Vorsa, P. P. Edger, et al. 2018. Breeding trait priorities of the blueberry industry in the United States and Canada. *HortScience* 53: 1021–1028.

Gan, W., H. Zhang, N. Bostan, and L. W. DeVetter. 2020. Pollen performance differs among cultivars of northern highbush blueberry (*Vaccinium corymbosum* L.). *Journal of the American Pomological Society* 74: 66–75.

Gardner, K. E., R. L. Foster, and S. O'Donnell. 2007. Experimental analysis of worker division of labor in bumblebee nest thermoregulation (*Bombus huntii*, Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology* 61: 783–792.

Gibbs, J., E. Elle, K. Bobiwash, T. Haapalainen, and R. Isaacs. 2016. Contrasting pollinators and pollination in native and non-native regions of highbush blueberry production. *PLoS ONE* 11: 1–24.

Giorno, F., M. Wolters-Arts, C. Mariani, and I. Rieu. 2013. Ensuring reproduction at high temperatures: the heat stress response during anther and pollen development. *Plants* 2: 489–506.

Gonzalez, V. H., K. Oyen, M. L. Aguilar, A. Herrera, R. D. Martin, and R. Ospina. 2022a. High thermal tolerance in high-elevation species and laboratory-reared colonies of tropical bumble bees. *Ecology and Evolution* 12: e9560.

Gonzalez, V. H., K. Oyen, O. Ávila, and R. Ospina. 2022b. Thermal limits of Africanized honey bees are influenced by temperature ramping rate but not by other experimental conditions. *Journal of Thermal Biology* 110: 103369.

González-Tokman, D., A. Córdoba-Aguilar, W. Dáttilo, A. Lira-Noriega, R. A. Sánchez-Guillén, and F. Villalobos. 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biological Reviews* 95: 802–821.

Gough, R., V. Shutak, and R. Hauke. 1978. Growth and development of highbush blueberry. II. reproductive growth, histological studies. *Journal of the American Society for Horticultural Science* 103: 476–479.

Graham, K. K., M. O. Milbrath, M. Killewald, A. Soehnlen, Y. Zhang, and R. Isaacs. 2023. Identity and diversity of pollens collected by two managed bee species while in blueberry fields for pollination. *Environmental Entomology* 52: 907–917.

Graham, K. K., M. O. Milbrath, Y. Zhang, A. Soehnlen, N. Baert, S. McArt, and R. Isaacs. 2021. Identities, concentrations, and sources of pesticide exposure in pollen collected by managed bees during blueberry pollination. *Scientific Reports* 11: 16857.

Grandison, R. C., M. D. W. Piper, and L. Partridge. 2009. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* 462: 1061–1064.

Greenop, A., N. Mica-Hawkyard, S. Walkington, A. Wilby, S. M. Cook, R. F. Pywell, and B. A. Woodcock. 2020. Equivocal evidence for colony level stress effects on bumble bee pollination services. *Insects* 11: 1–17.

Groh, C., J. Tautz, and W. Rössler. 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. *Proceedings of the National Academy of Sciences of the United States of America* 101: 4268–4273.

De Groot, A. P. 1952. Amino acid requirements for growth of the honeybee (*Apis mellifica* L.). *Experientia* 8: 192–194.

Guiné, R. P. F., C. Gonçalves, F. Gonçalves, and D. Costa. 2016. Some factors that may affect the physical-chemical properties of blueberries. *Agricultural Engineering International: CIGR Journal* 18: 334–342.

Gündüz, K., S. Serçe, and J. F. Hancock. 2015. Variation among highbush and rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics. *Journal of Food Composition and Analysis* 38: 69–79.

Gutierrez, G. M., K. A. LeCroy, T. H. Roulston, D. J. Biddinger, and M. M. López-Uribe. 2023. *Osmia taurus* (Hymenoptera: Megachilidae): A non-native bee species with invasiveness potential in North America D. *Environmental Entomology* 52: 149–156.

Hamblin, A. L., E. Youngsteadt, M. M. López-Uribe, and S. D. Frank. 2017. Physiological thermal limits predict differential responses of bees to urban heat-island effects. *Biology Letters* 13: 20170125.

Hamidou, F., O. Halilou, and V. Vadez. 2013. Assessment of groundnut under combined heat and drought stress. *Journal of Agronomy and Crop Science* 199: 1–11.

Han, M., C. Zhang, P. Suglo, S. Sun, M. Wang, and T. Su. 2021. l-Aspartate: an essential metabolite for plant growth and stress acclimation. *Molecules* 26: 1887.

Hancock, J. F., K. Haghighi, S. L. Krebs, J. A. Flore, and A. D. Draper. 1992. Photosynthetic heat stability in highbush blueberries and the possibility of genetic improvement. *HortScience* 27: 1111–1112.

Hao, L., L. Guo, R. Li, Y. Cheng, L. Huang, H. Zhou, M. Xu, et al. 2019. Responses of photosynthesis to high temperature stress associated with changes in leaf structure and biochemistry of blueberry (*Vaccinium corymbosum* L.). *Scientia Horticulturae* 246: 251–264.

Harrison, E., T. Drake, and R. Pius. 2023. finalfit: Quickly Create Elegant Regression Results Tables and Plots when Modelling. 10.32614/CRAN.package.finalfit

Hashida, S., H. Takahashi, M. Kawai-Yamada, and H. Uchimiya. 2007. *Arabidopsis thaliana* nicotinate/nicotinamide mononucleotide adenyltransferase (AtNMNAT) is required for pollen tube growth. *The Plant Journal* 49: 694–703.

Hashida, S., H. Takahashi, K. Takahara, M. Kawai-Yamada, K. Kitazaki, K. Shoji, F. Goto, et al. 2013. NAD+ accumulation during pollen maturation in *Arabidopsis* regulating onset of germination. *Molecular Plant* 6: 216–225.

Hashida, S., H. Takahashi, and H. Uchimiya. 2009. The role of NAD biosynthesis in plant development and stress responses. *Annals of Botany* 103: 819–824.

Hatfield, J. L., J. Antle, K. A. Garrett, R. C. Izaurralde, T. Mader, E. Marshall, M. Nearing, et al. 2020. Indicators of climate change in agricultural systems. *Climatic Change* 163: 1719–1732.

Hatfield, J. L., K. J. Boote, B. A. Kimball, L. H. Ziska, R. C. Izaurralde, D. Ort, A. M. Thomson, and D. Wolfe. 2011. Climate impacts on agriculture: Implications for crop production. *Agronomy Journal* 103: 351–370.

Hatfield, J. L., and J. H. Prueger. 2015. Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes* 10: 4–10.

Hayes, T., and G. López-Martínez. 2021. Resistance and survival to extreme heat shows circadian and sex-specific patterns in a cavity nesting bee. *Current Research in Insect Science* 1: 100020.

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

Hedhly, A., J. I. Hormaza, and M. Herrero. 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., J. I. Hormaza, and M. Herrero. 2009. Global warming and sexual plant reproduction. *Trends in Plant Science* 14: 30–36.

Hedhly, A., J. I. Hormaza, and M. Herrero. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology* 7: 476–483.

Hedhly, A., J. I. Hormaza, and M. Herrero. 2003. The effect of temperature on stigmatic receptivity in sweet cherry (*Prunus avium* L.). *Plant, Cell & Environment* 26: 1673–1680.

Hedhly, A., A. Nestorova, A. Herrmann, and U. Grossniklaus. 2020. Acute heat stress during stamen development affects both the germline and sporophytic lineages in *Arabidopsis thaliana* (L.) Heynh. *Environmental and Experimental Botany* 173: 103992.

Hegland, S. J., A. Nielsen, A. Lázaro, A. L. Bjerknes, and Ø. Totland. 2009. How does climate warming affect plant-pollinator interactions? *Ecology Letters* 12: 184–195.

Helm, B. R., J. P. Rinehart, G. D. Yocum, K. J. Greenlee, and J. H. Bowsher. 2017. Metamorphosis is induced by food absence rather than a critical weight in the solitary bee, *Osmia lignaria*. *Proceedings of the National Academy of Sciences* 114: 10924–10929.

Hemberger, J. A., N. M. Rosenberger, and N. M. Williams. 2023. Experimental heatwaves disrupt bumblebee foraging through direct heat effects and reduced nectar production. *Functional Ecology* 37: 591–601.

Herbert, E. W., W. E. Bickley, and H. Shimanuki. 1970. The brood-rearing capability of caged honey bees1 fed dandelion and mixed pollen diets. *Journal of Economic Entomology* 63: 215–218.

Hernández-Fuentes, C., J. Galmés, L. A. Bravo, and L. A. Cavieres. 2023. Elevation provenance affects photosynthesis and its acclimation to temperature in the high-Andes alpine herb *Phacelia secunda*. *Plant Biology* 25: 793–802.

Herrero, M., and A. Arbeloa. 1989. Influence of the pistil on pollen tube kinetics in Peach (*Prunus persica*). *American Journal of Botany* 76: 1441–1447.

Herrero, M., and J. I. Hormaza. 1996. Pistil strategies controlling pollen tube growth. 9: 343–347.

Hoover, S. E. R., H. A. Higo, and M. L. Winston. 2006. Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition. *Journal of Comparative Physiology B* 176: 55–63.

Hothorn, T., F. Bretz, P. Westfall. 2023. Simultaneous Inference in General Parametric Models. *Biometrical Journal* 50: 346-363.

Howell, A. D., and R. Alarcón. 2007. *Osmia* bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Animal Behaviour* 74: 199–205.

Human, H., S. W. Nicolson, K. Strauss, C. W. W. Pirk, and V. Dietemann. 2007. Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera* scutellata). *Journal of Insect Physiology* 53: 649–655.

Hüve, K., I. Bichele, B. Rasulov, and U. Niinemets. 2011. When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and $H_2O_2$ formation. *Plant, Cell & Environment* 34: 113–126.

IPCC 2018. Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA.

IPCC 2021. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IPCC 2023. *Climate Change 2023: Synthesis Report.* Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland, pp. 35-115.

Iqbal, M. Z., T. Jia, T. Tang, M. Anwar, A. Ali, M. J. Hassan, Y. Zhang, et al. 2022. A heat shock transcription factor TrHSFB2a of white clover negatively regulates drought, heat and salt stress tolerance in transgenic *Arabidopsis*. *International Journal of Molecular Sciences* 23: 12769.

Iwahori, S. 1966. High temperature injuries in tomato fertilization and development of embryos with special reference to the abnormalities cause by high temperature. *Journal of the Japanese Society for Horticultural Science* 35: 379–386.

Jain, M., P. S. Chourey, K. J. Boote, and L. H. Allen. 2010. Short-term high temperature growth conditions during vegetative-to-reproductive phase transition irreversibly compromise cell wall invertase-mediated sucrose catalysis and microspore meiosis in grain sorghum (*Sorghum bicolor*). *Journal of Plant Physiology* 167: 578–582.

Jain, M., P. V. V. Prasad, K. J. Boote, A. L. Hartwell, and P. S. Chourey. 2007. Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). *Planta* 227: 67–79.

Janousek, W. M., M. R. Douglas, S. Cannings, M. A. Clément, C. M. Delphia, J. G. Everett, R. G. Hatfield, et al. 2023. Recent and future declines of a historically widespread pollinator linked to climate, land cover, and pesticides. *Proceedings of the National Academy of Sciences* 120: e2211223120.

Jeannerod, L., A. Carlier, B. Schatz, C. Daise, A. Richel, Y. Agnan, M. Baude, and A.-L. Jacquemart. 2022. Some bee-pollinated plants provide nutritionally incomplete pollen amino acid resources to their pollinators. *PLoS ONE* 17: e0269992.

Jegadeesan, S., A. Beery, L. Altahan, S. Meir, E. Pressman, and N. Firon. 2018. Ethylene production and signaling in tomato (*Solanum lycopersicum*) pollen grains is responsive to heat stress conditions. *Plant Reproduction* 31: 367–383.

Jin, Y., H. Yang, Z. Wei, H. Ma, and X. Ge. 2013. Rice male development under drought stress: phenotypic changes and stage-dependent transcriptomic reprogramming. *Molecular plant* 6: 1630–1645.

Johnson, M. G., J. R. Glass, M. E. Dillon, and J. F. Harrison. 2023. Chapter One - How will climatic warming affect insect pollinators? *In* J. F. Harrison [ed.], Advances in Insect Physiology, Environmental Threats to Pollinator Health and Fitness, 1–115. Academic Press.

Jones, L. J., R. P. Haugland, and V. L. Singer. 2018. Development and characterization of the NanoOrange® Protein Quantitation Assay: A fluorescence-based assay of proteins in solution. *BioTechniques* 34: 850–861.

Jorquera-Fontena, E., M. Génard, A. Ribera-Fonseca, and N. Franck. 2017. A simple allometric model for estimating blueberry fruit weight from diameter measurements. *Scientia Horticulturae* 219: 131–134.

Julian, C., J. Rodrigo, and M. Herrero. 2011. Stamen development and winter dormancy in apricot (*Prunus armeniaca*). *Annals of Botany* 108: 617–625.

Jumrani, K., V. S. Bhatia, and G. P. Pandey. 2018. Screening soybean genotypes for high temperature tolerance by in vitro pollen germination, pollen tube length, reproductive efficiency and seed yield. *Indian Journal of Plant Physiology* 23: 77–90.

Juricic, P., S. Grönke, and L. Partridge. 2020. Branched-chain amino acids have equivalent effects to other essential amino acids on lifespan and aging-related traits in *Drosophila*. *The Journals of Gerontology: Series A* 75: 24–31.

Kammerer, M., S. C. Goslee, M. R. Douglas, J. F. Tooker, and C. M. Grozinger. 2021. Wild bees as winners and losers: Relative impacts of landscape composition, quality, and climate. *Global Change Biology* 27: 1250–1265.

Kazenel, M. R., K. W. Wright, T. Griswold, K. D. Whitney, and J. A. Rudgers. 2024. Heat and desiccation tolerances predict bee abundance under climate change. *Nature* 628: 342–348.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado.

Kehrberger, S., and A. Holzschuh. 2019. Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. *PLOS ONE* 14: e0218824.

Kierat, J., H. Szentgyörgyi, M. Czarnoleski, and M. Woyciechowski. 2017. The thermal environment of the nest affects body and cell size in the solitary red mason bee (*Osmia bicornis* L.). *Journal of Thermal Biology* 68: 39–44.

Kilian, R. 2016. Lacy Phacelia, *Phacelia tanacetifolia* Benth. A native annual forb for conservation use in Montana and Wyoming. *USDA Technical Bulletin* MT-113.

Kim, J. G., H. L. Kim, S. J. Kim, and K.-S. Park. 2013. Fruit quality, anthocyanin and total phenolic contents, and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea. *Journal of Zhejiang University. Science. B* 14: 793–799.

Kim, S. Y., C. B. Hong, and I. Lee. 2001. Heat shock stress causes stage-specific male sterility in *Arabidopsis thaliana*. *Journal of Plant Research* 114: 301–307.

Knauer, A. C., C. Alaux, M. J. Allan, R. R. Dean, V. Dievart, G. Glauser, T. Kiljanek, et al. 2022. Nutritional stress exacerbates impact of a novel insecticide on solitary bees' behaviour, reproduction and survival. *Proceedings of the Royal Society B: Biological Sciences* 289: 20221013.

Koltunow, A. M., J. Truettner, K. H. Cox, M. Wallroth, and R. B. Goldberg. 1990. Different temporal and spatial gene expression patterns occur during anther development. *The Plant Cell* 2: 1201–1224.

Kopecká, R., M. Kameniarová, M. Černý, B. Brzobohatý, and J. Novák. 2023. Abiotic stress in crop production. *International Journal of Molecular Sciences* 24: 6603.

Kopit, A. M., E. Klinger, D. L. Cox-Foster, R. A. Ramirez, and T. L. Pitts-Singer. 2022. Effects of provision type and pesticide exposure on the larval development of *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology* 51: 240–251.

Kozai, N., K. Beppu, R. Mochioka, U. Boonprakob, S. Subhadrabandhu, and I. Kataoka. 2004. Adverse effects of high temperature on the development of reproductive organs in 'Hakuho' peach trees. *The Journal of Horticultural Science and Biotechnology* 79: 533–537.

Kraemer, M. E., F. D. Favi, and C. E. Niedziela. 2014. Nesting and pollen preference of *Osmia lignaria lignaria* (Hymenoptera: Megachilidae) in Virginia and North Carolina Orchards. *Environmental Entomology* 43: 932–941.

Krebs, S. L., and J. F. Hancock. 1988. The consequences of inbreeding on fertility in *Vaccinium corymbosum* L. *Journal of the American Society for Horticultural Science* 113: 914–918.

Krishna, P., G. Pandey, R. Thomas, and S. Parks. 2023. Improving blueberry fruit nutritional quality through physiological and genetic interventions: A review of current research and future directions. *Antioxidants* 12: 810.

Kumar, S., M. Thakur, R. Mitra, S. Basu, and A. Anand. 2022. Sugar metabolism during pre- and post-fertilization events in plants under high temperature stress. *Plant Cell Reports* 41: 655–673.

Kumarihami, H. M. P. C., H.-G. Park, S.-M. Kim, J.-I. Park, E.-J. Lee, H. L. Kim, and J. G. Kim. 2021. Flower and leaf bud density manipulation affects fruit set, leaf-to-fruit ratio, and yield in southern highbush 'Misty' blueberry. *Scientia Horticulturae* 290: 110530.

Ladurner, E., J. Bosch, W. P. Kemp, and S. Maini. 2008. Foraging and nesting behavior of *Osmia lignaria* (Hymenoptera: Megachilidae) in the presence of fungicides: Cage studies. *Journal of Economic Entomology* 101: 647–653.

Ladurner, E., B. Maccagnani, D. Tesoriero, M. Nepi, and A. Felicioli. 1999. Laboratory Rearing of *Osmia cornuta* (Latreille) (Hymenoptera, Megachilidae) on artificial diet. *Bollettino dell'Istituto di entomologia '"G. Grandi"', Universita` di Bologna* 53: 133–146.

Lang, G. A., and E. J. Parrie. 1992. Pollen viability and vigor in hybrid southern highbush blueberries (*Vaccinium corymbosum* L. ×spp.). *HortScience* 27: 425–427.

Larkindale, J., and E. Vierling. 2008. Core genome responses involved in acclimation to high temperature. *Plant Physiology* 146: 748–761.

Lau, P., P. Lesne, R. J. Grebenok, J. Rangel, and S. T. Behmer. 2022. Assessing pollen nutrient content: a unifying approach for the study of bee nutritional ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences* 377: 20210510.

LeCroy, K. A., G. Savoy-Burke, D. E. Carr, D. A. Delaney, and T. H. Roulston. 2020. Decline of six native mason bee species following the arrival of an exotic congener. *Scientific Reports* 10: 18745.

Ledesma, N. A., and S. Kawabata. 2016. Responses of two strawberry cultivars to severe high temperature stress at different flower development stages. *Scientia Horticulturae* 211: 319–327.

Ledesma, N., and N. Sugiyama. 2005. Pollen quality and performance in strawberry plants exposed to high-temperature stress. *Journal of the American Society for Horticultural Science* 130: 341–347.

Lee, E., Y. He, and Y.-L. Park. 2018. Effects of climate change on the phenology of *Osmia cornifrons*: implications for population management. *Climatic Change* 150: 305–317.

Lee, K.-T., H.-S. Liao, and M.-H. Hsieh. 2023. Glutamine metabolism, sensing and signaling in plants. *Plant and Cell Physiology* 64: 1466–1481.

Lenth, R. V., B. Bolker, P. Buerkner, I. Giné-Vázquez, M. Herve, M. Jung, J. Love, et al. 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means. 10.32614/CRAN.package.emmeans

Levin, M. D., and M. H. Haydak. 1957. Comparative value of different pollens in the nutrition of *Osmia Lignaria*. *Bee World*.

Lin, H., A. L. Kwan, and S. K. Dutcher. 2010. Synthesizing and salvaging NAD+: Lessons learned from *Chlamydomonas reinhardtii*. *PLOS Genetics* 6: e1001105.

Lin, Y., G. Huang, Q. Zhang, Y. Wang, V. P. Dia, and X. Meng. 2020. Ripening affects the physicochemical properties, phytochemicals and antioxidant capacities of two blueberry cultivars. *Postharvest Biology and Technology* 162: 111097.

Linskens, H. F., and J. Schrauwen. 1969. The release of free amino acids from germinating pollen. *Acta Botanica Neerlandica* 18: 605–614.

Lobos, G. A., and J. F. Hancock. 2015. Breeding blueberries for a changing global environment: A review. *Frontiers in Plant Science* 6: 1–14.

Lohani, N., M. B. Singh, and P. L. Bhalla. 2020. High temperature susceptibility of sexual reproduction in crop plants. *Journal of Experimental Botany* 71: 555–568.

Lu, Y., and T. D. Sharkey. 2004. The role of amylomaltase in maltose metabolism in the cytosol of photosynthetic cells. *Planta* 218: 466–473.

Lu, Y., J. M. Steichen, S. E. Weise, and T. D. Sharkey. 2006. Cellular and organ level localization of maltose in maltose-excess Arabidopsis mutants. *Planta* 224: 935–943.

Lüdecke, D., D. Makowski, M. S. Ben-Shachar, I. Patil, P. Waggoner, B. M. Wiernik, R. Thériault, et al. 2024. performance: Assessment of Regression Models Performance. 10.32614/CRAN.package.performance

MacKenzie, K. E. 1997. Pollination requirements of three highbush blueberry (*Vaccinium corymbosum* L.) Cultivars. *Journal of the American Society for Horticultural Science* 122: 891–896.

Maebe, K., A. F. Hart, L. Marshall, P. Vandamme, N. J. Vereecken, D. Michez, and G. Smagghe. 2021. Bumblebee resilience to climate change, through plastic and adaptive responses. *Global Change Biology* 27: 4223–4237.

Malikov, E., R. Miao, and J. Zhang. 2020. Distributional and temporal heterogeneity in the climate change effects on U.S. agriculture. *Journal of Environmental Economics and Management* 104: 102386.

de Manincor, N., A. Fisogni, and N. E. Rafferty. 2023. Warming of experimental plant–pollinator communities advances phenologies, alters traits, reduces interactions and depresses reproduction. *Ecology Letters* 26: 323–334.

Martinet, B., S. Dellicour, G. Ghisbain, K. Przybyla, E. Zambra, T. Lecocq, M. Boustani, et al. 2021a. Global effects of extreme temperatures on wild bumblebees. *Conservation Biology* 35: 1507–1518.

Martinet, B., T. Lecocq, J. Smet, and P. Rasmont. 2015. A protocol to assess insect resistance to heat waves, applied to bumblebees (*Bombus latreille*, 1802). *PLoS ONE* 10: 1–9.

Martinet, B., E. Zambra, K. Przybyla, T. Lecocq, A. Anselmo, D. Nonclercq, P. Rasmont, et al. 2021b. Mating under climate change: Impact of simulated heatwaves on the reproduction of model pollinators. *Functional Ecology* 35: 739–752.

Masoomi-Aladizgeh, F., U. Najeeb, S. Hamzelou, D. Pascovici, A. Amirkhani, D. K. Y. Tan, M. Mirzaei, et al. 2021. Pollen development in cotton (*Gossypium hirsutum*) is highly sensitive to heat exposure during the tetrad stage. *Plant, Cell & Environment* 44: 2150–2166.

Matsuda, H., and H. Higuchi. 2020. Effect of temperatures on passion fruit flowering: *Tropical Agriculture and Development* 64: 54–60.

Mattioli, R., M. Biancucci, A. El Shall, L. Mosca, P. Costantino, D. Funck, and M. Trovato. 2018. Proline synthesis in developing microspores is required for pollen development and fertility. *BMC Plant Biology* 18: 356.

Mattioli, R., M. Biancucci, C. Lonoce, P. Costantino, and M. Trovato. 2012. Proline is required for male gametophyte development in *Arabidopsis*. *BMC Plant Biology* 12: 236.

McAfee, A., A. Chapman, H. Higo, R. Underwood, J. Milone, L. J. Foster, M. M. Guarna, et al. 2020. Vulnerability of honey bee queens to heat-induced loss of fertility. *Nature Sustainability* 3: 367–376.

Medina, R. G., R. J. Paxton, E. De Luna, F. A. Fleites-Ayil, L. A. Medina Medina, and J. J. G. Quezada-Euán. 2018. Developmental stability, age at onset of foraging and longevity of Africanized honey bees (*Apis mellifera* L.) under heat stress (Hymenoptera: Apidae). *Journal of Thermal Biology* 74: 214–225.

Melone, G. G., C. Stuligross, and N. M. Williams. 2024. Heatwaves increase larval mortality and delay development of a solitary bee. *Ecological Entomology*. 49: 433-444.

Memmott, J., P. G. Craze, N. M. Waser, and M. V. Price. 2007. Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* 10: 710–717.

Mesihovic, A., R. Iannacone, N. Firon, and S. Fragkostefanakis. 2016. Heat stress regimes for the investigation of pollen thermotolerance in crop plants. *Plant Reproduction* 29: 93–105.

Mirgorodskaya, O. E., N. K. Koteyeva, A. V. Volchanskaya, and E. A. Miroslavov. 2015. Pollen development in Rhododendron in relation to winter dormancy and bloom time. *Protoplasma* 252: 1313–1323.

Mitropolova, L., E. Korotkikh, O. Pavlova, and O. Ivleva. 2023. Study of *Phacelia Tanacetifolia* Benth as a green manure crop in the conditions of primorsky krai. *In* A. Beskopylny, M. Shamtsyan, and V. Artiukh [eds.], XV International Scientific Conference “INTERAGROMASH 2022”, 2455–2461. Springer International Publishing, Cham.

Moore, C. E., K. Meacham-Hensold, P. Lemonnier, R. A. Slattery, C. Benjamin, C. J. Bernacchi, T. Lawson, and A. P. Cavanagh. 2021. The effect of increasing temperature on crop

photosynthesis: from enzymes to ecosystems. *Journal of Experimental Botany* 72: 2822–2844.

Motha, R. P., and W. Baier. 2005. Impacts of present and future climate change and climate variability on agriculture in the temperate regions: North America. *Climatic Change* 70: 137–164.

Müller, F., and I. Rieu. 2016. Acclimation to high temperature during pollen development. *Plant Reproduction* 29: 107–118.

Naumchik, M., and E. Youngsteadt. 2023. Larger pollen loads increase risk of heat stress in foraging bumblebees. *Biology Letters* 19: 20220581.

Nepi, M., L. Cresti, B. Maccagnani, E. Ladurner, and E. Pacini. 2005. From the anther to the proctodeum: Pear (*Pyrus communis*) pollen digestion in *Osmia cornuta* larvae. *Journal of Insect Physiology* 51: 749–757.

Nicholson, C. C., and P. A. Egan. 2020. Natural hazard threats to pollinators and pollination. *Global Change Biology* 26: 380–391.

Nicolson, S. W. 2011. Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two. *African Zoology* 46: 197–204.

Nicolson, S. W., and R. W. Thornburg. 2007. Nectar chemistry. *In* S. W. Nicolson, M. Nepi, and E. Pacini [eds.], Nectaries and Nectar, 215–264. Springer Netherlands, Dordrecht.

Ogilvie, J. E., and J. R. Forrest. 2017. Interactions between bee foraging and floral resource phenology shape bee populations and communities. *Current Opinion in Insect Science* 21: 75–82.

Ostap-Chec, M., J. Kierat, K. Kuszewska, and M. Woyciechowski. 2021. Red mason bee (*Osmia bicornis*) thermal preferences for nest sites and their effects on offspring survival. *Apidologie* 52: 707–719.

Owayss, A. A., M. A. Shebl, J. Iqbal, A. M. Awad, H. S. Raweh, and A. S. Alqarni. 2020. *Phacelia tanacetifolia* can enhance conservation of honey bees and wild bees in the drastic hot-arid subtropical Central Arabia. *Journal of Apicultural Research* 59: 569–582.

Oyen, K. J., and M. E. Dillon. 2018. Critical thermal limits of bumble bees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age, or feeding status. *Journal of Experimental Biology*: jeb.165589.

Ozga, J. A., H. Kaur, R. P. Savada, and D. M. Reinecke. 2017. Hormonal regulation of reproductive growth under normal and heat-stress conditions in legume and other model crop species. *Journal of Experimental Botany* 68: 1885–1894.

Palanivelu, R., L. Brass, A. F. Edlund, and D. Preuss. 2003. Pollen tube growth and guidance is regulated by POP2, an *Arabidopsi*s gene that controls GABA levels. *Cell* 114: 47–59.

Palma, M. J., J. B. Retamales, E. J. Hanson, and C. M. Araya. 2023. Relationship between cane age and vegetative and reproductive traits of northern highbush blueberry in Chile and United States. *Scientia Horticulturae* 310: 111775.

Paoli, P. P., D. Donley, D. Stabler, A. Saseendranath, S. W. Nicolson, S. J. Simpson, and G. A. Wright. 2014. Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. *Amino Acids* 46: 1449–1458.

Parker, L. E., A. J. McElrone, S. M. Ostoja, and E. J. Forrestel. 2020. Extreme heat effects on perennial crops and strategies for sustaining future production. *Plant Science* 295: 110397–110397.

Parrotta, L., C. Faleri, M. Cresti, and G. Cai. 2016. Heat stress affects the cytoskeleton and the delivery of sucrose synthase in tobacco pollen tubes. *Planta* 243: 43–63.

Peet, M. M., S. Sato, and R. G. Gardner. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. *Plant, Cell & Environment* 21: 225–231.

Pelletier, D., and J. R. K. Forrest. 2023. Pollen specialisation is associated with later phenology in *Osmia* bees (Hymenoptera: Megachilidae). *Ecological Entomology* 48: 164–173.

Perdomo, J. A., S. Capó-Bauçà, E. Carmo-Silva, and J. Galmés. 2017. Rubisco and Rubisco Activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. *Frontiers in Plant Science* 8.

Pinilla-Gallego, M. S., and R. Isaacs. 2018. Pollen use by *Osmia lignaria* (Hymenoptera: Megachilidae) in Highbush blueberry fields. *Annals of the Entomological Society of America* 111: 335–340.

Pirk, C. W. W., C. Boodhoo, H. Human, and S. W. Nicolson. 2010. The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera* scutellata). *Apidologie* 41: 62–72.

Pitts-Singer, T. L., D. R. Artz, S. S. Peterson, N. K. Boyle, and G. I. Wardell. 2018. Examination of a managed pollinator strategy for almond production using *Apis mellifera* (Hymenoptera: Apidae) and *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology* 47: 364–377.

Pitts-Singer, T. L., and R. R. James. 2009. Prewinter management affects *Megachile rotundata* (Hymenoptera: Megachilidae) prepupal physiology and adult emergence and survival. *Journal of Economic Entomology* 102: 1407–1416.

Polowick, P. L., and V. K. Sawhney. 1993. Differentiation of the tapetum during microsporogenesis in tomato (*Lycopersicon esculentum* Mill.), with special reference to the tapetal cell wall. *Annals of Botany* 72: 595–605.

Porch, T., and M. Jáhn. 2001. Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. *Plant, Cell & Environment* 24: 723–731.

Pressman, E., M. M. Peet, and D. M. Pharr. 2002. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Annals of Botany* 90: 631–636.

Quinlan, G. M., and C. M. Grozinger. 2023. Chapter Five - Honey bee nutritional ecology: From physiology to landscapes. *In* J. F. Harrison [ed.], *Advances in Insect Physiology, Environmental Threats to Pollinator Health and Fitness*, 289–345. Academic Press.

R Core Team. 2023. R: A Language and Environment for Statistical Computing. Website https://www.r-project.org/.

Raja, M. M., G. Vijayalakshmi, M. L. Naik, P. O. Basha, K. Sergeant, J. F. Hausman, and P. S. S. V. Khan. 2019. Pollen development and function under heat stress: from effects to responses. *Acta Physiologiae Plantarum* 41.

Raven, P. H., and D. L. Wagner. 2021. Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences* 118: e2002548117.

Retamales, J. B., and J. F. Hancock. 2012. Blueberries. 1st ed. CABI, Wallingford, UK.

Retamales, J. B., and J. F. Hancock. 2018. Blueberries, 2nd ed. CABI, Wallingford, UK.

Rodrigo, J., and M. Herrero. 2002. Effects of pre-blossom temperatures on flower development and fruit set in apricot. *Scientia Horticulturae* 92: 125–135.

Roulston, T. H., and J. H. Cane. 2000. Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution* 222: 187–209.

Rowland, L. J., D. J. Bell, N. Alkharouf, N. V. Bassil, F. A. Drummond, L. Beers, E. J. Buck, et al. 2012. Generating genomic tools for blueberry improvement. *International Journal of Fruit Science* 12: 276–287.

Ruedenauer, F. A., N. W. Biewer, C. A. Nebauer, M. Scheiner, J. Spaethe, and S. D. Leonhardt. 2021. Honey bees can taste amino and fatty acids in pollen, but not sterols. *Frontiers in Ecology and Evolution* 9.

Ruedenauer, F. A., S. D. Leonhardt, K. Lunau, and J. Spaethe. 2019. Bumblebees are able to perceive amino acids via chemotactile antennal stimulation. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 0: 0–0.

Ruedenauer, F. A., J. Spaethe, and S. D. Leonhardt. 2015. How to know which food is good for you: bumblebees use taste to discriminate between different concentrations of food differing in nutrient content. *Journal of Experimental Biology* 218: 2233–2240.

Russell, K. A., and Q. S. McFrederick. 2021. Elevated temperature may affect nectar microbes, nectar sugars, and bumble bee foraging preference. *Microbial Ecology*.

Russo, L., C. W. de Keyzer, A. N. Harmon-Threatt, K. A. LeCroy, and J. S. MacIvor. 2021. The managed-to-invasive species continuum in social and solitary bees and impacts on native bee conservation. *Current Opinion in Insect Science* 46: 43–49.

Russo, L., A. D. Vaudo, C. J. Fisher, C. M. Grozinger, and K. Shea. 2019. Bee community preference for an invasive thistle associated with higher pollen protein content. *Oecologia* 190: 901–912.

Ryder, J. T., A. Cherrill, H. M. Thompson, and K. F. A. Walters. 2021. Lower pollen nutritional quality delays nest building and egg laying in *Bombus terrestris* audax micro-colonies leading to reduced biomass gain. *Apidologie* 52: 1033–1047.

Sakata, T., H. Takahashi, I. Nishiyama, and A. Higashitani. 2000. Effects of high temperature on the development of pollen mother cells and microspores in Barley *Hordeum vulgare* L. *Journal of Plant Research* 113: 395–402.

Salvucci, M. E., and S. J. Crafts-Brandner. 2004. Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco Activase in plants from contrasting thermal environments. *Plant Physiology* 134: 1460–1470.

Sampson, B. J., S. J. Stringer, and D. A. Marshall. 2013. Blueberry floral attributes and their effect on the pollination efficiency of an oligolectic bee, *Osmia ribifloris* Cockerell (Megachilidae: Apoidea). *HortScience* 48: 136–142.

Santiago, J. P., and T. D. Sharkey. 2019. Pollen development at high temperature and role of carbon and nitrogen metabolites. *Plant, Cell & Environment* 42: 2759–2775.

Santiago, J. P., A. Soltani, M. M. Bresson, A. L. Preiser, D. B. Lowry, and T. D. Sharkey. 2021. Contrasting anther glucose-6-phosphate dehydrogenase activities between two bean varieties suggest an important role in reproductive heat tolerance. *Plant, Cell & Environment* 44: 2185–2199.

Santiago, J. P., and M. Tegeder. 2016. Connecting source with sink: the role of *Arabidopsis* AAP8 in phloem loading of amino acids. *Plant Physiology* 171: 508–521.

Satake, T., and S. Yoshida. 1978. High temperature-induced sterility in indica rices at flowering. *Japanese journal of crop science* 47: 6–17.

Sato, S., M. Kamiyama, T. Iwata, N. Makita, H. Furukawa, and H. Ikeda. 2006. Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. *Annals of Botany* 97: 731–738.

Scaven, T., and N. Rafferty. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current zoology* 59: 418–426.

Schwacke, R., S. Grallath, K. E. Breitkreuz, E. Stransky, H. Stransky, W. B. Frommer, and D. Rentsch. 1999. LeProT1, a transporter for proline, glycine betaine, and γ-Amino butyric acid in tomato pollen. *The Plant Cell* 11: 377–391.

Sedivy, C., A. Müller, and S. Dorn. 2011. Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen. *Functional Ecology* 25: 718–725.

Seker, H., D. E. Rowe, and G. E. Brink. 2003. White clover morphology changes with stress treatments. *Crop Science* 43: 2218–2225.

Sepúlveda, Y., and D. Goulson. 2023. Feeling the heat: Bumblebee workers show no acclimation capacity of upper thermal tolerance to simulated heatwaves. *Journal of Thermal Biology* 116: 103672.

Settele, J., J. Bishop, and S. G. Potts. 2016. Climate change impacts on pollination. *Nature Plants* 2: 16092–16092.

Sgolastra, F., X. Arnan, T. L. Pitts-Singer, S. Maini, W. P. Kemp, and J. Bosch. 2016. Pre-wintering conditions and post-winter performance in a solitary bee: does diapause impose an energetic cost on reproductive success? *Ecological Entomology* 41: 201–210.

Sharkey, T. D. 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell & Environment* 28: 269–277.

Sheffield, C. S. 2014. Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Annapolis Valley, Nova Scotia, Canada. *Journal of Pollination Ecology* 12: 120–128.

Simpson, B. B., and J. L. Neff. Evolution and diversity of floral rewards, In: Handbook of Experimental Pollination Biology. Van Nostrand Rienhold Co. New York.

Sjoberg, D. D., M. Baillie, C. Fruechtenicht, S. Haesendonck, and T. Treis. 2023. ggsurvfit: Flexible Time-to-Event Figures. 10.32614/CRAN.package.ggsurvfit

Slominski, A. H., and L. A. Burkle. 2021. Asynchrony between solitary bee emergence and flower availability reduces flower visitation rate and may affect offspring size. *Basic and Applied Ecology* 56: 345–357.

Smrke, T., R. Veberic, M. Hudina, V. Zitko, M. Ferlan, and J. Jakopic. 2021. Fruit quality and yield of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars grown in two planting systems under different protected environments. *Horticulturae* 7: 591.

Smyth, D. R., J. L. Bowman, and E. M. Meyerowitz. 1990. Early flower development in *Arabidopsis*. *The Plant Cell* 2: 755–767.

Snider, J. L., and D. M. Oosterhuis. 2011. How does timing, duration and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant Signaling and Behavior* 6: 930–933.

Snider, J. L., D. M. Oosterhuis, B. W. Skulman, and E. M. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. *Physiologia Plantarum* 137: 125–138.

Somme, L., M. Vanderplanck, D. Michez, I. Lombaerde, R. Moerman, B. Wathelet, R. Wattiez, et al. 2015. Pollen and nectar quality drive the major and minor floral choices of bumble bees. *Apidologie* 46: 92–106.

Song, Y., L. Liu, H. Cui, W. Guo, S. Lv, B. Ye, L. Li, et al. 2023. Evaluation of *Osmia excavata* (Hymenoptera: Megachilidae) sensitivity to high-temperature stress. *Frontiers in Sustainable Food Systems* 7.

Soroye, P., T. Newbold, and J. Kerr. 2020. Climate change contributes to widespread declines among bumble bees across continents. *Science* 367: 685–688.

Spendal, R. C., and J. H. Cane. 2022. Multiple daily brood cells define the fecundity of *Osmia lignaria* bees in a semi-natural setting. *Apidologie* 53: 54.

Stabler, D., P. P. Paoli, S. W. Nicolson, and G. A. Wright. 2015. Nutrient balancing of the adult worker bumblebee (*Bombus terrestris*) depends on the dietary source of essential amino acids. *Journal of Experimental Biology* 218: 793–802.

Stanley, R. G., and H. F. Linskens. 1965. Protein diffusion from germinating pollen. *Physiologia Plantarum* 18: 47–48.

Strik, B. C., and A. J. Vance. 2019. Highbush blueberry cultivars differ in the relationship between seed number and berry weight during the harvest season. *HortScience* 54: 1728–1736.

Strik, B. C., A. J. Vance, and C. E. Finn. 2017. Northern highbush blueberry cultivars differed in yield and fruit quality in two organic production systems from planting to maturity. *HortScience* 52: 844–851.

Stuligross, C., and N. M. Williams. 2020. Pesticide and resource stressors additively impair wild bee reproduction: Stressors additively impair wild bees. *Proceedings of the Royal Society B: Biological Sciences* 287.

Tafi, E., S. Sagona, F. Coppola, V. Meucci, M. Galloni, L. Bortolotti, G. Bogo, et al. 2023. Effects of proline on survival, locomotion and amino acid haemolymph composition of *Osmia cornuta* (Latreille, 1805). *Physiological Entomology* 48: 161–170.

Tang, R.-S., J.-C. Zheng, Z.-Q. Jin, D.-D. Zhang, Y.-H. Huang, and L.-G. Chen. 2008. Possible correlation between high temperature-induced floret sterility and endogenous levels of IAA, GAs and ABA in rice (*Oryza sativa* L.). *Plant Growth Regulation* 54: 37–43.

Taurino, M., S. Costantini, S. De Domenico, F. Stefanelli, G. Ruano, M. O. Delgadillo, J. J. Sánchez-Serrano, et al. 2018. SEIPIN proteins mediate lipid droplet biogenesis to promote pollen transmission and reduce seed dormancy. *Plant Physiology* 176: 1531–1546.

Thalmann, M., and D. Santelia. 2017. Starch as a determinant of plant fitness under abiotic stress. *New Phytologist* 214: 943–951.

Therneau, T. M. 2024. coxme: Mixed Effects Cox Models. 10.32614/CRAN.package.coxme

Therneau, T. M., T. L. A. Elizabeth, and C. Cynthia. 2024. survival: Survival Analysis. 10.32614/CRAN.package.survival

Thomson, D. M., R. A. King, and E. L. Schultz. 2017. Between invaders and a risky place: Exotic grasses alter demographic tradeoffs of native forb germination timing. *Ecosphere* 8: e01987.

Thomson, D. M., J. W. Kwok, and E. L. Schultz. 2018. Extreme drought alters growth and interactions with exotic grasses, but not survival, for a California annual forb. *Plant Ecology* 219: 705–717.

Trovato, M., D. Funck, G. Forlani, S. Okumoto, and R. Amir. 2021. Editorial: Amino acids in plants: regulation and functions in development and stress defense. *Frontiers in Plant Science* 12.

Tuell, J. K., J. S. Ascher, and R. Isaacs. 2009. Wild bees (Hymenoptera: Apoidea: Anthophila) of the Michigan highbush blueberry agroecosystem. *Annals of the Entomological Society of America* 102: 275–287.

Tuell, J. K., and R. Isaacs. 2010. Weather during bloom affects pollination and yield of highbush blueberry. *Journal of Economic Entomology* 103: 557–562.

Tzin, V., and G. Galili. 2010. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Molecular Plant* 3: 956–972.

Vanderplanck, M., B. Martinet, L. G. Carvalheiro, P. Rasmont, A. Barraud, C. Renaudeau, and D. Michez. 2019. Ensuring access to high-quality resources reduces the impacts of heat stress on bees. *Scientific Reports* 9: 12596.

Vanderplanck, M., R. Moerman, P. Rasmont, G. Lognay, B. Wathelet, R. Wattiez, and D. Michez. 2014. How does pollen chemistry impact development and feeding behaviour of polylectic bees? *PLoS ONE* 9: 1–9.

van Es, S. W. 2020. Too hot to handle, the adverse effect of heat stress on crop yield. *Physiologia Plantarum* 169: 499–500.

Vara Prasad, P. V., P. Q. Craufurd, R. J. Summerfield, and T. R. Wheeler. 2000. Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypogaea* L.). *Journal of Experimental Botany* 51: 777–784.

Vaudo, A. D., H. M. Patch, D. A. Mortensen, J. F. Tooker, and C. M. Grozinger. 2016. Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences of the United States of America* 113: E4035–E4042.

Vaudo, A. D., J. F. Tooker, C. M. Grozinger, and H. M. Patch. 2015. Bee nutrition and floral resource restoration. *Current Opinion in Insect Science* 10: 133–141.

Vaudo, A. D., J. F. Tooker, H. M. Patch, D. J. Biddinger, M. Coccia, M. K. Crone, M. Fiely, et al. 2020. Pollen protein: Lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects* 11.

Wagner, D. L. 2020. Insect declines in the Anthropocene. *Annual Review of Entomology* 65: 457–480.

Waheeda, K., H. Kitchel, Q. Wang, and P.-L. Chiu. 2023. Molecular mechanism of Rubisco activase: Dynamic assembly and Rubisco remodeling. *Frontiers in Molecular Biosciences* 10: 1125922.

Wahid, A., S. Gelani, M. Ashraf, and M. R. Foolad. 2007. Heat tolerance in plants: An overview. *Environmental and Experimental Botany* 61: 199–223.

Walters, J., and R. Isaacs. 2023. Pollen germination and tube growth in northern highbush blueberry are inhibited by extreme heat. *HortScience* 58: 635–642.

Walters, J., J. Zavalnitskaya, R. Isaacs, and Z. Szendrei. 2022. Heat of the moment: extreme heat poses a risk to bee–plant interactions and crop yields. *Current Opinion in Insect Science* 52: 100927–100927.

Wang, Y., M. Q. Shahid, F. Ghouri, S. Ercişli, F. S. Baloch, and F. Nie. 2019. Transcriptome analysis and annotation: SNPs identified from single copy annotated unigenes of three polyploid blueberry crops. *PLOS ONE* 14: e0216299.

Wasielewski, O., T. Wojciechowicz, K. Giejdasz, and N. Krishnan. 2013. Overwintering strategies in the red mason solitary bee—physiological correlates of midgut metabolic activity and turnover of nutrient reserves in females of *Osmia bicornis*. *Apidologie* 44: 642–656.

Watanabe, M., R. Goto, M. Murakami, S. Komori, and A. Suzuki. 2021. Interaction between ethylene and abscisic acid and maturation in highbush blueberry. *The Horticulture Journal* 90: 14–22.

Vander Weide, J., R. Isaacs, T. Miles, P. Edger, C. Sloan, and C. Garcia-Salazar. 2024. Blueberry varieties for Michigan. MSU Extension article E3490.

Westreich, L. R., and P. C. Tobin. 2024. Effect of protein and lipids in pollen on the developmental success of the native solitary bee *Osmia lignaria* Say. *Journal of Apicultural Research* 0: 1–9.

Wickham, H., W. Chang, L. Henry, T. L. Pedersen, K. Takahashi, C. Wilke, K. Woo, et al. 2024. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. 10.32614/CRAN.package.ggplot2

Wickham, H., R. François, L. Henry, K. Müller, D. Vaughan, P. Software, and PBC. 2023. dplyr: A Grammar of Data Manipulation. 10.32614/CRAN.package.dplyr

Williams, N. M. 2003. Use of novel pollen species by specialist and generalist solitary bees (Hymenoptera: Megachilidae). *Oecologia* 134: 228–237.

Wilson, E. S., C. E. Murphy, J. P. Rinehart, G. Yocum, and J. H. Bowsher. 2020. Microclimate Temperatures impact nesting preference in *Megachile rotundata* (Hymenoptera: Megachilidae). *Environmental Entomology* 49: 296–303.

Woodard, S. H., M. A. Duennes, K. M. Watrous, and S. Jha. 2019. Diet and nutritional status during early adult life have immediate and persistent effects on queen bumble bees. *Conservation Physiology* 7: coz048.

Wright, S. J., D. M. Goad, B. L. Gross, P. R. Muñoz, and K. M. Olsen. 2022. Genetic trade-offs underlie divergent life history strategies for local adaptation in white clover. *Molecular Ecology* 31: 3742–3760.

Yang, F. H., D. R. Bryla, S. T. Orr, B. C. Strik, and Y. Zhao. 2020a. Thermal cooling with sprinklers or microsprinklers reduces heat damage and improves fruit quality in northern highbush blueberry. *HortScience* 55: 1365–1371.

Yang, F. H., D. R. Bryla, and B. C. Strik. 2019a. Critical temperatures and heating times for fruit damage in northern highbush blueberry. *HortScience* 54: 2231–2239.

Yang, F.-H., D. R. Bryla, S. T. Orr, B. C. Strik, and Y. Zhao. 2020b. Thermal cooling with sprinklers or microsprinklers reduces heat damage and improves fruit quality in northern highbush blueberry. *HortScience* 55: 1365–1371.

Yang, H., Y. Wu, C. Zhang, W. Wu, L. Lyu, and W. Li. 2022. Growth and physiological characteristics of four blueberry cultivars under different high soil pH treatments. *Environmental and Experimental Botany* 197: 104842.

Yang, Q., E. Liu, Y. Fu, F. Yuan, T. Zhang, and S. Peng. 2019b. High temperatures during flowering reduce fruit set in rabbiteye blueberry. *Journal of the American Society for Horticultural Science* 144: 339–351.

Yang, Q., D. Zhao, and Q. Liu. 2020c. Connections between amino acid metabolisms in plants: lysine as an example. *Frontiers in Plant Science* 11.

Yang, W. Q., J. Harpole, C. E. Finn, and B. C. Strik. 2009. Evaluating berry firmness and total soluble solids of newly released highbush blueberry cultivars. *Acta Horticulturae*: 863–868.

Zaleski, A. 1964. Effect of density of plant population, photoperiod, temperature and light intensity on inflorescence formation in white clover. *Grass and Forage Science* 19: 237–247.

Zdzisław, W., K. Giejdasz, and M. Fliszkiewicz. 2004. The influence of food amount consumed during the larval development on the body weight of the imago of the red mason bee (*Osmia rufa* L., Megachilidae). *Journal of Apicultural Science* 48: 38–47.

Zhang, C., G. Li, T. Chen, B. Feng, W. Fu, J. Yan, M. R. Islam, et al. 2018. Heat stress induces spikelet sterility in rice at anthesis through inhibition of pollen tube elongation interfering with auxin homeostasis in pollinated pistils. *Rice* 11: 14.

Zhang, L., Y. Dai, L. Yue, G. Chen, L. Yuan, S. Zhang, F. Li, et al. 2022. Heat stress response in Chinese cabbage (*Brassica rapa* L.) revealed by transcriptome and physiological analysis. *PeerJ* 10: e13427.

Zhang, S., and D. D. Fernando. 2005. Structural, histochemical, and protein analysis of male reproductive development in willow. *Sexual Plant Reproduction* 18: 37–46.

Zhao, H., G. Li, D. Guo, H. Li, Q. Liu, B. Xu, and X. Guo. 2021. Response mechanisms to heat stress in bees. *Apidologie* 52: 388–399.

Zhu, T., C. F. Fonseca De Lima, and I. De Smet. 2021. The heat is on: How crop growth, development, and yield respond to high temperature. *Journal of Experimental Botany* 72: 7359–7373.

Zinn, K. E., M. Tunc-Ozdemir, and J. F. Harper. 2010. Temperature stress and plant sexual reproduction: Uncovering the weakest links. *Journal of Experimental Botany* 61: 1959–1968.

## **APPENDIX A**: SUPPLEMENTARY MATERIAL FOR CHAPTER 2

**Table S2.1.** Mean pollen tube length (± SE) of four northern highbush blueberry cultivars after two durations of exposure to 10, 20, 30, or 40°C. Values within each cultivar and sampling time with a common letter are not statistically different at $P < 0.05$, based on a Sidak post-hoc test. Length data could not be taken on pollen tubes that failed to successfully germinate and were excluded from statistical analysis, indicated by NA. Statistical comparisons of the mean pollen tube lengths were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data. Non-significant results ($P > 0.05$) are indicated by NS.

| | | Mean ± SE pollen tube length (mm) | |
|---|---|---|---|
| Cultivar | Temperature (°C) | 4 h | 24 h |
| 'Bluecrop' | 10 | 0.04 ± 0.01 b | 0.27 ± 0.02 b |
| | 20 | 0.13 ± 0.02 a | 0.43 ± 0.04 a |
| | 30 | 0.20 ± 0.03 a | 0.43 ± 0.04 a |
| | 40 | NA | 0.09 ± 0.01 c |
| | | $\chi^2 = 35.96$, df = 2, $P < 0.001$ | $\chi^2 = 65.95$, df = 3, $P < 0.001$ |
| 'Elliott' | 10 | 0.04 ± 0.01 b | 0.33 ± 0.02 b |
| | 20 | 0.15 ± 0.02 a | 0.42 ± 0.03 ab |
| | 30 | 0.17 ± 0.02 a | 0.45 ± 0.03 a |
| | 40 | NA | NA |
| | | $\chi^2 = 12.08$, df = 2, $P < 0.01$ | $\chi^2 = 10.35$, df = 2, $P < 0.01$ |
| 'Jersey' | 10 | NA | 0.18 ± 0.04 a |
| | 20 | 0.15 ± 0.01 b | 0.31 ± 0.06 a |
| | 30 | 0.24 ± 0.02 a | 0.28 ± 0.06 a |
| | 40 | 0.06 ± 0.01 c | NA |
| | | $\chi^2 = 37.96$, df = 2, $P < 0.001$ | $\chi^2 = 3.70$, df = 2, NS |
| 'Liberty' | 10 | NA | 0.17 ± 0.05 a |
| | 20 | 0.12 ± 0.01 b | 0.23 ± 0.07 a |
| | 30 | 0.17 ± 0.01 a | 0.30 ± 0.09 a |
| | 40 | 0.03 ± 0.01 c | NA |
| | | $\chi^2 = 70.35$, df = 2, $P < 0.001$ | $\chi^2 = 3.92$, df = 2, NS |

**Table S2.2.** Mean pollen tube length (± SE) of four northern highbush blueberry cultivars after two durations of exposure to 30, 32.5, 35, 37.5, or 40°C. Values within each cultivar and sampling time with a common letter are not statistically different at $P < 0.05$, based on a Sidak post-hoc test. Length data could not be taken on pollen tubes that failed to successfully germinate, indicated by NA. Statistical comparison of mean pollen tube lengths were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data. Non-significant results ($P > 0.05$) are indicated by NS.

| | | Mean ± SE pollen tube length (mm) | |
|---|---|---|---|
| Cultivar | Temperature (°C) | 4 h | 24 h |
| 'Bluecrop' | 30 | 0.34 ± 0.02 a | 0.45 ± 0.08 a |
| | 32.5 | 0.28 ± 0.02 a | 0.34 ± 0.06 ab |
| | 35 | 0.16 ± 0.01 b | 0.27 ± 0.05 ab |
| | 37.5 | 0.09 ± 0.01 c | 0.19 ± 0.04 b |
| | 40 | NA | NA |
| | | $\chi^2 = 20.91$, df = 3, $P < 0.001$ | $\chi^2 = 10.73$, df = 3, $P < 0.05$ |
| 'Elliott' | 30 | 0.35 ± 0.04 a | 0.49 ± 0.09 a |
| | 32.5 | 0.23 ± 0.03 a | 0.43 ± 0.08 ab |
| | 35 | 0.25 ± 0.03 a | 0.40 ± 0.07 ab |
| | 37.5 | 0.08 ± 0.01 b | 0.22 ± 0.04 b |
| | 40 | NA | 0.04 ± 0.02 c |
| | | $\chi^2 = 62.57$, df = 3, $P < 0.001$ | $\chi^2 = 24.52$, df = 4, $P < 0.001$ |
| 'Jersey' | 30 | 0.28 ± 0.06 a | 0.44 ± 0.11 a |
| | 32.5 | 0.25 ± 0.06 ab | 0.48 ± 0.12 a |
| | 35 | 0.11 ± 0.02 bc | 0.27 ± 0.07 ab |
| | 37.5 | 0.07 ± 0.02 c | 0.15 ± 0.04 b |
| | 40 | NA | NA |
| | | $\chi^2 = 24.24$, df = 3, $P < 0.001$ | $\chi^2 = 11.33$, df = 3, $P < 0.05$ |
| 'Liberty' | 30 | 0.28 ± 0.06 a | 0.43 ± 0.13 a |
| | 32.5 | 0.22 ± 0.05 ab | 0.32 ± 0.10 a |
| | 35 | 0.22 ± 0.05 ab | 0.37 ± 0.11 a |
| | 37.5 | 0.11 ± 0.02 b | 0.18 ± 0.05 a |
| | 40 | NA | NA |
| | | $\chi^2 = 10.12$, df = 3, $P < 0.05$ | $\chi^2 = 3.35$, df = 3, NS |

**Table S2.3.** Mean pollen tube length (± SE) of 'Bluecrop' northern highbush blueberry after three durations of exposure to three different temperature regimes (CT = 25°C, HT = 37.5°C, RT = 37.5°C for 4 h and 25°C for 20 h). Mean values with a common letter are not statistically different at $P < 0.05$ within each sampling time, based on a Sidak post-hoc test. Statistical comparison of mean pollen tube length was derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data.

| Sampling time (h) | Temperature regime | Mean ± SE pollen tube length (mm) |
|---|---|---|
| 4 | CT | 0.43 ± 0.05 a |
| | HT | 0.11 ± 0.01 b |
| | RT | 0.10 ± 0.01 b |
| | | $\chi^2 = 124.74$, df = 2, $P < 0.001$ |
| 10 | CT | 0.70 ± 0.07 a |
| | HT | 0.25 ± 0.02 b |
| | RT | 0.29 ± 0.03 b |
| | | $\chi^2 = 72.24$, df = 2, $P < 0.001$ |
| 24 | CT | 0.70 ± 0.07 a |
| | HT | 0.21 ± 0.02 c |
| | RT | 0.31 ± 0.03 b |
| | | $\chi^2 = 86.11$, df = 2, $P < 0.001$ |

**Data analysis for Tables S2.4-S2.6**

All data were analyzed in RStudio (Version 4.2.2). The proportion of germinated pollen tetrads that produced more than one pollen tube among temperature treatments was analyzed separately for each cultivar and assessment time. The mean proportion of tetrads with two or more pollen tubes was calculated within each experimental unit (i.e., Petri dishes) resulting in 60 total tetrads observed for each cultivar, temperature, and assessment time. A generalized linear model (GLM) with a Gaussian distribution was used to compare pollen germination among temperatures (glm(mean germination success ~ temperature, family= gaussian)). Models were tested for normality by comparing deviance residual values and using the Shapiro-Wilk test. For normally distributed data, a one-way analysis of variance (ANOVA) was used to determine if temperature had a significant effect on pollen germination for each cultivar and assessment time. For data that were not normally distributed, a Kruskal Wallis test of significance was used. If these tests were significant ($P < 0.05$), a Sidak post-hoc test was used for pairwise comparisons among means. Statistically different means are represented by different letters in tables and figures ($P < 0.05$). The number of pollen tetrads that produced 2, 3, or 4 pollen tubes was also summarized out of 60 total pollen tetrads observed for each cultivar, temperature, and assessment time.

**Table S2.4.** The number of pollen tetrads that produced 2, 3, or 4 pollen tubes per tetrad and the mean (± SE) proportion of pollen tetrads that produced more than one pollen tube of four northern highbush blueberry cultivars after two durations of exposure to 10, 20, 30, or 40°C. Values within each cultivar and sampling time with a common letter are not statistically different at $P < 0.05$, based on a Sidak post-hoc test. Statistical comparison of mean proportion of pollen tetrads that produced more than one pollen tube were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data. Non-significant results ($P > 0.05$) are indicated by NS.

| | | 4 h | | | | 24 h | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cultivar | Temp (°C) | No. pollen tubes per tetrad | | | Proportion of tetrads with two or more pollen tubes | No. pollen tubes per tetrad | | | Proportion of tetrads with two or more pollen tubes |
| | | 2 | 3 | 4 | Mean (± SE) | 2 | 3 | 4 | Mean (± SE) |
| 'Bluecrop' | 10 | 0 | 0 | 0 | 0.00 ± 0.00 b | 10 | 8 | 2 | 0.33 ± 0.03 b |
| | 20 | 6 | 0 | 0 | 0.10 ± 0.01 b | 23 | 16 | 4 | 0.72 ± 0.02 a |
| | 30 | 10 | 8 | 4 | 0.37 ± 0.03 a | 18 | 22 | 8 | 0.80 ± 0.03 a |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 b | 0 | 0 | 0 | 0.00 ± 0.00 c |
| | | | | | $\chi^2 = 18.10$, df = 3, $P<0.001$ | | | | $\chi^2 = 61.24$, df = 3, $P<0.001$ |
| 'Elliott' | 10 | 0 | 0 | 0 | 0.00 ± 0.00 b | 6 | 0 | 0 | 0.10 ± 0.02 bc |
| | 20 | 0 | 0 | 0 | 0.00 ± 0.00 b | 13 | 1 | 1 | 0.25 ± 0.02 ab |
| | 30 | 8 | 2 | 0 | 0.17 ± 0.02 a | 17 | 5 | 1 | 0.38 ± 0.02 a |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 b | 0 | 0 | 0 | 0.00 ± 0.00 c |
| | | | | | $\chi^2 = 13.66$, df = 3, $P<0.01$ | | | | $\chi^2 = 36.00$, df = 3, $P<0.001$ |
| 'Jersey' | 10 | 0 | 0 | 0 | 0.00 ± 0.00 b | 5 | 0 | 0 | 0.08 ± 0.01 b |
| | 20 | 10 | 1 | 0 | 0.18 ± 0.02 a | 18 | 2 | 1 | 0.35 ± 0.02 a |
| | 30 | 6 | 2 | 0 | 0.13 ± 0.01 ab | 15 | 3 | 0 | 0.30 ± 0.02 a |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 b | 0 | 0 | 0 | 0.00 ± 0.00 b |
| | | | | | $\chi^2 = 15.16$, df = 3, $P<0.01$ | | | | $\chi^2 = 29.71$, df = 3, $P<0.001$ |
| 'Liberty' | 10 | 0 | 0 | 0 | 0.00 ± 0.00 a | 0 | 0 | 0 | 0.00 ± 0.00 b |
| | 20 | 2 | 0 | 0 | 0.03 ± 0.01 a | 9 | 3 | 1 | 0.22 ± 0.03 a |
| | 30 | 2 | 0 | 0 | 0.03 ± 0.01 a | 6 | 0 | 0 | 0.10 ± 0.01 ab |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 a | 0 | 0 | 0 | 0.00 ± 0.00 b |
| | | | | | $\chi^2 = 4.60$, df = 3, NS | | | | $\chi^2 = 13.89$, df = 3, $P<0.01$ |

**Table S2.5.** The number of pollen tetrads that produced 2, 3, or 4 pollen tubes per tetrad and the mean (± SE) proportion of pollen tetrads that produced more than one pollen tube of four northern highbush blueberry cultivars after two durations of exposure to 30, 32.5, 35, 37.5, or 40°C. Values within each cultivar and sampling time with a common letter are not statistically different at $P < 0.05$, based on a Sidak post-hoc test. Statistical comparison of mean proportion of pollen tetrads that produced more than one pollen tube were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data. Non-significant results ($P > 0.05$) are indicated by NS.

| | | 4 h | | | | 24 h | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cultivar | Temp (°C) | No. pollen tubes per tetrad | | | Proportion of tetrads with two or more pollen tubes | No. pollen tubes per tetrad | | | Proportion of tetrads with two or more pollen tubes |
| | | 2 | 3 | 4 | Mean (± SE) | 2 | 3 | 4 | Mean (± SE) |
| 'Bluecrop' | 30 | 10 | 22 | 17 | 0.82 ± 0.03 a | 12 | 21 | 15 | 0.80 ± 0.02 a |
| | 32.5 | 18 | 20 | 8 | 0.77 ± 0.03 a | 16 | 28 | 6 | 0.83 ± 0.02 a |
| | 35 | 29 | 12 | 3 | 0.73 ± 0.02 a | 20 | 19 | 3 | 0.70 ± 0.02 a |
| | 37.5 | 12 | 0 | 1 | 0.22 ± 0.02 b | 19 | 4 | 0 | 0.38 ± 0.03 b |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 b | 0 | 0 | 0 | 0.00 ± 0.00 c |
| | | | | | $\chi^2 = 22.93$, df = 4, $P<0.001$ | | | | $\chi^2 = 22.33$, df = 4, $P<0.001$ |
| 'Elliott' | 30 | 13 | 3 | 1 | 0.28 ± 0.02 ab | 16 | 10 | 6 | 0.53 ± 0.03 a |
| | 32.5 | 14 | 6 | 1 | 0.35 ± 0.02 a | 23 | 8 | 2 | 0.55 ± 0.02 a |
| | 35 | 17 | 2 | 0 | 0.32 ± 0.03 ab | 20 | 9 | 1 | 0.50 ± 0.02 a |
| | 37.5 | 3 | 2 | 0 | 0.08 ± 0.01 bc | 2 | 0 | 0 | 0.03 ± 0.01 b |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 c | 0 | 0 | 0 | 0.00 ± 0.00 b |
| | | | | | $\chi^2 = 28.93$, df = 4, $P<0.001$ | | | | $\chi^2 = 98.20$, df = 4, $P<0.001$ |
| 'Jersey' | 30 | 5 | 1 | 0 | 0.10 ± 0.02 a | 11 | 0 | 0 | 0.18 ± 0.02 a |
| | 32.5 | 6 | 1 | 0 | 0.12 ± 0.02 a | 10 | 2 | 1 | 0.22 ± 0.03 a |
| | 35 | 5 | 0 | 0 | 0.08 ± 0.01 a | 7 | 0 | 0 | 0.12 ± 0.02 a |
| | 37.5 | 1 | 0 | 0 | 0.02 ± 0.01 a | 1 | 0 | 0 | 0.02 ± 0.01 a |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 a | 0 | 0 | 0 | 0.00 ± 0.00 a |
| | | | | | $\chi^2 = 7.64$, df = 4, NS | | | | $\chi^2 = 10.31$, df = 4, NS |
| 'Liberty' | 30 | 18 | 2 | 0 | 0.33 ± 0.02 a | 18 | 4 | 0 | 0.37 ± 0.01 a |
| | 32.5 | 15 | 6 | 0 | 0.35 ± 0.03 a | 26 | 3 | 0 | 0.48 ± 0.02 a |
| | 35 | 14 | 6 | 0 | 0.33 ± 0.01 a | 14 | 8 | 1 | 0.38 ± 0.02 a |
| | 37.5 | 3 | 0 | 0 | 0.05 ± 0.01 b | 2 | 0 | 0 | 0.03 ± 0.01 b |
| | 40 | 0 | 0 | 0 | 0.00 ± 0.00 b | 0 | 0 | 0 | 0.00 ± 0.00 b |
| | | | | | $\chi^2 = 21.15$, df = 4, $P<0.001$ | | | | $\chi^2 = 23.20$, df = 4, $P<0.001$ |

**Table S2.6.** The number of pollen tetrads that produced 2, 3, or 4 pollen tubes per tetrad and the mean (± SE) proportion of pollen tetrads that produced more than one pollen tube of 'Bluecrop' northern highbush blueberry after three durations of exposure to three different temperature regimes (CT = 25°C, HT = 37.5°C, RT = 37.5°C for 4 h and 25°C for 20 h). Mean values with a common letter are not statistically different at $P < 0.05$ within each sampling time, based on a Sidak post-hoc test. Statistical comparison of mean proportion of pollen tetrads that produced more than one pollen tube were derived from an ANOVA test for normally distributed data or a Kruskal Wallis test for non-normally distributed data. Non-significant results ($P > 0.05$) are indicated by NS.

| Sampling time (h) | Temperature regime | No. pollen tubes per tetrad | | | Proportion of tetrads with two or more pollen tubes |
|---|---|---|---|---|---|
| | | 2 | 3 | 4 | Mean (± SE) |
| 4 | CT | 8 | 14 | 20 | 0.70 ± 0.04 a |
| | HT | 24 | 0 | 0 | 0.40 ± 0.03 ab |
| | RT | 13 | 5 | 0 | 0.30 ± 0.02 b |
| | | | | | $\chi^2 = 6.20$, df = 2, $P<0.05$ |
| 10 | CT | 7 | 12 | 30 | 0.82 ± 0.03 a |
| | HT | 23 | 2 | 0 | 0.42 ± 0.03 a |
| | RT | 16 | 12 | 1 | 0.48 ± 0.04 a |
| | | | | | $\chi^2 = 5.91$, df = 2, NS |
| 24 | CT | 3 | 13 | 30 | 0.77 ± 0.04 a |
| | HT | 18 | 5 | 1 | 0.40 ± 0.02 b |
| | RT | 12 | 12 | 1 | 0.42 ± 0.03 ab |
| | | | | | $\chi^2 = 7.27$, df = 2, $P<0.05$ |

## **APPENDIX B:** SUPPLEMENTARY MATERIAL FOR CHAPTER 4

**Table S4.1.** Model type, model response, response type, error distribution, link function, and model R syntax for all models in manuscript. Reference A-P is used to refer to each model, with greater detail in the following text.

| Reference | Model type | Model response | Response type | Error distribution | Link function | Model R syntax |
|---|---|---|---|---|---|---|
| A | GLMM | Number of open flowers | Count | Negative binomial | N/A | glmer.nb(num.open.fl~temp+(1\|obs.date)+(1\|initial.placement.date)+ (1\|plant.ID)+(1\|block.ID)+(1\|block.ID:cage.num),data=data) |
| B | GLMM | Number of flowers visited (per 30-min) | Count | Negative binomial | N/A | glmer.nb(num.open.fl~temp+(1\|obs.date)+(1\|obs.date:start.time)+ (1\|initial.placement.date)+(1\|plant.ID)+(1\|block.ID)+ (1\|block.ID:cage.num),data=data) |
| C | GLMM | Number of eggs laid (initiated egg laying) | Count | Negative binomial | N/A | glmer.nb(total.num.eggs.laid~temp+(1\|Bee.ID)+(1\|BlockID)+ (1\|BlockID:field.cage.num),data =data) |
| D | GLMM | Number of eggs laid (lived >6 days) | Count | Negative binomial | N/A | glmer.nb(total.num.eggs.laid~temp+(1\|Bee.ID)+(1\|BlockID)+ (1\|BlockID:field.cage.num),data =data) |
| E | GLMM | Pollen provision weight (g) | Positive, continous | Gamma | Inverse | glmer(poll.prov.WT.g~temp+(1\|BeeID)+(1\|date.egg.laid)+ (1\|loco.in.stem)+(1\|BlockID)+(1\|BlockID:cage.loco), family=Gamma(link="inverse"), data=data) |
| F | GLMM | Larval development duration (days) | Count | Negative binomial | N/A | glmer.nb(num.days~dev.stage*temp+(1\|MotherBeeID)+(1\|MotherBeeID: well.plate.code)+(1\|BlockID)+(1\|BlockID:cage.num),data=data) |
| G | Mixed effect Cox model | Larval survival (time, survival) | Proportion | Proportional hazards regression | N/A | coxme(Surv(time.days, survival.binary) ~ temp.group+(1\|BlockID/cage.num)+(1\|Mother.Bee.ID)+ (1\|well.plate.num), data=data) |
| H | GLMM | Pre-winter cocoon weight (g) | Positive, continous | Gamma | Inverse | glmer(pre.winter.WT.g~temp+(1\|well.plate.num)+(1\|BlockID)+ (1\|BlockID:cage.num), family=Gamma(link="inverse"), data=data) |
| I | GLMM | Post-winter cocoon weight (g) | Positive, continous | Gamma | Inverse | glmer(post.winter.WT.g~temp+(1\|well.plate.num)+(1\|BlockID)+ (1\|BlockID:cage.num), family=Gamma(link="inverse"), data=data) |
| J | GLMM | Weight lost after winter (g) | Positive, continous | Gamma | Inverse | glmer(WT.lost.after.winter.g~temp+(1\|well.plate.num)+(1\|BlockID)+ (1\|BlockID:cage.num), family=Gamma(link="inverse"), data=data) |
| K | Mixed effect Cox model | Adult emergence (time, emergence) | Proportion | Proportional hazards regression | N/A | coxme(Surv(time.days, emergence.binary)~temp.group+ (1\|BlockID/cage.num)+(1\|Mother.Bee.ID)+(1\|well.plate.num), data=data) |
| L | GLMM | Adult emergence timing (days) | Count | Poisson | Log | glmer.nb(num.days.till.emerge~temp+ (1\|BlockID)+(1\|BlockID:cage.num)+(1\|well.plate.num), family=poisson(link="log"), data =data) |
| M | Mixed effect Cox model | Adult survival (time, survival) | Proportion | Proportional hazards regression | N/A | coxme(Surv(time.days, survival.binary)~temp.group+ (1\|BlockID/cage.num)+(1\|Mother.Bee.ID)+(1\|well.plate.num), data=data) |
| N | GLMM | Adult longevity (days) | Count | Poisson | Log | glmer.nb(num.days.alive.adult~temp+ (1\|BlockID)+(1\|BlockID:cage.num)+(1\|well.plate.num), family=poisson(link="log"), data =data) |
| O | GLMM | Intertegular distance (ITD) (mm) | Positive, continous | Gamma | Inverse | glmer(tegular.distance.mm~temp+(1\|well.plate.num)+(1\|BlockID)+ (1\|BlockID:cage.num), family=Gamma(link="inverse"),data = data) |
| P | GLMM | Adult body weight (g) | Positive, continous | Gamma | Inverse | glmer(adult.body.WT.g~temp+(1\|well.plate.num)+(1\|BlockID)+ (1\|BlockID:cage.num), family=Gamma(link="inverse"),data = data) |

## MODEL DESCRIPTIONS

### *Bee behavior*

**Model A**. I used a generalized linear mixed model (GLMM) with a negative binomial distribution to test the effects of host plant temperature exposure on floral access, where the response variable was the number of open flowers. Observation date, initial plant placement date, plant identity, block identity/cage identity were included as random effects. Each plant type (blueberry, phacelia, clover) was analyzed separately to account for inherent differences in flowering morphology and phenology.

**Model B**. To test the effects of temperature regime on flower visitation, I used a GLMM with a negative binomial distribution. The response variable was the number of flowers visited (per 30-

minute observation) and observation date/observation time, plant identity, initial plant placement date, block identity/cage identity were included as random effects. Each plant type (blueberry, phacelia, and clover) was analyzed separately to account for inherent differences in flowering morphology and phenology.

**Model C and D.** To test the effects of host plant temperature exposure on female fecundity, a GLMM with a negative binomial distribution was used. The response variable was the total number of eggs laid by each bee and mother bee identity and block identity/cage identity were included as random effects. A unique identifier for each mother bee was made using the 'dplyr' package, considering the thorax paint color associated with each bee, the field cage it was placed in, and the date when it was released into a field cage, ensuring each mother bee was associated with the egg(s) it laid. This model structure was used for two sets of the data. In the first model, we excluded all bees who failed to produce eggs, testing whether temperature treatment affected fecundity of individuals who successfully initiated egg laying. In the second model, we excluded all bees who lived for < 6 days, as these bees did not live long enough to begin egg laying, but otherwise included all bees.

**Model E.** A GLMM with a Gamma distribution and inverse link function was used to test the effects of host plant temperature exposure on the amount of pollen provisions collected by female bees, where the response variable was the weight (g) of pollen provided to each egg and mother bee identity, egg laying date, cavity location, block identity/cage identity were included as random effects.

***Larval development and survival***

**Model F.** To determine whether temperature exposure of host plants affected the duration of development for larvae consuming host plant pollen, a GLMM with negative binomial distribution was used where the response variable was time (days) for each larva to reach a given development stage (egg/1$^{st}$ instar, 2$^{nd}$/3$^{rd}$ instar, 3$^{rd}$/4$^{th}$ instar, 5$^{th}$ instar, cocoon spinning, or fully spun cocoon). Development stages and temperature regime were included as fixed effects and mother bee identity/larva identity, block identity/cage identity were included as random effects.

**Model G**. To test the effects of host plant temperature exposure on larval survival, we used a mixed effect Cox model, where we created a survival object that included time (days) and a binary indicator for larval survival as the response variable. Mother bee identity, well plate number, block identity/cage identity were included as random effects. Well plate number refers to the 48-well cell culture plate (1-9) a given offspring was placed and reared.

**Model H.** Progeny that successfully pupated were evaluated for the effects of host plant temperature exposure on pre-winter cocoon weight using a GLMM with a Gamma distribution and inverse link function. Pre-winter cocoon weight (g) was included as the response variable and well plate number, block identity/cage identity were included as random effects.

**Model I.** Progeny that successfully pupated were evaluated for the effects of host plant temperature exposure on post-winter cocoon weight using a GLMM with a Gamma distribution and inverse link function. Post-winter cocoon weight (g) was included as the response variable and well plate number, block identity/cage identity were included as random effects.

**Model J.** Progeny that successfully pupated were evaluated for the effects of host plant temperature exposure on the pupae weight lost during overwintering, using a GLMM with a Gamma distribution and inverse link function. Weight lost during overwintering (g) was included as the response variable and well plate number, block identity/cage identity were included as random effects.

***Adult emergence, survival, body weight and size***

**Model K**. A mixed effects Cox model was used to determine whether host plant temperature exposure affected adult emergence, where an object that included time (days) and a binary indicator for emergence was included as the response variable and mother bee identity, well plate number, block identity/cage identity were included as random effects.

**Model L**. To determine the effect of host plant temperature on progeny emergence timing, we used a GLMM with a Poisson distribution and log link function where the number of days until emergence was included as the response variable and well plate number, block identity/cage identity were included as random effects.

**Model M**. To test the effects of host plant temperature exposure on adult bee survival, we again used a mixed effect Cox model. I created a response variable object that included time (days) and a binary indicator for adult survival and mother bee identity, well plate number, block identity/cage identity were included as random effects.

**Model N**. To test the effects of host plant temperature on the longevity of adult bees, we used a GLMM with a Poisson distribution and log link function where the number of days alive was included as the response variable and well plate number, block identity/cage identity were included as random effects.

**Model O**. To test the effects of host plant temperature exposure on the intertegular distance (ITD) of adult bee progeny, we used a GLMM with a Gamma distribution and inverse link function where ITD (mm) was included as the response variable and well plate number, block identity/cage identity were included as random effects.

**Model P**. To test the effects of host plant temperature exposure on the body weights of adult bee progeny, we used a GLMM with a Gamma distribution and inverse link function where body weight (g) was included as the response variable and well plate number, block identity/cage identity were included as random effects.