HortScience 58(6):635–642. 2023. https://doi.org/10.21273/HORTSCI17075-23

Pollen Germination and Tube Growth in Northern Highbush Blueberry are Inhibited by Extreme Heat

Jenna Walters and Rufus Isaacs

Department of Entomology, Michigan State University, East Lansing, MI 48824, USA; and Program in Ecology, Evolution, and Behavior, Michigan State University, East Lansing, MI 48824, USA

Keywords. blueberries, climate change, crop, extreme heat, pollination, reproduction

Abstract. **The increasing intensity and frequency of extreme heat events threaten crop productivity globally. Certain phases of plant reproduction necessary for fertilization are highly sensitive to extreme heat, particularly during pollen development, germination, and tube elongation. However, few studies have assessed the effects of extreme heat on pollen performance in perennial crop plants. To understand how northern highbush blueberry pollen responds to high temperatures, we quantified pollen germination and pollen tube growth in vitro using four commercially relevant cultivars (Bluecrop, Elliott, Jersey, and Liberty) in climate-controlled chambers. We also tested recovery from high heat in 'Bluecrop' to determine whether pollen tubes can still germinate and grow after short bursts of extreme heat. We found the highest proportion of germinated pollen tetrads and the greatest pollen tube growth at 20 and 30 °C, and the lowest levels at 10 and 40 °C, with nearly complete inhibition at 40 °C. Exposure to between 30 and 40 °C revealed significant reduction in pollen germination and tube growth 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 pollen tube growth, even after pollen was moved to optimal conditions of 25 °C. Extreme heat exposure, even for a short duration, significantly limits blueberry pollen germination and tube development. This is expected to have cascading effects on fruit set and crop yield. The nonreversibility of the effects on pollen highlights the need to prevent fields reaching damaging temperatures by developing crop monitoring and management strategies to protect crops during bloom.**

The intensity and frequency of extreme heat events are increasing in every region of the globe [Intergovernmental Panel on Climate Change (IPCC) 2021], with negative consequences for agricultural production (Hatfield et al. 2020; IPCC 2021; Lohani et al. 2020; Mesihovic et al. 2016; van Es 2020). Global yields are expected to decline by 2.5% to 10% across various crops in the 21st 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 to 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 (Hatfield and Prueger 2015; Mesihovic et al. 2016; Walters et al. 2022; Zinn et al. 2010). Although 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 by acute heat extremes compared with 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 (Hatfield et al. 2020; Lohani et al. 2020; Müller and Rieu 2016; Zinn et al. 2010). 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 (Lohani et al. 2020; Mesihovic et al. 2016; Snider and Oosterhuis 2011; Zinn et al. 2010). 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; Lohani et al. 2020; Mesihovic et al. 2016; Snider and Oosterhuis 2011). 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 (Mesihovic et al. 2016; Snider and Oosterhuis 2011).

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 to 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 (Mesihovic et al. 2016; Raja et al. 2019; Zinn et al. 2010). 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 ×ananassa*), pollen germination was significantly reduced when exposed to 30 °C for just 4 h compared with 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 (Hamidou et al. 2013; Hedhly et al. 2005; 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

Received for publication 4 Jan 2023. Accepted for publication 21 Mar 2023.
Published online 28 Apr 2023.
We thank Henry Pointon, Jacquelyn Perkins, Andie Veach, Alexandrea Peake, McKenna Barlass, Robin Fisher, and Michael Killewald for technical assistance with this research. We also thank Madeline Henrickson and Allison Zahorec for technical assistance and resources provided for this study, and Chrislyn Particka for greenhouse support. Funding for this study was provided by Project GREEEN, The Roger and Barbara Hoopingarner Endowed Graduate Fellowship, and by the US Department of Agriculture through the National Institute of Food and Agriculture (award no. 2020-51181-32155) via the Specialty Crops Research Initiative.
J.W. was involved in conceptualization, experimental work, formal analysis, and prepared the original draft. R.I. was involved in conceptualization, supervision, providing resources, reviewing, and editing.
Datasets used for analysis are accessible online at Figshare (DOI: 10.6084/m9.figshare.20755780).
J.W. is the corresponding author. E-mail: walte309@msu.edu.

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 that affect blueberry fruit size and thus affect yields (Dogterom et al. 2000; Dogterom and Winston 1999; Gan et al. 2020). However, pollen performance in northern highbush blueberry is not well characterized (Gan et al. 2020; Yang et al. 2019b), 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 with '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 preceding 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 et al. (2019b) 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 greater than 90% at 20 and 25 °C, whereas at 30 and 35 °C, the percentage of styles with germinating pollen was reduced to 63% and 57%, respectively. Yang et al. (2019b) 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 (Malikov et al. 2020; Motha and Baier 2005), but the negative impacts of extreme heat on crop production are already evident. In May 2018, the main blueberry production region in Michigan experienced midday conditions of <32 °C for more than 4 h 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% to 50% lower than normal. Reductions in blueberry production due to extreme heat have also been observed globally, with Yang et al. (2019b) listing temperature stress as the biggest hindrance to rabbiteye blueberry flowering and fruit set in 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.

Materials and Methods

Plant material and maintenance

Dormant 2-year-old northern highbush blueberry bushes were purchased in late winter (Hartmann Nursery, Grand Junction, MI, and DeGrandchamp Farms, South Haven, MI, USA). Four commercially relevant cultivars were selected for these studies, including Bluecrop, Elliott, Jersey, and Liberty. Dormant bushes were immediately placed in dark cold storage (Michigan State University Horticultural Teaching and Research Center, Holt, MI, USA) 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 laboratory 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, CO, USA) after removal from cold storage to reduce pest infestation. Plants were watered regularly to maintain moist soil and soil pH was monitored every 3 to 4 weeks to ensure it was <6.0. When necessary, bushes were treated with Jobe's Organics soil acidifier [calcium sulfate (80%), sulfur (18%), and bentonite clay (2%); Easy Gardener Products, Inc., Waco, TX, USA] 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, OH, USA] granules were also added to potted blueberry bushes following the manufacturer's label.

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, CO, USA) onto the outside of the corolla. The pollen was collected on the surface of a 16 × 60-mm petri dish (Fisher Scientific, Hampton, NH, USA) 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 subsequently for each experiment. To record temperatures in growth chambers, HOBO data loggers (HOBO Pendant Temperature/Light Data Logger; Onset Computer Corporation, Bourne, MA, USA) were placed in each chamber, and these recorded temperature every 30 min.

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 100× light microscope. For each sample, a randomized sample of 10 pollen tetrads was observed per petri dish using an eyepiece graticule (10 mm/100 Division Reticle eyepiece with crossline; ACCU-SCOPE, Commack, NY, USA). 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 (Jumrani et al. 2018; Lang and Parrie 1992). 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.

In Expt. 1, we investigated how pollen germination and growth responded to a range of temperatures between 10 and 40 °C. For each cultivar described earlier, 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 earlier, 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 Expt. 1, we conducted Expt. 2 to determine the temperature threshold inhibiting pollen germination and tube growth. We exposed pollen from cultivars described earlier to 30, 32.5, 35, 37.5, or 40 °C. This experiment followed similar methods described in Expt. 1, but due to the COVID-19 pandemic, some bushes for this experiment were moved from cold storage to our greenhouse (before the Mar 2020 shutdown), and some bushes were moved into our laboratory space (in late Apr 2020) and grown under grow lights (SolarFlare 220 LED Grow Light; California Lightworks, Canoga Park, CA, USA). No significant differences were found among pollen samples taken from the two conditions, and thus they were analyzed together. In this experiment, we used the same experimental design and replication as described earlier.

We conducted Expt. 3 to determine the potential for blueberry pollen germination and tube growth to recover after brief exposure to extreme heat. We compared the Bluecrop cultivar pollen 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, we assessed the proportion of successfully germinated pollen grains and the length of pollen tubes as described earlier. After 4, 10, and 24 h of exposure, petri dishes were removed from growth chambers and evaluated as described earlier.

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 whether 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 ($P < 0.05$).

Results

Expt. 1: Pollen response to 10 to 40 °C

Pollen germination. Across all cultivars and sampling times, temperature significantly affected pollen germination (Table 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 with 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 limited and significantly lower than at 20 or 30 °C across all cultivars and sampling times (Table 1). Across all cultivars and sampling times, no pollen tetrads exposed to 40 °C produced more than one pollen tube (Supplemental Table 4).

Pollen tube length. Pollen tube length was significantly affected by temperature for most cultivars and sampling times (Fig. 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 ~7- to 8-fold (respectively) from 4 to 24 h (Supplemental Table 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 to 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.

Expt. 2: Pollen response to 30 to 40 °C

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). For each of the tested cultivars and assessment times, the highest germination proportions were observed at 30 to 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 with 30 to 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 to 35 °C, whereas at 37.5 °C, germination ranged from 0.20 to 0.73 (Table 2). 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 with 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 5).

Pollen tube length. Temperature had a significant effect on pollen tube length for all cultivars and sampling times except for 'Liberty' at 24 h (Fig. 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 with 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 with 30 °C at 24 h for 'Bluecrop', 'Elliott', 'Jersey', and 'Liberty', respectively (Supplemental Table 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 with all other temperatures sampled (Supplemental Table 2).

Expt. 3: Recovery from extreme heat

Pollen germination. Germination rates of 'Bluecrop' pollen were significantly affected

Table 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.

Cultivar	Temperature (°C)	Mean ± *SE* proportion germination	
		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 analysis of variance test for normally distributed data or a Kruskal–Wallis test for nonnormally distributed data.

by CT, HT, or RT temperature regimes at 4 and 24 h, but not at 10 h (Table 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 with CT conditions, the proportion of pollen tetrads with more than one pollen tube was also reduced for HT and RT groups by ~30% to 40% across all sampling times (Supplemental Table 6).

Pollen tube length. Temperature regime had a significant effect on pollen tube length at all assessment times (Fig. 3). Pollen tube lengths were significantly shorter in HT and RT groups compared with 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 3). At 4 h, pollen tube lengths for HT and RT groups were 74% and 77% shorter, respectively, compared with the CT group. This trend continued at 10 and 24 h, where pollen tube lengths were shorter in the HT and RT groups compared with 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.

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 (Snider and Oosterhuis 2011; Yang et al. 2019b; Zinn et al. 2010). 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. We 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, we found that short bursts of extreme heat, only 4 h 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*) (Austin et al. 1998; Cerovlć and Ružić 1992; 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 et al. (2019b) 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 et al. 2019b). It is possible that the in situ methods used by Yang et al. (2019b) with 'Gardenblue' pollen measured in 'Brightwell' pistils contributed to a greater

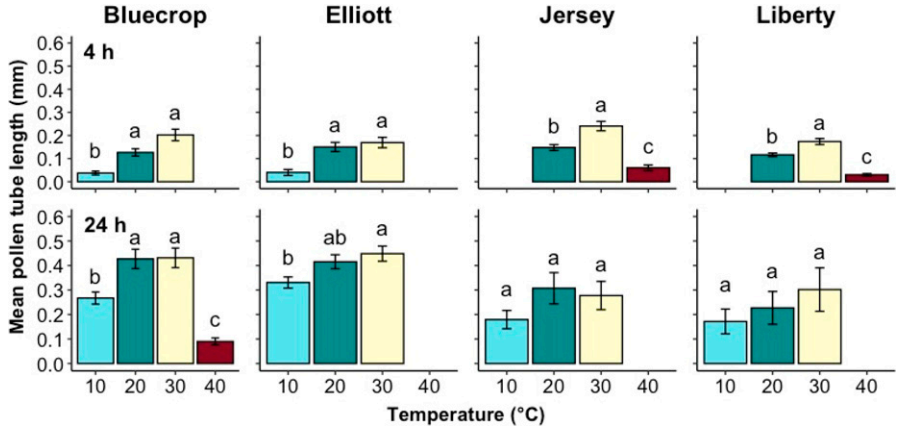

Fig. 1. Mean pollen tube length of four northern highbush blueberry cultivars after 4 and 24 h of exposure to 10, 20, 30, or 40 °C. Each bar represents the mean value of six independent replicates shown with standard error bars. Bars with a common letter, based on a Sidak post-hoc test, are not statistically different at $P < 0.05$ within each graph.

Table 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 analysis of variance test for normally distributed data or a Kruskal–Wallis test for nonnormally distributed data.

sensitivity to heat than in our study. As Yang et al. (2019b) suggested, 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 (Hedhly et al. 2003, 2005; Herrero and Hormaza 1996; 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 et al. (2019b) reported that the percentage of rabbiteye styles with germinated pollen at 10 °C was ~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. Germination was greatest between 30 and 35 °C, whereas 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 to 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 with cooler temperatures. Although pollen germination is a necessary prerequisite 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 because temperatures above 35 °C result in significant and often irreversible consequences in pollen performance. These results align with observations from Yang et al. (2019b), who reported significantly reduced pollen

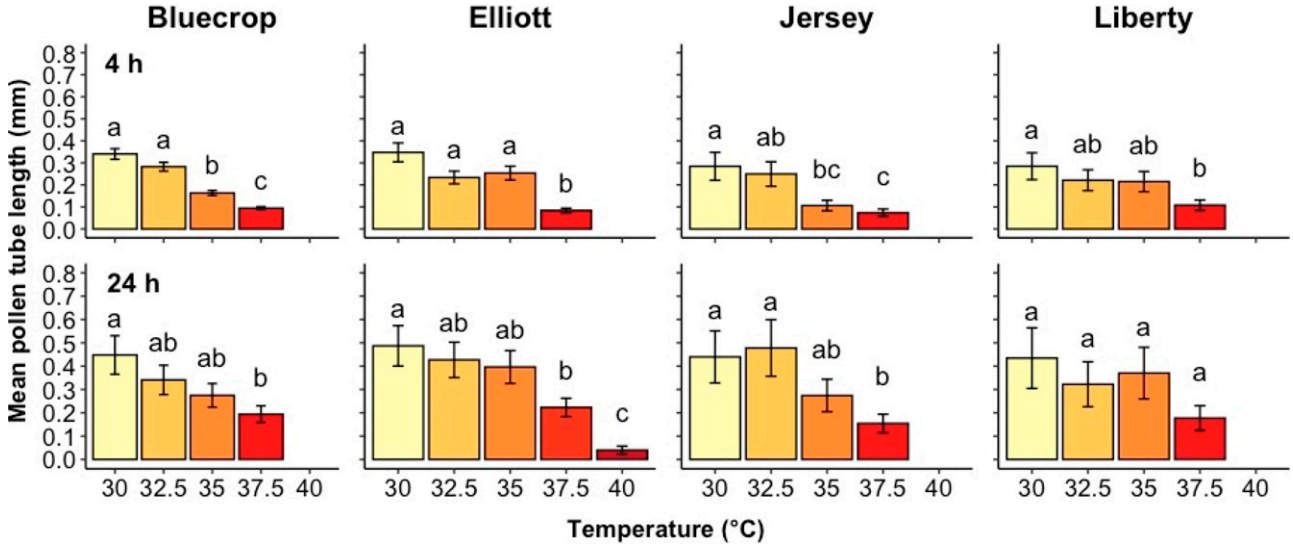

Fig. 2. Mean pollen tube length of four northern highbush blueberry cultivars after 4 and 24 h of exposure to 30, 32.5, 35, 37.5, or 40 °C. Each bar represents the mean value of six independent replicates shown with standard error bars. Bars with a common letter, based on a Sidak post hoc test, are not statistically different at $P < 0.05$ within each graph.

Table 3. Mean proportion germinated pollen tetrads (± *SE*) of the northern highbush blueberry cultivar Bluecrop under three temperature regimes.[i]

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, $P < 0.01$

[i] CT (control temperature) = 25 °C, HT (high temperature) = 37.5 °C, RT (relieving temperature) = 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. NS = nonsignificant comparisons ($P > 0.05$).

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 little recovery. In this third experiment, pollen tube lengths did not differ between the HT and RT groups for the first two sampling times and increased 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 because pollen germination rates did not significantly differ between HT and RT groups across all sampling times. Compared with 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 before pollination completely inhibited in vitro fertilization, although 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 ~10 °C (Yang et al. 2019a, 2020). Although 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 acute extreme heat on blueberry pollen, and the impact a few degrees can have on pollen performance. Thus, strategies that reduce blueberry field temperatures during this developmentally sensitive period could help prevent losses in

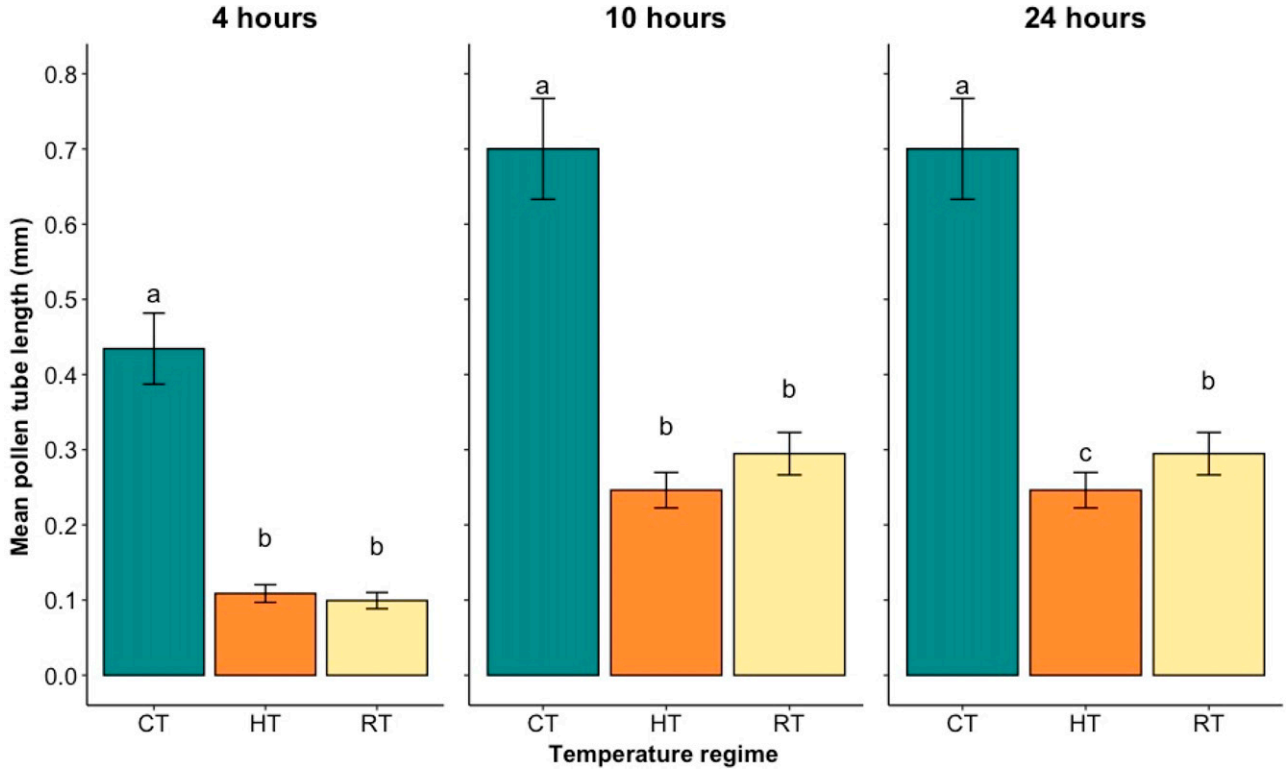

Fig. 3. Mean pollen tube length of the Bluecrop blueberry cultivar after 4, 10, and 24 h of exposure to three different temperature regimes (control temperature = 25 °C, high temperature = 37.5 °C, relieving temperature = 37.5 °C for 4 h and 25 °C for 20 h). Each bar represents the mean value of six 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.

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 highlight 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 (Lobos and Hancock 2015; Rowland et al. 2012; 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 (Driedonks et al. 2016; Lobos and Hancock 2015). Using in vitro techniques, our research provides novel information on the thermotolerance of four northern highbush blueberry cultivars that 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 et al. 2019b) 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 (IPCC 2021; Lobos and Hancock 2015; Yang et al. 2019b; van Es 2020; 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.

References Cited

Acar I, Kakani VG. 2010. The effects of temperature on *in vitro* pollen germination and pollen tube growth of *Pistacia* spp. Scientia Hortic. 125(4):569–572. https://doi.org/10.1016/j.scienta.2010.04.040.

Austin PT, Hewett EW, Noiton D, Plummer JA. 1998. Self incompatibility and temperature affect pollen tube growth in 'Sundrop' apricot (*Prunus armeniaca* L.). J Hortic Sci Biotechnol. 73(3):375–386. https://doi.org/10.1080/14620316.1998.11510988.

Brewer JW, Dobson RC. 1969. Seed count and berry size in relation to pollinator level and harvest date for the highbush blueberry, *Vaccinium corymbosum*. J Econ Entomol. 62(6):1353–1356. https://doi.org/10.1093/jee/62.6.1353.

Cerovlć R, Ružić D. 1992. Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. J Hortic Sci. 67(3):333–340. https://doi.org/10.1080/00221589.1992.11516256.

Dogterom MH, Winston ML. 1999. Pollen storage and foraging by honey bees (Hymenoptera: Apidae) in highbush blueberries (Ericaceae), cultivar 'Bluecrop'. Can Entomol. 131(6):757–768. https://doi.org/10.4039/Ent131757-6.

Dogterom MH, Winston ML, Mukai A. 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. 'Bluecrop' (*Vaccinium corymbosum*; Ericaceae). Am J Bot. 87(11):1584–1591. https://doi.org/10.2307/2656734.

Driedonks N, Rieu I, Vriezen WH. 2016. Breeding for plant heat tolerance at vegetative and reproductive stages. Plant Reprod. 29(1):67–79. https://doi.org/10.1007/s00497-016-0275-9.

Dupuis I, Dumas C. 1990. Influence of temperature stress on in vitro fertilization and heat shock protein synthesis in maize (*Zea mays* L.) reproductive tissues. Plant Physiol. 94(2):665–670. https://doi.org/10.1104/pp.94.2.665.

Eaton GW. 1966. Production of highbush blueberry pollen and its germination *in vitro* as affected by pH and sucrose concentration. Can J Plant Sci. 46(2):207–209. https://doi.org/10.4141/cjps66-032.

van Es SW. 2020. Too hot to handle, the adverse effect of heat stress on crop yield. Physiol Plant. 169(4):499–500. https://doi.org/10.1111/ppl.13165.

Gan W, Zhang H, Bostan N, DeVetter LW. 2020. Pollen performance differs among cultivars of northern highbush blueberry (*Vaccinium corymbosum* L.). J Am Pomol Soc. 74(2):66–75.

Hamidou F, Halilou O, Vadez V. 2013. Assessment of groundnut under combined heat and drought stress. J Agron Crop Sci. 199(1):1–11. https://doi.org/10.1111/j.1439-037X.2012.00518.x.

Hatfield JL, Boote KJ, Kimball BA, Ziska LH, Izaurralde RC, Ort D, Thomson AM, Wolfe D. 2011. Climate impacts on agriculture: Implications for crop production. Agron J. 103(2):351–370. https://doi.org/10.2134/agronj2010.0303.

Hatfield JL, Prueger JH. 2015. Temperature extremes: Effect on plant growth and development. Weather Clim Extrem. 10:4–10. https://doi.org/10.1016/j.wace.2015.08.001.

Hatfield JL, Antle J, Garrett KA, Izaurralde RC, Mader T, Marshall E, Nearing M, Philip Robertson G, Ziska L. 2020. Indicators of climate change in agricultural systems. Clim Change. 163(4):1719–1732. https://doi.org/10.1007/s10584-018-2222-2.

Hedhly A. 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. Environ Exp Bot. 74:9–16. https://doi.org/10.1016/j.envexpbot.2011.03.016.

Hedhly A, Hormaza JI, Herrero M. 2003. The effect of temperature on stigmatic receptivity in sweet cherry (*Prunus avium* L.). Plant Cell Environ. 26(10):1673–1680. https://doi.org/10.1046/j.1365-3040.2003.01085.x.

Hedhly A, Hormaza JI, Herrero M. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). Am J Bot. 91(4):558–564. https://doi.org/10.3732/ajb.91.4.558.

Hedhly A, Hormaza JI, Herrero M. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. Plant Biol. 7(5):476–483. https://doi.org/10.1055/s-2005-865850.

Hedhly A, Hormaza JI, Herrero M. 2009. Global warming and sexual plant reproduction. Trends Plant Sci. 14(1):30–36. https://doi.org/10.1016/j.tplants.2008.11.001.

Herrero M, Hormaza JI. 1996. Pistil strategies controlling pollen tube growth. Sex Plant Reprod. 9:343–347. https://doi.org/10.1007/BF02441953.

Intergovernmental Panel on Climate Change. 2018. Summary for policymakers, p 3–24. In: Masson-Delmotte V, Zhai P, Pörtner H-O, Roberts D, Skea J, Shukla PR, Pirani A, Moufouma-Okia W, Pean C, Pidcock R, Connors S, Matthews JBR, Chen Y, Zhou X, Gomis MI, Lonnoy E, Maycock T, Tignor M, Waterfield T (eds). Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Cambridge University Press, Cambridge, UK, and New York, NY, USA. https://doi.org/10.1017/9781009157940.001.

Intergovernmental Panel on Climate Change. 2021. Summary for policymakers, p 3–32. In: Masson-Delmotte V, Zhai P, Pirani A, Conners SL, Pean C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis MI, Husang M, Leitzell K, Lonnoy E, Matthews JBR, Maycock TK, Waterfield T, Yelekçi O, Yu R, Zhou B (eds). Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, and New York, NY, USA. https://doi.org/10.1017/9781009157896.001.

Iwahori S. 1966. High temperature injuries in tomato fertilization and development of embryos with special reference to the abnormalities cause by high temperature. J Jpn Soc Hortic. 35(4):379–386. https://doi.org/10.2503/jjshs.35.379.

Jumrani K, Bhatia VS, Pandey GP. 2018. Screening soybean genotypes for high temperature tolerance by *in vitro* pollen germination, pollen tube length, reproductive efficiency and seed yield. Indian J Plant Physiol. 23(1):77–90. https://doi.org/10.1007/s40502-018-0360-1.

Kearns CA, Inouye DW. 1993. Techniques for pollination biologists. University Press of Colorado, Denver, CO, USA.

Kozai N, Beppu K, Mochioka R, Boonprakob U, Subhadrabandhu S, Kataoka I. 2004. Adverse effects of high temperature on the development of reproductive organs in 'Hakuho' peach trees. J Hortic Sci Biotechnol. 79(4):533–537. https://doi.org/10.1080/14620316.2004.11511801.

Lang GA, Parrie EJ. 1992. Pollen viability and vigor in hybrid southern highbush blueberries (*Vaccinium corymbosum* L. ×spp.). HortScience. 27(5):425–427. https://doi.org/10.21273/HORTSCI.27.5.425.

Larkindale J, Vierling E. 2008. Core genome responses involved in acclimation to high temperature. Plant Physiol. 146(2):748–761. https://doi.org/10.1104/pp.107.112060.

Ledesma N, Sugiyama N. 2005. Pollen quality and performance in strawberry plants exposed to high-temperature stress. J Am Soc Hortic. 130(3):341–347. https://doi.org/10.21273/jashs.130.3.341.

Lobos GA, Hancock JF. 2015. Breeding blueberries for a changing global environment: A review. Front Plant Sci. 6:1–14. https://doi.org/10.3389/fpls.2015.00782.

Lohani N, Singh MB, Bhalla PL. 2020. High temperature susceptibility of sexual reproduction in crop plants. J Expt Bot. 71(2):555–568. https://doi.org/10.1093/jxb/erz426.

Malikov E, Miao R, Zhang J. 2020. Distributional and temporal heterogeneity in the climate change effects on U.S. agriculture. J Environ Econ Manage. 104:102386. https://doi.org/10.1016/j.jeem.2020.102386.

Masoomi-Aladizgeh F, Najeeb U, Hamzelou S, Pascovici D, Amirkhani A, Tan DKY, Mirzaei M, Haynes PA, Atwell BJ. 2021. Pollen development in cotton (*Gossypium hirsutum*) is highly sensitive to heat exposure during the tetrad stage. Plant Cell Environ. 44(7): 2150–2166. https://doi.org/10.1111/pce.13908.

Mesihovic A, Iannacone R, Firon N, Fragkostefanakis S. 2016. Heat stress regimes for the investigation of pollen thermotolerance in crop plants. Plant Reprod. 29(1–2):93–105. https://doi.org/10.1007/s00497-016-0281-y.

Motha RP, Baier W. 2005. Impacts of present and future climate change and climate variability on agriculture in the temperate regions: North America. Clim Change. 70(1):137–164. https://doi.org/10.1007/s10584-005-5940-1.

Müller F, Rieu I. 2016. Acclimation to high temperature during pollen development. Plant Reprod. 29(1–2):107–118. https://doi.org/10.1007/s00497-016-0282-x.

Parrotta L, Faleri C, Cresti M, Cai G. 2016. Heat stress affects the cytoskeleton and the delivery of sucrose synthase in tobacco pollen tubes. Planta. 243(1):43–63. https://doi.org/10.1007/s00425-015-2394-1.

Raja MM, Vijayalakshmi G, Naik ML, Basha PO, Sergeant K, Hausman JF, Khan PSSV. 2019. Pollen development and function under heat stress: From effects to responses. Acta Physiol Plant. 41(4). https://doi.org/10.1007/s11738-019-2835-8.

Retamales JB, Hancock JF. 2012. Blueberries. CABI, Wallingford, UK. Exp Agric. 48(4): 598. https://doi.org/10.1017/S00144797120 0049X.

Rowland LJ, Bell DJ, Alkharouf N, Bassil NV, Drummond FA, Beers L, Buck EJ, Finn CE, Graham J, McCallum S, Hancock JF, Polashock JJ, Olmstead JW, Main D. 2012. Generating genomic tools for blueberry improvement. Int J Fruit Sci. 12(1–3):276–287. https://doi.org/10.1080/15538362.2011.619452.

Satake T, Yoshida S. 1978. High temperature-induced sterility in indica rices at flowering. Jpn J Crop Sci. 47(1):6–17. https://doi.org/10.1626/jcs.47.6.

Snider JL, Oosterhuis DM. 2011. How does timing, duration and severity of heat stress influence pollen-pistil interactions in angiosperms? Plant Signal Behav. 6(7):930–933. https://doi.org/10.4161/psb.6.7.15315.

Walters J, Zavalnitskaya J, Isaacs R, Szendrei Z. 2022. Heat of the moment: Extreme heat poses a risk to bee–plant interactions and crop yields. Curr Opin Insect Sci. 52:100927. https://doi.org/10.1016/j.cois.2022.100927.

Wang Y, Shahid MQ, Ghouri F, Ercişli S, Baloch FS, Nie F. 2019. Transcriptome analysis and annotation: SNPs identified from single copy annotated unigenes of three polyploid blueberry crops. PLoS One. 14(4):e0216299. https://doi.org/10.1371/journal.pone.0216299.

Yang FH, Bryla DR, Orr ST, Strik BC, Zhao Y. 2020. Thermal cooling with sprinklers or microsprinklers reduces heat damage and improves fruit quality in northern highbush blueberry. HortScience. 55(8):1365–1371. https://doi.org/10.21273/HORTSCI15119-20.

Yang FH, Bryla DR, Strik BC. 2019a. Critical temperatures and heating times for fruit damage in northern highbush blueberry. HortScience. 54(12):2231–2239. https://doi.org/10.21273/HORTSCI14427-19.

Yang Q, Liu E, Fu Y, Yuan F, Zhang T, Peng S. 2019b. High temperatures during flowering reduce fruit set in rabbiteye blueberry. J Am Soc Hortic. 144(5):339–351. https://doi.org/10.21273/JASHS04650-19.

Zhang C, Li G, Chen T, Feng B, Fu W, Yan J, Islam MR, Jin Q, Tao L, Fu G. 2018. Heat stress induces spikelet sterility in rice at anthesis through inhibition of pollen tube elongation interfering with auxin homeostasis in pollinated pistils. Rice (N Y). 11(1):14. https://doi.org/10.1186/s12284-018-0206-5.

Zinn KE, Tunc-Ozdemir M, Harper JF. 2010. Temperature stress and plant sexual reproduction: Uncovering the weakest links. J Expt Bot. 61(7):1959–1968. https://doi.org/10.1093/jxb/erq053.

Supplemental Table 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 analysis of variance test for normally distributed data or a Kruskal–Wallis test for nonnormally distributed data.

		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

NS = nonsignificant results ($P > 0.05$).

Supplemental Table 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 analysis of variance test for normally distributed data or a Kruskal–Wallis test for nonnormally distributed data.

		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

NS = nonsignificant results ($P > 0.05$).

Supplemental Table 3. Mean pollen tube length (± *SE*) of 'Bluecrop' northern highbush blueberry after three durations of exposure to three temperature regimes. 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 were derived from an analysis of variance 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, $P < 0.001$

CT = control temperature (25 °C); HT = high temperature (37.5 °C); RT = relieving temperature (37.5 °C for 4 h and 25 °C for 20 h).

Data Analysis for Supplemental Tables 4 through 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 was used to determine whether 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 two, three, or four pollen tubes was also summarized out of 60 total pollen tetrads observed for each cultivar, temperature, and assessment time.

Supplemental Table 4. The number of pollen tetrads that produced two, three, or four 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 analysis of variance test for normally distributed data or a Kruskal–Wallis test for nonnormally distributed data.

		4 h				24 h			
Cultivar	Temperature (°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, $P < 0.01$

NS = nonsignificant results ($P > 0.05$).

Supplemental Table 5. The number of pollen tetrads that produced two, three, or four 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 analysis of variance test for normally distributed data or a Kruskal–Wallis test for nonnormally distributed data.

		4 h				24 h			
		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
Cultivar	Temperature (°C)	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, $P < 0.001$

NS = nonsignificant results ($P > 0.05$).

Supplemental Table 6. The number of pollen tetrads that produced two, three, or four 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 temperature regimes. 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 analysis of variance test for normally distributed data or a Kruskal Wallis test for nonnormally distributed data.

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, $P < 0.05$

CT = control temperature (25 °C); HT = high temperature (37.5 °C); NS = nonsignificant results ($P > 0.05$); RT = relieving temperature (37.5 °C for 4 h and 25 °C for 20 h).