High Temperatures during Flowering Reduce Fruit Set in Rabbiteye Blueberry

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ABSTRACT. After nearly a decade of development, the scale of blueberry (*Vaccinium* sp.) cultivation has increased, particularly in south China; however, this region is becoming increasingly challenged by temperature changes during the flowering phenophase. Understanding the effects of temperature on pollen germination and pollen tube growth in blueberry is thus important. Using the rabbiteye blueberry (*V. ashei*) ‘Brightwell’, different temperature treatments were carried out during open pollination and cross-pollination with the pollen from rabbiteye blueberry ‘Garden-blue’ in field, greenhouse, and controlled temperature experiments over two consecutive years. The differences in pollen germination, pollen tube dynamics, and ovule viability following different treatments were analyzed, and the critical temperatures were calculated using quadratic and modified bilinear equations to quantify the developmental responses to temperature. The results showed that the fruit set of the artificially pollinated plants inside the greenhouse was significantly higher than that outside the greenhouse. Furthermore, pollen germination and pollen tube growth gradually accelerated under the appropriate high-temperature range, resulting in reduced pollen tube travel time to the ovule. However, the percentage of the style traversed by the pollen tube did not increase at temperatures greater than 30 °C, and a high-temperature range could accelerate ovule degeneration. Therefore, impairment of pollen tube growth in the upper half of the style following pollen germination and ovule degeneration constituted important factors leading to reduced fruit setting under short periods of high temperature during the flowering phenophase in rabbiteye blueberry. This work advances our understanding of the effect of temperature on pollen germination, pollen tube growth, ovule longevity, and fruit setting in rabbiteye blueberry, and provides a foundation for continued cultivation and breeding enhancement. The findings propose that the tolerance of rabbiteye blueberry to a certain high-temperature range in the flowering phenophase should inform breeding strategies for temperature resistance and that temperature range is also an important indicator of suitable environments for cultivation to mitigate potential temperature stress.

Blueberry is a perennial evergreen or deciduous shrub that is native to North America (Wang et al., 2017). Northern highbush blueberry (*Vaccinium corymbosum*), rabbiteye blueberry, and southern highbush blueberry (*V. corymbosum* interspecific hybrids) are the three most commercially important species cultivated (Yu et al., 2016). As not all of these species are self-compatible, the need for cross-pollination between botanical varieties is widely acknowledged by commercial growers (Miller et al., 2011; Müller et al., 2013), and pollination by bees is required to improve yield and quality (Nicholson and Ricketts, 2019).

Blueberry is known to contain appreciable levels of phenolic compounds, which have high biological activity and may provide health benefits as dietary antioxidants (Castagnini

et al., 2015; Lin et al., 2016; Shen et al., 2014). Blueberries are a rich source of flavonoids, phenolic acids, anthocyanins, stilbenes, and tannins, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals (Nile and Park, 2014). In addition, there has been a growing trend in the use of blueberry extracts as ingredients in functional foods and dietary supplements (Drózdź et al., 2018). Blueberry fruit have thus become increasingly popular because of their high nutritional value and desirable flavor (Chu et al., 2017).

After nearly a decade of development, China recently became one of the leading countries in blueberry cultivation. In particular, the scale of cultivation has dramatically increased in south China because of its advantages of a longer growing season and high economic revenue (Yu et al., 2016). Rabbiteye blueberry has become an important economic crop in Guizhou Province (Zhang et al., 2015). However, extreme high-temperature events are becoming more frequent in south China as a result of increasing temperature and climatic variability, particularly during the flowering phenophase, thereby influencing cultivation (Yang et al., 2015b). In southeast Guizhou province, the daytime ground temperatures can remain at 35°C for more than 4 h on a typical spring day, and the difference in

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temperature can exceed 30 °C within 3 h (Yang et al., 2017). Abnormal temperature changes occurred frequently during the 2014 to 2018 period and were associated with erratic fruit set in blueberry. Production practice in recent years has shown that temperature stress constitutes the biggest obstacle to flowering and fruit setting in rabbiteye blueberry in south China.

In flowering plants, environmental factors play an important role in the regulation of plant growth and development, and the reproductive phase is typically highly sensitive to temperature stress (Gao et al., 2014; Ohnishi et al., 2010; Saini et al., 2017; Yang et al., 2015a). Complications can include abnormal meiosis (Bitá et al., 2011; Draeger and Moore, 2017; Ohnishi et al., 2010; Yang et al., 2015a); accelerated pollen development and increased pollen abortion (Higuchi et al., 1998; Shen et al., 1999); accelerated flower bud development, resulting in a hastening of flowering time (Rodrigo and Herrero, 2002); reduced pollen germination for underdeveloped pistils (Hedhly et al., 2004; Koubouris et al., 2009; Pham et al., 2015); faster pollen tube growth in the style (Hedhly et al., 2004; Xu and Xu, 2014); inhibition of pollen tube elongation (Zhang et al., 2018); reduction in the number of pollen tubes (Radičević et al., 2016); and reduction in the percentage of the style traversed by the pollen tube (Gao et al., 2014; Koubouris et al., 2009; Pham et al., 2015; Song and Chen, 2018), all of which can result in low fruit set. The aforementioned phenotypic characteristics are closely related with the genotypes of the species and botanical varieties (Pham et al., 2015; Radičević et al., 2016; Sorkheh et al., 2011).

However, a limited number of studies have attempted to clarify the effects of temperature on pollination and fertilization in blueberry. Nesmith et al. (1999) found that the corollas were most sensitive to freeze damage, followed by the styles and then the ovaries. Pollen viability was extremely low or lacking following 3 d or more of rain, and the stigma was unreceptive under drip film irrigation (Yang et al., 2015b). In addition, pollen viability and stigma receptivity were significantly reduced with increases or decreases in optimum temperature (Yang et al., 2017). To date, no studies have determined whether temperature reduces pollen germination in situ in blueberry, inhibits pollen tube elongation, or reduces ovule viability, which would have consequences for fertilization and fruit setting.

Using the rabbiteye blueberry ‘Brightwell’ as the study material, field/greenhouse-based and controlled temperature experiments were carried out over two consecutive years in the present study to assess the influence of different temperatures during pollination on pollen germination, pollen tube growth, and ovule viability. This study aims to provide a basis for clarifying the mechanism of heat suppression in rabbiteye blueberry fruit production and seeks to determine the temperature conditions for improved production and decreased damage from temperature stress. In addition, the cardinal temperatures can inform breeding strategies for blueberry tolerance to heat stress, which is of great significance for breeding heat-tolerant germplasms.

Materials and Methods

PLANT MATERIAL. The experiments were carried out over two consecutive years, 2016 and 2017, on different trees from a 5-year-old orchard of ‘Brightwell’, which is particularly prone to erratic fruit set. The experiments were conducted at the

experimental orchard of the Practice and Training Center for Horticulture, Kaili University, Kaili, Guizhou, China (lat. 26°31′N, long. 107°53′E). The orchard is situated in a tropical/subtropical climate at an altitude of 689 m and experiences an average annual temperature of 16.2 °C, an average maximum temperature of 25 to 27 °C (July), an average minimum temperature of 4 to 7 °C (December), average annual sunshine of around 1290 h, average annual rainfall of 1240 mm, and at least 280 frost-free days in a year. The orchard was cultivated on the open ground with a plant spacing of 2 m and row spacing of 2.5 m and was irrigated with a drip irrigation system with underground water with a hardness of 325 mg·L⁻¹ (as CaCO₃) and pH of 7.28. The soil in the orchard was weakly acidic with a pH value of 5.68, the effective contents of N, P, and K were 145.80, 10.96, and 34.53 mg·kg⁻¹, respectively, and the organic matter content was 39.40 g·kg⁻¹. The ‘Gardenblue’ variety was used as the male parent in the same orchard, and the pollen was collected in a 2-mL centrifuge tube from directly under the corolla mouth by tapping the corolla with forceps on the day of pollination.

FIELD-BASED AND GREENHOUSE TEMPERATURE TREATMENTS. We aimed to elucidate whether the unstable fruit set was due to temperature changes. A total of 90 5-year-old blueberry trees exhibiting robust and consistent growth were randomly selected for the experimental temperature treatments, 45 of which were cultured in a greenhouse, whereas the other 45 were cultured in the field. The trees were managed according to local field practices. To maintain a soil pH suitable for blueberry growth requirements, the soil was irrigated with wood vinegar solution (pH 4.32) once every 20 d. Based on the methods of Hedhly et al. (2004) and Rodrigo and Herrero (2002), at the balloon stage, the temperatures inside and outside the greenhouse were monitored every 5 min with a temperature logger (ZDR-20; Hangzhou Zida Instrument Co., Hangzhou, China) placed at 60 cm above the soil level and orientated to the north during the experimental period.

At the beginning of the flowering phenophase, bushes with sufficient flowering capacity and with flowers exhibiting uniform growth were selected from the open field and the greenhouse. From 5 to 13 Mar. 2016, 360 flowers at the balloon stage from the greenhouse plants and 360 flowers at the balloon stage from the field-grown plants were emasculated and bagged to avoid selfing and open pollination, respectively. Before this, the early or late flowers were thinned daily. On the second day after emasculation, from 1030 to 1200 HR, pollination was facilitated by sweeping a small brush containing the pollen from ‘Gardenblue’ onto the stigma until the pollen was visible, and the pollinated flowers were isolated again with the bags, following pollination. Sixty pollinated flowers were then sampled from the inside or outside treatments at 120 h after pollination, and 20 flowers each from outside and inside the greenhouse were measured using the method described in the fixation and microscopic observation section, with three replications. The remaining flowers of the two treatments were used for calculating the fruit set at 30 d after pollination using 100 flowers each from outside and inside the greenhouse, and three replications were tested. Furthermore, to elucidate whether the erratic fruit set was due to a lack of pollinators, from 7 to 15 Mar. 2016, 300 flowers from the outside treatment at the balloon stage were marked daily and used to calculate the fruit set of the open-pollinated flowers as a control analysis.

CONTROLLED TEMPERATURE EXPERIMENTS. The controlled temperature experiments were carried out in 2017. In the full-bloom stage, to evaluate pollen germination, pollen tube growth, and ovule longevity under unfavorable temperatures during pollination and fertilization, branches containing inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase. The nutrient solution was changed daily. Before the controlled temperature treatments, the early or late flowers were thinned, and the balloon-stage flowers were emasculated. After 24 h of cultivation at a constant temperature, 540 flowers in each temperature treatment at the balloon stage were pollinated as described in the field experiment temperature treatment. Sixty pollinated flowers from each temperature treatment were sampled at 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination, and 20 flowers from each sampled treatment were measured by the method described in the fixation and microscopic observation section, with three replications.

To determine the critical temperature and duration of tolerance, branches containing inflorescences were cultured in a controlled climate chamber at 0, 5, 10, 15, 20, 25, 30, or 35 °C as described in the previous paragraph. Before the treatments, the early or late flowers were thinned, and the balloon-stage flowers were emasculated and pollinated as described in the aforementioned methods. In contrast to the previous section, 60 flowers from each temperature treatment were pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of constant temperature after cultivation or before the style became brown and withered. In addition, to elucidate the resistance of the ovule to temperature, an additional 60 emasculated flowers were collected after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at different constant temperatures before pollination, and 20 flowers from each collected treatment were measured by the method described in the fixation and microscopic observation section, with three replications.

FIXATION AND MICROSCOPIC OBSERVATION. In all treatments, the styles and ovaries were fixed immediately after sampling in FAA solution [5:5:90 (v/v/v) 38% formaldehyde: acetic acid: 70% (v/v) ethanol] and softened for fluorescence microscopy, as described by Yang et al. (2012). The pistils and ovaries were then washed in distilled water (three times, 2 h each), softened until they became transparent by immersion in 1 mol·L⁻¹ NaOH (25 °C), and stained in 0.1% aniline blue (0.1% K₃PO₄) for 48 h successively. Based on the method of Yang et al. (2012), the pollen germination performance at the stigma and the pollen tube performance at the style and the ovary were measured in compressed preparations with a fluorescence microscope (Olympus, Tokyo, Japan) equipped with a generic description filter (U-MWU; Olympus). Images were captured with a generic description camera (Olympus).

EVALUATION OF THE EFFECTS OF TEMPERATURE ON POLLEN PERFORMANCE AND POLLEN TUBE GROWTH. Pollen performance, expressed as pollen germination and pollen tube growth, was studied in all temperature treatments. The pollen was identified as germinating based on the pollen tube length being at least equal to or greater than the pollen grain diameter (Acar and Kakani, 2010), and the effects of temperature on pollen germination were evaluated based on the pollen germination rate and germination tendency. The effects of temperature on

pollen tube growth were determined based on the percentage of the style traversed by the pollen tube (upper third of the style, half of the style, and the base of the style and ovary) under various temperature conditions (Hedhly et al., 2004; Yang et al., 2015c). Ovule longevity was determined according to the method described by Rosellini et al. (2003); specifically, an ovule was considered to be degenerative when it had a callose layer at the chalazal end (Rodrigo and Herrero, 2002; Rosellini et al., 2003). The number of ovules with a callose layer at the chalazal end was recorded.

DATA ANALYSIS. All statistical analyses were performed in SPSS (version 16.0; IBM Corp., Armonk, NY). Percentages were subjected to angular transformation to ensure normality before analysis of variance at $P \leq 0.05$, followed by Student–Newman–Keuls multiple range tests. In addition, the maximum pollen germination rate, percentages of the ovules reached by the pollen tubes, and ovules with callose deposition recorded were analyzed by the quadratic and modified bilinear equations commonly used to quantify developmental responses to temperatures (Acar and Kakani, 2010). Based on the methods of the quadratic model described by Acar and Kakani (2010), the critical temperatures [minimum ($T_{\min}$), optimum (T_{opt}), and maximum ($T_{\max}$)] of pollen germination, pollen tube growth, and ovule longevity were estimated using the following equations:

$$y = a + bT - cT^2,$$

$$T_{\text{opt}} = \frac{-b}{2c},$$

$$T_{\min} = \frac{-b + \sqrt{b^2 - 4ac}}{2c},$$

$$T_{\max} = \frac{-b - \sqrt{b^2 - 4ac}}{2c}.$$

In the equations, T is the actual treatment temperature, and a , b , and c are constants generated using PROC NLIN in SPSS.

Results

TEMPERATURE DIFFERENCES BETWEEN OUTSIDE AND INSIDE THE GREENHOUSE. The minimum, maximum, and daily mean temperatures outside and inside the greenhouse from 7 to 20 Mar. 2016 are indicated in Table 1. The greenhouse induced a mean increase in the minimum temperature of 4.1 to 5.9 °C, maximum temperature of 4.4 to 6.4 °C, and daily mean temperature of 4.8 to 5.5 °C. During the pollination experiment, the lowest temperature outside the greenhouse was 1.2 °C, whereas the lowest temperature inside the greenhouse was 6.3 °C. The highest temperature outside the greenhouse was 28.4 °C, whereas the highest temperature inside the greenhouse was 33.6 °C. Furthermore, on 7 Mar., the maximum temperature reached 25 °C outside the greenhouse, whereas the maximum temperature reached 31.4 °C inside the greenhouse, where it was maintained in an excess of 3.5 h. The temperature outside the greenhouse dropped to 19.7 °C within 3 h on 8 Mar. The minimum temperature was 1.2 °C on 10 Mar., whereas the daily mean temperature was 2.4 °C.

POLLEN TUBE GROWTH AND FRUIT SET DIFFERENCES BETWEEN OUTSIDE AND INSIDE THE GREENHOUSE. During the pollination test, the average temperature from pollination to sampling

Table 1. Maximum, minimum, and daily mean temperatures outside and inside the greenhouse (lat. 26°31'N, long. 107°53'E) during the 14-d pollination period during Mar. 2016.

Temp (°C)	Date													
	7 Mar.	8 Mar.	9 Mar.	10 Mar.	11 Mar.	12 Mar.	13 Mar.	14 Mar.	15 Mar.	16 Mar.	17 Mar.	18 Mar.	19 Mar.	20 Mar.
Minimum outside	16.3 ^z	3.3	1.3	1.2	1.3	5.6	7.1	6.0	9.6	10.1	9.3	13.2	12.3	11.1
Minimum inside	21.3	8.5	7.2	6.3	6.5	10.8	12.3	10.8	13.7	14.9	14.6	18.5	17.2	16.3
Maximum outside	25.0	23.0	8.4	5.5	12.2	13.1	16.5	15.1	16.4	18.1	15.8	28.4	22.9	19.1
Maximum inside	31.4	28.4	12.9	9.9	17.2	18.2	21.9	20.5	21.6	23.2	20.7	33.6	28.3	24.3
Daily mean outside	20.1	12.1	4.6	2.4	7.3	9.7	9.7	11.8	12.8	14.1	12.1	20.3	17.7	15.4
Daily mean inside temp	25.3	17.3	9.5	7.5	12.6	14.6	15.2	16.6	17.6	19.1	17.1	25.4	22.7	20.2
Avg daily difference	5.2	5.2	4.9	5.1	5.3	4.9	5.5	4.8	4.8	5.0	5.0	5.1	5.0	4.8

^zThe air temperature was monitored every 5 min with a temperature logger (ZDR-20; Hangzhou Zida Instrument Co.) placed at 60 cm above the soil inside and outside the greenhouse.

exhibited a consistent daily trend inside and outside the greenhouse, with lower temperatures being recorded during the beginning of the treatment and increasing toward the end of the treatment (Table 2). The lowest average temperatures were 7.6 and 12.7 °C, respectively, recorded on 8 and 9 Mar., whereas the highest average temperatures were 15.4 and 20.4 °C, respectively, recorded on 15 Mar. Outside the greenhouse, the average temperature from pollination to sampling did not exceed 9.5 °C from 7 to 10 Mar., and the pollen tube failed to grow through the style and into the embryo sac. By contrast, the average temperatures of the corresponding treatment inside the greenhouse ranged from 12.7 to 14.5 °C, 23.3% to 58.3% of the style was traversed by the pollen tube, and 37.8% to 41.1% of the ovules were reached by the pollen tube.

During the pollination test, the effect of temperature on the percentage of the style and ovules that were traversed by the pollen tube was assessed. Outside the greenhouse, the highest percentages of the style traversed by the pollen tube and the percentage of the ovules traversed by the pollen tube were 81.7% and 44.4%, respectively, on 15 Mar. Inside the greenhouse, the highest percentages of the style and ovules traversed by the pollen tube were 91.7% and 54.4% on 11 to 12 and 13 Mar., respectively. These values dropped significantly to 78.8% and 48.9%, respectively, when the average temperature increased to 20.4 °C on 15 Mar.

The fruit set during artificial pollination inside the greenhouse was significantly higher than that outside the greenhouse. The fruit set outside the greenhouse ranged only from 5.0% to 12.7% on 7 to 11 Mar., and the lowest fruit set of 5.0% was recorded on 9 Mar., whereas the highest (53.7%) was recorded on 15 Mar. By contrast, the fruit set inside the greenhouse ranged from 43.0% to 52.3% from 7 to 11 Mar., with the lowest value of 43.0% recorded on 9 Mar. and the highest value of 75.0% recorded on 13 Mar. The fruit sets of the artificially pollinated and open-pollinated plants differed. Both treatments were low from 7 to 11 Mar. and differed significantly from each other from 7 to 9 Mar. The fruit sets of both treatments increased significantly from 12 to 15 Mar., but did not differ significantly from one another. Both were significantly lower than the fruit set of the artificially pollinated plants inside the greenhouse.

EFFECTS OF TEMPERATURE ON POLLEN GERMINATION. Pollen germination gradually accelerated with the increase in temperature, but the percentage of the style traversed by the pollen tube did not increase gradually with the increase in temperature (Table 3). The pollen did not germinate until 120 h after pollination at 0 °C, and very little germinated pollen was observed until 96 h after pollination at 5 °C. Germinated pollen was observed at 2 h after pollination from 15 to 35 °C, but was only observed at 4 h after pollination at 10 °C. The percentage of the style with germinated pollen peaked at 24 h or 48 h after pollination.

In addition, comparative analysis indicated that the percentage of the style with germinated pollen was higher than 90% at 20.0 or 25.0 °C, which was significantly higher than the percentage of the style with germinated pollen at 10, 15, 30, and 35 °C. In particular, the percentage of the style with germinating pollen was lower by ≈56% in the 10, 15, and 30 °C treatments, whereas the pollen did not germinate at 35 °C. Only a few styles had germinating pollen at 5 °C. Based on the quadratic equation $Y = -7.292 + 9.455 T - 0.221 T^2$ ($r^2 = 0.931$)

Table 2. Air temperature, pollen tube growth and ovary penetration, and fruit set data for rabbiteye blueberry ‘Brightwell’ plants grown outside and inside the greenhouse, and with and without hand pollination during Mar. 2016.

Parameter	Date									
	7 Mar.	8 Mar.	9 Mar.	10 Mar.	11 Mar.	12 Mar.	13 Mar.	14 Mar.	15 Mar.	
Temp (°C) ^z	9.37 ^v 14.47	7.63 12.78	7.58 12.67	8.95 14.02	10.90 15.95	11.70 16.70	13.47 18.50	14.80 19.75	15.40 20.35	
Style traversed by pollen tube (%) ^y	Outside 53.33 ± 1.67 d	0.00 38.33 ± 1.67 e	0.00 23.33 ± 1.67 g	0.00 58.33 ± 1.67 c	16.67 ± 1.67 h	33.33 ± 1.67 f	51.67 ± 1.67 d	61.67 ± 1.67 c	81.67 ± 1.67 b	
Ovaries traversed by pollen tube (%) ^x	Outside 40.00 ± 1.92 de	0.00 37.78 ± 1.11 ef	0.00 37.78 ± 1.11 ef	0.00 41.11 ± 1.11 de	33.33 ± 1.93 f	36.67 ± 1.93 ef	37.78 ± 1.11 ef	40.00 ± 1.92 de	44.44 ± 1.11 cd	
Fruit set (%) ^w	Hand outside 11.00 ± 0.58 hi	12.67 ± 1.45 h	5.00 ± 1.16 k	5.67 ± 0.88 jk	5.67 ± 1.20 jk	32.00 ± 1.73 g	36.67 ± 0.88 f	40.00 ± 0.58 ef	53.67 ± 1.76 cd	
	Open-pollinated 3.00 ± 0.58 k	3.33 ± 0.88 k	9.67 ± 0.88 hij	5.00 ± 1.16 k	7.33 ± 0.88 ijk	32.00 ± 1.53 g	40.67 ± 1.45 ef	40.00 ± 1.53 ef	54.67 ± 0.88 c	
	Hand inside 52.33 ± 0.88 cd	43.33 ± 0.88 e	43.00 ± 1.53 e	50.33 ± 0.88 d	51.33 ± 1.20 cd	68.67 ± 0.88 b	75.00 ± 1.16 a	71.67 ± 0.88 b	69.00 ± 1.16 b	

^zDaily average air temperature from pollination to sampling, and the air temperature was monitored every 5 min with a temperature logger (ZDR-20; Hangzhou Zida Instrument Co.) placed at 60 cm above the soil inside and outside the greenhouse.

^yPercentage of the style traversed by the pollen tube of flowers on plants inside and outside the greenhouse.

^xPercentage of the ovaries penetrated by the pollen tube of flowers on plants inside and outside the greenhouse.

^wFruit set percentage for hand-pollinated flowers on plants grown inside and outside the greenhouse, as well as fruit set percentage of open-pollinated flowers on plants outside the greenhouse.

^vDifferent letters indicate significant differences within a parameter across dates by Student–Newman–Keuls tests ($P \leq 0.05$).

Table 3. Percentage of styles with germinated pollen of the flowers of rabbiteye blueberry ‘Brightwell’ under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 24 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination in 2017.

Temp (°C) ^z	Time after pollination (h) ^y									
	2	4	8	12	24	48	72	96	120	
0	0 ^x	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	0	
10	0	11.67 ± 1.67 e	21.67 ± 1.67 d	31.67 ± 1.67 f	43.33 ± 1.67 e	53.33 ± 1.67 d	58.33 ± 1.67 d	66.67 ± 1.67 c	73.33 ± 1.67 c	
15	23.33 ± 1.67 e	31.67 ± 1.67 d	51.67 ± 2.89 c	73.33 ± 1.67 c	76.67 ± 1.67 b	76.67 ± 1.67 b	78.33 ± 1.67 b	83.33 ± 2.89 b	88.33 ± 1.67 b	
20	31.67 ± 1.67 d	43.33 ± 2.89 c	58.33 ± 1.67 b	83.33 ± 2.89 b	93.33 ± 2.89 a	93.33 ± 2.89 a	95.00 ± 2.89 a	95.00 ± 2.89 a	96.67 ± 2.89 a	
25	41.67 ± 2.89 c	56.67 ± 1.67 b	68.33 ± 2.89 a	88.33 ± 1.67 a	91.67 ± 1.67 a	90.00 ± 2.89 a	91.67 ± 2.89 a	91.67 ± 2.89 a	91.67 ± 1.67 b	
30	58.33 ± 2.89 b	66.67 ± 2.89 a	68.33 ± 2.89 a	68.33 ± 1.67 d	68.33 ± 2.89 c	68.33 ± 1.67 c	66.67 ± 1.67 c	68.33 ± 2.89 c	66.67 ± 2.89 d	
35	53.33 ± 1.67 a	56.67 ± 1.67 b	56.67 ± 1.67 b	56.67 ± 2.89 e	58.33 ± 1.67 d	56.67 ± 1.67 d	56.67 ± 1.67 d	58.33 ± 1.67 d	56.67 ± 1.67 e	

^zIn the full-bloom stage, the branches containing inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 24 h of cultivation at a constant temperature, 540 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from ‘Gardenblue’ onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

by linear regression analysis, the predicted T_{min} , T_{opt} , and T_{max} of pollen germination of 'Gardenblue' in 'Brightwell' pistils were 0.8, 21.4, and 42.0 °C, respectively.

EFFECTS OF TEMPERATURE ON POLLEN TUBE GROWTH. Pollen tube growth increased with increasing temperature within a certain temperature range (Table 4). No pollen germinated at 0 °C, and pollen tube growth was extremely slow at 5.0 °C, with the pollen tube only reaching the upper third of the style at 120 h after pollination (Table 4). At 10 °C, only 8.3% of the upper third of the style had been traversed by the pollen tube at 12 h after pollination (Table 4), whereas 11.7% of the upper half of the style had been traversed at 72 h after pollination (Table 5). At temperatures of 15, 20, 25, and 30 °C, the time required for the pollen tube to reach the upper third of the style, the upper half of the style, and the base of the style was correspondingly reduced. The style was traversed by the pollen tube at 48 h after pollination at these temperatures (Table 6).

At 120 h after pollination, the percentage of the style traversed by the pollen tube at 15 and 20 °C was significantly higher than at 25 °C and was significantly higher at 25 °C than at 10.0 and 30 °C. Based on the quadratic equation of $Y = -8.519 + 5.420 T - 0.147 T^2$ ($r^2 = 0.882$), the T_{min} , T_{opt} , and T_{max} of the pollen tube growth of 'Gardenblue' in 'Brightwell' pistils were predicted to be 1.6, 18.4, and 35.2 °C, respectively.

The pollen tubes required at least 96 h of growth to reach the ovules at 10, 15, 20, 25, and 30 °C (Table 7). However, there was a significant difference in the pollen tube growth percentage between the different temperature treatments. At 15 and 20 °C, the pollen tubes had reached 40% of the ovules, whereas at 25 and 10 °C, the corresponding percentages were 36% $\approx$ 33%, respectively, and at 30 °C, this value was only $\approx$ 22%. The pollen tube did not reach the ovules in the 0, 5, and 35 °C treatments. The aforementioned results showed that temperatures of 15 and 20 °C were associated with improved pollen tube growth, whereas temperatures below or above these values were associated with reduced pollen tube growth.

EFFECTS OF CULTIVATION DURATION AND TEMPERATURE ON POLLEN GERMINATION. To elucidate the critical temperature and duration of tolerance, cut flowers were cultured in a controlled climate chamber at 0, 5, 10, 15, 20, 25, 30, or 35 °C and pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of controlled temperature culture (Table 8). No pollen germination occurred at 0 °C, and at 5 °C, only 16.7% or 18.3% of the style contained germinated pollen at 4, 8, 12, and 24 h after cultivation, and there was no significant difference between the time treatments (Table 8). However, at temperatures of 10, 15, 20, 25, 30, and 35 °C, there were significant differences between the different temperatures or treatment durations after cultivation under the same temperature.

Generally, the percentage of the style with germinated pollen increased initially with time but then decreased, with the maximum percentage of the style with germinating pollen appearing earlier under all the temperature treatments. The maximum percentage of the style with germinated pollen was 73.3% and 93.3% after 48 h of cultivation at 10 and 15 °C, respectively, and 93.3% after 24 h cultivation at 20 and 25 °C. By contrast, at 30 and 35 °C, these values were 66.7% and 61.7%, respectively, after 48 h of cultivation. After 72 h of cultivation, the style had become brown and withered in all treatments and could not be pollinated.

EFFECTS OF CULTIVATION DURATION AND TEMPERATURE ON POLLEN TUBE GROWTH. There were significant differences in the

Table 4. Percentage of the first-third of the style traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 24 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination in 2017.

Temp (°C) ^z	Time after pollination (h) ^y										
	2	4	8	12	24	48	72	96	120	First-third of style traversed by pollen tube [mean $\pm$ SE (%)]	
0	0 ^x	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
15	0	0	21.67 $\pm$ 1.67 d	8.33 $\pm$ 1.67 e	13.33 $\pm$ 1.67 e	31.67 $\pm$ 1.67 f	48.33 $\pm$ 1.67 e	66.67 $\pm$ 1.67 c	73.33 $\pm$ 1.67 c	73.33 $\pm$ 1.67 c	73.33 $\pm$ 1.67 c
20	0	13.33 $\pm$ 1.67 d	33.33 $\pm$ 1.67 c	38.33 $\pm$ 1.67 d	53.33 $\pm$ 1.67 d	78.33 $\pm$ 2.89 c	98.33 $\pm$ 1.67 a	98.33 $\pm$ 1.67 a	98.33 $\pm$ 1.67 a	98.33 $\pm$ 1.67 a	98.33 $\pm$ 1.67 a
25	0	23.33 $\pm$ 1.67 c	48.33 $\pm$ 2.89 b	71.67 $\pm$ 2.89 a	86.67 $\pm$ 1.67 a	85.00 $\pm$ 2.89 b	83.33 $\pm$ 2.89 b	81.67 $\pm$ 2.89 b	83.33 $\pm$ 2.89 b	83.33 $\pm$ 2.89 b	83.33 $\pm$ 2.89 b
30	8.33 $\pm$ 1.67 b	33.33 $\pm$ 1.67 b	58.33 $\pm$ 2.89 a	61.67 $\pm$ 1.67 b	61.67 $\pm$ 2.89 c	63.33 $\pm$ 1.67 d	61.67 $\pm$ 2.89 c	63.33 $\pm$ 2.89 c	58.33 $\pm$ 2.89 d	58.33 $\pm$ 2.89 d	58.33 $\pm$ 2.89 d
35	31.67 $\pm$ 1.67 a	53.33 $\pm$ 1.67 a	56.67 $\pm$ 1.67 a	58.33 $\pm$ 2.89 b	58.33 $\pm$ 1.67 c	56.67 $\pm$ 1.67 e	53.33 $\pm$ 1.67 d	56.67 $\pm$ 1.67 d	56.67 $\pm$ 1.67 d	56.67 $\pm$ 1.67 d	56.67 $\pm$ 1.67 d

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 24 h of cultivation at a constant temperature, 540 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

Table 5. Percentage of half of the style traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 24 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination in 2017.

Temp (°C) ^z	Time after pollination (h) ^y									
	2	4	8	12	24	48	72	96	120	
0	0 ^x	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	11.67 ± 1.67 d	21.67 ± 1.67 e	28.33 ± 1.67 d	28.33 ± 1.67 d
15	0	0	0	6.67 ± 1.67 d	18.33 ± 1.67 b	28.33 ± 2.89 e	58.33 ± 1.67 c	83.33 ± 2.89 b	98.33 ± 1.67 a	98.33 ± 1.67 a
20	0	0	13.33 ± 1.67 d	36.67 ± 2.89 c	58.33 ± 2.89 a	76.67 ± 1.67 b	91.67 ± 2.89 a	98.33 ± 1.67 a	98.33 ± 1.67 a	98.33 ± 1.67 a
25	0	0	21.67 ± 2.89 c	41.67 ± 1.67 b	63.33 ± 2.89 a	83.33 ± 2.89 a	81.67 ± 2.89 b	81.67 ± 2.89 b	83.33 ± 2.89 b	83.33 ± 2.89 b
30	0	6.67 ± 1.67 a	28.33 ± 1.67 b	56.67 ± 1.67 a	61.67 ± 2.89 a	61.67 ± 2.89 c	61.67 ± 2.89 c	61.67 ± 2.89 c	58.33 ± 2.89 c	58.33 ± 2.89 c
35	0	8.33 ± 1.67 a	33.33 ± 1.67 a	58.33 ± 2.89 a	58.33 ± 1.67 a	56.67 ± 1.67 d	58.33 ± 1.67 c	53.33 ± 1.67 d	58.33 ± 1.67 c	58.33 ± 1.67 c

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 24 h cultivation at a constant temperature, 540 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

Table 6. Percentage of the entire style that was traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 24 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination in 2017.

Temp (°C) ^z	Time after pollination (h) ^y									
	2	4	8	12	24	48	72	96	120	
0	0 ^x	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	18.33 ± 2.89 d	48.33 ± 1.67 c	11.67 ± 1.67 e	23.33 ± 1.67 d	23.33 ± 1.67 d
20	0	0	0	0	0	43.33 ± 2.89 a	81.67 ± 2.89 a	76.67 ± 2.89 c	96.67 ± 2.89 a	96.67 ± 2.89 a
25	0	0	0	0	0	31.67 ± 1.67 b	71.67 ± 2.89 b	96.67 ± 1.67 a	98.33 ± 1.67 a	98.33 ± 1.67 a
30	0	0	0	0	0	26.67 ± 1.67 c	31.67 ± 1.67 d	81.67 ± 2.89 b	78.33 ± 2.89 b	78.33 ± 2.89 b
35	0	0	0	0	0	0	0	28.33 ± 1.67 d	31.67 ± 2.89 c	31.67 ± 2.89 c

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 24 h cultivation at a constant temperature, 540 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

Table 7. Percentage of the ovules traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 24 h cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at the 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination in 2017.

Temp (°C) ^z	Time after pollination (h) ^y								
	2	4	8	12	24	48	72	96	120
	Ovules traversed by pollen tube [mean ± SE (%)]								
0	0 ^x	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	15.56 ± 1.113 d	33.33 ± 1.925 b
15	0	0	0	0	0	0	0	37.78 ± 1.110 ab	42.22 ± 1.110 a
20	0	0	0	0	0	0	0	38.89 ± 1.110 a	43.33 ± 1.925 a
25	0	0	0	0	0	0	0	35.56 ± 1.113 b	36.67 ± 1.925 b
30	0	0	0	0	0	0	0	21.11 ± 1.110 c	22.22 ± 1.110 c
35	0	0	0	0	0	0	0	0	0

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 24 h of cultivation at a constant temperature, 540 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

Table 8. Percentage of styles with germinated pollen of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 120 h after pollination in 2017.

Temp (°C) ^z	Time after cultivation (h) ^y							
	4	8	12	24	48	72	96	120
0	0 ^x	0	0	0	0	0	0	0
5	18.33 ± 1.67 a	18.33 ± 1.67 a	16.67 ± 1.67 a	16.67 ± 1.67 a	73.33 ± 1.67 a	66.67 ± 1.67 b	48.33 ± 1.67 c	26.67 ± 1.67 d
10	46.67 ± 1.67 c	48.33 ± 1.67 c	53.33 ± 1.67 c	71.67 ± 1.67 a	93.33 ± 1.67 a	63.33 ± 1.67 c	46.67 ± 1.67 f	21.67 ± 1.67 g
15	53.33 ± 1.67 e	58.33 ± 1.67 d	71.67 ± 2.89 b	91.67 ± 2.89 a	93.33 ± 1.67 b	58.33 ± 1.67 d	36.67 ± 2.89 e	16.67 ± 1.67 f
20	58.33 ± 1.67 d	63.33 ± 2.89 d	73.33 ± 2.89 c	93.33 ± 2.67 b	86.67 ± 1.67 b	36.67 ± 1.67 d	13.33 ± 2.89 e	— ^w
25	63.33 ± 1.67 c	68.33 ± 1.67 c	78.33 ± 2.89 b	93.33 ± 1.67 a	78.33 ± 1.67 b	36.67 ± 1.67 d	—	—
30	58.33 ± 1.67 b	63.33 ± 2.89 ab	66.67 ± 1.67 a	63.33 ± 1.67 ab	43.33 ± 1.67 c	16.67 ± 1.67 d	—	—
35	56.67 ± 1.67 a	58.33 ± 2.89 a	61.67 ± 1.67 a	56.67 ± 1.67 a	16.67 ± 1.67 b	—	—	—

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, 60 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

^wThe style became brown or withering when pollinated or sampled.

percentage of the style (upper third of the style, upper half of the style, and the whole style) that was traversed by the pollen tube among the different cultivation durations, with the exception of the 0 °C treatment, where no pollen tube growth was observed (Tables 9–12). At temperatures of 10, 15, 20, and 25 °C, the percentage of the style traversed by the pollen tube had begun declining following pollination and cultivation for more than 48 h. At 10, 15, and 20 °C, the pollen tube had not reached the upper half of the style, but had traversed the upper third of the style at 120 h following pollination. At all treatment temperatures, the pollen tube did not traverse the entire style at 96 h following pollination. Notably, at 35 °C, the percentage of the style traversed by the pollen tube was only 3.3% and 1.7% at 4 and 8 h after pollination, respectively, and the pollen tube did not reach the ovules.

EFFECTS OF CULTIVATION DURATION UNDER CONSTANT TEMPERATURE ON OVULE LONGEVITY. The results of the effect of temperature on ovule degeneration indicated that the percentage of ovule degeneration accelerated with increasing temperature after 120 h of cultivation (Table 13). It is worth noting that the percentage of the ovule with callose deposition was increased significantly after only 4 h culture at 30 and 35 °C, and the percentage of degenerated ovules was ≈74% and 100%, respectively, at 120 h culture, which was significantly higher than the percentage of degenerated ovules under other temperature conditions. However, at 0 and 5 °C, the percentage of degenerated ovules differed slightly between the different cultivation durations, and the percentage of degenerated ovules was ≈35% after 120 h cultivation, which was significantly lower than the percentage of degenerated ovules at 25 °C. At 10, 15, 20, and 25 °C, the ovule degeneration percentages were ≈40%, 43%, 44%, and 63%, respectively, indicating that higher temperatures accelerated ovule degeneration in blueberry.

Discussion

Temperature is among the most important climatic factors affecting plant reproductive processes such as pollen germination, pollen tube growth, and fruit setting (Acar and Kakani, 2010), and its consequences on reproductive success may increase with global warming (Hedhly et al., 2004). The effects of temperature on pollen germination in different species or different botanical varieties in the same species are diverse, as has been found in almond [*Prunus* sp. (Sorkheh et al., 2011)], apple [*Malus domestica* (Xu and Xu, 2014)], longan [*Dimocarpus longan* (Pham et al., 2015)], loquat [*Eriobotrya japonica* (Yang et al., 2015c)], mango [*Mangifera indica* (Sukhvibul et al., 2000)], and sweet cherry [*Prunus avium* (Radičević et al., 2016)]. The optimal temperature range for pollen germination in the aforementioned species was often close to the average temperature of their flowering phenophase. The results of this study corroborate these previous studies, with significantly higher pollen germination percentages recorded under average temperatures of 15, 20, and 25 °C during the flowering phenophase, and the predicted T_{min} , T_{opt} , and T_{max} of pollen germination of ‘Gardenblue’ in ‘Brightwell’ pistils were 0.8, 21.4, and 42.0 °C, respectively. This might be because higher or lower temperatures inhibit stigmatic mucus secretion, which reduces pollen germination as a result of the altered physiological conditions (Hedhly et al., 2003). In addition, research on the influence of temperature on pollen tube growth in pear

Table 9. Percentage of the first-third of the style traversed by the pollen tube of the flowers of rabbiteye blueberry ‘Brightwell’ under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 120 h after pollination in 2017.

Temp (°C) ^z	Time after cultivation (h) ^y							
	4	8	12	24	48	72	96	120
	First-third of style traversed by pollen tube [mean ± SE (%)]							
0	0 ^x	0	0	0	0	0	0	0
5	13.33 ± 1.67 a	6.67 ± 1.67 b	0	71.67 ± 1.67 a	71.67 ± 1.67 a	66.67 ± 2.89 a	48.33 ± 1.67 c	26.67 ± 2.89 d
10	43.33 ± 1.67 c	43.33 ± 1.67 c	53.33 ± 1.67 b	71.67 ± 1.67 a	91.67 ± 1.67 a	61.67 ± 1.67 c	41.67 ± 1.67 f	18.33 ± 1.67 g
15	51.67 ± 1.67 e	56.67 ± 1.67 d	71.67 ± 2.89 b	88.33 ± 2.89 a	88.33 ± 1.67 b	56.67 ± 1.67 d	33.33 ± 1.67 e	13.33 ± 1.67 f
20	56.67 ± 2.89 d	61.67 ± 1.67 d	71.67 ± 1.67 c	91.67 ± 2.89 a	88.33 ± 1.67 b	56.67 ± 1.67 d	33.33 ± 1.67 e	13.33 ± 1.67 f
25	61.67 ± 2.89 b	66.67 ± 1.67 b	76.67 ± 2.89 a	81.67 ± 1.67 a	76.67 ± 1.67 a	31.67 ± 1.67 c	11.67 ± 1.67 d	— ^w
30	46.67 ± 1.67 c	51.67 ± 2.89 ab	53.33 ± 1.67 a	46.67 ± 1.67 c	38.33 ± 1.67 d	11.67 ± 1.67 e	—	—
35	43.33 ± 1.67 a	46.67 ± 2.89 a	45.00 ± 1.67 a	33.33 ± 1.67 b	11.67 ± 1.67 c	—	—	—

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, 60 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from ‘Gardenblue’ onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student-Newman-Keuls tests ($P \leq 0.05$).

^wThe style became brown or withering when pollinated or sampled.

Table 10. Percentage of half of the style traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 120 h after pollination in 2017.

Temp (°C) ^z	Time after cultivation (h) ^y						
	4	8	12	24	48	72	120
0	0 ^x	0	0	0	0	0	0
5	0	0	0	0	0	0	0
10	38.33 ± 1.67 c	41.67 ± 2.89 c	51.67 ± 1.67 b	68.33 ± 1.67 a	53.33 ± 2.89 b	33.33 ± 1.67 d	23.33 ± 2.89 e
15	48.33 ± 2.89 c	53.33 ± 1.67 c	68.33 ± 1.67 b	86.67 ± 2.89 a	48.33 ± 1.67 c	31.67 ± 2.89 d	13.33 ± 1.67 e
20	56.67 ± 1.67 cd	60.00 ± 2.89 c	68.33 ± 1.67 b	88.33 ± 2.89 a	53.33 ± 1.67 d	33.33 ± 1.67 e	11.67 ± 1.67 f
25	58.33 ± 2.89 c	58.33 ± 1.67 c	66.67 ± 2.89 b	81.67 ± 1.67 a	56.67 ± 1.67 c	11.67 ± 1.67 d	— ^w
30	38.33 ± 1.67 a	36.67 ± 2.89 a	38.33 ± 1.67 a	28.33 ± 2.89 b	13.33 ± 2.89 c	0	—
35	33.33 ± 2.89 a	31.67 ± 1.67 a	31.67 ± 1.67 a	21.67 ± 1.67 b	0	—	—

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 4, 8, 12, 24, 48, 72, 96, and 120 h cultivation at a constant temperature, 60 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

^wThe style became brown or withering when pollinated or sampled.

Table 11. Percentage of style traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at 120 h after pollination in 2017.

Temp (°C) ^z	Time after cultivation (h) ^y						
	4	8	12	24	48	72	120
0	0 ^x	0	0	0	0	0	0
5	0	0	0	0	0	0	0
10	36.67 ± 1.67 c	36.67 ± 1.67 c	41.67 ± 1.67 bc	63.33 ± 1.67 a	43.33 ± 1.67 b	23.33 ± 1.67 d	0
15	41.67 ± 1.67 c	46.67 ± 1.67 bc	48.33 ± 1.67 b	88.33 ± 1.67 a	41.67 ± 1.67 c	18.33 ± 1.67 d	0
20	46.67 ± 1.67 b	48.33 ± 1.67 b	51.67 ± 1.67 b	86.67 ± 1.67 a	38.33 ± 1.67 c	16.67 ± 1.67	0
25	18.33 ± 1.67 a	21.67 ± 1.67 a	21.67 ± 1.67 a	23.33 ± 1.67 a	13.33 ± 1.67 b	0	— ^w
30	11.67 ± 1.67 a	11.67 ± 1.67 a	11.67 ± 1.67 a	6.67 ± 1.67 a	0	0	—
35	3.33 ± 1.67 a	1.67 ± 1.67 a	0	0	0	—	—

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 4, 8, 12, 24, 48, 72, 96, and 120 h cultivation at a constant temperature, 60 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student–Newman–Keuls tests ($P \leq 0.05$).

^wThe style became brown or withering when pollinated or sampled.

Table 12. Percentage of the ovules traversed by the pollen tube of the flowers of rabbiteye blueberry 'Brightwell' under 0, 5, 10, 15, 20, 25, 30, or 35 °C controlled temperature treatments. They were pollinated after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, and then the pollinated flowers of each temperature treatment were sampled at the 120 h after pollination in 2017.

Temp (°C) ^z	Time after cultivation (h) ^y							
	4	8	12	24	48	72	96	120
	Ovules traversed by pollen tube [mean ± SE (%)]							
0	0 ^x	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	17.78 ± 1.11 c	16.67 ± 0.67 c	21.11 ± 1.11 b	33.33 ± 1.93 a	22.22 ± 1.11 b	8.89 ± 1.11 e	0	0
15	43.33 ± 1.93 b	44.44 ± 1.11 b	48.89 ± 1.11 a	52.22 ± 1.11 a	35.56 ± 1.11 c	5.56 ± 1.11 d	0	0
20	44.45 ± 2.22 b	47.78 ± 1.11 b	52.22 ± 1.11 a	55.56 ± 1.11 a	34.44 ± 1.11 c	24.44 ± 1.11 d	0	0
25	30.00 ± 1.92 a	31.11 ± 1.11 a	28.89 ± 1.11 ab	27.78 ± 1.11 ab	25.56 ± 1.11 b	0	0	— ^w
30	21.11 ± 1.11 a	25.56 ± 1.11 a	21.11 ± 2.22 a	11.11 ± 2.22 b	0	0	—	—
35	0	0	0	0	0	—	—	—

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose, and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^yAfter 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at a constant temperature, 60 flowers were emasculated in each temperature treatment at the balloon stage. Pollination was facilitated by sweeping a small brush containing the pollen from 'Gardenblue' onto the stigma until the pollen was visible. Sixty pollinated flowers of each temperature treatment were then sampled at the 120 h after pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student-Newman-Keuls tests ($P \leq 0.05$).

^wThe style became brown or withering when pollinated or sampled.

Table 13. Percentage of the ovules with callose of the flowers rabbiteye blueberry 'Brightwell' collected after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at different constant temperatures before pollination in 2017.

Temp (°C) ^z	Time after cultivation (h) ^y							
	4	8	12	24	48	72	96	120
	Ovules with callose [% (mean ± SE)]							
0	25.56 ± 1.11 c ^x	25.56 ± 1.11 e	28.89 ± 1.11 d	28.89 ± 1.11 e	32.22 ± 1.11 e	34.44 ± 1.11 e	35.56 ± 1.11 e	35.56 ± 1.11 e
5	25.56 ± 1.11 c	27.78 ± 1.11 de	31.11 ± 1.11 d	33.33 ± 1.93 de	35.56 ± 1.11 de	35.56 ± 1.11 e	37.78 ± 1.11 de	37.78 ± 1.11 e
10	25.56 ± 1.11 c	28.89 ± 1.11 de	32.22 ± 1.11 d	34.45 ± 2.22 de	36.67 ± 1.93 de	37.78 ± 1.11 de	38.89 ± 2.22 de	40.00 ± 1.92 de
15	25.56 ± 1.11 c	31.11 ± 1.11 cd	32.22 ± 1.11 d	35.56 ± 1.11 de	38.89 ± 1.11 d	38.89 ± 2.22 de	41.11 ± 2.22 de	43.33 ± 1.93 d
20	27.78 ± 1.11 c	31.11 ± 1.11 cd	32.22 ± 1.11 d	36.67 ± 1.93 d	38.89 ± 1.11 d	41.11 ± 1.11 d	42.22 ± 1.11 d	44.44 ± 1.11 d
25	28.89 ± 1.11 c	34.44 ± 1.11 c	37.78 ± 1.11 c	45.56 ± 1.11 c	51.11 ± 1.11 c	55.89 ± 1.31 c	61.11 ± 1.11 c	63.33 ± 1.93 c
30	38.89 ± 1.11 b	53.33 ± 1.93 b	60.00 ± 1.92 b	64.33 ± 2.17 b	64.44 ± 1.11 b	67.78 ± 1.11 b	71.11 ± 1.11 b	74.44 ± 1.11 b
35	47.78 ± 1.11 a	57.78 ± 1.11 a	64.78 ± 1.45 a	73.33 ± 1.93 a	90.00 ± 1.92 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a

^zIn the full-bloom stage, the branches with entire inflorescences were placed in a controlled climate chamber at a humidity of 80%. Their stem bases were immersed in 1% (w/v) sucrose and they were subjected to 0, 5, 10, 15, 20, 25, 30, or 35 °C treatments, which covers the typical temperature range during the flowering phenophase.

^ySixty emasculated flowers were collected after 4, 8, 12, 24, 48, 72, 96, and 120 h of cultivation at different constant temperatures before pollination, and 20 flowers each from each temperature treatment were measured in compressed preparations with a fluorescence microscope, with three replications.

^xDifferent letters indicate significant differences within a parameter across the sampling times by Student-Newman-Keuls tests ($P \leq 0.05$).

[*Pyrus* sp. (Vasilakakis and Porlingis, 1985)], sour cherry [*Prunus cerasus* (Cerović and Ružić, 1992)], apricot [*Prunus armeniaca* (Austin et al., 1998)], and apple (Petropoullou and Alston, 1998) indicates that pollen tube growth can be accelerated under the appropriate high-temperature range, resulting in a reduced travel time of the pollen tube to the ovules.

In this study, increased temperature could accelerate pollen tube growth in 'Brightwell' blueberry, but pollen tube growth was generally halted at the middle of the style when the temperature was 30 °C, whereas no pollen tube growth was observed in the style at 35 °C. Furthermore, the percentage of the style traversed by the pollen tube at 30 °C was significantly lower than at 15, 20, and 25 °C. These results indicated that the suitable temperature range of pollen tube growth of 15 to 25 °C was consistent with that of pollen germination, suggesting that this temperature range during the flowering phenophase should positively influence pollination, fertilization, and fruit setting. However, it must be assumed that the ovule is still viable when the pollen tube reaches the ovule (i.e., the time required for the pollen tube to grow to the ovule is shorter than the period for which the ovule maintains its viability). More than 55% ovule viability was recorded at 96 h after cultivation at 15, 20, and 25 °C, which is consistent with the percentage of ovules with pollen tubes at this time.

Low temperatures and wet, cold weather are frequent in spring during the blueberry flowering phenophase and are the result of the changes in global climate in recent years, which has become a constraint on the sustainable development of blueberries in some regions (Yang et al., 2015b, 2017). In this study, the predicted T_{min} , T_{opt} , and T_{max} of the pollen tube growth of 'Gardenblue' in 'Brightwell' pistils were predicted to be 1.6, 18.4, and 35.2 °C, respectively, which imply that the suitable pollination and fertilization temperatures were in the range of 15 to 25 °C in blueberry. However, at temperatures of 0 and 5 °C, it is worth noting that only ≈35% of the ovules exhibited callose deposition, indicating that ≈65% of the ovules were still viable. This may be due to the ability of blueberries to adapt to low temperatures through long-term natural selection, enabling them to mitigate the effects of frequent cold weather on their pollination and fertilization. However, compared with the frequent cold weather in some regions, as the greenhouse effect intensifies, the impact of global temperature rise on the reproductive process of plants is also of concern. Some studies have shown that high temperature during the flowering phenophase adversely affects pollination and fertilization in some blueberry cultivation regions in China (Yang et al., 2015b, 2017; Yu et al., 2016). In this study, the percentage of ovules with callose was increased significantly after only 4 h of culture at 30 and 35 °C, and the percentage of degenerated ovules was ≈74% and 100%, respectively, at 120 h culture, which suggests that high temperatures could accelerate ovule degeneration.

The findings of this study demonstrated that 'Brightwell' is sensitive to a certain range of high temperatures during the flowering phenophase. The ability of blueberry to tolerate a certain high-temperature range during the flowering phenophase should thus become a focus for the breeding of temperature-resistant blueberries and should also become an important indicator for the ecological regionalization of blueberry cultivation. Of course, the influence of temperature on the reproductive process of plants is important not only during the flowering phenophase but also during the formation and development stage of the flower buds of fruit trees, such as pear,

peach (*Prunus persica*), and apricot. Therefore, further research on the influence of preflowering temperature on the reproductive process of blueberries is required.

Conclusions

In this article, a comprehensive analysis of the effect of temperature on pollen germination, pollen tube growth, fruit set, and ovule longevity showed that temperature significantly affects pollen germination, pollen tube growth, and ovule longevity. Furthermore, pollen tube growth was accelerated by the appropriate high temperature in the appropriate temperature range, resulting in a reduced travel time of the pollen tube to the ovule. However, the pollen tubes mostly stopped growing to the middle of the style at 30 °C, and no pollen tubes traversed the style at 35 °C. In addition, high temperature also accelerated ovule degeneration. The results of the regression analysis showed that the T_{opt} of pollen germination and pollen tube growth were 21.4 and 18.4 °C, respectively, in blueberry. Therefore, the tolerance of blueberry to a certain high-temperature range in the flowering phenophase should inform future breeding strategies for temperature resistance and is also an important indicator of suitable environments for blueberry cultivation to mitigate potential future temperature stress.

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