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Pollen Performance Differs Among Cultivars of Northern Highbush Blueberry (*Vaccinium corymbosum* L.)

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Abstract

Pollen performance (i.e., germination, viability, and tube growth) in highbush blueberry (*Vaccinium corymbosum* L.) is not well characterized across commercially important cultivars and under environmental conditions typical for pollination-limited western Washington, U.S. Cultivars may differ in pollen performance and how they respond to environmental conditions, which may impact ovule fertilization and subsequent berry development. Knowing intrinsic cultivar differences in tetrad germination rates and threshold environmental conditions that impact pollen performance may help growers implement targeted strategies that are being tested and developed to improve pollination and berry development. In this study, the main objective was to evaluate pollen performance by measuring pollen germination rate, tube length, and tube number per tetrad among four commercially important highbush blueberry cultivars under five different temperature conditions. 'Aurora', 'Draper', 'Duke', and 'Liberty' blueberry pollen were collected every week from 5-100% bloom in 2018 and incubated *in vitro* at 2, 7, 13, 18, and 24 °C for 4 days, which was when pollen growth ceased. Tetrads were observed using a microscope every 24 hours. Pollen germination rate, tube length, and tube number per tetrad were determined. Results showed that the optimal temperature range to reach maximum pollen germination, tube length, and tube number per tetrad *in vitro* is 13-24 °C for 'Aurora', 'Draper', and 'Duke', but 13-18 °C for 'Liberty'. 'Liberty' had a relatively lower pollen germination rate and tube growth than the other evaluated cultivars. 'Liberty' was also more sensitive to low and high temperatures. These observations suggest that some of the pollination and fruit development challenges with 'Liberty' may be due to the biology of the pollen itself, as it exhibited a reduced capacity to germinate and grow. These data also demonstrate pollen performance differs across commercially important cultivars of highbush blueberry and suggest developing cultivar-specific effective pollination period models may be useful. Additionally, these findings indicate breeders should consider phenotyping pollen characteristics to better understand adaptation and potential intrinsic pollination constraints at the genetic level.

Introduction

Northern highbush blueberry (*Vaccinium corymbosum* L.) is a commercially important crop globally with the United States (U.S.) leading worldwide production [Food and Agriculture Organization of the United Nations Statistics (FAOSTAT), 2019]. Washington State is a leading producer of highbush blueberries in the U.S. and produced 24% of the U.S.'s total yield (255 million kg) in 2018 [U.S. Department of Agriculture National Agricultural Statistics Service (USDA NASS), 2019]. Blueberry yields tend to be lower in western Washington, and poor pollination is considered a contributing factor causing lower average yields (Brady et al., 2015; DeVetter et al., 2016).

Pollen performance (i.e., germination, vi-

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ability, and tube growth) in highbush blueberry is not well characterized across commercially important cultivars and under environmental conditions typical for western Washington. Cultivars may differ in pollen performance and how they respond to environmental conditions, particularly among cultivars with more southern highbush or other *Vaccinium* species in the germplasm. Differences in pollen performance may impact the effective pollination period (EPP) and ultimately yield and fruit quality attributes.

Highbush blueberry requires insect pollination to optimize fruit set and yields (MacKenzie, 1997). Honey bees (*Apis mellifera* L.) are the primary pollinator in commercial blueberry systems in the Pacific Northwest, including Washington. Cool temperature (< 15 °C) during bloom can be unconducive for honey bee foraging (Julianna and Issacs, 2010). Unfortunately, the bloom period of many commercially important blueberry cultivars is short, sometimes only 5-12 days or less (Pritts et al., 1992). Such a short bloom window leads to a limited period of time for honey bees to successfully transfer pollen, which is critical for fertilizing ovules and initiating fruit development.

Blueberry pollen contains four microspores per tetrad (Stushnoff and Palser, 1969). Pollen viability has been positively correlated with increased blueberry fruit size and seed counts (Vander Kloet, 1984). Fertilized ovules and developing seeds contribute to blueberry fruit development, with higher seed counts associated with increased fruit size, although to some extent this will be influenced by cultivar and horticultural management (Dogterom et al., 2000; Lang and Danka, 1991; Payne et al., 1989). Few data exist describing carpel and seed number across highbush blueberry cultivars, but Dogterom et al. (2000) reported an average of 6 carpels per flower and ~18 ovules per carpel in 'Bluecrop', meaning a single flower has the potential to develop 106 seeds in a berry. Because of pollen competition (Mulcahy and Mulcahy, 1987), ovule and seed abortion (Davis et al., 1987), pollen tube attrition (Smith-Huerta, 1997), and physical blockage of pollen grains (Snow, 1986), high but not excessive numbers of pollen grains can lead to optimal seed set and fruit production. Therefore, the number of functional pollen grains received by a stigma is critical for successful fertilization and fruit set.

Temperature conditions are known to influence the growth, development, and performance of pollen in several horticultural species (Hedhly et al., 2004), but this has not been verified in blueberry. Hedhly et al. (2004) evaluated the pollen performance of 'Sunburst' and 'Cristobalina' sweet cherry (*Prunus avium* L.) under different temperature conditions and found reduced pollen germination and accelerated pollen tube growth at 30 °C. The study also showed that the different cultivars of sweet cherry responded differently to temperatures during the reproductive phase. 'Sunburst' had a reduced microgametophyte population at 30 °C, while the population of microgametophytes was reduced at 10 °C in 'Cristobalina'. Despite warmer temperatures generally being favorable for pollen performance by increasing the speed of metabolic reactions, excessive temperatures can be detrimental and shorten the effective pollination period (De Vries and Dubois, 1987; Hedhly et al., 2004). By comparison, cool and wet conditions can slow growth while promoting the decay of floral tissues and increase the susceptibility of these tissues to fungal and bacterial infections (Daykin and Milholland, 1990; Ngugi and Scherm, 2004 and 2006; Verma et al., 2006).

There are few studies that examined blueberry pollen and even less that have evaluated it in current commercially important northern highbush blueberry cultivars. Eaton et al. (1966) found that tetrad abortion and percent germination differed among 'Pemberton', 'Berkeley', 'Jersey', 'Rancocas', 'Dixi', and 'Weymouth' highbush blueberry. For example, tetrad germination ranged from 5.5% to

70.6% in 'Weymouth' and 'Pemberton', respectively. While none of these cultivars are currently being grown on a large commercial scale in Washington and the greater Pacific Northwest, this study indicates cultivars differ greatly in pollen performance.

Because the pollination period is short in highbush blueberry, combined with other factors like unfavorable weather conditions that limit honey bee activity, learning more about the biology and performance of blueberry pollen across different commercially important cultivars is essential. Knowing intrinsic cultivar differences in tetrad germination rates and threshold environmental conditions that may impact pollen performance could help growers implement targeted pollination strategies that are being tested and developed through research (Arrington and DeVetter, 2018). Furthermore, knowledge of these cultivar differences can help breeders, growers, and crop advisors determine whether or not selections and/or cultivars are adapted to a region and may even be a phenotyping trait for breeders. The objective of this study was to evaluate pollen performance with an emphasis on *in vitro* germination and tube growth (pollen tube number per tetrad and tube length) among four important cultivars of highbush blueberry and to understand how temperature impacts pollen germination and growth.

Materials and Methods

Sample collection and preparation. Pollen tetrads of 'Aurora', 'Draper', 'Duke', and 'Liberty' highbush blueberry were collected from a random sample of plants along transects in fields under commercial production and managed by the same grower within Skagit County, Washington in May 2018. 'Draper' and 'Liberty' were adjacent to each other in the field, while 'Aurora' and 'Duke' were less than 10 km away from each other and the remaining sites. All pollen samples were collected weekly from completely open blossoms for a total of three weeks (except for 'Aurora', which was only collected for two weeks because of difficulties with pollen release and subsequent collection during the first week). This sampling period spanned 5-100% bloom for all cultivars considered.

A minimum of 120 tetrads were dusted onto pollen-specific media poured into Petri dishes. The media contained 0.23% (w/v) Murashige and Skoog (MS) salts, 0.01% (w/v) inositol, 0.0004% (w/v) thiamine HCl, 2% (w/v) sucrose, and 0.4% (w/v) agar (Arrington, 2017). The pH of the media was 5.0. Petri dishes containing pollen were placed in incubators (Boekel 132000, Boekel Scientific, Feasterville, PA; Ambi-Hi-Lo Chamber, Lab-Line Instruments, Melrose Park, IL; I30BLL, Percival, Perry, IA) and a refrigerated cooler at the Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA. Tetrads were held at five different temperature treatments (2, 7, 13, 18, and 24 °C) for four days, which was after pollen tube growth ceased. No supplemental light was provided during incubation. These temperatures were selected because they represented the range of temperatures flowers may be exposed to within the region.

Data collection and analysis. Each Petri dish was divided into quadrants. Thirty clearly visible tetrads were randomly selected in each quadrant for further measurement. Tetrads were observed under a compound microscope (Eclipse 50i, Nikon, Japan) at 40x magnification every 24 hours. Pollen germination rate, tube length, and tube number per tetrad were determined for each quadrant containing 30 tetrads. A tetrad that produced one or more pollen tubes was considered germinated.

Statistical analyses were conducted using Statistical Analysis Software (SAS; SAS Institute Inc., Cary, NC). The experimental design was completely randomized, and data were evaluated for normality and equal variance before conducting regression analysis using PROC REG. An analysis of covariance approach was first used to determine if there was a cultivar x temperature interaction for

the variables measured with cultivar as the indicator variable and temperature as the covariate. After determining the interaction was significant (with $\alpha = 0.05$), linear and quadratic models were fitted for each cultivar. The significance of the model (*P*-value), coefficient of determination (R^2), and adjusted coefficient of determination (R^2_{adj}) were calculated and are presented for each cultivar. Only pollen germination, tube length, and tube number per tetrad data collected on the fourth day were used in the analyses, which was when pollen growth ceased for all cultivars. All data are presented in original units.

Results

Pollen germination, tube length, and tube number per tetrad had significant cultivar x temperature interactions ($P = < 0.0001$), so all variables were analyzed by cultivar.

Furthermore, all pollen growth stopped at the 4th day, showing *in vitro* longevity of pollen for the cultivars in this study was less than 4 days.

Pollen germination rate. Pollen germination rates of all cultivars showed a quadratic relationship when incubated at different temperatures (Fig. 1). Overall, germination rates increased with increasing temperature, but the response curve varied by cultivar (*P*-value = 0.0234). 'Liberty' had the lowest germination rate but peaked at 13 and 18 °C. The remaining cultivars showed a similar quadratic relationship with 'Duke' having the highest germination above 2 °C. Germination in 'Duke' peaked at 18 °C and declined at lower and higher incubation temperatures.

Each cultivar was fitted to the quadratic model and had a *P*-value <0.0001, indicating the models accounted for a significant pro-

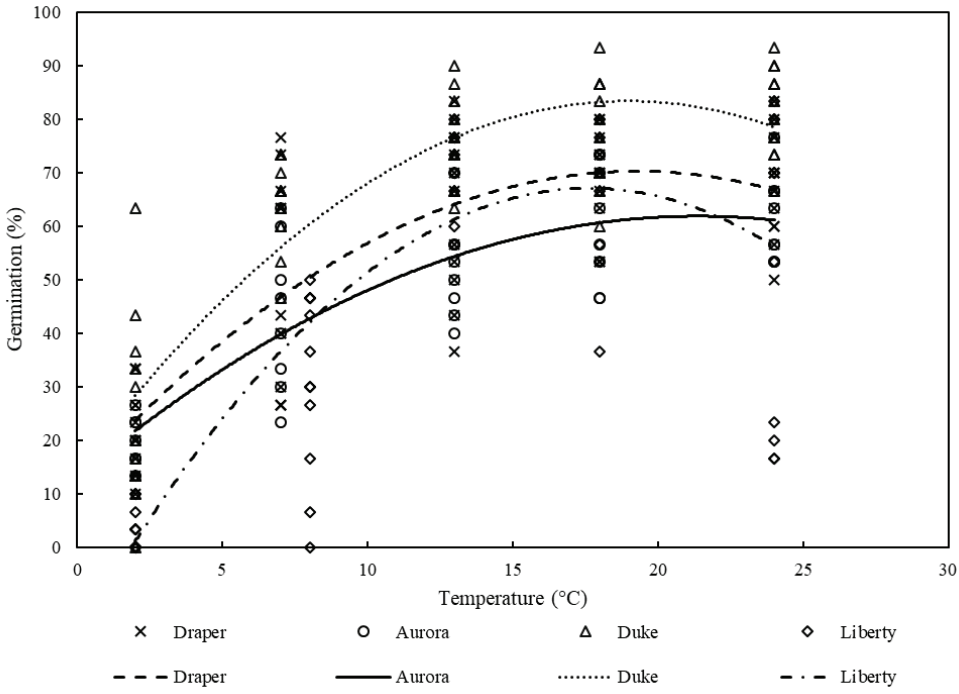

Figure 1. Pollen tube germination for 'Aurora', 'Draper', 'Duke', and 'Liberty' pollen tetrads collected from highbush blueberry plants grown in western Washington, U.S., and incubated *in vitro* at different temperatures, 2018.

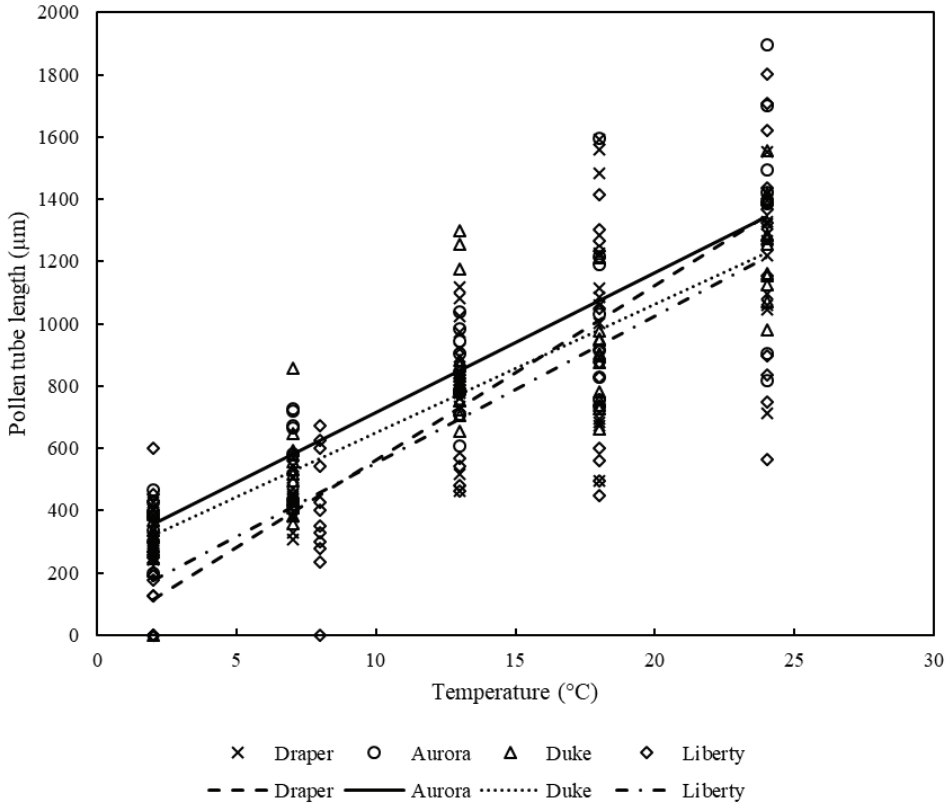

Figure 2. Pollen tube length for 'Aurora', 'Draper', 'Duke', and 'Liberty' pollen tetrads collected from highbush blueberry plants grown in western Washington, U.S., and incubated *in vitro* at different temperatures, 2018.

portion of the variation in the data. The R^2, R^2_{adj}, and formula for each cultivar are presented in Table 1. The R^2 ranged from 0.7497 in 'Duke' to 0.6373 in 'Liberty' while the R^2_{adj} were slightly lower ranging from 0.7407 in 'Duke' to 0.6241 in 'Liberty'. 'Aurora' had a slightly lower R^2 and R^2_{adj} compared to 'Duke' at 0.7281 and 0.7134, respectively. 'Draper' R^2 was 0.6562, while the R^2_{adj} was 0.6441.

Pollen tube length. Differences were observed in average pollen tube length by cultivar (P = 0.002) and there was a positive linear relationship with increasing temperature (Fig. 2). 'Draper' and 'Liberty' had comparatively shorter pollen tubes than 'Aurora' and 'Duke' at 2 and 7 °C, while 'Aurora' tended to produce longer pollen tubes across the incubation temperatures. At 24 °C, 'Aurora' and 'Duke' had reached their maximum pollen tube length and were on average longer than 'Draper' and 'Liberty'. The R^2 and R^2_{adj} for 'Aurora' was 0.7212 and 0.7138, respectively (Table 1). Similarly, the R^2 and R^2_{adj} was 0.7278 and 0.7231, respectively, for 'Draper' and 0.7772 and 0.7733, respectively, for 'Duke'. 'Liberty' had lower coeffecients with an R^2 at 0.6391 and R^2_{adj} 0.6326.

Pollen tube number per tetrad. Overall pollen tube number per tetrad was low across all cultivars and incubation temperatures (Fig. 3). The response curve was quadratic and

Table 1. Coefficients of determination (R^2) and adjusted coefficients of determination (R^2_{adj}) of 'Aurora', 'Draper', 'Duke', and 'Liberty' pollen tetrads collected from highbush blueberry plants grown in northwestern Washington, USA, and incubated in *vitro* at different temperatures (2, 7, 13, 18, and 24 °C) for four days, 2018. Data were fitted to linear and quadratic models including temperature and significant models with *P*-values <0.05 are presented.

		Cultivar			
Variable	Parameter	Aurora	Draper	Duke	Liberty
Germination	R^2	0.7281	0.6562	0.7497	0.6373
	R^2_{adj}	0.7134	0.6441	0.7407	0.6241
	Formula[z]	$y=-0.11x^2+4.74x+13.6$	$y=-0.16x^2+6.02x+12.29$	$y=-0.18x^2+6.86x+17.43$	$y=-0.27x^2+9.48x-16.52$
Tube length	R^2	0.7212	0.7278	0.7772	0.6391
	R^2_{adj}	0.7138	0.7231	0.7733	0.6326
	Formula[y]	y=44.85x+266.6	y=45.13x+192.87	y=40.35x+253.77	y=47.24x+94.3
Tube	R^2	0.6765	0.4362	0.6879	0.6373
number/tetrad	R^2_{adj}	0.659	0.4165	0.6768	0.6241
	Formula[z]	$y=-0.001x^2+0.05x+0.14$	$y=-0.002x^2+0.09x+0.09$	$y=-0.002x^2+0.08x+0.17$	$y=-0.003x^2+0.1x-0.16$

[z] Quadratic formulas for percent germination (y) and pollen tube number where x = temperature.
[y] Linear formulas for pollen tube length (y) where x = temperature.

varied by cultivar (*P*-value = 0.01). 'Liberty' had the lowest tube number at 2°C compared to the remaining cultivars. Pollen tube number per tetrad across the cultivars reached its maximum at 18°C, but was never greater than one pollen tube per tetrad. However, 'Liberty' and 'Aurora' produced the least pollen tube growth at temperatures above 2 °C. 'Draper' had the lowest R^2 at 0.4362 and R^2_{adj} 0.4165 (Table 1). The R^2 was greater for 'Aurora', 'Duke', and 'Liberty' at 0.6765, 0.6879, and 0.6373, respectively. Likewise, the R^2_{adj} was greater for these cultivars ('Aurora' = 0.659, 'Duke' = 0.6768, and 'Liberty' = 0.6241) compared to 'Draper'.

Discussion

Pollen performance measured as germination rate and tube growth differed by cultivar and generally increased with increasing temperature. Among the cultivars included in this study, 'Duke' tended to have the greatest pollen germination rate and higher average tube number per tetrad, although no cultivars reached an average pollen tube number greater than one (Fig. 3). Because of these pollen characteristics, 'Duke' may be easier fertilized and set fruit than the other three cultivars included in the study. 'Liberty' tended to have a lower pollen germination rate potential at cool (2 to 7 °C) and high (24 °C) temperatures, indicating the pollen of this cultivar may be more sensitive to temperature conditions relative to the other cultivars considered in this study (Fig. 1). Pollen germination in 'Liberty' maximized at 13 and 18 °C and exceeded that of 'Aurora', suggesting this cultivar may have a narrow optimal range for pollen performance. Such a narrow optimal range may impose constraints for fertilization and berry development and may partially explain some of the challenge growers in the Pacific Northwest express regarding growing 'Liberty' (Lisa DeVetter's personal communication with growers and crop advisors in the region). Thus, higher temperatures between 13-24 °C may increase pollen performance in 'Aurora', 'Draper', and 'Duke', but 13-18 °C is optimal for 'Liberty'. Pollen tube length showed similar trends across all cultivars with length increasing linearly as temperature increased (Fig. 2). In 'Liberty', although pollen germination rate decreased when the temperature increased

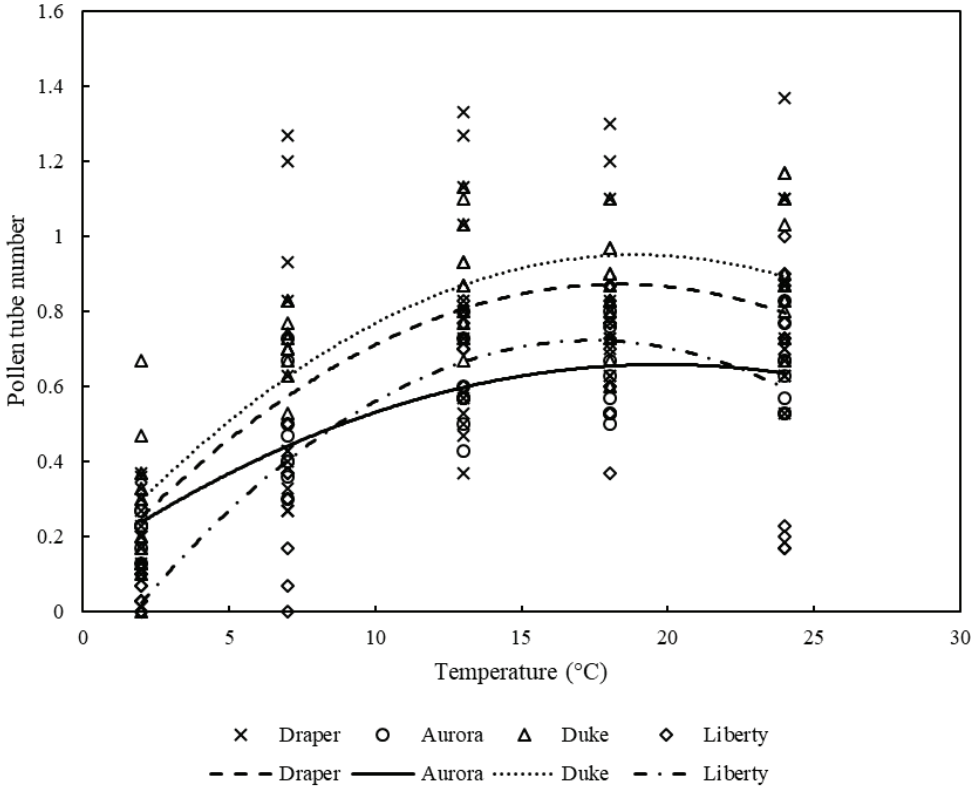

Figure 3. Pollen tube germination for 'Aurora', 'Draper', 'Duke', and 'Liberty' pollen tetrads collected from highbush blueberry plants grown in western Washington, U.S., and incubated *in vitro* at different temperatures, 2018.

to 24 °C (Fig. 1), average pollen tube length was highest at this temperature (Fig. 2). This discrepancy could indicate that the optimal temperatures for pollen germination and pollen tube growth are different for 'Liberty' and possibly poses additional challenges for pollinating this cultivar. Hedhly et al. (2004) similarly reported that pollen germination decreased while pollen tube growth increased when temperature was increased to 30 °C in sweet cherry. Therefore, although higher temperatures increase pollen tube growth, there is a risk that overall germination may be reduced in certain cultivars of highbush blueberry, which may reduce ovule fertilization, fruit set, and berry development depending on the number of seeds that must develop for commercially acceptable fruit size. The incubation conditions in this study did not go above 24 °C, so we are unable to infer how higher temperatures affect these variables.

In general, average pollen tube number per tetrad increased with increasing temperature. However, average pollen tube number per tetrad was low and never exceeded one tube per tetrad. *In vivo* studies show blueberry has the potential to produce 3-4 pollen tubes per tetrad (Dogterom et al., 2000). The low pollen tube number observed in our study could be due to the effects of our *in vitro* conditions. Dogterom et al. (2000) similarly observed reduced pollen tube number per tetrad in 'Bluecrop' and 'Patriot' blueberry when

grown on agar plates (1.43 and 1.66 tubes per tetrad, respectively). While our media may have led to overall low pollen germination and growth relative to *in vivo* conditions, we are still able to compare relative differences across cultivars because all of the pollen were incubated on the same media.

This study is overall consistent with the results generated by Eaton et al. (1966) and highlight cultivars differ in pollen performance. Other factors that contribute to successful ovule fertilization and berry development, such as ovule longevity and number of ovules needing to be fertilized for continued berry development of commercially acceptable fruit, may also respond to cultivar, temperature, and their interactions. These findings suggest cultivar-specific EPP models may aid growers and crop specialists in selecting suitable cultivars for their regions and implementing targeted pollination practices to enhance ovule fertilization and fruit development. The EPP concept was first described for 'Worcester Pearmain' apple (*Malus ×domestica*) by Williams (1965) and is defined as the number of days during which pollination is effective. The EPP is determined from ovule longevity minus the number of days between pollination and fertilization. To develop an EPP for blueberry, ovule longevity and pollen kinetics will need to be further characterized among different cultivars. Additional studies on stigmatic loading of pollen grains among different cultivars will also aid in improving the understanding of cultivar differences regarding optimization of pollination and fertilization.

Climate change is a concern for agricultural systems because of impacts on cropping system productivity and resiliency through increasing temperatures, short- and long-term droughts, and extremes in weather (USDA, 2013). Understanding the relationship between crop development and specific weather variables in conjunction with projected climate changes may foreshadow how cultivation of different plants may be impacted under changing climate scenarios. Pollination and fertilization are two important aspects of crop development to consider when projecting the impacts climate change may have on productions systems. Climate is projected to change in important blueberry production regions such as western Washington (Portmann et al., 2009). Additionally, average air temperatures in May (the typical pollination period for blueberry in western Washington) has already started to rise within the past ten years between 2009-2018 (Fig. 4). The average overall air temperature during the May pollination period for blueberry grown in western Washington has risen from 12.1 °C to 14.8 °C in Whatcom County and from 12.1 °C to 13.8 °C in Skagit County (AgWeatherNet data, 2009-2018) Both of these counties are economically significant contributors to blueberry production in Washington. Skagit County reached the highest overall air temperature in 2018 at 13.8 °C. Average overall air temperature in Whatcom County also peaked at 14.8 °C in 2018. If air temperatures continue to rise, this may promote pollen performance of certain cultivars. However, overall higher average temperatures could accelerate crop development and the bloom period, which could pose additional constraints with sourcing an adequate supply of honey bee hives and compress the overall bloom period. A compressed bloom period may make pollination more vulnerable, as there are fewer days for pollination. Our study suggests an increased air temperature to 18 °C should be beneficial for the pollen performance of 'Aurora', 'Draper', 'Duke', and 'Liberty' highbush blueberry, but there may be risks that overall pollen germination may be reduced at higher temperatures.

Conclusion

In vitro evaluations of pollen performance among several commercial cultivars of highbush blueberry showed that pollen germination and tube growth was greatest between 13 – 24 °C for 'Aurora', 'Duke', and 'Draper', and 13-18 °C for 'Liberty'. Among the

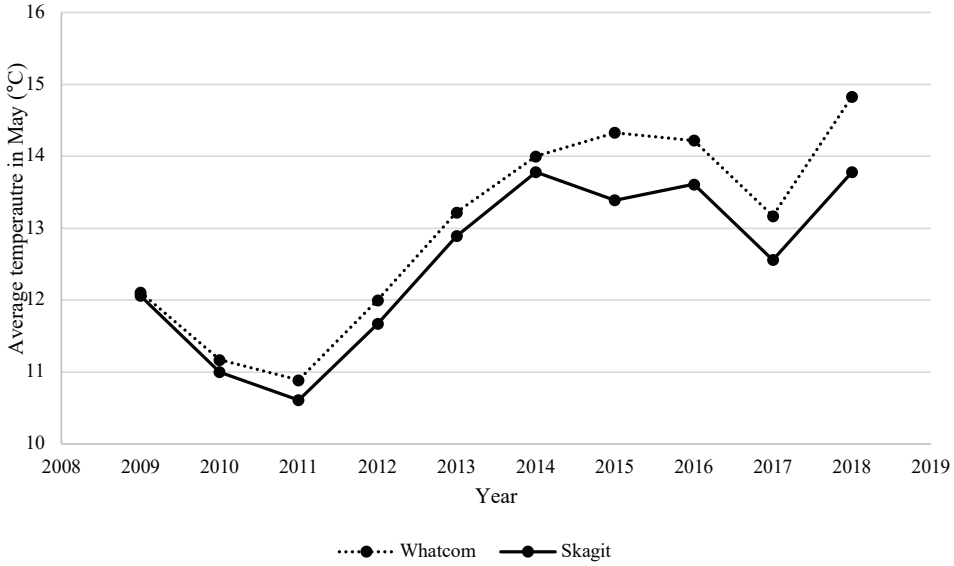

Figure 4. Average overall air temperatures in Whatcom and Skagit counties, northwestern Washington, U.S., during the May pollination period from 2009 to 2018. Data courtesy of WSU AgWeatherNet.

cultivars evaluated, 'Duke' pollen had the potential to have the greatest pollen germination rate. 'Liberty' pollen was more sensitive to temperature conditions and has a narrow range for optimal growth and development compared to the other cultivars in this study. We demonstrated these important commercial cultivars differ in pollen performance according to temperature. These differences may influence adaptation and commercial success, particularly in regions like western Washington where the air temperature during the bloom period can be cool. Knowledge of such differences can aid grower, advisors, and plant breeders in making recommendations for adapted cultivars or selections. Pollen characteristic may also be a useful phenotyping tool for plant breeders.

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Table 1. Coefficients of determination (R^2) and adjusted coefficients of determination (R^2_{adj}) of 'Aurora', 'Draper', 'Duke', and 'Liberty' pollen tetrads collected from highbush blueberry plants grown in northwestern Washington, USA, and incubated *in vitro* at different temperatures (2, 7, 13, 18, and 24 °C) for four days, 2018. Data were fitted to linear and quadratic models including temperature and significant models with P-values <0.05 are presented.