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Cross-pollination Improves Fruit Quantity and Quality Across Northern and Southern Highbush Blueberry Cultivars

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Abstract. **Cultivated blueberries (*Vaccinium* spp.) are partially self-sterile because of early-acting inbreeding depression and, as such, they may set fruit with self-pollination, but with a lower quantity and quality than that with cross-pollination. A lack of recent research of the pollination requirements of modern cultivars as well as shifting management and harvesting priorities have contributed to the increasing prevalence of single-cultivar blocks. However, cross-pollen limitation may be a significant factor that contributes to reduced pollination levels and low yields in these contexts. In this study, we applied hand-pollination treatments in a greenhouse and compared the effects of cross-pollination and self-pollination on numerous fruit responses related to both quantity and quality of 20 cultivars of both northern highbush (NHB) (*Vaccinium corymbosum*) blueberry and southern highbush (SHB) (*V. corymbosum* hybrids) blueberry. Cross-pollination improved the resulting fruit weight per pollinated flower for all SHB cultivars and 7 of 10 NHB cultivars. Increases ranged from 18% to 380% because of greater fruit set and/or weight of the set berries. Cross-pollination also reduced times to ripen for 9 of 10 SHB cultivars and 7 of 10 NHB cultivars. Cross-pollination reduced time to ripen by 3 to 27 days and increased seed set in all but one NHB cultivar. Effects on fruit quality were more variable and included fruit firmness and sugar:acid ratios, but there was some evidence of increased fruit firmness under cross-pollination in six NHB cultivars. Finally, effects were generally consistent across three to eight cross-pollen donors tested for each recipient cultivar, with a few exceptions. These results show that cross-pollination has significant and positive impacts on yields and ripening time of cultivated blueberry; however, the magnitude of the effect varies across cultivars. Furthermore, the consistency of effects across tested cross-pollen donors suggests that the selection of cultivars to plant together for optimal pollination outcomes should be based on management and overlapping phenology.**

Introduction

Many fruit, nut, and seed crops are partially to fully self-sterile and require cross-pollination for maximum fertility, yield, and quality. A recent review identified 156 global crops that benefit from cross-pollination but have varying degrees of self-sterility and mechanisms that contribute to self-sterility (Chabert and Mallinger 2025). In some fully self-sterile crops, such as Brazil nut (*Bertholletia excelsa*) and robusta coffee (*Coffea canephora*), pollination requirements are consistent across cultivars. In these crops, interplanting cultivars within the same field is necessary to obtain seeds and fruits. However, for many partially self-sterile crops, there can be considerable variation in self-sterility across cultivars of the same species (Chabert and Mallinger 2025). Thus, for these crops, the benefits of interplanting cultivars will depend on their relative degree of self-sterility and may or may not exceed the costs associated with interplanting (Chabert et al. 2024). Therefore, it is important to determine pollination requirements for individual cultivars of partially self-sterile crops to inform planting and management decisions.

Cross-pollination can affect not only seed and fruit set but also various aspects of fruit quality that are linked to fertilization and seed set. For instance, cross-pollination can result in larger, heavier fruit, as has been identified in apple (*Malus domestica*) (Vizzotto 2018), raspberry (*Rubus* spp.) (Żurawicz et al. 2018), strawberry (*Fragaria* ×*ananassa*) (Dung et al. 2021), and blueberry (*Vaccinium* spp.) (DeVetter et al. 2022; Martin et al. 2024). Additionally, cross-pollination can decrease ripening time because successful fertilization and seed set increase phytohormone production and the rate of fruit growth (Huang et al. 1997; Nagasaka et al. 2022). Although other fruit quality attributes have been less well-studied, there is some evidence of increased fruit firmness with cross-pollination in strawberries (Klatt et al. 2014), apples (Arafat et al. 1994), and some blueberry cultivars (Liu et al. 2022). Additionally, fruit firmness was correlated with the number of fertilized achenes in strawberries, suggesting that this trait is affected by pollination and the resulting seed set (Dung et al. 2021; Klatt et al. 2014). Finally, the effects of cross-pollination on fruit sugar and acid content have been studied in cherries (*Prunus* spp.), strawberries, and blueberries; however, the results have been mixed (Ansari and Davarynejad 2008; Dung et al. 2021, 2023; Klatt et al. 2014; Liu et al. 2022; Nagasaka et al. 2022; Tuttle 2023). Additionally, sugar concentration and acidity were not consistently correlated with seed set in blueberries, suggesting that these qualities may not be directly affected by pollination and fertilization (Nagasaka et al. 2022).

Highbush blueberry includes two crops, northern highbush (NHB) blueberry (mostly *V. corymbosum* with small amounts of *V. angustifolium*) (Edger et al. 2022), which are adapted to cooler climates, and southern highbush (SHB) blueberry (hybrids between mainly *V. corymbosum*, *V. darrowii*, and *V. virgatum*, but also *V. angustifolium*, *V. contablaei*,

V. elliottii, and *V. tenellum*) (Brevis et al. 2008), which are grown in warmer regions worldwide (Mainland 2012; Retamales and Hancock 2018). Both NHB and SHB are partially self-sterile because of an early-acting inbreeding depression (EID) that is widespread in the *Vaccinium* genus (Guillaume and Jacquemart 1999; Hokanson and Hancock 2000; Krebs and Hancock, 1990, 1991; Nuortila et al. 2006; Raspé et al. 2004). The EID occurs when inbreeding results in the accumulation of recessive, deleterious (and typically lethal) alleles expressed during embryo development, resulting in embryo abortion and reduced viable seed production (Husband and Schemske 1995, 1996; Keller and Waller 2002). Consequences of such self-sterility in cultivated blueberry production can be exacerbated by single-cultivar blocks in which all plants within the field are clones, resulting in self-pollination despite pollen flow between individual plants. Although the extent to which cultivated blueberry is planted in single-cultivar blocks varies across production regions, there is an increasing trend toward single-cultivar blocks for ease of management and machine harvesting (DeVetter et al. 2022). At the same time, modern cultivars may be increasingly prone to EID and, thus, increasingly self-sterile as breeding programs have created increasingly inbred lines over time (Ehlenfeldt 1994; Hancock and Siefker 1982). Past studies have found that fruit set, seed set, and the size and mass of individual fruit were greater and the time to ripen was shorter under cross-pollination as compared with self-pollination in both NHB and SHB (DeVetter et al. 2022, Martin et al. 2024); however, the results were sometimes variable across cultivars and years within studies (Czesnik et al. 1989; Doi et al. 2021; Gupton and Spiers 1994; Taber and Olmstead 2016). Additionally, the benefits of cross-pollination were found to vary across the studied metrics; for example, some studies found no effect of cross-pollination on fruit set, whereas seed set and fruit size were increased with cross-pollination (DeVetter et al. 2022; Gupton 1984; Gupton and Spiers 1994; Kendall et al. 2020; Lang and Danka 1991).

Both pollination and maternal resource availability affect seed and fruit set because, without sufficient resources to fuel seed and fruit growth, even well-pollinated flowers may set no or small seeds and fruits. Therefore, mixed results regarding blueberry pollination could be attributable to how maternal resource availability and reallocation were accounted for or controlled for in the study design. The relative importance of maternal resources and pollination to seed and fruit outcomes can vary across plants depending on resource ability (Casper and Niesenbaum 1993). Maternal resources can also be reallocated across flowers within a plant, thereby affecting the degree to which pollen limitation impacts pollination outcomes for individual flowers (Harder and Aizen 2010; Knight et al. 2006; Wesselingh 2007). Although some studies have suggested that pollen limitation should be assessed at the whole-plant level to avoid maternal resource reallocation effects within the plant (Knight et al. 2006; Webber et al. 2020), this may not be feasible for plants with many flowers and relatively long bloom periods like highbush blueberry. An alternative approach is to consider using integrated physiological units or physiological and morphological subunits within a plant in which resources can be reallocated relatively easily within but less easily across (Casper and Niesenbaum 1993; Watson 1986; Wesselingh 2007). For example, applying pollination treatments at the inflorescence or branch level can be an alternative to applying at the whole plant level because resource reallocation is less likely to occur across inflorescences or branches (Casper and Niesenbaum 1993). Indeed, experiments conducted at the inflorescence level reported degrees of pollen limitation comparable to those conducted at the whole plant level for fruit set, while experiments conducted at the individual flower level reported significantly higher degrees of pollen limitation compared with experiments conducted at the whole plant level (Knight et al. 2006).

In this study, we compared relative degrees of self-sterility and related cross-pollination benefits across 20 modern NHB and SHB cultivars. We conducted hand pollination experiments by controlling for maternal resource limitation and reallocation and using multiple cross-pollen donors per recipient cultivar. We compared self-pollination and cross-pollination outcomes for numerous metrics associated with crop quantity and quality including fruit and seed set, fruit weight, fruit ripening time, fruit firmness, and the sugar and acid contents of fruit, including the sugar:acid ratio. We hypothesized that cross-pollination would increase fruit set, seed set, and fruit weight while decreasing fruit ripening time, but that it would have less consistent or significant effects on the sugar:acid ratio because of the lack of relationships between seed set and sugar concentration or acidity found previously in blueberries (Nagasaka et al. 2022). This study expands past research that was conducted with older cultivars with limited commercial production, tested only a few recipient cultivars or recipient cultivars with few cross-pollen donors, or did not report accounting for maternal resource limitation and/or reallocation. Our results can inform future farm design decisions regarding interplanting blueberry cultivars for better pollination and increased fruit quantity and quality.

Methods

Cultivars and plants. We selected 10 cultivars of SHB and 10 cultivars of NHB that are currently in production, including older and recently developed cultivars. The SHB cultivars were Arcadia, Avanti, Chickadee, Colossus, Emerald, Farthing, Kestrel, Meadowlark, Optimus, and Sentinel. The NHB cultivars were Aurora, Bluecrop, Calypso, Draper, Duke, Elliott, Last Call, Legacy, Liberty, and Nelson. We purchased SHB plants from Island Grove Nursery (Island Grove, FL, USA) and NHB plants from Hartmann's Nursery (Grand Junction, MI, USA). The SHB plants were transported from the nursery to the greenhouse in late Fall 2021 before pollination experiments in Winter/Spring 2022. Dormant NHB plants were shipped to Florida from Michigan (with the appropriate permit) in Mar 2022 in a climate-controlled truck (5 °C) in preparation for pollination experiments the following year. All plants were kept in 3.8-L pots in standard soil medium in a greenhouse located in Gainesville FL, USA, at the University of Florida (lat. 29°38′00.88″N, long. 82°21′56.95"W). Plants were adequately pruned and fertilized before the experimental period and watered with an automatic drip system throughout the experiment, with rates adjusted depending on the time of year and temperature. Plants were fertilized with Jobe's Organics soil acidifier [calcium sulfate (80%), sulfur (18%), and bentonite clay (2%); Easy Gardener Products, Inc., Waco, TX, USA] and Osmocote Smart-Release Plant Food Flower and Vegetable granules [nitrogen (14%), available phosphate (14%), soluble potash (14%); The Scotts Company, Marysville, OH, USA] according to the manufacturers' labels.

Pollination experiments were conducted in 2022 for SHB and in 2023 for NHB. For SHB, pollination treatments were performed between January and April, when bushes were flowering, and fruits were harvested from April to June. For NHB, pollination treatments were performed between February and April when bushes were flowering, with fruits harvested from April to June. During the experimental period, the greenhouse was completely closed and free of flower-visiting insects. Additionally, an automatic cooling and heating system loosely regulated temperatures within the greenhouse so that they never fell below 0 °C or exceeded 37 °C. Finally, because Florida is not within the climatic niche of NHB, the NHB plants required a chilling treatment to make them bloom in 2023. In 2022, from mid-October to mid-December, the plants were kept in growth chambers for 8 weeks at 22 °C/18 °C day/night temperatures with an 8-h/16-h photoperiod. Then, they were kept in the dark for 6 weeks at 5 °C until early Feb 2023 to accumulate their required chilling hours.

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Pollination treatments. Twenty SHB plants per cultivar and three to 15 NHB plants per cultivar were selected for pollination treatments (Supplemental Table S1). All selected plants showed significant flower bud formation by January to February of the experimental year. Replication at the plant level was lower for NHB than for SHB because of low flower bud formation and limited plant availability for some NHB cultivars. When plants began to flower, they were observed every 2 d to record and label newly opened flowers. All pollination treatments described were used on recipient flowers that had opened the day before or that same day to ensure complete pistil receptivity according to a study of the cultivated blueberry pollination period (Chabert unpublished data). When plants had sufficient newly opened flowers, hand self-pollination and cross-pollination treatments were applied. Each pollen donor was applied to three flowers per plant belonging to different flower clusters on the same branch, and with each pollination treatment applied to a separate branch. The pollination treatments were applied on independent branches to avoid maternal resource reallocation between treatments. Applying pollination treatments to flowers within different clusters (and only pollinating one flower per cluster) was performed to avoid competition and limitation for maternal resources among flowers. Additionally, all flowers that were not pollinated were removed before or during bloom to avoid maternal resource limitation effects on seed and fruit production. When the number of open flowers and pollen donors available allowed, multiple different cross-pollen donors were tested on the same day for a given recipient plant, with three flowers treated per pollen donor per plant as described; otherwise, the different pollen donors were tested over time for a given plant. The number of individual flowers and plants tested for each recipient cultivar and pollination treatment (self-pollination and cross-pollination by each donor) can be found in Supplemental Table S1. Depending on overlap in bloom time between recipient and donor cultivars, recipient cultivars were tested with between three and eight different cross-pollen donors (Supplemental Table S1). Comparisons were made between self-pollination and cross-pollination treatments (all cross-pollen donors combined), and between self-pollination donors and each individual cross-pollen donor for each recipient cultivar.

Pollen used for hand-pollination was collected from at least three different plants for each pollen donor cultivar on each day to ensure pollen viability. "Self-pollen" was pollen collected from the same cultivar but different plants than those on which it was applied. Collection was performed by vibrating flowers and releasing pollen from the anthers with a VegiBee Deluxe (VegiBee Deluxe Garden Pollinator; MD Heights, MO, USA) into 1.5-mL Eppendorf tubes (Eppendorf, Hamburg, Germany). Recipient stigmas were saturated with pollen using a dark tiny brush (size 18/0). After pollinating every flower, brushes were cleaned and dried. Separate brushes were used for each pollen donor to prevent contamination. Each hand-pollinated flower was identified with a colored Cherry Marker (catalog no. 6200s; Paskal Group, Ma'alot, Israel), and treatment information was recorded with a permanent black marker on tags.

Metrics of fruit quantity and quality. Berries were checked every 2 d for signs of ripening and harvested when fully ripe (i.e., when they turned completely blue and the tip of the peduncle was also turning blue) (Supplemental Figure S1). The berries were weighed with a scale to the nearest 0.01 g. The flowers that did not set fruit were also recorded so that fruit set for each flower was 0 (no fruit) or 1 (set fruit). Time to ripen was calculated for each berry as the Julian date of maturity (when they were completely ripe) minus the Julian date of pollination. The firmness of individual harvested berries was measured (g/mm) on the day of harvest soon after being picked and at room temperature using a FruitFirm 1000 (CVM Inc., Pleasanton, CA, USA). After measuring firmness, fruits were crushed to separate seeds from juice. Seeds were counted and recorded, and mature seeds were separated from immature seeds. Mature seeds were characterized by their brown color, distinctly large size, and rounded, full appearance. Immature or unfertilized seeds were lighter in color, distinctly smaller, and typically disfigured with an irregular shape (Hokanson and Hancock 2000). We calculated seed set as the number of mature seeds per fruit. Finally, the sugar concentration (Brix; varying from 0% to 90%) and acidity (varying from 0.1% to 4.0%) of fruit juice from each fruit was measured using a pocket Brix/acidity meter for blueberries (PAL-BX|ACID7; Atago Co., LTD, Minato-ku, Tokyo, Japan).

Statistical analysis. To analyze the overall effect of cross-pollination relative to self-pollination on fruit metrics for SHB and NHB across the tested recipient cultivars, we used mixed models with pollination treatment (self and cross) and its interaction with recipient cultivar as fixed effects and included the individual recipient plant as a random effect. Separate models were run for SHB and NHB. Response variables and distributions of residuals were as follows: fruit weight per flower including flowers that did and did not set fruit [linear mixed model (lmm)], fruit set [generalized linear mixed model (glmm) with binomial distribution], fruit weight per berry including only set berries (lmm with fruit weight square root-transformed), mature seed set (glmm with negative binomial distribution for SHB and Poisson distribution for NHB), fruit ripening time (glmm with gamma distribution and log link function for SHB and lmm for NHB), fruit firmness (lmm), and fruit sugar:acid ratio (lmm with sugar:acid ratio log-transformed). To account for the effect of temperature variation across the experimental period on ripening time, an additional fixed effect of the Julian pollination date was included in fruit ripening time models. All models were computed with R software (version 4.0.3; R Core Team 2020) an the lme4 package (Bates et al. 2015).

A type III analysis of variance (ANOVA) (car package) (Fox and Weisberg 2018) was used to test the significance of fixed effects in models including the recipient cultivar × pollination treatment interaction. In the absence of evidence of an interaction ($P > 0.10$), results of main effects were reported with a type II ANOVA. When there was evidence of a recipient cultivar × pollination treatment interaction, the results of the type III ANOVA were reported, and contrasts for each cultivar were conducted using the lsmeans function (emmeans package) (Lenth and Piaskowski 2026) to assess the effect of pollination treatment for each recipient cultivar. Then, to compare the effects of individual cross-pollen donors with self-pollen donors, we conducted separate models for each recipient cultivar with pollen donor as the fixed effect including all self and cross donors individually and individual recipient plant as a random effect. Model types, distributions, and transformations were the same as those described previously. In these analyses, only pollen donors that were tested on at least two plants per recipient cultivar were included. After some evidence of an overall effect of donor (at $P \leq 0.10$), each cross-pollen donor was compared with self-pollination donors using planned post hoc pairwise comparisons with Dunnett's P value adjustment for multiple comparisons (emmeans package) (Lenth and Piaskowski 2026). For analyses of individual pollen donors for individual recipient cultivars, we only examined fruit weight per flower and time to ripen, which were the response variables most consistently affected by cross-pollination relative to self-pollination when cross-pollen donors were analyzed together (as described) and the most important production variables that affect yield (fruit weight per flower) and market access (time to ripen).

Statistical significance is generally reported using the language of evidence outlined by Muff et al. (2022), with weak evidence indicated by $0.05 \leq P < 0.10$, moderate evidence indicated by $0.01 \leq P < 0.05$, strong evidence indicated by $0.001 \leq P < 0.01$, and very strong evidence indicated by $P < 0.001$. The exceptions to this were post hoc pairwise comparisons among individual pollen donors for each recipient cultivar for which $P = 0.05$ is used to report significance.

Results

Overall effects of cross-pollination on NHB and SHB. There was very strong evidence that fruit weight per flower, which is a metric influenced by both fruit set and berry weight, was affected by the pollination treatment × cultivar interaction in both NHB and SHB (Table 1). For NHB, there was evidence that cross-pollination increased fruit weight per flower in seven of 10 cultivars, with increases ranging from 27% to 380% (Fig. 1). There was no evidence for 'Aurora' or 'Nelson'; however, for 'Legacy', there was strong evidence that self-pollination resulted in higher fruit weight per flower compared with cross-pollination, with in fruit weight with cross-pollination in eight of 10 cultivars, with increases ranging from 13% to 150% (Supplemental Fig. S2C). Fruit weight did not increase with cross-pollination for ‘Aurora’; however, for ‘Legacy’, fruit weight was higher with self-pollination than with cross-pollination by 15% (Supplemental Fig. S2C). In SHB, there was evidence of an increase in fruit weight per berry with cross-pollination in nine of 10 cultivars (all but ‘Kestrel’), with increases ranging from 6.5% to 58% (Supplemental Fig. S2D).

There was also very strong evidence of a cultivar × pollination treatment interaction on seed set in both NHB and SHB (Table 1). Although the magnitude of difference varied across cultivars, there was evidence that mature seed set was higher with cross-pollination in every SHB cultivar and in nine of 10 NHB cultivars (all but ‘Legacy’), with increases in mature seed set under cross-pollination ranging from 100% to 990% relative to self-pollination (Fig. 2).

For time to ripen, there was very strong evidence of a cultivar × pollination treatment interaction in both NHB and SHB (Table 1). For NHB, ripening time decreased in seven of 10 cultivars (all except ‘Aurora’, ‘Duke’, and ‘Legacy’), with magnitudes ranging from 2.5 to 17 d (Fig. 3). For SHB, the time to ripen decreased with cross-pollination for nine of 10 cultivars (all but ‘Emerald’), with magnitudes ranging from 3 to 27 d (Fig. 3).

Finally, for fruit quality, there was strong to very strong evidence of a pollination treatment × cultivar interaction on berry firmness in both NHB and SHB (Table 1). For NHB, there was evidence that firmness increased with cross-pollination in six cultivars and decreased in one cultivar (Legacy). For SHB, there was evidence that firmness increased with cross-pollination in three cultivars and decreased in one cultivar (Farthing) (Supplemental Fig. S3A and S3B). For the sugar:acid ratio, there was little to no evidence of an overall effect of pollination treatment or pollination treatment × cultivar interaction in NHB (Table 1; Supplemental Fig. S3C), but there was

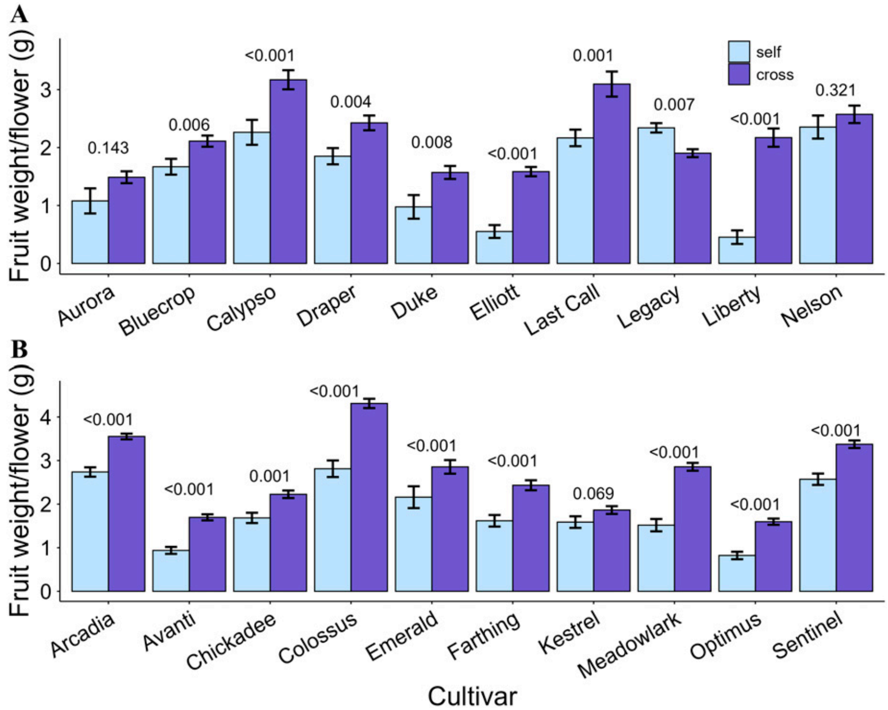

Fig. 1. Fruit weight (grams) per pollinated flower for northern highbush (**A**) and southern highbush blueberry cultivars (**B**) under cross-pollination and self-pollination treatments. Fruit weight per flower includes flowers that did not set fruit (0 g) and all flowers that did set fruit. Bars represent mean fruit weight per flower with standard errors. *P* values indicate the significance of the pollination treatment effect for individual cultivars.

an average increase of 23% (Fig. 1). For SHB, there was evidence that cross-pollination increased fruit weight per flower for all 10 cultivars, with increases ranging from 18% to 94% (Fig. 1); however, the evidence was weak for ‘Kestrel’ (Fig. 1).

Increases in fruit weight per flower under cross-pollination were driven by increases in fruit set and/or fruit weight. In NHB, there was very strong evidence of an overall effect of pollination treatment on fruit set, with no significant pollination treatment × cultivar interaction (Table 1, Supplemental Fig. S2A). Fruit set was 12% higher under cross-pollination (mean ± standard error averaged across cultivars: 92% ± 1.19%) than under self-pollination (80% ± 2.61%). For SHB, there was no evidence of an overall effect of pollination treatment on fruit set, but moderate evidence of a treatment × cultivar interaction was observed (Table 1). Five of 10 SHB cultivars showed evidence of increased fruit set under cross-pollination, with increases ranging from 11% to 38% depending on cultivar (Supplemental Fig. S2B).

There was also very strong evidence of a cultivar × pollination treatment interaction on fruit weight per berry (among successfully set berries) in both NHB and SHB (Table 1). For NHB, there was evidence of an increase

Table 1. Model results of the overall effect of pollination treatment (cross-pollination vs. self-pollination), recipient cultivar, and their interaction on seven response variables related to fruit quantity, size, and quality in both northern highbush (NHB) blueberry and southern highbush (SHB) blueberry.

	Cultivar	Treatment	Cultivar × treatment
NHB response variable			
Fruit weight per flower	χ^2 = 79.28, df = 9, *P* < 0.001****	χ^2 = 2.15, df = 1, *P* = 0.142	χ^2 = 68.90, df = 9, *P* < 0.001****
Fruit set	χ^2 = 12.24, df = 9, *P* = 0.200	χ^2 = 13.46, df = 1, *P* < 0.001****	χ^2 = 10.48, df = 9, *P* = 0.313
Fruit weight per berry	χ^2 = 96.58, df = 9, *P* < 0.001****	χ^2 = 1.71, df = 1, *P* = 0.191	χ^2 = 89.43, df = 9, *P* < 0.001****
Seed set per berry	χ^2 = 29.39, df = 9, *P* < 0.001****	χ^2 = 119.57, df = 1, *P* < 0.001****	χ^2 = 434.85, df = 9 *P* < 0.001****
Time to ripen	χ^2 = 61.05, df = 9, *P* < 0.001****	χ^2 = 1.33, df = 1, *P* = 0.249	χ^2 = 117.60, df = 9, *P* < 0.001****
Firmness	χ^2 = 100.25, df = 9, *P* < 0.001****	χ^2 = 1.05, df = 1, *P* = 0.305	χ^2 = 48.93, df = 9, *P* < 0.001****
Sugar:acid	χ^2 = 30.39, df = 9, *P* < 0.001****	χ^2 = 0.17, df = 1, *P* = 0.680	χ^2 = 14.38, df = 9, *P* = 0.110
SHB response variable			
Fruit weight per flower	χ^2 = 392.10, df = 9, *P* < 0.001****	χ^2 = 25.48, df = 1, *P* < 0.001****	χ^2 = 47.42, df = 9, *P* < 0.001****
Fruit set	χ^2 = 53.85, df = 9, *P* < 0.001****	χ^2 = 2.03, df = 1, *P* = 0.154	χ^2 = 18.81, df = 9, *P* = 0.027**
Fruit weight per berry	χ^2 = 453.62, df = 9, *P* < 0.001****	χ^2 = 29.53, df = 1, *P* < 0.001****	χ^2 = 48.22, df = 9, *P* < 0.001****
Seed set per berry	χ^2 = 202.97, df = 9, *P* < 0.001****	χ^2 = 121.50, df = 1, *P* < 0.001****	χ^2 = 125.07, df = 9, *P* < 0.001****
Time to ripen	χ^2 = 523.24, df = 9, *P* < 0.001****	χ^2 = 57.05, df = 1, *P* < 0.001****	χ^2 = 154.02, df = 9, *P* < 0.001****
Firmness	χ^2 = 512.85, df = 9, *P* < 0.001****	χ^2 = 0.677, df = 1, *P* = 0.411	χ^2 = 24.72, df = 9, *P* = 0.003***
Sugar:acid	χ^2 = 169.95, df = 9, *P* < 0.001****	χ^2 = 3.80, df = 1, *P* = 0.051*	χ^2 = 22.75, df = 9, *P* = 0.007***

Evidence of an effect is denoted as follows: ****very strong evidence (*P* < 0.001); ***strong evidence (0.01 > *P* > 0.001); **moderate evidence (0.05 > *P* > 0.01); and *weak evidence (0.10 > *P* > 0.05).
df = degrees of freedom.

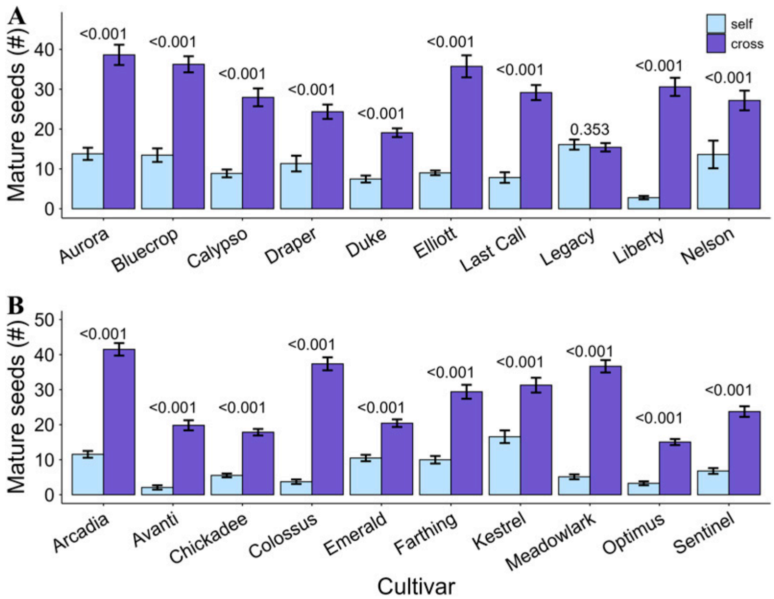

Fig. 2. Number of mature seeds per set berry for northern highbush (**A**) and southern highbush blueberry cultivars (**B**) under cross-pollination and self-pollination treatments. Bars represent the mean number of mature seeds with standard errors. *P* values indicate the significance of the pollination treatment effect for individual cultivars.

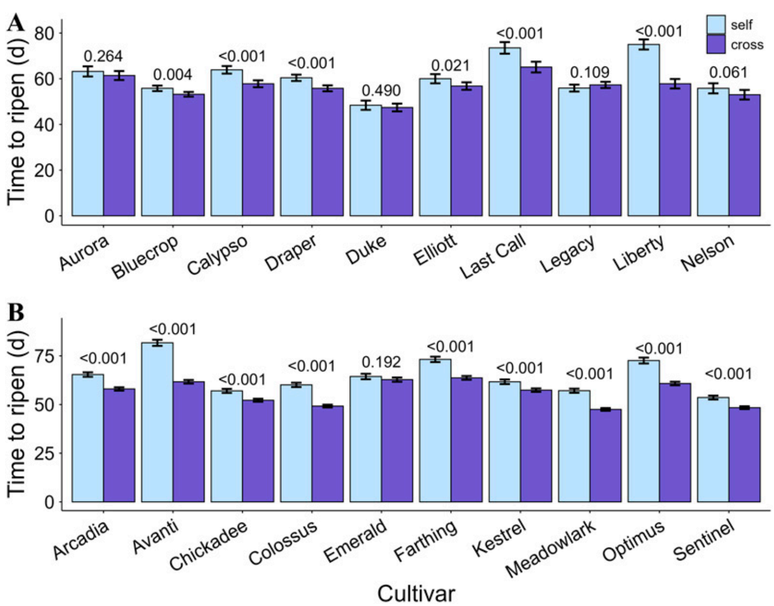

Fig. 3. Time to ripen (days) measured as the period of time between pollination and full berry ripening, for northern highbush (**A**) and southern highbush blueberry cultivars (**B**) under cross-pollination and self-pollination treatments. Bars represent the estimated mean number of days to ripen with standard errors (estimated marginal means accounting for the effect of pollination date). *P* values indicate the significance of the pollination treatment effect for individual cultivars.

strong evidence of this interaction in SHB (Table 1; Supplemental Fig. S3D). In SHB, sugar:acid ratios decreased with cross-pollination for four cultivars and increased in one cultivar, but evidence of the increase was weak (Supplemental Fig. S3D). Changes in sugar:acid ratios were generally driven by changes in both sugar and acid contents (Supplemental Table S2).

Effects of specific pollen donors on recipient cultivars. There was moderate to very strong evidence of an effect of individual pollen donor on fruit weight per flower for eight of 10 NHB cultivars, with weak evidence of an effect on 'Aurora' and no evidence of an effect on 'Nelson' (Table 2). For 'Liberty' and 'Elliott', all cross-pollen donors resulted in greater fruit weight per flower relative to self-pollination, while for 'Calypso', 'Duke', and 'Last Call', two of three cross-pollen donors tested resulted in greater fruit weight per flower relative to self-pollination. For 'Bluecrop' and 'Draper', one of three cross-pollen donors tested resulted in greater fruit weight per flower relative to self-pollination. Finally, for 'Legacy', fruit weight per flower following cross-pollination was equal to or, in the case of cross-pollination with 'Bluecrop', lower than that resulting from self-pollination (Table 2). There was also very strong evidence that individual pollen donor identity affected time to ripen for five NHB cultivars as well as moderate evidence in an additional cultivar (Legacy) (Table 3). For 'Calypso', 'Liberty', 'Last Call', and 'Draper', all cross-pollen donors decreased time to ripen relative to self-pollination; however, for 'Bluecrop', only one cross-pollen donor decreased time to ripen relative to self-pollination (Table 3). For 'Legacy', cross-pollination with 'Bluecrop' increased time to ripen relative to self-pollination, while other cross-pollen donors did not differ from self-pollination.

There was very strong evidence of an effect of individual pollen donor on fruit weight per flower for eight of 10 SHB cultivars and weak evidence of an additional cultivar (Emerald). Only 'Kestrel' showed no evidence of an effect of individual pollen donor, although there was weak evidence of an overall effect when cross-pollen donors were combined. For 'Arcadia', 'Colossus', 'Meadowlark', 'Optimus', and 'Sentinel', all cross-pollen donors resulted in greater fruit weight per flower relative to self-pollination (Table 2). For 'Avanti', 'Farthing', and 'Chickadee', two to three of the five to six cross-pollen donors tested resulted in increased fruit weight per flower relative to self-pollination; however, for 'Emerald', only pollination with 'Arcadia' resulted in a significant increase relative to self-pollination (Table 2). There was also very strong evidence of an effect of individual pollen donor on time to ripen for all SHB cultivars (Table 3). For most (seven of 10 cultivars), all cross-pollen donors resulted in reduced time to ripen relative to self-pollination; for 'Kestrel', all but one cross-pollen donor reduced time to ripen relative to self-pollination (Table 3). For 'Farthing', only cross-pollination with 'Emerald" reduced time to ripen relative to self-pollination; for 'Emerald', no cross-pollen donors reduced time to ripen relative to self-pollination, consistent with the results when all cross-pollen donors were combined, but pollination with 'Meadowlark' increased ripening time relative to self-pollination (Table 3).

Discussion

As hypothesized, cross-pollination increased fruit weight per flower through increased fruit set and/or individual berry weight and decreased time to ripen across most NHB and SHB blueberry cultivars. The few exceptions to these patterns were 'Legacy', which had equal or better outcomes under self-pollination, and 'Aurora', which showed no positive response to cross-pollination. In particular, 'Legacy' was the only cultivar to show no increase in seed set under cross-pollination, suggesting very high self-fertility. Such self-fertility may be attributable to its complex pedigree involving multiple NHB cultivars and the southern blueberry species *V. darrowii* (US Department of Agriculture 1993), characterizing it more appropriately as a mid-chill blueberry and resulting in a relatively low inbreeding coefficient (Ehlenfeldt 1994).

Table 2. Effect of individual pollen donors on fruit weight per flower (g) of recipient cultivars in northern highbush (NHB) blueberry and southern highbush (SHB) blueberry.

	NHB pollen donor cultivar and resulting berry wt (g) (mean ± *SE*)									
NHB recipient cultivar and overall model result	Aurora	Bluecrop	Calypso	Draper	Duke	Elliot	Last Call	Legacy	Liberty	Nelson
Aurora (χ^2 = 7.21, df = 3, *P* = 0.065*)	1.17 ± 0.26 a	NA	NA	1.41 ± 0.29 a	1.59 ± 0.27 a	NA	NA	1.77 ± 0.30 a	NA	NA
Bluecrop (χ^2 = 10.33, df = 3, *P* = 0.02**)	NA	1.72 ± 0.17 b	NA	2.34 ± 0.17 a	1.91 ± 0.17 b	NA	NA	2.12 ± 0.19 b	NA	NA
Calypso (χ^2 = 20.08, df = 3, *P* < 0.001****)	NA	NA	2.31 ± 0.32 b	3.25 ± 0.39 a	2.87 ± 0.34 b	NA	NA	3.48 ± 0.35 a	NA	NA
Draper (χ^2 = 11.28, df = 3, *P* = 0.01**)	NA	NA	NA	1.85 ± 0.17 b	2.17 ± 0.19 b	NA	NA	2.69 ± 0.21 a	NA	2.46 ± 0.22 b
Duke (χ^2 = 18.97, df = 3, *P* < 0.001****)	NA	NA	NA	1.63 ± 0.27 a	0.98 ± 0.27 b	NA	NA	1.73 ± 0.27 a	NA	1.21 ± 0.28 b
Elliott (χ^2 = 98.42, df = 3, *P* < 0.001****)	NA	NA	NA	1.56 ± 0.19 a	1.72 ± 0.21 a	0.50 ± 0.16 b	NA	1.49 ± 0.18 a	NA	NA
Last Call (χ^2 = 38.33, df = 3, *P* < 0.001****)	NA	NA	NA	4.06 ± 0.26 a	2.19 ± 0.25 b	NA	2.17 ± 0.24 b	3.33 ± 0.35 a	NA	NA
Legacy (χ^2 = 25.24, df = 3, *P* < 0.001****)	NA	1.59 ± 0.11 b		2.03 ± 0.10 a				2.34 ± 0.11 a	NA	2.06 ± 0.11 a
Liberty (χ^2 = 63.67, df = 2, *P* < 0.001****)	NA	NA	NA	1.88 ± 0.32 a	2.29 ± 0.26 a	NA	NA	NA	0.45 ± 0.23 b	NA
Nelson (χ^2 = 2.31, df = 3, *P* = 0.510)	NA	2.72 ± 0.33	NA	2.30 ± 0.27	NA	NA	NA	2.69 ± 0.23	NA	2.35 ± 0.21
	SHB pollen donor cultivar and resulting berry weight (g) (mean ± *SE*)									
SHB recipient cultivar and overall model result	Arcadia	Avanti	Chickadee	Colossus	Emerald	Farthing	Kestrel	Meadowlark	Optimus	Sentinel
Arcadia (χ^2 = 55.78, df = 5, *P* < 0.001****)	2.74 ± 0.10 b	NA	3.22 ± 0.15 a	NA	3.63 ± 0.15 a	3.81 ± 0.15 a	NA	NA	3.47 ± 0.15 a	3.62 ± 0.15 a
Avanti (χ^2 = 61.59, df = 5, *P* < 0.001****)	1.34 ± 0.16 b	0.94 ± 0.10 b	NA	1.87 ± 0.16 a	1.84 ± 0.16 a	1.45 ± 0.14 b	NA	NA	1.99 ± 0.15 a	NA
Chickadee (χ^2 = 25.73, df = 5, *P* < 0.001****)	2.18 ± 0.21 b	NA	1.69 ± 0.15 b	NA	1.83 ± 0.22 b	2.32 ± 0.21 a	2.06 ± 0.21 b	NA	NA	2.63 ± 0.21 a
Colossus (χ^2 = 73.86, df = 5, *P* < 0.001****)	3.93 ± 0.28 a	4.42 ± 0.28 a	4.44 ± 0.28 a	2.79 ± 0.21 b	4.61 ± 0.29 a	4.22 ± 0.28 a	NA	NA	NA	NA
Emerald (χ^2 = 13.65, df = 8, *P* = 0.092*)	3.62 ± 0.51 a	3.34 ± 0.50 b	3.10 ± 0.49 b	NA	2.17 ± 0.28 b	2.60 ± 0.49 b	2.60 ± 0.49 b	2.80 ± 0.50 b	2.54 ± 0.50 b	2.34 ± 0.48 b
Farthing (χ^2 = 36.05, df = 4, *P* < 0.001****)	2.80 ± 0.24 a	1.93 ± 0.18 b	NA	NA	2.56 ± 0.25 a	1.62 ± 0.17 b	NA	NA	2.87 ± 0.24 a	NA
Kestrel (χ^2 = 4.36, df = 5, *P* = 0.499)	1.87 ± 0.22	2.02 ± 0.22	1.92 ± 0.22	NA	1.84 ± 0.22	1.70 ± 0.22	1.60 ± 0.15	NA	NA	NA
Meadowlark (χ^2 = 87.82, df = 5, *P* < 0.001****)	2.99 ± 0.23 a	NA	NA	NA	2.69 ± 0.23 a	3.02 ± 0.24 a	2.55 ± 0.24 a	1.54 ± 0.17 b	NA	3.09 ± 0.24 a
Optimus (χ^2 = 52.52, df = 5, *P* < 0.001****)	1.43 ± 0.17 a	NA	NA	NA	1.68 ± 0.16 a	2.00 ± 0.17 a	1.52 ± 0.16 a	NA	0.83 ± 0.12 b	1.35 ± 0.16 a
Sentinel (χ^2 = 26.32, df = 4, *P* < 0.001****)	3.32 ± 0.14 a	NA	3.30 ± 0.20 a	NA	3.42 ± 0.20 a	3.52 ± 0.20 a	NA	NA	NA	2.57 ± 0.14 b

The estimated marginal mean ± standard error (*SE*) of fruit weight per flower (grams) for each pollen donor tested is provided. Self-pollen values are highlighted in gray. The overall significance of the pollen donor (model results) and results of post hoc pairwise comparisons between each cross-pollen donor and self-pollen donor when there was evidence of an overall effect of pollen donor ($P < 0.05$) are presented.
Letters represent significant differences between the self-pollen donor and each cross-pollen donor at $P < 0.05$ as determined by planned post hoc comparisons.
df = degrees of freedom; NA = not applicable (the pollen donor was not tested against the recipient cultivar).

Additionally, while benefits of cross-pollination were present across a large majority of cultivars, they varied in magnitude, with lower effect sizes on seed set, yield, and/or time to ripen, in Kestrel, Emerald, Duke, and Nelson. Notably, these cultivars are older than other cultivars of the same species tested in this study and, thus, may have lower inbreeding coefficients as compared with modern cultivars if inbreeding has increased over time within blueberry breeding programs (Ehlendfeldt 1994). However, with incomplete information about inbreeding coefficients for all tested cultivars, we cannot thoroughly test this hypothesis. Future research should be conducted to determine inbreeding coefficients in older and newer cultivars and relate them directly to pollination outcomes (Keller and Waller 2002). Regardless, benefits of cross-pollination for yield along with time to ripen for a large majority of cultivars showed the potential for significant improvements in blueberry production in settings where cross-pollination is encouraged through planting designs.

Fruit weight per flower, a metric that is most closely related to yield, was higher under cross-pollination than under self-pollination for most cultivars because of increases in both the weight of set berries and fruit set. Fruit set was found to be relatively high (>70%) for most cultivars under both self- and cross-pollination in this study as well as similar to the high levels of fruit set found under variable field conditions for both NHB and SHB in previous studies (Brouwer et al. 2025; Cavigliasso et al. 2021; Cromie et al. 2024; Isaacs and Kirk 2010; Mallinger et al. 2021; Tuell and Isaacs 2010). This relatively high fruit set reflects the partial self-fertility of blueberries and the relatively low number of pollen tetrads and mature seeds required to set blueberry (Dogterom et al. 2000; Komatz et al. 2025; Sun et al. 2021). As such, effects of cross-pollination on yield may be driven more by differences in individual fruit weight than by those in fruit set. However, in some cases, and particularly with cultivars that performed relatively better with self-pollination, fruit set was more affected than fruit weight (e.g., Emerald, Duke, and Kestrel). Therefore, it is important for future work to consider both fruit set and weight per berry as well as the composite variable fruit weight per flower, which represents the total effect of pollination on yield.

In addition to increasing yield, the strong effect of cross-pollination on time to ripen suggests that it is highly influenced by pollination success. Indeed, increases in auxins, gibberellic acid, and cytokinins following successful pollination and fertilization can lead to an increased rate of fruit development, thus accelerating ripening time (Nagasaka et al. 2022). Ripening time can significantly impact profits because early-ripening crops receive significantly higher prices than crops harvested later in the season, particularly for SHB (Zapien-Macias et al. 2025). Furthermore, a shorter time to ripen can reduce the likelihood of damage or yield loss from weather events, natural disasters, pest pressure, and pathogen pressure that

Table 3. Effect of individual pollen donors on berry ripening time (days) of recipient cultivars in northern highbush (NHB) blueberry and southern highbush (SHB) blueberry.

	NHB pollen donor cultivar and resulting time to ripen (days) (mean ± *SE*)									
NHB recipient cultivar and overall model result	Aurora	Bluecrop	Calypso	Draper	Duke	Elliot	Last Call	Legacy	Liberty	Nelson
Aurora (χ^2 = 3.31, df = 3, *P* = 0.347)	62.2 ± 1.28	NA	NA	60.4 ± 1.49	61.5 ± 1.32	NA	NA	59.1 ± 1.50	NA	NA
Bluecrop (χ^2 = 22.70, df = 3, *P* < 0.001****)	NA	55.4 ± 1.00 b	NA	50.9 ± 0.97 a	53.3 ± 1.00 b	NA	NA	55.4 ± 1.03 b	NA	NA
Calypso (χ^2 = 54.10, df = 3, *P* < 0.001****)	NA	NA	63.2 ± 2.00 b	57.6 ± 2.17 a	55.1 ± 2.04 a	NA	NA	57.7 ± 2.13 a	NA	NA
Draper (χ^2 = 21.47, df = 3, *P* < 0.001****)	NA	NA	NA	60.8 ± 1.40 b	56.3 ± 1.50 a	NA	NA	55.9 ± 1.53 a	NA	55.2 ± 1.58 a
Duke (χ^2 = 3.99, df = 3, *P* = 0.263)	NA	NA	NA	48.2 ± 1.97	48.5 ± 2.02	NA	NA	46.7 ± 2.00	NA	47.7 ± 2.10
Elliott (χ^2 = 1.91, df = 3, *P* = 0.591)	NA	NA	NA	58.6 ± 1.38	58.4 ± 1.70	57.2 ± 1.54	NA	56.5 ± 1.35	NA	NA
Last Call (χ^2 = 57.20, df = 3, *P* < 0.001****)	NA	NA	NA	63.9 ± 1.33 a	68.5 ± 1.37 a	NA	74.1 ± 1.20 b	66.1 ± 1.72 a	NA	NA
Legacy (χ^2 = 8.63, df = 3, *P* = 0.035**)	NA	NA	60.3 ± 1.13 b	57.4 ± 0.97 a	NA	NA	NA	56.6 ± 1.04 a	NA	58.4 ± 0.97 a
Liberty (χ^2 = 162.69, df = 2, *P* < 0.001****)	NA	NA	NA	56.5 ± 2.79 a	58.1 ± 2.65 a	NA	NA	NA	74.7 ± 2.54 b	NA
Nelson (χ^2 = 4.22, df = 3, *P* = 0.239)	NA	54.3 ± 3.67	NA	54.5 ± 3.67	NA	NA	NA	53.4 ± 3.56	NA	57.0 ± 3.51

	SHB pollen donor cultivar and resulting time to ripen (days) (mean ± *SE*)									
SHB recipient cultivar and overall model result	Arcadia	Avanti	Chickadee	Colossus	Emerald	Farthing	Kestrel	Meadowlark	Optimus	Sentinel
Arcadia (χ^2 =118.92, df = 5, *P* < 0.001****)	64.3 ± 0.76 b	NA	58.2 ± 0.96 a	NA	56.5 ± 0.91 a	56.7 ± 0.91 a	NA	NA	58.5 ± 0.95 a	57.1 ± 0.91 a
Avanti (χ^2 = 135.76, df = 5, *P* < 0.001****)	70.3 ± 2.92 a	87.8 ± 2.60 b	NA	67.1 ± 2.60 a	64.7 ± 2.84 a	76.0 ± 2.84 a	NA	NA	56.2 ± 2.18 a	NA
Chickadee (χ^2 = 101.38, df = 5, *P* < 0.001****)	48.8 ± 0.86 a	NA	53.7 ± 0.76 b	NA	50.3 ± 0.92 a	48.0 ± 0.85 a	49.3 ± 0.86 a	NA	NA	48.7 ± 0.84 a
Colossus (χ^2 = 150.30, df = 5, *P* < 0.001****)	52.6 ± 1.12 a	56.4 ± 1.20 a	58.2 ± 1.24 a	67.9 ± 1.07 b	54.4 ± 1.17 a	56.9 ± 1.23 a	NA	NA	NA	NA
Emerald (χ^2 = 27.72, df = 8, *P* < 0.001****)	43.4 ± 1.18 a	44.2 ± 1.26 a	44.2 ± 1.17 a	NA	46.3 ± 0.81 a	43.7 ± 1.25 a	44.8 ± 1.32 a	51.0 ± 1.56 b	47.4 ± 1.31 a	45.2 ± 1.35 a
Farthing (χ^2 = 120.27, df = 4 *P* < 0.001****)	58.6 ± 1.23 b	65.6 ± 1.10 b	NA	NA	63.6 ± 1.41 a	75.1 ± 1.21 b	NA	NA	63.9 ± 1.37 b	NA
Kestrel (χ^2 = 33.71, df = 5, *P* < 0.001****)	48.9 ± 0.82 a	47.4 ± 0.73 a	48.7 ± 0.81 a	NA	49.8 ± 0.78 b	48.6 ± 0.84 a	52.0 ± 0.59 b	NA	NA	NA
Meadowlark (χ^2 = 222.05, df = 5, *P* < 0.001****)	48.6 ± 1.10 a	NA	NA	NA	49.1 ± 1.11 a	49.6 ± 1.13 a	46.7 ± 1.12 a	58.2 ± 1.08 b	NA	47.0 ± 1.07 a
Optimus (χ^2 =101.85, df = 5, *P* < 0.001****)	68.5 ± 1.48 a	NA	NA	NA	70.3 ± 1.56 a	66.6 ± 1.48 a	66.3 ± 1.48 a	NA	81.7 ± 1.50 b	70.0 ± 1.65 a
Sentinel (χ^2 = 53.40, df = 4, *P* < 0.001****)	56.2 ± 0.80 a	NA	55.9 ± 1.11 a	NA	53.8 ± 1.05 a	54.3 ± 1.06 a	NA	NA	NA	61.3 ± 0.88 b

The estimated marginal mean ± standard error (*SE*) of time to ripen (days) for each pollen donor tested is presented. Self-pollen values are highlighted in gray. The overall significance of pollen donor (model results) and results of post hoc pairwise comparisons between each cross-pollen donor and self-pollen donor when there was evidence of an overall effect of pollen donor ($P < 0.05$) are presented.
Letters represent significant differences between the self-pollen donor and each cross-pollen donor at $P < 0.05$ as determined by post hoc comparisons.
df = degrees of freedom; NA = not applicable (pollen donor was not tested against the recipient cultivar).

build during the ripening season (Hampton et al. 2014; Rodriguez-Saona et al. 2019). Additionally, because blueberry fields must be harvested multiple times, a more concentrated harvest window could increase harvest efficiency and reduce labor costs.

Although some studies have shown a relationship between pollination success and blueberry firmness (Cavigliasso et al. 2021), this has not been well-studied in a controlled environment with hand pollination experiments. Our results provide some support for a positive effect of cross-pollination on fruit firmness, particularly for NHB, which could facilitate machine harvesting and storage; however, this result was cultivar-dependent. Finally, effects of cross-pollination on sugar and acid contents were also variable; however, overall, there was some evidence of a slight decline in sugar content and increase in acidity with cross-pollination. Regardless, at least for SHB cultivars, sugar:acid ratios were generally within the recommended range of 10 to 33 (Edger et al. 2022) under both cross-pollination and self-pollination treatments. Because consumers generally prefer blueberries with a balance of sweetness and acidity (Edger et al. 2022), high sugar:acid ratios are not necessarily desirable, as was found for ‘Meadowlark’ under self-pollination. For NHB, sugar:acid ratios were generally low under both self-pollination and cross-pollination, which may have been caused by the warm and humid climate in which they were grown (i.e., greenhouse conditions in Florida) outside of their climatic niche. Thus, cross-pollination had an overall negligible or inconsequential effect on sugar and acid contents in blueberries, particularly as they relate to consumer preferences.

Studies have indicated that certain cross-pollen donors have overall greater positive impacts on blueberry yield in both NHB (Cremins 1951; Doi et al. 2021; Nagasaka et al. 2022) and SHB (Gupton and Spiers 1994; Liu et al. 2022; Martin et al. 2025); however, we did not find strong evidence of this. Instead, our results are consistent with those of Ehlenfeldt (2003), who reported variation in outcomes depending on pollen donor and interactions with recipient cultivar, but no universally good or bad pollen donors. Additionally, for most recipient cultivars, all or a majority of cross-pollen donors improved fruit weight per flower or time to ripen in comparison with self-pollination, suggesting that mixed plantings should be integrated in new planting designs with cultivar selection primarily based on overlapping phenology or similarity of management. When cross-pollen donors did differ in their efficacy, relatedness between recipient and donor cultivar could explain differences in pollination outcomes (Krebs and Hancock 1990). However, we were unable to thoroughly test this hypothesis because of limited information regarding relatedness for all crosses. Finally, our study was designed to compare each cross-pollen donor with self-pollination, but it was not optimal for comparing cross-pollen donors with each other because not all of them were applied to the same recipient plants or on the same days. Variation in the timing of pollination relative to plant flowering stage and weather during fruit development could influence fruit size and time to ripen (Nesmith 2012) and could be confounded with pollen donor. Thus, future research could explicitly test this hypothesis by selecting plants along a gradient of relatedness and comparing multiple cross-pollen donors that differ in their relatedness to the recipient plant.

Our study provides strong evidence of a positive effect of cross-pollination on fruit quantity and quality across most NHB and SHB cultivars in a controlled environment and under high resource availability. To limit the effects of maternal resource limitation and reallocation, we pollinated a fraction of flowers on each plant and removed all unpollinated flowers. It is possible that the benefits of cross-pollination for yield would be reduced under maternal resource limitation in the field, but previous studies in field contexts have shown significant blueberry yield increases in mixed plantings where cross-pollination rates were higher than those in single cultivar plantings (Mallinger et al. 2024; Retamales et al. 2015). In some cases, increased blueberry yield in the field was nearly identical to that found in controlled greenhouse pollination experiments when comparing cross-pollination and self-pollination (Mallinger et al. 2024). Future studies with newer cultivars should investigate whether the increases in yield found in this study are as strong under variable field contexts and with varying degrees of resource limitation (Kumarihami et al. 2021). However, we expect that even if the benefits for yield are reduced under maternal resource limitation, the strong effects on time to ripen would remain consistent. Furthermore, mixing blueberry cultivars provides additional benefits such as insurance under conditions that disfavor certain cultivars (Chabert et al. 2024; Walters and Isaacs 2023) and could increase pollen availability given the variation in pollen production across blueberry genotypes (Mallinger et al. unpublished data). The results of this study could be incorporated in cost–benefit analyses that compare the monetary benefits associated with increases in yield and decreases in time to ripen with the costs of mixed planting designs. Such analyses would facilitate decision-making related to determining the cultivars and production contexts in which the benefits outweigh the costs, as may be likely for cultivars with very high yield increases and reduced times to ripen.

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