Pollen Sources Influence Early Fruit Growth of Southern Highbush Blueberry

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ABSTRACT. Ovule abortion occurred between 5 and 10 days after pollination (DAP) in self- and cross-pollinated 'Sharpblue' blueberry (*Vaccinium corymbosum* L.) fruit. More ovule abortion occurred with self-pollination (35%) than with cross-pollination (22% for 'Sharpblue' x 'O'Neal' and 29% for 'Sharpblue' x 'Gulfcoast'), and there were more poorly developed ovules with self-pollination (88.1%) than with cross-pollination (x 'O'Neal', 33.6%; x 'Gulfcoast' 50.8%). The increase in ovule area correlated exponentially with fruit growth during early developmental stages, regardless of pollination treatment. However, cross-pollination resulted in significantly greater ovule area and fruit mass during early fruit development as well as at ripening. Ovule area was maximum at 25 to 30 DAP for both pollination treatments, followed by exponential fruit growth (stage III). Cross-pollination resulted in greater fruit growth and a shorter stage III. At 10 DAP, ovules from cross-pollination were larger than those from self-pollination, suggesting that cross-pollination initiated ovule growth immediately after fertilization. This research suggests that southern highbush blueberry fruit growth and development is intimately associated with ovule growth and development, which is affected by pollen sources.

'Sharpblue' is a southern highbush (SHB) cultivar grown in the southern United States (Moore, 1993). It was released by the Univ. of Florida in the mid-1970s as an early ripening cultivar (Sharpe and Sherman, 1976) to exploit early markets, since its fruit development period is ≈ 30 d shorter than the native rabbiteye blueberries (Lyrene and Sherman, 1985).

Cross-pollination resulted in prominent effects on fruit mass, number, and size of mature ovules (seeds) when 'Sharpblue' was cross-pollinated with 'O'Neal' or 'Gulfcoast' (Huang and Lang, 1995). Previous studies on the effects of self-pollination, cross-pollination, and mixed-pollination of 'Sharpblue' blueberry have been conducted (Lang and Danka, 1991; Lyrene, 1989). Lyrene (1989) showed that self-pollination resulted in a much lower percentage of fruit set, delayed ripening, smaller berry size, and fewer mature seeds than cross- and mixed-pollination in a greenhouse. However, Lang and Danka (1991) reported that cross-pollination increased fruit size by 14%, mature seed count by 27%, and the harvest percentage of early ripening fruits by 140% but had no influence on fruit set.

'Sharpblue' has a complicated pedigree, consisting of 54% *V. corymbosum* L., 31% *V. darrowi* Camp., 2% *V. angustifolium* Ait., and 13% *V. ashei* Reade. (Lang, 1993). Compared with the other SHB cultivars, such as 'O'Neal' and 'Gulfcoast', this cultivar has a lower percentage of *V. corymbosum* germplasm, and is less self-compatible or self-fertile. 'Sharpblue' was originally recommended for interplanting with 'Flordablue' for cross-pollination to optimize fruit set and development (Sharpe and Sherman, 1976). On the other hand, solid-block 'Sharpblue' plantings were at one time recommended to commercial growers (Lyrene, 1986). The complex pedigree involving self-fertile and self-sterile species makes it difficult to predict the influence of pollination variables on the

developmental physiology of 'Sharpblue' fruit. Previous studies focused primarily on the effects of pollen source on the number of mature ovules (seeds), which correlated with fruit ripening, size, and set. No information is available about the effects of pollen source on early physiological and developmental events in 'Sharpblue' fruit and ovules. The objectives of this study were to 1) study the effects of 'Sharpblue' self- and cross-pollination on ovule and fruit development at early stages after pollination and 2) investigate the relationship of early ovule development to early fruit growth and development.

Materials and Methods

Seven-year-old 'Sharpblue' plants were used in this study. Pollen sources were 'O'Neal', 'Gulfcoast', and 'Sharpblue'. Plants of uniform size and vigor were transplanted into 37-L pots in middle January 1995. The plants were trimmed and stored at 7 °C to ensure sufficient chilling. On 21 Mar., the plants were fertilized with Osmocote (100 g/pot, 18N–6P–12K) and placed in a greenhouse to force flowering. Bloom was monitored daily.

To minimize the effects of plant age, vigor, and bud location on ovule and berry development, several criteria were taken into consideration during pollination: 1) plants of uniform size and vigor were designated as fruiting plants; 2) inflorescences located at the middle to outer canopy were chosen; 3) only flowers of similar physiological ages were pollinated; and 4) only four to five flowers were pollinated on a single inflorescence (the remaining flowers were removed).

Flowers were emasculated using forceps to remove the corolla and stamens. Fresh pollen was collected onto a thumbnail by shaking an opened flower in an inverse direction. Pollination was done by gently touching the stigmatic surface of an emasculated flower with the pollen grains loaded on the thumbnail.

Four 'Sharpblue' plants were used as four experimental blocks. The pollination treatments were 1) selfed with 'Sharpblue', 2) crossed with 'O'Neal', and 3) crossed with 'Gulfcoast'. All pollination treatments were applied randomly on a single plant. Several parameters compared between pollination treatments as well as at various developmental stages were ovule number per berry, ovule area per berry, mean fruit mass, and mean individual ovule area. Samples were collected at anthesis, designated as 0, 5, 10, 15, 20,

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25, 30 days after pollination (DAP) and ripening. At each sampling date, four berries from each plant were picked for each pollination treatment. Ovules were separated from fresh fruit tissue with the aid of a scalpel and forceps and counted under a dissecting microscope. Ovules from various developmental stages were photographed on Kodak technical pan film and printed onto Ilford polycontrast paper at identical magnifications. Ovule area was calculated using standard morphometric techniques (Sundberg, 1984). The experimental model for data analysis was a randomized complete-block design. Data analysis was carried out by a general linear model procedure (SAS institute, Cary, N.C.). Mean separations were conducted by Duncan's multiple range test at $P \leq 0.05$. The pooled means of ovule area and fruit mass were fitted to an exponential regression model to describe the correlation of ovule growth with fruit growth at early developmental stages (5 to 30 DAP).

At ripening, the percentage of poorly developed ovules was calculated from 15 berries for each pollination treatment. Ovules were classified into two groups: well and poorly developed, based on size, appearance, surface texture, color, and plumpness, using a binary dissecting microscope. Well-developed ovules were large and plump with a brown surface texture, whereas poorly developed ovules were small and flat with a whitish surface appearance.

Results

Berry growth patterns were differed between self- and cross-pollinations. There was no significant mass increase in self-pollinated berries from 5 to 15 DAP, whereas a difference was observed at 10 DAP in 'Sharpblue' cross-pollinated with either 'O'Neal' or 'Gulfcoast' (Fig. 1). There was a significant increase in berry mass from 15 to 20 and 25 to 30 DAP in all treatments, with a greater increase as a result of cross-pollinations. Fruit mass showed no significant increase from 20 to 25 DAP (stage II). An exponential fruit growth rate occurred after 30 DAP (stage III). Cross-pollinated fruit needed ≈ 10 d less to complete stage III, with a more rapid fruit growth rate compared with self-pollination (Fig. 1).

There was no difference in ovule number at 5 DAP in self- and cross-pollinated fruit compared with anthesis (Fig. 2). Although ovule number did not differ significantly between self- and cross-pollination at 10 DAP, ovule number was dramatically decreased in both treatments at 10 DAP, with a greater decrease in self-pollinated fruit (Fig. 2). There was no obvious change in ovule number in either treatment during the subsequent developmental stages. These results suggested that ovule abortion occurred between 5 and 10 DAP, with more severe abortion in self-pollinated fruit.

Significant differences in ovule area per berry and mean individual ovule area were observed at 10 DAP between self- and cross-pollination treatments (Fig. 3). Pollen from other cultivars resulted in a greater total ovule area, as well as average ovule size at 10 DAP.

Cross-pollination positively affected early ovule growth and survival. At corresponding DAP, cross-pollination always resulted in a significantly greater total ovule area and average ovule size than self-pollination (Fig. 3 A and B). Regardless of pollen sources, ovule area reached a maximum at 25 DAP, suggesting that ovule growth occurred at early developmental stages after pollination. At ripening, 88.1% of the ovules were poorly developed in selfed 'Sharpblue' fruit compared to 50.8% and 33.6% in fruit from 'Gulfcoast' and 'O'Neal' crosses, respectively. There was no significant increase in fruit growth at 25 DAP. Seedcoat lignification probably occurs between 20 and 25 DAP (stage II). Cross-pollinations with 'Gulfcoast' and 'O'Neal' brought about a 72% and 63% increase in ovule area and a 80.1% and 68.6% increase in fruit mass, respectively, compared with self-pollination. A greater early ovule growth led to double fruit mass in cross-pollination (Fig. 1). Total ovule area and mean ovule area did not change until fruit began to ripen (Fig. 3). A slight decrease was detected in ovule area at 30 DAP and at ripening, probably due to water loss from mature ovules (seeds). Fruit mass was correlated exponentially with total ovule area per berry at early developmental stages (5 to 30 DAP) in all pollination treatments (Fig. 4). Regressors of mean fruit mass versus total ovule area per

Fig. 1. Blueberry fruit growth (fresh mass) from pollination to ripening in 'Sharpblue' fruit derived from three pollen sources. Vertical bars represent standard errors. SB x SB = self-pollination with 'Sharpblue'; SB x GC and SB x ON = cross-pollination with 'Gulfcoast' and 'O'Neal', respectively.

berry were significantly different from 0 in cross- and self-pollination, indicating that early ovule growth can affect fruit mass, irrespective of pollination treatments. The regressors were different between self- and cross-pollination. A greater regressor showed in self-pollination meant that early ovule growth was more critical to early fruit growth in self-pollination than in cross-pollination.

Discussion

Blueberry ovule abortion was reported by Darrow (1941). He demonstrated that highbush blueberry (*Vaccinium corymbosum* L.) fruit potentially could have ≈ 115 seeds (ovules), but many developing ovules abort during fruit development. However, he did not identify when ovule abortion occurred. Ovule abortion also is common during fruit development in SHB. Parrie (1990) reported that a newly opened 'Sharpblue' flower has an average of 112 ovules, which is consistent with the data observed in this study (107 ± 6). Only ≈ 60 ovules (seeds) were found in ripe 'Sharpblue' fruit, with no difference in total ovule number as a result of either self- or cross-pollination. More mature ovules (seeds) and a greater

mature ovule size were observed in cross-pollinated 'Sharpblue' fruit (Huang, 1995). These facts imply that ovule abortion occurs during 'Sharpblue' fruit development. In this study, ovule abortion was observed to occur between 5 and 10 DAP for self- and cross-pollinated 'Sharpblue' berries, with more severe ovule abortion associated with self-pollination. Other studies have indicated that self-pollination of some SHB cultivars results in a significant decrease in seed number and fruit mass and an increase in fruit development period (Gupton and Spiers, 1994; Lang and Danka, 1991). Many factors could account for the difference between potential and observed mature ovule (seeds) number, including 1) geitonogamy and self-pollination (Vander Kloet and Lyrene, 1987), 2) nutrition competition between developing fruit on a single inflorescence (Gorchov, 1985), 3) low levels of cross-fertilization due to low pollinator activity at anthesis (McGregor, 1976), and 4) poor-quality pollen deposited on the stigma (Vander Kloet, 1983).

Cross-pollination resulted in a greater increase in ovule area and fruit mass at early stages after pollination. This indicates that pollen from different cultivars is genetically compatible for zygote formation and its subsequent growth, which is a prerequisite for fruit growth. Early ovule growth through ≈ 30 DAP correlated exponentially with young fruit growth for self- and cross-pollination treatments (Fig. 4). Ovule area reached its maximum at 25 or 30 DAP, and exponential fruit growth began at 30 DAP. The low rate of fruit growth occurring during early stages could be explained by 1) rapid cell division and 2) ovule growth concomitant with berry growth.

A correlation between seed content and fruit size also was reported in kiwifruit (*Actinidia chinensis* Planch.) (Lawes et al., 1990), and seed dry mass correlated better with fruit mass than seed number. For SHB, there was a better correlation between mature ovule (seeds) area or number and fruit mass than between total ovule area or number and fruit mass (Huang, 1995). This obvious implication is that ovule quality is critical to berry size and that poorly developed ovules are less effective stimulants of fruit growth than well-developed ovules (seeds). Ovules might affect fruit growth via production of plant hormones. A large number of mature ovules (seeds) may be a greater source of plant growth regulators and therefore become a stronger sink than a small number of mature ovules (seeds). This assumption can be supported by some experimental results. Gibberellic acid (GA_3) has been used for more than 15 years to increase fruit set of rabbiteye blueberries (Austin, 1979; Davies and Buchanan, 1979; Mainland et al., 1979). Recent studies (Williamson et al., 1995) indicated that fruit set and yield of rabbiteye blueberries were increased by repeated spraying of GA_3 at different stages during bloom in field experiments in Florida and in grower trials in Georgia. Percent fruit set was improved when GA_3 was applied to nonpollinated flowers under controlled-environmental conditions. Stimulatory effects of growth regulators on fruit growth and development have also been reported in other fruit species classified botanically as berries, such as kiwifruit (Hopping and Jerram, 1980).

Fig. 2. Comparison of ovule number per berry from anthesis to fruit ripening in 'Sharpblue' blueberries derived from three pollen sources. Arrow indicates period of apparent post-pollination ovule abortion. Vertical bars represent standard errors. SB x SB = self-pollination with 'Sharpblue'; SB x GC and SB x ON = cross-pollination with 'Gulfcoast' and 'O'Neal', respectively.

Fig. 3. Comparison of ovule development from pollination to fruit ripening in 'Sharpblue' blueberries derived from three pollen sources. (A) Period of differential growth in average ovule size. (B) Total ovule area per fruit during development. SB x SB = self-pollination with 'Sharpblue'; SB x GC and SB x ON = cross-pollination with 'Gulfcoast' and 'O'Neal', respectively.

Fig. 4. Correlation of mean fruit mass with total ovule area per berry at early developmental stages [5 to 30 d after pollination (DAP)] for three pollination treatments. The relationship is described by $Y = ae^{bx}$. Regressors are significantly different from 0 at $P \leq 0.05$. SB x SB = self-pollination with 'Sharpblue'; SB x GC and SB x ON = cross-pollination with 'Gulfcoast' and 'O'Neal', respectively.

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