Production and Viability of Pollen and Pollen–Ovule Ratios in Four Rabbiteye Blueberry Cultivars

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ABSTRACT. Fruit set of rabbiteye blueberries (*Vaccinium ashei* Reade) can be pollen-limited under certain conditions. The objective of this study was to determine production, release, and viability of pollen, as well as pollen–ovule ratios in the rabbiteye blueberry cultivars Austin, Brightwell, Climax, and Tifblue. In vitro tetrad germination varied among genotypes, although, values were high ($\geq 80\%$) in all cultivars. Pollen viability does not seem to contribute to reproductive failure in the cultivars studied. Total pollen production per flower averaged 8434 tetrads across all cultivars. On a per ovule basis, pollen production was very low relative to other xenogamous species. The low pollen–ovule ratio of rabbiteye blueberry (≈ 400) may be an indicator of the high efficiency of its pollen dispensing mechanism. Total pollen production varied among cultivars. Furthermore, a significant difference in pollen release was found between two cultivars with similar total pollen production per flower. The possible mechanism regulating pollen release in these cultivars is discussed.

It is common for xenogamous (outcrossed), hermaphroditic flowering plants to produce more flowers and ovules than fruits and seeds (Bawa and Webb, 1984). Two explanations for this pattern are 1) maternal resources available for fruit and seed maturation are limited, or 2) pollen delivery to the stigma is insufficient (Sutherland, 1987). Pollen limitation of seed set is widely reported in angiosperms (Burd, 1994; Larson and Barret, 2000).

According to Shutak and Marucci (1966), highbush blueberries (*Vaccinium corymbosum* L.) are capable of setting fruit for almost 100% of their flowers, and fruit set levels as high as 80% are required for adequate commercial production. In contrast, poor fruit set is one of the most important horticultural problems of the rabbiteye blueberry (*V. ashei*) industry (Scherm et al., 2001). For instance, estimates of fruit set as low as 8% to 10% have been recorded for the widely planted rabbiteye cultivar Tifblue under field conditions in the southeastern United States (Lyrene and Crocker, 1983; NeSmith and Adair, 2004). Rabbiteye blueberries have a limited degree of self-fertility and require cross-pollination with another rabbiteye cultivar (El-Agamy et al., 1981). In general, self-infertile plants exhibit more pollen limitation than those with the capacity to self-fertilize, because self-pollen cannot contribute to reproductive success (Burd, 1994; Larson and Barret, 2000). Therefore, it is likely that fruit set of rabbiteye blueberries can be pollen-limited, rather than resource-limited, under certain conditions.

Several studies have examined the role of pollen quality on the fruiting capacity of blueberry. Pollen germination is affected by genotype (Eaton, 1966; Goldy and Lyrene, 1983; Lang and Parrie, 1992), and low pollen viability has been linked to poor fruit set in some highbush blueberry cultivars (Brewer and Dobson, 1969; Stushnoff and Hough, 1968). Moreover, Vander Kloet (1983) reported significant correlation between pollen viability and fruit quality parameters such as fruit size. Pollen viability studies in rabbiteye blueberry are quite limited (Cockerham and Galleta, 1976), so the role of pollen quality on fruit set remains unclear.

High pollen production is desirable for bee-pollinated plants. Since bees constantly groom their bodies and pack pollen into scopae (specialized pollen-transporting areas), most of the pollen collected by these pollinators is unavailable for pollination (Harder and Wilson, 1997; Thorp, 2000). Therefore, pollen must be produced in abundance to compensate for its inefficient transfer by bees, and in fact, xenogamous flowers produce more pollen (on a per ovule basis) than those of autogamous (self-pollinated) plants (Cruden, 1977). Blueberry is a bee-pollinated crop, and cross-pollination is beneficial for fruit quality and yield. Although knowledge of pollen production and release could help to identify cultivars with outstanding performance as pollinizers, these variables have received little attention in *Vaccinium* L. section *Cyanococcus* A. Gray. Brewer and Dobson (1969) reported that ‘Jersey’, a highbush blueberry cultivar with problems of low fruit set, released less pollen than ‘Rubel.’ Reader (1977) quantified total pollen production per flower of *V. myrtilloides* Michx. growing in a peat bog. To our knowledge, there is no such information available for rabbiteye blueberries. The objective of this research was to determine viability, production, and release of pollen in selected rabbiteye blueberry cultivars. Additionally, the pollen–ovule ratio of rabbiteye blueberry was estimated to relate its pollen production to that of other flowering plants with similar breeding systems.

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Materials and Methods

PLANT MATERIAL AND LOCATIONS. Three-year-old blueberry plants of the rabbiteye cultivars Austin, Brightwell, Climax, and Tifblue were obtained from the blueberry breeding program at the Univ. of Georgia Griffin Campus. Plants were grown in 19-L containers using pine bark as a growing medium. Seven plants of each cultivar were kept from 6 Nov. 2001 until near the beginning of bloom in the following spring at Griffin and Alapaha in order to provide varied pre-bloom environments. Griffin is located in central Georgia, where the USDA plant hardiness zone is 7b. Alapaha is located in southern Georgia (hardiness zone 8a) and is more representative of the blueberry producing area of the state.

POLLEN PRODUCTION AND VIABILITY. Near the beginning of bloom, plants were brought into a greenhouse at the Univ. of Georgia Athens Campus to evaluate total pollen production and viability. Plants from Alapaha were moved into the greenhouse on 7 Mar. 2002, and those from Griffin were moved 2 weeks later. Plants were fertilized with a 15N-3.9P-10K controlled-release fertilizer and watered as needed.

Pollen viability was assessed by determining germination of tetrads in vitro. Germination tests were conducted using fresh pollen collected on the same day. Experimental units consisted of pollen samples extracted from 15 flowers at the stage of anthesis collected from the seven plants of each cultivar at each location. Pollen was extracted in the laboratory by rolling a flower between the thumb and index finger over a petri dish. The pollen germination medium consisted of 100 mg·L⁻¹ H₃BO₃, 300 mg·L⁻¹ Ca(NO₃)₂·H₂O, 200 mg·L⁻¹ MgSO₄·7H₂O, 100 mg·L⁻¹ KNO₃, and 10% sucrose (Garvey and Lyrene, 1987). Pollen from each sample was suspended in the germination medium at a rate of 100 mg·L⁻¹, and 200-μL aliquots of stirring suspension were added to six wells of a culture plate (model Microtest III; Falcon, Lincoln Park, N.J.). After 2 h of incubation in a dark growth chamber at 24 °C, pollen germination was examined using an inverted microscope (Nikon, Garden City, N.Y.). Pollen grains were considered germinated if pollen tube extension was greater than the diameter of the tetrad (Lang and Parrie, 1992). The number of tetrads containing zero, one, two, three, and four pollen tubes was recorded. Pollen viability was expressed as tetrad germination (percentage of tetrads with at least one pollen tube). The mean number of pollen tubes per germinated tetrad was also calculated.

In order to determine total pollen production, flowers just prior to anthesis were sampled. Experimental units consisted of 30 flowers collected from the seven plants of each cultivar at each location. Flowers were detached with forceps and placed upright on the wells of a tissue culture plate. Plates were carried to the laboratory and stored in a refrigerator before flower dissection. The distal half of the tubular corolla and the style were removed so that pollen could be released directly into a 150-mL sample container without interference from flower parts. A pair of toothpicks and a piece of double-coated adhesive tape were used to hang multiple flowers from their pedicels. Thus, flowers were stored at room temperature in a hanging position inside the sample container for several weeks. Blueberry anthers are poricidally dehiscent and require vibration for efficient pollen release (Buchmann, 1983; Cane and Payne, 1988); therefore, flowers were sonicated with a 512-Hz tuning fork (Indigo Instruments, Waterloo, Ont.). Sonication was repeated several times, until little or no pollen appeared to remain inside the dry anthers. The number of tetrads in the container was measured electronically using a particle

counter. Tetrads were suspended in 10 mL of 70% ethanol, and then 130 mL of Isoton II (Coulter Electronics, Hialeah, Fla.) were added. Repeated 0.5-mL aliquots of continuously stirred pollen suspension were counted with a Coulter (model ZB1; Coulter Electronics) counter using a 140-μm aperture.

The experimental design was a split-plot with location as the main plot and cultivar as the subplot. Experimental units for both evaluations were sampled on three separate days, with two replications per treatment combination per day. Data were analyzed using PROC MIXED of SAS (SAS Institute, Cary, N.C.).

OVULE NUMBER AND POLLEN RELEASE. Ovule number and pollen release were estimated in 2004 using containerized blueberry plants similar to those described previously. Three plants of each cultivar received their chilling requirement under natural conditions and then were brought into a greenhouse at the Univ. of Georgia Griffin Campus to force bloom. Samples of 25 flowers per cultivar were collected and fixed in 7:3, 95% ethanol:acetic acid. Ovaries were dissected and ovules were counted under a dissecting microscope.

Pollen release was evaluated under greenhouse conditions for 'Brightwell' and 'Climax' only. Twenty-five flowers of each cultivar were collected 1 d after anthesis. Pollen was extracted by rolling a flower between the thumb and index finger until no additional pollen was released. Tetrads from individual flowers were collected into a 20-mL blood cell-counter vial (Labcon, Petaluma, Calif.) and counted using the previously described particle counter. Ovule number and pollen release data were compared among cultivars using one-way analysis of variance.

Results and Discussion

POLLEN VIABILITY. Cultivar was the only significant main effect for in vitro tetrad germination (Table 1). Location had no effect on in vitro tetrad germination, nor did it interact with cultivar. Although tetrad germination varied among genotypes, values were ≥80% in all four cultivars. These results were similar to the mean percentage of stainable pollen previously reported for rabbiteye blueberry (Cockerham and Galleta, 1976). The viability results from this study were also similar to the level of pollen viability reported for southern highbush blueberry (*V. corymbosum* × spp.) cultivars (Lang and Parrie, 1992). The number of pollen tubes observed in germinated tetrads ranged from one to four. On average, germinated tetrads of all cultivars produced two pollen tubes per tetrad. These findings contrast with previous reports indicating that multiple pollen tubes per tetrad are infrequent in blueberry (Brewer and Dobson, 1969). According to Lang and Parrie (1992), the average number of pollen tubes per germinated tetrad ranged from 1.7 to 3.4 among southern highbush blueberry cultivars.

TOTAL POLLEN PRODUCTION AND POLLEN-OVULE RATIO. Total pollen production per flower averaged 8434 tetrads across all cultivars. This is about three times higher than the number reported for *V. myrtilloides* (Reader, 1977). Total pollen production was affected by cultivar and location (Table 1). Pollen numbers in 'Brightwell', 'Climax', and 'Tifblue' were significantly higher than in 'Austin'. Additional research would be needed to confirm a location effect and to identify the environmental factors that may have an influence on pollen production. There was no location × cultivar interaction.

The mean number of ovules per flower ranged between 71 and 103 (Table 2). Tetrad and ovule numbers per flower estimated in 2002 and 2004, respectively, were used to calculate tetrad-

Table 1. In vitro tetrad germination, number of pollen tubes per germinated tetrad, and total pollen tetrad production per flower in 'Austin', 'Brightwell', 'Climax', and 'Tifblue' rabbiteye blueberry from two locations.

Location	Tetrad germination (%)	Pollen tubes per germinated tetrad (no.)	Total pollen production (tetrads per flower)
Alapaha	87.5 a ^z	2.0 a	7613 b
Griffin	82.9 a	2.0 a	9254 a
Cultivar			
Austin	80.8 b	2.0 a	6638 b
Brightwell	90.2 a	2.1 a	9213 a
Climax	88.4 a	2.0 a	9065 a
Tifblue	81.5 b	2.0 a	8818 a
Significance			
Location (L)	NS	NS	*
Cultivar (C)	**	NS	***
L × C	NS	NS	NS

^zMeans were separated by LSD test at $P \leq 0.05$.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Number of ovules per flower, tetrad-to-ovule ratio, pollen grain-to-ovule ratio, and pollen release in four rabbiteye blueberry cultivars.

Cultivar	Ovules per flower (no.)	Tetrad: ovule ratio ^z	Pollen: ovule ratio ^z	Pollen release (tetrads per flower)
Austin	78 c ^y	85	340 ^x	--- ^w
Brightwell	88 b	105	419	8,339 a ^y
Climax	103 a	88	352	4,771 b
Tifblue	71 d	124	497	---
Mean ± SE	85 ± 7	101 ± 9	402 ± 36	

^zRatios were estimated based on overall means of tetrad production per flower. Tetrad production was evaluated in 2002 (Table 1). No. ovules per flower and pollen release were evaluated in 2004.

^yMeans were separated by LSD test at $P \leq 0.05$.

^xPollen grain-to-ovule ratio was calculated using the formula [(no. tetrads/flower) × (4 pollen grains/tetrad)] / (no. ovules/flower).

^wNot evaluated

to-ovule and pollen grain-to-ovule ratios. Rabbiteye blueberry cultivars had a pollen grain-to-ovule ratio of ≈ 400 . According to Cruden (1977), the pollen-ovule ratio of xenogamous species is ≈ 6000 . Clearly, pollen-to-ovule ratios in rabbiteye blueberry are much lower than in other flowering plants with similar breeding systems. It has been proposed that pollen-to-ovule ratios are inversely related to the likelihood of a pollen grain reaching the stigma (Cruden, 1977; Cruden and Jensen, 1979; Cruden and Lyon, 1989). Low pollen-to-ovule ratios in rabbiteye blueberry flowers indicate that pollen transfer might be more efficient than in other xenogamous entomophilous plants. Several aspects of the reproductive biology of blueberries support this idea. Blueberry flowers are adapted to buzz-pollination, which implies that their anthers are poricidal (apically dehiscent), and that sonication (bee-induced vibration of the androecium) is the most effective way to release pollen (Buchmann, 1983; Cane and Payne, 1988). Research has shown that poricidal anthers act as an efficient pollen dispensing mechanism, restricting removal per pollinator and controlling release according to the visitation rate (Harder and Barclay, 1994). Moreover, pollen grains in poricidal anthers are

Fig. 1. Pollen-ovule ratios (●) in xenogamous species that are buzz-pollinated ($n = 8$) and non buzz-pollinated ($n = 24$), based on a literature review. Mean ($\pm$ SE) pollen-ovule ratios for each group (□) are also presented. Buzz-pollinated species: *Moneses uniflora* (L.) A. Gray, *Orthilia secunda* (L.) House, *Pyrola minor* L., *P. rotundifolia* L., *P. chlorantha* Swartz (Knudsen and Olesen, 1993), *Rhexia virginica* L. (Larson and Barrett, 1999), *Vaccinium myrtilloides* [based on pollen and ovule numbers from Reader (1977) and Palser (1961)], and *V. ashei* (this study). Non buzz-pollinated species: 24 species from 10 families listed as xenogamous by Cruden (1977). These taxa are excluded from the list of poricidally dehiscent plants given by Buchmann (1983).

well protected from environmental hazards such as exposure to ultraviolet light and rain, as compared to those in longitudinally dehiscent anthers (Buchmann, 1983; Michener, 2000). The high efficiency of the pollen dispensing mechanism would justify a reduction in the pollen-ovule ratio of rabbiteye blueberries, relative to other xenogamous non-buzz pollinated species. Pollen-ovule ratios reported in other buzz-pollinated plants are also low, which further support this hypothesis (Fig. 1).

Previous studies have shown that species with pollen transporting mechanisms such as tetrads, polyads, and viscin threads have lower pollen-ovule ratios than species that simply release pollen as monads (Cruden, 1977; Cruden and Jensen, 1979; Vasek and Weng, 1988). The pollen dispersal unit for *Vaccinium* is a tetrad (Cockerham and Galleta, 1976; Stushnoff and Hough, 1968). This strategy also helps to explain the low pollen-ovule ratio of rabbiteye blueberry.

POLLEN RELEASE. 'Brightwell' flowers released almost twice as many tetrads as those of 'Climax' (Table 2), even though total pollen production per flower was similar in both cultivars (Table 1). Brewer and Dobson (1969) reported a similar difference between two highbush cultivars, although the regulatory mechanism responsible for this variation was not identified. In 'Brightwell' and 'Climax', the length and diameter of the anther tubes may have a direct or indirect effect on pollen release. Examination of 'Climax' stamens revealed longer, narrower anther tubes, relative to 'Brightwell' (Fig. 2). Anther tubes with such characteristics will likely offer a higher resistance to the flow of pollen, thus restricting its release. Pollen grains from poricidal anthers tend to form clumps due to either electrostatic interactions (Buchmann, 1983) or the presence of tapetal fluid (King and Ferguson, 1994). Therefore, narrow tubes would tend to clog more easily with these clumps. Alternatively, tube length and diameter may have an effect on the dehydration rate of pollen grains inside the anther locules. Shorter, wider tubes would allow better gas exchange, which would increase the rate of pollen dehydration. In poricidally dehiscent plants, pollen grains must be dry, smooth, and non-sticky for efficient release following

Fig. 2. Scanning electron micrographs of anther tubes from the rabbiteye blueberry cultivars Brightwell (left) and Climax (right). All micrographs are at the same scale. Scale bar: 0.5 mm.

sonication (Buchmann and Hurley, 1978). Hence, it is suggested that these anther characteristics might account for differences in pollen release observed among cultivars.

In summary, *in vitro* tetrad germination was high ($\geq 80\%$) in all cultivars studied. Pollen viability does not seem to be a limiting factor for fruit set in rabbiteye blueberry. The low pollen–ovule ratio of rabbiteye blueberry is apparently an indicator of the high efficiency of its pollen dispensing mechanism, characterized by poricidally dehiscent anthers. A significant difference in pollen release was found between flowers of two cultivars with similar pollen production. Additional research is needed to establish if different propensities for pollen release can influence cultivar preferences by pollen-collecting bees.

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