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Pollen Fertility of Hybrids Between Rabbiteye Blueberry and *Vaccinium constablaei*

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Abstract

Vaccinium ashei Reade, the cultivated rabbiteye blueberry of the southeastern United States, was hybridized with *V. constablaei* A. Gray, a lower-growing, highly rhizomatous blueberry native on the high mountain balds of western North Carolina and eastern Tennessee. Both species are hexaploid. The F_1 hybrids appeared to be fully male fertile based on copious pollen shed and on the high percentage of the pollen that could be stained with acetocarmine dye. Twelve F_1 plants representing three crosses averaged 97% pollen staining, compared to 96% for *V. ashei* and 95% for *V. constablaei*. Crosses of tetraploid southern highbush selections (largely *V. corymbosum* L.) with *V. constablaei* produced F_1 hybrids that shed little pollen and averaged only 48% pollen staining. The low pollen fertility was probably due to differences in chromosome numbers between the parental species. Unreduced gametes were detected in *V. constablaei* and were probably involved in the evolution of *V. constablaei* from diploid and tetraploid species. *Vaccinium constablaei* has several desirable traits needed in rabbiteye blueberry cultivars, including short stature, low bloom-to-ripe heat unit requirement, small seeds, and inconspicuous sclereids. Because the interspecific hybrids are highly fertile, it is likely that hexaploid blueberry cultivars of the future will increasingly contain a mixture of genes from the two species.

Introduction

Rabbiteye blueberry (*Vaccinium ashei* Reade) is a hexaploid species native in parts of the southeastern United States. The species has been domesticated during this century, and several thousand hectares are now under cultivation. Most of the hectares planted to rabbiteye blueberry are in the southeastern United States, but there is also commercial production in Chile, Australia, Japan, Spain and elsewhere. Breeding work was begun to improve rabbiteye blueberry in 1940, and the first two cultivars resulting from this work were released in 1950 (6). Since then, many new cultivars have been released.

Vaccinium constablaei A. Gray is a wild blueberry species native to the high mountain balds of western North Carolina and eastern Tennessee (3). Like rabbiteye, it is hexaploid, and is thus one of the few *Vaccinium* species that produces hexaploid hybrids when crossed with rabbiteye cultivars. Desirable characteristics in *V. constablaei* that could possibly be transferred to rabbit eye cultivars are a short flowering-to-ripening interval, a shorter plant stature, and fruit with thinner skins, smaller seeds, and less-prominent sclereids (2, 8, 15). Crosses between rabbiteye and *V. constablaei* were made as early as 1943 (9), and an F_1 hybrid has recently been released as the cultivar Little Giant (5).

The University of Florida blueberry breeding program has been using *V. constablaei* in crosses with low-chill rabbiteye selections for the past 15 years. Despite some promising results and the release of one cultivar ('Snowflake') from the backcross of an F_1 to a rabbiteye selection (7), there have been many problems with *V. ashei* x *V. constablaei* derivatives. Chief among these have been reduced plant vigor under Florida conditions and susceptibility to phytophthora root rot (*Phytophthora cinnamomi*), blueberry gall midge (*Dasineura oxycoccana*), and to various leaf diseases.

Fertility has not been a conspicuous problem in *V. ashei* x *V. constablaei* crosses in the Florida breeding program. The crosses have usually given a large number of F_1 seedlings and neither the F_1 hybrids nor backcross populations have given poorer fruit set than is normal for rabbiteye blueberries in Florida. Still, blueberries are capable of making many

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seeds per berry, and even plants with good fruit set in open-pollinated nurseries may have reduced pollen abundance or quality, which could be problematic in commercial production fields. Because blueberries are usually not parthenocarpic in the field, good pollination is necessary for maximum fruit set and highest yields. The purpose of this study was to compare pollen staining of *V. constablaei*, *V. ashei*, and their F_1 hybrids to see if there was any potential reduction in fertility in the hybrids.

Materials and Methods

In Jan. 1991, branches of several *V. constablaei* clones were sent by mail from the National Clonal Germplasm Repository in Corvallis, Oregon. These branches were forced in a laboratory to obtain flowers. Pollen from these flowers was applied to the stigmas of emasculated flowers on potted rabbiteye selections from the University of Florida blueberry breeding program. The crosses were made in a bee-proof greenhouse. Three crosses, involving three *V. constablaei* selections and three *V. ashei* clones, were made, and about 100 seedlings per cross were transplanted to a test field in Gainesville, Florida, in May 1992 (Table 1). Three *V. ashei* x *V. ashei* crosses were made for comparison, and the seedlings were transplanted at the same time. During the flowering season, when these plants were 2.3 years old, four plants were selected at random from each of the six crosses, and well-developed but unopened flowers were collected for pollen analysis from each.

In Dec. 1992, branches bearing flower buds were collected from several *V. constablaei* clones near Shining Rock Wilderness Area south of Asheville, North Carolina, and from Roan Mountain, Tennessee. These were collected from mountain balds at altitudes above 1600 meters. The clones were 1.1 to 1.7 meters tall and had hundreds of canes arising from extensive colonies 4 to 10 meters in diameter. Thus, they corresponded to the lower-growing, more rhizomatous elements of what Camp (3) described as *V. constablaei*. Our examinations of plants in the same area that seemed to fit the taller, less colonial element of Camp's *V. constablaei* have yielded only tetraploid or diploid plants. The branches we collected were brought to Gainesville, Florida in an ice chest, stored in the dark at about 7°C for 30 days, and then forced in a greenhouse by placing the cut ends in an aqueous preservative solution made of sucrose (5%) + citric acid (5.93 x 10^{-4}M) + 8-hydroxy quinoline hemisulfate salt (5.93 x 10^{-4}M).

Crosses were made in the greenhouse in the spring of 1993 between four tetraploid southern highbush selections from the Florida breeding program (FL90-175, FL90-178, FL90-182, and FL93-39) and four *V. constablaei* clones, using flowers from the cut branches as the pollen source. Due to the low number of seedlings obtained, seedlings from the four crosses were combined and planted at the University of Florida Horticultural Unit in May 1994. In March/April 1996, well-developed but still unopened flowers from six of these seedlings were collected for pollen analysis. Pollen from six southern highbush cultivars (Jubilee, Magnolia, Misty, Sharpblue, Southmoon, and Star) was collected for comparison.

Pollen staining was done with 2% aceto-carmine glycerol jelly (13). With this stain, pollen grains containing cytoplasm (potentially viable) are stained, and aborted pollen grains containing little or no cytoplasm do not stain. Pollen was prepared for examination as follows. Two drops of 2% aceto-carmine glycerol jelly were placed on a clean slide. Four flowers were picked at random from each plant, and a small portion of the apex of the corolla tube, if unopened, was cut off with scissors. Individual flowers were rolled between the indicator finger and the thumb to release the pollen. This was spread evenly onto the dye. The dye was then covered with a cover slip, and after 15 minutes the pollen was examined under a light microscope at 250X. For each sample, 100 sporads (tetrads, triads, dyads and monads) were observed so that

Table 1. Origin of the *V. ashei* and *V. ashei* x *V. constablaei* seedlings whose pollen staining is reported in Table 2.

Population	Seed parent[z]	Pollen parent
	(*V. ashei*)	(*V. ashei*)[y]
1	89-176	Powderblue
2	91-54	Premier
3	91-63	Brightwell
	(*V. ashei*)	(*V. constablaei*)[x]
4	85-97	Corvallis #5
5	85-109	Corvallis #8
6	91-2	Corvallis #11

[z]Six advanced rabbiteye selections from the Florida breeding program.
[y]Three rabbiteye blueberry cultivars.
[x]Three *V. constablaei* clones from the U. S. Dept. of Agriculture National Clonal Germplasm Repository in Corvallis, Oregon.

the number of stained and unstained pollen grains and the number of unreduced pollen grains that stained could be counted. Pollen grains that occurred as dyads or monads were considered unreduced. Dyads and monads were carefully examined at higher magnification to distinguish them from tetrads containing aborted spores.

Percentage of stainable pollen grains and percentage of unreduced pollen that stained were estimated for each plant. The data relating to unreduced pollen were transformed to the square root of (X + 1) before analysis. Differences among taxa on the two pollen characteristics were tested by analysis of variance, completely randomized design, and if significance was achieved, means were compared by the Tukey-test.

Results and Discussion

The 12 F_1 *V. ashei* x *V. constablaei* hybrid plants, representing three crosses, all shed copious pollen, and pollen staining averaged 97.1% (Table 2). This figure was as high as that for the two parental species (Table 2). These data, and the lack of fruit set problems in hybrid derivatives of *V. ashei* x *V. constablaei* suggest that these species are highly cross-compatible, despite their great morphological and physiological differences.

Despite our data, we disagree with Vander Kloet (14) who combined *V. constablaei* with *V. ashei* and several other highbush blueberry species under the name *V. corymbosum*. If all species that could be crossed readily in the greenhouse to make fertile hybrids were combined, section Cyanococcus would consist of only three species: one diploid, one tetraploid, and one hexaploid. If production of natural hybrids in the field were adopted as the criterion for combining taxa, the combinations would be even more extensive than those suggested by Vander Kloet, but *V. ashei* and *V. constablaei* would not be combined, because neither their ranges nor their habitat preferences, nor their seasons of flowering overlap. On the other hand, combining the taxa that naturally hybridize abundantly in the forest would certainly result in combining *V. myrsinites*

Table 2. Frequency of stainable pollen in seedlings or clones of various blueberry taxa.

Population	Number of genotypes examined	Amount of pollen shed	% Stainable pollen grains[z]	
			Mean	Range
V. ashei x *V. ashei*[y]	12	Copious	96.3[u]	87.1-100.0
V. const. (Shining Rock)[x]	4	Copious	94.0	84.9-99.7
V. const. (Roan Mt.)[x]	4	Copious	96.5	92.7-100.0
F_1 (*V. ashei* x *V. const.*)[y]	12	Copious	97.1	87.2-100.0
V. corymbosum[w]	6	Copious	98.2	92.3-100.0
F_1 (*V. corymb.* x *V. Const.*)[v]	6	Little	48.4[T]	3.9-63.9

[z]One hundred sporads were examined per clone.
[y]Four seedlings from each of the three crosses listed in Table 1.
[x]Flowers from branches cut from native clones in western North Carolina and eastern Tennessee and forced in a greenhouse in Gainesville, Florida.
[w]Florida southern highbush selections from the breeding program.
[v]Florida southern highbush selections crossed with *V. constablaei* from western North Carolina and eastern Tennessee.
[u]The first five means in this column did not differ at $P \leq 0.05$ using the Tukey-test.
[T]The last mean differed from the first five at $P \leq 0.05$ by the Tukey-test.

Table 3. Frequency of stainable, unreduced pollen in seedlings or clones of various blueberry taxa.

Population	Number of genotypes examined	% Stainable pollen grains[z]	
		Mean	Range
V. ashei x *V. ashei*	12[y]	0.00 a[u]	00.0
V. const. (Shining Rock)	4[x]	0.00 a	0.00
V. const. (Roan Mt.)	4[x]	1.96 b	0.00-4.86
F_1 (*V. ashei* x *V. const.*)	12[y]	0.31 a	0.00-3.20
V. corymbosum	6[w]	0.62[T]	0.00-2.13
F_1 (*V. corymb.* x *V. Const.*)	6[v]	5.65[T]	1.29-10.00

[z]One hundred sporads (mostly tetrads) were examined per clone.
[y]Four seedlings from each of the three crosses listed in Table 1.
[x]Flowers from branches cut from native clones in western North Carolina and eastern Tennessee and forced in a greenhouse in Gainesville, Florida.
[w]The southern highbush cultivars Jubilee, Magnolia, Misty, Sharpblue, Southmoon, and Star.
[v]Seedlings bulked from four crosses involving four southern highbush selections from the Florida breeding program and four *V. constablaei* clones from western North Carolina and eastern Tennessee.
[u]Among the first four populations, means within a column not followed by a common letter were significantly different at P ≤ 0.05 using the Tukey-test.
[T]These two means differed from each other at P ≤ 0.05 by the Tukey-test.

L. with tetraploid forms of *V. corymbosum* in north Florida, *V. darrowi* Camp with diploid forms of *V. corymbosum* in the central Florida peninsula, and *V. darrowi* with *V. elliottii* Chapm. in the Florida panhandle. We do not support the combination of these taxa, because, despite the numerous natural hybrids with intermediated characteristics that occur in areas where their ranges overlap, the core populations of these species are morphologically and ecologically distinct.

The six F_1 hybrids from *V. corymbosum* x *V. constablaei* shed little pollen, and this averaged only 48.4% staining. The most likely reason for this low fertility is a chromosome number difference between the parents. The *V. corymbosum* cultivars were tetraploid and the *V. constablaei* clones, which came from the mountain balds of western North Carolina and eastern Tennessee, were probably hexaploid. In their native habitat, these plants formed low-growing colonies with hundreds of shoots, 1.1 to 1.7 m tall, tightly packed into extensive clonal colonies. Several plants of this type from this area were found to be hexaploid by chromosome count (Lyrene, unpublished). Workers wishing to obtain hexaploid germplasm from this region should avoid the taller, more monopodial plants, several of which were found to be tetraploid by chromosome count (Lyrene, unpublished), although they fit the description for one form of *V. constablaei* as given by Camp (3). The upright, tetraploid plants are probably *V. simulatum* Small.

Unreduced gametes, which have the same chromosome number as the somatic tissues of the plant that produced them, instead of the normal haploid number, provide the most likely path for the evolution of polyploid blueberries from species of lower ploidy. Blueberry species and clones within species vary widely in the frequency of unreduced gamete production (1, 4, 10, 11, 12). The presence of one clone with relatively high 2n pollen production in the *V. constablaei* from Roan Mountain shows that 2n gametes could have played a part in the evolution of this species (Table 3). Both *V. ashei* and *V. corymbosum* hybrids with *V. constablaei* contained one or more plants with high 2n gamete frequency.

Because the morphological and physiological characteristics of *V. ashei* and *V. constablaei* are complementary in many ways, the weaknesses of one species being the strengths of the other, it is likely that genes from the two will increasingly be blended in the hexaploid cultivars of the future.

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Outcrossing in a Diverse Peach Rootstock Seed Block

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Abstract

Over a 3 year period incidence of evident outcrosses (heterozygous redleaf) was recorded in open-pollinated seedlots of 'Nemared' and 'Rutgers Redleaf' collected in a rootstock repository. Since this block is made up predominantly of green-leaf cultivars this technique should detect most outcrossing events. Outcrossing averaged 6 percent. However, rootstock cultivar had a highly significant effect on the number of outcrossing events; in 'Nemared' such events occurred on average at more than three times the rate observed in 'Rutgers Redleaf.' In the absence of sufficient isolation to prevent undesirable outcrossing, rootstock cultivars need to have some readily identifiable 'marker' to reveal outcrosses so that they may be discarded.

Introduction

Knowledge of the extent of outcrossing in a plant species is an important factor in the understanding of its breeding behavior and subsequently to the development of a breeding strategy for crop improvement. Currently, the United States peach industry is based almost entirely on seedling-type peach rootstocks. Commercial seed blocks are rarely sufficiently isolated from other peach orchards to prevent the introduction of 'off-type' pollen by bees and other pollinating insects. Such outcrossing is likely to have a detrimental effect on commercial rootstock performance by interfering with the uniform

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