

Level of Self- and Cross-fertility of Derivatives of *Vaccinium arboreum* x *Vaccinium* Section *Cyanococcus* Hybrids

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Abstract. The extent of self-fertility and self-fruitfulness was studied in *Vaccinium arboreum* Marsh, *V. darrowi* Camp, and in seedlings, termed MIKs, from open-pollination of *V. darrowi* (section *Cyanococcus*) x *V. arboreum* (section *Batodendron*) F₁ hybrids. The open pollinations that produced the MIKs occurred in a field containing tetraploid southern highbush selections (based largely on *V. corymbosum* L.), and the pollen parents of the MIKs are believed to be southern highbush selections. The MIKs that were studied had been selected for high fruit set after open pollination in the field. Both *V. arboreum* and *V. darrowi* exhibited very low self-fruitfulness and self-fertility when hand-pollinated in a greenhouse; the former produced no seedlings from more than 600 selfed flowers, and the latter produced only 13. By contrast, southern highbush clones averaged 70 seedlings per 100 pollinated flowers when selfed and 230 when crossed. Self-fertility and self-fruitfulness of the MIKs were higher than those of *V. arboreum* and *V. darrowi* but lower than those of southern highbush selections. MIK x MIK crosses gave fewer seedlings per 100 pollinated flowers (84) than highbush x highbush crosses (230), probably reflecting their hybrid ancestry. Although introduction of *V. arboreum* genes into southern highbush blueberry gives plants of excellent vigor and adaptation to north Florida, several generations of breeding will be needed to obtain cultivars with high fertility and berry quality.

Cultivated blueberries require cross-pollination to produce high fruit yields in Florida. This requires planting at least two different cultivars in a field, usually alternating cultivars by rows. Alternate-row planting may lead to harvest problems, as different cultivars usually do not ripen at exactly the same time. Therefore, commercial cultivars that produced a good yield via self-pollination or parthenocarpy would be highly desirable.

Self-incompatibility is common in *Vaccinium*. Furthermore, the structure of the flower reduces within-flower selfing (Ballington and Galletta, 1978; Eck and Mainland, 1971). The receptive surface of the stigma extends beyond the surrounding stamens and normally receives little pollen from the same flower. In addition, the sides of the stigma are angled so as to deflect pollen falling from the anthers (Eck and Mainland, 1971).

There is good agreement in the literature that the diploid species in *Vaccinium* section *Cyanococcus* and hexaploid *V. ashei* Reade are partially to highly self-incompatible. Ballington and Galletta (1978) examined self-incompatibility in four diploid species

(*Vaccinium atrococcum* Heller, *Vaccinium caesariense* Mackenzie, *V. tenellum* Aiton and *V. darrowi*). They found that self-pollinations were considerably less successful than intraspecific cross-pollinations; cross-pollination produced six times the amount of fruit, 37 times as many seeds, 46 times as many germinated seeds, and 52 times as many vigorous seedlings as did self-pollination.

Tetraploid *V. corymbosum*, the highbush blueberry, shows much variability in self-compatibility. Coville (1937) reported that experiments to self-pollinate highbush were a failure and he abandoned the technique. White and Clark (1939) found a wide range of variation among highbush cultivars from total self-incompatibility to total self-compatibility. El-Agamy et al. (1981) found that the average fruit set after selfing five cultivars and advanced selections was 67%, with a range of 43% to 95%. An average of 3.9 seeds per berry with a range of 2.2 to 5.4 were produced by these selfings. Cross-pollinations for these same clones averaged 82%, with an average of 11.2 seeds per berry.

Vander Kloet and Lyrene (1985) discussed several reasons why studies of self-incompatibility in *V. corymbosum* have given variable results. The first reason was that most studies have been done with cultivars or selections from breeding programs. These clones have been selected for their ability to fruit well in commercial blocks planted with only one or a few clones. Therefore, they are not representative of the species as a whole. The second issue was the method of self-pollination. Self-fertil-

ity could be measured by observing plants: 1) placed in a bee-proof greenhouse with no provision made to move pollen from the anthers to the stigmas; 2) worked by bees that had access to only one cultivar; or 3) self-pollinated by hand. Since the structure of the blueberry flower reduces the amount of self-pollination that occurs in the absence of insects, unassisted self-pollination would give results different from assisted self-pollination. A third point was the criteria used to determine success of the cross. Evaluating the results of self-pollination by different criteria (for example, percent fruit set, number of plump seeds produced per flower, or number of seedlings produced per pollinated flower) might lead to different conclusions regarding the same genotype.

In a study of native populations of diploid, tetraploid, and hexaploid *V. corymbosum*, self-pollinations were less successful than cross-pollinations in every experiment for each ploidy level (Vander Kloet and Lyrene, 1985). Percent fruit set for self-pollinations averaged less than one-fourth and the number of plump seeds per berry less than one-third that for cross-pollinations. Self-pollination also gave a lower percent seed germination. The authors cited earlier studies showing that only one to a few well-developed seeds are necessary for normal fruit development in the greenhouse (Lyrene and Sherman, 1983). This implies that data for percent fruit set may give a much higher estimate of self-fertility than data for viable seedling production.

In 1991, experiments were begun to investigate the properties of *V. darrowi* x *V. arboreum* F₁ hybrids (Brooks, 1996; Lyrene, 1991). This is an intersectional cross; *V. darrowi* is in section *Cyanococcus* and *V. arboreum* in section *Batodendron*. Also included in the study was a population of plants obtained by open-pollination of the F₁ hybrids in a field with highbush blueberry cultivars. These open-pollinated seedlings were termed MIKs. Study of the morphology and crossing behavior of the MIK population indicated that at least the more fertile plants were outcrosses with highbush selections, were tetraploid, and could be easily crossed with southern highbush cultivars to introduce *V. arboreum* genes into the cultivated gene pool (Brooks, 1996).

The original reason for attempting to introgress genes from *V. arboreum* into highbush blueberry was to increase the heat and drought tolerance of the cultivated highbush blueberry and to increase its vigor and ability to grow on soils low in organic matter (Lyrene and Brooks, 1995). Our early observations of selected MIK plants indicated that some of them might have a tendency toward parthenocarpic fruit production or increased self-fertility. We hypothesized that wide hybridity could have disrupted the genetic system that governs self-incompatibility or the system that causes seedless fruit to abscise early in development. Because parthenocarpy and high self-fertility would be valuable in commercial production fields, the selfing behavior of selected MIK plants was studied further. The question of whether MIK plants had reduced cross-fertil-

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ity as a result of their hybrid ancestry was also studied. The principal goals of the study were: 1) to estimate the self-fertility of three clones of *V. arboreum*, three of *V. darrowi*, 20 MIK clones, and three southern highbush clones; 2) to compare the fertility of 10 MIK x MIK crosses with that of 10 highbush x highbush crosses; and 3) to study the ability of MIK clones to produce fruit without hand-pollination in a bee-proof greenhouse.

Materials and Methods

The plant material used in this study included three clones of *V. arboreum* and three of *V. darrowi* that originated from native stands in the north-central Florida peninsula near Gainesville. Twenty MIK clones were also used. These were obtained by planting seed harvested from F_1 *V. darrowi* x *V. arboreum* hybrids that had been open-pollinated in a field containing many southern highbush selections from the Florida breeding program. The MIKs that were used had been selected from a larger MIK population (about 100 plants) based on high fruitfulness when open-pollinated in field nurseries. Five clones that had been derived by backcrossing MIK clones to southern highbush selections, and 10 southern highbush clones were also used. Each clone was represented by one plant.

The crosses were made on plants that were dug from high-density field nurseries at the Univ. of Florida Horticultural Unit near Gainesville, in Dec. 1991, 1992, and 1993. The plants were potted in peat and placed in an unlighted cooler at $\approx 5^\circ\text{C}$ for at least a month to satisfy their chilling requirement. After chilling, the plants were moved to a bee-proof greenhouse where the temperature was maintained between 5°C and 27°C . Any old leaves remaining on the plants were removed when they were moved to the greenhouse.

Flowers were pollinated on or near the day of anthesis. On the day of pollination, the corolla and anthers were removed. Pollen was applied to the stigma from the thumbnail. Before pollination, the male parent was checked for pollen viability to ensure that low fruit set would not be due to inviable pollen. This was accomplished by staining pollen grains with 1% acetocarmine and examining them under a microscope (Brooks, 1996). The number of flowers pollinated per cross varied, but averaged about 200. The pollinations made are listed in Table 1.

Ripe berries were harvested daily, counted and stored at 7°C if necessary. Fruit set, berry weight, and number of plump seeds per berry were recorded. The first 30 berries harvested from each cross were kept separate from the rest of the harvest. These berries were squashed individually on paper towels, the seeds were dried overnight on a laboratory bench, and the number of plump seeds per berry was counted the next day. As the remaining berries were harvested, each was individually opened and scored for the presence or absence of plump seeds.

Percentage fruit set was compared for the crosses by using the adjusted Chi-square test

of independence in 2×2 contingency tables (Steel and Torrie, 1960). Means for the other parameters were compared among crosses using pair-wise *t* tests.

The MIK clones FL89-107 and FL91-333 had produced many berries without assisted pollination in the greenhouse in 1991. Their need for pollination was studied in Spring 1994. One large plant of each clone was potted, chilled, and placed on a bench in a bee-proof greenhouse. The berries produced on these plants were counted at harvest, the seed content was determined, the seed planted, and the resulting seedlings were counted.

Results and Discussion

Vaccinium arboreum and *V. darrowi*, each represented by three different clones, were very low in all self-fertility parameters (Table 1). By contrast, the three selfed highbush clones, which represented the cultivated gene pool, averaged almost as high in percentage fruit set as the highbush crosses. Selfing in highbush did, however, reduce berry weight, the number of plump seeds per pollinated flower, and the number of seedlings per pollinated flower. The last two parameters were reduced to about one-third the values for cross-pollination.

The 20 MIK clones that were selfed averaged only 37% fruit set compared to 77% for the 10 MIK clones cross-pollinated with other MIKs (Table 1). The number of seedlings per pollinated flower was also greatly reduced for the selfed MIKs.

Fruit set of the 10 MIK x MIK crosses averaged almost as high as that of the 10 highbush x highbush crosses (Table 1). The other parameters, however, indicated that full fertility had not been recovered in the MIK population. Plump seed-number per 100 cross-pollinated flowers averaged only 312 for the MIK crosses compared with 1991 for the highbush; the number of seedlings per 100 pollinated flowers averaged 84 for the MIK crosses and 230 for the highbush. Berry weight after cross pollination averaged 0.71 g for the MIK crosses and 1.73 g for the highbush. This probably reflects the fact that both the *V.*

darrowi and *V. arboreum* in the ancestry of the MIKs have fruit much smaller than cultivated highbush. The lower fertility of the MIKs may be due in part to the fact that they contain genes of highly disparate origin. (*V. darrowi* and highbush are in section *Cyanococcus*, whereas *V. arboreum* is in section *Batodendron*). Despite the fact that we have obtained extremely vigorous and upright plants after *V. arboreum* introgression into cultivated southern highbush, berry size and quality in the best MIKs and in seedlings from the first backcross to southern highbush cultivars are still below commercial standards.

The two MIK clones that had produced fruit without pollination in 1991 again produced some ripe fruit in 1994 without assisted pollination (Table 2). However, fruit set was only 4% and 13%, respectively, for the two clones. Most of the berries contained one or more well-developed seeds, despite the fact that the flowers were not pollinated by hand or by bees. Parthenocarpy was rare in these clones under the conditions that prevailed.

One of the two MIK clones, 91-333, while setting only 12.8% of its flowers, produced an average of 41.5 plump seeds per berry in the first 30 berries that matured. Whether the flowers that developed into berries had more pollen deposited on their stigmas than the flowers that aborted was not determined. If the flower structure could be modified by breeding to permit large amounts of pollen to fall on the stigmas in the absence of insect visitation, fruit set might be greatly increased.

We conclude from this study that the intersectional hybrid material examined was not high in self-fertility or in self-fruitfulness in comparison with highbush cultivars, nor did the intersectional hybrids have a strong tendency toward parthenocarpy. The extreme variability for many features that has been observed in segregating generations offers the possibility of selecting exceptional plants with increased self-fruitfulness. The extremely desirable plant characteristics that can be obtained after *V. arboreum* introgression into highbush offer much promise for the long-range utility of this type of hybridization, but fertility and berry quality must be improved before cultivars can be obtained.

Table 1. Summary of data on effects of self- and cross-pollination in controlled crosses using *Vaccinium arboreum*, *V. darrowi*, MIK (open-pollinated progeny of F_1 *V. darrowi* x *V. arboreum* hybrids), southern highbush (HB), and MIK x HB clones of blueberry.

Cross	n ^a	Mean no. pollinations per cross	Mean fruit set (%)	Berry wt (g)	Plump seeds per 100 pollinated flowers	Seedlings per 100 pollinated flowers
<i>V. arboreum</i> selfed	3	221.7	7.8 a ^b	0.27 a	1.8 a	0.0 a
<i>V. darrowi</i> selfed	3	200.3	7.0 a	0.18 a	28.5 a	2.5 a
MIK ^c selfed	20	192.6	37.2 b	0.67 a	168.2 ab	19.5 a
MIK x HB ^d selfed	5	176.4	41.7 b	0.92 a	214.3 ab	11.4 a
HB selfed	3	100.0	76.7 c	1.22 a	602.0 ab	70.5 ab
MIK x MIK	10	278.1	77.2 c	0.71 a	312.4 ab	83.7 b
HB x HB	10	99.3	81.4 c	1.73 b	1991.4 c	230.5 c

^aNumber of crosses.

^bMean separation within columns by pair-wise *t* tests ($P = 0.05$).

^cOpen-pollinated progeny of F_1 *V. darrowi* x *V. arboreum* hybrids.

^dSouthern highbush (cultivated taxon based largely on *V. corymbosum*, which also contains genes from other *Vaccinium* species).

Table 2. Fruit, seed, and seedling yields from two MIK' blueberry plants after open pollination in a bee-proof greenhouse. Spring 1994.

Plant	No. flowers on plant	Fruit set (%)	Berry wt [†] (g)	Plump seed per berry [‡]		No. berries with:		No. seedlings per 100 flowers
				(No.)	wt (mg)	One or more plump seeds	No plump seeds	
89-107	1325	4.2	0.40	8.1	3.4	53	3	3.8
91-333	1450	12.8	1.06	41.5	21.6	172	14	34.6

[†]Open-pollinated progeny of *V. darrowi* x *V. arboreum* F₁ hybrids.

[‡]Average from the first 30 berries that ripened.

[§]Average of 50 berries.

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