

First Report of *Vaccinium arboreum* Hybrids with Cultivated Highbush Blueberry

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Abstract. Over several years, we obtained no hybrids after pollinating thousands of flowers of cultivated tetraploidighbush blueberry (*Vaccinium corymbosum* L. hybrids, section *Cyanococcus*) with pollen from diploid *V. arboreum* Marshall (section *Batodendron*, sparkleberry), a drought-tolerant blueberry relative native in the southeastern United States. In an effort to produce tetraploid *V. arboreum* that could be crossed withighbush blueberry, more than 30,000 seeds were soaked in aqueous colchicine (0.1% to 0.2%) for 24 h or more. The seeds were germinated, and putative tetraploid plants (selected based on morphological characteristics) were grown long enough to obtain pollen for microscopic examination. Twelve selected seedlings that produced unusually large pollen tetrads were used as pollen parents in crosses with more than 40 different tetraploidighbush cultivars and advanced selections. Eighty-six crosses, in which a total of 17,968 flowers were pollinated, gave 1,569 plants that were verified as hybrids after one growing season in the field. Hybrids varied from very weak to quite vigorous, some equalingighbush cultivars in vigor. A few vigorous hybrids were male-sterile, but most had at least some pollen fertility. Of the most vigorous F₁ hybrids, 12 of the most fertile, based on the amount of pollen shed and on the microscopic appearance of the pollen, were backcrossed toighbush cultivars, and 3919 backcross seedlings were obtained. These varied widely in vigor but averaged higher in vigor than their F₁ interspecific hybrid parents.

Vaccinium arboreum (sparkleberry; section *Batodendron*) is a widespread and abundant diploid blueberry species native in the southeastern United States (Stockton, 1976; Vander Kloet, 1988). It flowers in April in north Florida and produces numerous small, shiny black berries that ripen from mid-September through October. The berries are barely edible, having large seeds, grit-like sclerids, and little juice. *Vaccinium arboreum* interests blueberry breeders for several reasons. In particular, it has a wide, deep root system, which makes the plants drought-tolerant, and it can grow well on soils of higher pH than any species in section *Cyanococcus*. Cultivatedighbush blueberries require acid soil and have shallow, fibrous roots that require continuous moisture in the top meter of soil.

Several intersectional crosses have produced hybrids in *Vaccinium* (Ballington, 1980, 2001; Darrow and Camp, 1945; Rousi, 1963) but our attempts to cross diploid *V. arboreum* with tetraploidighbush cultivars have failed. In each of 4 different years, we obtained no hybrids after pollinating several hundred to several thousand flowers of southernighbush cultivars with pollen from *V. arboreum* (Lyrene,

unpublished data). These failures were probably attributable in part to the triploid block, which is strong in *Vaccinium* (Darrow et al., 1944), along with other genetic crossing barriers between sections *Batodendron* and *Cyanococcus*. In 1981, we crossed *V. arboreum* as a pollen parent with several clones of *V. darrowii* Camp (section *Cyanococcus*), a diploid close relative ofighbush blueberry (Lyrene, 1991). These crosses produced hundreds of seedlings, some quite vigorous. Most of the hybrid seedlings that were grown to maturity flowered heavily each year but produced little or no fruit or seed when open-pollinated in the field.

Chavez and Lyrene (2009) studied meiosis in 10 of the hybrids and confirmed that they were diploid. Despite considerable chromosome pairing, meiotic irregularities were common, including univalents and multivalents at Metaphase I and a high frequency of irregular chromosome distributions at Anaphase I.

The fertility of wide-hybrid diploid plants can sometimes be increased by doubling the chromosome number (Clausen et al., 1945). Increased fertility at the tetraploid level may be the result of a higher frequency of bivalent chromosome pairing (amphidiploid pairing) or greater genetic buffering resulting from genetic redundancy in polyploids.

This study had two goals: 1) to see if hybrids could be obtained by crossing tetraploidighbush blueberry cultivars with tetraploid *V. arboreum* produced using colchicine and 2) to see if the hybrids would be sterile or fertile.

Beginning in 2004, a composite of open-pollinated berries was harvested in each of 3 years from 30 or more *V. arboreum* trees growing in a 100-ha forest in Clay County, in northeast Florida, near Jacksonville. *Vaccinium arboreum* seedlings sometimes form large colonies from root sprouts. The trees from which the berries were harvested were widely dispersed, variable in berry size and shape, and clearly included many genotypes. Seeds were extracted and dried. In November of each harvest year, ≈10,000 dry seeds were imbibed in aqueous colchicine (either 0.1% or 0.2%) for ≈110 h and then planted without rinsing on peat in a greenhouse under mist. The colchicine treatment appeared to have little detrimental effect on germination, and several thousand seedlings were grown each year. Starting when the first seedlings reached the two- to six-leaf stage, the germination pots were examined weekly, and seedlings that had unusually thick hypocotyls, asymmetrical leaf shape, or other morphological indications of possible polyploidy were transplanted to trays of peat. In each of the 3 years, 200 to 500 seedlings were selected and transplanted. As the seedlings grew, they were repeatedly re-selected, and those that maintained morphological indications of polyploidy were potted and saved. When these plants flowered, pollen tetrad diameter was measured for each plant using a compound microscope at 250×, and 12 putative tetraploid plants were selected based on pollen size for use in crosses with tetraploidighbush cultivars. Pollen tetrads of the selected plants were conspicuously larger than those of diploid *V. arboreum*, but pollen size varied considerably from one putative tetraploid to another (data not shown).

During the crossing seasons of 2007, 2008, and 2009 (February through April), 17,968 emasculated flowers on tetraploid southernighbush selections were pollinated in a bee-proof greenhouse using pollen from the 12 putative tetraploid *V. arboreum* plants. Three diploid *V. arboreum* clones from seed not treated with colchicine were used to pollinate 500 additional flowers onighbush cultivars. The seeds obtained were extracted, dried, and planted the next November on peat under mist in a greenhouse. Seedlings were transplanted to trays of peat in January and February and then to a fumigated high-density field nursery in late April or early May. In November and December, the seedlings in the field were evaluated subjectively for vigor and were classified as hybrid or not hybrid. Leaf color, leaf and stem pubescence, and the color and patterning of the bark on older wood were the most useful traits for distinguishing hybrids from nonhybrids. Hybridity was confirmed in late winter by the size and shape of inflorescence buds. By the time the plants flowered (mostly in March and April), no questions about hybrid identity remained.

Flower bud formation on the hybrids in the field contrasted strongly with that ofighbush blueberries. By late December, flower buds were conspicuous on allighbush seedlings

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that flowered the next spring. On hybrid seedlings, no flower buds were apparent before January, and from 1 Feb. through 1 Apr., many hybrid plants that appeared to have no flower buds would have flowers nearing anthesis 2 weeks later. Throughout Feb. and Mar. 2009, plants were examined weekly, and 61 hybrids that showed signs of flowering were dug, potted, and brought to the greenhouse for backcrossing to southern highbush cultivars.

The first few flowers that opened on each hybrid plant were harvested, and pollen was examined under a microscope at 250 \times . Pollen was dusted onto a drop of water on a microscope slide by rotating a flower between the thumb and index finger. The amount of pollen shed per flower was estimated subjectively compared with the amount of pollen typically obtained from highbush flowers. A minimum of 25 pollen tetrads (100 microspores) was examined for each plant, and the percentage of the spores that appeared to be large, plump, and potentially viable was estimated. Additional F₁ hybrids, mostly from the second year's crosses, were dug, potted, and assessed for pollen fertility in late winter and spring of 2010. In all, 91 F₁ hybrid seedlings were examined for pollen fertility. Twelve F₁ hybrids were chosen for use in backcrosses to tetraploid highbush cultivars. Selection was based on the number of flowers available, the amount of pollen shed, and the apparent viability of the pollen as judged by the appearance of the pollen under the microscope. Three of the F₁ hybrids that had the most flowers were used both as pollen and seed parents in backcrosses to highbush.

Crosses were made in a greenhouse from which pollinating insects were excluded. Flowers were emasculated before or shortly after anthesis. Pollen from the male parent, collected as previously described, was transferred immediately to stigmas of emasculated flowers of the seed parent.

Results

Pollination of tetraploid highbush flowers with pollen from V. arboreum. Pollination of 500 highbush flowers with pollen from three diploid *V. arboreum* plants in 2008 produced no seed (Table 1). This was consistent with the results of previous years. The 17,968 highbush flowers pollinated using pollen from the putative tetraploid *V. arboreum* plants gave 1,569 plants that were verified in the field as hybrids (Fig. 1), an average of 87 hybrids per 1,000 pollinated flowers (Table 1). Approximately 60% of the hybrids had medium to high vigor. The other 40% were weak and will probably die before flowering.

Although the crosses were not designed to test differences in hybrid production using different *V. arboreum* and highbush clones as parents, *V. arboreum* clones appeared to differ in effectiveness as pollenizers. For example, in 2009, *V. arboreum* clone 08-478 produced no hybrids after pollination of 2692 flowers on seven different highbush cultivars, whereas *V. arboreum* clone 07-544 produced 173 hybrids after pollination of 2272 flowers on eight different highbush clones (Table 1). Because of the failure of 08-478 to produce hybrids, its pollen was re-examined the next year and was found

to be larger than that of the diploid control plants but the smallest of the putative tetraploids.

When several crosses were made using the same *V. arboreum* pollen parent, the identity of the highbush female parent seemed to strongly affect the number and vigor of the F₁ hybrids obtained (data not shown). The experimental design did not permit statistical testing of these differences.

Fertility of F₁ hybrids. The 91 F₁ hybrids from 2007 and 2008 crosses from which pollen was examined microscopically in 2009 and 2010 ranged from completely male-sterile to having 98% of the microspores well developed and presumably viable. The median percent microspore viability for the 91 hybrids was \approx 40%. By comparison, typical microspore viability estimated in the same way for Florida highbush cultivars and for *V. arboreum* ranges from 95% to 99%.

The 16 backcrosses in which highbush was the seed parent gave 3290 seedlings from 2658 pollinated flowers, an average of 1238 hybrids per 1000 pollinated flowers (Table 2). The nine reciprocal backcrosses, in which the F₁ hybrid was the seed parent and highbush the pollen parent, yielded 620 seedlings from 766 pollinated flowers, an average of 809 seedlings per 1000 pollinated flowers. The difference in rate of seedling production for the two types of backcrosses was significant at 1% according to the chi-square test.

Discussion

Vigorous hybrids can be obtained by pollinating tetraploid highbush cultivars with

Table 1. Results of crosses in 2007, 2008, and 2009 between tetraploid highbush cultivars and selected *Vaccinium arboreum* clones.

<i>V. arboreum</i> pollen source	Crosses (no.) ^z	Total flowers pollinated	Percent set (avg)	Berries sampled (no.)	Plump seeds/berry (mean) SD		Number of hybrids after 1 year in the field			Hybrids per flower (no.)	Plants not hybrid (no.)
							Vigorous	Medium vigor	Weak		
2007											
06-730	8	426	50.0	140	4.3	3.5	51	34	31	0.27	3
06-753	8	1619	33.7	130	8.4	5.4	82	88	164	0.21	12
06-754	4	347	14.7	34	2.9	2.3	2	7	9	0.05	0
2008											
06-730	12	2805					167	159	266	0.21	61
06-753	2	461					14	13	10	0.08	0
06-754	5	1157					30	10	11	0.04	14
06-748	1	110					0	0	0	0.00	0
BAQ 3-11	3	981					12	12	9	0.03	10
BAQ 9	2	441					0	2	17	0.04	0
06-734	5	962					13	16	38	0.07	9
06-765-2x ^y	1	117					0	0	0	0.00	0
06-772-2x	1	202					0	0	0	0.00	0
06-776-2x	1	181					0	0	0	0.00	0
2009											
06-730	4	762	42.9	40	6.0	4.4	16	14	25	0.07	59
06-748	3	762	34.6	40	3.4	2.6	16	10	7	0.04	43
BAQ 3-11	4	616	10.9	62	0.8	1.0	0	0	0	0.00	0
BAQ 9	1	44	100.0 ^x	20	0.0	0.0	0	0	0	0.00	0
07-544	8	2272	17.8	70	9.8	6.8	102	37	34	0.08	36
07-505	2	344	6.4	20	5.4	3.7	19	8	11	0.11	4
07-131	1	479	14.8	20	3.2	1.7	0	0	0	0.00	37
07-149	3	688	17.2	38	1.0	1.5	1	1	1	0.00	46
08-478	7	2692	6.0	51	3.4	5.5	0	0	0	0.00	74

^zEach cross had a different tetraploid highbush clone as seed parent.

^yClones marked -2x were known diploids.

^xThe female parent produced many parthenocarpic berries.

pollen from *V. arboreum* plants grown from colchicine-treated seed. Because similar crosses using diploid *V. arboreum* had repeatedly failed to produce hybrids, it appears that the triploid block is an important barrier to crossing these species. However, our most successful *V. arboreum* parent gave an average of only 0.27 hybrid seedlings per highbush flower pollinated. By comparison, highbush × highbush crosses in our greenhouse typically average 10 to 20 seedlings per pollinated flower. Most of the 12 *V. arboreum* plants used as pollen sources appeared to have high pollen fertility. Of the 11 clones for which data were recorded, three had ≈50% well-developed microspores and the other eight had more than 90%. The low rate of hybrid production does not

appear to be the result of low fertility in the *V. arboreum* parents. *Vaccinium arboreum* and *Vaccinium* section *Cyanococcus* apparently have diverged enough genetically to make crosses difficult even when chromosome numbers are equal. Five of the tetraploid *V. arboreum* parent plants were planted together in a garden plot without other *V. arboreum* plants nearby. Four plants flowered heavily and produced berries in 2010. One produced numerous berries, which had a median of nine well-developed seeds per berry, about the same as for diploid *V. arboreum* berries collected from the woods at the same time (Lyrene, unpublished data). The other three putative tetraploids produced numerous berries but had median seed-per-berry counts of only one, three, and four, respectively.

Both predictive models and experimental evidence indicate that, during the differentiation of two species from a common ancestor, crossing barriers arise when alleles evolve that work well within each species but interact destructively when the species are crossed (Matute et al., 2010; Moyle and Nakazato, 2010). As two species diverge over time, there is an increase in the number of loci bearing alleles that give unfavorable interactions when the two species are crossed. Many of the loci involved in cross-incompatibility are polymorphic for alleles that differ in their effects in hybrids (Kermicle and Evans, 2010). High heterozygosity in the parents we used and allelic polymorphism at many loci in each

parent species probably explains why we were able to obtain some vigorous hybrids although most pollinated flowers gave nothing. The same considerations can explain the high variability observed in vigor and fertility of the F₁ hybrids.

The F₁ highbush × *V. arboreum* hybrids varied greatly in male fertility. Some plants produced no pollen. Others shed pollen abundantly but with most of the pollen obviously aborted when viewed at 250×. Still other plants produced abundant pollen with a high percentage of the microspores apparently well developed. Surprisingly, one F₁ hybrid that seemed to be totally male-sterile based on microscopic examination of squashed anthers produced a full crop of highly seedy berries when placed outside the greenhouse with various highbush and *V. arboreum* plants. Thus, male sterility did not always indicate female sterility in these hybrids.

The F₁ hybrids selected for use in backcrosses (a sample biased toward higher fertility) were far more fertile in crosses to highbush than were any of the *V. arboreum* parents. Large numbers of backcross (to highbush) seedlings can easily be obtained using the F₁ hybrids as either the male or female parent. The rates of production of backcross seedlings shown in Table 2 are minimum estimates for these crosses, because crossing conditions in the greenhouse were poor when the crosses were being made. The F₁ hybrid plants flowered very late, when the greenhouse was hot, and flower thrips (*Frankliniella* spp.) were visibly damaging many of the flowers. Very late flowering was characteristic of the F₁ hybrids, even when both the *V. arboreum* and the highbush parents had low chilling requirements. Very late flowering in *V. arboreum* was a major obstacle in producing the F₁ hybrids, and late flowering seems to have come through strongly in the hybrids.

In many years of observing large populations of wild blueberry plants in the southeastern United States, I have never seen a plant that looked like a hybrid between *V. arboreum* and any other species. In north Florida, *V. arboreum* frequently occurs in association with *V. darrowii* and *V. elliottii* Chapman, both of which are diploid species in section *Cyanococcus*. The absence or infrequency of hybridization is undoubtedly attributable in part to the late flowering of *V. arboreum* but probably has additional genetic components.

With considerable effort, vigorous, fertile tetraploid hybrids and backcrosses can be made between cultivated highbush blueberry and tetraploid *V. arboreum*. Production of useful cultivars from this introgression will require much additional work. However, many potential benefits could come from *V. arboreum* introgression, including plants with increased drought tolerance, tolerance to higher soil pH, late flowering, late ripening, upright canes, long peduncles and pedicels, and loose berry clusters. Segregating generations from these intersectional hybrids are expected to provide many opportunities for selecting novel plant types.



Fig. 1. Foliage and maturing fruit of an F₁ hybrid (highbush cultivar × *Vaccinium arboreum*) after flowers were pollinated with pollen from a highbush cultivar.

Table 2. Results of backcrossing selected F₁ (highbush × *Vaccinium arboreum*) hybrids to highbush in 2009.

Highbush as female parent					
Parents of cross		Flowers pollinated (no.)	Fruit set (%)	Seedlings (no.)	Seedlings per flower (no.)
Highbush	F ₁ (highbush × <i>V. arboreum</i>)				
Windsor	08-445	66	50.0	120	0.6
Sweetcrisp	08-447	293	46.8	250	0.9
Farthing	08-447	131	67.2	120	0.9
05-613	08-447	91	42.9	100	1.1
Windsor	08-444	139	53.2	200	1.4
00-116	08-444	43	65.1	150	3.5
05-264	08-444	56	82.1	200	3.6
03-291	08-454	149	50.3	200	1.3
06-600	08-454	251	43.4	300	1.2
Southern Belle	08-467	325	71.4	300	0.9
Abundance	08-467	274	42.3	400	1.5
Farthing	08-467	172	63.0	300	1.7
08-14	08-467	213	52.1	300	1.4
Farthing	08-461	146	64.4	200	1.4
05-323	08-461	187	22.5	100	0.5
Southern Belle	08-479	122	27.9	50	0.4
Total of all crosses		2658		3290	1.2

F ₁ (highbush × <i>Vaccinium arboreum</i>) as female parent					
F ₁ (highbush × <i>V. arboreum</i>)	Highbush parent	Flowers pollinated (no.)	Fruit set (%)	Seedlings (no.)	Seedlings per flower (no.)
08-479	01-233	84	22.6	40	0.5
08-464	00-211	33	6.1	15	0.5
08-443	98-325	51	19.6	60	1.2
08-442	98-325	104	6.7	40	0.4
08-447	01-233	192	65.6	150	0.8
08-488	Sweetcrisp	64	35.9	100	1.6
08-483	Sweetcrisp	44	47.4	30	0.7
08-467	Abundance	76	34.2	150	2.0
08-470	01-233	118	15.3	35	0.3
Total of all crosses		766		620	0.8

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