

Breeding Cultivars from Blueberry × Deerberry Hybrids: Progress and Prospects

Paul M. Lyrene

Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Additional index words. blueberry breeding, intersectional hybrids, introgression, tetraploid highbush blueberries, *Vaccinium stamineum*, wide hybridization

Abstract. *Vaccinium stamineum* (deerberry) is a highly variable diploid species in section *Polycodium*. Deerberry is native on excessively drained sandy soils from southeastern Ontario, south through the Florida peninsula to Lake Okechobee, west to eastern Texas and southeastern Kansas. The *V. stamineum* used in this study were tall plants (2–4 m) native in north Florida, with a plant architecture similar to rabbiteye blueberry (*V. virgatum*). Starting in 2013 with crosses between tetraploid highbush cultivars (section *Cyanococcus*) and colchicine-doubled *V. stamineum*, hundreds of F₁ and thousands of later-generation seedlings were grown and evaluated in high-density field nurseries at Citra in North Florida. The populations studied included F₁, F₂, backcrosses to each parent species, and BC₁ × BC₁ seedlings. The goal of the study was to assess the feasibility of introgressing into highbush blueberry cultivars desirable traits from *V. stamineum* (drought tolerance, red-flesh berries, new flavor components, open flowers with short corolla cups and exerted anthers and stigmas) without introducing horticulturally problematic characteristics (bitter skin, berries that shatter when ripe, difficult vegetative propagation). Vigor averaged very low in F₁ seedlings, higher in F₂ seedlings and in seedlings from backcrosses to *V. stamineum*, and highest in seedlings from backcrosses to highbush. Most crosses yielded numerous plump seeds, but crosses to produce F₁ hybrids yielded fewer than 10% as many seeds as highbush × highbush crosses. Most vegetative, flower, and fruit traits that differentiate highbush from *V. stamineum* were intermediate in F₁ seedlings. Backcross seedlings more closely resembled the recurrent parent. Variability in morphological characters was high in every generation, giving much opportunity for selection. Some seedlings from backcrosses to highbush (≈5%) appeared to have the vigor, berry quality, and yield potential required in commercial cultivars. Producing highbush cultivars that strongly express a particular *V. stamineum* trait might best be accomplished by growing large, segregating F₂ populations from which parents for backcrosses can be selected.

Cultivated highbush blueberries are clones that were developed by crosses involving *Vaccinium* section *Cyanococcus* species. Section *Cyanococcus* includes northern and southern highbush cultivars (complex hybrids based on *V. corymbosum* and related *Cyanococcus* species), lowbush (*V. angustifolium*), rabbiteye (*V. virgatum*), and various undomesticated diploid, tetraploid, and hexaploid species native in eastern North America.

The genus *Vaccinium* has wide distribution, with numerous native species in the tropical highlands of both the Old and New World. Vander Kloet (1997) divided the genus into 35 sections. Several sections, including *Cyanococcus*, include diploid, tetra-

ploid, and hexaploid species. Within sections, homoploid crosses are usually successful and produce vigorous, fertile hybrids. There is a strong triploid block, and intrasectional crosses between diploid and tetraploid species normally fail or give only a few seedlings. These may be triploid or tetraploid, with the tetraploids being the result of unreduced gametes from the diploid parent.

A few intersectional *Vaccinium* hybrids have been discovered or created. The most widely studied natural intersectional hybrid {[*V. intermedium* = *V. myrtilloides* (section *Myrtilloides*)] × *V. vitis-idaea* (section *Vitis-idaea*)} was first reported from northern Germany in 1826 (Ritchie, 1955). *V. intermedium* and both parent species are diploid. The hybrid, which has arisen at various times and places in Europe, is vigorous, sometimes even heterotic, and partially fertile (Hiirsalmi, 1977; Rousi, 1966). By contrast, most experimentally produced diploid intersectional *Vaccinium* hybrids have been weak and sterile.

At the tetraploid level, intersectional *Vaccinium* hybrids can be fertile. The most widely studied has been the cross between

the highbush blueberry cultivar Rancocas and tetraploid *V. uliginosum* (section *Vaccinium*; Rousi, 1963). The hybrids were fertile enough to allow the production of thousands of seedlings in backcrosses to tetraploid highbush. Some, but not all, tetraploid F₁ hybrids between highbush cultivars and colchicine-produced *V. arboreum* (section *Batodendron*) were both male and female fertile (Lyrene, 2013). Tsuda et al. (2013) obtained fertile hybrids by crossing colchicine-produced tetraploids of the Japanese native blueberry (*V. bracteatum*) with the highbush blueberry cultivar Spartan. Ehlenfeldt and Polashock (2014) reported fertile hybrids between highbush cultivars and tetraploid *V. padifolium* (section *Hemimyrtillus*). A self-fertile F₁ hybrid between *V. corymbodendron* (section *Pyxothamnus*) and diploid *V. vitis-idaea* (section *Vitis-idaea*) was reported by Ehlenfeldt et al. (2018).

Vaccinium stamineum (deerberry) is the only species in *Vaccinium* section *Polycodium*, but it is highly polymorphic. Deerberries are endemic in North America and are found from southwestern Ontario to central Florida and west to eastern Texas (Vander Kloet, 1988). As noted by Vander Kloet (1988), Ballinger et al. (1979) found that deerberries resemble cranberries (*V. macrocarpon* section *Oxycoccus*) more than blueberries (section *Cyanococcus*), not only in fresh-market and culinary qualities, but also in the types of anthocyanins and flavanols the berries contain. Deerberry plants in Florida are capable of maturing full crops of fruit in the forest, even under xeric conditions (Sharpe and Sherman, 1971). Ballington (1995) carefully studied large populations of *V. stamineum* in North Carolina and South Carolina, and concluded that deerberries had a high potential for domestication as a new crop.

Intersectional hybrids involving sections *Cyanococcus* and *Polycodium* have been reported previously. Coville produced viable diploid hybrids between *V. stamineum* and the diploid *Cyanococcus* species *V. myrtilloides* (Darrow and Camp, 1945). Uttal (1987) reported a natural hybrid between *V. pallidum* and *V. stamineum*. Several F₂ hybrids from *V. darrowii* × *V. stamineum* yielded tetraploid seedlings when crossed to tetraploid highbush in North Carolina (Ballington et al., 1997).

Tetraploid hybrids between highbush blueberry cultivars and colchicine-induced tetraploid *V. stamineum* have been reported previously (Lyrene, 2016, 2018). This article describes further progress in our efforts to recombine the genes of highbush cultivars and *V. stamineum*. The results of additional crosses involving F₁, F₂, and backcross parents are given, along with a description of the phenotype of hybrid and backcross populations after several years in the field. Prospects for developing cultivars with novel characteristics, such as red flesh, adaptation to droughty soils, flowers that are accessible to a wider range of pollinating insects, and exotic new flavors, are discussed in light of

Received for publication 3 Dec. 2020. Accepted for publication 15 Jan. 2021.

Published online 16 February 2021.

P.M.L. is the corresponding author. E-mail: Lyrene@ufl.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

what has been seen in hybrid and backcross populations.

Materials and Methods

Crossing and greenhouse procedures. All crosses were made in a bee-proof greenhouse between 2013 and 2020 using potted plants. The crosses were made to study the fertility and crossing behavior of various parental combinations and to produce seedlings for evaluation in the field. BC₁ seedlings in this study were of two types, depending on whether the recurrent parent was highbush or tetraploid *V. stamineum*. Unless otherwise specified, BC₁ hereafter refers to seedlings in which highbush was the recurrent parent, because they were most numerous and most extensively studied. Except in cases when the stigmas were far exerted from the corolla (e.g., in *V. stamineum*, some F₁ hybrids, and BC₁ seedlings when *V. stamineum* was the recurrent parent), the flowers on the seed parent were emasculated before anthesis; flowers not used in crosses were removed as they opened. Pollen was transferred to the stigmas by thumbnail. The F₁ plants used in crosses were selected from high-density field nurseries after examination throughout the year to make sure they were valid hybrids. Their identity as valid intersectional hybrids was confirmed by flower and inflorescence morphology when they flowered in the greenhouse (data not shown). As would be expected from intersectional hybrids, the hybrids were easily distinguishable from seedlings of the parental species after they flowered and fruited.

Unilateral cross compatibility has been reported in *Vaccinium* (Hiirsalmi, 1977; Wenslaff and Lyrene, 2003; Zeldin and McCown, 1997). Before 2019, all of our F₁ hybrids were produced using *V. stamineum* as the pollen parent. In 2019 and 2020, some crosses were made using tetraploid *V. stamineum* as the seed parent, and highbush cultivars or F₁ hybrids as pollen parents (Table 1). The reciprocal crosses were attempted to determine whether they would give seed and whether the seedling phenotype would differ, depending on the direction in which the cross was made.

Most crosses in which *V. stamineum* was the seed parent were made using second-generation tetraploid *V. stamineum* seedlings. These were produced by intercrossing tetraploid *V. stamineum* plants produced using colchicine. The advantages of using second-generation tetraploids were that they could easily be produced in large numbers and they avoided sectorial chimeras (some branches diploid, others tetraploid), which had been a problem with first-generation colchicine tetraploids (Lyrene, 2016). The *V. stamineum* plants used in the 2019 and 2020 crosses were confirmed as tetraploids based on pollen tetrad size. Both the flowers and the pollen tetrads from tetraploid plants were substantially larger than those from diploids (e.g., pollen tetrad diameters, typically 60 vs. 40 nm).

An experiment was conducted in 2020 to test the possibility that selfing might have occurred while highbush pollen was being applied to the stigmas of unemasculated *V. stamineum* flowers. The experiment was conducted because a surprisingly large number of seeds had been obtained in 2019 by pollinating tetraploid *V. stamineum* stigmas with highbush or F₁ pollen. This raised the question of whether some second-generation tetraploid *V. stamineum* plants might be self-compatible, producing selfed seedlings rather than hybrids after highbush pollen was placed on the stigmas of unemasculated flowers. *V. stamineum* stigmas extend far beyond the anthers and the corolla, and highbush pollen was applied easily without emasculation. Although care was taken to avoid selfing while applying highbush pollen, some self-pollination could have occurred if the seed parents were self-compatible. Diploid *V. stamineum* has been considered highly self-incompatible (Cane et al., 1985), but tetraploids have never been tested.

In the 2020 experiment, branches on each of seven tetraploid *V. stamineum* plants were divided into two groups. Flowers on some branches were pollinated with an abundance of self pollen, and those on others with an abundance of highbush pollen. None of the flowers was emasculated. Most of the flowers were pollinated multiple times over a period of a week or more.

Five crosses of a new type were attempted in 2020. Flowers of tetraploid *V. stamineum* were pollinated with pollen from tetraploid *Vaccinium arboreum* (section *Batodendron*). Like *V. stamineum*, *V. arboreum* (sparkleberry) is a diploid blueberry native in Florida and throughout the southeastern United States. Plants of the two species sometimes occur together in the forest, but *V. stamineum* normally flowers earlier and occurs on drier sites. Pollen used in these crosses was a composite from three colchicine-produced tetraploid *V. arboreum* plants that had been planted in a garden between 2005 and 2010 (Lyrene, 2011). The plants had previously been determined to be tetraploid by pollen size and stomate size, and by the production of large numbers of seedlings when crossed as pollen parent to tetraploid highbush cultivars (Lyrene, 2011). The branches from which the pollen was taken in 2020 were verified as tetraploid based on pollen size a few days before pollen was collected for use in crosses. *V. stamineum* flowers were pollinated over a period of 6 weeks starting in early Mar. 2020. Fresh *V. arboreum* pollen was collected from the garden for use each week.

Data gathered from the greenhouse. The number of flowers pollinated and the number of plump seeds obtained from each cross in 2019 and 2020 were recorded (Table 1). The first 10 berries that ripened from each cross were opened individually and the plump seeds in each berry were counted.

Various plant and berry characteristics were observed in the greenhouse on plants used as seed parents. Observed characteris-

tics included flower and inflorescence morphology, plant vigor, berry size, berry color (wax, skin, and flesh), picking scar, stemminess, firmness, flavor, and tendency for berries to shatter when ripe.

Seed germination and planting of the field nurseries. The seeds from each cross were planted on the surface of peat in 4-L pots in a greenhouse in November of the year the cross was made. For crosses that produced large numbers of seed, only enough seeds were planted to provide at least 120 seedlings. During January and February (2–3 months after sowing the seed), young seedlings were transplanted to trays of peat. For crosses that produced numerous seedlings, no more than 120 seedlings were saved per cross. The seedlings were grown in the greenhouse for ≈3 months.

Planting and evaluation of seedlings in the field nurseries. In May of each year, from 2015 through 2020, seedlings from the previous year's crosses were transplanted to a high-density field evaluation nursery at the University of Florida Plant Science Unit in Citra, FL (Table 2). The nurseries were prepared by mixing a 3-cm layer of pine bark chips into the top 20 cm of the sandy soil. Seedlings were spaced ≈20 cm apart in rows that were ≈12 m long, with 45 cm between rows. The nurseries were irrigated with overhead irrigation and fertilized to maximize the growth of highbush blueberry seedlings planted in the same nursery. About 10,000 highbush and 400 *V. stamineum* seedlings were planted along with the hybrids in each of the nurseries.

Each nursery was maintained for 4.3 years, allowing the seedlings in the older nurseries to be observed through four fruiting seasons. The nurseries were observed weekly throughout the year, and more frequently during the months of flowering and fruiting. The goal was to assess vigor, fruitfulness, and other characteristics of each type of seedling. During November, December, and January of each year, selected plants were dug, potted, and moved to the greenhouse for use as parents. Night-time temperature in the greenhouse was kept above 5 °C, but was otherwise allowed to vary with weather conditions, and fell to less than 10 °C on many nights in December, January, and February. It was discovered that many F₁ plants that had no visible flower buds in the field nursery in early November and appeared to be too small and weak to flower the next spring, would flower if dug and potted in early November, chilled at 5 °C for 6 weeks in a dark cooler, and then moved to the greenhouse in late December. On most plants, flowers, sometimes numerous, were evident on the new leafy shoots that appeared from January through March.

Despite the close spacing of plants in the evaluation nurseries, vigor of F₁ hybrids and BC₁ hybrids in which *V. stamineum* was the recurrent parent was so low that plants had enough space to grow and develop during 4 years in the nursery. F₁ populations were

Table 1. Number of plump seeds produced by various types of controlled crosses in a greenhouse in 2019 and 2020.

Type of cross	No. of crosses	Total flowers pollinated	Total plump seeds	Avg seeds per pollinated flower	Seeds per berry (first 10 ripe)	
					Mean	SD [†]
2019 Crosses						
4xST × 4xST ^z	5	1,130	4,696	4.16	12.4	5.76
4xST × HB ^y	3	800	2,630	3.29	6.5	0.35
4xST × F ₁ ^x	2	550	1,400	2.55	5.7	1.82
HB × 4xST	22	5,100	4,086	0.80	5.5	3.21
F ₁ × F ₁	7	960	2,290	2.39	8.9	6.08
F ₁ × HB	6 ^w	1,260	13,000	10.32	15.4	8.14
BC _{1S} ^v × HB	4	280	579	2.07	7.6	2.39
2020 Crosses						
4xST × 4xST	2	300	1,606	5.35	11.2	0.19
4xST × HB	15	2,200	2,829	1.29	3.5	1.49
4xST self-pollinated	9	900	1,779	1.98	4.1	0.72
4xST × 4xAR ^u	5	900	1,663	1.85	4.4	1.44
F ₁ × F ₁	3	450	2,948	6.55	15.8	2.41
F ₁ × HB	17 ^w	2,000	10,211	5.11	12.5	8.40
BC _{1S} × HB	3	300	192	0.64	5.3	4.35

^zTetraploid *V. stamineum*.

^yTetraploid highbush cultivar.

^xTetraploid hybrid from highbush cultivar × tetraploid *V. stamineum*.

^wIn some of these crosses, HB was the seed parent; in others, the F₁ was the seed parent. In all other crosses, the seed parent is stated first and the pollen parent second.

^vBackcross seedlings produced by pollinating F₁ hybrids with pollen from tetraploid *V. stamineum*.

^uTetraploid *V. arboreum* from colchicine treatment of seed.

[†]Sample SD.

Table 2. Number of seedlings and number of crosses (in parentheses) for various types of highbush-*V. stamineum* hybrids planted in high-density field evaluation nurseries from 2014 to 2020.

Type of cross	2014	2015	2016	2017	2018	2019	2020	Total
2xST ^z × 2xST	99 (2)	0	149 ^p	106 ^p	149 ^p	0	0	503
2xFus ^y × 2xST	82 (2)	0	0	0	0	0	0	82 (2)
2xEll ^x × 2xST	0	0	0	95 (2)	0	0	0	95 (2)
2xDar ^w × 2xST	0	0	0	50 (1)	0	0	0	50 (1)
HB ^y × 2xST	6 (2)	0	0	0	0	0	0	6 (2)
HB × 4xST ^u	457 (11)	1,077 (23)	429 (11)	882 (17)	524 (10)	646 (10)	849 (15)	4,864 (97)
HB × F ₁ ^{x,s}	0	0	2,247 (31)	1,883 (27)	858 (14)	425 (8)	519 (7)	5,932 (87)
F ₁ × F ₁	0	0	169 (6)	659 (7)	204 (3)	890 (12)	582 (8)	2,504 (36)
F ₁ × 4xST	0	0	45 (3)	459 (9)	312 (7)	82 (1)	0	898 (20)
BC _{1H} × BC _{1HF}	0	0	0	0	1,820 (23)	492 ^o	342 ^o	2,654
BC _{1H} × HB	0	0	0	0	74 (1)	37 (1)	88 (1)	199 (3)
BC _{1H} × F ₁	0	0	0	0	0	0	87 (1)	87 (1)
BC _{1S} × BC _{1S^a}	0	0	0	0	91 (1)	0	0	91 (1)
BC _{1S} × HB	0	0	0	0	0	123 (2)	288 (4)	411 (6)
BC _{1S} × F ₁	0	0	0	0	0	0	226 (2)	226 (2)
BC _{1S} × 4xST	0	0	0	0	0	9 (1)	0	9 (1)
4xST × 4xST	67 (1)	119 (3)	351 (6)	108 (2)	234 (3)	223 (3)	614 (8)	1,716 (23)
4xST × HB	0	0	0	0	0	0	195 (3)	195 (3)
4xST × F ₁	0	0	0	0	0	0	176 (2)	176 (2)

^zDiploid *V. stamineum*.

^yDiploid *V. fuscatum* from a forest near Frostproof, FL.

^xDiploid *V. elliotii* from southwestern Alabama.

^wDiploid *V. darrowii* from a forest near Lake Placid, FL.

^vTetraploid highbush cultivar.

^uTetraploid *V. stamineum*.

^sF₁ is the tetraploid hybrid from HB × 4xST.

^aIn some of the crosses on this line, HB was the seed parent; in others, the F₁ was the seed parent. In all other crosses, the seed parent is stated first and the pollen parent second.

^pF₁ × HB.

^qF₁ × 4xST.

^rGrown from open-pollinated seed from the forest.

^oSeeds from open pollination of 30 BC_{1H} plants in an isolation block with no other pollen sources.

examined at the end of the first growing season to remove plants that did not appear to be the intended hybrids. This was necessary because highbush plants that appeared with the hybrids in some populations were so vigorous comparatively that they crowded out nearby F₁ plants after the second year in the nursery if not removed. In BC₁ populations in which highbush was the recurrent

parent, and in BC₁ × BC₁ populations, there was much variation in vigor, and after the first growing season in the field, competition among plants began to eliminate the weaker plants or prevent them from flowering. After the second year in the field nurseries, observations of fruit characteristics could only be made from the strongest, most competitive plants.

Results and Discussion

Seed production from greenhouse crosses

Most of the crosses made in the greenhouse in 2019 and 2020 produced numerous seeds (Table 1). Exceptions were highbush × tetraploid *V. stamineum* crosses and crosses in which one parent was a Backcross-1 of the

Table 3. Production of plump seeds by seven tetraploid *V. stamineum* seedlings after self-pollination and after pollination with pollen from tetraploid highbush cultivars.

<i>V. stamineum</i> female parent	Pollen parent	Flowers pollinated (n)	Fruit set (%)	Seeds per pollinated flower	Seeds per berry (first 10 ripe) ^z	
					Mean	SD ^y
20-519	Selfed	50	18	0.58	4.75	3.96 NS ^w
20-519	12-179 HB ^x	120	8	0.30	3.00	1.76
18-726	Selfed	70	59	1.24	4.70	2.64 ^v
18-726	05-185 HB	100	59	1.07	3.09	1.47
20-518	Selfed	120	69	1.12	3.59	2.26 ^v
20-518	05-185 HB	110	45	0.59	2.12	1.70
18-727	Selfed	70	69	1.36	3.09	2.15 NS ^w
18-727	13-143 HB	300	100	1.97	2.71	1.62
20-501	Selfed	80	69	2.13	4.80	3.69 NS ^w
20-501	Kestrel HB	40	30	1.20	5.60	3.84
20-505	Selfed	200	28	5.36	4.75	1.65 NS ^w
20-505	Emerald HB	200	24	4.40	5.10	1.77
20-513	Selfed	100	64	1.36	3.40	2.28 NS ^w
20-513	13-143 HB	120	78	0.94	2.62	1.69

^zPairwise *t* tests were done to compare mean number of seeds per berry in the first 10 ripe berries when each female was selfed or pollinated with highbush cultivar pollen.

^ySample SD.

^xTetraploid highbush cultivar.

^wThe means did not differ at *P* = 5%.

^vThe means differed at *P* = 5%.

NS = not significant.

type (highbush × *V. stamineum*) × *V. stamineum*. Similar crosses had given low seed numbers in previous years (Lyrene, 2016, 2018; and unpublished).

In the 2020 experiment, which involved seven tetraploid *V. stamineum* plants used as female parents, both self-pollinated flowers and flowers that received highbush pollen produced berries and seeds (Table 3). On most *V. stamineum* females, the number of seeds obtained did not differ significantly for the selfed and crossed branches. This suggests that most of the seedlings from the highbush pollen were true hybrids, not seedlings from self-pollination. Had most of the seedlings from crossed flowers been the result of unintended self-pollination, more seed would have been expected from flowers that were intentionally self-pollinated with an abundance of pollen than from flowers where efforts were made to avoid self-pollination and highbush pollen was applied in abundance. Both the selfed and the intersectional pollinations gave fewer seeds than crosses between tetraploid *V. stamineum* genotypes (Table 1), suggesting that partial self-incompatibility reduced seed production from self-pollinated flowers, and intersectional incompatibility reduced seed production from flowers pollinated with highbush pollen.

After one growing season in the field, most seedlings from the *V. stamineum* × highbush and *V. stamineum* × F₁ crosses made in 2019 had phenotypes consistent with their hybrid origins. Seedlings for which F₁ hybrids had been the pollen parent were more vigorous than those with highbush as the pollen parent. This would not be expected if seedlings from both types of pollinations had resulted from selfing.

Evaluation of horticultural characters in the field nursery

Vigor. Except for having larger flowers, second-generation tetraploid *V. stamineum* seedlings were similar to diploids in vigor

and macroscopic morphology. Both were as vigorous as seedlings from highbush × highbush crosses growing in the same nursery.

Crosses in which a diploid section *Cyanococcus* species (*V. darrowii*, *V. elliotti*, and *V. fuscatum* were tried) was the seed parent and diploid *V. stamineum* was the pollen parent gave numerous hybrid seedlings, but these were invariably very weak. Some of these seedlings probably would have survived to flower had they been potted and placed in a greenhouse, but this was not attempted.

With rare exceptions, F₁ hybrids obtained by crossing tetraploid highbush cultivars with tetraploid *V. stamineum* were far weaker than either parent (Fig. 1). F₁ hybrids varied greatly in vigor depending on the particular highbush and *V. stamineum* plants that were crossed. Sibling seedlings within some crosses were highly variable in vigor, but in other crosses all the seedlings were very weak. About half the F₁ seedlings from the most vigorous half of the crosses could probably have been brought to flowering if potted and grown in a greenhouse. When the strongest F₁ plants were dug, potted, and placed in the greenhouse, most of them increased in vigor but remained less vigorous than highbush seedlings. The weakness of the F₁ hybrids in the high-density field nurseries was probably a result, in part, to excess soil moisture. Overhead irrigation scheduling was adjusted to maximize the growth of highbush seedlings planted in other parts of the same nursery. These were far more vigorous and required much more water than the hybrids. Year after year, F₁ seedlings were much more susceptible to leaf mites (*Eotetranychus clitus*) in the greenhouse than either parent growing in the same greenhouse. To prevent defoliation, it was necessary to control mites. Of the original four tetraploid *V. stamineum* plants used repeatedly in crosses, FL13-1145 consistently



Fig. 1. Seedlings in the 2019 high-density nursery photographed 22 Oct. 2020. The nursery was planted 30 May 2019 using greenhouse-grown seedlings from seed planted in Nov. 2018. The four weakest rows in the center contain F₁ hybrids from highbush × tetraploid *V. stamineum* crosses. The more vigorous rows on the left contain seedlings obtained by intercrossing BC₁ seedlings that had *V. stamineum* as the recurrent parent, and those on the right contain F₂ seedlings from F₁ × F₁ crosses.

produced the most vigorous F₁ seedlings, although nothing about its appearance distinguished it as special.

F₂ seedlings, produced by intercrossing unrelated F₁ hybrids, were much more vigorous than F₁ seedlings (Fig. 1). In part, this was probably because only the strongest F₁ plants were used as parents, but gametic and zygotic selection were also probably important, because only the most functional gametes from the F₁ parents were likely to make zygotes and only the most functional zygotes were likely to make viable seed.

An additional increase in vigor was seen in BC₁ seedlings if highbush was the recurrent parent. Like F₁ seedlings, BC₁ seedlings were highly variable in vigor, both within and between crosses. Only about 10% of the thousands of BC₁ seedlings grown in the field for 3 years or more appeared to be as vigorous as typical highbush seedlings. Poor

leaf health, resulting from diseases and physiological disorders, was one reason for the weakness of many seedlings. Seedlings from BC₁ × BC₁ crosses were no more vigorous than seedlings of the parent generation, and vigor continued to be highly dependent on which particular plants served as parents.

BC₁ seedlings from crosses in which *V. stamineum* was the recurrent parent were less vigorous than those with highbush as the recurrent parent, but they were extremely variable in vigor. About 30% of these seedlings, after three growing seasons in the field, had medium vigor and healthy leaves. The *V. stamineum* backcross seedlings were more vigorous and looked more like *V. stamineum* than the most vigorous F₁ plants. In growth habit and vegetative appearance, the BC₁ seedlings were very distinct from *V. stamineum*. In the field, some of the plants flowered profusely but produced little fruit. This may have been the result of low male or female fertility and the fact that they flowered late, when little pollen was available from other tetraploid blueberries growing nearby. Several of the BC₁-to-*V. stamineum* seedlings produced defective flowers in which the styles were missing or poorly developed.

In the 2019 greenhouse, four *V. stamineum* backcross seedlings that were pollinated with highbush pollen produced berries of the expected phenotype (more like deerberries than blueberries) and made a considerable amount of seed (Table 1). Seedlings from these four crosses were substantially more vigorous than most highbush × *V. stamineum* F₁ hybrids, and their leaves had a healthier green color.

Plant architecture. The deerberry plants used as parents resembled highbush blueberries in plant architecture (crown forming, with 2–10 major canes arising from a small base, reaching a height of 2–3 m after several years in field nurseries). Except for low vigor, the bush structure of the F₁ hybrids resembled the parents. Seedlings from backcrosses to highbush generally resembled highbush in architecture. After 3 years in the field, a few F₂ and backcross seedlings (especially those with *V. stamineum* as the recurrent parent) produced upright canes bearing large numbers of short, stiff side branches oriented perpendicular to the upright canes. These seemed analogous to fruiting spurs in apricots, almonds, apples, and pears. Branches of this kind are rare in highbush and not common in deerberry. The apical 65 cm of one vigorous, upright cane on a BC₁ plant examined in November bore 50 spurlike branches (Fig. 2). These spurs were stiff, woody, and 2 to 6 cm long. Each had four to six nodes, with a bud at each node. The previous May, several canes on the plant shown in Fig. 2 had produced clusters of ripe berries extending basipetally from the apex for 40 to 60 cm on the major canes, giving the appearance of cattail (*Typha*) spikes.

Fertility. Most tetraploid *V. stamineum* plants were highly fertile, whether they had been produced by colchicine treatment or by intercrossing colchicine-derived tetraploids.

An exception was FL13-872, which was widely used as a pollen parent because it was one of the first tetraploids available, in which only about half of the microspores appeared to be normal under the microscope at ×250 magnification. F₁ hybrids varied widely in fertility (Lyrene, 2016). The most vigorous seedlings obtained by backcrossing F₁ hybrids to highbush were typically very fruitful and produced berries with numerous seeds when open-pollinated in the field. An open-pollinated polycross involving 25 BC₁ plants in 15-L pots placed outside of the greenhouse, in which the only pollen source was other BC₁ hybrids, produced an abundance of berries, and these contained numerous well-developed seeds. The increased fertility in the BC₁ population compared with the F₁ was probably a result, in part, of the use of the most fertile F₁ plants as parents and, in part, to gametic and zygotic selection for increased fertility.

Ease of vegetative propagation. Highbush blueberry cultivars are clones, propagated by stem cuttings or in vitro. *V. stamineum* of the type used in this study (2–4 m tall with a few stout canes) is nearly impossible to propagate from stem cuttings except using highly juvenile cuttings. Juvenile cuttings are not available from plants that have been selected for superior fruit. Juvenile shoots suitable for rooting in a mist propagation bed can be produced in vitro from mature plants of *V. stamineum* (Lubell and Brand, 2018; A. Garcia, personal communication), but efficient methods for producing field-ready plants from *V. stamineum* microcuttings need to be developed. Nonjuvenile softwood cuttings of most F₁ hybrids rooted readily under mist, but cuttings of others did not (Lyrene, 2016). Cuttings from some hybrids fail to root because they defoliate quickly when placed under mist, but cuttings from other hybrids maintain healthy leaves for several months under mist but do not form roots. Some seedlings from backcrosses to highbush are also slow to root from stem cuttings.

Ripening date. The highbush cultivars used in the crosses ripen from 10 Apr. through 15 May when grown in the field in North Florida. Berries on the diploid *V. stamineum* plants from which the tetraploids

were derived ripen in the forest in North Florida from late June through late July. BC₁ seedlings with highbush as the recurrent parent typically ripened 3 to 4 weeks later than highbush cultivars when the two were grown together in field plots at the research station at Citra, FL, but there was much variation among the BC₁ seedlings. BC₁ hybrids that have a low-chill requirement but late flowering and ripening might be used to extend the blueberry harvest season in low-chill production areas. In Florida, late flowering decreases losses from freezes but increases problems with flower thrips, which often destroy late flowers and prevent pollination.

Inflorescence, raceme, and flower structure. Highbush flowers are borne on short peduncles that do not persist after the berries are harvested. Flowers and berries on some cultivars are tightly clustered. Tight berry clusters can become a problem as berry size is increased through breeding. In *V. stamineum*, both the pedicels and peduncles are much longer, with much more space between the berries. In F₁ and BC₁ hybrids, peduncle and pedicel length range widely between the parental types, but the racemes of most hybrids are more open than in highbush (Figs. 3–5).

Flowers of blueberry species in section *Cyanococcus* are adapted to buzz pollination by native bees such as *Bombus* and



Fig. 3. BC₁ seedlings with inflorescence structure intermediate between that of highbush and *V. stamineum*. Peduncles elongate well before the flowers open. Photographed 22 Oct. 2020.

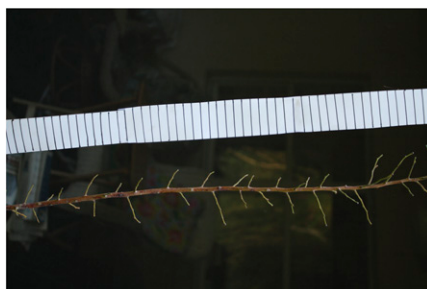


Fig. 2. Spurlike branches on an upright cane of a BC₁ seedling (highbush the recurrent parent) transplanted to the field nursery in May 2017. Photographed 4 Nov. 2020. The scale is in centimeters.



Fig. 4. Clusters of berries on an F₁ hybrid in the greenhouse showing long pedicels and peduncles. The flowers had been pollinated with pollen from a highbush cultivar. The plant had been defoliated by mites during berry development.



Fig. 5. Loose clusters of berries on an F₁ hybrid in the greenhouse after the flowers had been pollinated with pollen from a highbush cultivar.

Habropoda. Achieving adequate pollination using honeybees has been a problem for growers in commercial blueberry plantations in Florida and Georgia. Flowers of most highbush cultivars have a long corolla tube, which has a constricted aperture and anthers located well inside the tube. By contrast, in *V. stamineum*, the corolla cup is short, with a wide opening, and both the stigma and anthers extend well beyond the end of the corolla cup (Cane et al., 1985). The pollen and stigmas are readily accessible to honeybees and other nonsonicating pollinators. Flower structure in F₁ and F₂ hybrids was surprisingly variable. All F₁ plants had flower morphology somewhere between that of the parents; but, in some, flowers were more like those of *V. stamineum* and, in others, more like those of highbush. Flowers on both F₁ and BC₁ plants tended to be smaller than on highbush. Flowers of all F₁ plants had anther awns, which are absent in highbush and conspicuous in *V. stamineum*, but these were invariably smaller than in *V. stamineum*. Flower structure remained quite variable in BC₁ (to highbush) hybrids, and plants could be selected in which the pollen is readily accessible to honeybees. In many BC₁ hybrids, the pollen discharge tubes extend all the way to the stigma, and both are near the aperture of the corolla tube. In highbush flowers, the anthers are situated deep inside the corolla tube.

Berry size. Deerberries in the forest are larger than those of any other wild North American *Vaccinium* species (Fig. 6). Mean weight of 100 individually weighed berries selected randomly from each of three heavily loaded *V. stamineum* plants in the forest in Alachua County, FL, on 8 July 2020 were 1.24, 1.37, and 1.15 g, respectively, with SEMs of 0.057, 0.035, and 0.023. Mean berry weight for 23 wild tetraploid highbush blueberry plants (*V. fuscatum*) from Alachua County, FL, ranged from 0.14 to 0.54 g, with an overall mean for the 23 plants of 0.25 g (Lyrene and Sherman, 1980). Berries of the highbush cultivars grown in Florida are large because of 6 to 10 generations of selection for large berries. Berries on the most vigorous and fertile BC₁ plants averaged about as large as berries from random seedlings from highbush × highbush cultivar crosses, indicating that *V. stamineum* introgression had not reduced berry size.



Fig. 6. Internal berry color of freshly sliced mature berries of tetraploid selection FL 15-669 (a highbush cultivar × tetraploid *V. stamineum* F₁ hybrid) and highbush cultivar FL06-96.

Berry color. Ripe berries from highbush cultivars typically have black skin, green to white flesh, and variable amounts of surface wax that gives the berries of some varieties a powdery blue appearance. Mature berries from the wild *V. stamineum* plants used in this study had skin that was black after the surface wax was removed. Because of variations in berry-surface waxes, mature *V. stamineum* berries in the forest ranged from shiny black to powdery blue depending on the plant.

Unlike the section *Cyanococcus* species and the cultivars bred from them, *V. stamineum* is polymorphic for flesh color. About half the plants in the forest from which our parents came had a flesh color of white–green, like that of highbush, and about half had flesh color ranging from light pink through bright red to dark purple. These colors were consistent over years on individual plants, some of which have been observed yearly for 8 years or more. The tetraploid *V. stamineum* plants used in this study were obtained by colchicine treatment of seeds or seedlings with a flesh color that was unknown at the time of colchicine treatment. By chance, two of the first four tetraploid plants used in crosses had pink to red flesh in fully ripe berries.

Some F₁ hybrids had pink to red flesh (Fig. 6; Lyrene, 2016). The F₁ plants used in backcrosses were selected for vigor and fertility, without regard to flesh color. The berry flesh of some BC₁ plants in the field was somewhat pink, but the pink was less intense than in the pigmented F₁ hybrids and only appeared in berries that were fully mature. Mature berries from numerous BC₁ plants were compared with those from numerous highbush seedlings growing in the same nursery. Pink flesh was much more common among seedlings in the BC₁ populations. None of the BC₁ plants had enough pigment to be considered commercially purple fleshed. A project to obtain highbush-type cultivars with deep-red flesh should start with the selection of *V. stamineum* parents having the most intense purple flesh, because the character is extremely variable in *V. stamineum*.

When extracting seeds from crosses, it was noticed that a 50:50 (by volume) mix of water and fresh berries homogenized in a



Fig. 7. Large berries from a diploid plant of *V. stamineum* (clone FL15-695) harvested from a forest in Alachua County, FL, July 2015. Pedicels remain attached to the berries when they abscise from the peduncle. The scale is in centimeters.

food blender produced a puree that was normally much brighter red with F₁ and BC₁ berries than with highbush berries. When berries from wild diploid *V. stamineum* plants were processed in the same way, the puree was bright red, even if the berries had white–green flesh. These bright colors were stable for more than a week at room temperature. The difference between highbush and *V. stamineum* puree pigment intensity may be a result of the thicker skin of *V. stamineum* berries or a greater pigment concentration in the skin.

Berry scar, stemminess, and shattering. When highbush blueberries ripen, an abscission zone forms between the berry and the pedicel, and harvested berries are free of pedicels (i.e., they are not stemmy). In *V. stamineum*, the abscission zone is at the opposite end of the pedicel, and the berries come off the bush with the pedicel attached (Fig. 7). The F₁ hybrids tended to be intermediate, with much variation from plant to plant. Most BC₁ plants resembled highbush in stem separation, and the berries were mostly not stemmy. In wild highbush and in some of the earlier released cultivars, the skin tended to tear when the berry was separated from the pedicel. These cultivars were said to have a bad scar. In *V. stamineum*, the pedicel is long and very thin compared with highbush. When the pedicels are forcibly removed from the berries, only a small scar is formed. The scar on most BC₁ plants was small and dry.

The diploid *V. stamineum* population from which the parents came was polymorphic for berry shattering. Some plants that seemed to be loaded with berries at the start of the ripening season never held a large number of ripe berries, because the berries fell as they ripened. On only 20% to 30% of the plants were most of the ripe berries retained long enough on the bush so the plant appeared, at some time, to be loaded with ripe fruit. Berry shattering was highly variable among both the first- and second-generation tetraploid *V. stamineum* plants. Most F₁ and F₂ seedlings and nearly all backcross seedlings held their berries well, but shattering was a problem with a few.

Flavor. Four aspects seemed variable in F₁ populations: sugar, acidity, bitterness, and the presence of aromatic or spicy flavors not found in section *Cyanococcus* species. Deerberries in the source population had high Brix. Ten plants growing in the forest whose average Brix was determined using a hand refractometer on 16 July 2020 (stated as the average of three single-berry samples per plant), and ranged from 15.0 to 22.0 °Brix. Typical values for cultivated highbush range from 13 to 16 °Brix (DeGraw et al., 2016). Acidity was not measured but, by taste, ranged from low to medium compared with the average for highbush cultivars. Berries of *V. stamineum* are sometimes stated in the literature to be inedible or barely edible because of their astringency or bitterness. In our deerberry source population, berries never seemed astringent, but ranged from slightly bitter to strongly bitter. Bitterness seemed to be concentrated in the skin and was less noticeable in the flesh. Bitterness was not detected in the berries of any BC₁ plants in which highbush was the recurrent parent. The berries of some F₂ and BC₁ plants had pleasant aromatic or spicy flavors not found in section *Cyanococcus* species. Deerberries from the forest, when dried at room temperature and stored for 3 months without refrigeration, were sweet, somewhat bitter, and had a flavor component suggestive of pine.

Texture. *V. stamineum* seeds are slightly larger than those of highbush, but the berries are large and juicy and do not seem more gritty than highbush berries. Deerberries have thick skin. This gives the berries a pleasant “pop” or crunch when eaten, but the skin residues that persist during chewing sometimes elicit complaints. Thick skin did not seem problematic in the BC₁ plants.

Potential postharvest qualities. No formal studies were done, but deerberries held in paper bags at room temperature for 10 d seemed to stay firm without shriveling or becoming moldy just as well as the best highbush cultivars. Berries collected from the ground beneath the plants in the forest remain firm for a week or more after they fall.

Difficulties encountered when trying to derive cultivars from highbush × *V. stamineum* hybrid populations

Obtaining plants that meet the collective requirements of growers, marketers, and consumers starting with weak intersectional hybrids will not be easy. It was not hard to get hybrid seedlings by crossing highbush cultivars with tetraploid *V. stamineum* plants. Many (but not all) of the tetraploid F₁ hybrids had surprisingly high fertility and fruitfulness, and, in some cases, high berry quality, but nearly all of them were weak, and many were susceptible to disease, insect, and physiological problems. In September, October, and December, when highbush cultivars often make their best growth in Florida, *V. stamineum* plants in the forest are often losing their leaves. In October, in high-density nurseries, the foliage on many (but not all) F₂ and BC₁ seedlings appeared less healthy than

on highbush seedlings. In the greenhouse, defoliation by leaf mites was much more severe on F₁ blueberry–deerberry hybrids than on the parent taxa.

Fall flowering was a problem with some *V. stamineum*–highbush advanced-generation hybrids. Fall flowering in North Florida usually results in loss of berries resulting from winter freezes, and berries that ripen in early winter can have high acidity and low sugar contents. Many hybrids that lost flowers and berries from winter freezes still produced numerous berries in the spring because of the flower buds that remained dormant until late winter.

A problem in the introgression program has been the occasional appearance of highbush plants and plants of unknown origin among the F₁ seedling populations. Because large numbers of flowers must be pollinated to generate relatively few F₁ hybrids, even a low level of self-pollination can produce a substantial percentage of nonhybrid plants. Even with careful emasculation and removal of all unpollinated flowers from female parents in the greenhouse, some nonhybrid plants appeared in some populations of hybrid seedlings. In the high-density field evaluation nursery, these plants were almost always far more vigorous than the F₁ hybrids. Because of the close spacing of the plants, a single highbush plant could overshadow and kill several true hybrids. It was necessary to identify and remove nonhybrid plants from the nursery before the second growing season. On rare occasions, a plant appeared among the F₁ seedlings that resembled highbush in most aspects, but seemed to have one or more traits that could only have come from *V. stamineum*. These anomalous seedlings are a puzzle, but southern highbush cultivars have a complex genetic background, and it is not always safe to conclude that a particular seedling with surprising characteristics could not be a highbush seedling.

Summary and Outlook

The phenotypic variation observed indicates that commercial cultivars could probably be selected from seedlings in BC₁ or BC₂ populations following highbush × *V. stamineum* crosses. In addition to the characters already discussed, several useful traits might be obtained in highbush cultivars using genes from *V. stamineum*. Improved drought tolerance is likely to be found in the hybrids. Deerberries grow naturally on excessively drained, coarse-textured upland sands; highbush are native on moist flatwoods soils where peat accumulates.

Cultivars with a flower structure that makes the pollen readily accessible to honeybees could be selected. The ability to flower on new spring growth flushes rather than from overwintering flower buds formed the previous autumn might be useful in some environments. Highbush blueberries that lose their leaves prematurely in the fall as a result of insects, leaf diseases, hurricanes, or other causes, form few flower buds, and few flowers open in the spring. During winter,

highbush flower buds can be damaged by freezes, blueberry bud mites, or gall midges. In Florida forests, *V. stamineum* normally begins to defoliate early in the fall as a result of insects, diseases, drought, low soil nutrients, and genetic factors. No flower buds are visible on *V. stamineum* in January, but the plants can still flower heavily 3 months later and produce heavy berry crops on the spring growth flushes. Many of the F₁ hybrids appeared to have inherited the ability to form flowers on spring growth flushes even when no flower buds could be seen in midwinter.

In addition to adaptation to excessively drained mineral soils, open flower structure, and red flesh, *V. stamineum* could be the source of various nutraceutical compounds, such as iridoids. Iridoids are secondary metabolites, present in various plant families, that may exhibit a range of pharmacological activities, such as cardiovascular, hypoglycemic, anti-inflammatory, hepatoprotective, antimutagenic, antispasmodic, antitumoral, antiviral, immunomodulatory, and purgative effects (Viljoen et al., 2012). Plants that produce iridoids have a history of use in traditional medicine in treating inflammatory conditions (Viljoen et al., 2012). Leisner et al. (2017) measured iridoid content in the berries of 84 *Vaccinium* accessions, including many *Cyanococcus* cultivars and several wild species from other sections. Of the 71 *Cyanococcus* cultivars sampled, ‘Ozarkblue’ had the greatest iridoid content measured as monotropein (113 ng·mg⁻¹ dry fruit weight). Of the non-*Cyanococcus* species sampled, *V. stamineum* had the greatest content of monotropein (2371 ng·mg⁻¹ berry dry weight), and *V. arboreum* had 1692 ng·mg⁻¹. If iridoids can contribute to human health, both *V. stamineum* and *V. arboreum* could serve as gene sources for increasing iridoids in highbush cultivars.

In attempting to move single traits, such as red flesh, wide-open flowers, or exotic flavors, from *V. stamineum* into highbush cultivars, growing large F₂ populations might be the key to success. F₂ seedlings are relatively easy to produce in large numbers, and the plants are much more vigorous than F₁ seedlings. F₂ seedlings are vigorous and fertile enough to be evaluated for plant and fruit characteristics in high-density field nurseries. F₂ seedlings are extremely variable for many of the traits that distinguish highbush and *V. stamineum*. This high variability indicates that at least some homoeologous chromosome pairing is occurring in the F₁ hybrids (Ramsey and Ramsey, 2014). To minimize inbreeding and maximize diversity, the F₁ parents should be chosen so that every F₂ seedling has four distinct genotypes as grandparents. Superior F₂ seedlings can be used in backcrosses to produce cultivars.

Literature Cited

Ballinger, W.E., G.J. Galletta, and E.P. Maness. 1979. Anthocyanins of fruits of *Vaccinium* subgenera *Cyanococcus* and *Polycodium*. J. Amer. Soc. Hort. Sci. 104:554–557.

- Ballington, J.R. 1995. The deerberry (*Vaccinium stamineum* L. *Vaccinium* section *Polycodium* (Raf.) Sleumer): A potential new small fruit crop, p. 21–28. In: R.E. Gough and R.F. Korcak (eds.). *Blueberries: A century of research*. Haworth Press, Inc., New York, NY, doi: 10.1300/J065v03n02_03.
- Ballington, J.R., S.D. Rooks, W.O. Cline, J.R. Meyer, and R.D. Milholland. 1997. The North Carolina State University blueberry breeding program: Toward *V. × covilleum*? *Acta Hort.* 446:243–250, doi: 10.17660/ActaHortic.1997.446.36.
- Cane, J.H., G.C. Eickwort, F.R. Wesley, and J. Spielholz. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae). *Amer. J. Bot.* 72:135–142, doi: 10.1002/j.1537-2197.1985.tb05351.x.
- Darrow, G.M. and W.H. Camp. 1945. *Vaccinium* hybrids and the development of new horticultural material. *Bull. Torrey Bot. Club* 72:1–21.
- DeGraw, C., S. Watkinson, and L. DeVetter. 2016. Highbush blueberry cultivar trial in northwestern Washington. *Whatcom Ag Monthly* 5(9). 8 Feb. 2021. <<https://extension.wsu.edu/wam/past-issues-2016/>>.
- Ehlenfeldt, M.K. and J.J. Polashock. 2014. Highly fertile intersectional blueberry hybrids of *Vaccinium padifolium* section *Hemimyrtillus* and *V. corymbosum* section *Cyanococcus*. *J. Amer. Soc. Hort. Sci.* 139:30–38, doi: 10.21273/JASH.139.1.30.
- Ehlenfeldt, M.K., J.J. Polashock, and J.R. Ballington. 2018. *Vaccinium corymbodendron* Dunal as a bridge between taxonomic sections and ploidies in *Vaccinium*: A work in progress. North American Blueberry Research and Extension Workers Conference: 15. 8 Feb. 2021. <<https://digitalcommons.library.umaine.edu/nabrew2018/proceedingpapers/proceedingpapers/15>>.
- Hiirsalmi, H. 1977. Inheritance of characters in hybrids of *Vaccinium uliginosum* and highbush blueberries. *Ann. Agr. Fenn.* 16:7–18.
- Leisner, C.P., M.O. Kamileen, M.E. Conway, S.E. O'Connor, and R. Buell. 2017. Differential iridoid production as revealed by a diversity panel of 84 cultivated and wild blueberry species. *PLoS One*, doi: 10.1371/journal.pone.0179417.
- Lubell, J. and M. Brand. 2018. Softwood cutting propagation and micropropagation of deerberry (*Vaccinium stamineum*). *Native Plants J.* 10:216–223, doi: 10.3368/npj.19.3.216.
- Lyrene, P.M. 2011. First report of *Vaccinium arboreum* hybrids with cultivated highbush blueberry. *HortScience* 46:563–566, doi: 10.21273/HORTSCI.46.4.563.
- Lyrene, P.M. 2013. Fertility and other characteristics of F₁ and backcross₁ progeny from an intersectional blueberry cross (highbush cultivar × *Vaccinium arboreum*) × highbush cultivar. *HortScience* 48:146–149, doi: 10.21273/HORTSCI.48.2.146.
- Lyrene, P.M. 2016. Phenotype and fertility of intersectional hybrids between tetraploid highbush blueberry and colchicine-treated *Vaccinium stamineum*. *HortScience* 51:15–22, doi: 10.21273/HORTSCI.51.1.15.
- Lyrene, P.M. 2018. Hybridization of cultivated highbush blueberry with *Vaccinium stamineum*: Phenotype and fertility of backcross₁ populations. *HortScience* 53:159–166, doi: 10.21273/HORTSCI.12650-17.
- Lyrene, P.M. and W.B. Sherman. 1980. Horticultural characteristics of native *Vaccinium darrowii*, *V. elliottii*, *V. fuscatum*, and *V. myrsinites* in Alachua County, Florida. *J. Amer. Soc. Hort. Sci.* 105:393–396.
- Ramsey, J. and T.S. Ramsey. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Phil. Trans. R. Soc. Britain.* 369: 20130352, doi: 10.1098/rstb.2013.0352.
- Ritchie, J.C. 1955. A natural hybrid in *Vaccinium* I: The structure, performance and chorology of the cross *Vaccinium intermedium* Ruthe. *New Phytol.* 54:49–67, doi: 10.1111/j.1469-8137.1955.tb06157.x.
- Rousi, A. 1963. Hybridization between *Vaccinium uliginosum* and cultivated blueberry. *Ann. Agr. Fenn.* 2:12–18.
- Rousi, A. 1966. Cytological observations on some species and hybrids of *Vaccinium*. *Der Zuechter* 36:352–359, doi: 10.1007/BF00631138.
- Sharpe, R.H. and W.B. Sherman. 1971. Breeding blueberries for low chilling requirement. *Hort-Science* 6:145–147.
- Tsuda, H., H. Kunitake, M. Yamasaki, H. Komatsu, and K. Yoshioka. 2013. Production of intersectional hybrids between colchicine-induced tetraploid Shashanbo (*Vaccinium bracteatum*) and highbush blueberry 'Spartan'. *J. Amer. Soc. Hort. Sci.* 138:317–324, doi: 10.21273/JASHS.138.4.317.
- Uttal, L.J. 1987. The genus *Vaccinium* L. (Ericaceae) in Virginia. *Castanea* 52:231–255.
- Vander Kloet, S.P. 1988. The genus *Vaccinium* in North America. *Pub.* 1828, Agriculture Canada.
- Vander Kloet, S.P. 1997. The nature and variation of dichotomous characters in *Vaccinium* L. *Amer. J. Bot.* 84(6) (Supplement to p. 239) (abstr.).
- Viljoen, A., N. Mncwangi, and I. Vermaak. 2012. Anti-inflammatory iridoids of botanical origin. *Curr. Med. Chem.* 19:2104–2127, doi: 10.2174/092986712800229005.
- Wenslaff, T. and P. Lyrene. 2003. Unilateral cross compatibility in *Vaccinium elliottii* × *V. arboreum*, an intersectional blueberry hybrid. *Euphytica* 131:255–258, doi: 10.1023/A:1024034219248.
- Zeldin, E.L. and B.H. McCown. 1997. Intersectional hybrids of lingonberry (*Vaccinium vitis-idaea*, section *Oxycoccus*) and cranberry (*V. macrocarpon*, section *Macropelma*). *Acta Hort.* 446:235–238, doi: 10.17660/ActaHortic.1997.446.34.