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Fertile Intersectional F_1 Hybrids of 4*x* *Vaccinium meridionale* (Section *Pyxothamnus*) and Highbush Blueberry, *V. corymbosum* (Section *Cyanococcus*)

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Abstract. ***Vaccinium meridionale*** **(section *Pyxothamnus*), a tetraploid species native to higher altitude locations in Jamaica, Colombia, and Venezuela, is of considerable interest to blueberry breeders for its profuse, concentrated flowering and monopodial plant structure, both of which may be useful in breeding for mechanical harvest. In this study, tetraploid *V. meridionale* was successfully hybridized as a male with 4*x* *V. corymbosum* (section *Cyanococcus*, highbush blueberry). The first-generation hybrids with highbush blueberry selections were intermediate in morphology and notably vigorous. The 4*x* F_1 hybrids displayed variable branching structure, dormancy, prolificacy, fruit wax, etc.; however, most appear to be deciduous to semi-evergreen, with small, dark-colored fruit. The F_1 hybrids displayed good fertility as females in backcrosses to 4*x* highbush and these crosses have produced numerous offspring morphologically indistinguishable from 4*x* highbush at the seedling stage. Evaluations of male fertility found variation for pollen production and quality but, significantly, found some clones with very good shed, high stainability, and almost complete tetrad production. The fertility suggests that these hybrids, despite being derived from intersectional crosses, might be conventionally used without significant difficulty. These hybrids also have potential value for the nascent *V. meridionale* breeding efforts occurring in Colombia, South America.**

This study follows up two previous publications (Ehlenfeldt and Ballington, 2017; Ehlenfeldt et al., 2018) that identified the species material used as *V. corymbodendron*. That material had been tentatively identified as *V. corymbodendron* (Dunal, 1839) when originally collected and deposited at the U.S. Department of Agriculture-Agriculture Research Service (USDA-ARS) National Clonal Germplasm Repository (NCGR) (Corvallis, OR). Further taxonomic study by one of the current authors (J.L. Luteyn, personal communication) has determined the original identification to be erroneous and has determined that the material should be correctly identified as *V. meridionale* (Swartz, 1788).

Distribution and habitat. V. meridionale (section *Pyxothamnus*) is part of a taxonomic complex that includes *V. consanguineum* Klotzsch (Costa Rica and adjacent Panama), *V. floribundum* Kunth (Costa Rica to northern Argentina), and probably also *V. puberulum* Klotzsch (Venezuelan Guayana Highland) (J.L. Luteyn, personal communication).

V. meridionale is known mostly from the Caribbean-facing watershed slopes of northern Venezuela (Coastal Cordillera and Andes), westwards into the Andes of northern Colombia where it is common at mid-elevations, and then disjunct to the Caribbean island of Jamaica from where the type was described (Swartz, 1788). It is widespread in its range, but only locally common. Its habitat includes isolated populations in high montane cloud forest to sub-páramo thickets, where it is a shrub up to 3.5 m tall. Its elevational range stretches from ca. 1000 to 2800 m. Notably, flowering material has been collected (for herbarium specimens) in nearly every month of the year; mature fruit have been collected in Jan.–Feb., July–Aug., and Nov.–Dec.

V. meridionale is characterized by its small leaves, the blades of which are pinnately nerved and minutely crenate-serrate margined, and by its racemose inflorescences with small, 4- to 5-merous, white-to-pink, cylindric to ovoid-cylindric, glabrous flowers, with typically as many as 15 to 25 flowers per inflorescence. Its fruit is usually spherical or, more rarely, slightly oblong or flattened, and ≈14 to 20 mm in diameter. Fruit develop as dark reddish, to dark maroon-black, to blue-black berries in which the top of the ovary is prominently convex or dome-shaped (Fig. 1A and B). The fruit is uniform in size when properly pollinated. The fruit is relatively thick-skinned, and the interior flesh pulpy. The thick skins are reflective of their high levels of antioxidants (Gaviria et al., 2009). In very ripe fruit, the interior locule surfaces begin to accumulate pigment, suggesting that it might be possible to accentuate flesh pigmentation in the future with carefully planned and selected crosses.

Utility for breeding. There is current interest in South America (primarily Colombia) in developing *V. meridionale* as a commercial crop and, through breeding and selection, essentially "domesticating" *V. meridionale*, much as was done with *V. corymbosum*. The fruits of *V. meridionale* have economic potential as a local, high-elevation Andean small-fruit crop and additional potential for international markets due to its high content of polyphenolic compounds known for beneficial effects on human health, including anticarcinogenic properties (Gaviria et al., 2009; Ligarreto, 2009).

For our purposes, this species is of interest because *V. meridionale* is notable for its high number of flowers per bud, as well as its loose inflorescence structure that may be amenable to machine harvest (Luby et al., 1991). Plants of *V. meridionale* also have the potential to develop an upright, tree-like bush structure with a monopodial base. Such a narrow-based plant structure also lends itself to the use of mechanical harvesters.

In a previous study, 4*x* *V. meridionale* was found to produce high numbers of triploids in crosses with 2*x* *V. corymbosum*; however, these hybrids had low fertility, and subsequent backcrosses to 4*x* *V. corymbosum* had modest but very limited success (Ehlenfeldt and Ballington, 2017). It has also been reported that *V. meridionale* had the potential to act as a bridge between taxonomic sections and ploidies in *Vaccinium* (Ehlenfeldt et al., 2018). In this study, we sought to determine the feasibility of crossing 4*x* *V. meridionale* with 4*x* *V. corymbosum* to facilitate incorporation of the desired characters from *V. meridionale*.

Materials and Methods

Plant material. The genotype of *V. meridionale* described here derived from seed collected in Colombia by Dr. James L. Luteyn (Curator, New York Botanical Garden) in 1990. Two of these clones are currently held at the USDA-ARS NCGR. Our particular clone of interest, NC 3735, is maintained as CVAC 1146. Among these two clones, NC 3735/CVAC 1146 has a slightly waxy fruit surface and is the more

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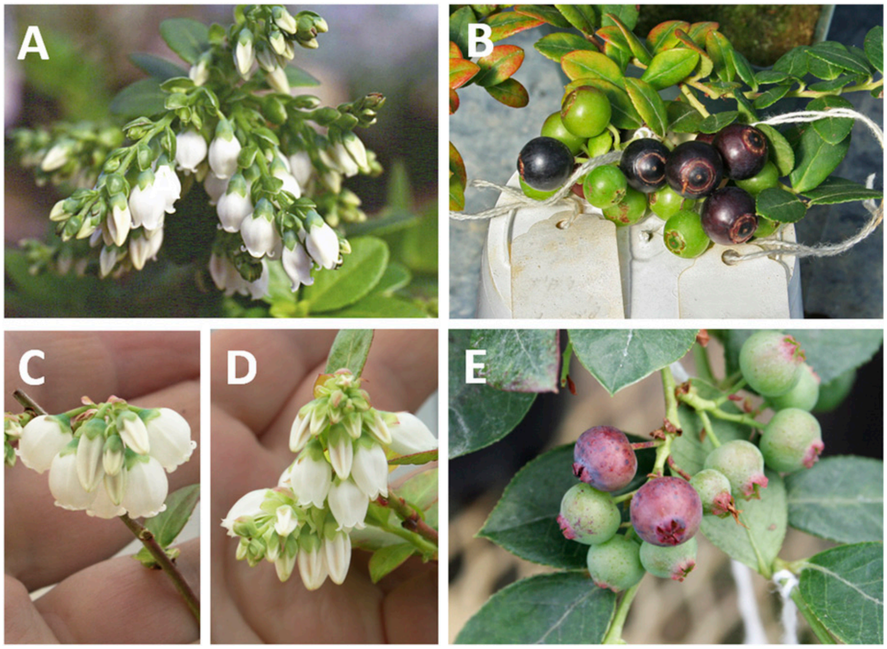

Fig. 1. Flowers and fruit of *V. meridionale* and *V. corymbosum* × *V. meridionale* hybrids: (**A**) Flowers of *V. meridionale*, NC 3737; (**B**) ripe and unripe fruit of *V. meridionale* (US 2381); (**C, D**) Flowers of F_1 *V. corymbosum* × *V. meridionale* (US 2452-A & US 2453-F); and (**E**) unripe and ripening fruit of F_1 *V. corymbosum* × *V. meridionale* (US 2453-I).

male fertile of the two as judged by pollen shed. NC 3737/CVAC 1148 has darker fruit and in our experience has almost no pollen shed.

The *V. corymbosum* genotypes used as females and males are either commercially available cultivars or are USDA-ARS breeding selections. ARS 99-72 is a cross of 'Elliott' × 'Bluegold' and is a promising, midlate ripening northern highbush selection. 'Bluecrop' (Darrow et al., 1952) and 'Duke' (Draper et al., 1987) are northern highbush cultivars primarily composed of *V. corymbosum* germplasm. 'Sharpblue' is a southern highbush cultivar composed primarily of *V. corymbosum* (slightly <60%), with its remaining ancestry split between *V. darrowii* (≈29%) and *V. virgatum* (syn. *V. ashei*) (≈13% to 15%) (Ehlenfeldt, 1994; Sharpe and Sherman, 1976;). 'Cara's Choice' is a mixed species cultivar that is considered northern highbush but contains germplasm of *V. corymbosum*, *V. darrowii*, *V. virgatum*, and *V. constablaei*. It was selected for use as a parent in this project because of its diverse background and its excellent fruit quality (Ehlenfeldt, et al., 2005).

Crossing. We initially had no *V. meridionale* plants to use as females. Thus, for our initial crosses, *V. meridionale* was used as a male with 4*x* cultivars and selections. To this end, we acquired flower clusters from plants growing at the NCGR and collected pollen from these flowers in New Jersey. Due to the nature of the flowers (small with narrow corolla openings), only limited amounts of pollen could be collected. To get pollen from the 4*x* cultivars and selections used, canes were cut from the field and put in vases in a heated greenhouse. Pollen was collected from newly opened flowers over a period of 1 to 2 weeks.

For both *V. meridionale* and other 4*x* selections, pollen was extracted from open flowers by manual manipulation and collected on glassine weighing paper. If pollen was needed for longer term work, pollen was stored for up to a month under refrigerated, desiccated conditions.

To perform pollinations, a graphite pencil tip was dipped into the collected pollen and then used to apply the pollen to the stigmas of unemasculated flowers in an insect-free greenhouse. Pollinations were made on what were judged to be mature stigmas.

Because all pollinations were performed in an insect-free greenhouse, and because it was expected that hybrids would be morphologically recognizable, the female cultivar parents were not emasculated.

Ploidy determinations. Because previous crosses with *V. meridionale* had produced anomalous triploids, it was decided to test a sample group of F_1 hybrids for ploidy verification. For flow cytometry, sampled leaf material (1 cm^2/20 to 50 mg) together with leaf material of an internal standard with known DNA content (*Zea mays* L.) was chopped with a sharp razor blade in 500 mL of extraction buffer (CyStain PI absolute P buffer, catalog number 05-5502; Partec, Münster, Germany) containing RNA-se, 0.1% dithiothreitol (DTT), and 1% polyvinylpyrrolidone (ice cold) in a plastic petri dish. After 30 to 60 s of incubation, 2.0 mL staining buffer (CyStain PI absolute P buffer) containing propidium iodide (PI) as fluorescent dye, RNA-se, 0.1% DTT, and 1% polyvinylpyrrolidone was added. The sample, containing cell constituents and large tissue remnants of the sample and the internal standard, was then filtered through a 50-mm mesh nylon filter. After an incubation of at least 30 min at room temperature, the filtered solution with stained nuclei was measured with the flow cytometer [CyFlow ML (Partec) with a green diode laser 50 mW 532 nm (for use with PI); software: Flomax Version 2.4 d (Partec)]. The DNA amount of the unknown samples was calculated by multiplying the DNA amount of the internal standard with the DNA ratio of the relative DNA amount of the unknown sample and the internal standard. DNA amounts were measured and compared with a set of standards covering a diploid to hexaploid range (2*x* *V. darrowii* 'Fla 4B', 4*x* *V. corymbosum* cv. Duke, and 6*x* *V. virgatum* cv. Powderblue) to determine basic ploidy levels.

Female fertility. Cross numbers varied depending upon flower availability. Pollinations and fruit set were recorded. Fruit was collected when ripe and measured for fruit size (mm) at the time of seed extraction. Extraction was performed manually under a dissecting microscope, and the seed were evaluated for number and quality. For our purposes, seed were classified as good, good/fair, fair, fair/poor, poor, or aborted. "Good" and "fair" described seed that subjectively ranged from those considered fully normal to those somewhat reduced in size and/or development but nonetheless were judged likely to be capable of germination. "Poor" described seed that displayed reduced size or development, often flattened or brown, and judged less likely to be capable of germination. "Aborted seed" were those that were flat and brown, and generally translucent. Notes were made of the size of aborted seed.

Seed were germinated on a greenhouse mist bench in a soil mix composed of 50:50, peat:sand mixture. At approximately a three true-leaf stage, seedlings were transplanted to 36-cell flats. All primary hybrids were transferred to 3-L pots in their second season.

Male fertility. Pollen samples were stained with acetocarmine jelly (75% acetic acid with iron acetate) prepared according to the recipe of Jensen (1962). Pollen samples were assayed for quantity, stainability, and general condition. For general condition, our ratings were as follows: very good = almost all tetrads, good = tetrads and triads, fair = almost exclusively triads, and poor = mostly aborted grains.

Results

Primary crossing success. As noted, initial crosses used *V. meridionale* as a male with several cultivars. These crosses were made in two cycles, the first in 2015 with ARS 99-72, 'Bluecrop', and 'Duke' as females and the second cycle in 2016 with 'Sharpblue' as the female parent. In the subsequent text, these families are given series numbers, such that US 2452 numbers denote 'Bluecrop' ancestry, US 2453 numbers denote ARS 99-72 ancestry, and US 2454 numbers denote 'Duke' ancestry.

Table 1. Seed set of primary hybridizations of highbush/southern highbush cultivars × *V. meridionale*.

Pedigree	Pollinations	Fruit	Total seed (good + good/fair)	Avg seed/fruit	Range seed/fruit	Hybrids extant	F_1/poll.
ARS 99-72 × NC 3735	66	46	158 (8 fruit)	19.8 (8 fruit)	8–30 (8 fruit)	116	1.8
Bluecrop × NC 3735	36	20	175	8.8	0–34	27	0.8
Duke × NC 3735	17	5	21[z]	4.2[z]	0–21	2	0.1
Sharpblue × NC 3735	15	12	50	4.2	0–35	25[y]	1.7

[z]Good/fair only.
[y]Insufficiently mature to verify true hybridity.

Fig. 2. Leaf morphology of *V. corymbosum*, F_1 hybrid, and *V. meridionale*.

Several results are worth noting in the first cycle crosses (Table 1). First, the female cultivar parent significantly affected success. Crosses with ARS 99-72 had the highest level of success, with the production of 1.8 hybrids for every pollination. 'Bluecrop' was only about half as successful with 0.8 hybrids for every pollination, and 'Duke' was least successful with only 0.1 hybrids per pollination. In tabulations of presumed viable seed, ARS 99-72 and 'Bluecrop' both had seed quality rated as good and good/fair, whereas for 'Duke' crosses, only good-fair seed quality was recorded. Seed from these crosses was generally viable in appearance but reduced in size. It has been our experience that if a seed appears plump and viable, it is likely to germinate, no matter how small. Such small seed is typically slower to germinate and results in seedlings of reduced size; however, once established, the resultant seedlings typically appear to be normal in growth.

In the second cycle, a limited number of crosses were done with 'Sharpblue' as female. The averages suggest that 'Sharpblue' performed comparably to ARS 99-72, producing 1.7 (putative) hybrids per pollination (see below). These second cycle crosses aimed to combine *V. meridionale* with southern highbush material.

Hybrid morphology. From the first cycle of crossing 145 hybrids were produced. Once these plants reached an ≈20 cm height, the leaf morphology of these hybrids was distinctive (Fig. 2). Leaves were intermediate in size to the parents and lance-shaped, with smooth, nonserrated edges. The leaves possessed a considerably flatter texture than highbush leaves (but not as fully featureless as rabbiteye leaves). Foliage was medium green and nonwaxy. Well-expanded leaves were uniform in size within a clone and typically averaged ≈4.5 cm (length) × 2.5 cm (width). At the time of this writing, the second cycle plants of 'Sharpblue' × *V. meridionale* were at the 20-cm stage; however, unlike the crosses with northern highbush, these crosses are not yet unequivocally recognizable as hybrids. This seems most likely due to the genetics and morphology that the *V. darrowii* contribution brings to southern highbush.

Hybrid plants were relatively slower growing than comparable *V. corymbosum* seedlings. As young plants (and into their current growth), they exhibited a range of stature, ranging from brushy to those with stronger canes and more upright stature (Table 2). Potted plants growing outdoors retained their foliage in a green state considerably longer than highbush plants growing alongside under the same conditions. In late November of their second season (2019), plants were moved into a nonfreezing cold greenhouse for the winter, and most subsequently entered dormancy and dropped their leaves.

In the spring of their third season (2020), the still relatively young plants were rated for their bush morphology (Table 2). In the US 2452 series ('Bluecrop' ancestry) 7 of 27 or ≈26% were rated as brushy, and 15 of 27 or ≈56% were rated as nonbrushy. The remaining genotypes were either intermediate, or plants were too weak to make an adequate determination. In the US 2453 series (ARS 99-72) ancestry, 32 of 116 or ≈28% were considered brushy, and 62 of 116 or ≈53% were considered nonbrushy. The US 2454 series ('Duke' ancestry) split evenly for bush stature (only 2 plants). It should be noted that as young plants *V. darrowii*–*V. corymbosum* plants often exhibit brushy stature. In southern highbush, plants usually outgrow this brushy stage but may retain a more branched morphology than northern highbush, even as mature plants. It is our experience that our relatively young plants of pure *V. meridionale* lack upright cane growth; however, these same clones at the NCGR have strong upright cane development.

In the spring of their third season, plants were also assayed for evergreen characteristics (Table 2). In the US 2452 series ('Bluecrop' ancestry), 25 of 27 or ≈93% were rated as deciduous, and only 2 of 27 or ≈7% rated as semi-evergreen. Semi-evergreen plants were considered to be those that shed some, but not all, leaves under the given greenhouse overwintering conditions. In the US 2453 series (ARS 99-72 ancestry), 112 of 116 or ≈97% were considered deciduous, whereas one of 116 or ≈1% was considered evergreen, and three plants were inconclusive. The US 2454 series ('Duke' ancestry) split evenly with one deciduous and one semi-evergreen plant.

In the third year of growth, a modest number of plants from each of the two larger families flowered. Flowers showed variability for form, with the flowers of some clones possessing morphologies very much like highbush, but about half the size (Fig. 1C). Other clones had flowers displaying greater *V. meridionale* morphology influence (Fig. 1D), but again approximately half the size of highbush flowers. All flowers observed possessed slightly recessed stigmas. Much like southern highbush, these hybrids had early and rapid bud emergence.

Ballington (2001) noted that an F_1 between *V. corymbodendron* (herein, *V. meridionale*) and cultivated highbush was extremely susceptible to powdery mildew (*Microsphaerea vaccinii*). Unlike the findings of Ballington, our primary hybrids (more than 200 plants) in three parental genotype combinations have thus far exhibited no extraordinary susceptibility to powdery mildew. As might be expected, the next generation BC_1 hybrids to highbush thus far

Table 2. Plant phenotypes of *V. corymbosum* × *V. meridionale* clones.

			Morphology				Foliage retention			Floral buds	
Series	Female parent	Extant plants	Brushy	Nonbrushy	Int.	Unk.[z]	Deciduous	Evergreen	Unk.[z]	2019–20 (no. plants)	2019–20 (% of family)
US 2452	Bluecrop	27	7	15	1	4	25	2 semi	—	7/4	26/15
US 2453	ARS 99-72	116	32	62	20	2	112	1	3	10/14	9/12
US 2454	Duke	2	1	1	—	—	1	1 semi	—	0/1	—

[z]Unknown determinations were typically on genotypes too weak to make an adequate determination.

Table 3. Female fertility of *V. corymbosum* × *V. meridionale* clones.

Year	Quantity and identity	Poll.	Fruit	Set (%)	Seed	Seed/fruit	Seed/fruit range
2019	17 genotypes, combined	160	97	61	797	8.3	0–28
	7 genotypes, US 2452-# (Bluecrop)	68	34	50	233	5.9	0–16
	10 genotypes, US 2453-# (ARS 99-72)	92	63	68	564	10.0	1–28
2020	13 genotypes, combined	173	112	65	1168	12.6	0–31
	4 genotypes, US 2452-# (Bluecrop)	47	30	64	289	15.0	0–28
	9 genotypes, US 2453-# (ARS 99-72)	126	82	65	879	11.5	1–31
Plants flowering both years	7 genotypes, combined	—	—	60	—	10.4	—
	4 genotypes, US 2452-# (Bluecrop)	—	—	55	—	10.8	—
	3 genotypes, US 2453-# (ARS 99-72)	—	—	66	—	9.8	—

also appear within the range of normal powdery mildew susceptibility.

Flow cytometry. In our previous study, with *V. meridionale* (Ehlenfeldt and Ballington, 2017), we found hybrids from 4*x* *V. meridionale* × 2*x* *V. corymbosum* crosses to be triploid. Triploidy was an unusual result considering that typically intraspecific 4*x* × 2*x* crosses result in cross failure (Cooper and Brink, 1945) or the production of tetraploids through the functioning of 2*n* gametes (Hanneman and Peloquin, 1968). Triploids in *Vaccinium* generally are exceedingly rare (Dweikat and Lyrene, 1988; Vorsa and Ballington, 1991); however, it should be pointed out that our previous crosses were not intraspecific crosses. All of our current crosses were 4*x* × 4*x*; however, in light of the high levels of anomalous triploids found in *V. meridionale* crosses in the earlier study, we decided to sample our populations to verify that our hybrids were the tetraploids we expected. A random sample of 20 plants were tested from each of our largest two families, the US 2452 series and the US 2453 series. In all cases, hybrids were found to be 4*x*, suggesting balanced chromosome sets and likely fertility.

Evaluations of female fertility. Several plants from each large series displayed flower bud development in their second (2019) and third (2020) seasons (Table 3); however not all buds in either year developed fully to maturity. In 2019, 26% of the plants in the US 2452 series developed buds, whereas 15% of the plants in the US 2453 series developed buds. In 2020, the percentages for these same groups were 15% and 12%, respectively.

In 2019, a decision was made to prioritize the use of these hybrids as females only to advance germplasm and ensure provenance of offspring. In that year, we evaluated female fertility by pollinating any flowering plant with pollen from 'Cara's Choice'. 'Cara's Choice' was chosen for its diverse species background, which we believed would facilitate crosses, and for its high fruit quality in terms of color, firmness, flavor, sweetness, and volatiles (Ehlenfeldt et al., 2005).

In 2019, 17 genotypes flowered across our two main families (Table 3). In that year, 160 pollinations were made producing 97 fruit (61% fruit set) with an average seed production of 8.3 seed per fruit.

In 2020, 13 genotypes flowered adequately to use for crosses. In 2020, 173 pollinations were made, producing 112 fruit (65% fruit set) with an average seed production of 12.6 seed/fruit.

Regardless of female parentage or year, fruit was relatively uniform-sized, with fruit slightly oblate ranging from 12.0 ± 0.6 mm (width) × 9.8 ± 0.7 mm (length) for larger fruited clones, to 8.3 ± 1.0 mm (width) × 7.0 ± 0.8 mm (length) for somewhat smaller-fruited clones. In all cases, ripe fruit was nearly black with relatively tough skins. Stem attachment scars in both the *V. meridionale* parent and the hybrids were small, ≈0.5 mm. Unlike the *V. meridionale* parent, the hybrids displayed a calyx development more typical of *V. corymbosum* (Fig. 1E). Among the tested offspring, only one clone (US 2453-P) was observed to produce slightly waxy/blue fruit. Its seed set with 'Cara's Choice' was not very high (48% set, 60 seed, 6.23 seed/fruit), but these crosses did succeed.

Several hybrids were also noted as having tendencies to reflower in the fall, if kept under greenhouse conditions. It is unclear what expression of this trait might be under outdoor conditions, but as noted previously, *V. meridionale* under natural conditions has been observed in flower in nearly every month

All of the seed from these crosses was planted, and at time of writing, there were 77 hybrids from the US 2452-series × 'Cara's Choice' crosses ('Bluecrop' ancestry), and 482 hybrids from the US 2453-series × 'Cara's Choice' crosses (ARS 99-72 ancestry).

Male fertility of hybrids. In 2020, any plant that flowered was sampled for pollen shed. Pollen condition varied widely (Table 4). In the US 2452 series ('Bluecrop' ancestry), only three pollen samples were collected. Among these, there was one individual each of good, fair, and poor ratings. Even the "good" selection among these (US 2452-A) demonstrated indications of irregular meiosis, having mostly triads, and some dyads and monads (Fig. 3). In the same series, the one clone considered "poor" due to very light pollen shed and many aborted pollen grains (US 2452-I) produced elevated levels of stainable dyads.

Of the 13 samples of the US 2453 series (ARS 99-72 ancestry), six (46%) were rated very good, and four (13%) were rated good for pollen quality. Of the remaining plants that flowered, one (8%) was rated poor, and two (15%) had negligible pollen shed. It is worth noting that even in this relatively modest sample, we found plants with high shed, high stainability, and nearly all viable-appearing tetrads, among them US 2453-S and US 2453-W (Fig. 3). Other clones reflected an apparent range of genetic incompatibilities between these species, resulting in triads.

Discussion

There are three salient points to be made about these hybrids:

1) Despite being intersectional crosses, it was possible to generate hybrids of *V. corymbosum* with *V. meridionale*, using *V. corymbosum* as females. These hybrids were relatively easy to make and were generated with several *V. corymbosum* genotypes. Although *V. meridionale* is not in section *Cyanococcus*, Powell and Kron (2002) placed *V. meridionale* immediately adjacent to *V. tenellum* and *V. corymbosum* on a subbranch of a DNA-based mat-K taxonomic tree of 50 *Vaccinium* species, suggesting a potentially close relationship with section *Cyanococcus* despite geographic separation and taxonomic classification. We are currently in the process of broadening the genetic base of our materials further by crossing

Table 4. Male fertility of *V. corymbosum* × *V. meridionale* clones.

Clone	Shed	Stainability	Quality; condition
US 2452 series ('Bluecrop' ancestry)			
US 2452-A	Good	High	Good; mostly triads, some dyads and monads (Fig. 2)
US 2452-B	Good	High	Fair; mostly triads, rare monads
US 2452-I	Light	Low-medium	Poor; many aborted grains but also dyads (Fig. 2)
US 2453 series (ARS 99-72 ancestry)			
US 2453-A	Good	High	Very good; tetrads and triads
US 2453-B	Light	High	Good; tetrads, triads, aborted grains
US 2453-E	Nil	—	—
US 2453-F	Light	Low-medium	Poor; triads, tetrads, aborted grains
US 2453-J	Nil	—	—
US 2453-P	Light	Medium	Good; mostly triads, a few tetrads
US 2453-Q	Light	High	Good; tetrads and triads
US 2453-S	Good	High	Very good; mostly tetrads (Fig. 2)
US 2453-W	Good	High	Very good; mostly tetrads, some triads and dyads (Fig. 2)
US 2453-X	Good	High	Very good; tetrads, triads, aborted grains
US 2453-AU	Good	High	Good; tetrads and triads, some aborted grains
US 2453-AY	Good	High	Very good; tetrads, triads, dyads
US 2453-BD	Good	High	Very good; tetrads and triads

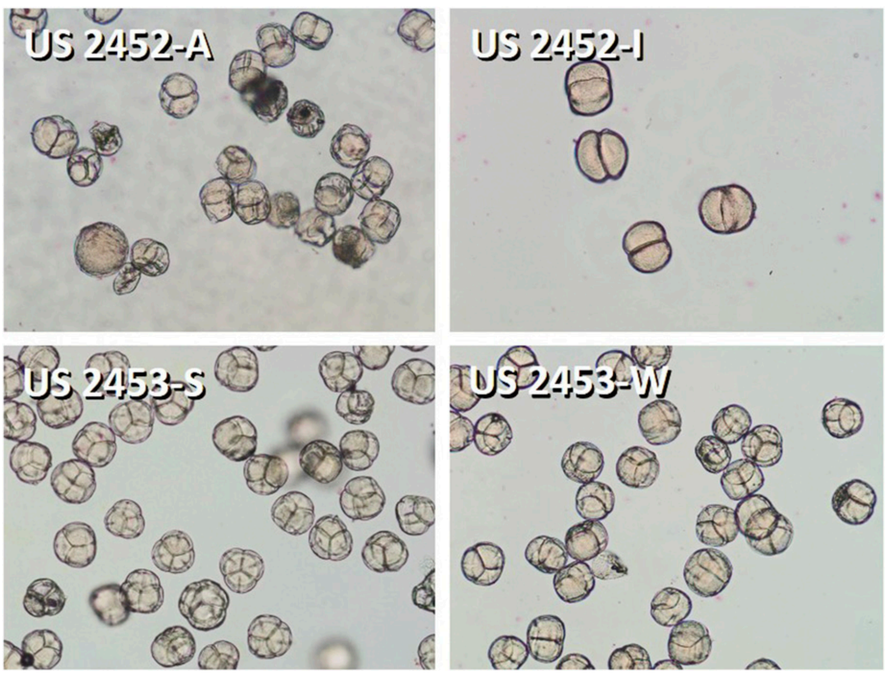

Fig. 3. Pollen images of selected clones of *V. corymbosum* × *V. meridionale* (×200).

V. meridionale to an even wider range of cultivated genotypes.

2) These hybrids are tetraploid, and most are substantially female fertile. In tetraploid hybrids such as these, a significant degree of fertility due to allopolyploidy might be expected (Clausen and Goodspeed, 1925). However, the relative ease of producing primary hybrids in quantity suggests that no strong genetic incompatibilities exist between these genomes. A critical question for future utilization may be the subsequent degree of hybrid fertility of BC_1 selections made using other 4*x V. corymbosum* cultivars as the backcross parent. Using our initial hybrids as females and *V. corymbosum* ('Cara's Choice') as the male parent, we have produced the aforementioned BC_1 hybrid families and have offspring numbering in the hundreds. The question of BC_1 fertility should be answerable in 1 to 2 years.

3) Among the primary hybrids are genotypes that are highly male fertile. Fertility in a few of our clones is judged to be greater than 90% viable pollen production. Other clones have been observed with low fertility and disrupted or incomplete meiosis. Among these low-fertility clones are individuals that produce apparently viable dyad and monad microspores, but even these genotypes may have potential in transferring this germplasm to crosses involving 6*x V. virgatum.*

The fertility found in these hybrids is a positive trait for potential breeding in both *V. corymbosum* and *V. meridionale*. *V. corymbosum* stands to benefit from the prolificacy and high flower-bud number of *V. meridionale*. *V. corymbosum* may also benefit from *V. meridionale'*s concentrated flowering and good upright bush stature. It is expected that *V. meridionale* may also enhance pigmentation and antioxidant levels.

Conceptually, our population of 'Bluecrop' × *V. meridionale* 'NC 3735' genotypes (i.e., the US 2452 series) are equivalent to US 75 (= 'Bluecrop' × *V. darrowii* 'Fla 4B'). US 75 is a selection that gave rise to several early southern highbush cultivars, among them: 'Cape Fear' (= US 75 × 'Patriot'), 'Cooper' (= G-180 × US 75), 'Georgiagem' (= G 132 × US 75), and other later released southern highbush as well: 'Legacy' (= 'Elizabeth' × US 75) and 'Dixieblue' (= G-144 × US 75). Looking at the plants and flowers of our F_1s, we believe they may have similar parental potential. At least some clones are upright, floriferous, and fertile. They appear to be adapted toward low-chill physiology much like *V. darrowii*, with variability for factors such as bloom time, evergreen foliage, and surface wax. When such F_1 hybrids are backcrossed to yield three-quarter highbush hybrids, it is expected they could be essentially equivalent to commercial types. A major question is how *V. meridionale* may affect fruit quality and what other beneficial or detrimental traits may be inherited. *V. meridionale* has also had virtually no evaluation for insect or disease resistance, although under natural conditions there is virtually no apparent herbivory.

With respect to *V. meridionale* breeding/domestication, that species stands to benefit notably from *V. corymbosum* in terms of larger fruit size and better fruit quality. Although waxiness may not be considered a need for *V. meridionale*, cuticular wax enhances storage ability of *Vaccinium* fruit, and this may become an important concern, especially if future selection occurs for more tender (i.e., thinner) skin in *V. meridionale* introgressants.

Conclusions

Much remains to be understood about intersectional hybrids in *Vaccinium*; however, given these results and the results of several other studies (Ehlenfeldt and Polashock, 2014; Lyrene, 2011, 2016; Morozov, 2007), intersectional crossing barriers appear to be modest at best. The hybridization of *V.*

padifolium (Ehlenfeldt and Polashock, 2014) would have seemed to be an extraordinarily difficult one based on modern molecular-based taxonomic determinations that listed sect. *Hemimyrtillus* species among the furthest outliers from section *Cyanococcus* (Powell and Kron, 2002), yet these hybrids were made with only moderate difficulty, and the resultant tetraploid hybrids were highly fertile. There seems to be no doubt that diploid intersectional hybrids present fertility issues, but if hybrids can be made at the tetraploid level, allotetraploid fertility and apparent tetraploid buffering almost surely guarantee some level of success will exist in the backcrossing of intersectional hybrids to tetraploid cultivated germplasm. To be sure, in wide crosses, even after seed is produced, not all genetic combinations are fully viable. After germination, seedlings may manifest morphology ranging from viable, to sublethal, to lethal. Among sublethal types we see, there are those that display reddish pigmentation and develop slowly, others that are deeply pigmented and barely grow beyond the cotyledonary stage, and still others that stagnate and die within a brief period. Occasionally, sublethal genotypes transition from stagnation to relatively normal growth. Of true value, however, are the plants that display relatively normal growth and a vigorous genotype.

On the basis of our studies, we feel that *V. meridionale* will play a critical role in producing wide hybrids because it appears to be a very accepting parent. We believe we are on the brink of opening up access to a wide array of tertiary blueberry germplasm that will benefit both conventional and molecular aspects of blueberry breeding.

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