Ploidy, Fertility, and Phenotypes of F₁ Hybrids between Tetraploid Highbush Blueberry Cultivars and Diploid *Vaccinium elliotii*

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Abstract. A progeny of 55 blueberry seedlings produced by pollinating 4301 flowers of tetraploid highbush blueberry cultivars with pollen from 19 different diploid *Vaccinium elliotii* plants was studied to determine hybridity and ploidy. Of the 21 seedlings whose phenotypes were intermediate between parental types, indicating hybridity, 18 were triploid and three were tetraploid. Pollen of the triploids, when viewed at $\times 250$, was almost all shrunken and aborted, although some triploid hybrids produced a few large, plump microspores in dyads or monads. Triploids produced no seed when pollinated with pollen from 4x highbush or 2x *V. elliotii* or when open-pollinated outside the greenhouse in the presence of fertile diploid and tetraploid blueberries. Tetraploid hybrids produced large populations of vigorous seedlings when intercrossed. Both triploid and tetraploid F₁ hybrids were intermediate between the parents in leaf size and flower size. The triploids produced no berries; the tetraploids were intermediate between the parents in berry size but averaged lower in Brix and berry firmness than either parent. Seven additional F₁ hybrids from reciprocal crosses were obtained by pollinating 2309 flowers of 2x *V. elliotii* with pollen from tetraploid highbush cultivars. Although five *V. elliotii* clones served as female parents in these crosses, only one produced any seedlings. Six of the seven hybrids flowered and were fertile tetraploids; one was a sterile triploid.

Vaccinium section *Cyanococcus* (Ericaceae) consists of diploid, tetraploid, and hexaploid species (Camp, 1945). Except for one diploid species, *Vaccinium myrtilloides*, whose native range extends from Nova Scotia to Vancouver Island and southwestern British Columbia, all species in the section are native to eastern North America (Vander Kloet, 1988). The species in section *Cyanococcus* are closely related. Homoploid interspecific crosses are easy to make, and thousands of vigorous, fertile seedlings can be obtained. Tetraploid blueberry cultivars have been produced by interspecific crosses in section *Cyanococcus*, followed by recurrent selection for desirable horticultural characteristics and clonal propagation of the best seedlings (Coville, 1937; Ballington et al., 1996).

A strong triploid block makes it hard to cross diploid and tetraploid species within the section, but tetraploid plants of the diploid species, produced with colchicine (Dweikat and Lyrene, 1991; Perry and Lyrene, 1984), can readily be crossed with the tetraploid species, and the resulting hybrids are vigorous and fertile.

Unreduced gametes also can enable the production of fertile tetraploid hybrids from crosses between diploid and tetraploid species in section *Cyanococcus* (Lyrene and Ballington, 1986; Lyrene et al., 2003; Sharpe and Darrow, 1959). The frequency of unreduced gametes is low in *Vaccinium*, and varies widely both among and within species (Chavez and Lyrene, 2009; Megalos and Ballington, 1988; Ortiz et al., 1992).

V. elliotii, a diploid highbush species, is locally abundant on acid, sandy, and sandy-clay soils in the southeastern United States from north Florida to east Texas and as far north as North Carolina. Although Vander Kloet (1980) lumped *V. elliotii* with other highbush blueberries under the name *Vaccinium corymbosum*, most regional biologists who have taken into consideration range, plant abundance, morphology, phenology, ecology, and crossing behavior, consider *V. elliotii* a distinct species (Camp, 1945; Uttal, 1987; Ward, 1974). *V. elliotii* was easily distinguishable by simple sequence repeat

analysis from diploid *Vaccinium fuscum*, the highbush species it most closely resembles (Bassil et al., 2018).

V. elliotii has been used to a limited extent in blueberry breeding programs in North Carolina and Florida. The intent has been to obtain cultivars that incorporate the ability of *V. elliotii* to grow on drought-prone upland soils that have low organic-matter content (Lyrene, 1997; Lyrene and Sherman, 1980). *V. corymbosum*, the principal species used in domesticating highbush blueberry, occurs naturally on high-organic soils having poorly drained subsoils, and is poorly adapted to uplands. *V. elliotii* plants flower early and require relatively few days between flowering and fruit ripening. The berries ripen very early in the spring. *V. elliotii* berries are small, approximately the size of berries harvested commercially from *Vaccinium angustifolium* in Maine and eastern Canada, but are juicy, fragrant, and have small seeds. In architecture, the plants are upright, and typically form compact colonies 2 to 4 m tall, with canes that range from stiffly upright on some plants to rather weeping on others. The leaves are small and deciduous. The flowers are small and differ from other highbush blueberries by having a short style, with the stigma normally located well inside the corolla tube (Lyrene, 1994).

A highbush blueberry cultivar, Carteret, which has *V. elliotii* in its pedigree, was released by North Carolina State University in 2009 (Ballington and Rooks, 2009). In the late 1970s, crosses were made at the University of Florida between low-chill Florida highbush cultivars and *V. elliotii* plants propagated from forests in southwest Alabama and northeast Florida. Two cultivars with *V. elliotii* in their pedigrees, Snow-chaser and Kestrel, were eventually released by the Florida blueberry breeding program. Both ripen early and produce berries that have pleasant, aromatic flavors. The success of these cultivars resulted in renewed interest in using *V. elliotii* as a parent in the Florida blueberry breeding program.

In 2014, 175 seeds were obtained by pollinating 4301 emasculated flowers on 19 tetraploid highbush cultivars with pollen bulked from ≈ 30 different *V. elliotii* genotypes that had been propagated as cuttings from native populations in southwest Alabama (Lyrene, 2014). From these seeds, 55 plants were grown to the age of flowering. The purpose of this paper was to describe the ploidy, phenotypes, pollen fertility, and crossing behavior of plants from these and similar crosses.

Materials and Methods

The seeds from the interspecific crosses were planted on the surface of Canadian peat in a greenhouse under mist in Nov. 2014. In Jan. 2015, the resulting seedlings were transplanted to a tray of peat, which was maintained in a greenhouse until 28 May 2015, at which time the seedlings were transplanted to a high-density field nursery (Sherman et al., 1973) at Citra, FL. On 1 Dec. 2015, the 55

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putative F_1 hybrids that survived in the nursery were examined to eliminate plants that did not look like valid hybrids. Leaf size and shape; presence or absence of pubescence on the stems, petioles, and leaves; bark pattern on the oldest wood; plant architecture; and plant vigor were used to judge hybridity. In total, 38 plants were selected as putative hybrids. These were dug, retaining as much root soil as possible, and were fit into 20-L plastic pots by packing peat around the root ball. The plants were placed in a cooler at 4 °C until 1 Jan. 2016 when they were moved to a bee-proof greenhouse. The plants began to flower in late Jan. 2016. Pollen from the first two flowers that opened on each plant was shed onto a drop of 45% acetic acid on a microscope slide and examined at $\times 250$ magnification. Abundance of pollen shed, diameter of fully formed tetrads, and percent fully formed pollen tetrads were recorded. The next 10 flowers that opened on each plant were used to measure corolla length, corolla diameter, stigma position vs. corolla length, and style length.

When vegetative growth began, tender tissue was collected from 24 of the hybrids and placed between wet napkins until it was processed (Norden, 2017). Flow cytometry analysis (Costich et al., 1993) was done using the BD Accuri C6 instrument (BD Biosciences, Franklin Lake, NJ) at the University of Florida's Interdisciplinary Center for Biotechnology Research, Gainesville, FL. Tissue was prepared using the Sysmex CyStain PI Absolute P kit (Kobe, Japan). Before tissue preparation, Ribonuclease A (RNaseA) stock solution and staining solution were prepared. The RNaseA stock solution was prepared by adding 1.5 mL H_2O to one tube of RNaseA. The stock solution was stored at -20 °C. The staining solution was prepared by combining 20 mL staining buffer, 120 μ L propidium iodide, and 60 μ L RNaseA stock solution. The staining solution was stored at 4 °C in bottles wrapped in aluminum foil to exclude light until used. Etiolated leaf tissue (500 mg) from the apex of rapidly growing vegetative shoots was harvested from each plant. Within 8 h, the tissue was chopped for 60 s in 500 μ L of nuclei-extraction buffer on a 7.5-cm petri dish using a microtome blade. Tissue of diploid *Vigna radiata* (genome size 1.06 pg) was co chopped with the blueberry tissue as an internal standard. The solution was filtered through 50- μ m mesh into a 5-mL glass tube. Staining solution (2 mL) was added to each 5-mL tube, and the tube was immediately placed on ice in the dark to incubate for 1 to 4 h until analyzed.

The equipment settings were held constant for all samples. Each sample measured 10,000 events at a medium flow rate of 35 μ L/min and a maximum fluorescence emission threshold of 1000 on FL2-H. The threshold was set according to each sample. Samples were vortexed before being analyzed. To determine the ploidy of each sample, a *Vaccinium elliotii* nuclei solution was prepared and used as an external diploid standard. The standard was run first, followed by the un-

known samples. Because a tetraploid was expected to have doubled the DNA content of a diploid, and the diploid relative fluorescence intensity (RFI) peak was known, any peak from an unknown sample that had an RFI peak twice as large as the diploid standard was considered a potential tetraploid.

In Jan. 2016, 28 putative F_1 hybrid plants that had been moved from the cooler to the bee-proof greenhouse were selected for crossing (Table 1). Plants that were thought to be triploid, based on flow cytometry and low pollen fertility, were used as female parents in crosses with diploid *V. elliotii*. It was hoped that diploid or near-diploid plants could be obtained that contained some genetic material from the highbush cultivar parents. Some of the hybrids that were thought to be fertile tetraploids were intercrossed to generate segregating F_2 populations, one was pollinated with pollen from diploid *V. elliotii*, and five were backcrossed to highbush cultivars. In making the crosses, unopened flowers on plants that were used as female parents were emasculated by removing the corolla and anthers with forceps. Pollen from the selected pollen parents was shed onto the fingernail and applied to the stigma of emasculated flowers. The goal was to pollinate at least 50 flowers per plant, but some plants chosen as females produced fewer than 50 flowers.

Berries began to ripen in late March of 2017. The first 10 berries that ripened on each plant were evaluated for phenotypic characteristics. Fruit firmness and diameter were measured using the Firmtech 2 instrument (BioWorks, Inc., Wamego, KS). Berries were cut in half and juice was squeezed onto an American Optical Company (Buffalo, NY) hand refractometer to measure degrees Brix. Seeds were harvested by squashing individual berries onto paper towels. Mean number of plump seeds per berry was estimated based on 10 berries per cross.

The putative hybrid plants that had been used in crosses in 2016 were maintained in large pots in the open during the flowering season of 2017. Many other fertile diploid and tetraploid plants (highbush cultivars, *V. elliotii*, and *Vaccinium darrowii*) on the same mat served as pollen sources. Honeybee and bumblebee activity was sufficient to set full crops of seedy berries on these fertile plants. Fruit set and seed content were observed on the putative hybrids, and the phenotype of each plant was carefully observed many times during the year.

In Aug. 2017, leaf length and width were measured on highbush, *V. elliotii*, and F_1 hybrid plants growing in pots in full sun outside the greenhouse.

In 2015 and 2016, five *V. elliotii* plants were used as seed parents in crosses with highbush cultivars. Seeds from these crosses were germinated on peat in a greenhouse under mist, and the seedlings were grown to the age of flowering in pots. Pollen was examined from the four hybrids obtained in 2015, and the three plants that appeared to produce good pollen were used in crosses with fertile tetraploids from the 2014 crosses.

Results

Of the 26 plants selected as hybrids from the highbush cultivar $\times$ *V. elliotii* crosses made in 2014, 17 proved to be triploid, evidenced by flow cytometry and by high levels of pollen abortion (Tables 1 and 2). One plant, N32, appeared to be triploid based on flow cytometry data but produced 40% starch-filled pollen tetrads. When pollinated with tetraploid highbush pollen, 337 flowers of this plant produced no viable seed, and the plant is now believed to be triploid. All of the triploid plants were unequivocal hybrids, strongly showing characteristics of the *V. elliotii* pollen parent, including small leaves, small flowers, pubescent stems, and pubescent leaves. Of the eight tetraploid seedlings from these crosses, three clearly had F_1 phenotypes, but five had ambiguous phenotypes, seeming to have some hybrid characteristics but resembling highbush in most characteristics. These were considered uncertain hybrids.

The seven seedlings obtained by pollinating *V. elliotii* clone #8 with pollen from tetraploid hybrid cultivars in 2015 and 2016 (Table 3) were clearly hybrids, each showing obvious phenotypic characteristics from the highbush parent. The four hybrid plants from the 2015 crosses flowered in 2017. Pollen from three (N60, N63, and N64, Table 2) was plump and starch-filled, indicating that the plants were tetraploid; pollen from the fourth (N61) was almost completely aborted, indicating that N61 was probably triploid. The three additional hybrids from 2016 crosses flowered in 2018. All three produced abundant pollen, more than 95% of which was plump and starch-filled.

The triploid hybrids were highly sterile in all crosses. The 17 triploid hybrids (highbush $\times$ *V. elliotii*) that were pollinated in the greenhouse with pollen from diploid *V. elliotii* produced no berries from a total of 2063 flowers pollinated (first 17 crosses in Table 1). The one cross in which a triploid was pollinated with pollen from a tetraploid cultivar (N24 $\times$ 15-108) also produced no seed. Of the three crosses in which both parents appeared to be tetraploid hybrids (N1 $\times$ N4, N10 $\times$ N4, and N4 $\times$ N32), the first two gave high fruit set and berries with numerous seeds, but the last resulted in low fruit set and few seeds (Table 1).

The triploid plants fell into several classes based on the amount of pollen shed and its appearance at $\times 125$ magnification. With some plants, no pollen could be obtained by rotating open flowers over a microscope slide, and when the aborted anthers were smashed in a drop of acetic acid, not even aborted pollen could be seen. Other triploid plants shed pollen abundantly, but virtually every pollen grain was empty. Still other triploid plants produced pollen that was mostly aborted but included a few well-developed spores. These spores frequently occurred as dyads of two plump spores or as monads with one very large plump spore.

Table 1. Results of crosses made in 2016 using the highbush cultivar × *Vaccinium elliotii* F₁ hybrids, which are designated by N-numbers.

Seed parent	Ploidy	Pollen parent	Ploidy	Flowers pollinated (no.)	Berries harvested	Fruit set (%)	PPF ^z
N9 ^y	3x	VE ^x	2x	190	0	0	0
N6 ^y	3x	VE	2x	97	0	0	0
N12 ^y	3x	VE	2x	223	0	0	0
N22 ^y	3x	VE	2x	43	0	0	0
N23 ^y	3x	VE	2x	121	0	0	0
N8 ^y	3x	VE	2x	169	0	0	0
N5 ^y	3x	VE	2x	20	0	0	0
N11 ^y	3x	VE	2x	42	0	0	0
N29 ^y	3x	VE	2x	29	0	0	0
N3 ^y	3x	VE	2x	28	0	0	0
N39 ^y	3x	VE	2x	15	0	0	0
N15 ^y	3x	VE	2x	31	0	0	0
N30 ^y	3x	VE	2x	55	0	0	0
N13 ^y	3x	VE	2x	217	0	0	0
N21 ^y	3x	VE	2x	79	0	0	0
N35 ^y	3x	VE	2x	146	0	0	0
N33 ^y	3x	VE	2x	68	0	0	0
N24 ^y	3x	15-108 ^w	2x	100	0	0	0
N4 ^v	4x	N32 ^y	3x	223	44	19.7	0.1
N37 ^v	4x	VE	2x	22	0	0	0
N36 ^v	4x	14-15 ^w	4x	54	22	40.7	14.8
N1 ^v	4x	N4 ^v	4x	59	38	64.4	24.0
N10 ^v	4x	N4 ^v	4x	42	33	78.6	29.8
N38 ^u	4x	11-67 ^w	4x	150	146	97.3	29.2
N2 ^u	4x	15-155 ^w	4x	67	19	28.4	2.1
N7 ^u	4x	14-28 ^w	4x	81	20	24.7	1.9
N28 ^u	4x	N4	4x	185	67	36.2	15.3

^zPPF = average number of plump seeds per pollinated flower.^yTriploid F₁ hybrid (highbush cultivar × *V. elliotii*).^x*V. elliotii* clone 13-1163^wTetraploid highbush cultivar.^vTetraploid F₁ hybrid (highbush cultivar × *V. elliotii*).^uA putative F₁ hybrid but it resembled highbush in many features; validity is in doubt.Table 2. Assessment of ploidy of F₁ hybrids from tetraploid highbush × diploid *Vaccinium elliotii* crosses. Diploid *Vigna radiata* (genome size 1.06 pg) was used as an internal standard.

Clone	Flow cytometry 2C value (pg)	Pollen ^z size (μm)	Estimated ploidy		Amount of pollen shed ^y	Fully formed tetrad %
			Cytometry	Pollen		
N3	1.83	51.1	3x	NA ^x	9	0.1
N5	1.79	49.8	3x	3X	5	5.0
N9	1.80	52.0	3x	3X	0	0.1
N11	2.11	NA	3x	NA	1	0.0
N12	1.88	NA	3x	NA	1	0.0
N13	2.02	NA	3x	NA	5	0.0
N14	2.03	NA	3x	NA	0	0.0
N15	2.05	52.4	3x	3x	1	1.0
N21	2.06	51.7	3x	3x	9	1.0
N22	1.90	49.3	3x	3x	8	10.0
N23	2.00	52.2	3x	3x	1	0.1
N24	2.06	50.9	3x	3x	2	0.1
N30	1.95	NA	3x	NA	0	0.0
N32	1.99	50.3	3x	4x	8	40.0
N33	2.05	NA	3x	NA	1	0.0
N39	1.87	NA	3x	NA	1	0.0
N1	2.82	56.4	4x	4x	9	15.0
N4	2.67	59.7	4x	4x	9	60.0
N10	2.46	56.0	4x	4x	9	25.0
N40	2.58	57.3	4x	4x	9	80.0
N19 ^w	2.58	58.1	4x	4x	9	60.0
N28 ^w	2.60	54.8	4x	4x	9	90.0
N36 ^w	2.85	56.8	4x	4x	9	5.0
N2 ^x	2.68	57.6	4x	4x	9	90.0
N25 ^x	2.43	55.6	4x	4x	9	75.0
N60	2.44	58.1	4x	4x	9	90.0
N63	2.36	57.8	4x	4x	9	90.0
N64	2.34	59.0	4x	4x	9	90.0
<i>V. elliotii</i>	1.16–1.48	48.2–53.9	2x	2x	8–9	80–90
Highbush cultivars	2.31–2.73	50.7–60.0	4x	4x	8–9	5–90

^zDiameter of well-formed pollen tetrads.^y9 = Abundant; equivalent to the shed of fully fertile highbush cultivars; 0 = no pollen shed.^xData not available.^wPutative F₁ hybrid, but resembled highbush in most leaf, stem, and berry features.

Table 3. Results of pollinating five diploid *Vaccinium elliotii* clones with pollen from tetraploid highbush cultivars.

Yr of cross	Female parent ^z	Pollen parent ^y	Flowers pollinated (no.)	Fruit harvested (no.)	Hybrid seedlings obtained (no.)
2015	'Shill Yellow'	Emerald	128	0	0
2015	Ell #6	05-603	262	0	0
2015	Ell #1	12-76	222	0	0
2015	Ell #35	05-603	301	0	0
2015	Ell #8	00-180	451	177	4 ^x
2016	Ell #8	Emerald	786	281	3 ^w
2016	Ell #8	Ell #8	159	3	0
Totals			2309	461	7

^zAll were diploid *V. elliotii* selections from the forest.^yAll were tetraploid highbush cultivars except for Ell #8.^xAll had hybrid phenotype after 1 year; three were tetraploid and one was diploid.^wAll had hybrid phenotype after 1 year, shed an abundance of pollen tetrads, more than 95% of which were plump and starch-filled and are very probably tetraploid.Table 4. Leaf, flower, and berry characteristics of three taxa: *Vaccinium elliotii* (VE), southern highbush (HB), and F₁ hybrids.

		Taxa ^z		
Samples		VE	HB	F ₁ (HB×VE)
Leaf characteristics (cm)	No. leaves PG ^y	1	1	2
	No. genotypes	10	10	10
	Length	2.6 ^x (0.35) a ^w	5.9 (1.88) b	4.4 (0.36) c
	Width	1.1 (0.35) a	3.6 (0.47) b	2.3 (0.38) c
Flower characteristics (mm)	No. flowers PG	10	10	10
	No. genotypes	2	2	30
	Corolla length	6.2 (0.57) a	10.1 (0.82) b	7.8 (0.84) c
	Corolla diameter	3.3 (0.47) a	6.8 (0.46) b	5.7 (1.10) c
Berry characteristics	Distal end of style to distal end of corolla ^v	1.7 (0.54) a	1.3 (0.33) b	0.97 (0.71) b
	Style length	4.9 (0.68) a	8.3 (0.72) b	7.50 (0.69) c
	No. berries PG	5	5	10
	No. genotypes	10	10	38
Berry characteristics	Weight (g)	0.12 (0.04) a	2.3 (0.43) b	1.40 (0.72) c
	Diameter (mm)	11.1 (1.40) a	17.8 (1.53) b	14.2 (3.20) c
	Firmness (g/mm)	314.7 (29.9) a	253.3 (55.5) b	220.1 (58.1) c
	Brix	18.7 (1.10) a	12.7 (1.30) b	12.2 (2.40) c

^zVE = *Vaccinium elliotii*. HB = Tetraploid highbush cultivars based on *Vaccinium corymbosum*. F₁ = Highbush cultivars × *V. elliotii*.^yPG = per genotype.^xMeans and (standard deviations).^wSimilar letters within a row indicate means not significantly different, Tukey's honestly significant difference test, $\alpha = 0.05$.^vPositive values indicate the corolla is longer than the style.

Seven large triploid plants in 20-L pots, which were placed outside for open pollination in a potted *Vaccinium* nursery containing numerous plants of diploid *V. darrowii*, diploid *V. elliotii*, and tetraploid highbush cultivars, flowered heavily but produced only a few berries, all of which were seedless. The diploid and tetraploid plants in the same nursery set full crops of well-seeded berries.

The means for leaf, flower, and berry characteristics for F₁ plants were intermediate to those of the two parents except for stigma position (Table 4). Compared with both parental taxa, the F₁ population had lower Brix and berry firmness and shorter styles relative to the corolla. Leaves, flowers, and berries were much smaller on *V. elliotii* than on the highbush cultivars, and the F₁ hybrids were intermediate for all of these measurements.

Three tetraploid hybrids (N60, N63, and N64) produced by crossing *V. elliotii* #8 as female parent with highbush cultivar FL00-80 were quite fertile when crossed with tetraploid hybrids from the original highbush × *V. elliotii* crosses (Table 5). Most of the F₂ hybrids obtained from these crosses were highly vigorous as seedlings 18 months after they had been transplanted to a high-density field nursery. F₂ seedlings within crosses were

variable in vigor and architecture, and most crosses contained a few weak plants. In the 2017 crosses, four triploid F₁ hybrids produced only one seed from a total of 810 flowers that were pollinated with pollen from tetraploid highbush cultivars (Table 5).

Discussion

In view of the known strength of the triploid block in *Vaccinium*, it was surprising that most of the F₁ hybrids obtained by pollinating 4301 flowers of tetraploid highbush cultivars with pollen from diploid *V. elliotii* were triploid, rather than tetraploid. Apparently, none of the 30 *V. elliotii* plants used as pollen parents produced many unreduced gametes (Kreiner et al., 2017). The seeds from all of the crosses in which *V. elliotii* was the seed parent were planted as one bulked lot, so it could not be determined how many different *V. elliotii* plants gave one or more seedlings.

The reciprocal crosses, in which *V. elliotii* was the seed parent and highbush cultivars were the pollen parents, gave seven seedlings from 2309 pollinated flowers (Table 4). Examination of the pollen showed that six of the hybrids were tetraploid and one was triploid. All seven of the hybrid seedlings came from

the cross in which *V. elliotii* #8 was the female parent. The other four *V. elliotii* plants used in the experiment produced no seeds. When *V. elliotii* clone #8 was emasculated and self-pollinated, no seedlings resulted from 159 pollinated flowers, indicating that the clone is highly self-incompatible.

The number of hybrid plants we obtained by pollinating 4301 flowers of tetraploid highbush blueberries with pollen from diploid *V. elliotii* (≈ 18 triploids and three tetraploids) was extremely low compared with the results of tetraploid × diploid *Solanum* crosses, in which Graebner et al. (2019) obtained 59 triploid and 32 tetraploid hybrids from only 200 pollinated flowers. The number of hybrid seedlings we obtained from tetraploid highbush × diploid *V. elliotii* crosses was also very low compared with the number of hybrids Chavez and Lyrene (2009) obtained from tetraploid highbush × diploid *V. darrowii* crosses.

A mixture of triploid and tetraploid hybrids has been reported by previous workers following diploid-tetraploid crosses in *Vaccinium* (Lyrene and Sherman, 1983; Megalos and Ballington, 1988). Too few hybrids were obtained from these crosses to determine with confidence whether it is better, in terms of the number of hybrids obtained and the ratio of triploid to tetraploid hybrids, to use

Table 5. Results from 2017 crosses, including intercrosses between F₁ hybrids to obtain F₂ populations and backcrosses to tetraploid highbush cultivars.

Type of cross ^z	Female parent and ploidy	Pollen parent and ploidy	Flowers pollinated	Fruit set ^y (%)	PPF ^x	Seedlings in nursery ^w
F ₁ × F ₁	N1 ^v (4x)	N10 (4x)	132	49.2	6.0	110
F ₁ × F ₁	N4 (4x)	N60 (4x)	331	70.4	10.0	98
F ₁ × F ₁	N10 (4x)	N63 (4x)	250	91.6	12.0	113
F ₁ × F ₁	N37 (4x)	N4 (4x)	173	38.2	0.9	NP ^v
F ₁ × F ₁	N60 (4x)	N4 (4x)	11	63.6	20.0	NP
F ₁ × F ₁	N63 (4x)	N10 (4x)	99	64.6	25.6	NP
F ₁ × F ₁	N64 (4x)	N4 (4x)	10	40.0	25.4	22
F ₁ × F ₁	N2 ^u (4x)	N28 ^u (4x)	234	99.2	63.0	251
F ₁ × F ₁	N19 ^u (4x)	N2 ^u (4x)	75	44.0	1.0	NP
F ₁ × F ₁	N28 ^u (4x)	N2 ^u (4x)	254	96.1	21.0	118
F ₁ × F ₁	N36 (4x)	N1 (4x)	34	64.7	2.0	NP
F ₁ × F ₁	N40 (4x)	N36 ^u (4x)	41	43.9	12.9	NP
F ₁ × HB ^t	N64 (4x)	05-603 ^t (4x)	27	44.4	6.2	16
HB × F ₁	05-603 ^t (4x)	N64 (4x)	143	81.8	16.4	101
F ₁ × HB	N30 (3x)	13-168 ^t (4x)	100	99.0	0.1	0
F ₁ × HB	N32 (3x)	11-199 ^t (4x)	337	16.3	0.0	0
F ₁ × HB	N21 (3x)	13-121 ^t (4x)	115	80.0	0.0	0
F ₁ × HB	N22 (3x)	13-16 ^t (4x)	258	11.2	0.0	0

^zF₁ = Highbush × *Vaccinium elliotii*; HB = highbush cultivar.^yNumber of ripe berries harvested/number of flowers pollinated × 100.^xPPF = average number of plump seeds per pollinated flower.^wNumber of seedlings surviving 5 months after they were transplanted to a field nursery. For crosses that produced large numbers of seedlings, only some of the seedlings were saved. For crosses that had more than 100 surviving seedlings in the nursery, the numbers represent only a fraction of the seedlings produced.^vNP = Seeds were not planted.^uPutative F₁ hybrid but resembled highbush in many features; validity is in doubt.^tHighbush cultivar.

the diploid plant as the male parent or as the female parent. Megalos and Ballington (1988) obtained two hybrid seedlings by pollinating 930 flowers of tetraploid highbush cultivars with pollen from *V. elliotii* and six hybrids by pollinating 750 flowers of *V. elliotii* with pollen from highbush cultivars. All of these hybrids were tetraploid. In the same study, they obtained four triploid and three tetraploid hybrids from crosses between tetraploid highbush cultivars and what they described as “diploid pubescent forms of *V. corymbosum*.”

Of the five *V. elliotii* clones whose flowers received pollen from tetraploid highbush, only *V. elliotii* #8 produced seedlings. Previous work has shown that diploid clones within section *Cyanococcus* vary as to whether they produce unreduced gametes, and the clones that produce unreduced gametes vary in the frequency of production (Chavez and Lyrene, 2009; Megalos and Ballington, 1988; Ortiz et al., 1992). Furthermore, some clones produced unreduced microspores but no unreduced megaspores, whereas other clones did the reverse.

Megalos and Ballington (1988) examined the relationship between the frequency of pollen shed in dyads and the ability of various *V. elliotii* and diploid *Vaccinium atrococcum* plants to make seed when crossed with tetraploid highbush cultivars. Diploid plants that produced no unreduced gametes as judged by microscopic examination of the pollen produced no hybrids when crossed with tetraploid highbush. The low frequency of unreduced gametes and the few hybrids obtained made it hard to evaluate the strength of the correlation.

Crosses in which both parents are colchicine-derived tetraploid *V. elliotii* plants produce large numbers of tetraploid seedlings (Norden, 2017). However, diploid

V. elliotii is highly variable in many features, including plant architecture (weeping vs. fastigate), berry size, and berry glaucousness, and it would be desirable to produce fertile highbush × *V. elliotii* hybrids using a diversity of *V. elliotii* genotypes. These diverse hybrids could be produced either by searching in the forest for *V. elliotii* plants that make high frequencies of unreduced gametes or by producing 10 or more tetraploid *V. elliotii* plants using colchicine or oryzalin.

Our tetraploid F₁ hybrids were vigorous and quite fertile when intercrossed, and large F₂ populations could be produced. Rather than use the few available tetraploid F₁ hybrids in backcrosses to highbush cultivars, as was done in previous introgression efforts, we plan to produce and evaluate large F₂ seedling populations and select superior segregates for use in backcrossing. In selecting these plants, emphasis will be given to large, firm berries with aromatic flavors.

Vorsa and Ballington (1991) previously found triploid blueberry hybrids to be either sterile or very low in fertility. In their studies, triploid × diploid crosses and the reciprocals gave no seedlings. Highbush flowers pollinated with pollen from triploids also gave no seedlings. The reciprocal, triploids pollinated with pollen from highbush, gave an average of 0.2 seedlings per pollinated flower. The highest fertility, 0.3 seedlings per pollinated flower, was seen when triploids received pollen from hexaploid hybrids, but the reciprocal crosses produced no hybrids. Whether any of the triploids used in these crosses had *V. elliotii* in their pedigrees is not clear. The diploids were designated as “*V. corymbosum* based on Vander Kloet’s (1980) taxonomic treatment,” which would include Camp’s *V. atrococcum*, *V. elliotii*, and other diploid highbush taxa.

The recessed stigma of *V. elliotii* has been noted previously (Lyrene, 1994; Sampson et al., 2013; Vander Kloet, 1998). This flower structure seems to be unique in *V. elliotii* compared with other section *Cyanococcus* species and might be used as a diagnostic character to separate *V. elliotii* from other highbush *Cyanococcus* species. The inserted stigma and small corolla aperture could make *V. elliotii* dependent on sonicating bees for pollination (Sampson, et al., 2013).

The results of our study show that vigorous, fertile, tetraploid hybrids can be obtained by crossing diploid *V. elliotii* with tetraploid highbush cultivars. The process is not easy, and not many fertile hybrids are obtained. Fortunately, the tetraploid F₁ hybrids produce large F₂ populations when intercrossed and can be easily backcrossed to highbush cultivars, allowing breeders to use *V. elliotii* genes in breeding highbush cultivars.

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