Field Performance of Backcross (BC₁) Blueberry Hybrids of *Vaccinium padifolium* (Section *Hemimyrtilus*) with *V. corymbosum*/*V. angustifolium*

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Abstract. There is ongoing interest in transferring new characteristics into commercial blueberry from other blueberry species. *Vaccinium padifolium* is a species distantly related to commercial blueberry that has traits of notable value to conventional blueberry development. Among these traits are upright structure, strong growth, abundant flowering and fruiting, superior self-fertility, fruit-cluster structure suited to mechanical harvesting, and repeat/continuous flowering. Previously produced F₁ hybrids of *V. padifolium* × *V. corymbosum* were used in crosses with a variety of conventional blueberry selections to generate 13 backcross (BC₁) families. The backcross families were evaluated under field conditions to determine their performance and to guide further use of this germplasm. The offspring varied considerably, but most families produced several individuals of acceptable commercial quality. The recovery of *V. padifolium* characteristics varied. Many plants bore evidence of *V. padifolium* plant structure, but none showed indications thus far of continuous or repeat flowering. The best selections from these families have been saved and will be intermated to generate the next cycle of this material. These clones will also be crossed to hybrid combinations of *V. arctostaphylos* and *V. cylindraceum* to further diversify and recombine this germplasm.

Blueberries (family *Ericaceae*, genus *Vaccinium*, commonly section *Cyanococcus*) are a diverse taxonomic group, and blueberries currently in commercial production represent three major *Vaccinium* species and two ploidy levels: 4x *V. angustifolium* (low-bush blueberry), 4x *V. corymbosum* (high-bush blueberry), and 6x *V. virgatum* (syn. *V. ashei*; rabbiteye blueberry). As such, these three types may be considered the primary gene pool of blueberry. Two other commercial types of blueberry, half-high blueberry cultivars and southern highbush cultivars, are mixtures using these species. Half-highs have been produced by hybridization of 4x *V. corymbosum* with 4x *V. angustifolium* and retain a significant but variable percentage contribution from each species. Southern highbush cultivars have been developed by the introgression of low-chilling-requirement species, primarily 2x *V. darrowii* and 6x *V. virgatum* into 4x *V. corymbosum* at contribution levels that average around 25% in modern cultivars. Several additional species from

the secondary gene pool have also contributed small amounts of germplasm to named blueberry cultivars—among them, 2x *V. elliotii*, 6x *V. constablaei*, and 2x *V. tenellum*.

Section *Hemimyrtilus* and Its Species

Vander Kloet and Dickinson (1992) considered the species of section *Hemimyrtilus* to be the remnants of a once widely distributed taxon. Today, six species comprise section *Hemimyrtilus*, and three of these are found in limited localities. The species and their ranges are *V. cylindraceum* (Azores, Portugal), *V. padifolium* (Madeira Islands, Portugal), *V. arctostaphylos* (Caucasus Region), *V. smallii* (Korea, Japan, Russia), *V. yakushimense* (Yakushima Island, Kyushu, Japan), and *V. hirtum* (Japan, South Korea).

V. padifolium, our primary species of concern, is native only to the Madeira Islands and is typically found in subalpine landscapes at altitudes of 1220 to 1700 m. Plants are bushy to treelike, and typically 1 to 4 m tall; under native conditions, plants possess an evergreen habit. Under our conditions, *V. padifolium* is insufficiently cold-hardy to thrive outdoors. In general, plants have tough, semiglossy leaves with a finely reticulated texture. Plants produce creamy, pink-tinged, bell-shaped flowers that give rise to medium-blue, ovate fruit (M.K. Ehlenfeldt, personal observations). Vander Kloet and Dickinson (2005) noted the development of

a nonperenniating floral buds as a character of *V. padifolium*. The occurrence of nonperenniating buds has implications for the season of flower bud development, the differentiation time of flowers within a bud, and the location of such buds (often as adventitious buds on older wood). Our *V. padifolium* parental clone is notable for its profuse and continuous flowering and its high number of flower buds located on both young and old wood. Continuous/repeat flowering is a trait that, if introgressed into *V. corymbosum*, may allow multiple crops or continuous cropping. Other useful traits of *V. padifolium* include its general vigor, large mature plant size, excellent fertility, good fruit size, and general self-fertility. Its evergreen nature may be a useful trait under some conditions but might also present management problems in terms of physiology and/or allowing the plants to act as insect or pathogen reservoirs under field conditions.

Utilization Value of the *Hemimyrtilus* Species

Ballington (2001) reported several inter-sectional crosses of *V. cylindraceum* with both 2x and 4x germplasm of section *Cyanococcus* and other sections. Ballington generated BC₁ hybrids with *V. corymbosum*, several of which were grown under field conditions to observe their performance. Of the limited number of hybrids produced, the majority failed to thrive; however, a few BC₂ and F₂ selections continued to be evaluated on an ongoing basis (Ehlenfeldt and Ballington, 2012). These hybrids used only *V. cylindraceum* and *V. corymbosum* germplasm.

A significant challenge in breeding with exotic species hybrids is the recovery of the necessary horticultural plant type, and especially deriving plants with fruit morphology that is neither too small nor too dark to meet commercial standards. In a previous paper, we reported on the generation of fertile 4x hybrids between *V. padifolium* and *V. corymbosum* and subsequent backcrosses to cultivated germplasm (Ehlenfeldt and Polashock, 2014). The goal in working with these species is to incorporate germplasm from this section into cultivated germplasm and transfer the desirable traits that these species possess for mechanical harvesting and commercial production. The objective we address here is the evaluation of hybrids and BC₁ derivatives under field conditions to understand strengths and weaknesses and to determine where further effort needs to be applied.

Materials and Methods

Materials. We generated 13 families using US 1896 (= *V. padifolium* US 908 × *V. corymbosum* US 1825) as either a female or a male. The details of the origin of US 1896 have been described previously (Ehlenfeldt and Polashock, 2014). Of the 13 families, two used US 1896 as a female and 4x *V. corymbosum* ('Bluecrop' and ARS 98-15, respectively) as males. Eleven families were

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generated using US 1896 as a male in crosses using commercial cultivars and selections as females. These cultivars were Biloxi, Bluecrop, Brunswick (lowbush), Cara's Choice, Duke, Hannah's Choice, Legacy, Northsky (half-high), Sharpblue, Sweetheart, and TH 622. The details of these crosses were described previously (Ehlenfeldt and Polashock, 2014). All of the parents listed are named cultivars except for ARS 98-15 and TH 622. ARS 98-15 is a breeding selection of the pedigree 'Duke' × 'Nelson'. The selection TH 622 is a sibling of the southern highbush cultivars Camellia (NeSmith and Draper, 2007) and Gupton (Stringer et al., 2012) and comes from the cross of MS 122 × MS 6. The cross MS 122 × MS 6 was notable for productive, vigorous plants with large, pale blue fruit.

To confirm that the plants evaluated were true hybrids, we evaluated a subsample of 34 genotypes using a genotyping by sequencing (GBS) approach (Elshire et al., 2011). DNA extraction and GBS library construction were as detailed in Daverdin et al. (2017). The libraries were sent to Novogene Corporation Inc. (Sacramento, CA) and sequenced on the Illumina (San Diego, CA) HiSeq platform (PE150). GBS sequencing data were demultiplexed using STACKS process_radtags, and contigs were generated using the default STACKS pipeline denovo_map.pl (Catchen et al., 2011). BWA-mem and samtools were used to perform read alignment and call single nucleotide polymorphisms (SNPs) against contigs generated by the STACKS de novo pipeline (Li, 2011; Li and Durbin, 2009). Homogeneous SNPs were filtered out, SNPs unique to each parent were cataloged, and SNPs from offspring matching parent-type SNPs were subsequently binned and counted. In all cases, the maternal parent is known, so the focus of the data analyses was to confirm that all progeny inherited at least some SNPs from the putative pollen parent.

Progeny from all crosses were grown for 9 months in the greenhouse and were planted to the field in June 2015 (Table 2). Families were planted in three adjacent rows spaced at 2 feet within rows and 8 feet between rows, with rows averaging 650 feet long. For this evaluation, families were planted in consecutive order with no replication. Chatsworth, NJ, is located at 39.8° N latitude and 74.5° W longitude. New Jersey has a moderate climate, with cold winters and warm, humid summers. Chatsworth's average midsummer high (July) is 30.5 °C; the average wintertime low (January) is -5.0 °C. The planting was made on Berryland soil and maintained using standard growing practices. Evaluations were made in 2018.

To assess general field performance, plants were evaluated for survival, vigor, height, bloom density, percent bloom, 50% ripe date, fruit size, and fruit color. Limited evaluations were made for fruit firmness and flavor/volatiles.

- Survival was assayed at the first evaluation date, 18 Apr. 2018. Any plant that had

resumed growth no matter how poor was considered a survivor.

- Vigor was evaluated in mid-June. Rating was done on a scale of 0 to 3, where 0 = struggling, 1 = fair vigor, 2 = average vigor, and 3 = above average vigor.
- Height was determined at the end of the summer season (22 Aug. 2018) by measuring each plant to the nearest 5 cm.
- At the time of first inspection, bloom density data were also taken. Flowering intensity was rated with subjective notations of no flowers, almost no flowers, light flowering, average flowering, and vigorous flowering, but for the purposes of this report, flowering intensity was reduced to a binary (0–1) scale with 0 = no flowers or almost no flowers, and 1 = categories from light flowering to vigorous flowering.
- Bloom timing was compared by measuring percent bloom at a single date, 17 May 2018.
- A 50% ripe date was determined by evaluating/rating the percent of ripe fruit weekly from 20 June to 26 July. To calculate a 50% ripeness date, the dates bracketing a potential 50% value were converted to ordinal dates and linearly interpolated to determine a 50% ripeness date. Values were rounded to the nearest whole day. After averaging across clones to derive a family mean, ordinal dates were back-converted to calendar dates.
- Fruit color was rated on a 1 to 5 scale, where 1 = light blue, 2 = light-medium blue, 3 = medium blue, 4 = dark blue, and 5 = any one of several shades including very dark blue-black, dusky black, or black. Commercial cultivars would be expected to rate as 2 or lower. Values were averaged across families, and for reporting purposes, average color values were additionally back-converted to subjective color descriptions.
- Fruit size was rated on a 1 to 4 scale, where 1 = very small, 2 = small, 3 = medium, and 4 = large. For fruit size, 0.5 increments were used as needed. Commercial cultivars would be expected to rate as 3 or greater. As was done for fruit color values, fruit size values were averaged across families, and for reporting purposes, average fruit size values were also back-converted to subjective descriptions.

To better understand the families we had generated, the evaluations rated the plants, calculated means, and determined standard deviations where meaningful. Additionally, not every evaluation could be made on every clone; for example, no 50% ripeness date could be generated for a plant that failed to flower, so observation numbers are listed parenthetically in both tables and text.

Results and Discussion

Propagules of our two original *V. padifolium* × *V. corymbosum* hybrids, US 1896 and US 1897, were set into the field in 2012.

Neither proved to be winter-hardy; both showed substantial dieback. After a year in the ground, US 1897 was no longer alive. US 1896 continues to persist but is winter damaged each year to a considerable extent. Propagules of both of these selections were subsequently sent to U.S. Department of Agriculture, Agricultural Research Service in Poplarville, MS, for field planting and evaluation (still underway).

The BC₁ generation seed, when first germinated and planted out to seedling flats, showed considerable segregation from weak to vigorous. It was expected that this level of segregation might occur in the progeny of such wide hybrids, and it was additionally expected that many of these combinations might be suboptimal. When seedlings were planted in the field, the weakest and most dwarfed types were eliminated.

Genotyping by sequencing. Of 952,013,696 total sequencing reads, 755,924,283 were available after de-multiplexing and filtering for matching barcodes. The STACKS de novo pipeline generated 737,894 contigs. After read alignment and SNP calling of reads back to the de novo generated contigs, the sum total of unique SNPs from all female parents was 16,990, whereas the male parent, US 1896, exhibited 1,583 unique SNPs (Table 1). On average, individual offspring matched ≈652 total unique SNPs across all varieties used as females in this study, whereas the bulk of the matching SNPs were identified from at least one parent in the pedigree (Table 1). Most of the unique SNPs could be attributed to the pollen parent, US 1896. This suggests that for all of the clones evaluated, the progeny are not selfs and are indeed true hybrids.

Morphology. In the BC₁ cross generation, ancestry was 25% *V. padifolium* and 75% cultivated germplasm (i.e., *V. corymbosum*, *V. angustifolium*, and lesser amounts of *V. darrowii* and *V. virgatum*) thus much of the morphology tended toward cultivated phenotypes (*V. corymbosum*/*V. angustifolium*). A casual observer of these families in the field would not recognize any significant differences from highbush. The offspring with 'Northsky' as a parent tended to have shorter, bushier, and more branching plant structure; offspring with 'Cara's Choice' were intermediate in stature; offspring with TH 622 were more upright, robust plants. Bush structure appeared little different from comparable highbush type crosses; however, on larger plants, canes were more robust, but possibly also more brittle. Some, but not excessive, breakage was observed.

The two main features that generally distinguished these hybrids, were leaf morphology and variant shoot development. Regarding leaf morphology, although similar in size to cultivated germplasm, leaves were generally flatter and less textured than those of highbush, but venation appeared slightly more prominent against this flatter texture (Fig. 1). The parent US 1896 possesses a noticeably patterned leaf surface, and its leaves are relatively coarse; thus some of this morphology appears to have been inherited.

Table 1. Unique parental single nucleotide polymorphisms (SNP) variants exhibited in a subset of parents and progeny selections (parents of a given selection are identified by gray shading).

	Unique SNPs								Male US 1896
	Females								
	Biloxi	Cara's Choice	Duke	Legacy	Northsky	Sharpblue	Sweetheart	TH 622	
Selections	1507	2177	3030	2725	111	275	6313	852	1583
US 2403	10	28	18	29	2	2	24	16	158
US 2405	2	20	2	3	2	3	1	1	44
US 2406	11	130	23	22	0	1	43	11	261
US 2407	13	215	32	29	5	4	100	12	376
US 2408	30	284	38	55	0	1	98	18	388
US 2409	30	22	81	26	0	7	37	7	256
US 2410	15	11	55	20	2	8	12	7	205
US 2412	16	25	29	250	0	2	73	7	317
US 2413	21	21	27	176	1	2	53	16	284
US 2415	13	34	20	17	11	3	44	5	213
US 2416	4	12	7	16	14	1	17	1	106
US 2417	17	31	28	25	21	2	53	22	207
US 2418	1	30	37	22	21	1	50	1	203
US 2419	5	9	10	13	9	3	22	2	81
US 2420	6	14	14	6	17	3	24	10	134
US 2426	0	1	2	4	18	1	2	3	45
US 2428	16	24	38	22	14	0	38	6	182
US 2429	3	9	17	18	11	1	20	13	123
US 2430	64	21	28	28	1	23	41	21	218
US 2431	56	20	15	19	2	14	34	16	214
US 2432	29	9	10	19	0	16	27	8	151
US 2433	20	5	7	4	2	11	6	0	110
US 2434	100	41	57	55	1	19	82	33	327
US 2435	147	45	65	65	0	31	120	30	395
US 2436	44	54	80	69	0	5	1188	24	461
US 2437	26	29	36	53	0	1	514	5	414
US 2438	37	59	95	106	0	2	1461	19	488
US 2439	28	32	39	44	0	4	593	10	404
US 2440	62	124	200	199	0	7	406	138	462
US 2441	49	66	79	78	3	9	108	114	386
US 2442	25	33	31	46	0	8	52	92	293
US 2443	39	77	72	72	0	6	139	127	350
US 2444	34	64	43	53	1	10	77	128	337
US 2446	42	60	75	50	0	7	107	116	364

Table 2. Survival, vigor ratings, and height measurement averages for *V. padifolium* backcross (BC₁) families to 4x *V. corymbosum*/*V. angustifolium* material at Chatsworth, NJ, 2018 (sample sizes denoted in parentheses).

Pedigree	Planted (no.)	Surviving (no.)	Survival (%)	Vigor rating ± sd (0–3 scale) ²	Mean plant ht ± sd (cm) ³	Tallest selection (cm)
US 1896 × Bluecrop	13	0	0.0	—	—	—
US 1896 × ARS 98-15	20	0	0.0	—	—	—
Biloxi × US 1896	75	4	5.3	1.8 ± 1.3 (4)	53 ± 29	90
Bluecrop × US 1896	68	8	11.8	2.3 ± 1.0 (8)	62 ± 16	85
Brunswick × US 1896	7	0	0.0	—	—	—
Cara's Choice × US 1896	108	42	38.9	1.9 ± 1.3 (41)	55 ± 19	100
Duke × US 1896	105	40	38.1	2.4 ± 1.0 (39)	58 ± 13	90
Hannah's Choice × US 1896	65	2	3.1	1.3 ± 1.5 (3)	53 ± 26	75
Legacy × US 1896	103	34	33.0	1.5 ± 1.3 (34)	51 ± 21	95
Northsky × US 1896	100	62	62.0	2.0 ± 1.1 (62)	40 ± 13	75
Sharpblue × US 1896	94	34	36.2	1.4 ± 1.3 (36)	51 ± 17	85
Sweetheart × US 1896	135	29	21.5	1.6 ± 1.3 (29)	61 ± 26	120
TH 622 × US 1896	103	29	28.2	2.0 ± 1.3 (31)	70 ± 21	115
Average	996	284	28.5			

²Rating scale of 0 to 3, where 0 = struggling, 1 = fair vigor, 2 = average vigor, and 3 = above average vigor.

³Height was measured to the nearest 5 cm at the end of the summer season (22 Aug. 2018).

Also unusual was the development of variegated, and sometimes totally chlorophyll deficient shoot development (Fig. 2A and B). This is believed to be the result of nuclear-cytoplasmic interactions of this exotic material with cultivated germplasm (Correns, 1909). In conventional crosses, variegation, although not frequent, is not unusual in combinations that mix early-maturing germplasm (generally containing greater lowbush ancestry) with midseason or late-season maturing germplasm. What

was somewhat unusual in these offspring was the vigor of the totally chlorophyll deficient shoots derived from some crosses (Fig. 2B). Flowers on these hybrids did not appear notably different from *V. corymbosum* material.

Survival. The field plot was not uniform in its drainage or soil conditions, and these plants were not planted in replicates; thus, a uniform estimate of survival was not feasible. Nonetheless, the survival rankings across families appeared generally to represent the

potential relative responses typical for the families (Table 2). Across all families (a total of 996 plants), survival averaged 28.5% (284 plants). Notably, for the two crosses with US 1896 as a female parent, no offspring survived (both were relatively small families). The reasons for this are unclear, but it is a distinct possibility that these were selfs of US 1896 (these were not genotyped). As noted, it was recognized that US 1896 had poor survival under New Jersey field conditions. Among families using US 1896 as a male,

survival across families ranged from 0.0% ('Brunswick' × US 1896) to 62.0% ('Northsky' × US 1896). Of potential interest in these two contrasting families is that lowbush introgression via 'Brunswick' produced a family apparently totally unadapted to our test conditions (although sample size was small, 7 individuals). This cross had also been less successful in terms of fruit set and total seed generated initially (Ehlenfeldt and Polashock, 2014). In contrast, the family with lowbush-highbush introgression via 'Northsky' exhibited superior survival capability (62% survival, 62 individuals).

Vigor. A number of plants had cane die-back (presumably from cold damage), but often these plants were growing back from ground level or from a branch near ground level. On the rating scale of 0 to 3, vigor ratings ranged from a high of 2.4 ± 1.0 (39 individuals) in a family of 'Duke' × US 1896 to lows of 1.3 ± 1.5 (three individuals) for a small family of 'Hannah's Choice' × US 1896, and similarly, 1.4 ± 1.3 (36 individuals) for the family of 'Sharpblue' × US 1896 (Table 2). The 2.4 value represents a family of average to above average vigor. The 1.3/1.4 values represent fair to average vigor.

Plant height. Height was measured to the nearest 5 cm at the end of the summer season (22 Aug 2018) (Table 2). Average height ratings ranged from 40 ± 13 cm (62 individuals) for 'Northsky' × US 1896 to 70 ± 21 cm

(31 individuals) for TH 622 × US 1896. The best plant in the TH 622 × US 1896 family had a height of 120 cm. The best plant in 'Northsky' × US 1896 had a height of 75 cm. A height of 140 to 170 cm might be considered ideal (approximately chest high to head high); a height of 100 cm would also be considered acceptable (approximately waist high).

Bloom intensity. Bloom intensity was summarized on a 0 to 1 scale with 0 = plants with no flowers or almost no flowers and 1 = light flowering to vigorous flowering. This value essentially described how many plants within the family bloomed. Bloom ratings ranged from 0.2 (17 individuals) for the family of 'Sweetheart' × US 1896 (i.e., ≈20% total flowering) to 0.8 (33 individuals) for 'Cara's Choice' × US 1896 (≈80% total flowering) (Table 3). Although many factors can influence flowering, for our purposes flowering, is considered at least a partial proxy for winter floral-bud hardiness, and plants with weak flowering were ultimately discarded.

Bloom timing. Bloom timing should be examined with the proviso that for plants with light flowering, it was often hard to judge flowering percentages critically. As a result, the listed data reflect only clones for which flowering percentage could be reasonably estimated (Table 3); in a number of families, this resulted in observation numbers lower than those subsequently used for fruit color ratings and other factors. Bloom timing was examined on 17 May 2018, a date that was considered to be mid-late bloom, allowing relative ranking of families. Upon observation, it was apparent that a number of individual plants in several families had already reached 100% bloom before this date. These are noted separately from the plants that were not yet at 100% bloom and are listed as 100+. Bloom on 17 June 2018 ranged from 61% [61% (five individuals); 100+ (one individual)] for the family TH 622 × US 1896% to 90% [90% (two individuals); 100+ (three individuals)] for the family 'Sharpblue' × US 1896. As a check on these estimates, bloom percentage was also calculated including the 100+ values as exactly 100%. Although it shifted values

slightly, rankings remained essentially unchanged (data not shown). On the basis of the observed bloom, it can be concluded that these materials flowered relatively early, and their bloom timing may be similar to that of southern highbush; thus, climate adaptation may be a concern in selecting for their further utilization.

Ballington (2001) reported several inter-sectional crosses of *V. cylindraceum* with both 2x and 4x germplasm of section *Cyanococcus* and other sections. Ballington generated BC₁ hybrids with *V. corymbosum*, several of which were grown under field conditions in North Carolina to observe their performance. These BC₁ hybrids used only *V. cylindraceum* germplasm from section *Hemimyrtillus*. The observed hybrids exhibited good fertility but were early flowering and often sustained frost damage.

Fifty percent ripeness dates. Ripeness was rated weekly from 20 June to 26 July 2018. By the last evaluation date, only five plants had not completed ripening, and all five of these were at 90% ripeness or greater. These late ripening selections were distributed across four families. Fifty percent (50%) ripeness dates ranged from 26 June ± 5 (12 individuals) for 'Duke' × US 1896 to 5 July ± 4 (seven individuals) for TH 622 × US 1896. The cultivar Duke was rated at 60% ripeness on 20 June 2018. Thus, the family with 'Duke' might be considered relatively early ripening; however, most of these families would be considered, on average, to be midseason, and some clones were considerably later to ripen. For families of sufficient size, most standard deviation values were relatively equivalent.

Time and practical constraints did not allow specific assays for fertility, but ripeness estimates may be considered a partial/limited proxy for fertility because to estimate ripeness, it was necessary not only to have a parent with relatively good flowering but also to have a plant that subsequently set adequate fruit to allow a ripeness estimation. Pollination was not expected to be an issue because many seedling families of typical highbush were grown in adjacent evaluation rows.

Fruit color. Fruit color was rated on a 1 to 5 scale with 1 being lightest and 5 darkest. Across families, fruit color tended toward darker than commercially standard values (2 = commercially acceptable). Our F₁ parent, US 1896, had relatively dark fruit and a mottled wax coating, and on the current scale, it would be considered 3 to 3.5. For offspring families, fruit color means ranged from 2.5 ± 0.8 (eight individuals; light-medium blue/medium blue) for the TH 622 × US 1896 family to 3.5 ± 0.7 (11 individuals; medium/dark blue) for the 'Duke' × US 1896 family. Although the family means were all greater than 2, five of 13 families had individual clones with acceptable commercial ratings of 2 (Table 3; Fig. 3A and B). It is worth noting that only the TH 622 × US 1896 family had any clones receiving a rating of 1 (light blue). As a southern highbush selection, the TH 622 parent had particularly pale blue fruit. In

Fig. 1. Leaf morphology of *V. corymbosum* 'Bluecrop' (left) and a backcross (BC₁) clone of *V. padifolium*, US 2445 (= TH 622 × US 1896) (right) at Chatsworth, NJ, 2018.

Fig. 2. Variegation and chlorophyll deficient shoots in backcross (BC₁) families of *V. padifolium* to 4x *V. corymbosum*/*V. angustifolium* material at Chatsworth, NJ, 2018: (A) US 2425 (= 'Northsky' × US 1896) and (B) US 2428 (= 'Northsky' × US 1896).

Table 3. Averaged bloom ratings, 50% ripeness date, fruit color, and fruit size ratings for *V. padifolium* backcross (BC₁) families to 4x *V. corymbosum*/*V. angustifolium* material at Chatsworth, NJ, 2018 (sample sizes denoted in parentheses).

	Pedigree	Bloom Rating 2018 (0–1 scale) ^z	Bloom on 17 May 2018 (%) ^y	50% ripe date ± sd (days)	Fruit color ratings (1–3) ^x			Fruit size ratings (1–4) ^w			
					Color range (1–3 scale)	Mean value ± sd	Equivalent color (blue)	Size range (1–4 scale)	Mean size value ± sd	Equivalent size	
Biloxi Bluecrop Cara's Choice	×	US 1896	0.3 (3)	10 (1)	3	3 (1)	Medium	3.25	3.25 (1)	m-ml	
	×	US 1896	0.6 (7)	58 (2); 100+ (2)	5 July ± 8 (3)	3	3 (2)	Medium	2.5–3.0	2.8 ± 0.3 (3)	ms-m
	×	US 1896	0.8 (33)	87 (14); 100+ (6)	30 June ± 5 (25)	2–4	2.9 ± 0.7 (27)	Medium	1.0–3.0	2.6 ± 0.5 (26)	ms
Duke Hannah's Choice Legacy Northsky Sharpblue Sweetheart TH 622	×	US 1896	0.3 (36)	78 (4); 100+ (4)	26 June ± 5 (12)	2–4	3.5 ± 0.7 (11)	Medium-dark	2.0–3.5	2.9 ± 0.5 (9)	m
	×	US 1896	0.5 (3)	100+ (1)	7 July (1)	4	4 (1)	Dark	3.5	3.5 (1)	ml
	×	US 1896	0.3 (27)	63 (4); 100+ (3)	5 July ± 6 (9)	3–4	3.2 ± 0.4 (9)	Medium	2.0–3.5	2.8 ± 0.6 (9)	ms-m
	×	US 1896	0.6 (54)	74 (22); 100+ (7)	2 July ± 4 (33)	2–5	3.2 ± 1.1 (34)	Medium	1.0–3.5	2.5 ± 0.5 (35)	ms
	×	US 1896	0.4 (22)	90 (2); 100+ (3)	3 July ± 4 (22)	3–4	3.4 ± 0.6 (15)	Medium-dark	2.5–3.5	2.8 ± 0.3 (13)	ms-m
	×	US 1896	0.2 (27)	85 (3); 100+ (2)	3 July ± 6 (6)	2–4	2.8 ± 0.8 (6)	Medium	2.0–3.0	2.3 ± 0.4 (6)	s-ms
	×	US 1896	0.3 (25)	61 (5); 100+ (1)	5 July ± 4 (7)	1–3	2.5 ± 0.8 (8)	Light medium-medium	2.5–3.5	3.0 ± 0.3 (8)	m

^yFlowering was rated on a 0 to 1 scale: 0 = plants with no flowers or almost no flowers, 1 = light flowering to vigorous flowering.

^zThe cultivar Duke was rated at 60% bloom on 17 June 2018.

^wFruit color was rated on a 1 to 5 scale: 1 = light blue, 2 = light-medium blue, 3 = medium blue, 4 = dark blue, and 5 = any one of several ratings including: very dark blue/black, dusky black, or black.

^wFruit size was rated on a 1–4 scale: 1 = very small, 2 = small, 3 = medium, and 4 = large.
s = small; m = medium, l = large.

contrast, only the 'Northsky' × US 1896 family had any individuals receiving a rating of 5 (blue-black to black).

Fruit size. Fruit size was rated on a 1 to 4 scale with 1 smallest and 4 largest. Family means for fruit size ranged from 2.5 ± 0.5 (35 individuals; small) for 'Northsky' × US 1896 to 3.0 ± 0.3 (eight individuals; medium) for TH 622 × US 1896. Five families had individual clones that rated as high as 3.5 (medium-large). The parents of these families were 'Duke', 'Legacy', 'Sharpblue', TH 622, and, rather notably, 'Northsky'.

Fruit quality. Fruit quality was given only limited, subjective evaluation, and no clones were noted that had exceptional fruit firmness. Most clones appeared to have firmness comparable to traditional northern highbush. Similarly, fruit flavor/volatiles exhibited no perceptible unusual qualities; in fact, in early ripening stages, it was a concern that the fruit was undistinguished with regard to volatiles; however, when sufficiently ripened, fruit quality was also within normal ranges for northern highbush flavor.

Perhaps the most interesting question to pose is whether any clones exhibited unusual flowering patterns similar to *V. padifolium*. Vander Kloet and Dickinson (2005) labeled the flowering mode of *V. padifolium* as indeterminate. Manifestations of indeterminate flowering by their delineation include inflorescences developing without perennation, ongoing flower differentiation within or along a floral axis even after initial bloom, and, as an outcome of this, high flower numbers. Our concern in this respect was monitoring mainly for nonspecific, continuous, or possibly a second flowering/cropping cycle. Thus far, under our growth conditions and cultural practices, no return bloom or similar manifestations have been observed in any of these clones, but such behavior may depend on maturity and specific seasonal conditions.

Conclusions

A survey of 13 BC₁ families having US 1896 as a male parent showed that several families had individual clones that produced fruit of acceptable commercial quality. This is perhaps not surprising because these offspring are 75% cultivated germplasm. Among these families, the best combinations with respect to plant stature and fruit characteristics were those with southern highbush or intermediate northern highbush clones with higher percentages of introgressed *V. darrowii*.

With respect to recovery of useful *V. padifolium* traits, morphological observations demonstrated that *V. padifolium* influences were inherited and exhibited with respect to leaf morphology and some aspects of plant structure. With respect to continuous or repeat flowering, however, no obvious expressions were observed. As of now, little is known about dilution or inheritance of these traits, so it may be expected that further recombination

Fig. 3. Examples of retained clones of backcross (BC_1) families of *V. padifolium* to $4x$ *V. corymbosum* material at Chatsworth, NJ, 2018: (A) US 2408 (= 'Cara's Choice' $\times$ US 1896) and (B) US 2411 (= 'Legacy' $\times$ US 1896).

among *Hemimyrtilus* hybrids, without further dilution of the *V. padifolium* component, might be needed to achieve the desired goals. It was beyond the scope of this initial study to assay any broader range of parental combinations or possibilities.

The best selections from these families have been retained (Fig. 3A and B), and it is planned to intermate among these clones to generate the next cycle of this material. Among experimental materials, the breeding program has several clones of 'Sharpblue' $\times$ *V. padifolium* (US 2157, US 2312, US 2315-A, US 2315-B), a clone of *V. padifolium* $\times$ *V. arctostaphylos* (US 1971), and a clone of *V. cylindraceum* $\times$ *V. arctostaphylos* (US 2318) that are planned to be integrated into these breeding

populations to further diversify and further recombine this germplasm.

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