Interspecific Crosses and Backcrosses between Diploid *Vaccinium darrowii* and Tetraploid Southern Highbush Blueberry

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ABSTRACT. Diploid *Vaccinium darrowii* Camp has been used in breeding tetraploid southern highbush blueberry (*Vaccinium corymbosum* L.) as a source of reduced chilling requirement, adaptation to hot, wet summers, and resistance to leaf diseases. *V. darrowii* in Florida is quite variable, but most crosses have involved only one *V. darrowii* clone, Fla. 4B. The use in breeding of a wider range of *V. darrowii* accessions would provide beneficial diversity in the blueberry cultivated gene pool. The purpose of this research was to determine the functional 2n gamete frequency of numerous *V. darrowii* genotypes when crossed with tetraploid *V. corymbosum*, and to study the pollen fertility and backcross ability of the interspecific (*V. darrowii* × *V. corymbosum*) hybrids to *V. corymbosum*. Crosses between diploid *V. darrowii* and tetraploid highbush blueberry cultivars had low fruit set compared with the *V. darrowii* × *V. darrowii* and highbush × highbush crosses. The unusually high number of hybrids per pollinated flower (HPF) in certain 4x-2x or 2x-4x crosses was attributed to high functional 2n gamete production in certain *V. darrowii* genotypes. Diploid *Vaccinium fuscatum* Aiton and diploid *V. darrowii* × *V. fuscatum* hybrids, when crossed with southern highbush blueberry cultivars, were equally productive of hybrids whether used as male or female parents. Variation in frequency of functional 2n gametes in *V. darrowii*, expressed as high HPF, was present within plants (megaspores vs. microspores) and among *V. darrowii* plants. Of the 114 interspecific (*V. darrowii* × *V. corymbosum*) hybrids studied, 106 had pollen stainability >50%. This indicated that most of these hybrids were tetraploid, because triploid blueberries, like most triploid plants, are highly sterile. Twenty-two *V. darrowii* × *V. corymbosum* hybrids were backcrossed to tetraploid highbush blueberry cultivars. Fruit set was variable, but large populations of vigorous hybrids were obtained. Lower fruit set was associated with hybrids that had lower pollen fertility. It should be possible to obtain plants of cultivar quality in a few generations of backcrosses.

Vaccinium L. section *Cyanococcus* L. includes diploid (2n = 2x = 24), tetraploid (2n = 4x = 48), and hexaploid (2n = 6x = 72) species native to North America (Camp, 1945; Vander Kloet, 1983, 1988). The main cultivated groups are lowbush blueberry (*Vaccinium angustifolium* Aiton), highbush blueberry (*Vaccinium corymbosum* and their hybrids), and rabbiteye blueberry (*Vaccinium virgatum* Aiton). Since the establishment of blueberry breeding programs, genes from various wild blueberry relatives have been introgressed into the cultivated gene pool. In the blueberry breeding program at the University of Florida, *Vaccinium myrsinites* Lam. (4x), *Vaccinium darrowii* (2x), and *V. virgatum* (6x), were successfully used as parents to reduce the chilling requirement of northern highbush blueberries (Goldy and Lyrene 1984; Moore, 1965; Sharpe, 1953; Sharpe and Darrow, 1959).

Hybridization between tetraploid northern highbush blueberry cultivars and diploid *V. darrowii* was first reported by Sharpe and Darrow (1959). *V. darrowii* clones Fla. 4A and Fla. 4B were used in these foundation crosses (Sharpe and Sherman, 1971). From 1600 pollinations, 31 fertile hybrids were obtained (Sharpe and Darrow, 1959). The 2n gametes in *V. darrowii* and a strong triploid block in *Vaccinium* allowed tetraploid hybrids to be obtained from these crosses (Cockerham and Galleta, 1976; Den Nijs and Peloquin, 1977; Goldy and Lyrene, 1984; Johnston et al., 1980; Lyrene and Sherman, 1983; Marks, 1966;

Moore, 1965; Ortiz et al., 1992a, 1992b; Sharpe and Darrow, 1959; Woodell and Valentine, 1961).

Undesired characteristics from *V. darrowii* (i.e., short, twiggy plant structure, small fruit, and long fruit development period) were eliminated by crossing and selection. Acceptable fruit and plant quality were obtained. Phenotypically, the F₁ interspecific hybrids were intermediate between the parents, and when the hybrids were backcrossed to tetraploid southern highbush blueberry cultivars, seedlings with variable morphology and high fertility were obtained (Lyrene, 1997). All selections from the southern highbush blueberry program at Gainesville, FL, have *V. darrowii* in their pedigree (Sharpe and Sherman, 1971).

Obtaining fertile tetraploid hybrids by crossing *V. darrowii* with tetraploid highbush blueberry depends on 2n gamete production in *V. darrowii*, which is infrequent and confined to certain *V. darrowii* genotypes. Several methods have been used to determine the 2n gamete frequency of diploid species. Stelly and Peloquin (1983) estimated the frequency of 2n gamete production in various diploid *Solanum* L. plants by microscopic examination of pollen sporads (2n pollen) and whole-ovule preparations (cell nuclei size and 2n eggs), and by crossing diploid and tetraploid species. No correlation was found between the results of microscopic examination and the results of the crosses. Megalos and Ballington (1988) made crosses between diploid and tetraploid blueberry species. They found that diploid *Vaccinium elliottii* Chapm. clones that produced numerous tetraploid seedlings when crossed with one tetraploid highbush blueberry did not necessarily produce numerous tetraploid hybrids when crossed with a different

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tetraploid plant. Bretagnolle and Thompson (1995) counted 4x seeds or seedlings after 2x-4x and 4x-2x crosses to assess 2n pollen and/or 2n egg production in individual diploid plants of potato and other genera. The purpose of our research was to study the functional 2n gamete frequency in plants of *V. darrowii* present in Florida when crossed with tetraploid *V. corymbosum* by determining the number of hybrids produced per cross, their level of fertility, and their ability to cross with tetraploid southern highbush blueberry and other *Vaccinium* species.

Materials and Methods

Plant material

HYBRIDIZATION STUDIES. Diploid *V. darrowii* clones, diploid *V. darrowii* × *V. fuscatum* hybrids, and tetraploid southern highbush blueberry (*V. corymbosum*) cultivars were used in hybridization. *V. darrowii* clones selected in the Florida panhandle and in the central Florida peninsula and *V. darrowii* × *V. fuscatum* hybrids selected from a moist wood in the peninsula near Davenport, FL, were propagated by softwood cuttings. The selected plants were vigorous and healthy and had highly glaucous foliage. The *V. darrowii* panhandle clones (Lyrene, 1986) were from Cotton Landing, Sumatra, Wilma, and Chattahoochee, FL, all in or near the Apalachicola National Forest. The *V. darrowii* clones from the Florida peninsula (Lyrene, 1986) were selected from Davenport, near Haines City, and from Highlands County, FL. Softwood cuttings of the *V. darrowii* and *V. darrowii* × *V. fuscatum* hybrids were rooted in trays of sphagnum peat and perlite (1:1). Rooted cuttings were transplanted into 4-L nursery containers containing sphagnum peat and perlite (1:1) and were maintained in a greenhouse in Gainesville, FL. *V. darrowii* and *V. darrowii* × *V. fuscatum* hybrids are evergreen, and have no chilling requirement.

Tetraploid southern highbush blueberry (*V. corymbosum* hybrids) cultivars or advanced selections were selected from the University of Florida blueberry breeding program's field nursery. The plants were dug from the field in Dec. 2006, were transplanted into 8-L nursery containers filled with sphagnum peat and perlite, and were chilled for more than 1000 h in a walk-in cooler at 5 °C with no light. After being chilled, the southern highbush blueberry plants were placed inside a bee-proof greenhouse.

POLLEN STAINABILITY. Plant material used to assess pollen fertility included two *V. darrowii* × *V. corymbosum* hybrids from crosses made in 2004, three from crosses made in 2005, and 109 from crosses made in 2006. Plants were grown in Citra, FL, in a high-density nursery. These plants were selected from the University of Florida blueberry germplasm collection because they were the only seedlings from these crosses whose vegetative morphology clearly showed they were hybrids. Plants were transplanted to 4-L nursery containers and placed in a cooler at 5 °C with no light on 20 Dec. 2007. In late Jan. 2008, they were moved to a bee-proof greenhouse in Gainesville, FL.

BACKCROSSING EXPERIMENTS. Plants used in crosses with southern highbush blueberry cultivars (4x) included two *V. darrowii* × *V. corymbosum* hybrids from crosses made in 2004, three interspecific hybrids from crosses made in 2005, and 17 interspecific hybrids from crosses made in 2006. The plants were collected from field nurseries at University of Florida

Plant Science Unit in Citra, FL, transplanted to 8-L nursery containers of sphagnum peat, and placed in a cooler at 5 °C with no light in December. The interspecific plants from crosses in 2006 were not given additional chilling beyond what they received in the field. In late January, the selected interspecific plants were placed in a bee-proof greenhouse in Gainesville, FL.

Plant material used in reciprocal crosses with *V. virgatum* (6x) included two *V. darrowii* × *V. corymbosum* hybrids with low pollen fertility from crosses made in 2004 and 2005, and one interspecific hybrid with high pollen fertility from crosses made in 2005. The interspecific hybrids were transplanted from the field and placed in a cooler at 5 °C with no light on 20 Dec. 2006. In late Jan. 2007, they were placed in a bee-proof greenhouse as described before.

Plant material used in crosses with *V. darrowii* (2x) included two of the *V. darrowii* × *V. corymbosum* hybrids that had high pollen fertility, FL07-111-F₁ and FL07-112-F₁, derived from crosses made in 2005. The interspecific hybrids were moved from a greenhouse in Gainesville, FL, and were placed in a cooler at 5 °C with no light in Dec. 2007. The *V. darrowii* clones were selected from the germplasm collection available at the University of Florida in Gainesville. They were evergreen and were not chilled. In late Jan. 2008, the selected interspecific hybrids and the *V. darrowii* clones were placed in a bee-proof greenhouse.

Hybridization studies

Open flowers from the plants selected as females were removed to avoid unplanned pollination. Female plant-to-plant variation was eliminated as a confounding factor by dividing individual plants into two parts to compare intraspecific crosses (*V. darrowii* × *V. darrowii* and *V. corymbosum* × *V. corymbosum*) with interspecific crosses [*V. darrowii* × *V. corymbosum* and reciprocal, and (*V. darrowii* × *V. fuscatum*) hybrids × *V. corymbosum* and reciprocal] (Morrow, 1943). On each plant, one side was randomly assigned to an interspecific cross and the other to an intraspecific cross. Flowers were emasculated before pollination by removing the corolla and anthers with forceps. Pollination was performed by touching the stigma with pollen that had been collected a few minutes earlier on the thumbnail. For *V. darrowii* × *V. darrowii* crosses, ≈250 flowers were pollinated per plant, and 150 flowers were pollinated per plant for highbush × highbush crosses. For interspecific crosses, ≈500 flowers were pollinated per plant.

Berries were harvested when fully ripe. The first 20 berries that ripened were opened individually to determine seed content. Seeds from additional berries were extracted using a food blender, after which they were dried at room temperature on a desk top and stored in coin envelopes at 5 °C for 3 months.

Determination of functional 2n gamete frequency

Seeds from each cross were counted and classified into two categories: plump or shriveled. The number of plump seeds per pollinated flower was calculated for each cross. In Nov. 2007, seed packages of crosses made in 2007 were treated with a fungicide (captan at 0.1 g per package) and planted on the top layer of 4-L nursery containers of sphagnum peat. The nursery containers were maintained in a greenhouse with intermittent mist for 2 to 3 months, until most seeds had germinated. The number of seedlings per pollinated flower for each cross was calculated.

In Feb. 2008, when the seedlings from the interspecific crosses were 1 to 2 cm high, they were transferred to trays of sphagnum peat, 100 seedlings each. In May 2008, when the seedlings were 4 to 8 cm high, they were planted in a high-density nursery in Citra, FL, with plant-to-plant spacing of 10 cm and row-to-row spacing of 40 cm. The high-density nursery was maintained with overhead irrigation and fertilization when required. After 60 d in the high-density nursery, the seedlings were counted and classified into two categories: hybrids or selfs based on visual comparisons of morphology or resemblance to the female parent (Figs. 1–3). The strong triploid block in *Vaccinium* and the relatively low frequency of 2n gametes required that a very large numbers of flowers be pollinated (Cockerham and Galleta, 1976; Goldy and Lyrene, 1984; Marks, 1966; Moore, 1965; Lyrene and Sherman, 1983; Ortiz et al., 1992a, 1992b; Sharpe and Darrow, 1959; Woodell and Valentine, 1961). With so many flowers pollinated, low levels of selfing may have occurred and the morphology of seedlings was examined to confirm that they were in fact interspecific hybrids.

Pollen stainability of *V. darrowii* × *V. corymbosum* hybrids

Open flowers were collected to determine pollen stainability in January and Feb. 2008. Flowers were dried and pollen was stained with 1% aceto-carmin solution (1 g of carmine boiled in 45 mL of glacial acetic acid + 55 mL of H₂O). Microphotographs were taken using a Moticam 1000 1.3-megapixel microscope digital camera with the Motic Images Plus Version 2.0ML software (Motic Instruments, Richmond, British Columbia, Canada) mounted on a phase-contrast microscope (Leitz, Wetzlar, Germany). About 80 random pollen grains were examined for each plant, and the percentage of stained grains was determined.

Sterile pollen grains in water or aceto-carmin appeared shriveled, unstained, or abnormal in shape, and had varying degrees of pollen inflation (Dermen, 1940). Abnormal pollen grains may result from chromosome pairing abnormalities during meiosis (Goldy and Lyrene, 1984; Lyrene and Sherman, 1983).

Fig. 1. Comparative differences in leaf morphology between diploid *Vaccinium darrowii* (left), interspecific (*V. darrowii* × *Vaccinium corymbosum*) hybrids (center), and tetraploid *V. corymbosum* (right). Leaves were taken from 2-year-old plants.

Fig. 2. Comparative differences in flower and fruit morphology for diploid *Vaccinium darrowii* (left), interspecific (*V. darrowii* × *Vaccinium corymbosum*) hybrids (center), and tetraploid *V. corymbosum* (right). Flowers and fruits were taken from 2-year-old plants.

Fig. 3. Comparative differences in plant architecture of diploid *Vaccinium darrowii* (left), interspecific (*V. darrowii* × *Vaccinium corymbosum*) hybrid (center), and tetraploid *V. corymbosum* (right). The plastic container (not including the plant) on the left is 30 cm tall. Plants are 2 years old.

Backcrossing experiments

INTERSPECIFIC (*V. DARROWII* × *V. CORYMBOSUM*) HYBRIDS × SOUTHERN Highbush BLUEBERRY. The selected interspecific plants were placed in a bee-proof greenhouse for use as parents in crosses with tetraploid southern highbush blueberry cultivars or advanced selections. The interspecific hybrids were used as female parents with southern highbush blueberry cultivars, except for one from crosses made in 2005 that was also used as male parent. Emasculation and pollination were done as described above. The interspecific hybrids from crosses made in 2004 and 2005 were divided into two sections. One section was pollinated with pollen from tetraploid southern highbush blueberry cultivars. The other section was pollinated with pollen from hexaploid *V. virgatum* 'Florida Rose' (Clark and Finn, 2006). About 250 flowers were pollinated per section for each plant in Feb. 2007. The interspecific hybrids from crosses

made in 2006 were not divided into sections. Between 30 and 500 flowers on each plant were pollinated in Feb. 2008. Fruit harvest, seed extraction, seed planting, and seedling identification were as described in the hybridization studies.

INTERSPECIFIC HYBRIDS $\times$ *V. VIRGATUM* AND RECIPROCALLS. Two *V. darrowii* $\times$ *V. corymbosum* hybrids that had low pollen fertility were crossed as male and female parents with hexaploid *V. virgatum* cv. Florida Rose. The idea was that the low-fertility *V. darrowii* $\times$ *V. corymbosum* hybrids might be triploid, and might produce 2n gametes that, when fused with normal gametes from hexaploid *V. virgatum*, would produce hexaploid hybrids. One *V. darrowii* $\times$ *V. corymbosum* hybrid with high pollen fertility, from crosses made in 2005, was also used as male and female parent in crosses with 'Florida Rose'. About 250 flowers were pollinated per section for each plant. Fruit harvest, seed extraction, seed planting, and seedling identification were as described above.

INTERSPECIFIC HYBRIDS $\times$ *V. DARROWII* AND RECIPROCALLS. *Vaccinium darrowii* $\times$ *V. corymbosum* hybrids FL07-111-F₁ and FL07-112-F₁, which had high pollen fertility, were selected as male and female parents for backcross to diploid *V. darrowii*. The two *V. darrowii* clones used as male and female parents in these crosses were known from earlier crosses to be high 2n egg producers. The goal was to obtain tetraploid plants with 75% of their genes derived from *V. darrowii*. About 500 flowers were pollinated for each cross. Fruit harvest and seed extraction were as described above.

Data analysis

Data collected for each cross included fruit set percentage, number of plump seeds per pollinated flower (PPF), number of seedlings per pollinated flower (SPF), and number of hybrids per pollinated flower (HPF). Means for PPF, SPF, and HPF for different treatments were separated using least squares means by Tukey's test, with a significance level of 5%. Means for fruit set percentage were separated using chi-square "test of independence," with a significance level of 5%. In these chi-square tests, each pollinated flower was considered an observation with two possible responses: set or nonset. The null hypothesis of the chi-square "test of independence" was that the ratio of set to nonset was the same for the two crosses being compared. Data analysis and analysis of variance (ANOVA) were performed using the PROC GLM, and PROC FREQ procedures of SAS (version 9.1; SAS Institute, Cary, NC).

Results and Discussion

Hybridization studies

Several diploid *V. darrowii* clones produced a high frequency of functional 2n gametes when crossed to tetraploid southern highbush blueberry cultivars. No association was found between functional 2n pollen and 2n egg production for *V. darrowii* clones—that is, clones that frequently produced 2n pollen were not those that frequently produced 2n eggs. Variation in frequency of functional 2n gamete production in diploid *V. darrowii* was present within plants (megaspores vs. microspores) and among plants within *V. darrowii* races. *V. darrowii* clones that produced numerous fertile hybrids when used as males in 4x-2x crosses did not necessarily produce many hybrids when used as females in 2x-4x crosses, and vice versa. Exceptional clones allowed us to produce many hybrids from 4x-2x and 2x-4x crosses.

Number of seedlings per pollinated flower and HPF for *V. darrowii* (2x) $\times$ *V. corymbosum* (4x) and reciprocals varied widely among crosses. Previous *V. darrowii* (2x) $\times$ *V. corymbosum* (4x) crosses [in 2001 and 2005 (P.M. Lyrene, unpublished data)] produced numerous seedless berries, and most of the seedlings obtained were not hybrids. From 4739 pollinations of *V. darrowii* clones from Lake Istokpoga (Istokpoga race), only three interspecific hybrids were obtained [0.001 HPF (data not shown)]. This compares with 133 hybrids obtained from 7340 pollinations when *V. darrowii* clones from the Ocala National Forest (Ocala race) were used [0.021 HPF (data not shown)]. No cross in 2001 or 2005 produced more than 0.100 HPF. Fruit set ranged from 0% to 93.4% in the interspecific crosses made in 2006 (P.M. Lyrene, unpublished data). One *V. darrowii* clone from the Florida panhandle race (FL03-421-P) crossed with a composite of pollen from highbush blueberry cultivars gave unusually high values for PPF, SPF, and HPF (140 interspecific hybrids were obtained by pollinating 1406 flowers) compared with the eight other crosses made in 2006 (three interspecific hybrids from 2498 pollinated flowers for all other crosses combined). The higher production of hybrids was attributed to high functional 2n egg production by FL03-421-P.

Three *V. darrowii* clones, FL03-421-P, FL03-422-P, and FL06-660-I, averaged higher in PPF, SPF, and HPF than the other seven *V. darrowii* clones used as females in *V. darrowii* $\times$ *V. corymbosum* crosses in 2007 ($P < 0.05$) (Table 1). No association between higher fruit set and higher HPF was found in 2007. This was due to high levels of parthenocarpic berry production in some *V. darrowii* clones. Crosses made in 2007 confirmed the results of 2006. FL03-421-P produced a high functional 2n egg frequency when crossed with southern highbush blueberry cultivars. Although pollinations were a laborious method of screening for functional 2n gamete production, they provided repeatable results.

Fruit set ranged from 0.9% to 44.0% (Table 1) in *V. corymbosum* (4x) $\times$ *V. darrowii* (2x) crosses made in 2007. Low fruit set was expected in these crosses because of the difference in ploidy levels between the parents. Three interspecific crosses, FL06-446-HB $\times$ FL03-419-I, FL07-92-HB $\times$ FL03-418-I, and FL-95-50-HB $\times$ FL03-423-P, gave surprisingly high PPF, SPF, and HPF compared with the mean of the seven other *V. corymbosum* (4x) $\times$ *V. darrowii* (2x) crosses made in 2007 ($P < 0.0001$). Unusually high levels of functional 2n gamete production in the diploid *V. darrowii* parents are the most likely reason for the high success rate in these crosses. Fruit set from intraspecific crosses averaged 78.1% for *V. corymbosum* and 74.3% for *V. darrowii* (Table 2) in 2007.

OVERALL COMPARISONS. Six diploid *V. darrowii* clones were used as pollen and seed parents in crosses with tetraploid highbush blueberry cultivars in 2007 (Table 3). One *V. darrowii* clone (FL03-421-P) produced far more hybrid seedlings when used as seed parent. Three others (FL03-419-I, FL03-418-I, and FL03-423-P) gave more hybrids when used as pollen parents. *Vaccinium darrowii* FL03-421-P gave 40.8 hybrids per 100 pollinated flowers when used as seed parent, but only 5.9 when used as pollen parent ($P < 0.0001$). FL03-419-I gave only 4.5 hybrids per 100 pollinated flower when used as a seed parent compared with 34.2 when used as pollen parent. Two other *V. darrowii* clones, FL03-418-I and FL03-423-P, also produced far more hybrids when used as pollen parents [$P < 0.0001$ in each case (Table 3)]. When *V. darrowii* clones were compared, a clone's success as a pollen parent was not

Table 1. Number of flowers pollinated, and fruit, seed, and hybrid seedling production in interspecific crosses between *Vaccinium darrowii* and southern highbush blueberry (*Vaccinium corymbosum*) cultivars and reciprocals.

Female		Male	Flowers (no.)	Berries (no.)	Fruit set (%)	PPF (no.) ^x	SPF (no.) ^x	HPF (no.) ^x
<i>V. darrowii</i>	×	Highbush						
FL03-414-I ^z	×	FL03-228-HB	540	94	17.4	0.44	0.078	0.022
FL03-404-I	×	FL03-286-HB	667	112	16.8	0.35	0.024	0.000
FL03-421-P	×	FL00-59-HB	544	220	40.4	1.24	0.426	0.408
FL03-419-I	×	FL92-84-HB	644	237	36.8	1.97	0.087	0.045
FL03-418-I	×	FL01-297-HB	554	38	6.9	0.11	0.023	0.000
FL03-422-P	×	FL88-53-HB	541	225	41.6	1.80	0.153	0.125
FL03-416-I	×	Jewel-HB	573	268	46.8	0.79	0.058	0.003
FL03-423-P	×	FL01-63-HB	701	229	32.7	1.09	0.023	0.003
FL06-660-I	×	Southern Belle-HB	551	302	54.8	6.23	0.161	0.152
FL03-402-I	×	FL07-49-HB	551	7	1.3	0.19	0.087	0.000
Highbush	×	<i>V. darrowii</i>						
FL06-446-HB ^y	×	FL03-419-I	569	138	24.3	2.33	0.342	0.342
FL02-16-HB	×	FL03-421-P	596	29	4.9	0.15	0.059	0.059
FL07-92-HB	×	FL03-418-I	400	133	33.3	1.22	0.460	0.460
FL06-468-HB	×	FL06-712-P	549	11	2.0	0.01	0.004	0.004
FL07-108-HB	×	FL03-404-I	557	5	0.9	0.03	0.011	0.011
FL06-25-HB	×	FL06-716-P	528	46	8.7	0.06	0.002	0.002
FL95-50-HB	×	FL03-423-P	549	155	28.2	0.35	0.093	0.093
FL01-277-HB	×	FL06-702-P	505	27	5.4	0.10	0.081	0.079
Southern Belle-HB	×	FL03-414-I	533	28	5.3	0.14	0.043	0.043
FL00-45-HB	×	FL06-719-I	359	158	44.0	0.25	0.042	0.042

^zDiploid *V. darrowii*, -P = Florida panhandle race, -I = Istokpoga race.

^y-HB = tetraploid *V. corymbosum*.

^xPPF = plump seeds per pollinated flower, SPF = seedlings per pollinated flower, HPF = hybrids per pollinated flower.

Table 2. Results of crossing diploid and tetraploid Florida *Vaccinium* species in section *Cyanococcus* measured by fruit set (%), number of plump seeds per pollinated flower (PPF), seedlings per pollinated flower (SPF), and hybrids per pollinated flower (HPF).

Cross ^z	Ploidy of parents	Flowers (no.)	Fruit set (%)	PPF (no.) ^w	SPF (no.) ^w	HPF (no.) ^w
HB × HB	4x × 4x	652	78.1 a ^y	7.92 ab	17.518 a	— ^x
HB × DFNH	4x × 2x	506	4.4 i	0.12 b	0.010 b	0.010 a
HB × VD-P	4x × 2x	2,727	9.8 h	0.13 b	0.046 b	0.046 a
HB × VD-I	4x × 2x	2,418	21.5 g	0.79 b	0.178 b	0.178 a
DFNH × HB	2x × 4x	499	67.8 b	0.43 b	0.020 b	0.020 a
VD-P × HB	2x × 4x	1,786	38.2 e	1.38 ab	0.200 b	0.180 a
VD-I × HB	2x × 4x	4,080	25.8 f	1.44 ab	0.074 b	0.031 a
VD × VD	2x × 2x	1,496	74.3 a	15.28 a	4.535 ab	—
F ₁ × HB	? × 4x	1,351	57.5 c	9.66 ab	2.464 ab	—
HB × F ₁	4x × ?	280	49.3 d	6.81 ab	1.925 ab	—

^zHB = *Vaccinium corymbosum*, DFNH = natural hybrid (*Vaccinium darrowii* × *Vaccinium fuscatum*), VD-P = *V. darrowii* Florida panhandle race, VD-I = *V. darrowii* Istokpoga race, F₁ = interspecific hybrid (*V. darrowii* × *V. corymbosum*).

^ySimilar letters within a column indicate means not significantly different. Tukey's test for PPF, SPF, and HPF at $\alpha = 0.05$; chi-square "test of independence" for fruit set at $\alpha = 0.05$.

^x— = no data. Because the parents are fully cross-compatible, almost all the seedlings from controlled crosses are expected to be hybrids.

^wPPF = plump seeds per pollinated flower, SPF = seedlings per pollinated flower, HPF = hybrids per pollinated flower.

related to its success as a seed parent. Because most of the hybrids produced from our crossing experiments have been fertile (and presumably tetraploid), hybrid production rate from these crosses indicated the rate of functional 2n gamete production in the *V. darrowii* parent. Megalos and Ballington (1988) reported similar results. They found that the frequency of 2n gametes varied among *Vaccinium* species and among clones within species. They also found that the frequency of 2n gametes production differed for megaspores and microspores in the same plant.

Two diploid natural hybrids of *V. darrowii* × *V. fuscatum*, collected from a moist forest in the central Florida peninsula,

were used in reciprocal crosses with tetraploid highbush blueberry cultivars. Fruit set percentage was 15 times higher when the natural hybrids were used as the seed parent compared with the reciprocals, but the berries had very few seeds ($P < 0.05$) (Table 2). Lyrene (P.M. Lyrene, unpublished data) obtained no hybrid seedlings after pollinating ≈ 1000 flowers of tetraploid cultivars with pollen from two diploid *V. fuscatum* plants from eastern Alachua County, FL. Overall, diploid *V. darrowii*, diploid *V. fuscatum*, and diploid interspecific (*V. darrowii* × *V. fuscatum*, natural hybrids), when crossed with southern highbush blueberry cultivars, were equally productive of hybrids whether used as male or female parents.

Table 3. Results of reciprocal interspecific crosses between *Vaccinium darrowii* and southern highbush blueberry (*Vaccinium corymbosum*) cultivars.^a The goal was to see whether it was better to use *V. darrowii* as the seed parent or the pollen parent. *V. darrowii* clone names end in –I or –P and tetraploid highbush blueberry names end in HB.

Female ^b		Male	Flowers (no.)	Berries (no.)	Fruit set (%)	P:100F ^c (no.)	S:100F ^c (no.)	H:100F ^c (no.)
FL03–414-I	×	FL03–228-HB	540	94	17.4	44.9	7.8	2.2
Southern Belle-HB	×	FL03–414-I	533	28	5.3	13.9	4.3	4.3
				<i>P</i> value	<0.0001	<0.0001	0.3281	0.4021
FL03–404-I	×	FL03–286-HB	667	112	16.8	35.4	2.4	0.0
FL07–108-HB	×	FL03–404-I	557	5	0.9	3.4	1.1	1.1
				<i>P</i> value	<0.0001	<0.0001	0.5974	0.2513
FL03–421-P	×	FL00–59-HB	544	220	40.4	124.2	42.6	40.8
FL02–16-HB	×	FL03–421-P	596	29	4.9	15.3	5.9	5.9
				<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
FL03–419-I	×	FL92–84-HB	644	237	36.8	196.7	8.7	4.5
FL06–446-HB	×	FL03–419-I	569	138	24.3	233.3	34.2	34.2
				<i>P</i> value	<0.0001	0.0088	<0.0001	<0.0001
FL03–418-I	×	FL01–297-HB	554	38	6.9	10.6	2.3	0.0
FL07–92-HB	×	FL03–418-I	400	133	33.3	121.9	46.0	46.0
				<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
FL03–423-P	×	FL01–63-HB	701	229	32.7	108.8	2.3	0.3
FL95–50-HB	×	FL03–423-P	549	155	28.2	35.0	9.3	9.3
				<i>P</i> value	0.0917	<0.0001	0.0133	0.0010
Totals:								
VD	×	HB	3,650 ^w	930	25.2 ^u	86.7	11.0	7.9
HB	×	VD	3,204	488	16.1	70.5	16.8	16.8
				<i>P</i> value	<0.0001 ^v	0.6370	0.1490	0.0345

^aNote that the *V. darrowii* clone in each case is identical in the reciprocal pairs, but the southern highbush blueberry clones change. All the southern highbush blueberry clones used are highly fertile.

^bDiploid *V. darrowii* (VD), –P = Florida panhandle race, –I = Istokpoga race; tetraploid *V. corymbosum* (HB) represented by –HB.

^cP:100F = plump seeds per 100 pollinated flower, S:100F = seedlings per 100 pollinated flower, H:100F = hybrids per 100 pollinated flower.

^wTotal values per cross type.

^vChi-square “test of independence” at $P \leq 0.01$. Comparisons were made within columns and between reciprocal crosses.

^uMean averages per cross type.

As expected, the crosses between species of the same ploidy level made in 2007, *V. darrowii* × *V. darrowii* and *V. corymbosum* × *V. corymbosum*, had higher fruit set (74.3% and 78.1%, respectively) than the tetraploid × diploid crosses ($P < 0.05$, Table 2). The homoploid crosses had higher PPF and SPF than the 4x-2x or the 2x-4x crosses. Although the means differed substantially, they were not significantly different at $P = 0.05$ due to the low number of replicates.

Pollen stainability of *V. darrowii* × *V. corymbosum* hybrids

Vaccinium darrowii × *V. corymbosum* hybrid pollen stainability was highly variable among clones. Of the five interspecific hybrids produced in crosses made in 2004 and 2005, FL06–105-F₁ and FL07–110-F₁ had low pollen stainability (14.5% and 21.4%, respectively), and FL06–107-F₁, FL07–111-F₁, and FL07–112-F₁ had high pollen stainability (99.4%, 95.5%, and 98.3%, respectively). Crosses made in 2006 produced 109 hybrids whose average pollen stainability was 88.1%. Pollen stainability less than 50% was found in 5.57% (six plants) of the 109 interspecific hybrids made in 2006 (Fig. 4). These plants had shriveled pollen with abnormal shapes that failed to stain with 1% aceto-carmin (Dermen, 1940). Pollen stainability was greater than 50% in 103 of the 109 hybrid plants (94.9%). The interspecific hybrids with high pollen stainability were probably tetraploids, and interspecific hybrids with low pollen stainability may have been triploids or aneuploids (Cockerham and Galleta, 1976; Goldy and Lyrene, 1984; Lyrene and Sherman, 1983). The tetraploid interspecific hybrids should be easy to backcross to the tetraploid gene pool. The putative triploid interspecific hybrids could potentially be backcrossed to hexaploid *V. virgatum* through 2n gamete production. In this way, genes from *V. darrowii* and *V. corymbosum* could be moved to the *V. virgatum* gene pool (Ehlenfeldt and Vorsa, 1993).

Fig. 4. Histogram showing the distribution of plants for percentage of pollen staining in a population of 109 interspecific (*Vaccinium darrowii* × *Vaccinium corymbosum*) hybrids.

Hybrid FL07-110-F₁ (*V. darrowii* × *V. corymbosum*) produced numerous 2n microspores and unstained tetrads as seen under the microscope. Meiotic abnormalities during pollen formation probably produced the many abnormal tetrads (unstained sporads) and the 2n pollen sporads. FL07-110-F₁ was probably triploid because of the reduced pollen fertility and the high 2n gamete production (Cockerham and Galleta, 1976; Goldy and Lyrene, 1984; Lyrene and Sherman, 1983). In a preliminary study, hybrid FL06-105-F₁ was determined to be triploid (2n = 3x = 36) by cytogenetic studies of pollen mother cells during metaphase I and prophase I (data not shown).

Backcrossing experiments

INTERSPECIFIC (*V. DARROWII* × *V. CORYMBOSUM*) HYBRIDS × SOUTHERN Highbush BLUEBERRY. The interspecific (*V. darrowii* × *V. corymbosum*) hybrids were backcrossed to southern highbush blueberry cultivars and selections. Fruit set for these crosses was variable. Lower fruit set was associated with interspecific hybrids with low pollen fertility, FL06-105-F₁ and FL07-110-F₁. Fruit set for the backcrosses made in 2007 ranged from 3.2% to 97.8% (Table 4). Low fruit set, 3.2% and 36.3%, occurred in crosses when the interspecific hybrids FL07-110-F₁ and FL06-105-F₁ were the seed parents. These two interspecific hybrids had low pollen stainability. The other four backcrosses had fruit set from 49.3% to 97.8%. The interspecific hybrids used in these backcrosses had high pollen stainability. PPF and SPF were associated with pollen stainability of the interspecific hybrids (Table 4).

Fruit set values for *V. darrowii* × *V. corymbosum* hybrids pollinated with pollen from tetraploid highbush blueberry cultivars in 2008 ranged from 0.3% to 86.9%. SPF for these

crosses averaged 4.41, twice the SPF value for backcrosses made in 2007. Pollen stainability for the population of these interspecific hybrids was highly variable (Fig. 4). Of the 17 interspecific hybrids used in these backcrosses, three hybrids had low pollen stainability (data not shown). These results were similar to those in 2007. The diploid × tetraploid crosses, *V. darrowii* × *V. corymbosum* and reciprocals, as expected, had much lower fruit set and PPF than the homoploid crosses (Table 2). These results are consistent with the findings of the previous studies (Darrow et al., 1949; Meader and Darrow, 1944; Rousi, 1966; Sharpe, 1953; Sharpe and Darrow, 1959).

Berry weight was highly variable depending on the female parent. The largest berries were produced by highbush blueberry parents (1.1–2.0 g) and the smallest by *V. darrowii* (0.3–0.4 g). Berry weight of the interspecific hybrids was intermediate (mean = 1.0 g) and probably too small for a commercial blueberry cultivar. The number of large seeds per berry was highest for highbush × highbush crosses (31.7 large seeds per berry), lowest for the interploid crosses (3.1–8.7 large seeds per berry), and intermediate for crosses between highbush blueberry and the interspecific hybrids (11.8–13.1 large seeds per berry). Seed production in highbush blueberry × interspecific hybrids and reciprocal was sufficient to produce large backcross populations.

INTERSPECIFIC HYBRIDS × *V. VIRGATUM* AND RECIPROCALLS. Crosses of the interspecific (*V. darrowii* × *V. corymbosum*) hybrids × *V. virgatum* gave few seeds. Even though the number of seedlings obtained was low, the interspecific hybrids with lower pollen stainability were successfully crossed with the hexaploid *V. virgatum* ‘Florida Rose’ (Table 4). Fruit set was

Table 4. Result of hybridization of *Vaccinium darrowii* × *Vaccinium corymbosum* hybrids with other Florida *Vaccinium species*, measured by fruit set (%), and number of plump seeds (PPF) and seedlings per pollinated flower (SPF).

Cross ^z			Flowers (no.)	Berries (no.)	Fruit set (%)	PPF (no.) ^w	SPF (no.) ^w
F ₁ seedlings	×	Highbush ^y					
FL06–105-F ₁	×	Jewel-HB	281	102	36.3	0.45	0.050
FL 07–110-F ₁	×	Jewel-HB	254	8	3.2	0.04	0.012
FL07–111-F ₁	×	FL 95–12-HB	270	264	97.8	23.17	5.160
FL 06–107-F ₁	×	FL 95–12-HB	282	179	63.5	11.41	2.773
FL 07–112-F ₁	×	Jewel-HB	264	229	86.7	13.23	4.328
Highbush	×	F ₁ seedlings ^y					
FL 02–16-HB	×	FL 07–112-F ₁	280	138	49.3	6.81	1.925
F ₁ seedlings	×	<i>Vaccinium virgatum</i> ^y					
FL 06–105-F ₁	×	Florida Rose-R	270	44	16.3	0.14	0.044
FL 07–110-F ₁	×	Florida Rose-R	279	5	1.8	0.08	0.039
FL 07–112-F ₁	×	Florida Rose-R	244	81	33.2	1.28	0.131
<i>V. virgatum</i>	×	F ₁ seedlings ^y					
Florida Rose-R	×	FL 06–105-F ₁	211	0	0.0	0.00	0.000
Florida Rose-R	×	FL 07–110-F ₁	259	4	1.5	0.16	0.012
Florida Rose-R	×	FL 07–112-F ₁	289	5	1.7	0.08	0.007
F ₁ seedlings	×	<i>V. darrowii</i> ^x					
FL 07–111-F ₁	×	FL 06–660-I	528	47	8.9	0.25	0.047
FL 07–112-F ₁	×	FL 06–660-I	293	13	4.4	0.19	0.092
<i>V. darrowii</i>	×	F ₁ seedlings ^x					
FL 03–421-P	×	FL 07–111-F ₁	507	166	32.7	0.36	0.055
FL 03–421-P	×	FL 07–112-F ₁	491	165	33.6	0.67	0.096

^z-F₁ = interspecific hybrid [*V. darrowii* × southern highbush blueberry (*V. corymbosum*) cultivars], -HB = highbush blueberry cultivars. R = hexaploid *V. virgatum* (syn = *Vaccinium ashei*) cv. Florida Rose, I = *V. darrowii* Istokopoga race, P = *V. darrowii* Florida panhandle race.

^yCrosses made in 2007.

^xCrosses made in 2008.

^wPPF = plump seeds per pollinated flower, SPF = seedlings per pollinated flower.

16.3% for FL06–105-F₁ and 1.8% for FL07–110-F₁ when crossed as seed parents with *V. virgatum*. FL07–112-F₁ fruit set was 33.2%. PPF and SPF were proportional to fruit set. SPF was very low for all of these crosses, but highest for the cross with FL07–112-F₁. It is not known whether these *V. darrowii* × highbush blueberry hybrids were triploid, tetraploid, or aneuploid. Some of these hybrids crossed with *V. virgatum* could produce hexaploid, pentaploid, or near pentaploid progeny (Ehlenfeldt and Vorsa, 1993; Lyrene, 1997). Most of the hybrid seedlings, after one growing season in the field, were highly vigorous and appeared to be valid hybrids, but their chromosome numbers have been not yet determined.

INTERSPECIFIC HYBRIDS × *V. DARROWII* AND RECIPROCALLS. The preliminary results in 2008 indicate that backcrosses to *V. darrowii* can be successful. The *V. darrowii* × *V. corymbosum* hybrids that had the highest pollen staining were just as successful as tetraploid highbush blueberry cultivars in backcrosses to diploid *V. darrowii* (Table 4).

Fruit set for interspecific (*V. darrowii* × *V. corymbosum*) hybrids × *V. darrowii* and reciprocal was lower when the interspecific hybrids were used as seed parents. Increased fruit set for *V. darrowii* × interspecific hybrids may have been due to 2n egg production by *V. darrowii* or might indicate a higher level of parthenocarpy (Table 4). PPF and SPF have not been a good estimate of the number of hybrid seedlings obtained, and until the seedlings are examined during flowering, we will not know how many tetraploid hybrids have been produced from the backcrosses.

Mean berry weight was highly variable depending on the female parent. The number of large seeds per berry was high (mean = 13.1 large seeds per berry) in the tetraploid homoploid crosses between F₁ (*V. darrowii* × *V. corymbosum*) hybrids and *V. corymbosum* and low in the tetraploid × diploid crosses between F₁ hybrids and *V. darrowii* (2x) and reciprocals (3.2 and 2.6 large seeds per berry, respectively). *Vaccinium corymbosum* (4x) × *V. darrowii* (2x) and reciprocal crosses produced an average of 3.1 and 8.7 large seeds per berry, respectively (data not shown).

Summary

Backcrossing *V. darrowii* × *V. corymbosum* hybrids to several *Vaccinium* species (including the cultivated gene pool) produced large populations of hybrids. These should make it possible to obtain plants of cultivar quality in a few generations of backcrosses with desired traits from wild species. The interspecific hybrids with high pollen fertility were easily backcrossed to southern highbush blueberry cultivars. (Cockerham and Galleta, 1976; Goldy and Lyrene, 1984; Lyrene and Sherman, 1983; Sharpe and Darrow, 1959).

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