Fertility of Triploid Highbush Blueberry

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Abstract. Eight highbush blueberry (V. corymbosum L.) triploids (2n = 3x = 36) were crossed with diploids (2n = 2x = 24), tetraploids (2n = 4x = 48), and hexaploids (2n = 6x = 72). No plants were recovered from 4021 3x × 2x crosses. One triploid was relatively fertile in 3x × 4x and 3x × 6x crosses, which is most likely attributable to 2n gamete production in the triploid. The lack of fertility of triploids, which do not produce 2n gametes, in crosses with diploids and tetraploids suggests that the production of gametes with numerically balanced (n = 12 or 24) chromosome numbers is extremely low. In addition, the inability to recover progeny from 3x × 2x crosses also suggests that aneuploid gametophytes and/or zygotes, including trisomics, are inviable in blueberry. Pollen stainability was also highly reduced in triploids. Frequency distributions of anaphase I pole chromosomal constitutions of three triploids were significantly different from one another. Two of the three distributions were shifted toward the basic chromosome number of 12, with one triploid having 25% poles with 12 chromosomes. However, the sterility of 3x × 2x and 2x × 3x crosses indicates that lagging chromosomes during meiotic anaphases are probably not excluded from gametes, resulting in unbalanced gametes in blueberry. Triploids can be used as a bridge to facilitate gene transfer from the diploid and tetraploid levels to the hexaploid level in blueberry.

Aneuploids have been useful in genetic studies involving gene localization and for gaining greater insight into the basic nature of the genome, with trisomies having been particularly useful (Khush, 1973; Khush et al., 1984). The most common and dependable source of trisomics in many plant species have been triploids. The frequency of trisomics recovered from triploid by diploid crosses depends on the species and, in many cases, the direction in which the cross is made (Levan, 1942). In some species, however, triploid by diploid crosses are either completely unsuccessful, as in aster (Avers, 1954), or produce only a few diploid progeny, as in watermelon (Kihara, 1951). Aneuploid series between the tetraploid and hexaploid levels have been developed in blueberry (Vorsa, 1988; Vorsa et al., 1986). However, the usefulness of these aneuploids for localizing genes and linkage groups is limited since these aneuploids are not phenotypically distinguishable from one another or from euploids (Vorsa et al., 1986). Polyploidy is considered to have a buffering effect on the imbalance caused by aneuploidy (Khush, 1973). Thus, the four-genome minimum present in these aneuploids may obscure any aneuploid effects.

A trisomic series derived from triploids in blueberry could be useful in gaining a better understanding of the genetics of this crop. Triploids in blueberry have only recently been realized (Dweikat and Lyrene, 1988; Megalos and Ballington, 1988; Vorsa, 1990). A cross between a complex tetraploid (2n = 4x = 48) hybrid derived from largely V. corymbosum L. ancestry and a wild diploid (2n = 2x = 24) V. corymbosum clone resulted in eight triploid (2n = 3x = 36) and three tetraploid hybrids (Vorsa, 1990). Three additional triploids derived from two unrelated crosses of tetraploid by diploid V. corymbosum were also available (Megalos and Ballington, 1988).

The purpose of our study was to determine the potential value of blueberry triploids in genetic studies and blueberry breeding programs. Specific objectives were to: 1) characterize the fertility of blueberry triploids in crosses with diploids, tetraploids, and hexaploids and 2) produce a trisomic series (2n = 2x + 1 = 25).

Materials and Methods

Eleven triploids derived from three different crosses were used in this study. Triploids NJ856-1, 2, 4, 5, 6, 7, 8, and 9 were derived from the cross G-434 (4x) × NJ85-CS (2x). Tetraploids NJ856-3, 10, and 11 from this same cross were included for the pollen stainability study. Triploids NC3100 and NC3101 were derived from the cross ‘Bluechip’ (4x) × NC79-34 (2x). Triploid NC3102 was derived from the cross ‘Jersey’ (4x) × NC80-18-9 (2x). ‘Bluechip’ and ‘Jersey’ are tetraploid V. corymbosum. Selection G-434 is a tetraploid derived from southern and northern V. corymbosum ancestry. Diploid clones NC79-34, NJ85-CS, and NC80-18-9 are considered V. corymbosum based on Vander Kloot’s (1980) taxonomic treatment.

All crosses were made in the greenhouse. Flowers were not emasculated for 3x × x, 2x × x, and 3x × 3x crosses. Only triploids NJ856-1 and NJ856-8 yielded pollen when flowers were disturbed. Self-pollinations of these two triploids were unsuccessful, indicating self-fertility was not a factor. Diploid blueberry is generally self-sterile. To ensure that this was a reasonable assumption, diploids used as female parents in 2x × 3x crosses were self-pollinated (50 to 100 flowers). In 4x × 3x and 6x × 3x crosses, flowers were emasculated = 1 to 2 days before anthesis. Flowers were pollinated with fresh pollen 2 to 4 days after emasculation. Species designation and hybrid origin of the diploids, tetraploids, and hexaploids used in crosses are given in Table 1. All diploid, tetraploid, and hexaploid parents used as females in crosses were also pollinated with pollen from unrelated pollen parents having the same chromosome number as the female to ensure that crossing procedures and flowers of plants used as females were functional. Most of the diploid and all the tetraploid and hexaploid parents used as pollen parents in crosses were also tested by crossing them, as pollen parents, with unrelated female parents having the same chromosome number. All of the above test crosses set fruit, with the mean
number of developed seeds per berry ranging from 4.4 to 40.7 in 2x × 2x crosses, 6.0 to 57.0 in 4x × 4x crosses, and 28.9 to 47.1 in 6x × 6x crosses.

Pollination and pollen collection procedures were as those given in Vorsa et al. (1987). However, in a few of the triploids, where difficulty was encountered in obtaining pollen, anthers were macerated in the stain. Pollen grains were considered potentially viable if they were well stained and plump at ×250 magnification. The frequency of 2n pollen (large dyads), as pollen stainability of normal meiosis pollen as compared to their tetraploid parents of these triploids ranged from 84.0% (NJ85-CS) to 94.4% (‘Jersey’). All triploids exhibited reduced pollen stainability of normal meiosis pollen as compared to their tetraploid parents of these triploids ranging from 84.0% (NJ85-CS) to 94.4% (‘Jersey’). All triploids exhibited reduced pollen stainability of normal meiosis pollen as compared to their tetraploid parents of these triploids ranging from 84.0% (NJ85-CS) to 94.4% (‘Jersey’).

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For cytological analyses and ovule counts, plants were brought into the greenhouse in late winter. The greenhouse was maintained between 20 to 30°C to initiate budbreak. Method of bud fixation, pollen mother cell (PMC) extraction, and cytological preparation are given in Vorsa et al. (1986). Chromosome constitutions of 56 anaphase I poles (28 PMCs) were determined for triploids NJ856-2, NC3101, and NC3102. Ovaries of flowers were collected 1 to 2 days before or at anthesis and fixed in a 2:1 (propionic: 9570 ethanol) solution. Ovaries were dissected under ×12 magnification and the number of ovules was determined on 10 flowers.

Results

Fertility of crosses

Triploid by diploid crosses. A total of 4021 3x × 2x pollinations were made during 1988 and 1989. Ten triploids were crossed as females with 21 diploid pollen parents, resulting in 61 different crosses over the 2 years. Six 1988 crosses were repeated in 1989. Fruit set ranged from 0% to 6% (Table 2). Five developed seeds were recovered. All five seeds failed to germinate.

Three triploids (NJ856-1, 4, and 9) having some pollen production were used as pollen parents in 2x × 3x crosses with seven diploids. A total of 705 pollinations were made, representing 17 different crosses. Although some fruit was set, no developed seed were recovered (Table 2).

Triploid by triploid crosses. A total of 112 3x × 3x pollinations were made, representing eight crosses. All crosses failed to set fruit (Table 2).

Triploid by tetraploid crosses. A total of 856 3x × 4x pollinations were made during 1988 and 1989, representing 18 different crosses. Eight crosses set fruit, with fruit set ranging from 0% to 35% (Table 2). Number of developed seeds per pollination ranged from zero to 0.3. Only one seed germinated from the 1988 crosses (NJ856-2 × ‘Earliblue’). The two crosses made in 1989, NJ856-1 × ‘Jersey’ and NJ856-1 × ‘Bluecrop’, yielded 15 and seven progeny, respectively, which represents a mean of 0.2 seedlings per flower pollinated (Table 2).

A total of 57 4x × 3x pollinations were made, representing three different crosses. One cross (‘Bluecrop’ × NJ856-9) set fruit, but no developed seeds were recovered.

Triploid by hexaploid crosses. A total of 20 different 3x × 6x crosses were made in 1988 and 1989 with eight triploids and eight hexaploid pollen parents. Triploid NJ856-1 exhibited the highest fruit and seed set and yielded the greatest number of progeny per pollination (Table 2). In 1988, NJ856-1 crosses with V. ashei clones ‘Tifblue’ and NC83-15-1, as pollen parents, yielded the greatest number of developed seeds and progeny per pollination (Table 2). Seed set and the number of progeny recovered was slightly lower when JU101 and US124 were used as pollen parents and considerably reduced with JU47 and JU56 as pollen parents.

Fruit and seed set with the other triploids was low. NJ856-2 and NJ856-4 were the only other triploids to yield progeny in crosses with hexaploids. Triploids NC3100 and NC3101 did not set fruit in any of the crosses, and NC3102 fruit set was <1% (Table 2).

Four 1988 3x × 6x crosses were repeated in 1989: NJ856-1 × ‘Tifblue’, NJ856-2 × ‘Tifblue’, NJ856-4 × ‘Tifblue’, and NJ856-4 × ‘Woodard’. The level of fruit and seed set was similar to that of 1988.

Triploids yielding visible amounts of pollen were NJ856-1, 4, and 9. These were used as pollen parents in crosses with four hexaploid females. A total of 449 6x × 3x pollinations were made, representing nine crosses. Of these nine, four set fruit and developed seed; only ‘Tifblue’ × NJ856-1 yielded progeny.

Pollen stainability

Stainability of pollen arising from normal meiosis, i.e., two-division meiosis, ranged from 0% to 3.5% in the 11 triploids of the study (Table 3). Only triploids NC3100, NC3102, NJ856-7, and NJ856-9 exhibited stainable pollen resulting from “normal” meiosis. Pollen stainability of the respective diploid and tetraploid parents of these triploids ranged from 84.0% (NJ85-CS) to 94.4% (‘Jersey’). All triploids exhibited reduced pollen stainability of normal meiosis pollen as compared to their respective parents. Pollen stainability of the tetraploid siblings (NJ856-3, 10, and 11) of the NJ856 series was also greater than that of the triploids, ranging from 58% to 99% (Table 3).

Frequency of 2n pollen ranged from 0% to 22% in the triploids. Only six triploids, all derived from the G434 × NJ85-CS cross, produced 2n pollen (Table 3). Stainability of 2n pollen ranged from 0% (NJ856-6) to 51% (NJ856-7). Tetraploid siblings NJ856-3, 10, and 11 did not produce 2n pollen.

Ovule number

The mean number of ovules per flower in these triploids ranged from 75 (NJ856-2) to 104 for NJ856-1 (Table 4). The lowest
Table 2. Percent fruit set (FS), mean total seeds/fruit (TS), developed seeds/fruit (DS), percent developed seeds/fruit (PCDS), developed seeds/pollination (DS/Poll), and scedlings/pollination (S/Poll) in triploid crosses with diploids, tetraploids, and hexaploids.

| Female parent(s) | x | Pollen parent(s) | Flowers polinated (no.) | FS (%) | TS | DS | PCDS (%) | DS/Poll | S/Poll |
|-------------------|---|------------------|-------------------------|--------|----|----|----------|---------|--------|
|                   | 3x x 2x |                  |                         |        |    |    |          |         |        |
| 1988 Crosses      |     |                  |                         |        |    |    |          |         |        |
| NJ856-1           |     | H1, C2           | 96                      | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-2           |     | C1,3,5,8, H1     | 149                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-3           |     | C2,6; D1; E1,3; H1,5 | 450               | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-4           |     | D2              | 56                      | 1.8    | 1.0| 1.0| 100      | 0.02    | 0      | 0      |
| NJ856-5           |     | C1,2; H1,4      | 183                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-6           |     | C2, H1          | 50                      | 6.0    | 0  | ---| ---      | ---     | 0      | 0      |
| NJ856-7           |     | C2, H1          | 76                      | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-8           |     | T1              | 42                      | 2.4    | 10.0| 3.0| 30       | 0.07    | 0      | 0      |
| NJ856-9           |     | C2; D1; E1,2; H1 | 311                  | 1.0    | 0  | ---| ---      | ---     | 0      | 0      |
| NJ856-10          |     | H5              | 75                      | 0.1    | 1.0| 1.0| 100      | 0.01    | 0      | 0      |
| NC3100            |     | C1,3; T1; H1,2  | 253                     | 3.6    | 0  | ---| ---      | ---     | 0      | 0      |
| NC3101            |     | C6, E2          | 92                      | 5.4    | 0  | ---| ---      | ---     | 0      | 0      |
| NC3102            |     | C2,5; E2; H1    | 482                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| Total             |     |                 | 2225                    | <0.01  |    |    |          |         | 0      | 0      |
| 1989 crosses      |     |                  |                         |        |    |    |          |         |        |
| NJ856-1           |     | C1,2; H6        | 204                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-2           |     | C3              | 53                      | 3.8    | 1.5| 2.1| 0        | 0       | 0      | 0      |
| NJ856-3           |     | C1,3,5,7; H2    | 366                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-4           |     | C1,3,5; D3; T2; H2,6 | 754            | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-5           |     | C1              | 29                      | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-6           |     | C2,3,5; T2      | 230                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NC3100            |     | T2              | 50                      | 0      | ---| ---| ---      | ---     | 0      | 0      |
| NC3101            |     | T2              | 50                      | 6.0    | 0  | ---| ---      | ---     | 0      | 0      |
| Total             |     |                 | 1786                    | 0      |    |    |          |         | 0      | 0      |
|                  | 2x x 3x |                  |                         |        |    |    |          |         |        |
| C2; H1,5          | NJ856-1 | 187               | 20.9                    | 0      | ---| ---| ---      | ---     | 0      | 0      |
| C2; H1,5          | NJ856-4 | 219               | 1.8                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| C1,2; D2; H1,3-5  | NJ856-9 | 299               | 11.7                    | 0      | ---| ---| ---      | ---     | 0      | 0      |
| Total             |       |                 | 705                     | 0      |    |    |          |         | 0      | 0      |
|                  | 3x x 3x |                  |                         |        |    |    |          |         |        |
| NJ856-1           | NJ856-4 | 32                | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-1           | Self   | 51                | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-1           | NJ856  | 24                | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NC3101            | NJ856-1 | 53                | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| Total             |       |                 | 112                     | 0      |    |    |          |         | 0      | 0      |
|                  | 3x x 4x |                  |                         |        |    |    |          |         |        |
| NJ856-1           | NJ856-11| 36                | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-1           | Bluejay | 51               | 29.4                    | 2.1    | 2.0| 1.1| 45       | 37      | 0.3    | 0      |
| NJ856-2           | Bluecrop | 11              | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-2           | Earlblue | 39              | 2.6                     | 1.0    | ---| 1.0| 100      | <0.1   | <0.1   |
| NJ856-3           | 290-2    | 98               | 1.0                     | 1.0    | ---| ---| 100      | 0.1     | 0      |
| NJ856-4           | 290-2    | 64               | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-6           | Bluecrop, NJ856-11, Earlblue | 91            | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NJ856-9           | Bluejay | 51               | 13.7                    | 1.2    | 1.0| 0.7| 67       | 43      | 0.1    | 0      |
| NJ856-9           | Bluecrop | 49              | 2.0                     | 1.0    | ---| 0  | 0       | 0       | 0      | 0      |
| NJ856-9           | NJ856-17, NJ856-11 | 44          | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NC3101            | NJ856-17 | 36               | 0                       | ---    | ---| ---| ---      | ---     | 0      | 0      |
| NC3102            | Bluecrop, Earlblue | 174          | 1.1                     | 0      | ---| ---| ---      | ---     | 0      | 0      |
| Total             |       |                 | 744                     | <0.1   |    |    |          |         | <0.1   | <0.1   |
| 1989 crosses      |     |                  |                         |        |    |    |          |         |        |
| NJ856-1           | Jersey  | 78                | 33.3                    | 1.5    | 1.5| 0.7| 49       | 43      | 0.2    | 0.2    |
| NJ856-1           | Bluecrop | 34              | 35.3                    | 1.6    | 1.8| 0.4| 37       | 16      | 0.2    | 0.2    |
| Total             |       |                 | 112                     | 0.2    |    |    |          |         | 0.2    | 0.2    |

(continued)
number of ovules per ovary was 60 (NJ856-2) and the highest was 115 (NJ856-1). Thus, significant differences exist for mean ovule number between these triploids (Table 4).

Anaphase I chromosome distribution

Frequency distributions of anaphase I pole chromosome constitutions (chromosomes undergoing equational division at the centromere not included) for triploids NC3100, NC3102, and NJ856-2 (Fig. 1) were all significantly different from one another \( (P < 0.001; \text{contingency chi-square; 5 df}) \). Anaphase I pole chromosome number ranged from 11 (NJ856-2 and NC3102) to 21 (NC3100). Although all three triploids exhibited anaphase I constitutions having 12 chromosomes, NJ856-2 exhibited the highest frequency (25%) for this class.

A mean chromosome number of 18 would be expected if assortment of the extra genome chromosomes were random. All three distributions were shifted toward the basic chromosome number of 12. The NJ856-2 distribution exhibited the greatest shift (mean = 13.4), whereas the distribution for NC3102 exhibited the smallest shift (mean = 15.7). Triploid NC3100 exhibited the broadest distribution, with chromosome numbers ranging from 12 to 21. The number of equationally dividing anaphase I chromosomes varied from one to 10 (NC3100), three to 12 (NC3102), and four to 16 (NJ856-2).

Discussion

Relative to other species, where progeny are generally recovered from crosses with triploids (Levan, 1942), the triploid blueberry exhibits one of the highest degrees of sterility. Apparently, all aneuploid gametes between the monoploid and diploid number are highly inviable and/or result in zygotes that...
crosses, which most likely is the result of relatively high 2n gamete production, as will be discussed later. If NJ856-1 is omitted from consideration, then only one progeny was recovered from 657 3x × 4x crosses (i.e., from NJ856-2 × 'Earliblue'). Thus, the production of n = 2x = 24 gametes in these triploids also appears to be low or lacking. In any event, the frequency of n = 24 megagametophytes is likely less than one in 4.9 × 10^3.

Univalent at metaphase I generally result in lagging chromosomes during the division phases of meiosis (Avers, 1954; Lesley, 1928; Mather, 1935; Punyasingh, 1947; Satina and Blakeslee, 1937), as appears to be the case in the three triploids examined cytologically in this study; the frequency distributions for the number of lagging anaphase I chromosomes did not differ significantly from the corresponding univalent number frequency distributions during metaphase I (N.V., unpublished data). Khush (1973) suggested that lagging chromosomes may misdivide during anaphase I and, if not included in telophase I nuclei, will be eliminated from gametes. Thus, anaphase I pole chromosome constitutions with 12 chromosomes have the potential of yielding gametes with numerically balanced chromosome counts. Of the three triploids examined, NJ856-2 had the highest frequency (25%) of anaphase I poles with 12 chromosomes. However, NJ856-2 exhibited essentially complete sterility, indicating that all the gametes are aneuploid. Thus, lagging chromosomes during anaphase I and II divisions are probably not always excluded from gametes. In other species, chromosomes not included in telophase II nuclei may also be eliminated through microcyte formation (Satina and Blakeslee, 1937).

In addition, since no progeny were recovered from 4021 3x × 2x pollinations in this study, gametes having the euploid number of n = x = 12 apparently were either not produced or of undetectable frequency. Considering mean ovule number per flower, the number of pollinations, and given complete fertilization, it can be reasonably assumed that between 3.0 × 10^5 and 4.2 × 10^5 ovules were fertilized by diploid pollen with the 4021 3x × 2x crosses. Thus, the frequency of megagametophytes having the balanced number of n = x = 12 is less than one in 3 × 10^4. All viable seed in these triploids are likely to be recovered since six of the triploids (NC3100, NC3101, NC3102, and NJ856-6, 7, and 9) set fruit parthenocarpically, and three triploids set fruit in other crosses having only one seed. Thus, ovaries having only one viable seed would probably be retained and develop into a mature fruit.

NJ856-1 was the only triploid exhibiting fertility in 3x × 4x crosses, which most likely is the result of relatively high 2n gamete production, as will be discussed later. If NJ856-1 is omitted from consideration, then only one progeny was recovered from 657 3x × 4x crosses (i.e., from NJ856-2 × 'Earliblue'). Thus, the production of n = 2x = 24 gametes in these triploids also appears to be low or lacking. In any event, the frequency of n = 24 megagametophytes is likely less than one in 4.9 × 10^3.

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### Table 3. Mean (±95% confidence interval) percent pollen stainability, percent 2n pollen, and percent 2n pollen stainability of triploids and tetraploids derived from 4x × 2x crosses and their parents.

| Plant      | No. sampled | Pollen stained (%) | 2n Pollen (%) | 2n Pollen sampled (no.) | Stainable 2n pollen (%) |
|------------|-------------|--------------------|---------------|-------------------------|-------------------------|
| Parents    |             |                    |               |                         |                         |
| Jersey (4x)| 1000        | 94.4 ± 1.4         | 0             | ...                     | ...                     |
| NC80-18-9 (2x) | 528        | 87.7 ± 2.8         | 0             | ...                     | ...                     |
| 3x Hybrid  |             |                    |               |                         |                         |
| NC3102     | 720         | 3.5 ± 1.3          | 0             | ...                     | ...                     |
| Parents    |             |                    |               |                         |                         |
| G434 (4x)  | 1108        | 84.6 ± 2.1         | 0             | ...                     | ...                     |
| NJ85-CS (2x)| 544         | 84.0 ± 3.1         | 0             | ...                     | ...                     |
| 3x Hybrids |             |                    |               |                         |                         |
| NJ856-1    | 680         | 0                  | 22.4 ± 5.5    | 656                     | 46.0 ± 3.8              |
| -2         | 880         | 0                  | 4.5 ± 3.9     | 386                     | 22.3 ± 4.2              |
| -4         | 424         | 0                  | 11.8 ± 4.1    | 180                     | 11.1 ± 4.6              |
| -5         | 840         | 0                  | 5.9 ± 3.0     | 122                     | 0                       |
| -6         | 880         | 0                  | 8.3 ± 2.4     | 360                     | 51.1 ± 5.2              |
| -7         | 1816        | 1.9 ± 0.6          | 0             | ...                     | ...                     |
| -9         | 1600        | 0                  | 2.9 ± 1.3     | 192                     | 15.1 ± 5.1              |
| 4x Hybrids |             |                    |               |                         |                         |
| NJ856-3    | 484         | 99.4 ± 0.7         | 0             | ...                     | ...                     |
| -10        | 580         | 57.9 ± 4.0         | 0             | ...                     | ...                     |
| -11        | 864         | 58.0 ± 3.3         | 0             | ...                     | ...                     |
| Parents    |             |                    |               |                         |                         |
| Bluechip (4x)| 324        | 87.9 ± 3.5         | 0             | ...                     | ...                     |
| NC79-34 (2x)| 488         | 86.8 ± 3.1         | 0             | ...                     | ...                     |
| 3x Hybrids |             |                    |               |                         |                         |
| NC3100     | 828         | 0.2 ± 0.3          | 0             | ...                     | ...                     |
| NC3101     | 1000        | 0                  | 0             | ...                     | ...                     |

*2n Pollen not included.

### Table 4. Mean (± se) ovule number per flower in six blueberry triploids.

| Triploid | Mean ovule no. | SNK | Range |
|----------|----------------|-----|-------|
| NC3100   | 87 ± 3.9       | b, c | 74–97 |
| NC3101   | 77 ± 1.6       | d   | 72–80 |
| NJ856-1  | 104 ± 2.3      | a   | 91–115|
| NJ856-2  | 75 ± 2.3       | d   | 60–87 |
| NJ856-4  | 92 ± 3.1       | b   | 83–108|
| NJ856-9  | 80 ± 2.2       | c, d| 67–89 |

*Mean separation based on Student–Newman–Keuls test at P = 0.05 level.

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The variability in fertility of 3x × 6x crosses may reflect the ancestry of the hexaploid parent used in the cross; crosses where the V. ashei Reade × V. amoenum Ait. hybrids (JU47 and JU56) were used as pollen parents were reduced in fertility in contrast to either V. ashei clones (‘Tifblue’, ‘Woodard’, NC83-15-1) or V. ashei × V. constablaei Gray (US124) and V. amoenum × V. constablaei (JU101) hybrids. This pattern is also reflected in the vigor of the resulting seedlings. Progenies derived from V. ashei × V. amoenum parents are also reduced in vigor relative to the progenies derived from either of the other two combinations. Thus, it appears that the combination of V. corymbosum with V. ashei and V. amoenum genomes in one cytoplasm may be partially incompatible.

The inability to recover any progeny from triploid by diploid crosses limits the use of triploids in developing a trisomic series for genetic studies in blueberry. The apparent formation of 2n gametes by triploids, in this and other studies with blueberry triploids (Dweikat and Lyrene, 1988), facilitates their use as a bridge to transfer genes from the diploid and tetraploid levels to the hexaploid level in blueberry breeding programs.

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