Utility of Parthenocarpic Interspecific Hybrids Between *Vaccinium corymbosum* and *Vaccinium virgatum* for Breeding Blueberry Cultivars Suitable for Cluster Harvesting

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Blueberries are handpicked because of irregular maturation of fruits within clusters. Therefore, harvesting is labor intensive and results in a short shelf life of the product. Cluster harvesting, as performed for grapes, could solve these problems. Previously, we produced many interspecific hybrids between the highbush blueberry (HB; *Vaccinium corymbosum* L.) and rabbiteye blueberry (RB; *Vaccinium virgatum* Aiton) and found that some individuals were parthenocarpic and maturation of berries within fruit clusters was relatively uniform. In the present study, we investigated the degree of parthenocarpy and suitability of the hybrids for cluster harvesting and considered the use of parthenocarpic hybrids to breed cultivars for cluster harvesting. The degree of parthenocarpy was evaluated in 21 blueberry plants (hybrids and cultivars) based on the average fruit set without pollination. It was found that 2 hybrid individuals had the highest frequencies of parthenocarpy and their fruit set and weight were close to those of pollinated fruits; moreover, pollinated fruits of these hybrids were seedless. Thereafter, the uniformity of flowering and fruit maturation was evaluated, and the 2 hybrids showed a relatively uniform maturity of fruits in a cluster; furthermore, fruit dropping occurred later. In addition, correlation analysis was performed for 5 parameters related to parthenocarpy and suitability for cluster harvesting. For seeded fruits, it was found that the flowers that bloomed earlier in a cluster formed a fruit containing more seeds and matured earlier. Comparison of cluster and individual harvesting showed that the percentages of mature fruits within clusters in the 2 hybrids were markedly higher than those in the HB cultivars. Moreover, cluster harvesting of the hybrids greatly shortened the working time needed for harvest and subsequent fruit sorting to 59% of that of individual harvesting. Thus, the 2 hybrids that had a higher degree of parthenocarpy than that in the existing blueberry cultivars were assessed to be highly suitable for cluster harvesting. Factors underlying this suitability may be related to the seedlessness of interspecific hybrids. These results suggest that interspecific hybridization between HB and RB is useful as a breeding method to produce cultivars suitable for cluster harvesting.

Key Words: harvest time reduction, highbush blueberry, rabbiteye blueberry, seedless fruits, uniform ripening.

Introduction

The harvesting of blueberries (*Vaccinium* spp.) involves hand-picking of fruits one by one, owing to irregular maturation of fruits within fruit clusters. Using this method, only 20–30 kg of fruit is harvested per person per day (Ishikawa and Koike, 2006). Therefore, in blueberry cultivation, harvesting constitutes a larger portion of working time than that required for other fruit trees, hindering the expansion of growth areas for this fruit and the chance to reduce labor costs. In addition, individual harvesting reduces the fruit shelf life because of water evaporation and fungal invasion due to the exposed pedicel scar (Cappellini et al., 1982). If cluster harvesting became possible in blueberries, similar to grapes, it could help reduce the working time needed for harvesting and increase the fruit shelf life. Kobayashi et al. (2011) evaluated the quality of fruits...
harvested by individual and cluster harvesting in 4 highbush blueberry (HB; *Vaccinium corymbosum* L., 2n = 4x = 48) cultivars and suggested that the following 3 requirements should be met for cluster harvesting: 1) fruits within clusters should mature concurrently, based on the concurrent blooming of flowers within clusters; 2) mature fruits should drop late, depending on a large fruit detachment force; and 3) variation in fruit quality within clusters should be small. However, to the best of our knowledge, blueberry cultivars suitable for cluster harvesting have not yet been developed.

In our previous study, we aimed to develop cultivars with the high fruit quality of HB and warm area adaptability of the rabbiteye blueberry (RB; *Vaccinium virgatum* Aiton, 2n = 6x = 60) and produced many interspecific hybrid individuals of these 2 species (Miyashita et al., 2012). The hybrids were shown to be pentaploid and were characterized by a low crossability, similar to that reported by Vorsa et al. (1987). The tree vigor was generally strong, and the harvest time was intermediate between that of the parent species (i.e., from the late rainy season until 1 month after the end of the rainy season in the southern part of the Kanto region in Japan). Some hybrid individuals had excellent fruit characteristics, such as parthenocarpy (Otsuki et al., 2016), a relatively large size, and delicious taste. Parthenocarpy is a useful characteristic in fruit trees because it can secure a stable yield without requiring insect pollination and produce seedless fruits with a good texture and suitable for processing. In HB (Ehlenfeldt and Vorsa, 2007) and RB (Ehlenfeldt and Hall, 1996), clones with parthenocarpy were evaluated, and the utilization of parthenocarpy for breeding has been studied (Ehlenfeldt, 2012).

The fruit weight and time to maturity are affected by the number of seeds present in blueberries (Iwasaki et al., 2006). Therefore, we evaluated the population of interspecific hybrids between HB and RB in a preliminary experiment, focusing on parthenocarpy, and found that all fruits within clusters tended to mature at approximately the same time in the parthenocarpic hybrids. Therefore, we proposed a hypothesis that the variability in fruit maturation within clusters is low in seedless parthenocarpic hybrids. In addition, we thought that this property could be used for breeding cultivars for cluster harvesting.

In the present study, we first investigated the degree of parthenocarpy in the interspecific hybrids of HB and RB and in cultivars of these 2 species. Thereafter, the uniformity of flowering and fruit ripening and the degree of fruit drop were evaluated in clusters. In addition, the uniformity of fruit quality, the fruit detachment force, and working time were investigated for the cluster and individual harvesting methods. Based on the results, we discuss the potential for utilization of parthenocarpic hybrids for breeding blueberry cultivars for cluster harvesting.

### Materials and Methods

#### Plant materials

From the population of interspecific pentaploid hybrids between HB and RB, produced by Miyashita et al. (2012), 4 individuals showing parthenocarpy were used in this study, namely, 1 individual of RB ‘Homebell’ × HB ‘Spartan’ (HoSp-S65G-13) and 3 individuals of HB ‘Elliot’ × RB ‘Tifblue’ (EITi-S65G-14, EITi-S65G-31, and EITi-S65G-38). For comparison, 14 cultivars of HB and 3 cultivars of RB were used (Table 1). As of 2016, the hybrids were 14-year-old seedlings, and the cultivars were 5–7-year-old clones. These plants were cultivated in a field in Tachikawa-shi, Tokyo, Japan.

#### Evaluation of parthenocarpy (Experiment 1)

The experiment was conducted twice, in 2016 and 2017, on the 4 hybrids, 14 HB cultivars, and 3 RB cultivars (Table 1). Two crossing treatments (no pollination and pollination) were performed in April of each year. In the period from the early stage to the full-bloom stage of each seed parent, buds just before blooming, with orange- or red-colored anthers, were selected and emasculated (all the petals and anthers were removed). In the no-pollination treatment, buds were covered with paraffin-paper bags. In the pollination treatment, buds were pollinated with pollen collected from the pollen parent just after flowering and then covered. As pollen parents, ‘Darrow’ was used for the HB cultivars and hybrids, and ‘Brightwell’ was used for the RB cultivars. However, for ‘Darrow’ and ‘Brightwell’, ‘Echota’ and ‘Tifblue’ were used as pollen parents, respectively. Each treatment comprised 2–3 replicates, with 6–10 buds each, and the buds in each treatment were covered with paraffin-paper bags for 2 weeks. Ripe fruits were harvested during each maturation period, and all the seeds (both developed and aborted) were collected from the fruits. The percentage of fruit set, maturity date, weight per fruit, and number of seeds per berry were recorded. The degree of parthenocarpy was evaluated using a score from 0 to 4, based on the average fruit set in the 2 years in the no-pollination treatment, as follows: score 0, average fruit set of 0%; score 1, >0% but <25%; score 2, 25% to <50%; score 3, 50% to <75%; and score 4, 75–100%. The rates of fruit set and weight per fruit (relative percentages in the no-pollination treatment, assuming that the percentage in the pollination treatment was 100%) were calculated.

#### Evaluation of flowering and maturation uniformity (Experiment 2)

Three interspecific hybrids (HoSp-S65G-13, EITi-S65G-14, and EITi-S65G-38) and ‘Echota’ were used. The experiment was conducted twice, in 2016 and 2017. In the period from the early to full-bloom stage of each seed parent in April, crossings were performed with no-pollination and pollination treatments.
Table 1. Evaluation of parthenocarpy in blueberry interspecific hybrids and cultivars (*Vaccinium* spp.).

| Accession and treatment | Average fruit set (%) | Parthenocarpy\(^y\) | Fruit set NDF-M\(^w\) | Weight per fruit | Seeds per berry (no.) |
|-------------------------|-----------------------|----------------------|------------------------|------------------|----------------------|
|                         | 2016 2017 2016 2017   | 2016 2017 2016 2017 | 2016 2017 2016 2017 | 2016 2017 2016 2017 | 2016 2017 2016 2017 |
| Interspecific hybrids    | % Rate\(^z\) % Rate | No. Rate No. Rate | g Rate g Rate | g Rate g Rate |
| HoSp-S65G-13            | NP 85 4              | 85 107 80 93       | 1.3 89 1.0 91 | 0.0 0.0 |
|                         | P 85 100             | 80 86             | 1.5 1.1 0.0 0.3 |
| EITI-S65G-14            | NP 83 4              | 107 123 100 105   | 1.5 89 1.1 86 | 0.0 0.0 |
|                         | P 73 92              | 86 96             | 1.7 1.2 0.5 0.0 |
| EITI-S65G-31            | NP 20 1              | 111 98           | 0.7 114 — 0.0 — | 0.0 — |
|                         | P 13 76              | 113 103          | 0.6 0.3 0.3 0.0 |
| EITI-S65G-38            | NP 57 3              | 98 114 95 100    | 0.8 72 0.5 35 | 0.0 0.0 |
|                         | P 86 87              | 86 94            | 1.2 1.5 14.7 2.3 |
| *V. corymbosum*         | Berkeley NP 7 1       | 67 107 59 104    | 1.0 57 0.9 48 | 0.0 0.0 |
|                         | P 32 67              | 62 57            | 1.7 2.0 28.3 43.0 |
|                         | Blueray NP 17 1       | 61 117          | 1.2 67 — 0.0 — | 0.0 — |
|                         | P 65 46              | 65 52            | 1.6 1.7 27.2 34.8 |
|                         | Brigitta Blue NP 28 2 | 75 134         | 1.3 59 — 0.0 — | 0.0 — |
|                         | P 35 58              | 66 56            | 1.7 2.1 5.7 5.8 |
|                         | Darrow NP 22 1        | 69 59            | 2.1 1.8 15.5 20.4 |
|                         | Denise Blue NP 14 1   | 52 90            | 1.5 73 — 0.0 — | 0.0 — |
|                         | P 46 61              | 68 58            | 1.6 2.0 31.9 29.7 |
|                         | Earlblue NP 10 1      | 54 44           | 1.2 1.3 38.9 40.8 |
|                         | Echota NP 57 3        | 73 118 59 109   | 1.3 57 1.7 86 | 0.0 0.0 |
|                         | P 67 90              | 61 54            | 2.3 1.9 42.4 35.8 |
|                         | Elliott NP 3 1        | 90 118          | 0.4 43 — 0.0 — | 0.0 — |
|                         | P 94 81              | 74 76            | 1.5 1.0 40.5 20.0 |
|                         | Herbert NP 0 0        | 67 73            | 1.3 57 1.7 86 | 0.0 0.0 |
|                         | P 55 64              | 66 54            | 1.5 1.8 23.5 38.7 |
|                         | O’Neal NP 0 0         | 81 132          | 0.3 46 — 0.0 — | 0.0 — |
|                         | P 47 48              | 62 55            | 1.5 2.2 30.9 48.5 |
|                         | Rubel NP 36 2         | 72 61           | 0.7 0.7 31.9 17.5 |
|                         | P 45 99              | 72 61            | 0.7 0.7 31.9 17.5 |
|                         | Spartan NP 36 2       | 61 117 51 102   | 1.5 98 1.4 68 | 0.0 0.0 |
|                         | P 79 67              | 52 49            | 1.5 2.0 27.7 34.0 |
|                         | Sunshine Blue NP 0 0  | 76 132          | 1.3 105 1.1 76 | 0.0 5.6 |
|                         | P 83 68              | 67 65            | 1.8 2.0 47.3 48.5 |
|                         | Weymouth NP 70 3      | 51 101 54 109   | 1.3 105 1.1 76 | 0.0 5.6 |
|                         | P 65 75              | 50 49            | 1.3 1.4 31.4 34.6 |
| *V. virgatum*           | Brightwell NP 22 1    | 92 112 91 116   | 1.0 65 0.8 54 | 0.0 0.0 |
|                         | P 83 70              | 82 78            | 1.5 1.4 40.3 46.2 |
|                         | Ochlockonee NP 21 1   | 99 103 106 138  | 1.0 75 0.6 42 | 0.0 0.0 |
|                         | P 73 66              | 96 77            | 1.4 1.3 11.3 12.3 |
|                         | Tifblue NP 5 1        | 117 133         | 0.8 48 — 0.0 — | 0.0 — |
|                         | P 73 51              | 88 72            | 1.7 1.6 42.0 31.9 |

\(^{z}\) NP: no pollination; P: pollination.

\(^{y}\) The degree of parthenocarpy was evaluated using scores 0 to 4 based on the average fruit set over 2 years in the no-pollination treatment: 0: 0%; 1: >0% but <25%; 2: 25% to <50%; 3: 50% to <75%; 4: 75% to 100%.

\(^{w}\) Number of days from flowering until maturity.

\(^{x}\) Values are relative percentages in the no-pollination treatment, assuming that the percentage of polination was 100%.

\(^{*}\) Number of days from flowering until maturity.
‘Darrow’ was used as the pollen parent in the pollination treatment. The number of buds in each cluster was adjusted to 5. Each bud was emasculated just before blooming and subjected to pollination/no-pollination treatments, as in Experiment 1, and the cluster was covered with a paraffin-paper bag. Each treatment comprised 2–3 replicates, with 5 flowers each. The flowers in a cluster were numbered 1–5 in the order of blooming. The blooming date, maturity date, and dropping date of each flower were recorded, and the number of days from blooming until maturity (hereafter referred to as NDF-M) and that from blooming until dropping (hereafter, NDF-D) were calculated. In the maturation period, a net bag was attached to each cluster, and the dropped fruits were collected. Subsequently, the numbers of developed seeds (dark brown, with the largest diameter of 1 mm or more) and aborted seeds (white or less than 1 mm) per berry were recorded. The evaluation parameters of the properties related to suitability for cluster harvesting were set as follows. The uniformity of blooming was defined as the time difference between the blooming of the first and the fifth flower in a flower cluster (hereafter, TD1-5F). The uniformity of maturation was defined as the time difference between the earliest and latest matured fruit in a cluster (hereafter, TDE-LM). The ease of dropping was defined as the time difference between the latest matured and earliest dropped fruit in a cluster (hereafter, TDLM-ED). Thereafter, to clarify the relationships among the parameters related to parthenocarpy and suitability for cluster harvesting, the following 5 parameters were analyzed using Spearman’s correlation: the flowering order, NDF-M, NDF-D, the total number of seeds per berry (i.e., the sum of developed and aborted seeds), and the number of developed seeds per berry.

**Evaluation of fruit quality and harvest time for cluster harvesting (Experiment 3)**

Two interspecific hybrids (HoSp-S65G-13 and ElTi-S65G-14) were evaluated, and HB cultivars (‘Echota’ and ‘Darrow’) were used as controls. In the period from the early to full-bloom stage of each seed parent in April 2016, the number of flowers just after blooming was adjusted to 6–10 per cluster. Thereafter, the flowers were pollinated, without emasculation, and covered with a paraffin-paper bag. ‘Darrow’ was used as a pollen parent, and ‘Echota’ was only used for ‘Darrow’. Cluster harvesting and individual harvesting were performed. During cluster harvesting, a cluster was harvested by cutting the branch just above the cluster or at the base of the pedicel when the pericarp of all fruits in the cluster turned completely blue or when one or more mature fruits dropped. During individual harvesting, mature fruits, in which the entire pericarp and the base of the pedicel turned blue, were picked one by one. Evaluation of fruit quality was conducted immediately after harvesting. The percentage of fruits at each maturity level in a cluster and the fruit detachment force were investigated only for cluster harvesting, while the weight per fruit was measured for both harvesting methods. Regarding the percentage of fruits at each maturity level, 3 levels of maturity were used (mature = the entire pericarp and the base of the pedicel are colored; semi-mature = the entire pericarp but not the pedicel is colored; and immature = the pericarp is not yet completely colored), and each percentage value was calculated based on the average value for 5 clusters. The detachment force between the fruit and pedicel was measured using a Fudoh rheometer (RT-3005D; Rheotech, Tokyo, Japan) according to the method of Kobayashi et al. (2011). The mean value and the coefficient of variation were calculated, and significance of differences between plants was analyzed by the Kruskal–Wallis H-test. To determine the weight per fruit, 10 fruits were randomly selected for each harvesting method and measured. The mean ± standard deviation (SD) were calculated, and significance of differences between the harvesting methods was analyzed by the Mann–Whitney U-test.

To evaluate the working time, HoSp-S65G-13 was used. During the optimum harvesting period in 2016, cluster harvesting and individual harvesting were performed, and the time needed for harvesting and subsequent fruit sorting was measured. Clusters of similar size and maturity level were selected beforehand and marked as harvest targets. All the work was performed by one skilled person. By cluster harvesting, 5 clusters were harvested. During individual harvesting, only mature fruits from 5 clusters were picked one by one. Sorting of the harvested fruits was carried out by hand indoors, and immature, wounded, or desiccated fruits were removed. Based on the time spent on 5 clusters (average weight per cluster was 84 g after sorting), the working time was calculated per kilogram of fruit harvested.

**Results**

**Parthenocarpy (Experiment 1)**

The degree of parthenocarpy was evaluated in 21 blueberry plants (4 hybrids, 14 HB cultivars, and 3 RB cultivars) based on the average fruit set in the no-pollination treatment. Among these, parthenocarpy (scores 1–4; Table 1) was found in 18 plants, but not in 3 HB cultivars. Only 2 hybrids (HoSp-S65G-13 and ElTi-S65G-14) had the highest score (4), and 3 plants (ElTi-S65G-38, ‘Echota’, and ‘Weymouth’) had a score of 3.

Comparison of the fruiting data from the no-pollination treatment with those from the pollination treatment for the 18 plants with parthenocarpy (scores 1–4) showed that, in general, the fruit set was lower, NDF-M was longer, and the weight per fruit was lower in the former treatment. The rates of fruit set and weight per fruit in HoSp-S65G-13 and ElTi-S65G-14
were high (84–123%) in both years. Meanwhile, in ‘Echota’ and ‘Weymouth’, the rates of fruit set were relatively high (67% or more) in both years. In ‘Spartan’ and ‘Weymouth’, the rates of weight per fruit were relatively high (68% or more) in both years.

Based on the number of seeds per berry, all cultivars and individuals were found to have seedless fruits in the no-pollination treatment. In the pollination treatment, all HB and RB cultivars and EITi-S65G-38 had seeded fruits, whereas HoSp-S65G-13, EITi-S65G-14, and EITi-S65G-31 were seedless.

As mentioned above, HoSp-S65G-13 and EITi-S65G-14 had a high frequency of parthenocarpy (score 4) and produced non-pollinated fruits, with a fruit-setting rate and weight close to those of pollinated fruits, while the pollinated fruits of these hybrids were almost seedless.

**Uniformity of flowering and maturation (Experiment 2)**

In the 4 plants (HoSp-S65G-13, EITi-S65G-14, EITi-S65G-38, and ‘Echota’) with high parthenocarpy scores (3–4), the TD1-5F values were in the range of 2.7–5.5 days, with no large difference (Table 2). In all these plants, the fruit set was high (80% or more) under both treatments, and pollinated fruits had seeds in EITi-S65G-38 and ‘Echota’, whereas the fruits were almost seedless in HoSp-S65G-13 and EITi-S65G-14 (data not shown). The dropped fruits generally had the pedicel attached, and the abscission occurred at the junction between the pedicel and peduncle. In 2016, the TDE-LM values were as short as 7–14 days in the 2 seedless-type plants (HoSp-S65G-13 and EITi-S65G-14) compared to 21–24 days in EITi-S65G-38 and ‘Echota’ (Table 2). In addition, the TDLM-ED values in 2016 were as long as 3–18 days in the 3 hybrids, compared to −14 to −4 days in ‘Echota’. Although the annual differences in the values were large between 2016 and 2017, the tendencies observed were consistent in both years. Comparison of the values for each parameter between the no-pollination and pollination treatments showed that TDE-LM generally tended to be shorter in the former treatment. As mentioned above, HoSp-S65G-13 and EITi-S65G-14, which had the highest frequencies of parthenocarpy, showed a relatively uniform fruit maturity within clusters and late fruit dropping after maturation.

Analysis of relationships among the 5 parameters related to parthenocarpy and suitability for cluster harvesting in all the 4 tested plants showed a significant positive correlation between the flowering order and NDF-M in the pollination treatment for ‘Echota’ in both years (Table 3). However, the other parameters were not significantly correlated in either year, or the direction of correlation differed between the 2 years. Subsequently, the relationships of the number of seeds with the other parameters were analyzed in the pollination treatment of the 2 plants with seeded-type fruits (EITi-S65G-38 and ‘Echota’). There was a significant negative correlation in both years between the flowering order and the number of developed seeds in both plants, between the flowering order and the total number of seeds in EITi-S65G-38, and between NDF-M and the total number of seeds in ‘Echota’ (Table 4). For the other combinations, the results of the correlation analysis differed between the 2 years. Thus, in the seeded type, a negative correlation was found between the flowering order and days to maturity, between the flowering order and the number of seeds, and between the time until maturity and the number of seeds. Hence, the flowers that bloomed earlier in the cluster formed a fruit containing more seeds and matured faster. However, in the seedless type, there were no clear relationships among the parameters studied.

**Fruit quality and harvest time (Experiment 3)**

The effects of the harvesting methods on fruit quality were evaluated in 2 hybrid individuals (HoSp-S65G-13 and EITi-S65G-14), which had a high frequency of parthenocarpy and a uniform fruit maturity within clusters, and in 2 HB cultivars (‘Echota’ and ‘Darrow’), which...

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**Table 2. Uniformity of flowering and maturation and the degree of dropping in blueberry interspecific hybrids and a cultivar.**

| Accession/Plant | Treatment | TD1-5Fz (days) 2016 | TD1-5Fz (days) 2017 | TDE-LMz (days) 2016 | TDE-LMz (days) 2017 | TDLM-EDz (days) 2016 | TDLM-EDz (days) 2017 |
|-----------------|-----------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| HoSp-S65G-13    | No pollination | 4.5                | 2.7                | 7                   | 5                   | 15                  | 39                  |
|                 | Pollination | 4.0                | 3.3                | 10                  | 7                   | 18                  | 11                  |
| EITi-S65G-14    | No pollination | 3.0                | 3.3                | 12                  | 4                   | 7                   | 20                  |
|                 | Pollination | 3.0                | 4.0                | 14                  | 5                   | 9                   | 27                  |
| EITi-S65G-38    | No pollination | 5.0                | 4.0                | 22                  | 10                  | 3                   | 27                  |
|                 | Pollination | 5.0                | 3.3                | 24                  | 26                  | 16                  | 15                  |
| 'Echota'        | No pollination | 5.5                | 4.7                | 23                  | 7                   | −14                 | 3                   |
|                 | Pollination | 5.0                | 4.3                | 21                  | 12                  | −4                  | 10                  |

* Time difference between the blooming of the first and the fifth flower in a flower cluster.
* Time difference between the earliest and latest matured fruit in a cluster.
* Time difference between the latest matured and earliest dropped fruit in a cluster.
were used as controls. The cluster harvesting method could be generally used for hybrid individuals when the entire pericarp of all fruits in a cluster turned blue (Fig. 1). However, in the HB cultivars, harvesting was often carried out when one or more fruits dropped. Regarding the ratio of each fruit maturity level in cluster harvesting, the percentages of mature fruits were very high (74–100%) in the 2 hybrids, whereas they were as low as 14% or less in the 2 HB cultivars (Fig. 2). The fruit detachment force of the cluster-harvested fruits was 67–117 g in the 4 plants studied, and there was no significant difference among the plants (Fig. 3). The coefficients of variation in the fruit detachment force tended to be larger in the 2 HB cultivars than in the 2 hybrids. The frequency of fruit dropping after cluster harvesting was low in all plants (data not shown), and the abscission occurred at the junction between the ped-

Table 3. Coefficients of correlation between the flowering order and days until maturity or days until dropping.

| Flowering order | No pollination | Pollination |
|-----------------|----------------|-------------|
|                 | 2016           | 2017        | 2016           | 2017        |
| HoSp-S65G-13    |                |             |                |             |
| NDF-M*          | 0.056 NS−      | −0.024 NS   | 0.990 **       | 0.100 NS    |
| NDF-D*          | 0.300 NS       | 0.023 NS    | 0.740 *        | 0.123 NS    |
| EITI-S65G-14    |                |             |                |             |
| NDF-M           | 0.455 NS       | −0.582 *    | 0.878 **       | −0.630 *    |
| NDF-D           | 0.366 NS       | −0.481 NS   | 0.725 *        | 0.069 NS    |
| EITI-S65G-38    |                |             |                |             |
| NDF-M           | 0.925 **       | 0.250 NS    | 0.753 *        | −0.704 **   |
| NDF-D           | 0.788 **       | 0.386 NS    | 0.786 **       | −0.361 NS   |
| Echota          |                |             |                |             |
| NDF-M           | 0.318 NS       | 0.170 NS    | 0.633 *        | 0.644 *     |
| NDF-D           | 0.830 *        | −0.256 NS   | −0.643 *       | 0.201 NS    |

* Number of days from flowering until maturity.
* Number of days from flowering until dropping.
" Significance: NS, not significant; *, *P ≤ 0.05; **, *P ≤ 0.01, according to Spearman’s correlation (n=8–15).

Gray shading indicates consistency in the correlation results between the 2 years.

Table 4. Coefficients of correlation between the number of seeds and flowering order, days until maturity, or days until dropping in the pollination treatment.

| Total number of seeds per berry | Developed seeds per berry |
|---------------------------------|---------------------------|
|                                 | 2016  | 2017  | 2016  | 2017  |
| EITI-S65G-38                    |       |       |       |       |
| Flowering order                 | −0.739 * | −0.662 ** | −0.780 ** | −0.594 * |
| NDF-M*                          | −0.921 ** | 0.409 NS | −0.964 ** | 0.422 NS |
| NDF-D*                          | −0.788 ** | −0.101 NS | −0.826 ** | −0.196 NS |
| Echota                          |       |       |       |       |
| Flowering order                 | −0.830 ** | −0.458 NS | −0.866 ** | −0.609 * |
| NDF-M                           | −0.664 * | −0.684 ** | −0.939 NS | −0.782 ** |
| NDF-D                           | 0.434 NS | −0.030 NS | 0.751 * | −0.109 NS |

* Number of days from flowering until maturity.
* Number of days from flowering until dropping.
" Significance: NS, not significant; *, *P ≤ 0.05; **, *P ≤ 0.01, according to Spearman’s correlation (n=8–15).

Gray shading indicates consistency in the correlation results between the 2 years.

Fig. 1. Uniformity of maturation within fruit clusters of the highbush blueberry cultivar ‘Echota’ (A) and interspecific hybrid EITI-S65G-14 (B). In each harvesting season, 2 to 3 adjacent clusters on a branch were photographed when the pericarp of all the fruits in the clusters turned blue or when one mature fruit dropped. In ‘Echota’, the variation in maturation within clusters was large, so the capturing timing was when one fruit dropped. In EITI-S65G-14, the fruits within clusters matured at the same time without dropping.
Fig. 2. Percentage of each fruit maturity level at cluster harvesting. Values are the means (n = 5).

Fig. 3. Fruit detachment force at cluster harvesting. Values are the coefficients of variation in the fruit detachment force (n = 10). NS, Not significant according to the Kruskal–Wallis H-test.

Discussion

Parthenocarpy and suitability of interspecific hybrids for cluster harvesting

The interspecific hybrids HoSp-S65G-13 and ElTi-S65G-14 were found to have the highest frequency of parthenocarpy among the 21 blueberry individuals and cultivars tested. In addition, in these 2 hybrids, the fruit set and fruit weight after no-pollination treatment were similar to those of pollinated fruits, and the pollinated fruits were almost all seedless. We found that these 2 hybrids produced seedless fruits, with stable fruit set and weight, regardless of pollination.

In addition, these 2 hybrids showed a relatively uniform fruit maturity within clusters, regardless of pollination, and moreover, fruit dropping occurred late. Thus, 2 of the 3 requirements mentioned by Kobayashi et al. (2011) for cultivars suitable for cluster harvesting were met. Regarding the fruit quality as the third requirement, the percentage of mature fruits was very high in these 2 hybrids, and the weight of cluster-harvested fruits was similar to that of individually harvested fruits. These results suggest only a small variation in fruit quality in these 2 hybrids.
et al. (2011) investigated 4 HB cultivars and proposed ‘Darrow’ as suitable for cluster harvesting. In this study (Experiment 3), it was shown that the 2 hybrids were more suitable for cluster harvesting than ‘Darrow’. Therefore, we conclude that the 2 hybrids are more suitable for cluster harvesting than the existing blueberry cultivars.

Regarding the influence of the harvest methods on the fruit weight, no difference was found, likely because of the small number of fruits measured in this study. It is necessary to investigate the potential effects in more detail, including fruit quality parameters such as the sugar content, acidity, and hardness.

Relationship between interspecific hybrids and parthenocarpy

The interspecific hybrids HoSp-S65G-13 and ElTi-S65G-14 were shown to have a higher frequency of parthenocarpy than the existing cultivars; moreover, the fruit growth was good, and all the fruits were seedless, regardless of pollination. All 3 parents (HB ‘Spartan’, HB ‘Elliott’, and RB ‘Tifblue’) of the 2 hybrids used in Experiment 1 showed lower frequencies of parthenocarpy (scores 1–2) than the 2 hybrids and were inferior in terms of the weight of non-polliinated fruits. Although knowledge about the inheritance pattern of parthenocarpy in the blueberry is still poor, it has been reported that the inheritance in HB is recessive (Ehlenfeldt and Vorsa, 2007). The 2 hybrids may have better characteristics than those of the parents owing to the inheritance of some recessive genes affecting parthenocarpy and the growth of non-polliinated fruits. Interspecific pentaploid hybrids between HB and RB often exhibit low crossability (Vorsa et al., 1987). Female sterility of the hybrids may cause the seedlessness of polliinated fruits.

The weight per fruit in the 2 hybrids was generally 2 g in Experiment 3, whereas it was lower (1.0–1.7 g) in Experiment 1. These differences were also observed in the control HB cultivars and may have been caused by emasculation, which was carried out in both treatments in Experiment 1, but not in Experiment 3. Thus, emasculation may affect fruit growth.

Relationship between parthenocarpy and suitability for cluster harvesting

In this study, it was suggested that the interspecific hybrids HoSp-S65G-13 and ElTi-S65G-14 were more suitable for cluster harvesting than the existing blueberry cultivars based on the factors affecting each property required for cluster harvesting. Regarding the uniformity of maturation, TD1-5F was no more than 6 days in any individual or cultivar tested. If the variation in the flowering date is the main factor causing the variation in fruit maturation, TDE-LM should be close to TD1-5F. However, TDE-LM in ‘Echota’ and ElTi-S65G-38 was long (maximum 23–24 days). Therefore, factors other than flowering may increase the variation.

In the blueberry, fruits containing many seeds generally show early maturation (Gupton and Spiers, 1994; Iwasaki et al., 2006; Lang and Danka, 1991), and the number of seeds is thought to be an important factor that affects fruit maturation. In this study, in the seeded fruits of ‘Echota’ and ElTi-S65G-38, the larger the seed number, the shorter the period until maturity. In addition, TDE-LM generally tended to be shorter in non-polliinated fruits than in fruits produced after polliination treatment. These results suggested that fruits within a cluster competed with each other for assimilation products and fruits containing more seeds had some competitive advantage so that their maturation was promoted, increasing the variation in maturation within clusters. However, it was considered that competition among fruits within clusters was low in the seedless hybrid individuals, which reduced the variation in maturation. However, in ‘Echota’ and ElTi-S65G-38, the TDE-LM values were large (maximum 22–23 days) even for non-polliinated fruits. Therefore, in these plants, there are some other factors that increase the variation in maturation in addition to the number of seeds. There may be a difference in growth regulation between parthenocarpic fruits induced by no-polliination treatment and fruits of parthenocarpic plants, such as HoSp-S65G-13 and ElTi-S65G-14. Thus, it is necessary to investigate factors affecting the uniformity of maturation in detail.

Regarding fruit dropping before harvesting, the difference in the data between the 2 years was generally large, as shown by the correlation analysis in Experiment 2, and we could not find any factors that had a clear relationship with fruit dropping. These results suggested that fruit dropping was affected by a combined effect of the flowering order, number of seeds, and the environment. Moreover, because the 3 hybrid individuals investigated showed fruit dropping at a later date than ‘Echota’, regardless of the degree of parthenocarpy and the use of polliination, these hybrids may have a common genotype. In addition, we attempted to evaluate the difficulty in fruit dropping after cluster harvesting based on the fruit detachment force from the peduncle. However, the data were not significantly different among the hybrids and HB cultivars tested, although the HB cultivars showed more variation than the hybrids. In HB (Akase, 2000) and RB (Yamagishi et al., 2002), as the maturation of fruits progressed, the fruit detachment force from the peduncle decreased. The large variation in the fruit detachment force in HB may be due to different maturity levels of fruits within clusters. To evaluate the difficulty in fruit dropping after cluster harvesting in the hybrids and cultivars, it is necessary to investigate changes in the fruit detachment force and in the degree of dropping due to vibrations during shipment with the passage of time.
Development of cultivars for cluster harvesting by interspecific hybridization

The 2 interspecific hybrids between HB and RB investigated in this study had higher frequencies of parthenocarpy than the existing blueberry cultivars. Furthermore, it was suggested that these interspecific hybrids were more suitable for cluster harvesting than the existing cultivars. The factors affecting this suitability appeared to be related to the seedlessness of the interspecific hybrids. Moreover, cluster harvesting of the hybrids revealed that the time needed for harvest was greatly shortened compared with that required for individual harvesting and that the labor-saving effect was large. Ehlenfeldt and Vorsa (2007) reported that HB parthenocarpic individuals generally showed low vigor and were not suitable for practical use. However, both of the parthenocarpic hybrids tested in the present study showed vigorous growth, which has been maintained for 14 years or more. These results suggest that interspecific hybridization between HB and RB is useful as a breeding method to produce cultivars suitable for cluster harvesting.

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