Does Inducing Tetraploidy in *Vaccinium ovatum* Improve Fruit Traits and Plant Architecture?

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Abstract. *Vaccinium ovatum* (evergreen huckleberry) is an evergreen shrub native to the Pacific Northwest. Evergreen huckleberry is diploid ($2n = 2x = 24$), but unreduced gametes have been reported that facilitated in interspecific tetraploids. To our knowledge, tetraploid forms of evergreen huckleberry have not previously been evaluated. There is interest in this species as a native, edible, evergreen landscape shrub, but it requires improvement of the fruit and plant qualities for an eventual cultivar release. To obtain variation in plant qualities, we induced polyploidy in a collection of plants in 2013. The purpose of this study was to assess the impacts of polyploidy on the fruit and plant qualities of *V. ovatum*. This fruit and plant quality study provides a contribution to the scientific knowledge base that is currently lacking for evergreen huckleberries. Plant qualities were determined by measuring plant height and width, obtained in Fall 2017. The fruit volume (mm^3) and for soluble solids content (SSC, °Brix) were measured using a digital caliper and a digital refractometer, respectively. Measurements were taken on diploid, mixoploid, and tetraploid ($2x$, $2x + 4x$, $4x$) cytotypes, once in 2017, five times over 9 weeks in 2018, and three times over 9 weeks in 2019. Tetraploids had larger fruit than diploids in 2017 ($P < 0.0001$), suggesting there was a gigas effect from polyploidy in evergreen huckleberries. However, during 2018 and 2019, tetraploid fruit was smaller than that of diploid and mixoploid genotypes. Differences were observed in diploid fruit volume among all years ($P < 0.0001$) such that 2019 was largest and 2017 was smallest. It is unclear what led to this variation. In tetraploids, SSC was statistically significant among years ($P = 0.0002$) such that 2017 was highest and 2019 was lowest. Although our preliminary data suggested that induced polyploidy may result in larger fruit, this was not observed in subsequent years, and it does not appear that tetraploids necessarily will have larger or sweeter fruit. However, these tetraploids may facilitate crossing with other species at the tetraploid level as a means for improvement of various traits.

The genus *Vaccinium* contains two subgenera: subgenus *Oxycoccus*, which contains cranberries, and subgenus *Vaccinium*, which has 21 sections. Evergreen huckleberry (*Vaccinium ovatum*) falls into the section *Pycnothamnus*, which is the only North American species in the section. Of the 450 species of

Vaccinium, 40 are native to North America, and ≈ 15 are found in the Pacific Northwest, where *V. ovatum* is native (Richards and Alexander, 2006; Vander Kloet, 1988). In the Eastern United States, the common name huckleberry often refers to plants in the genus *Gaylussacia*, which contains more than 50 species, of which at least four produce edible berries. In the Pacific Northwest, all huckleberries are members of *Vaccinium* because they are all five-chambered fruit as opposed to the 10 chambers in *Gaylussacia* (Nicholson, 2011; Richards and Alexander, 2006).

Vaccinium ovatum is native to understories in the Pacific Northwest and found in abundance west of the Cascade Mountains from Canada (British Columbia) to the northern coast of California (Hitchcock and Cronquist, 1973; Postman, 2004; Vander Kloet, 1988). Evergreen huckleberries are often found growing in conjunction with red huckleberry (*V. parvifolium*) and common snowberry (*Symphoricarpos albus*). Evergreen huckleberries are rarely cultivated in large numbers but are a niche/novelty food item in the Pacific Northwest. Although still not a crop that is mass-produced, huckleberries have extensive history of use in the Pacific

Northwest. Huckleberries have been collected by Native American tribes for eating and cultural use, and some stands have begun to be commercially harvested (Richards and Alexander, 2006).

Vaccinium ovatum is an erect evergreen shrub that varies in height from 0.5 to 3 m tall. It has leathery, alternately arranged ovate leaves, with serrate margins that are 2 to 5 cm long. The foliage is evergreen with new growth emerging in colors from bright pink to a burnt orange. The inflorescence emerges from the leaf axil in early spring through early summer, bearing bright white to pink urceolate flowers. The fruit ripens throughout summer into the fall, continuing to hang on the plant into late winter. Berries are rarely stripped from plants, despite being eaten by various bird species. The berries of *V. ovatum* tend to develop asynchronously and when ripe often persist for a month or longer (Vander Kloet, 1988). The berries hang on the shrub far longer into the fall and winter than blueberries, extending into early spring in landscape plantings, making this an ideal ornamental and edible species for landscape use. Although the fruit are edible, they are small and many find them to be too tart. Like most other *Vaccinium*, evergreen huckleberries thrive in either the sun or shade and require acidic soil (Tamanda, 2002).

As native and edible plants continue to gain popularity, there is potential for improved forms of evergreen huckleberry to become popular garden plants for their evergreen foliage and edible fruit. For many years evergreen huckleberry has been popular among florists for use of cut branches in bouquet designs (Schlosser et al., 1992; Vander Kloet, 1988), and although there are at least six cultivars, they are not widely found at garden centers, and seedling forms are common in the trade. Unselected seedling forms often have sparse branching and irregular habit that is undesirable.

Polyploidy, having more complete chromosome sets than the diploid chromosome complement, is common among diverse plant groups. Polyploids occur naturally by unreduced gametes and through human manipulation of the mitotic cycle using chemicals (Sleeper and Poehlman, 2006). Tetraploids contain four chromosome copies, whereas mixoploids, or cytochimera (when occurring as periclinal chimeras), have different ploidy levels in different histogenic layers. Polyploid plants are selected and sought out by plant breeders to improve agricultural traits like organ sizes (including fruit size), blooming time, and pest resistance. In ornamental crops where little breeding has been done, polyploidization may be used to develop novel crops and increase the number of genetic variants (Manzoor et al., 2019). There is an increase in cell size associated with polyploidy; this effect is known as the gigas effect, which results in increasing organ size, including flowers and fruit, as well as altering plant shape (Sattler et al., 2016).

Blueberry breeders have used *V. ovatum* as a parent in interspecific and interploidy

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crosses. The North Carolina State University breeding program crossed ‘NC 2267’ (a diploid hybrid that is 1/4 *V. corymbosum*, 3/4 *V. darrowi*) with a selection of diploid *V. ovatum*. Seedling ‘NC 3048’ was the only one that grew from 275 pollinations and is tetraploid. According to Ballington et al. (1997), this selection was at least partly fertile and was successfully backcrossed to tetraploid section *Cyanococcus* genotypes, proving successful as a male parent in producing seedlings (Ballington, 2001). A tetraploid F1 was developed between *V. corymbosum* ‘Coville’ and *V. ovatum* via unreduced gametes in the latter (Lyrene and Ballington, 1986). This tetraploid intersectorial hybrid was successfully backcrossed to both northern and southern highbush. *Vaccinium ovatum* is potentially useful as a parent for habitat adaptation and drought resistance (Ballington, 2001). Viable but largely sterile diploid hybrids have been produced between *V. ovatum* and *V. darrowi*, and *V. ovatum*, and *V. crassifolium*, and one partially fertile tetraploid hybrid between ‘Coville’ (*V. corymbosum*) and *V. ovatum* was produced through an unreduced gamete (Luby et al., 1991).

Degree Brix (°Brix) is a measure of the mass ratio of soluble solids to water and is a widely used approximation for sugar content. Winemakers, vegetable processors, and many other members of the food industry use degrees Brix to express the level of SSC, which is sugars plus all other dissolved solids as amount per unit of the solvent water (Coombe, 1992; Kleinhenz and Bumgarner, 2012). In many crops, SSC mainly measures sugars and acids making the refractometer a good predictor of sugar content. SSC has been used in many studies including as a rapid postharvest method to determine soluble solids in sweet corn (Hale et al., 2005) and grape sugar content (Song et al., 2015). It is important to note that sugar content or sweetness can be overwhelmed by other aspects of flavor (such as acidity); therefore, a high SSC is not a guarantee of sweet flavor (Kleinhenz and Bumgarner, 2012). Jayasena and Cameron (2008) used a degrees Brix-to-acid ratio as a good prediction for consumer acceptability of grapes.

Berries contain phenolic compounds that accumulate during ripening including tannins, anthocyanins, and flavanols that all contribute to color and taste or flavor of fresh fruit (Seymour et al., 1993). Little research has been done on the development of *V. ovatum* berries over the course of a season, although several studies have looked at anthocyanin development in several species of huckleberries (Ballington et al., 1988; Lee et al., 2004). Lee et al. (2004) determined that *V. ovatum* had greater total anthocyanin content, higher pH, and SSC than *V. membranaceum*. Blueberry fruit development in two species was monitored at three ripening stages to determine the effect of fruit maturation on the development of sugars and acid composition of the berries (Ayaz et al., 2001). There have been several studies in both highbush and lowbush blueberries measuring various berry composition changes at different physiological

stages of the berry (Ballinger and Kushman, 1970; Kader et al., 1993; Kalt and McDonald, 1996; Kalt et al., 1995). Ballinger and Kushman (1970) found that, in general, percent soluble solids and berry weight both increased over berry development stages. Berries are generally measured over time because the different stages of berry fruit development are highly influenced by temperature (Darnell

et al., 1992). To quantify this relationship, heat units or degree-day accumulation are used in fruits to estimate developmental time (DeJong and Goudriaan, 1989; Godoy et al., 2007). However, data are lacking for *V. ovatum* for optimal harvest stage and thus any related degree-day accumulation.

Although there are several blueberry studies using various physiological ages of berries

Table 1. *Vaccinium ovatum* accessions, genome size calculated by analyzing DAPI-stained nuclei using flow cytometry with *Pisum sativum* ‘Ctirad’ (2C = 8.76 pg) as an internal standard, plant height, and width of plants growing in 21-L containers at Lewis Brown Research farm in Corvallis, OR.

Accession	Ploidy ^z	Genome size 2C (pg) ± SEM	Ht (cm)	Width (cm)
12-0018-002	2x	1.22 ± 0.01	76.2	66.0
12-0018-003	2x	1.21 ± 0.01	53.3	55.9
12-0018-005	2x	1.25 ± 0.01	33.0	66.0
12-0018-007	2x	1.18 ± 0.01	40.6	76.2
12-0018-011	2x	1.25 ± 0.01	30.5	55.9
12-0018-012	2x	1.22 ± 0.01	33.0	50.8
12-0018-015	4x	2.40 ± 0.03	30.5	30.5
12-0018-020	2x	1.23 ± 0.00	50.8	45.7
12-0018-021	2x	1.23 ± 0.02	35.6	43.2
12-0018-023	4x	2.48 ± 0.01	15.2	35.6
12-0018-026	2x + 4x	1.22 ± 0.01 2.49 ± 0.03	33.0	30.5
12-0018-027	2x + 4x	1.25 ± 0.05 2.52 ± 0.08	12.7	35.6
12-0018-028	2x	1.19 ± 0.04	33.0	55.9
12-0018-030	4x	2.59 ± 0.04	17.8	35.6
12-0018-031	2x + 4x	1.26 ± 0.01 2.59 ± 0.02	25.4	38.1
12-0018-035	2x + 4x	1.26 ± 0.00 2.56 ± 0.02	60.9	45.7
12-0018-040	2x	1.22 ± 0.00	35.6	38.1
12-0018-052	2x + 4x	1.13 ± 0.01 2.28 ± 0.03	43.2	63.5
12-0018-058	2x + 4x	1.26 ± 0.02 2.60 ± 0.03	38.1	25.4
12-0018-061	2x + 4x	1.21 ± 0.00 2.46 ± 0.01	30.5	35.6
12-0018-063	4x	2.53 ± 0.02	15.2	48.3
12-0018-064	4x	2.52 ± 0.02	38.1	35.6
12-0018-065	4x	2.48 ± 0.04	22.9	22.9
12-0018-066	4x	2.47 ± 0.03	38.1	53.3
12-0018-070	2x	1.25 ± 0.01	60.9	40.6
12-0018-072	2x	1.20 ± 0.05	53.3	38.1
12-0018-073	4x	2.56 ± 0.03	25.4	27.9
12-0018-078	4x	2.55 ± 0.06	22.9	30.5
12-0018-084	4x	2.58 ± 0.03	33.0	33.0
12-0018-088	2x	1.25 ± 0.00	22.9	45.7
12-0018-089	4x	2.51 ± 0.04	25.4	40.6
12-0018-095	4x	2.51 ± 0.02	38.1	45.7
12-0018-096	4x	2.51 ± 0.02	30.5	30.5
12-0018-098	2x	1.25 ± 0.01	55.9	30.5
12-0018-100	2x	1.24 ± 0.00	55.5	53.3
12-0018-101	4x	2.51 ± 0.04	30.5	25.4
12-0018-107	2x	1.29 ± 0.00	50.8	55.9
12-0018-108	4x	2.62 ± 0.04	27.9	40.6
12-0018-112	4x	2.39 ± 0.02	12.7	20.3
12-0018-121	2x	1.14 ± 0.03	38.1	55.9
12-0018-124	4x	2.52 ± 0.03	27.9	40.6
12-0018-128A	2x	1.21 ± 0.03	40.6	53.3
12-0018-128B	4x	2.54 ± 0.03	22.9	40.6
12-0018-130	4x	2.48 ± 0.02	33.0	60.9
12-0018-137	4x	2.57 ± 0.02	27.9	38.1
12-0018-144	4x	2.40 ± 0.02	25.4	33.0
12-0018-145	2x	1.20 ± 0.01	45.7	43.2
12-0018-147	4x	2.44 ± 0.02	20.3	35.6
12-0018-148	4x	2.52 ± 0.03	25.4	27.9
12-0018-159	4x	2.48 ± 0.02	38.1	38.1
12-0018-163	2x	1.21 ± 0.02	55.9	55.9
12-0018-165	2x	1.23 ± 0.02	45.7	60.9
12-0018-171	2x	1.22 ± 0.01	48.3	66.0
12-0018-173	2x	1.21 ± 0.02	35.6	63.5
12-0018-176	2x	1.25 ± 0.01	55.9	58.4
12-0018-177	2x + 4x	1.23 ± 0.03 2.57 ± 0.03	76.2	50.8
12-0018-178	2x	1.13 ± 0.04	22.9	43.2

^zSplits the 2x and 4x values.

^yMean ± SEM; means based on average of taxa means, three samples of the accession were used to calculate the mean.

and skin color as indices, no studies were found measuring sugar levels and fruit volume in *V. ovatum* over the course of a season. The objectives of the present work were to analyze fruit size and SSC of three cytotypes of containerized evergreen huckleberry over the course of a season to assess the impact of ploidy and harvest date on fruit traits and evaluate how polyploidy influenced plant architecture.

Materials and Methods

Plant material. Tetraploids and mixoploids of normally diploid *Vaccinium ovatum* were developed by Contreras and Friddle (unpublished data) in 2012–13. Fruit was collected from unimproved shrubs grown from seed that were found on Oregon State University Corvallis campus in Oct. 2012. Fruit was macerated in a blender with dulled blades and then spread to dry for 24 h. Seeds were sown uniformly using a saltshaker on the surface of 4 douglas-fir bark : 1 peat growing media. Trays were watered, covered with a plastic dome to retain humidity, and placed into cold stratification at 4 °C for 90 d. Trays were removed from stratification in February and placed into a glasshouse with day/night set temperatures of 24/18 °C with a 16-h daylength. Seedlings, starting at the cotyledon stage, were sprayed daily for 20 d with 150 µm oryzalin plus 0.1% Triton X-100. Leaves of seedlings were tested using flow cytometry during Mar. 2014 to determine ploidy. All plants used in this study were single replicates of each genotype that maintained in 21-L containers filled with 100% unaged douglas-fir bark (Lane Forest Products, Eugene, OR) under an unheated polyhouse with regular overhead irrigation.

Flow cytometry. Flow cytometry was used to confirm ploidy level of study plants. We calculated holoploid (2C) genome size of individual accessions of *Vaccinium ovatum* by comparing mean relative fluorescence of the sample against an internal standard, *Pisum sativum* ‘Ctirad’, with a known genome size of 8.76 pg (Greilhuber et al., 2007). A total of 57 accessions were evaluated using flow cytometric analysis of nuclei stained with 4',6'-diamidino-2-phenylindole (DAPI). For each sample, three young, fully expanded leaves were collected to provide a random sample of nuclei. Each sample was prepared by cohomopping 1 to 2 cm² of tissue from both *V. ovatum* and the internal standard with a double-sided razor blade in a polystyrene petri dish containing 400 µL of nuclei extraction buffer (Cytastain Ultraviolet Precise P Nuclei Extraction Buffer; Sysmex, Görlitz, Germany). Buffer containing chopped leaf tissue was passed through a 50-µm gauze filter (Partec Celltrics, Münster, Germany) into a 3.5-mL plastic tube (Sarstedt Ag & Co., Nümbrecht, Germany). Next, 1.6 mL of DAPI stain was added to the nuclei suspension. All samples were analyzed using a Partec PA II flow cytometer. A minimum of 3000 nuclei were analyzed per sample with average cv for each fluorescence histogram

Fig. 1. Flow cytometry histograms of three *Vaccinium ovatum* cytotypes. More than 4000 DAPI-stained nuclei extracted from leaves were included in each analysis and all peaks had coefficient of variance (cv%) < 10%. (A) Peaks represent diploid *V. ovatum* (a, 2C = 1.22 pg) and the internal standard *Pisum sativum* ‘Ctirad’ (b, 2C = 8.76 pg). (B) Peaks of tetraploid *V. ovatum* (a, 2C = 2.45 pg) and the internal standard (b). (C) Peaks represent mixoploid *V. ovatum* with a diploid (a) and a tetraploid peak (b) as well as internal standard (c).

less than 10. Relative 2C genome size was calculated as:

$$2C = \text{DNA content of standard} \times \frac{\text{mean fluorescence value of sample}}{\text{mean fluorescence value of standard}}$$

Monoploid genome sizes were calculated by dividing each sample's 2C genome size by inferred ploidy.

Plant measurements. Plant height and width were measured during Fall 2017 to the nearest centimeter of plants that had been grown for four growing seasons. Fruit size was measured once during 2017 (31 Aug.), biweekly over 9 weeks starting 30 Aug. 2018, and three times over 9 weeks starting 11 Sept. 2019. The decision on when to begin collecting observations was made on apparent ripeness based on what was considered fully ripe color. Berry sampling was initiated when, through a visual inspection, over half of the fruit on the plant appeared to be at peak ripeness. Before initial measurements, no prior data were collected (e.g., firmness or chemistry measurements). To compare volume and SSC among years, the first week of testing

during each year were compared. Measurements taken in 2018 and 2019 over the course of 9 weeks were compared in a separate analysis. Two perpendicular diameters of three berries were measured using a steel digital caliper (General Tools, UltraTech, Secaucus, NJ). These replicates were used to calculate mean berry volume. Using the mean of the two diameters, the following formula was used to calculate relative volume:

$$\text{Volume of a sphere} = \frac{4}{3} \pi r^2.$$

In 2017, SSC was measured once using a combination of three berries, the juices were strained through a fine mesh filter into the sample well of a digital refractometer (Atago, Japan). In both 2018 and 2019, SSC was assessed by squeezing the juice of three berries, individually through a fine mesh filter onto a digital refractometer. These replicates were used to calculate mean berry degrees Brix. Twenty-eight accessions were tested in 2017 (2x, n = 21; 2x + 4x, n = 4; 4x, n = 3). Thirty-four accessions were tested in 2018 (2x, n = 24; 2x + 4x, n = 4; 4x, n = 6). Twenty-five accessions were tested in 2019 (2x, n = 17; 2x + 4x, n = 5; 4x, n = 3). Due to

fruiting differences across the years, not all accessions were tested each year. A low fruit set in 2019 limited the number of plants that were tested.

Statistical analysis. All response variables were evaluated for normality (PROC UNIVARIATE, SAS 9.4). Analysis of variance (ANOVA) was conducted using a general linear model (PROC GLM using SAS Version 9.4, SAS Institute Inc., Cary, NC) to determine if ploidy had a significant impact on either volume or SSC and means were separated using Tukey's honestly significant difference (HSD; $\alpha = 0.05$), where appropriate. A *t* test (PROC TTEST, SAS 9.4) was used to compare 2018 to 2019 at each ploidy and each collection time (weeks). Bar graph, scatterplot, regression equations, and multiple correlation coefficients (R^2) were prepared in Excel (Microsoft Corp., Redmond, WA) to visualize relationships among independent and response variables.

Results

Genome size and plant size. Genome size of the diploid *Vaccinium ovatum* accessions in our collection ranged from 1.13 ± 0.04 pg to 1.29 ± 0.00 pg. Tetraploid accessions in our collection ranged from 2.39 ± 0.02 pg to 2.62 ± 0.04 pg (Table 1). Mixoploids were identified from cell cycling (G2 peaks) by having similar populations of $2x$ and $4x$ cells (Fig. 1). Tetraploids were shorter than both diploids and mixoploids ($P < 0.0001$). Diploids were wider than mixoploids and tetraploids ($P < 0.0001$) (Table 2).

Fruit measurements on a single date. SSC and volume were normally distributed in all 3 years. There were no consistent trends for SSC across years for all ploidy levels. Because data were only collected on one date, we could not assess change over time during 2017. In 2017, tetraploid fruit volume was larger than diploid and mixoploid with a minimum significant difference of 28.1 mm^3 ($P = < 0.0001$) (Fig. 2). There was a difference of 55 mm^3 between diploid and tetraploid fruit volume. SSC exhibited a trending increase from 15.3°Brix in diploids to 18.0°Brix in tetraploids; however, ploidy levels were not significantly different from each other ($P = 0.3431$).

In 2018, volume ($P = 0.3984$) and SSC ($P = 0.1427$) measurements were not different among ploidy levels. Although not statistically significant, volume ranged among the ploidy levels from 246.6 mm^3 in tetraploids to 279.9 mm^3 in mixoploids. There were smaller variations in SSC across ploidy levels with the biggest difference being between diploids with 15.5°Brix and mixoploids with 14.1°Brix (Fig. 2).

In 2019, fruit volume was not significant ($P = 0.2237$) across ploidy levels during the first week of testing. Tetraploids had a volume of 264.8 mm^3 , whereas mixoploids had a volume of 321.2 mm^3 , and diploids had a volume of 301.2 mm^3 . SSC measurements in 2019 were significant ($P = 0.0163$). Diploids and mixoploids had a very similar average of

Table 2. Mean height and width measurements from three cytotypes of *Vaccinium ovatum*. Means within columns followed by different letters are significant based on Tukey's honestly significant difference ($\alpha = 0.05$). Plants of each cytotype included $n = 25$ accessions of diploids ($2x$), $n = 8$ accessions of mixoploids ($2x + 4x$), and $n = 24$ accessions of tetraploids ($4x$).

Ploidy	Ht (cm)	Width (cm)
$2x$	44.4 a	52.7 a
$2x+4x$	40.0 a	40.6 b
$4x$	26.9 b	36.3 b

$\approx 14.0^\circ\text{Brix}$, whereas tetraploids had significantly less sugar content of 11.0°Brix . SSC exhibited a decreasing trend across ploidy levels for a single date measurement (Fig. 2).

Volume was significantly different across all years in diploid plants ($P < 0.0001$). Volume of mixoploid plants in 2017 was significantly different from both 2018 and 2019 mixoploid plants ($P < 0.0001$), and volume of tetraploid plants in 2019 was significantly different from tetraploid plants in 2017 ($P = 0.0045$). SSC was only significantly different according to Tukey's HSD across all years in

tetraploid plants ($P = 0.0002$); however, when testing with an overall ANOVA, SSC was significantly different in diploids as well ($P = 0.0173$) (Fig. 2).

Fruit measurements over time in 2018 and 2019. Across all ploidy levels SSC decreased over time in 2018 (Fig. 3). Volume of diploids ($P = 0.0391$) and tetraploids ($P = 0.0222$) in 2018 was different between weeks 5 and 7. Although statistically there was no difference in mixoploids due the large variance, there was a visual difference between fruit volume of 262.68 mm^3 and 338.58 mm^3 . SSC values in 2018, unlike volume, showed a decrease over time within ploidy levels (Fig. 3). In mixoploids there was a significant reduction in SSC ($P = 0.0055$) from 14.1°Brix in week 1 to 11.0°Brix in both week 7 and week 9 (Fig. 3).

During 2019, there was no clear trend for SSC over time; however volume decreased across weeks in all ploidy levels. Volume showed a significant decrease from week 1 to week 9 in both diploid ($P = 0.0021$) and tetraploid ($P = 0.0135$). Diploids showed a significant difference in SSC values between week 5 and week 9 ($P = 0.0371$) (Fig. 4). SSC for all

Fig. 2. Fruit volume (A–C) and soluble solids content (D–F) measurements collected on a single date of collection from diploid ($2x$, A and D), mixoploid ($2x + 4x$, B and E), and tetraploid ($4x$, C and F) cytotypes of *Vaccinium ovatum* during 2017, 2018, and 2019. Mean separation using Tukey's honestly significant difference ($\alpha = 0.05$). Comparisons within year, among cytotypes with the same capital letter were not statistically different. Comparison within cytotype, among years with the same lowercase letter were not statistically different.

Fig. 3. Fruit volume and soluble solids content (SSC) measured biweekly for 9 weeks during 2018 from three from (A) diploid (2x, n = 24), (B) mixoploid (2x + 4x, n = 4), and (C) tetraploid (4x, n = 6) cytotypes of *Vaccinium ovatum*. Bars represent fruit volume and closed circles represent SSC. Same letters within bars indicates volume not statistically different over weeks within year and cytotype. Same letters outside bars indicates SSC not statistically different over weeks within cytotype. Linear trendlines fitted over time for diploids ($y = -0.1208x + 15.502$, $R^2 = 0.83$), mixoploids ($y = -0.365x + 13.86$, $R^2 = 0.8074$), and tetraploids ($y = -0.2428x + 14.567$, $R^2 = 0.8175$).

ploidy levels exhibited a trend of decreasing in the fifth week and then increasing again in the ninth week (Fig. 4).

There was a significant difference in diploid volume in weeks 1 ($P = 0.0044$), 5 ($P = 0.0078$), and 9 ($P = 0.0252$) between 2018 and 2019. Mixoploids during week 5 exhibited a different volume between 2018 and 2019 ($P = 0.0477$). In week 1, diploid fruit in 2019 had a significantly larger volume than that in 2018, but in week 9, fruit volume in 2019 was significantly smaller than in 2018. Diploid and tetraploid SSC values were significantly different between 2018 and 2019 in both week 1 (2x $P = 0.0079$; 4x $P < 0.0001$) and week 5 (2x $P = 0.0258$; 4x $P = 0.0010$). SSC values of mixoploids ($P = 0.001$) were significantly different between 2018 and 2019 in week 9.

In 2018, there was a significant difference in SSC values in week 3 ($P = 0.0053$), 7 ($P = 0.0022$), and 9 ($P = 0.0002$) between mixoploids and diploids (Fig. 3). Diploids had higher SSC than mixoploids and tetraploids across all weeks. Mixoploids had consistently larger volumes and lower SSC across the weeks compared with the other ploidy levels (Fig. 3). Diploids had intermediate volumes

and consistently the highest SSC. Across the weeks, diploids and tetraploids had relatively similar volumes. Like 2018, mixoploids in 2019 had a consistently higher volume across weeks, whereas tetraploids had the smallest volume and lowest SSC (Fig. 4). There was a significant difference in SSC of diploids and tetraploids in week 9 ($P = 0.0049$). In both week 5 ($P = 0.0028$) and week 9 ($P = 0.0048$), tetraploids had significantly lower volumes than both diploids and mixoploids. Tetraploids had significantly lower SSC than both diploids and mixoploids all 3 weeks (Fig. 4). Mixoploids had the largest volume, but diploids and tetraploids were not different across the weeks in both years, although diploids appeared slightly larger than tetraploids. SSC did not follow a similar pattern for mixoploids between years.

Discussion

Plant height and width were statistically significant among cytotypes. Tetraploids were shortest and narrowest in width. This indicates that ploidy does affect plant size and architecture *V. ovatum*. A similar result was observed following induced polyploidization of *Hibiscus*

acetosella, which resulted in octoploids that were shorter, had reduced canopy volume, and shorter internodes (Contreras et al., 2009). The impact of induced polyploidy on plant size and architecture is variable, with some plants generally increasing in size at the tetraploid level compared with diploids (e.g., *Rhododendron* 'Fragrant Affinity', personal observation), others become more compact, as described in *Hibiscus acetosella* (Contreras et al., 2009), whereas still others appear to have little or no change in early observations of overall plant size following induced polyploidization (*Acer ginnala*, personal observation).

There are numerous examples where polyploidy has a gigas effect on fruit and plant qualities when increasing ploidy levels (Sattler et al., 2016; Wu et al., 2012), and we also observed impacts of polyploidy on fruit volume and sugar content. First, over the duration of the study, there was no consistent increase in fruit size from diploid to tetraploid. In 2017, measurements were taken on a single date in an early but unknown stage of fruit maturation. These measurements were able to be compared with the first week of measurements from the following 2 years. There was considerable variability in measurements across accessions within ploidy levels. This should be considered when reviewing these numbers as variation among seedlings was high. Overall, tetraploids were significantly different in fruit size from both diploids and mixoploids, however nothing else was statistically significant during 2017. Based on our first year of observation, polyploidy appeared to be a promising method to increase fruit size of evergreen huckleberry. Further, although the differences in SSC values were not statistically significant, tetraploids (15.0°Brix) were 36% higher than both diploid and mixoploids (<11.0°Brix). The results from 2017 indicated that higher ploidy increased fruit size and sugar content of *V. ovatum*. The data followed the gigas effect described by Manzoor et al. (2019), such that a doubled genome resulted in a larger fruit and higher sugar content. It is unclear why we observed this trend during a single year.

Conversely, fruit volume did not show the same trends related to ploidy level during either 2018 or 2019, and we did not observe the apparent gigas effect observed during 2017. Volume in diploids was different from other cytotypes across years ($P < 0.0001$), and SSC in tetraploids was significantly different across all years ($P = 0.0002$). In 2018 and 2019 fruit volume of tetraploid fruit was smaller than that of diploid and mixoploid. In both years mixoploids were larger than the two other cytotypes, but SSC levels varied each year. Although we can say that there is some effect of genome size on fruit volume, it is not the normal expected effect, with a higher ploidy level resulting in greater volume, and is not consistent over time. Our results indicate that there were factors other than just genome size affecting the fruit development. It is unclear how much plant status, inadvertent fruit selection, or other error factors may have

Fig. 4. Fruit volume and soluble solids content measured three times during 9 weeks of 2019 from (A) diploid (2x, n = 17), (B) mixoploid (2x + 4x, n = 5), and (C) tetraploid (4x, n = 3) cytotypes of *Vaccinium ovatum*.

contributed to our results. Timing of physiological maturity and optimal harvest date was a possible contributing factor, as flowering date and time to maturity has been observed to vary among ploidy levels.

To address the question of harvest timing, we investigated development of the fruit size and sugars over the growing season to identify optimal harvest date for comparison. SSC values were similar to each other among collection dates within seasons, which resulted in a lack of statistical significance within ploidy across weeks. There was no clear trend for volume development over the season in 2018, although it is unclear why. There are no studies that show similar results in either grapes or blueberries. The only statistically significant volume measurements in 2018, were between week 5 and 7. We have no explanation for the dip in berry volume development in the fifth week of testing beyond experimental error. In contrast to 2018, SSC increased across the weeks during 2019, as would typically be expected as fruit ripens, and starches turn to sugar. There were inconsistent results in measurements over years, indicating there may be more affecting

these measurements than just genome size. SSC did not follow a similar pattern for mixoploids between years, but for diploids and tetraploids SSC follows a similar pattern where SSC for diploids is just slightly higher than that of tetraploids over all the weeks. Like 2018, mixoploids in 2019 had larger fruit across weeks, whereas tetraploids had the smallest fruit and lowest SSC (Fig. 4). There was a significant difference in SSC between diploids and tetraploids in week 9.

Berry size was a poor indicator of ripeness in evergreen huckleberries. As displayed in several studies on highbush blueberry (Woodruff and Dewey, 1959) and lowbush blueberry (Barker et al., 1963; Collins et al., 1966; Ismail and Kender, 1974), a better determinant of a berry's ripeness could be color change rather than the diameter/volume measurement. If fruit had been tested from an earlier period through the later part of the season a typical double-sigmoid curve of growth would have been observed (Godoy et al., 2007; Tamanda, 2002). This double sigmoid growth is fruit growth occurring in three stages: stage 1 immature fruit that are growing rapidly after flowering, stage 2 fruit grow very

little, and stage 3 a period of rapid growth where fruit hit maturity and maximum growth. This double sigmoid growth pattern could explain some of the variability in our data, as we did not test early enough to see the stage 1 phase, samples could have been taken in different stages of fruit growth between the years. All berries were deeply colored by the time our observations began.

In several previous studies in cherries, kiwifruit, and peaches, high SSC is related to consumer acceptability (Crisosto et al., 2003; Gorini and Lasorella, 1990; Robertson et al., 1988). However, SSC alone are not the best indicator of consumer acceptability and preferences, as Jayasena and Cameron (2008) found that acids are important in human perceptions of sweetness. They determined that a °Brix-to-acid ratio was highly associated with consumer acceptability of 'Crimson' seedless table grapes. Berries in our study were not evaluated at immature, midripe, and ripe stages, as previously conducted (Ayaz et al., 2001). Berries were also being evaluated to determine correct time of ripeness for home consumers and, because this timeline did not need to be exact, rather a general guide to ripening would suffice. As determined from other sources and our own data inconsistencies, heat unit or degree-day accumulation offers a more accurate measure of the developmental time of the fruits and determines the optimal picking time for home gardeners (Godoy et al., 2007) and should be considered in future evaluation of evergreen huckleberry.

As a native, edible, evergreen shrub, *V. ovatum* remains an attractive option to Pacific Northwest gardeners. Our interest is to develop cultivars with compact habit and larger, more flavorful fruit. Among the plants evaluated during this study, some appear superior for one or more of these traits, but induced polyploidy alone is not a panacea. We will continue to measure production quality attributes but regarding flavor traits, a sensory panel is a logical next step, as quantitative measurements alone are often poor predictors of consumer preference.

These results are limited by our sampling technique and small number of plants tested. Destructive sampling was a limitation that contributed to our limited sampling number and likely contributed to sampling bias and some of the inexplicable data. Another limit that will be hard to overcome is in the determination of the SSC value in single fruits, which showed a rather high standard error. These limitations could be mitigated in the future through different sampling techniques such as near-IR spectroscopy, high-performance liquid chromatography, or a nondestructive technique that allows for repeated measures as described by Coombe (1992) (Godoy et al., 2007; Ventura et al., 1998). Along with using different methods for testing, Jayasena and Cameron (2008) proposed a method for testing over a season in grapes that should be used for future evaluation. Although each of these methods has their advantage, this relatively minor crop likely

does not warrant the necessary optimization of methods and likely is better served with semiformal sensory panels such as inviting visitors to the plots to blind taste accessions.

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