Highbush Blueberry Cultivars Differ in the Relationship between Seed Number and Berry Weight during the Harvest Season

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Abstract. The relationship between individual berry weight and viable seed number of small- (<15 mm), medium- (15–19 mm), and large-diameter (>19 mm) berries was studied over 2 years in nine cultivars (Aurora, Bluecrop, Bluegold, Draper, Duke, Liberty, Legacy, Ozarkblue, and Reka) through their harvest seasons. Plants were grown with two different preplant amendment-mulch treatments, but this treatment had no effect on the variables measured, so data were pooled. The highest average seeds/berry was in ‘Bluecrop’ (55.5) and ‘Duke’ (50.0) and the lowest in ‘Bluegold’ (17.1), ‘Aurora’ (22.5), and ‘Liberty’ (23.5). Average berry weight over the fruiting season ranged from 1.79 and 1.80 g for ‘Liberty’ and ‘Reka’, respectively, to 2.30 and 2.44 g in ‘Ozarkblue’ and ‘Draper’, respectively. The average number of seeds/berry was higher in 2010 than in 2009 for all cultivars, ranging from 14% higher in ‘Reka’ to 96% higher in ‘Liberty’. The flowering period was earlier in 2010 than in 2009, but bloom was concentrated in 2009 (28 days) compared with 2010 (45 days), likely affecting pollinator success. In general, seeds/berry and berry weight declined through the harvest season. Some cultivars had a considerable difference in the number of seeds in large-sized than in small-sized berries (e.g., 89%, 107%, 108%, and 147% more seeds in ‘Aurora’, ‘Reka’, ‘Bluegold’, and ‘Liberty’, respectively), whereas others had relatively little difference (14% and 36% in ‘Draper’ and ‘Bluecrop’, respectively). There was a linear relationship between berry weight and viable seeds/berry. Cultivars did not differ in the berry weight per seed (slope of the line) between the 2 years, except for ‘Draper’ (only significant in 2009), ‘Legacy’, and ‘Reka’. ‘Bluegold’ had the greatest berry weight per seed and ‘Reka’ the lowest. The weakest relationship between berry weight and seed number was in ‘Draper’. Cultivars that produced parthenocarpic fruit of marketable size were ‘Aurora’, ‘Bluegold’, and ‘Liberty’. ‘Duke’, ‘Legacy’, ‘Reka’, and ‘Ozarkblue’ produced commercially acceptable fruit (0.75–1.0 g) with fewer than 7 seeds/berry. By contrast, some cultivars required a relatively large number of seeds to produce a berry including ‘Bluecrop’ (28–40 seeds), ‘Draper’ (15–23 seeds), and ‘Legacy’ in 2010 (20 seeds). The number of seeds per berry accounted for as much as 87% of the variability in berry weight indicating the importance of good pollination and seed set for berry weight and yield in these cultivars.

In blueberry (*Vaccinium* spp.), issues related to poor pollination are considered as important production problems in many regions (e.g., Oregon Blueberry Commission, 2018; Northwest Center for Small Fruits Research, 2018; Washington Blueberry Commission, 2018). In particular, growers are concerned with poor fruit set (the proportion of flowers that become

berries), but seed set (the proportion of ovules that become seeds) and fruit size at harvest are also important. Commercial growers in the northwestern United States have reported poor fruit set in northern highbush blueberry (*V. corymbosum* L.) from insufficient pollinator activity, particularly in Washington State (DeVetter et al., 2016). In Oregon, commercial growers report fewer problems with fruit set, likely because of better weather and pollinator activity during bloom. In addition, Strik et al. (2017) found good fruit set (93%) over a 6-year study in a research trial in Oregon, with little difference between 10 cultivars.

Low fruit and seed set may be related to insufficient pollinator activity, a short period when individual flowers are open (5–12 d), limited pollinator access in some cultivars related to flower shape (Courcelles et al., 2013) and differential nectar or pollen quality. The even shorter effective pollination period, estimated at 2–4 d (Dogterom et al., 2000; Kirk and

Isaacs, 2012; Moore, 1964), coupled with impacts of weather on pollen deposition, pollen germination, pollen tube growth, and ovule fertilization (Stephenson et al., 1992) may further limit fruit and seed set. The main pollinator used in commercial blueberry fields is the honey bee (*Apis mellifera* L.). Reduced air temperature (<12 °C), wind speeds above 20 kph, and precipitation reduced honey bee activity and correspondingly the number of seeds/berry (Tuell and Isaacs, 2010). Honey bee visitation rates were correlated with the number of seeds per berry (seeds/berry) and correspondingly with berry weight (DeVetter et al., 2016). Nutrient deficiencies within the flower may limit seed set (Chen et al., 1998; May and Killingbeck, 1992).

Cultivars differ in percent fruit set or seed set because of differing bloom periods and associated differences in weather (Arrington and DeVetter, 2017), differences in attractiveness of the flowers to pollinators (Courcelles et al., 2013), and genetic differences in self- or cross-compatibility and pollen quantity or quality (e.g., Brewer and Dobson, 1969; Chavez and Lyrene, 2009; Ehlenfeldt and Kramer, 2012; MacKenzie, 1997; Rabaey and Luby, 1988; Vander Kloet, 1983). As a result, cultivars have been found to differ in the seeds/berry and, often as a result, berry size (Arrington and DeVetter, 2018; Brewer and Dobson, 1969; DeVetter et al., 2016; Eaton, 1967; Ehlenfeldt, 2001; Ehlenfeldt and Martin, 2010; Gupton, 1984; Gupton and Spiers, 1994; Jackson et al., 1972; Lang and Danko, 1991; Lyrene, 1989; Meader, 1936; Meader and Darrow, 1944, 1947; Moore et al., 1972; Morrow, 1943; Payne et al., 1989; Taber and Olmstead, 2016).

Cultivars differ in the berry weight per seed (Bailey, 1938; Brewer and Dobson, 1969; Eaton, 1967; Gupton, 1984; Knight and Scott, 1964; Lyrene and Goldy, 1983; Meader and Darrow, 1944; Rabaey and Luby, 1988; Taber and Olmstead, 2016). It is possible that cultivars vary in growth hormones produced by the fertilized ovules or developing seeds (Mainland and Eck, 1968; Meader and Darrow, 1944, 1947; Moore et al., 1972). With poor seed set, parthenocarpic fruit may be induced by exogenous application of gibberellic acid, but these berries remain small compared with pollinated fruit with seeds (Barker and Collins, 1965; Williamson et al., 1995).

There are many highbush blueberry cultivars grown in the northwestern United States (Strik et al., 2014), some of which are reported to have more variability in berry weight and fruit set than others (e.g., ‘Liberty’ and ‘Draper’). Our objectives were to determine 1) whether the commonly grown cultivars differ in the number of seeds/berry, and 2) the relationship between seeds/berry on berry diameter, harvest season, and berry weight. This information will enhance our understanding of the importance of good pollination and potentially explain differences observed among cultivars.

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Materials and Methods

The study was conducted in 2009 to 2010 in a planting of highbush blueberry established in Oct. 2006 at Oregon State University's North Willamette Research and Extension Center (NWREC, Aurora, OR; lat. 45°16'47"N, long. 122°45'23"W). Weather data for this site are available from an AgriMet weather station (U.S. Dept. Interior, 2014). Soil at the site was a Willamette silt loam (fine-silty mixed superactive mesic Pacific Ultic Argixeroll) and had a pH of 4.9% and 3.7% organic matter at planting. Plants were irrigated using a single line of polyethylene drip tubing (Netafim, Fresno, CA) with 2 L·h⁻¹ pressure-compensating, inline emitters spaced every 0.3 m. Plant spacing was 0.8 m by 3.0 m (4385 plants/ha). A permanent grass cover crop was grown in the row aisles and mowed during the growing season, as required. Additional details of planting establishment, management, fertilization, and leaf nutrient sufficiency levels are published elsewhere (Strik et al., 2017; Strik and Vance, 2015). Honey bees were introduced to the field at ≈5% to 10% bloom at a stocking rate of 10 hives/ha.

Nine cultivars (Aurora, Bluecrop, Bluegold, Draper, Duke, Liberty, Legacy, Ozarkblue, and Reka) were planted with two different preplant amendment-mulch treatments. The "compost + sawdust" treatment included a preplant amendment and a mulch of municipal yard debris compost and sawdust, while the "weed mat" treatment involved no preplant amendment but included a mulch of fresh douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*] sawdust topped with black, woven polyethylene groundcover. Additional information on amendment-mulch treatments are reported in Strik et al. (2017). There were two replicates of each cultivar-amendment-mulch treatment combination, and plots consisted of seven plants each. The design of the planting with nine cultivars and a good stocking rate of honey bees—ensured pollination (self- or cross-) was not limiting if weather was favorable. Data were collected on the date of 5% bloom and full bloom for each cultivar.

Whole plots of each cultivar were harvested by hand at a commercial stage of ripeness at ≈7- to 10-d intervals, between two and five times per year, depending on the cultivar and the associated length of the fruiting season. On each of harvest 1–5, a subsample of fruit from each plot was divided into three berry size categories, small (<15 mm), medium (15–19 mm), and large (>19 mm) diameter, and 10 berries from each size category were individually weighed and frozen for later analysis of seed number.

Seeds were separated from skin and pulp and prepared for counting using the procedure published by Ehlenfeldt and Martin (2010). A 32 × 32 wire mesh screen (0.57-mm-diameter holes) was used to separate seeds considered viable (presumed to contain a live embryo) from smaller, unviable seeds

that passed through the sieve. Seed viability was determined using a tetrazolium chloride assay (Patil and Dadlani, 2009) of all seeds from a subsample of 10 large-diameter 'Bluecrop' fruit. Seeds were separated into seven size categories, and live embryos were only found in seeds larger than 0.57 mm (100%); these seeds were plump and darker in color. Seeds smaller than 0.57 mm were flat and lighter colored and were determined to be unviable; these were not included in the seed counts and data reported herein. Our perception of the visible differences between viable and nonviable seeds agreed with others (Dogterom et al., 2000; Ehlenfeldt and Martin, 2010; Morrow, 1943), but we found that viable seeds were smaller than previously reported (Dogterom et al., 2000).

For each berry, the seeds remaining after sieving were weighed before counting using an electronic seed counter (Model 850-2; The Old Mill Co., Savage, MD). The machine was calibrated to confirm accurate seed counts against a known number of seeds. Stone cells, which were separated from the pulp along with the viable seeds, were counted and subtracted from the number of seeds counted. The number of seeds/berry and berry weight were recorded for individual fruit within berry size categories for each harvest, as described previously. The seed number per gram of berry was calculated.

Statistical analyses were performed using SAS 9.3 (Cary, NC). An initial analysis using PROC MIXED was performed to determine the effects of year, amendment-mulch, and cultivar. Amendment-mulch had no significant effects on seed number or berry weight (data not shown) and did not interact with the significant effects of year and cultivar, so the data were pooled providing four replicates for the remaining analyses. A split-plot analysis was performed by cultivar to evaluate the year (main plot, *n* = 2), harvest number (subplot, *n* = 2 to 5, depending on cultivar), and berry size category effects (nested subplot, *n* = 3). PROC REG was used to de-

termine the linear relationship between seeds/berry and berry weight by cultivar. The effect of year and cultivar on the minimum and maximum number of seeds/berry was analyzed using PROC MIXED. Means were separated at the 5% level using Tukey's honest significant difference test.

Results and Discussion

Year and cultivar effects. There was an effect of cultivar and a year by cultivar interaction (*P* < 0.0001) on the average number of viable seeds/berry, berry weight, and seeds per gram of berry weight but no effect of amendment-mulch (data not shown). The highest average seeds/berry was found in 'Bluecrop' (55.5) and 'Duke' (50.0) and the lowest in 'Bluegold' (17.1), 'Aurora' (22.5), and 'Liberty' (23.5). Average berry weight ranged from as low as 1.79 and 1.80 g for 'Liberty' and 'Reka', respectively, to 2.30 and 2.44 g in 'Ozarkblue' and 'Draper', respectively. On average, 'Bluecrop', 'Duke', 'Legacy', and 'Reka' had the highest number of seeds per gram of berry weight (21.7–28.1) and 'Aurora', 'Bluegold', 'Liberty', and 'Ozarkblue' the fewest (8.0–13.7).

There was an effect of year on the average minimum and maximum number of viable seeds/berry, across all cultivars, harvest numbers, and berry size categories with lower levels in 2009 than in 2010 (Table 1). 'Bluecrop', 'Duke', and 'Reka' had the highest maximum number of seeds/berry, whereas 'Bluegold' and 'Aurora' had the lowest. 'Bluecrop' also had the highest minimum number of seeds/berry, whereas 'Aurora', 'Bluegold', 'Liberty', and 'Reka' had the lowest. Most cultivars had little difference between years for the maximum number of seeds/berry. However, 'Bluecrop' and 'Legacy' had a higher maximum seeds/berry in 2010 than in 2009 (data not shown).

The weight of seeds/berry was greater in 2010 than in 2009 and cultivars differed in

Table 1. The effect of northern highbush blueberry cultivar and year on the number of seeds, seed weight, and the number of stone cells per berry, averaged over harvest number and amendment mulch.

	Seeds/berry		Seed wt (mg/berry)	Stone cells/berry (no.)
	Maximum	Minimum		
Year				
2009	46 b ^y	11 b	9.5 b	0.8
2010	55 a	21 a	14.0 a	0.9
Cultivar				
Aurora	37 d	6 d	9.2 e	0.2 g
Bluecrop	65 a	38 a	19.4 a	1.3 ab
Bluegold	26 e	7 d	4.4 f	0.3 fg
Draper	45 c	26 b	11.6 cd	1.3 ab
Duke	69 a	19 bc	13.2 c	0.7 de
Legacy	54 b	20 bc	15.3 b	1.4 a
Liberty	41 cd	7 d	9.7 de	0.9 cd
Ozarkblue	53 b	13 cd	12.0 c	0.5 ef
Reka	64 a	10 d	11.8 c	1.1 bc
Significance ^z				
Year	<0.0001	<0.0001	<0.0001	NS
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001
Year × cultivar	<0.0001	NS	<0.0001	<0.0001

^zResults from analysis of variance. Nonsignificant (NS) or actual *P* value provided when significant (*P* < 0.05).

^yMeans followed by the same letter within treatment are not significantly different (LSMEANS) (*P* > 0.05).

Table 2. The main effects of year, harvest number (earliest, 1, to latest, 5, depending on the cultivar) and berry size category (small, medium, and large diameter) on the seeds/berry and berry weight for nine northern highbush blueberry cultivars.

Treatment	Aurora			Bluecrop			Bluegold			Draper			Duke			Legacy			Liberty			Ozarkblue			Reka		
	Seeds/berry ^z	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry	Seeds/berry	wt (g)	Berry
Year																											
2009	22.4 b	2.14	1.78 b	15.0 b	1.92 b	2.05 b	31.5 b	2.05 b	43.6 b	1.89 b	27.9 b	1.67 b	15.9 b	1.48 b	25.4 b	2.00 b	39.7 b	1.61 b									
2010	26.2 a	2.05	2.41 a	18.9 a	2.37 a	2.83 a	42.0 a	2.83 a	56.5 a	2.32 a	48.8 a	2.36 a	31.1 a	2.10 a	34.9 a	2.61 a	47.0 a	2.03 a									
Harvest number																											
1	28.3 a ^y	2.28 a	2.21 a	17.9	2.29 a	2.61 a	38.3	2.61 a	58.0 a	2.20 a	45.0 a	2.41 a	29.8 a	2.03 a	40.9 a	2.76 a	47.6 b	1.94 a									
2	27.9 a	2.19 a	1.98 b	16.2	2.00 b	2.45 b	36.9	2.45 b	54.4 a	2.12 a	41.9 a	2.06 b	25.2 b	1.87 b	35.9 b	2.35 b	51.4 a	1.97 a									
3	23.9 b	2.14 a	na ^x	na	na	2.26 c	35.0	2.26 c	46.4 b	2.18 a	36.5 b	1.99 b	21.8 c	1.77 b	30.0 c	2.45 b	41.4 c	1.79 b									
4	17.6 c	1.85 b	na	na	na	Na	na	Na	41.3 b	1.92 b	30.0 c	1.62 c	17.1 d	1.49 c	24.8 d	2.04 c	32.9 d	1.58 c									
5	na	na	na	na	na	Na	na	Na	na	na	na	na	na	na	16.9 e	1.81 d	na	na									
Berry size ^w																											
Small	16.7 c	1.14 c	1.35 c	11.4 c	1.37 c	1.55 c	34.8 b	1.55 c	38.1 c	1.31 c	31.5	1.21 c	13.2 c	0.92 c	23.2 c	1.35 c	27.5	1.20 c									
Medium	25.9 b	2.08 b	2.06 b	17.5 b	2.08 b	2.37 b	35.7 b	2.37 b	53.3 b	2.07 b	38.9	1.99 b	24.6 b	1.77 b	30.6 b	2.23 b	45.6	1.87 b									
Large	29.9 a	3.06 a	2.87 a	22.5 a	3.05 a	3.39 a	39.7 a	3.39 a	58.8 a	2.94 a	44.7	2.85 a	32.6 a	2.68 a	36.2 a	3.31 a	56.8	2.39 a									
Significance ^v																											
Year (Y)	<0.0001	0.7198	<0.0001	0.0011	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001									
Harvest (H)	<0.0001	<0.0001	0.0059	<0.000001	<0.0001	<0.0001	0.1409	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001									
Y × H	0.0892	<0.0001	0.3651	0.0076	<0.0001	0.209	0.7236	0.0015	0.0453	0.0453	0.0014	<0.0001	0.0077	0.001	0.0004	<0.0001	0.0022	0.0453									
Berry size (S)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.013	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001									
Y × S	0.8717	0.1183	0.9714	0.4504	0.0045	0.3087	0.387	<0.0001	0.4173	0.0762	0.0686	<0.0001	0.3366	0.0155	0.1004	0.0004	0.0246	0.3475									
H × S	0.4452	0.2190	0.7505	0.2805	0.696	<0.0001	0.2135	0.001	0.0117	0.0026	0.7405	0.0009	0.07	0.4899	0.0106	0.0001	<0.0001	0.0117									
Y × H × S	0.1422	0.2760	0.5415	0.5637	0.7198	0.1627	0.578	0.1038	0.4939	0.0628	0.9808	0.7484	0.0158	0.1496	0.4319	0.6744	0.0544	0.3631									

^zAverage number of viable seeds/berry.

^yMeans followed by the same letter within treatment are not significantly different (LSMEANS) ($P > 0.05$).

^xData not available as no fruit were harvested or harvest took place in only one out of 2 years.

^wBerry size dimensions per category. Small (<15 mm in diameter); medium (15–19 mm); and large (>19 mm).

^v P values represent the analysis for harvests that occurred in both years, excluding data from harvest numbers that occurred in only 1 year. NS indicates not significant ($P > 0.05$).

seed weight/berry with ‘Bluecrop’ having the most and ‘Bluegold’, ‘Aurora’, and ‘Liberty’ the least. Moore et al. (1972) found that seed size differed among highbush blueberry cultivars, but this was not related to berry weight. On average, there was less than one stone cell per berry, but cultivars differed with ‘Legacy’ having the highest and ‘Aurora’ and ‘Bluegold’ the fewest (Table 1).

Others have reported cultivar differences in seeds/berry in blueberry (e.g., Darrow, 1958; Ehlenfeldt and Kramer, 2012; Taber and Olmstead, 2016). The average numbers of seeds/berry reported here are higher than what has been reported for the same cultivars in other studies. For example, MacKenzie (1997) reported an average of 20 to 24 seeds/berry in open pollinated ‘Bluecrop’, depending on year, whereas Dogterom et al. (2000) found 48 seeds/berry in ‘Bluecrop’. Ehlenfeldt and Martin (2010) reported 24–50 and 21–42 seeds/berry in ‘Bluecrop’ and ‘Duke’, respectively, with a minimum seed number of 8–30, depending on year. In our study, minimum seed number also varied greatly among cultivars. In Washington, Arrington and DeVetter (2018) found fewer seeds/berry in ‘Duke’ (11–33) than in our study, but also reported lower fruit set in their region, likely because of adverse weather reducing pollinator activity (Arrington and DeVetter, 2017). Increased pollinator activity through stocking with more hives/ha increased seeds/berry (Arrington and DeVetter, 2018). Although Ehlenfeldt and Martin (2010) reported that an early-ripening cultivar had fewer seeds and smaller berries than a later ripening cultivar, we found no such relationship. The earliest fruiting cultivar in our study was ‘Duke’, which had the highest average seeds/berry and the largest berry weight. By contrast, the latest ripening cultivar Aurora had large berries with lower average seeds/berry.

Year, harvest number, and berry size category effects by cultivar. There was a significant effect ($P \leq 0.05$) of year on the average seeds/berry in all cultivars and on berry weight in all except for ‘Aurora’ (Table 2). Weather, particularly rain and temperature, affects the activity of honey bees and can, thus, affect fruit set or seed set (DeVetter et al., 2016; Tuell and Isaacs, 2010). Although we did not measure percent fruit set, Strik et al. (2017) reported an average fruit set of 93% (2008–13) in this planting, and we observed good pollinator activity during the study. Multiple visits by bees to a flower are required for pollination in blueberry because deposition of higher numbers of pollen tetrads increase seed set or seeds/berry (Dogterom et al., 2000). They found that providing excess pollen to ‘Bluecrop’ flowers with an average of 106 ovules led to 48 large, plump seeds for a seed set of 45%. Moore et al. (1972) reported a seed set ranging from 22% to 52%, depending on cultivar, with 52% in ‘Bluecrop’.

The average number of seeds/berry was higher in 2010 than in 2009 for all cultivars, ranging from 14% higher in ‘Reka’ to 96%

higher in ‘Liberty’. The flowering period started earlier (5% bloom from 24 Mar. to 20 Apr.) and full bloom was earlier (18 Apr.–6 May) in 2010 than in 2009 (5% bloom from 15 to 30 Apr.; full bloom 2–15 May) because of a warmer late-winter period in 2010 (data not shown). In addition, bloom was concentrated in 2009 (28 d) compared with 2010 (45 d). Although there were more rain events during the bloom season in 2010 than in 2009 (30 and 14 d, respectively) and a higher volume of rain (190 and 104 mm, respectively), it was considerably warmer in 2009 than in 2010 (Fig. 1). If temperatures get too warm (potentially above 20 °C), the effective pollination period or pollen fertility may be reduced (Kirk and Isaacs, 2012; Stephenson et al., 1992), potentially decreasing the seed set. The more concentrated bloom period in

2009 may also have limited pollinator effectiveness with more flowers open at once, yet the same number of hives per hectare coupled with a possibly shorter effective pollination period due to warmer temperatures. Although ‘Legacy’ and ‘Reka’ were the earliest to reach 5% bloom in both years, all nine cultivars overlapped considerably in the bloom period (data not shown).

Harvest number had a significant effect on seeds/berry in all cultivars, except for ‘Bluegold’ ($P = 0.06$) and ‘Draper’ ($P = 0.14$), and on berry weight in all cultivars, both declining through the harvest season (Table 2). There was a year by harvest number interaction on seeds/berry in six cultivars (Fig. 2). There were more harvests in 2010 than in 2009 in ‘Bluegold’, ‘Legacy’, and ‘Reka’, likely because of the

prolonged bloom period (Fig. 1). In ‘Bluegold’, ‘Legacy’, ‘Duke’, and ‘Liberty’, the seeds/berry was more consistent among harvest dates in 2010 than in 2009, when seeds/berry declined more rapidly (Fig. 2). In ‘Ozarkblue’, the seeds/berry declined from the first through the fifth harvests in both years (Fig. 2E). Our results agree with others who reported a decrease in seeds/berry with progressively later harvests (Brewer and Dobson, 1969; Darrow, 1958; Ehlenfeldt and Martin, 2010; Moore et al., 1972). The decrease in seeds/berry over the harvest season may be related to less adequate pollination in later opening flowers (Brewer and Dobson, 1969), fewer ovules in later opening flowers (Moore et al., 1972), or a decrease in the percent seed set as shown by Meader and Darrow (1947).

Fig. 1. Maximum and minimum daily air temperature (A) and precipitation (B) in 2009 and 2010 from 20 Mar. to 22 May at Oregon State University's North Willamette Research and Extension Center, Aurora, OR. The earliest date for 5% bloom and the latest date for full bloom are indicated. Weather data from an on-site AgriMet weather station (U.S. Dept. Interior, 2014).

Fig. 2. Relationship of harvest number (1–5, depending on the cultivar) and year (2009 and 2010) on the seeds/berry of (A) 'Bluegold', (B) 'Duke', (C) 'Legacy', (D) 'Liberty', (E) 'Ozarkblue', and (F) 'Reka', averaged over berry size category. Error bars represent ± 1 SE.

There was a year by harvest number interaction on berry weight in all of the cultivars (Fig. 3) except for 'Draper' (Table 2). In most cultivars, berry weight was greater in 2010 than in 2009 for all harvests, but differences between years were greater in the second and subsequent harvests than for the first harvest (Fig. 3).

Because berries were divided into categories by their diameter to ensure a range in berry weights and potential seed number, seeds/berry and berry weight differed by size category in all cultivars (Table 2). In general, large-diameter berries had more seeds than small-diameter berries as found by Jackson et al. (1972). There was, however, quite a range in seeds/berry by size category. For example, some cultivars had a considerable difference in the number of seeds in large-sized (>19 mm) compared with small-sized

berries (<15 mm) (e.g., 89%, 107%, 108%, and 147% more seeds in 'Aurora', 'Reka', 'Bluegold', and 'Liberty', respectively), whereas others had relatively little difference (14% and 36% in 'Draper' and 'Bluecrop', respectively); 'Legacy' and 'Duke' were in between (45% and 54%, respectively). On average, medium-sized berries had from 3% ('Draper') to 86% ('Liberty') more seeds than small-sized berries. There was a year by berry size category interaction for seeds/berry for 'Bluegold', 'Legacy', and 'Reka' and for berry weight in 'Bluegold', 'Draper', 'Legacy', and 'Liberty' (Table 2). In 'Bluegold', although the seeds/berry was similar for large- and medium-sized fruit in both years, small-sized fruit had almost double the seeds in 2010 compared with 2009 (data not shown). In 'Reka', small- and medium-sized fruit had slightly more seeds/berry in 2010

than in 2009, but large fruit were similar between years. In 'Legacy', berries in all size categories had more seeds in 2010 than in 2009, but particularly in large fruit (data not shown).

There was a harvest number by berry size category interaction on seeds/berry for 'Duke', 'Ozarkblue', and 'Reka' and for berry weight in 'Bluegold', 'Draper', 'Duke', 'Legacy', 'Ozarkblue', and 'Reka' (Table 2). 'Duke' fruit maintained a high number of seeds/berry for medium- and large-sized fruit through the harvest season, as did 'Reka' (Fig. 4). However, the seeds/berry declined through the harvest period for all size categories in 'Ozarkblue'. In general, for cultivars where there was an interaction between harvest number and berry size category for berry weight, there was more of a difference between the weight of small-, medium-, and

Fig. 3. The relationship of harvest number (1–5, depending on the cultivar) and year (2009 and 2010) on the berry weight of (A) 'Aurora', (B) 'Bluecrop', (C) 'Bluegold', (D) 'Duke', (E) 'Legacy', (F) 'Liberty', (G) 'Ozarkblue', and (H) 'Reka', averaged over berry size category. Error bars represent ± 1 SE.

large-sized fruit on the last harvest date than earlier harvests (data not shown).

Seed number effect on berry weight. There was a significant linear relationship between berry weight and viable seeds/berry for all

cultivars in both years, except for 'Draper' in 2010 (Table 3; Fig. 5). Cultivars did not differ in the berry weight per seed (slope of the line) between the 2 years, except for 'Draper' (only significant in 2009), 'Legacy', and

'Reka'. 'Bluegold' had the greatest berry weight per seed (94–102 mg/seed). A large group of cultivars had intermediate slopes, including 'Aurora', 'Bluecrop', 'Draper', 'Legacy' (2010), 'Liberty', and 'Ozarkblue'.

Fig. 4. The relationship of harvest number (1–5, depending on cultivar) and berry size category (small = <15 mm diameter; medium = 15–19 mm; large = >19 mm) on the seeds/berry for (A) 'Duke', (B) 'Ozarkblue', and (C) 'Reka', averaged over year. Error bars represent ± 1 SE.

'Reka' had the smallest slope of the line, particularly in 2009 (29 mg/seed). Both 'Reka' and 'Liberty' had the strongest relationship between berry weight and seed number in both years (high R^2 ; Table 3). The weakest relationship between berry weight and seed number was in 'Draper' where it was only significant in 2009 and was relatively weak (low R^2), e.g., berries with 30 seeds produced anywhere from 0.75 to 3.0 g fruit (Fig. 5D). Our findings are counter to those of Ehlenfeldt and Martin (2010), who found no significant linear relationship between berry weight and seeds/berry in 'Duke'. Our results agree with others, who reported an increase in the number of fully developed seeds was associated with an increase in berry size in some or all of the highbush blueberry cultivars studied (DeVetter et al., 2016; Ehlenfeldt, 2001; Ehlenfeldt and Martin, 2010; Gupton, 1984; Gupton and Spiers, 1994; Lang and Danka, 1991; Lyrene, 1989; Meader, 1936; Meader and Darrow, 1944; Moore et al., 1972).

The berry weight per seed (slope of line in Table 3) ranged from 29–102 mg/seed depending on cultivar, similar to values reported by others (Ehlenfeldt, 2001; Moore et al., 1972) but greater than what was reported by Eaton (1967). Similar prior work found that cultivars differed in the berry weight developed per seed (Bailey, 1938; Brewer and Dobson, 1969; Eaton, 1967; Gupton, 1984; Knight and Scott, 1964; Lyrene and Goldy, 1983; Meader and Darrow, 1944; Rabaey and Luby, 1988; Taber and Olmstead, 2016). Fruit enlargement in blueberry is thought to be stimulated by growth hormones produced by the fertilized ovules or developing seeds (Mainland and Eck, 1968; Meader and Darrow, 1944, 1947). Cultivars may differ in the amount of stimulating growth hormone produced per seed (Meader and Darrow, 1944; Moore et al., 1972) leading to the observed differences. In the case of no or little seed set, parthenocarpic fruit may be induced by exogenous application of gibberellic acid, but in such cases, fruit do not attain the size of pollinated fruit with seeds (Barker and Dobson, 1969; Williamson et al., 1995). Differences in size between pollinated and parthenocarpic fruit treated with gibberellic acid were due to more cell enlargement in pollinated fruit rather than more cell division (Cano-Medrano and Darnell, 1997).

Results of regression analysis indicated that cultivars with a positive intercept ('Bluegold', 'Duke', 'Ozarkblue', and 'Reka', and 'Aurora', 'Legacy', and 'Liberty' in 2009) may produce a berry without any seeds (parthenocarpic) (Table 3). However, based on the data collected, cultivars that did produce parthenocarpic fruit of marketable size (0.5 g) were 'Aurora', 'Bluegold', and 'Liberty' (all in 2009 only) (Fig. 5). In addition, 'Duke', 'Legacy', 'Reka', and 'Ozarkblue' produced commercially acceptable fruit (0.75–1.0 g) in 2009 with fewer than 7 seeds/berry. By contrast, some cultivars required a relatively large number of seeds to

produce a berry, including ‘Bluecrop’ (28–40 seeds), ‘Draper’ (15–23 seeds), and ‘Legacy’ in 2010 (20 seeds) (Fig. 5). Our findings of ‘Bluecrop’ having low levels of parthenocarpy compared with ‘Duke’ agree with MacKenzie (1997) and Ehlenfeldt and Martin (2010).

In some cultivars, the stimulation of pollination and perhaps even fertilization of ovules with early abortion of embryos (no full seed development) may increase berry size (Meader and Darrow, 1944). However,

Moore et al. (1972) found a weak relationship between berry weight and total seed number (including those not fully developed) in various cultivars grown in a mixed planting (outcross pollination likely). Also Dogterom et al. (2000) found that excess pollen (outcross rather than self) did not result in 100% seed set or an increase in fruit weight. Although we found a strong impact of seeds/berry on berry weight, the amount of berry weight per seed varied with cultivar, and large berries among cultivars differed in

viable seed number. For example, 3.5 to 4.0 g fruit had as few as 30 seeds in ‘Aurora’ to 85 seeds in ‘Bluecrop’ (Fig. 5A and B). Darrow (1958) noted that cultivars with the largest berries did not always have the most seeds/berry.

In past studies, seeds/berry accounted for a relatively small percentage of the variability in berry weight [e.g., less than 40% in Eaton (1967); 37% to 47% (‘Rubel’) in Brewer and Dobson (1969); and 8% (in ‘Legacy’) to 46% (in ‘Duke’) in Ehlenfeldt (2001)]. In addition, Brewer and Dobson (1969) and Eaton (1967) hypothesized that a certain number of seeds was needed to produce a typical sized fruit for the cultivar, but seed numbers above this threshold led to no further increase in berry size. Although others have speculated that the majority of variation in berry weight may be caused by factors other than seeds/berry, we found that seeds/berry accounted for as much as 87% of the variability in berry weight (Table 3). Differences between our results and prior studies may be related to sampling because we measured individual berry weight with corresponding seed number (not pooled samples using averages) for berries of different size categories (30 berries per experimental unit) and harvest numbers to obtain a wide range of fruit per cultivar. We hypothesize that the total viable seeds/berry are more limited by ovule number per flower and the percent seed set. Although we do not know percent seed set, we found no evidence that there was a threshold in berry weight at a high number of viable seeds. Seed set may

Table 3. The results from linear regression analysis of berry weight on the number of viable seeds/berry for nine northern highbush blueberry cultivars in each of 2 years. Data include all harvest dates and berry size categories.

Cultivar	Yr	Linear regression equation ²	R ²	P value	95% Confidence interval of the slope	
					Lower limit	Upper limit
Aurora	2009	$y = 0.070x + 0.670$	0.5295	<0.0001	0.053	0.088
	2010	$y = 0.084x - 0.160$	0.7086	<0.0001	0.067	0.102
Bluecrop	2009	$y = 0.063x - 0.853$	0.6009	<0.0001	0.040	0.086
	2010	$y = 0.051x - 1.025$	0.5873	<0.0001	0.036	0.066
Bluegold	2009	$y = 0.094x + 0.502$	0.7634	<0.0001	0.070	0.118
	2010	$y = 0.102x + 0.444$	0.5468	<0.0001	0.070	0.134
Draper	2009	$y = 0.069x - 0.106$	0.3142	0.0004	0.033	0.104
	2010	—	0.0583	NS ³	—	—
Duke	2009	$y = 0.031x + 0.542$	0.5353	<0.0001	0.022	0.039
	2010	$y = 0.037x + 0.211$	0.4171	<0.0001	0.024	0.050
Legacy	2009	$y = 0.042x + 0.501$	0.4400	<0.0001	0.028	0.056
	2010	$y = 0.073x - 1.306$	0.6871	<0.0001	0.060	0.086
Liberty	2009	$y = 0.062x + 0.503$	0.7191	<0.0001	0.050	0.073
	2010	$y = 0.072x - 0.118$	0.8127	<0.0001	0.061	0.082
Ozarkblue	2009	$y = 0.063x + 0.391$	0.5559	<0.0001	0.048	0.078
	2010	$y = 0.055x + 0.677$	0.5175	<0.0001	0.041	0.070
Reka	2009	$y = 0.029x + 0.469$	0.8730	<0.0001	0.025	0.032
	2010	$y = 0.037x + 0.296$	0.8270	<0.0001	0.032	0.042

²Equation from linear regression y = berry weight (g); x = number of seeds per berry.

³Not significant ($P > 0.05$).

Fig. 5. Linear regression of berry weight on seeds/berry in 2009 and 2010 for (A) ‘Aurora’, (B) ‘Bluecrop’, (C) ‘Bluegold’, (D) ‘Draper’, (E) ‘Duke’, (F) ‘Legacy’, (G) ‘Liberty’, (H) ‘Ozarkblue’, and (I) ‘Reka’. Each point represents the average of 10 berries per replicate within each size category and harvest number.

be limited by levels of endogenous hormones or other resources with these limitations differing among cultivars. Breeders may wish to select for high-quality cultivars that produce a large berry weight per seed and can produce parthenocarpic fruit of marketable size.

The observed variable fruit set of 'Liberty' in our region seems likely due to a low percent fruit set rather than seed set based on our findings. This is especially important considering that the cultivars evaluated in this study were planted in a situation where cross-pollination was not limiting. By contrast, 'Draper' may have issues with fruit drop and variable fruit size because a relatively high number of seeds are required to produce a marketable berry and the relationship between seed number and berry weight varies by year. The very strong relationship between viable seeds/berry and berry weight in all cultivars but 'Draper' stresses the importance of good pollination and seed set for berry weight and yield in these cultivars.

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