FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Influence of cropload level on fruit size and quality of sweet cherry

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Total Project Request: $290,530 **Year 1: $**69,258 **Year 2:** $71,688 **Year 3:** $74,214

Other funding Sources: None

WTFRC Collaborative expenses: None

Budget 1 Todd Einhorn
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Item	2010	2011	2012
Salaries[1]	37,350	38,844	40,397
Benefits	21,758	22,628	23,533
Wages[2]	1,500	1,560	1,622
Benefits	150	156	162
Equipment			
Supplies[3]	6,500	6,500	6,500
Travel[4]	2,000	2,000	2,000
Miscellaneous			
Total	69,258	71,688	74,214

Footnotes: [1]Salaries are for: 1) 0.75 FTE of a postdoc salary. OPE is 57 %, and 2) 0.15 FTE for faculty research assistant factoring an OPE rate of 65 %. [2]Wages are for part-time employee to assist with data collection, OPE is 10%. [3]Supplies include GA, chemicals for cell activity assays, and rates for microscopy lab use at OSU-Corvallis. [4]Travel includes visits to sites for sampling and trips to WA for laboratory analyses, and is 59 cents/mile.

Objectives

1) Understand timing of mesocarp cell division & expansion cycles, and their relative role in fruit quality, and determine the influence of cropload on these processes
2) Determine effective application timings and rates of GA for improved cherry fruit quality

Significant Findings

1. Fruit growth, cell number and size

- Growth characteristics of 'Chelan', 'Bing' and 'Sweetheart' were remarkably similar during early- and mid-stage growth. The difference between cherry varieties was in the duration of the stage III- final fruit swelling.
- 'Sweetheart' fruit size was negatively affected by high cropload level. Reduced fruit size on heavily cropped spurs was observed by 18 days after full bloom. These effects were sustained through the remainder of the season, and at harvest. Heavily cropped spurs reduced the average fruit size by about 15%.
- Heavy croploads limited pit size (endocarp). Pit growth ceased by 38 days after full bloom, and was positively correlated with final fruit size.
- The cell division period of 'Sweetheart' fruit was completed during mid-stage I growth, about 20 days after full bloom. Cell numbers do not appear to be affected by cropload.
- Roughly half of the cells comprising an individual fruit at harvest are already present at full bloom.
- Cells of the inner region of the mesocarp were elongated at harvest. Cells of the outer region also were elongated, but during the final fruit swelling became rounded as they expanded tangentially.
- Final fruit size was more strongly correlated with cell size than cell number. These results suggest that growers have a longer time frame to alter cropload than if cell number was the dominate factor in fruit size. However, early season deficits in growth will also be evident at harvest.
- The majority of the nuclei of 'Chelan', 'Bing' and 'Sweetheart' fruit become polyploid (i.e., cells possess multiple copies of chromosomes compared to vegetative tissues). After full bloom, polyploidy in fruit increased rapidly to > 60% by 7-10 days, in step with cell division activity. Fruitlets from flowers that were bagged to prevent pollination were far less polyploid which emphasizes the role of fertilization in promoting cell division and growth.
- Cropload level, and genotype, did not influence the magnitude or timing of polyploidy during fruit development when fruit size was considered.

2. GA Experiments

- The largest differences among all tested GA concentrations (10, 20, 30, 40, 60 ppm) and timings (single applications at straw color, or multiple applications split between straw color and mid-stage III) were observed between 0 and 20 ppm. The quality attribute consistently affected by GA was fruit firmness (higher when provided GA, but not consistently with rates beyond 10 ppm). This effect was observed on all cultivars evaluated (Lapins, Skeena, Sweetheart and Staccato).
- Multiple applications did not result in higher quality fruit.
- In most trials fruit size was not increased with GA. In trials where improvements in fruit size were detected, the response was not influenced by rate beyond 20 ppm.
- Skin color (darkening) was delayed with the application of GA; however, beyond the 20 ppm rate, the effects were highly variable, and difficult to qualify.
- Pitting was reduced in Lapins and Sweetheart at 25 ppm GA; however, concentrations exceeding 25 ppm (up to 100 ppm) did not improve the response.

  Return bloom (floral buds per spur and flowers per floral bud) was not reduced by rates between 10 and 60 ppm.

Results and Discussion

Fruit growth: Better uniformity in the timing of pollination and a greatly increased number of experimentally 'set' fruit were obtained by our modified procedures (refer to methods section). We estimate that full bloom of experimental-tagged flowers occurred within 1 day. A large number of un-tagged flowers and fruits were also sampled in an effort to capture the full range of fruit size on a given day. These samples included both early and late blooming fruits as well as un-fertilized fruitlets. Results confirm that better uniformity in fruit size of 'set' fruit was obtained throughout the growing season compared to the 'range' or crop average. Because the 'range' material displayed wide variability of sizes during the first few weeks after bloom, we were able to identify fruitlets that were likely un-fertilized. Upon re-examination of the 'set' fruit we realized that most of the 'set' material we had sampled in the first few weeks of growth were in fact un-fertilized and small in size. Once June-drop occurred the range of fruit sizes was greatly diminished because only successfully fertilized fruit were sampled. From this time onward the differences in variability between 'set' and 'range' fruit were evident, and we attributed the difference to individual bloom time. However, the question remained—what are the relative effects of individual bloom time and size of the ovary to final fruit size? To address these questions we needed to assess ovary size before bloom. Beginning before dormancy break this year, we began sampling buds and dissecting the ovaries for volume measurements. We also set up a large amount of material in bee-exclusion netting to measure the growth of what would only be un-fertilized fruitlets.

Growth analysis: Growth analysis, simply put, is the application of a mathematical function to growth data using curve fitting techniques. A necessary step in the analysis is an assessment of the variability of data from the beginning to end of the observations—and we achieved this from bud dormancy to harvest by combining last year's 'bloom to harvest' with this year's 'dormancy to bloom' data (Figure 1). Furthermore, because data of this kind spans several orders of magnitude, the variability of the raw data between sampling dates cannot be adequately described without mathematical transformation of the data. The transformation most commonly used is the $\text{Log}_{(e)}$, or 'natural log' because plant growth, especially in determinate growth organs such as fruit, follows a logarithmic pattern. This type of growth is also commonly described as the principle of compound interest. A pleasing result of this data transformation as applied to cherry growth is the observation that the magnitude of the variability in harvest fruit size is very similar, if not identical to the variability observed in dormant buds and blooms. This spurred us to attempt to segregate the data into what would be either successful 'fruit' or 'failures'. After performing these analyses separately for each cultivar of this study, as well as the cropload study, we realized that the patterns of growth were very similar. Therefore, we re-evaluated the data by combining all the data (a total of 12,099 individuals) and refitting it to a polynomial curve. Much can be said about the choice and validity of various curve fitting techniques but it suffices to point out the good correlation of the actual data points to the fitted curve for each variety.

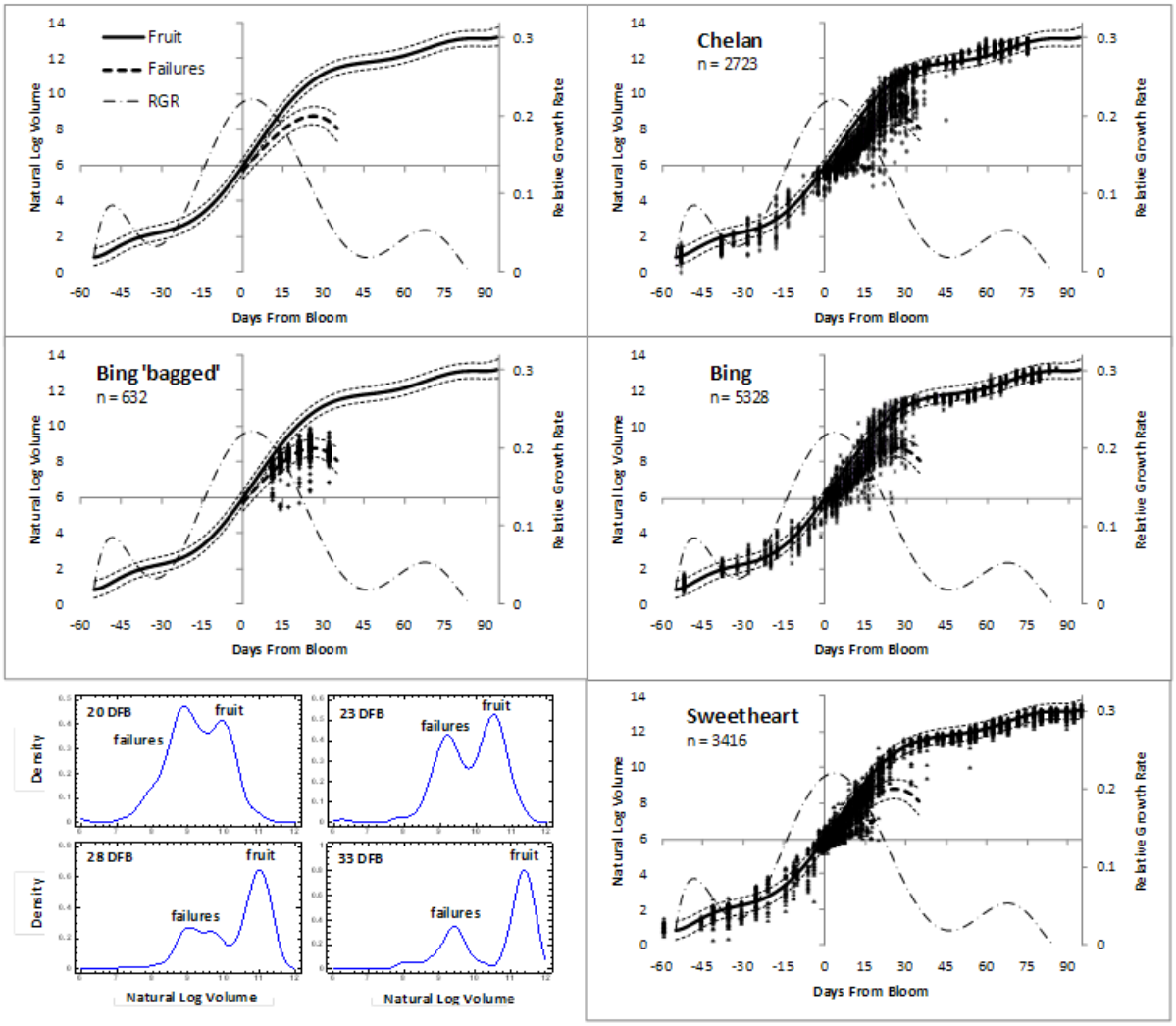


Figure 1. Growth analyses of 'Chelan', 'Bing' and 'Sweetheart' from bud-dormancy break to fruit harvest. Volume data were $Log_{(e)}$ transformed before polynomial regression of the means of 'fruit' (solid lines) and 'failures' (heavy dashed lines) with 95% prediction limits (thin dashed lines). Relative growth rates were calculated from the fitted curve (dot-dash lines). 'Fruit' and 'failure' data were distinguished by measurement of the ovaries in bee-exclusion 'bagged' limbs (i.e., unfertilized ovaries). Data from the time period when growth of 'fruit' and 'failures' overlap (4 to 17 DFB) were excluded from the polynomial regression. Data from 18 to 35 DFB were visually separated into 'fruit' and 'failures' using distribution density analysis (lower left panels), thereby defining the transition from rapid growth to pit hardening. Data for each cultivar are superimposed on the fitted curves.

Pit growth: Growth of the endocarp (pit) was completed by 38 DFB, and was negatively influenced by cropload level (Fig. 2). Differences in pit volume between heavy and light cropload treatments were observed by about 18 DFB (Fig. 2). Final fruit size was positively related to the size of the pit (Fig. 3). Approximately 94% to 95% of the final fruit volume was mesocarp, irrespective of cropload. These data support the contention that greater competition for carbohydrates by higher fruit load has an early, and marked, effect on fruit growth. They also imply that mechanisms controlling pit growth could be targeted to produce larger fruit.

We also evaluated the condition of the kernel throughout the growing season. Interestingly, kernel development ceased in about 50% of fruit during early-stage III growth and at harvest only about

50% of fruit had a full kernel. Therefore, once the pit has hardened a fertile kernel is not required for continued fruit growth.

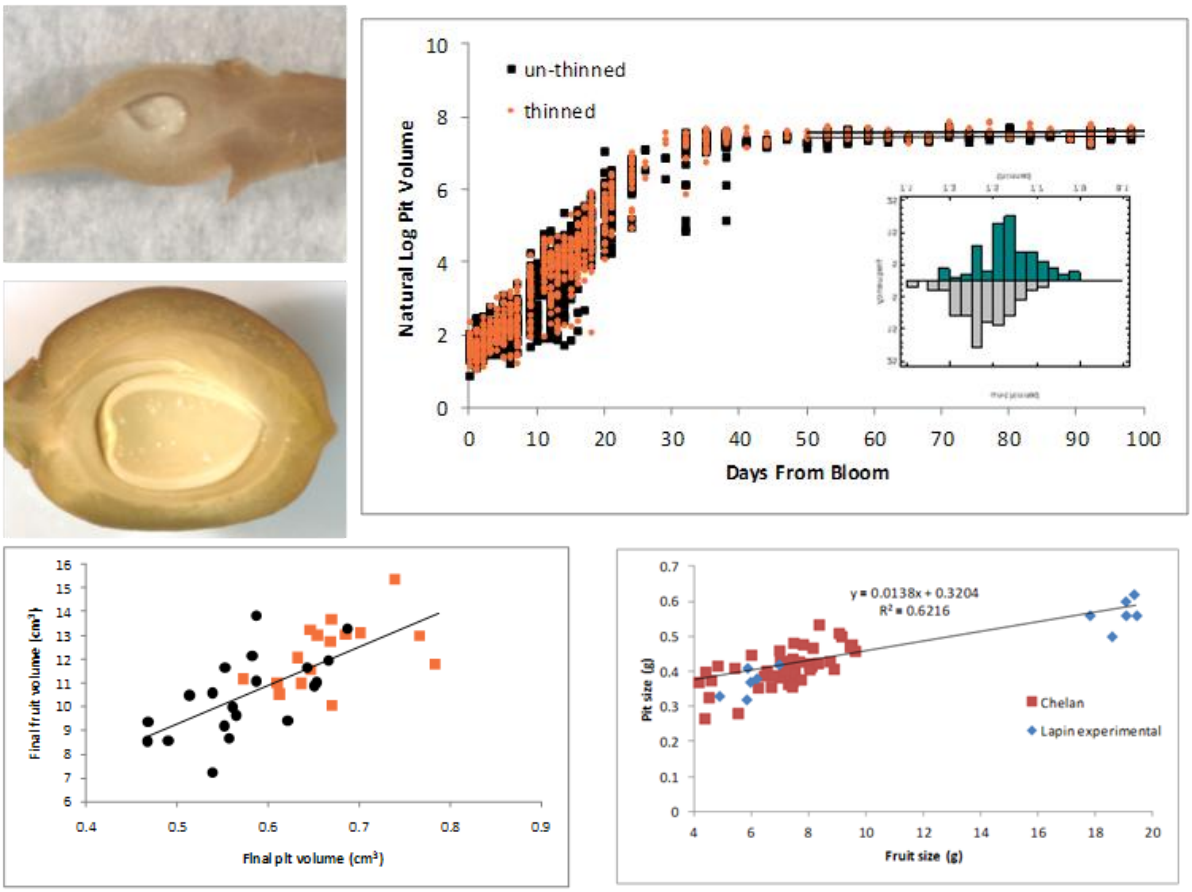


Figure 2. Pit size and fruit size are positively correlated. Ovaries were dissected (upper left panel) for measurement of pit volumes until pit hardening. After pit hardening, fruits were measured then the pits were cleaned for measurement of pit volume. Reduction in growth of the pit was observed as early as 18 DFB (upper right panel) in 'Sweetheart' when fruit per spur was greater than 12 as compared to less than 4. The average hardened pit size (inset histogram, 50-100 DFB) was significantly smaller with high cropload. Larger fruit had larger pits, irrespective of cropload in 'Sweetheart' (lower left panel). Comparison of average size Chelan, small Lapins and very large Lapins (lower right panel) demonstrate a positive pit to fruit size relationship.

SEM: High quality images were obtained using standard sample preparation techniques (Fig. 3). The region chosen for analysis was the widest breadth of the "cheek" perpendicular to the suture plane as has been done in several studies of stone fruit. Whereas most studies only observed a central region of the mesocarp for cell size measurements, we were able to obtain measurements across the entire mesocarp (Fig 3), and plot these measurements in relation to their position in the fruit (Fig. 4). From these relationships, it can be seen that cell divisions occur early and terminate early during fruit development. Further, cell expansion parallels the growth of the fruit throughout its entire development. With these measurements we determined that three regions (outer, inner and pit boundary) of the mesocarp showed differences in size, shape and growth. Cell shape was determined by the ratio of the radial to tangential diameter (data not shown). All cells at full bloom were slightly flattened tangentially then became isodiametric near the time of the cessation of cell division, about 20 days after full bloom. After cell divisions stopped, cells of the outer and inner mesocarp elongated about 20 times their original size by the middle of stage III. During the final fruit swelling of late-stage III, mesocarp cells continued to elongate, including those of the pit boundary. However, only

the outer mesocarp cells swelled tangentially forming once again, isodiametric cells. Furthermore, these outer mesocarp cells were the only cells that showed a treatment effect of flower thinning. Outer mesocarp cells of the thinned treatment were larger in both dimensions compared to cells of the unthinned treatment.

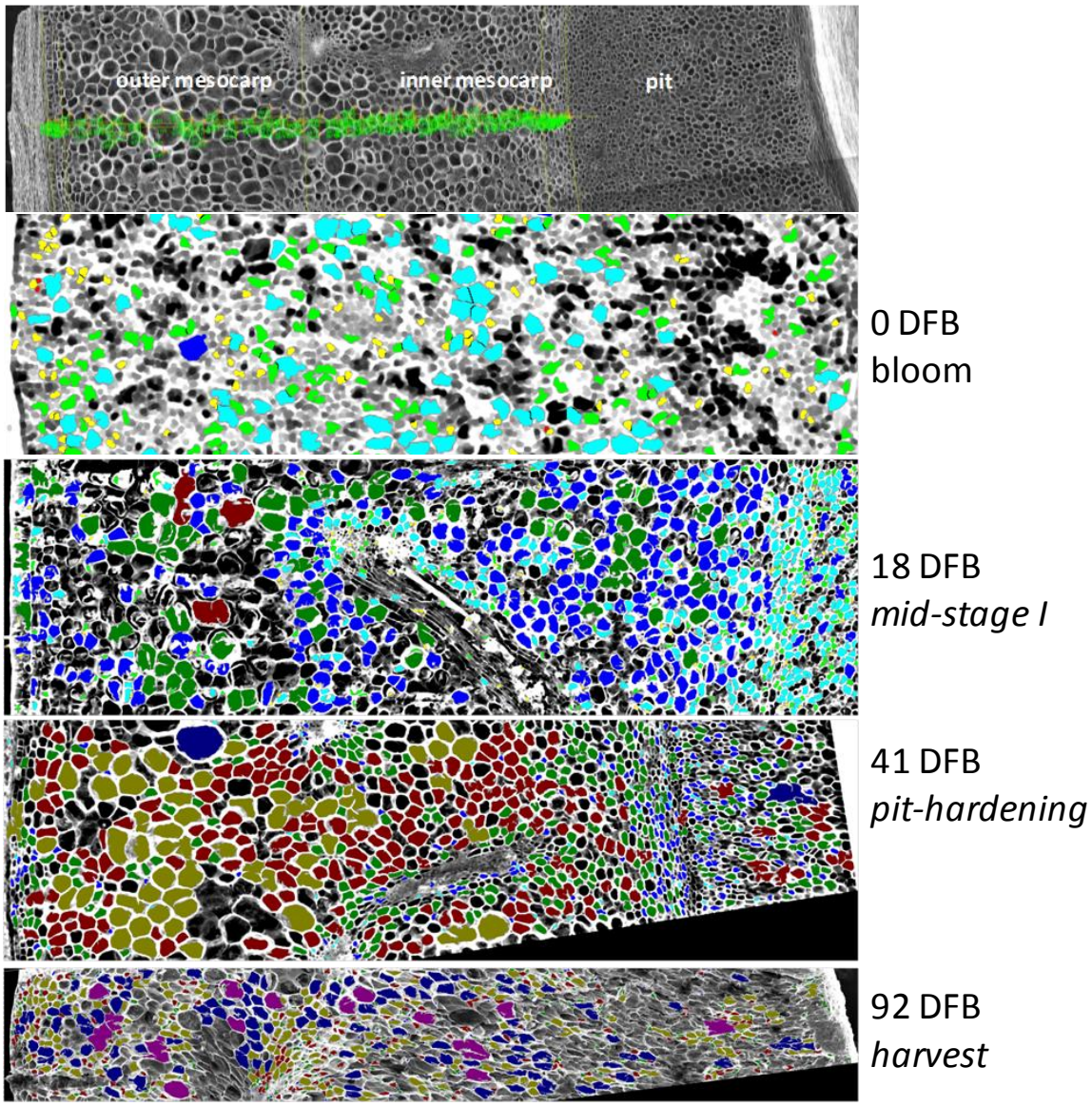


Figure 3. Image analysis for automatic detection of cell areas provided greater detail of cell size and position in cherry fruit compared to the 'line counting' method. The lower four panels show color coded size measurements of fruits 0, 18, 41 and 92 DFB.

Cell number was also determined in all regions revealing no differences between the cropload treatments of 'Sweetheart' (data not shown). However, insufficient SEM data in the earliest growth phase cannot rule out possible cropload effects on the timing of cell division; however, flow cytometry data do not indicate a cropload effect on the timing of cell division.

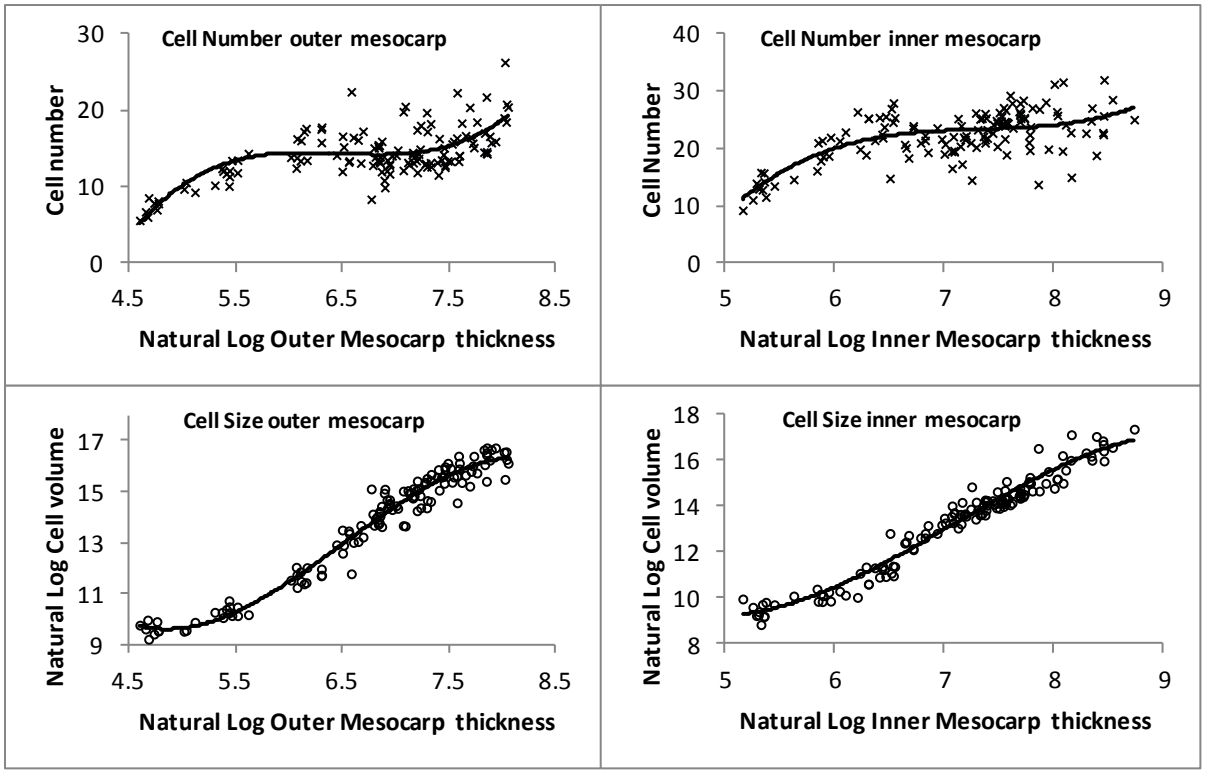
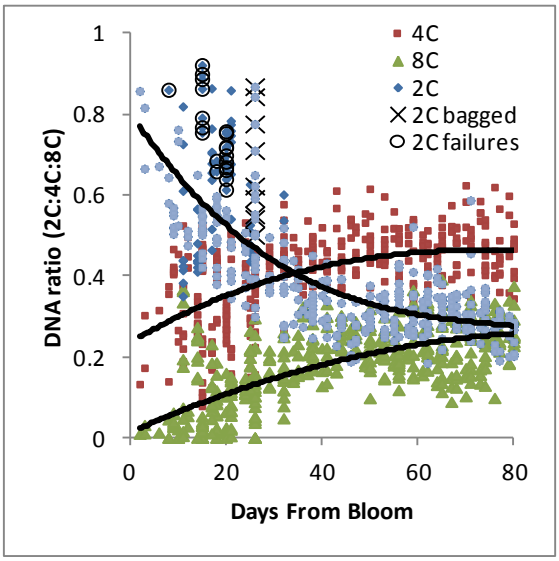


Figure 4. Estimates of cell number and cell size. Log transformation of the data allow comparison over several orders of magnitude.



	Ploidy distribution			
Green tissue	% 2C	% 4C	% 8C	% 16C
young leaf	80	20	0	0
ovary at bloom	80	20	0	0
	Ploidy distribution			
Ripe fruit	% 2C	% 4C	% 8C	% 16C
choke cherry	84	16	0	0
wild cherry	52	22	20	5
Chelan	32	39	27	2
Bing	26	49	24	1
Sweetheart	24	49	27	1
Lapin, small (6.0 g)	48	25	19	7
Lapin, very large (18.9 g)	39	20	26	13

Table 1. Sweet cherry endoreduplication

Figure 5. DNA content of nuclei

Flow cytometry: Heightened cell activity in 'Sweetheart' fruit can be observed immediately following bloom (decreasing 2C/increasing 4C and 8C), irrespective of cropload level (Fig. 5). These data indicate increased cell division activity, and suggest that cell divisions are completed very soon after full bloom (within the first 3 weeks). Interestingly, >60% of the nuclei of all three cultivars evaluated

were polyploid, beginning ~7-10 days after bloom, and remaining until harvest. When plant cells become polyploid they typically cease cell division. If this process, termed endoreduplication, could be delayed, then more cell divisions could potentially occur resulting in larger fruit. We are unclear as to whether cells with higher ploidy levels result in larger fruit size. Increased polyploidy in fruit was positively correlated with fruit size when comparing two strains of 'Gala' apple (Peter Hirst, personal communication); however, in this case the larger-fruited mutant was tetraploid. Though we did not observe significant differences in ploidy distribution among the cultivars that we investigated ('Chelan', 'Bing' and 'Sweetheart'), the finding that small-fruited *Prunus virginiana* (Chokecherry) were nearly entirely diploid [2C] (Table 1) prompted us to analyze ploidy levels for a few large-fruited and small-fruited genotypes in 2012 to determine whether differences in polyploidy occur at the extreme ends of the fruit size spectrum. Results from small-fruited wild cherry and very large Lapins did not support a positive correlation of endoreduplication and fruit size (Table 1).

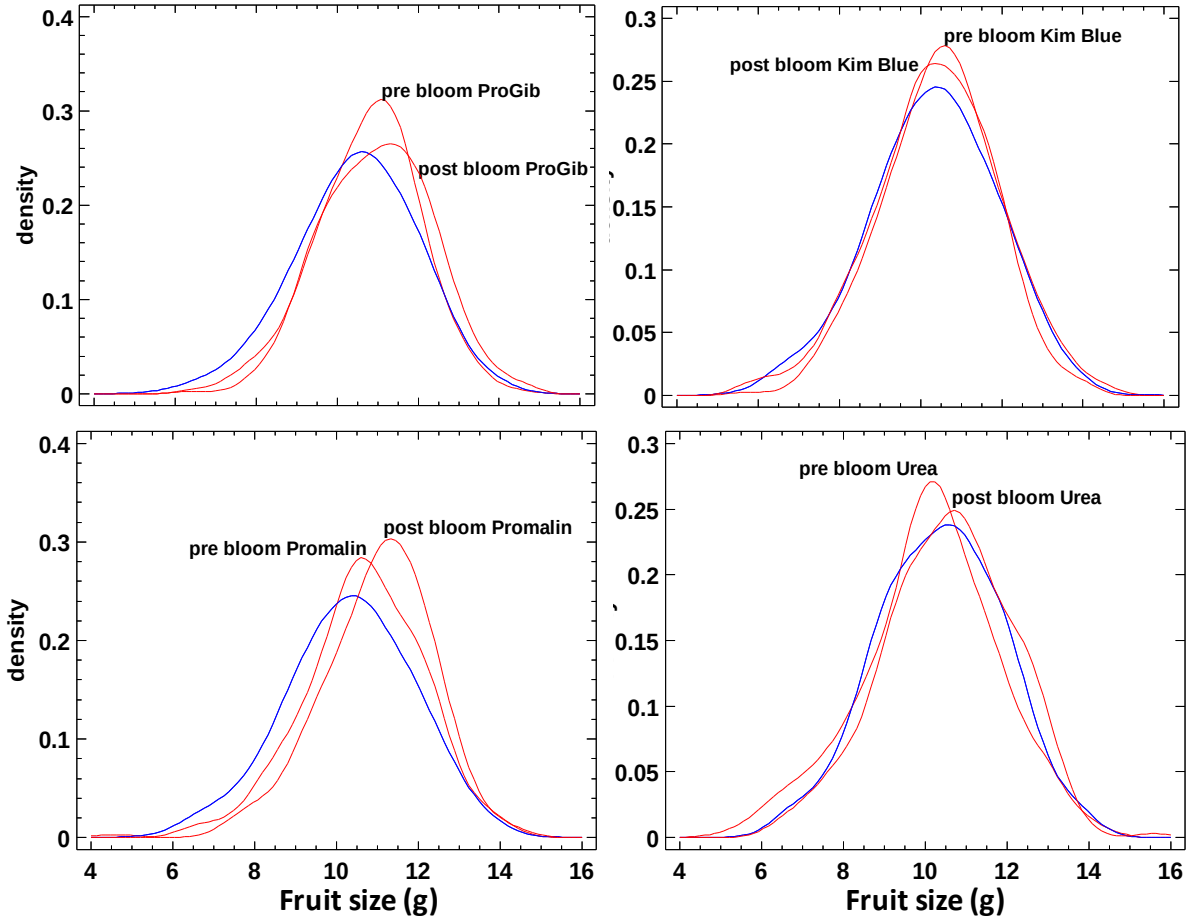


Figure 6. Distribution density (relative frequency) analysis of fruit size at harvest shows gibberellin containing PGR sprays (left panels) increased fruit size when applied soon after bloom. A synthetic cytokinin, Kim Blue and urea had little or no effect (right panels). Limbs were bud thinned two weeks prior to bloom to avoid high cropload, and PGR's were applied either -5 DFB or +12 DFB to six limbs per treatment. Fruit were collected at harvest time and weighed individually (n = 300 to 400 per treatment).

PGR trials: Because cell divisions occur early in cherry fruit growth we hypothesized growth regulators such as giberellin and cytokinin would be most effective around bloom time. In 2012, we conducted a preliminary investigation to test the effect of early applications of these different classes

of PGRs on final fruit size. PGRs were applied to individual fruiting limbs. Both GA and Promalin (GA+cytokinin) improved final fruit size (Figure 6). Interestingly, cytokinin alone (CPPU) did not induce growth resulting in larger fruit. These results seemed counterintuitive, given that the maximum growth rate of sweet cherry 4-5 days after full bloom is due to cell divisions, and cytokinins augment cell division activity. Alternatively, bio-activity of GA, and the constituents of Promalin may have affected cell division, cell expansion, or a combination of the two to shift fruit into larger size classes. More testing is required to determine if significant, consistent increases in fruit size can be made through early applications of PGRs. Our urea (foliar application) was intended to provide additional nitrogen for growth at a time when maximum growth of fruit is occurring (see relative growth curves in Figure 1). This treatment did not appear to improve growth of fruits.

GA Experiments. We have tested applications of intermediate rates of GA (10-60 ppm) on a range of late-season cultivars: Skeena (2010 & 2011; two sites per year), Sweetheart (2010, 2011, 2012), Staccato (2010), and Lapins (2012). Our aim was to determine if increased fruit size from either indirect (delayed harvests via lighter skin color), or direct effects were attainable. Rates of 100 ppm and higher have been shown to markedly reduce return bloom. All applications were applied at ~straw color to scaffold limbs (with the exception of the 2012 Lapins trial where whole canopies were sprayed). In some trials, multiple applications were split between straw color and ~two or three weeks prior to harvest. Rates of 10 ppm and higher significantly improved fruit firmness, irrespective of cultivar, site, or year. It is the one fruit quality attribute that was consistently affected by GA. However, rates exceeding 20 ppm did not result in greater improvements in fruit firmness. Fruit size varied in response to GA: Sweetheart (2010) and Staccato (2010) fruit size was improved at 20 ppm relative to controls (no GA); larger fruit size of Skeena (both years), Sweetheart (1 year) and Lapins were not detected for any of the GA treatments relative to the control. In the case where an increase in fruit size was observed for 20 ppm GA, there were no additional significant gains at higher rates. Multiple timings had no additional effects on fruit size or firmness in any year of application. Other fruit quality attributes (sugars, acids) were not consistently affected by GA timings or rates.

Table 2. 2012 return bloom from 2011 GA applications to 7th leaf 'Skeena' / 'Gi6' scaffolds. Data are means of 6 replicate limbs (n= 25 for spurs & buds).

2012 Skeena	Return buds/spur		Return flowers/bud	
GA trt (ppm)	Site 1	Site 2	Site 1	Site 2
Control	4.9	5.4	3.5 c	3.6
Surfactant	4.3	5.1	3.7 bc	4.1
10	5	4.3	3.9 ab	3.7
20	5	4.5	3.7 bc	3.7
30	4.5	4.9	3.8 b	3.6
40	4.5	4.6	3.8 b	4
60	4.8	4.3	3.7 bc	3.7
20 + 20	5.3	-	3.8 ab	-
20 + 40	4.5	-	4.1 a	-
P >F	0.06	0.35	0.005	0.38

Timings for GA rates 20/20 and 20/40 were at straw/mid Stage III. Single rates were all applied at straw.

Skin color was typically lighter at commercial harvest timing for GA-treated limbs, regardless of the cultivar treated, but lighter skin colors were not observed with higher rates (i.e., those exceeding either 10 or 20 ppm, depending on the trial); the exception was for the Skeena 2010 trial (40 ppm fruit were significantly lighter than 20 ppm fruit). Depending on the season, fruit color was difficult to assess. In cases where protracted bloom periods occurred, variability in color was too great to detect significant differences for higher rates.

Intermediate rates (30 to 60 ppm) did not affect the number of return floral buds per spur, or the number of flowers per bud (shown for 2011 Skeena in Table 2); however, given the fruit size and quality results in the year of application, there would be no value in applying rates exceeding 20 ppm. In 2012, as part of a larger study, we explored the role of GA on pitting using Lapins and Sweetheart (Table 3). GA did reduce pitting, but not at rates higher than 25 ppm. In fact, the fruit quality data from these trials agreed with our previous findings; low rates of GA are sufficient to saturate the response of measured variables.

Table 3. Fruit size and fruit quality attributes (FF, fruit firmness; SSC, soluble solids; TA, total acids; induced pitting on a 1-4 scale where 1 is no pitting, and 4 is severe pitting following pit induction; natural pitting of fruit from picking and handling [not run over a commercial line]; stem browning; and skin color, CTIFL [scale of 1-7 where 1 is light pink and 7 is black]*).

2012 Lapins	Fruit diam.	FF	SSC	TA	Induced pitting	Natural pitting	Stem Browning	Skin Color
GA (ppm)	mm	g mm^{-1}	%	%	1 to 4	%	%	CTIFL
0 Control	30.7	261 b	17.9	0.81 a	2.83 a	20.6 a	16.6 a	4.9
0 Surfactant	30.1	250 b	18	0.81 a	2.79 a	16.3 ab	15.5 a	5.0
25	30.7	297 a	17.9	0.74 b	2.61 b	10.1 b	9.1 b	5.2
50	30.0	281 a	17	0.74 b	2.6 b	12.2 b	9.6 b	4.9
100	29.6	262 b	16.7	0.74 b	2.68 ab	15.6 ab	8.5 b	4.9

2012 Sweetheart	Fruit diam.	FF	SSC	TA	Induced pitting	Natural pitting	Stem Browning	Skin Color
GA (ppm)	mm	g mm^{-1}	%	%	1 to 4	%	%	CTIFL
0 Control	27.1	298 b	19.3	0.89	2.61 a	12.8 a	20.5 a	4.0 a
0 Surfactant	28.0	305 b	21.1	0.91	2.65 a	8.3 ab	21.3 a	4.1 a
25	27.8	331 a	20.8	0.95	2.3 b	6.6 b	14.9 b	4.0 a
50	27.6	345 a	22.5	0.91	2.31 b	7.2 b	15.8 b	4.1 a
100	28.1	352 a	21.2	0.92	2.32 b	5.8 b	15 b	4.5 b

*In Lapins 25, 50 and 100 ppm fruit were lighter at commercial harvest timing, and were required an additonal 5 days to reach simialr CTIFL than control and surfactant fruit. In Sweetheart, skin color was lighter at harvest timing for 50 and 100 ppm fruit and were given 3 additional days to darken.

Methods

Fruit development studies: Methods were described in the 2010 report with the following changes. We did not hand pollinate flowers as done in 2010, due to the rather poor set that we observed. Alternatively, we selected flowers of similar age (within 1 day) borne on fruiting spurs of 2 and 3 year old sections of wood, located on adjacent trees within commercial blocks. Sections of wood were flagged for 'Bing' and 'Chelan', and all flowers in the balloon stage were left intact. Simultaneously, flowers that were either opened, or more tightly closed (less advanced) were removed. The following day we observed the flowers for uniformity, and removed all those that had not yet opened. At the completion of flower selection, all tagged spurs had roughly 2-4 flowers. For 'Sweetheart' two levels of cropload were established: 1) Heavy (unthinned), and 2) light (achieved by removing all but one of the reproductive buds per spur prior to bloom). Flower selection for the light cropload treatment was as described above. The heavy treatment flowers were selected at the balloon stage, and identified by lassoing the pedicel with a tag, being careful not to girdle the pedicel. All other flowers on the spur, either advanced or delayed in their development, were left intact so as to achieve a potential heavy fruit set.

Flower and fruit sampling occurred daily for the first 21 days after bloom, and then every 3 days until harvest. At each sampling date, 8 to 10 tagged fruit were selected for each cultivar. Half of the fruit were placed in fixative for imaging on the scanning electron microscope (SEM), while the other half were sectioned and stored in the freezer for flow cytometry analysis. At each sampling date, an additional 30 to 50 fruit from un-tagged spurs on similar 2 and 3 year old wood were stored in fixative. This second sampling revealed that two size populations, presumably fertilized or un-fertilized fruits, could be distinguished as early as 14 days after bloom. This finding prompted us to follow the growth characteristics of un-fertilized fruitlets during the 2012 season from limbs that were wrapped in bee-exclusion netting. During this season of 2012, we also collected floral buds that we dissected for measurement of ovaries.

Growth curves of whole fruit and its component tissues (mesocarp, endocarp and kernel) were obtained from measurements of photographs taken with a stereozoom microscope. Photos were taken of a whole fruit then the fruit (until pit hardening) was split lengthwise along the suture plane and photographed again. Next, a cross-section of one half of the fruit was obtained and photographed.

After pit hardening, photos of the whole fruit were taken then the pit was cleaned and photographed. The pits were split open and the condition of the kernel (full or shriveled) was noted. This method gave measurements of the length (x-axis) and maximal diameters (y- and z-axis) of the whole fruit and its tissues. These values were used to calculate the equivalent volume of an ellipsoid. This method avoided the difficulty of fresh weight and caliper measurements, especially in very small fruit.

We continued to investigate ploidy levels of 'Chelan', 'Bing' and 'Sweetheart' fruit using flow cytometry. These experiments were carried out in Corvallis, Oregon in collaboration with Dr. Ryan Contreras. Methods were modified from 2010 with greatly improved results. Fresh fruit mesocarp tissue was dissected on the day of sampling then quickly frozen and stored until analysis. Frozen tissue was finely chopped in buffers that separate and stain cell nuclei. A solution containing these nuclei was then injected into a cytometer and passed through a laser. The stained nuclei fluoresce in the light source, and the amount of fluorescence is proportional to the DNA content of the nuclei. The number of nuclei is also obtained which allows a calculation of the ploidy distribution.

Scanning electron microscopy was performed at the EM facility at OSU. Fixed whole-fruit were dissected to reveal the widest extent of the "cheek" region, perpendicular to the suture plane. Following dehydration in a graded series of acetone, samples were critical point dried, mounted on stubs and sputter coated with gold and palladium. A series of digital images from the epidermis to pit were obtained at the appropriate magnification then assembled as a montage. Measurements of cell diameters and position within the fruit were accomplished with digital image analysis software.

EXECUTIVE SUMMARY

Project Title: Influence of cropload level on fruit size and quality of sweet cherry

Fruit growth, cell number and size

- Fruit size was negatively affected on spurs with more than 12 fruit.
- Heavy croploads limited pit size.
- Pit size was positively correlated with final fruit size.
- Half the number of cells comprising an individual fruit at harvest were already present at full bloom.
- Most cell division were completed during mid-stage I, but likely continue into early-stage II.
- Cell numbers do not appear to be affected by cropload.
- Cells of the inner region of mesocarp were elongated at harvest.
- Cells of the outer region were more rounded especially during final fruit swelling.
- Final fruit size more strongly correlated with cell size than cell number.
- Majority of the nuclei of 'Chelan', 'Bing' and 'Sweetheart' fruit become polyploid.
- Leaf tissue and flowers at full bloom showed no polyploidy.
- After full bloom, polyploidy in fruit increased rapidly to > 75% at harvest time.
- Non-pollinated flowers did not show polyploidy indicating fertilization was required.
  → *Polyploidy and activity of the genes involved could be used as early markers of fruit set.*
- Cropload level and genotype did not influence magnitude or timing of polyploidy during fruit development.
- Fruit set can be determined by segregating ovary size, well before shuck drop.
  → *This is markedly earlier than current assessment of fruit set (i.e., after June drop)*

Giberellic Acid (GA) Experiments

- Moderate rates of GA were applied to Lapins, Skeena, Sweetheart and Staccato over multiple years.
- The largest differences among all tested GA concentrations (10, 20, 30, 40, 60 ppm) and timings (single applications at straw color, or multiple applications split between straw color and mid-stage III) were observed between 0 and 20 ppm. The quality attribute consistently affected by GA was fruit firmness (higher when provided GA, but not consistently with rates beyond 10 ppm).
- Multiple applications did not result in higher quality fruit.
- In most trials fruit size was not increased with GA. In trials where improvements in fruit size were detected, the response was not influenced by rate beyond 20 ppm.
- Skin color (darkening) was delayed with the application of GA; however, beyond the 20 ppm rate, the effects were highly variable, and difficult to qualify.
- Pitting was reduced in Lapins and Sweetheart at 25 ppm GA; however, concentrations exceeding 25 ppm (up to 100 ppm) did not improve the response.
- Return bloom (floral buds per spur and flowers per floral bud) was not reduced by rates between 10 and 60 ppm.