FINAL REPORT **YEAR: 2 of 2**

Project Title: Early season estimation of fruit set and size potential

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Budget 1-Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: L.J. Koong**
Telephone: 541 737-4866 **Email address: l.j.koong@oregonstate.edu**

Item	2013	2014
Salaries	28,784	29,648
Benefits	18,064	18,604
Wages	3520	3520
Benefits	352	352
Equipment		
Supplies	2310	1960
Travel	1000	1000
Miscellaneous		
Plot Fees		
Total	54,030	55,084

Footnotes: Salaries for 0.75 FTE postdoc (3% is added to year 2); benefits were calculated based on Actuals; wages are for 300 hours part-time summer employee for image analysis of cherry fruit ($11/hr); benefits for part-time (10%); supplies include fixative, PGRs, tubes for storage of fruit in fixative, bee exclusion netting (only factored into year 1), Ziploc plastic bags, flagging and lab tape for limb and fruit selection; travel includes 1,700 miles estimated for all sample collections and growth rate analyses at $0.55 per mile.

Budget 2- Long

Organization Name: OSU-MCAREC **Contract Administrator: L.J. Koong**
Telephone: 541 737-4866 **Email address: l.j.koong@oregonstate.edu**

Item	2013	2014
Salaries		
Benefits		
Wages	4800	4800
Benefits	480	480
Equipment		
Supplies	200	200
Travel	400	400
Plot Fees		
Miscellaneous		
Total	5880	5880

Footnotes: Wages are for 2.5 months of part-time summer employee for fruit sample collection ($12/hr); benefits for part-time (10%); supplies include Ziploc bags, flagging, and lab tape and dry ice for transport; travel includes 740 miles estimated for all sample collections for fruit set estimates and growth rate analyses at $0.55 per mile.

Objectives:

1) Develop sampling and measurement protocols at the tree, row and orchard scale for Rainier, Bing, Chelan, and Sweetheart. Define the number of fruitlets required for precise crop estimates
2) Analyze growth rates of unfertilized and fertilized fruit of Rainier, Bing, Chelan, and Sweetheart to strengthen our model
3) Develop models of fruit growth that incorporate calendar date and growing degree units so they may be broadly applicable to the cherry growing regions of the PNW
4) Time whole-tree PGR applications with early-season growth of cherry and determine their effect on fruit set, yield, harvestable fruit size, and fruit quality

Significant Findings:

1) The dry weight of 2000 to 3000 ovaries sampled randomly was sufficient for crop estimates by 18 days after bloom
2) Ovary length to width ratios improved detection of potential fruit versus developmentally failed fruit
3) Crop estimates based on fruit from 30 spurs per sampling date, when combined with ovary shape, provided estimates of fruit set by 20 days after bloom
4) Sweetheart grown in three locations with differing seasonal temperature indicated the Base Temperature for accumulation of Degree Days (43°F) is inappropriate and should be lowered
5) Pre-bloom ovary growth was significantly and positively related to temperature
6) The calendar day order for beginning of the Sweetheart season at five locations was The Dalles (BA, SK, JH), Hood River and Parkdale
7) 40°F was sufficient, and 50°F was near the upper limit of a growth response in the green tip phase
8) 70°F produced a large growth effect during the open cluster and first white phases
9) Flowers that bloom early, with respect to average bloom date, produce larger fruit at harvest
10) Pre-bloom (~first white) application of Promalin or cytokinin alone (CPPU) increased fruit size between 7% and 14% when sampled around pit hardening. Promalin significantly increased stem length and leaf area indicating absorption

Results:

Fruit Growth. Our first goal was to complete a growth analysis from dormancy to bloom. An essential component of these growth analyses was the segregation of fertilized fruit from non-fertilized fruit, *prior to their abscission*. These two populations cannot be statistically differentiated within the first 18-20 days from bloom based on their growth rates (Fig. 1).

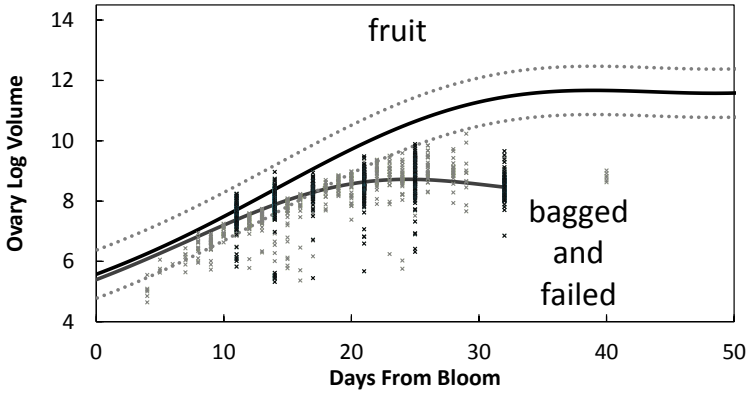

Figure 1. Ovary growth from bloom of a population of fruit comprising both fertilized fruits and fruits destined to abscise compared to non-fertilized fruits developing in bee-exclusion bags.

We then eliminated all fruit that were destined to drop through statistical procedures, of cluster and discriminate analysis, in order to only describe the growth of harvestable fruit of Chelan, Bing, and Sweetheart. Surprisingly, relative growth rates (and timing) defining growth of early developmental stages (First swelling through Stage II) did not differ among these three cultivars (Table 1).

Table 1. Days from bloom of growth phase transitions determined from the minima, maxima and up or down inflexion points of relative growth rate (RGR) curves (not shown).

Variety	**Growth phase**							
	FS,SG	SG,GT	GT,OC	I	I,II	II,III	III	Maturation
	Direction of relative growth rate curve							
	minimum	down	up	maximum	up	minimum	maximum	asymptote*
	Days from bloom							
'Sweetheart'	-39	-31	-17	11	29	44	60	75,79,88
'Bing'	-37	-29	-14	12	30	45	64	70,72,77
'Chelan'	-38	-29	-14	15	30	43	56	59,61,65

First swelling (FS), side green (SG), green tip (GT), open cluster (OC).
**Days from bloom of the additional 90, 95 and 99% increase in phase III volume as determined by logistic functions.*

Based on these similarities, we then developed sampling protocols that provide a good representation of fruit set and variability in fruit size. We attempted moderate (300) and large (3,000) fruit sampling protocols.

Fruit set. Set was determined in two ways. Recounting fruits per flower on flagged limbs at weekly intervals during the season yielded good results but was difficult (see last year's continuing report). A more random sampling proved to be more informative. Sampling at random for dry weight measurements was good but required a lot of sample (>>1000; Fig. 2). A convenient unit to base fruit set on is the spur. Spurs can be sampled as random units throughout the orchard and based on pre-determined average bud and flower numbers per spur (Table 2), the fruit remaining on a spur represents the percentage of fruit set (Table 3). In comparison to limb sampling, sampling entire spurs captured much of the variability and was possibly more accurate; this is attributed to each spur representing flowers at various stages of development so sampling by single spurs from many trees is more likely to represent the orchard as a whole.

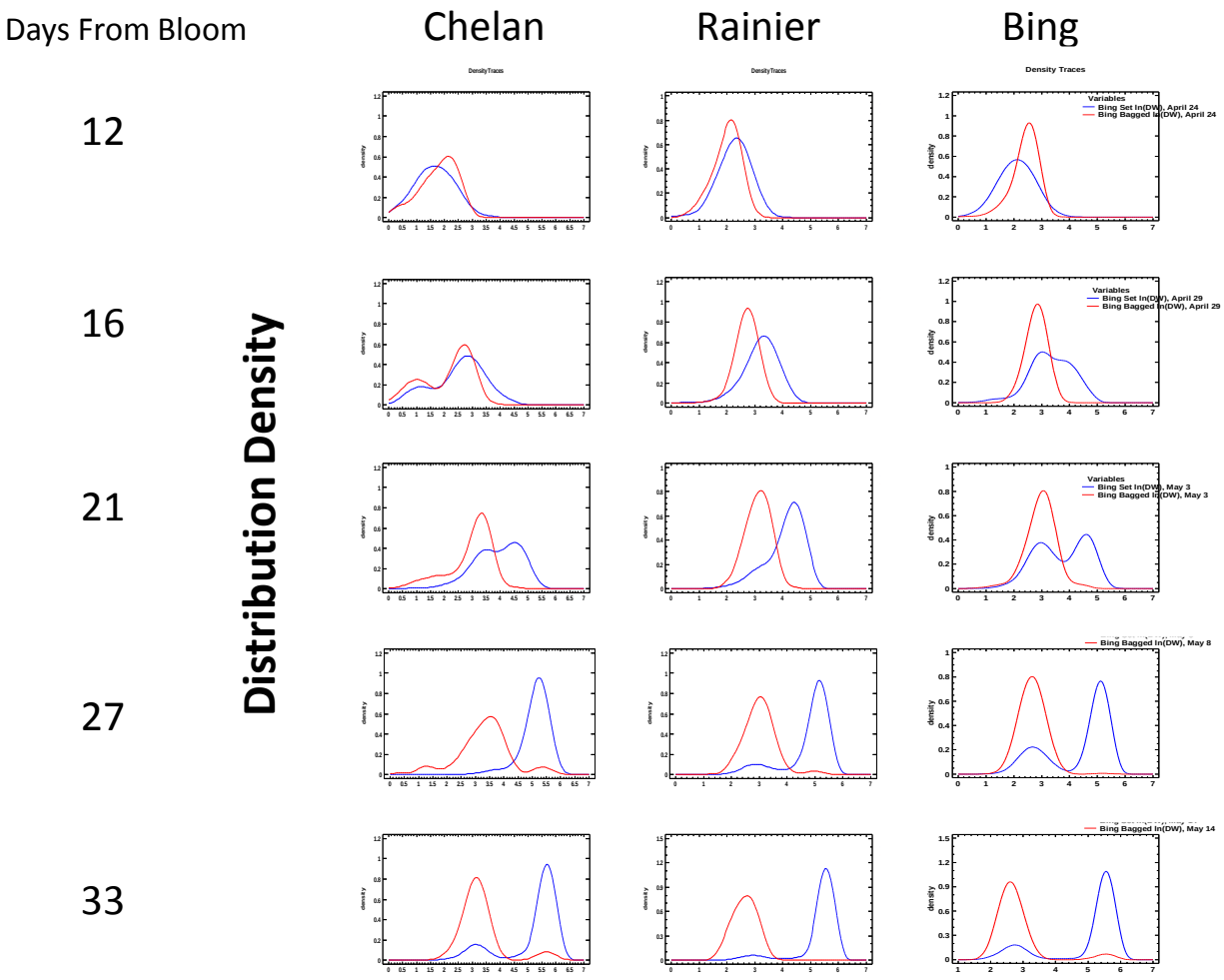

Figure 2. Dry weight gave a sufficient early estimate of fruit set, 16 to 21 DFB, but only if >1000 ovaries were measured. Populations of fruit form two distinct curves- the curve to the left of each graph is for bagged, non-fertilized fruit. The curve to the right is from a random sampling (it is comprised of both fertilized and unfertilized fruit, as can be seen by the bi-modal distribution beginning ~16 to 20 DFB).

Table 2. Spur data used for the fruit set and growth analysis of Sweetheart across multiple sites. This baseline data were best taken before bud break.

	The Dalles			Hood River	Parkdale
	BA	SK	JH	HR	PD
	Average of 30 spurs				
Flower per bud	2.87	3.06	3.48	3.06	3.69
Bud per spur	4.42	4.37	4.44	5.12	3.61
Potential Flowers per spur	12.6	13.3	15.4	15.5	12.8

Location, which includes biological variability attributed to tree age, rootstock, etc., affected flower and bud number.

To reduce sample size we developed a better sampling protocol. In addition, a more sensitive, discriminant measure of ovaries was conducted by integrating shape and volume estimates from digital images (data not shown). A fruit set estimate was reliably detected about 15 DFB from 200-300 fruit collected from sampling 30 spurs on separate trees (Table 3).

Table 3. Fruit set of Sweetheart determined from spur sampling and photographic analysis.

	The Dalles			Hood River	Parkdale
	BA	SK	JH	HR	PD
			% fruit set		
10 to 19 DFB	47	52	68	37	35
Harvest	41	46	56	37	42

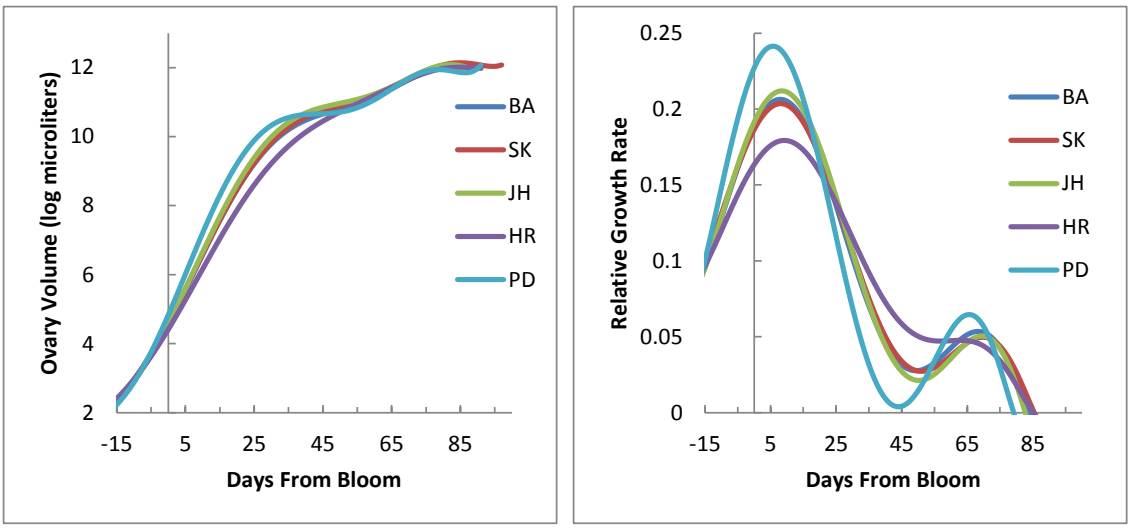

Figure 3. Growth curves of Sweetheart were derived from spur sampling twice weekly at five locations. Left: growth in volume expressed in logarithmic form. Right: Relative growth rates.

The similarity of the minima, maxima and inflection points (data not shown but see Table 1) on relative growth rate curves from 2014 (Fig. 3, right panel), and those of the previous year (provided in 2013 continuing report) show synchrony in development despite varied environments.

Sources of variability in growth and fruit size. Bloom dates have always presented a question mark with no uniformly agreed upon protocol for its determination. And this is surprising considering how important bloom date can be in determining fruit size. Given that cherries are typically harvested in one pass, bloom that is significantly behind the curve (as we have previously demonstrated) do not catch up and will be smaller at harvest. The most straight forward way to approach this question is to count blooms as they open (Fig. 4). As expected, a range in bloom progression and timing was observed at different sites. A consistent ranking of size on given dates was not found between sites; however, after pit hardening (45 DFB) fruit from sites in The Dalles were larger than Hood River and Parkdale where protracted bloom periods were observed (Fig. 4).

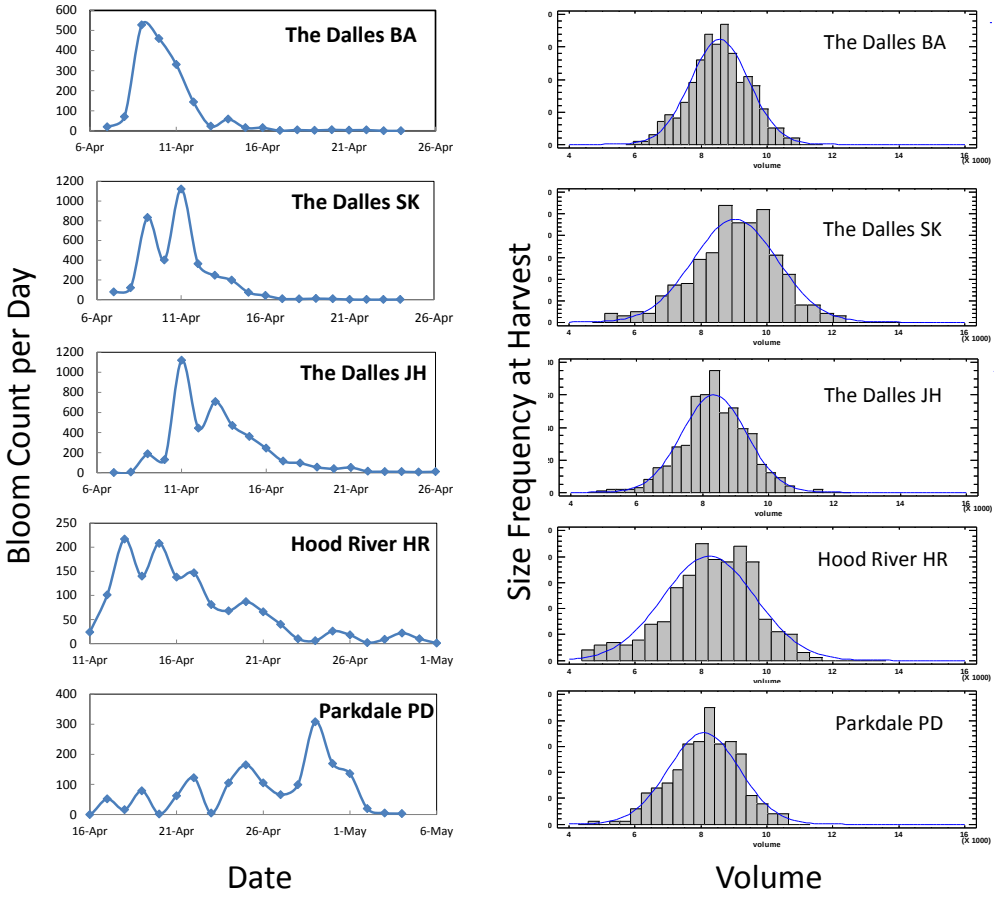

Figure 4. Bloom progression of Sweetheart at 5 sites may offer insights into fruit volume differences at harvest. Left panel: Blooms were removed and counted on the day they opened from portions of 15 limbs (of separate trees). Right panel: The narrowest distribution in fruit size was from site BA (10% variation) and the broadest was HR (17% variation) mirroring the bloom progression. PD had fewer large fruit than may be expected (poor pollination of early bloom) explaining the smaller size, but narrow distribution.

We've settled on an approximate 50% bloom to begin our fruit growth and set calculations, but this choice is debatable (HR) or delayed blooming (PD) could have a large effect on the variation of fruit size, and possibly detrimental to overall size if the early bloom was left unfertilized. Additionally, the prolonged bloom would have affected the fruit vs. failure determination adding to the variation. HR and PD were smaller and had long duration of bloom.

An experiment with Regina also tested the importance of bloom date and its relationship to final fruit size. In 2014, 250 flowers were tagged each day as they opened from the beginning to the end of the bloom period. At harvest the fruit were recovered to record the fruit size. As we have previously shown, early flowers yield the largest fruit (Fig. 5). Interestingly, fruit set of this orchard was quite low indicating that even under ample carbon supply, potential fruit size (of later blooming ovaries) cannot be made up.

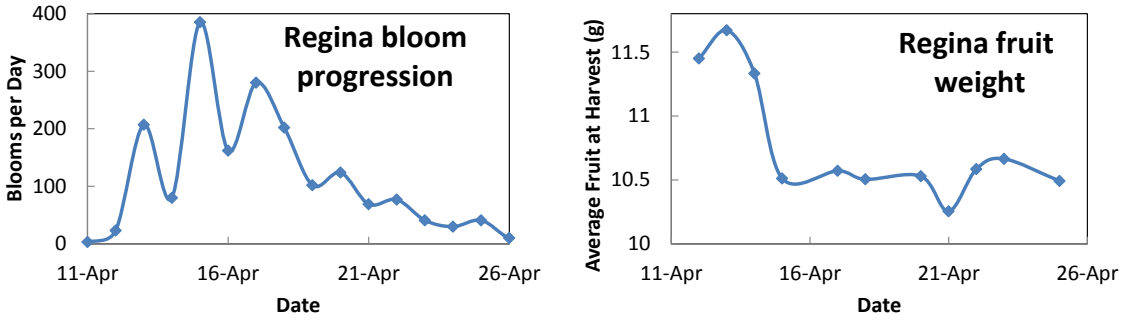

Figure 5. Relationship of bloom date to fruit size. The first three dates of bloom resulted in significantly larger fruit.

Growth models.

Temperature affects the progression of bloom and the growth of ovaries. We experimentally manipulated temperature prior to bloom in order to determine temperature optima for ovary growth. This is a necessary step toward model development. For these experiments, Bing and Regina whole limbs were harvested and placed in temperature controlled growth chambers. These two cultivars were selected based on their different developmental timelines in early spring. As low as 40°F was sufficient for growth effects approximately 22 DFB (i.e., in the green tip phase; Fig. 6). Near 50°F was probably the upper limit for growth but did appear to have a marked influence on Regina ovary growth.

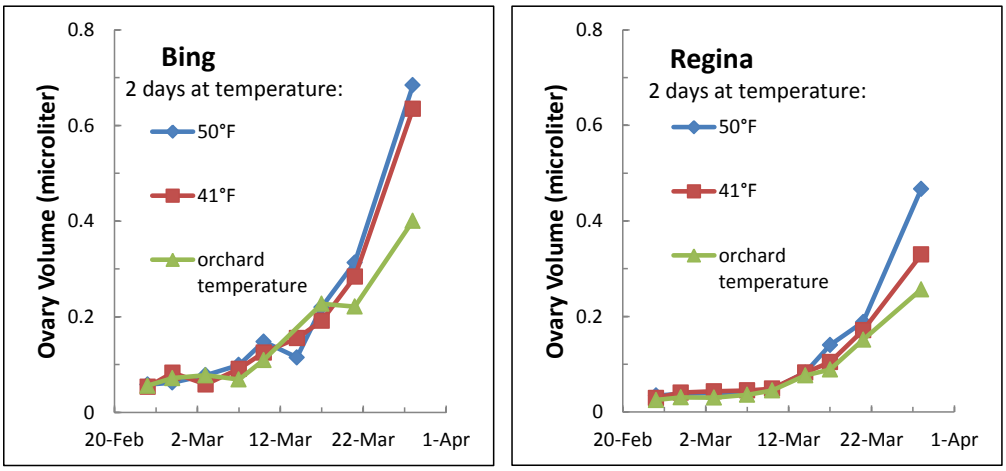

Figure 6. Growth of Bing and Regina ovaries between dormancy and green tip as affected by temperature.

However, for advanced stages of bud break, temperatures of 70°F produced a significant growth effect approx. 9 DFB when buds were in the open cluster to first white phases. These responses need to be expanded upon (see Einhorn New Proposal) in a systematic manner to determine how temperature optima for growth change with development. This is absolutely essential to the development of an accurate growth model.

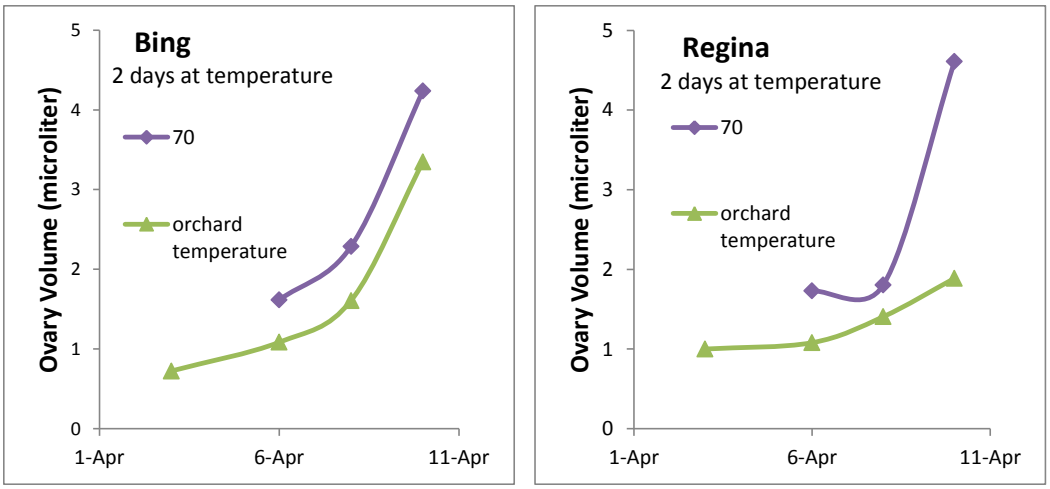

Figure 7. Growth of Bing and Regina ovaries in advanced stages of bud-break.

Adjusting model indices in step with the season

In addition to experimentally determining the optima of the growth response, temperature indices can be manipulated to explain the seasonality of growth, especially post-bloom. We created a spreadsheet with inputs for temperature data from the IFPnet, sunrise and sunset data from the Naval Observatory, and of course growth measurements. The spread sheet uses easily adjusted temperature indices for asymmetric curves of the growth response to temperature. The temperature response we observed in the pre-bloom phases (Fig 6 and 7) matches well with the empirical choice of temperature indices we used in our new model (Table 4).

Table 4. Adjusting temperature indices can result in a more accurate model.

Location, Year	Calendar Days From Bloom	Linear Degree Hour Model	Our NEW Adjusted Degree Model
The Dalles, 2013	91 (April 14)	20820	6129
Hood River, 2013	**92 (April 21)**	**27085**	**6077**
Parkdale, 2013	97 (April 27)	25252	6119
The Dalles2, 2011	**95 (May 2)**	**27026**	**6150**
Average Coefficient of Variation	94 3% (+/- 3 days)	25046 12% (+/- 11 days)	6119 0.5% (<1 day)

This model changes indices for day/night, and seasonal progression. Day and night indices are changed to account for photosynthesis and respiration, while indices are also adjusted seasonally to account for phenology and year to year variation.

PGRs

Stem growth is complete by pit-hardening; in nearly every case pre-bloom applications of solutions containing GA were highly effective in elongating stems (comparable results were observed, but not quantified, for leaf area- a process similarly completed in a relatively short time span). These data provided evidence of uptake and translocation when applied at first white; a possibly prohibitive time given the relatively limited supply of absorptive green tissue present. Fruit growth, however, appeared to be more greatly affected by cytokinins. Packout data (~2,000 fruit per treatment) of Sweetheart revealed a significant size improvement for the prebloom (-7 dfb) CPPU application producing 72% 9.5 row and larger fruit compared to 59% for the control.

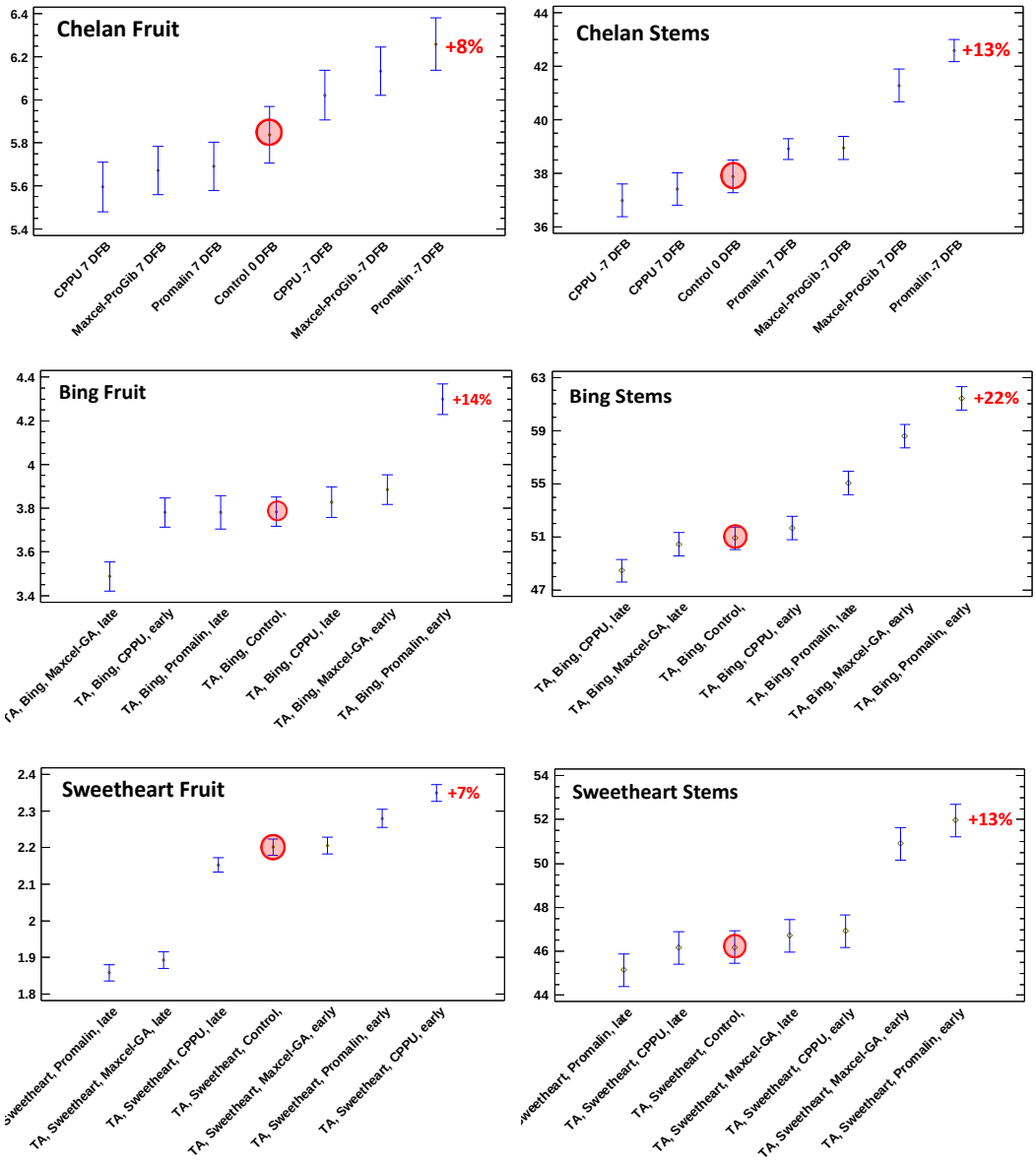

Figure 8. Pre-harvest sampling of Chelan, Bing, and Sweetheart fruit from a commercial orchard in WA. Fruit size data are grams (n=250); stem length data are mm (n=250). Treatments on the x-axis are

ascending with respect to the data and are therefore not consistently ordered across graphs. On each graph, controls are circled for comparisons and treatments showing the greatest percent increase relative to the control are indicated. Late applications were performed at ~7 days after bloom; early applications were made between open cluster and first white (-5 to -7 days from bloom).

These data aligned with our pre-season measurements, which were taken prior to pit hardening (in the case of Sweetheart). For Bing and Chelan, however, no significant differences at harvest were quantifiable- a perplexing outcome given a visibly noticeable size improvement in rows treated with early Promalin. Our pre-harvest sampling of individual fruit of Chelan, for example, was taken ~2.5 weeks prior to harvest. Chelan cropload (and yield) was exceptionally high, and could have increased the demand for carbon during the last few weeks of stage III growth, thus limiting the growth potential established early by CPPU and Promalin, relative to controls. The greater leaf area, produced by Promalin in particular, would have likely augmented carbon available to supply fruit. More work is needed on early-season PGRs before programs can be recommended.

Executive summary

Growers can use these guidelines for assessing their orchard:

- Sample one spur from at least 30 trees for a good size and set estimate
- Count bloom progression from one limb portion from 15 trees to set bloom date accurately
- 40°F is sufficient to enhance ovary growth at green tip phase
- 70°F at open cluster to first white advances growth considerably
- Good crop estimates can be made 20 days after bloom
- Pre-bloom PGR applications increased fruit size, stem length and/or leaf area
- Effort to set early bloom should be made; these flowers produce big fruit

Further work is proposed because:

- Maturation could be better qualified with photographic analysis of color
- A more descriptive model of growing degree units can, and needs, to be done
- Early season cytokinin sprays to enhance fruit size appear promising