**FINAL PROJECT REPORT**
**WTFRC Project Number:** CH-06-601

**Project Title:** Causes and prevention of pistil doubling


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|---|---|---|---|
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**Cooperators:** Del Feigal, AFC; Kent Waliser, Sagemoor Farms; Wild Willow Orchards; John Ferguson and Julie Tarara, USDA-ARS; Bhaskar Bondada, WSU Tri-cities

**Other funding Sources**

**Agency Name:** BASF
**Amount awarded:** $7,000
**Notes:** additional funding to support doubling research

**Total Project Funding:**

**Budget History:**

| **Item** | **Year 1:** 2006 | **Year 2:** 2007 | |
|---|---|---|---|
| **Salaries** | 18,310 | 19,508 | |
| **Benefits** | 1,738 | 1,844 | |
| **Wages** | 5,300 | 6,000 | |
| **Benefits** | 530 | 690 | |
| **Equipment** | 1,500 | 1,500 | |
| **Supplies** | 1,500 | 1,500 | |
| **Travel** | 1,500 | 2,000 | |
| | | | |
| | | | |
| | | | |
| **Miscellaneous** | | | |
| **Total** | 30,378 | 33,042 | |

**OBJECTIVES:**

For early-, mid-, and late-season sweet cherry varieties:

1. Elucidate the seasonal trends in flower bud initiation & organ differentiation

2. Determine seasonal susceptibility to pistil doubling

3. Determine seasonal relationship between tissue and air temperature and pistil doubling

4. Compare efficacy of practical means for reducing pistil doubling

**SIGNIFICANT FINDINGS:**

- cultivars are asynchronous in floral organ differentiation
- early-maturing cultivars exhibit advanced differentiation vs. late-maturing cultivars
- variability in organ differentiation among flowers exists within a tree, spur, and bud
- the system for manipulating tissue temperature *in situ* was able to heat and cool developing fruit buds by ± 5$^{o}$C from untreated
- both tissue temperature and timing are important factors in pistil doubling
- in 2005, 'Bing' flowers were more susceptible to doubling in late July vs. early July and early August
- buds kept below 34.3 C (ca. 94 F) throughout the 2005 and 2006 trials did not exhibit doubling
- over-tree evaporative cooling shows great potential for moderating tissue temperature (ca. 8.5 F reduction) and reducing pistil doubling
- under-tree microsprinklers are ineffective at reducing canopy tissue temperature
- shade and Surround® are moderately effective at reducing canopy temperature (ca. 3.5 to 4 F reduction)
- in grower cooperator trials, both over-tree evaporative cooling and Surround reflective treatments significantly reduced multiple pistils by about half

**RESULTS AND DISCUSSION:**

***Floral bud initiation and organ differentiation***

We documented in 2006 and 2007 the seasonal progression in floral organ differentiation for Chelan, Tieton, Bing, Skeena, and Sweetheart via scanning electron microscopy. We did not observe significant differences among these cultivars for the date of floral bud induction. We observed the initiation of reproductive buds in leaf axils during the first half of May, irrespective of cultivar (data not shown).

In contrast, we observed significant differences among cultivars in the initiation and seasonal progression of floral organ differentiation (e.g., sepals, pistil, stamens). Samples were collected at approximately 3-week intervals between late May and November. Our earliest samples reveal very little difference in organ differentiation among cultivars and buds were comprised primarily of rounded meristems with two to four bracts (images not shown). June samples too revealed buds with largely undifferentiated meristems. By mid-late July however, floral bud initiation was apparent, particularly in Chelan (Appendix A). A pentagonal whorl of sepal primordia was visible in Chelan buds on 31 July. In contrast, on the same sample date buds of other cultivars were less advanced, with Sweetheart exhibiting the least development (no floral primordia). Sweetheart did not exhibit the similar whorl of sepals until the middle of August – more than two weeks later. To our knowledge, this is the first report of asynchrony in sweet cherry flower bud development and organ

differentiation. Interestingly, organ differentiation was related to fruit harvest timing (i.e., Chelan the earliest, Sweetheart the latest), though not as discrepant. This suggests that apparent resistance or susceptibility to MP may be related, in part to variability in the stage of organ differentiation. From our studies of critical timings and temperatures for multiple pistils (MP) (see discussion below), it appears that Bing buds were most susceptible during late July and early August. At that stage, distinct floral meristems are present (Figure 1) though little organ differentiation has occurred. It should be noted that Tieton flower bud differentiation progresses similarly, or slightly advanced, to that of Bing and yet Tieton is significantly more susceptible to MP. This also suggests a strong genetic component to susceptibility/resistance to MP.

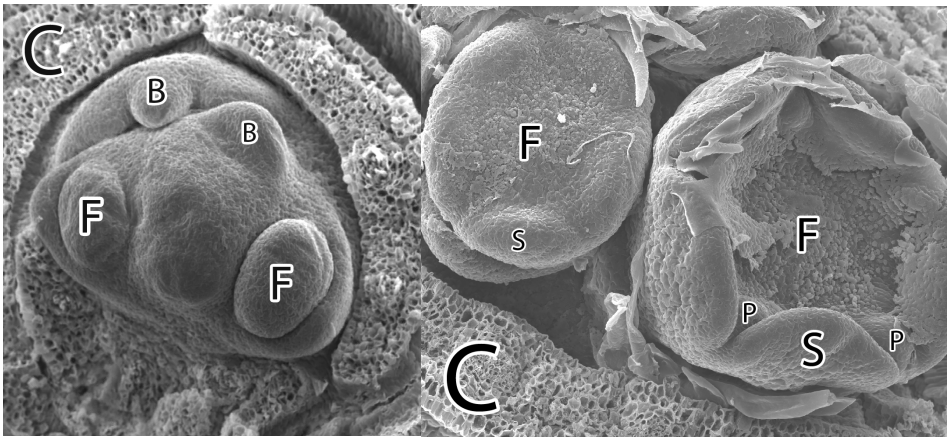



**Figure 1.** Scanning electron microscope images of Bing sweet cherry flower buds at a stage of high susceptibility to multiple pistils. Bud in left image collected on 31 July (1464 GDU), on right collected 17 August (1750 GDU). F = floral meristem; S = sepal; P = petal; B = bract.

Our histological studies identified variability in the stage of organ differentiation among spurs in a tree, among buds on a spur, and among individual floral meristems in a bud. For example, in the image on the right of Fig. 1, one bud has more developed sepal primordia and is at the beginning stages of petal differentiation whereas the other bud has only begun sepal differentiation. We estimate the floral meristem on the left to be approximately 1 week behind the other. To better characterize the variability among floral meristems in a tree, we initiated an additional trial in 2007 – entire spurs were harvested and each bud is being evaluated for meristem differentiation (i.e., from the oldest to youngest). In addition, we collected spurs from the youngest branches (i.e., one-year-old) and older branches (i.e., 4+ years old) and will compare stages of floral meristem differentiation. Our preliminary results on Bing show advanced differentiation for buds from older wood vs. those from younger wood. We observed a similar pattern for flower buds within a spur (i.e., oldest were further advanced). This variability in the stage of organ differentiation may explain why MP is variable within a spur/tree.

***Critical timing and temperatures***

This work utilized a novel heating/cooling system to manipulate tissue temperature at ca. 2-week intervals throughout bud differentiation. The heating/cooling apparatus worked well and was able to increase and reduce bud tissue temperature by 5$^{o}$C (ca. 9$^{o}$F) from ambient throughout the day (data not shown). These experiments were designed to elucidate the seasonal variability in susceptibility to multiple pistils. Knowing this, we will be able to effectively target preventative strategies.

In 2006, natural MP from 2005 conditions was about 4.5% for Bing. Our trials on Bing in 2005/2006 (heated in 2005 and assessed in 2006) showed that developing flower buds were

susceptible to doubling between 18 and 25 of July (i.e., about one month after harvest, 1079 to 1312 GDU base 50F). Flowers within buds artificially heated during this period exhibited 11% MP (ca. 6.5% more than control) in 2006. Buds were more susceptible between 2 and 14 of August (1435 to 1723 GDU) – we recorded 16% MP (ca. 11% more than control) in response to artificial heating. Timing (i.e., stage of bud differentiation) appears to have an effect because we recorded more MP from heat treatments in early August than from heat treatments in late July despite exposure to similar temperature regimes (58 and 76 $^{o}C \cdot HR$, respectively). However, temperatures were higher during the latter half of July and early August than they were during the earliest interval (July 5 – 14). It is not known how much doubling would have occurred in the early July timing in response to similar high temperatures. What is clear, is the role of high temperature – we did not record a single double pistil from cooled spurs, irrespective of timing. In addition, flowers that were cooler than ca. 37$^{o}$C (99$^{o}$F) throughout our trial period did not have doubled pistils – we only observed doubling, albeit variable (0 – 60%), when tissue temperatures exceeded 37$^{o}$C (data not shown). The variability in doubling above 37$^{o}$C also reinforces the need to analyze time-temperature threshold rather than a particular temperature alone.

The incidence of MP in 2007 was significantly lower than in the previous year. We recorded less than 2% MP in untreated Bing spurs despite warmer temperatures during key stages of bud differentiation (i.e., late July and August) in 2006 compared with 2005. Indeed, industry-wide, growers observed much less MP in 2007 than was anticipated. Moreover, our heated spurs did not exhibit MP in 2007 as we had expected, based on our results from 2006. Percent flowers exhibiting MP in 2007 never exceeded about 6% and from only two of the five heat treatments in 2006 did we observe an increase in MP vs. the control. Buds heated during 16 Aug to 25 Aug and from 27 Aug to 7 September had ca. 2.5% and 4% more MP than the control, respectively (Figure 2). Despite applying the greatest amount of heat to differentiating buds at a susceptible stage (157 $^{o}C \cdot HR$ in late July, 1230 to 1477 GDU; based on our observations in 2006 from 2005 heat treatments), we were unable to induce MP in 2006. Again, in the subsequent heat treatment (early August), we were unable to induce MP. In contrast, by applying a similar heat treatment in 2005, we induced significant (ca. 11%) doubling. Overall, our data support the general observation across the industry in 2007– that there were far fewer MP than expected. Our hypothesis is that a period of unusually high temperature in late June and early July acclimated the tissue to high temperature stress at a period when differentiating buds were not susceptible to MP. We initiated a trial in 2007 designed to evaluate the effect of a heat treatment applied in late June on incidence of MP (Figure 3). In late June to early July, buds were heated (to mimic the natural heat wave in 2006) or cooled (to avoid the potential natural heat stress). Buds were subsequently heated or cooled in late July to early August to induce or prevent MP, respectively. We will assess MP in dormant flower buds this winter and are particularly interested in the difference between those that received two heat applications vs. those cooled and then heated. The effect of hot temperatures during early stages of bud differentiation on the potential for MP in the subsequent season is an area deserving further investigation, particularly for developing models for predicting susceptibility to MP. Interestingly, we observed an increase, albeit minor, in MP from heat treatments in late August and early September (Fig. 2).

Interestingly, from both 2005 and 2006 experiments, buds treated with cool air for a single interval (e.g., 10 to 14 days) exhibited no multiple pistils, regardless of the timing of the application of cool air. This suggests that MP may be reduced by applying cooling treatments and, furthermore, that precise timing of cooling treatments may not be critical.

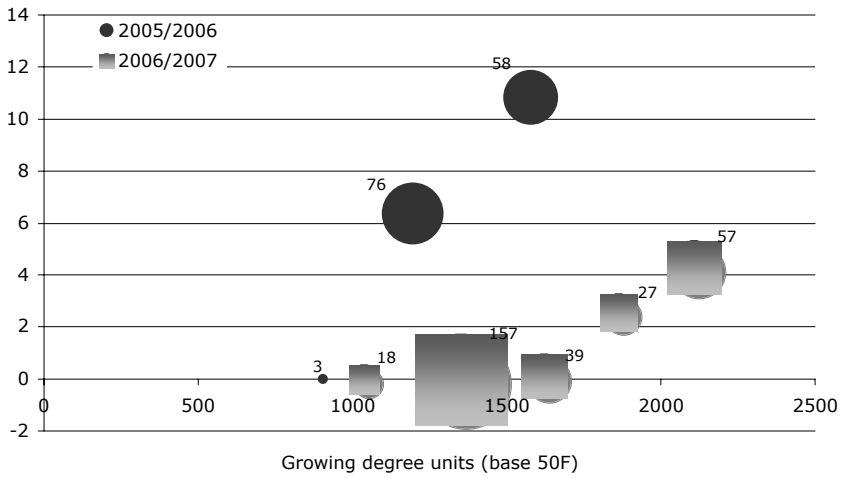



**Figure 2**. Relationship between timing of heat treatment and effect on multiple pistils in the subsequent season (% greater than for control). Each point is the mean of 8 replicate spurs on 2-year-old wood. The size of the circle is proportional to the hours accumulated at 99F (37C) during the heat treatment ($^{o}C \cdot HR$ indicated beside each circle). Natural (control) multiple pistil incidence was 4.6% in 2006 and 2.0% in 2007.

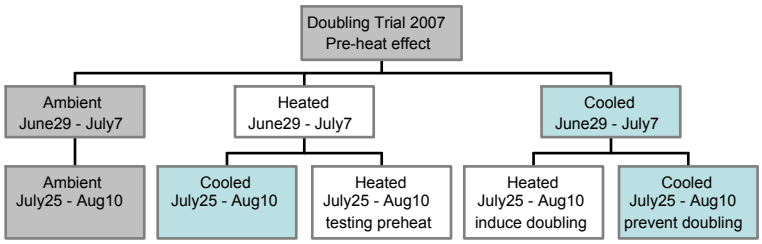



**Figure 3**. Flow chart outlining trial initiated in 2007 to investigate the effects of high temperature on susceptibility to multiple pistils.

***Practical strategies for reducing multiple pistils***

In 2006 we initiated several trials with grower cooperators to assess the efficacy of several methods for reducing multiple pistils. In a trial in a 7$^{th}$ leaf Tieton/Gisela 5 orchard, we compared applications of the kaolin reflective Surround® with over-tree evaporative cooling and shading (ca. 20% shade). We observed significant reductions in multiple pistils with each preventative treatment (Fig. 4). Surround, evaporative cooling, and shade were similarly effective, reducing the incidence of multiple pistils by about 45% on a whole-tree level. We also selected one limb per tree (from similar height/orientation in canopy) and counted multiple pistils at bloom and again at harvest. Our data suggest that assessing multiple pistils at bloom may accurately represent final incidence of multiple pistils (on that limb) since we observed little change in multiple pistils between bloom and harvest (Fig. 4). This suggests that fruit with multiple pistils are no more or less likely to drop throughout development than normal fruit. We also observed significantly higher MP (ca. 20% more) on selected limbs vs. the entire tree. Moreover, variability in MP among limbs was much greater than that among trees. These results demonstrate how variable MP can be within a canopy and suggest that further research should continue to assess doubling on a whole-tree basis at harvest. Variability in MP within a tree is likely related to differences in spur microclimate, particularly with respect to light

interception and tissue temperature. The limbs we analyzed in detail were in the upper 1/3 of the canopy and therefore, a high light environment. Potential preventative strategies may be prudently focused on the upper canopy regions where light interception and temperature are relatively high.

Incidence of MP on a whole-tree basis was variable, ranging between 8% and 42% (data not shown). This variability was not related to tree vigor (Fig. 5). Susceptibility to MP may be related to characteristics other than the overall vigor of the tree such as limb and leaf orientation, light distribution and interception, etc. Previous research in Japan had ruled out any role of water stress in MP but this has yet to be investigated for conditions and cultivars grown in the PNW.

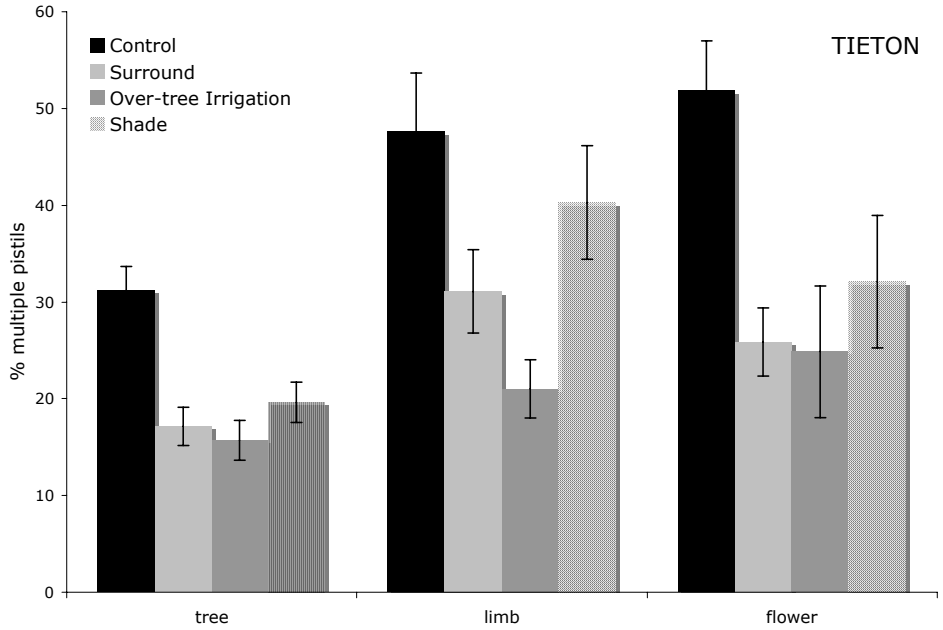



**Figure 4**. Percent multiple pistils on a whole-tree and limb level (at harvest and full bloom). Assessed on 7th leaf 'Tieton'/'Gisela®5' trees. Each bar is the mean of 8 replications ± standard error.

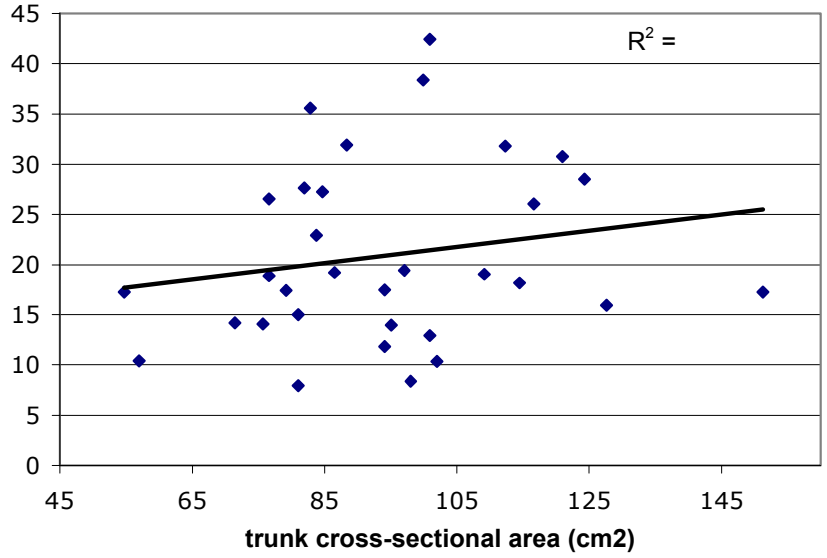



**Figure 5.** Percent multiple pistils on a whole-tree level in relation to tree vigor (trunk cross sectional area). Assessed on 7th leaf 'Tieton'/'Gisela®5' trees.

In trials on other cultivars, incidence of MP was significantly less than it was for Tieton. In mature Bing and Chelan orchards, we recorded only 4% and 1% MP, respectively on a whole-tree level. Regardless, each preventative treatment we evaluated reduced MP (Fig. 6). In the Bing orchard, both evaporative cooling and Surround treatments reduced MP by about half. In the Chelan trial, Surround and Raynox reduced MP by about 30% and 60%, respectively. Again, our data highlight the need to assess MP on a whole-tree basis – incidence on selected limbs was highly variable and even contradictory to whole-tree results.

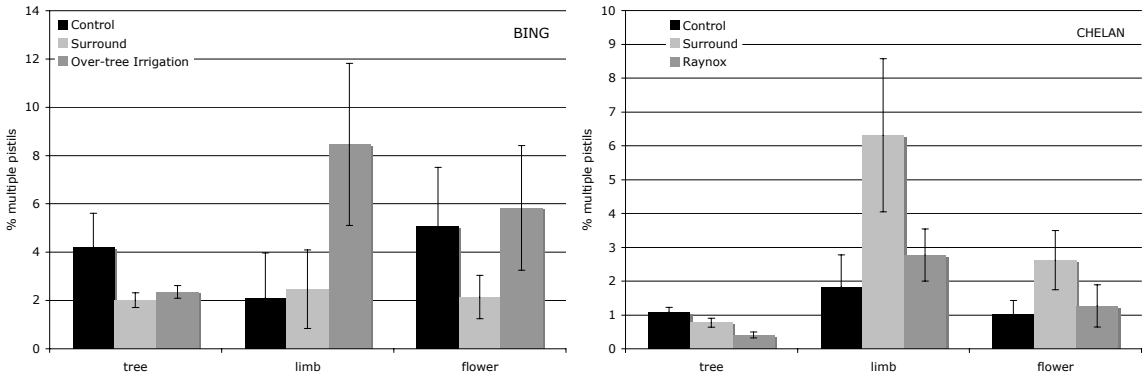



**Figure 6.** Percent multiple pistils on a whole-tree and limb level (at harvest and full bloom). Assessed on mature 'Bing'/Mazzard and 'Chelan'/Mazzard trees. Each bar is the mean of 4 or 8 replications ± standard error for Bing and Chelan, respectively.

## PROJECT OUTREACH:

### *Presentations*:

WTFRC/OSCC NW cherry research review. Richland, WA, November 16, 2006. "Causes and prevention of pistil doubling". Poster presentation. Attendance: ca. 75

Annual Meeting of WA Hort. Assoc. Yakima, WA, December 4-6, 2006. "Causes and prevention of pistil doubling". Poster presentation. Attendance: ca. 50

Cherry Institute Meeting, Yakima WA Jan. 12, 2007. "Blame it on the sun: Preliminary results from research into the causes and prevention of pistil doubling" Attendance: ca. 450

Annual grower meeting. Sambado Packing/Primavera Fruit, Linden CA. March 5, 2007. "Causes and prevention of multiple pistils and deep suture". Attendance: ca. 100

WSU-IAREC Annual Cherry Field Day. June 12, 2007. "Update on pistil doubling research" Attendance: ca. 200

Sunnyside Rotary Club meeting, Sunnyside WA, 22 August. "Research advances in the WSU sweet cherry program". Attendance: ca. 30

Annual Meeting of WA Hort. Assoc. Wenatchee, WA, December 3-5, 2007. "Practical strategies for reducing multiple pistils in sweet cherry". (Poster).

Cherry Institute Meeting, Yakima WA Jan. 11. 2008. “Research update on causes and prevention of multiple pistils”

### ***Articles in popular press*:**

Hansen, M. 2006. “The causes and cures of doubling”. Good Fruit Grower. Vol. 57

Hansen, M. 2006. “Research aims to help growers prevent doubling”. Good Fruit Grower. Vol. 57 (10)

Martin, R. and M. Whiting. 2008. “Practical strategies for reducing sweet cherry pistil doubling”. Good Fruit Grower. Vol 58. Diseases and disorders issue. 15 Feb. (*in preparation*)

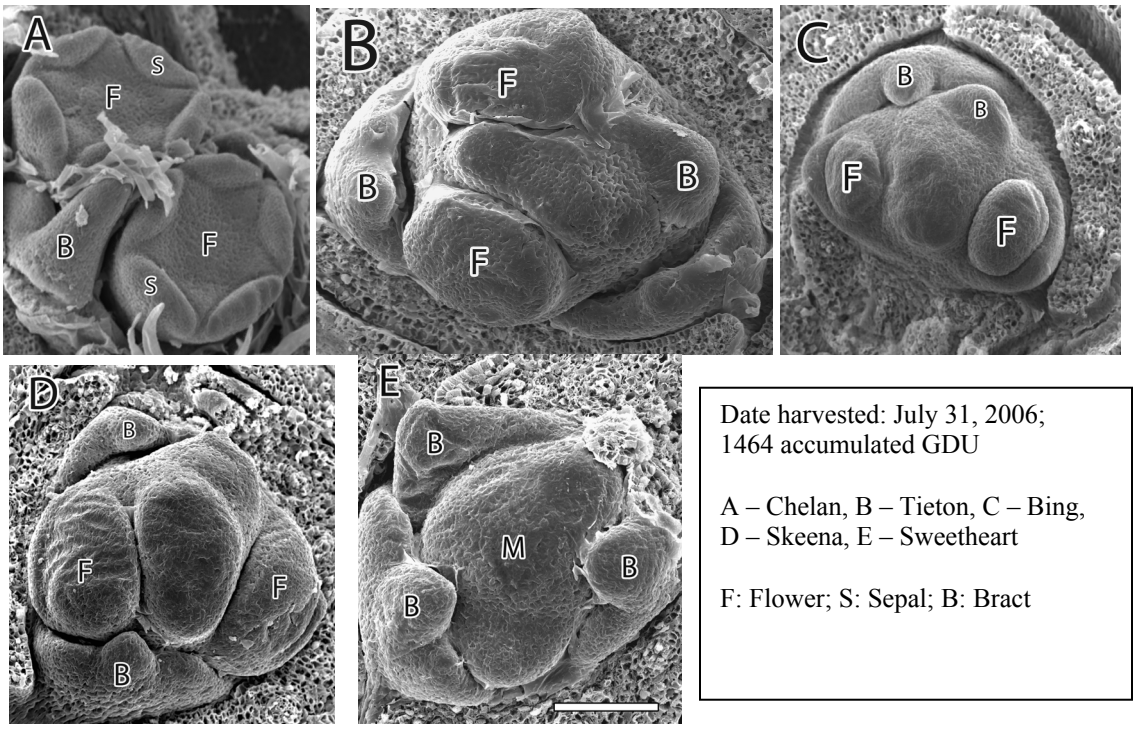
### ***Peer-reviewed manuscripts in preparation*:**

Martin, R. and M. Whiting. 2008. Asynchrony in sweet cherry floral organ differentiation among several cultivars. J. Am. Soc. Hort. Sci.

Martin, R. and M. Whiting. 2008. Susceptibility of ‘Bing’ sweet cherry to polycarpy varies seasonally and with tissue temperature. J. Am. Soc. Hort. Sci.

Martin, R. and M. Whiting. 2008. Practical strategies for reducing multiple pistils in sweet cherry. HortTechnol.

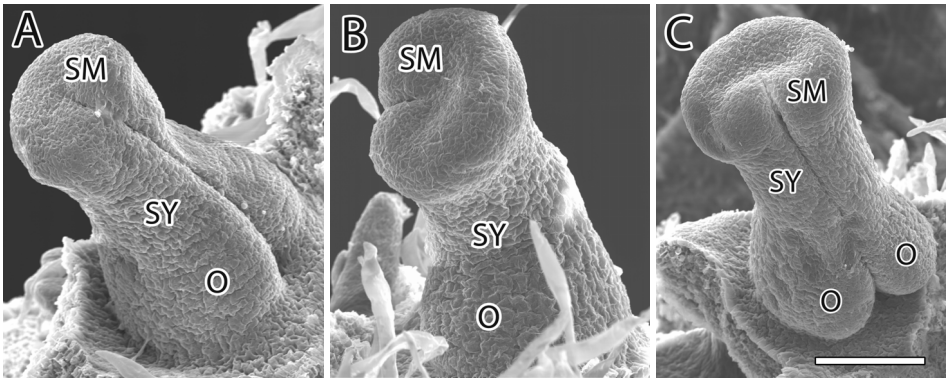
APPENDIX A. Scanning electron images of differentiating cherry flower buds.

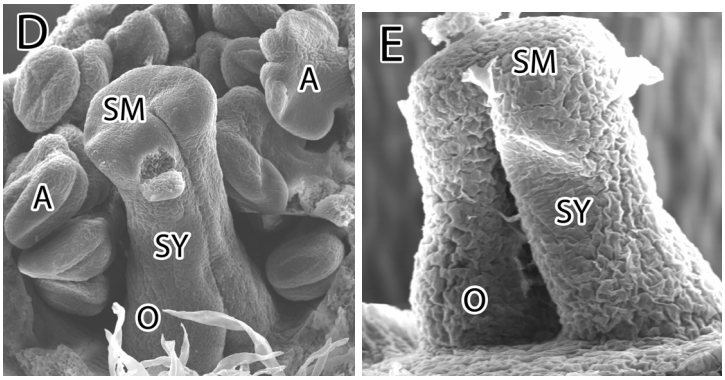



Date harvested: July 31, 2006;
1464 accumulated GDU

A – Chelan, B – Tieton, C – Bing,
D – Skeena, E – Sweetheart

F: Flower; S: Sepal; B: Bract




Date harvested: October 7, 2006;
2446 accumulated GDU

A – Chelan, B – Tieton, C – Bing,
D – Skeena, E – Sweetheart

SM: stigma; SY: style; O: ovary;
A: anther