FINAL PROJECT REPORT
WTFRC Project Number:

Project Title: Flowering and pollination of 'Regina' and 'Bing' sweet cherry trees

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Other Funding Sources

Total Project Funding:

Budget History:

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	8,300	8,300	8,300
Benefits	4,900	4,900	4,900
Wages	2,500	2,500	2,500
Benefits			
Equipment	500	500	500
Supplies			
Travel	1,500	1,500	1,500
Miscellaneous			
Total	17,700	17,700	17,700

Final Report to the Agricultural Research Foundation,
Oregon Sweet Cherry Commission, Washington Tree Fruit Research Commission, and
California Cherry Advisory Board

Project title: Flowering and pollination of 'Regina' and 'Bing' cherry trees

Objectives:

1. Determine ovule longevity of 'Regina' and 'Bing' flowers.

2. Assess pollen viability of 'Attika', 'Sam', 'Sandra Rose', 'Stark's Gold', 'Sylvia', 'Skeena', 'Regina' and 'Schneider's Späte Knorpel'.

3. Compare pollen tube growth rates and fruit set when 2-4 standard pollinizers are used in 'Regina' and 'Bing' plantings.

Significant findings and results:

Below are significant findings to date, however are incomplete because 7 funded months still remain to complete analysis.

- *In situ ovule longevity studies* – The ovules in 'Regina' flowers began declining substantively earlier than 'Bing' flowers in two of the three years (2005 and 2007) that were studied (Figs. 1-3). However, once the ovules began to senesce the rate of decline was relatively similar between each genotype each year. Across the years of study, the rate of decline differs. Ovule viability in 2005 began to decline in 'Bing' flowers after 746 growing degree hours (GDH) (12 days), while in 'Regina' flowers the decline began at 233 GDH (4 days) (Fig. 4). In 2006, flowers of both cultivars began to decline at approximately 350 GDH. This GDH accumulation across both locations occurred at 6 and 3 days for 'Bing' and 'Regina' flowers, respectively. In 2007, ovules began to senesce in 'Regina' flowers at 370 GDH (5 days in Corvallis and 4 days in The Dalles). The ovules in 'Bing' flowers remained viable over 1000 GDH longer than 'Regina' ovules before beginning to senesce. Senescence began at 1352 GDH (13 days) and 1072 GDH (13 days) at the two Mid-Columbia locations. In 2007, ovules remained viable longer in both cultivars before starting their decline compared to 2006 when ovule decline began much sooner. In general, 'Bing' ovules remained viable longer than 'Regina'.

- *Pollen tube growth and fruit set* - Pollen tube growth in 'Regina' appeared more fragile than in 'Bing'. In 2006, two pollen types never penetrated the style to produce tubes, but in 2007 all pollen types germinated on the style and penetrated but tube growth was slow often stopping in the first 15% of the style. Only 5-10% of all hand-pollinated flowers contained pollen tubes of which a few did reach the base of the style. Length of style and length/width of ovary in 'Regina' was significantly smaller than 'Bing' (Fig. 5 and Table 1). In order to ensure adequate numbers of flowers, the earliest blooming flowers were used. These 'Regina' flowers may be inferior to those maturing later and could potentially explain the poor pollen tube growth, especially since fruit set was very good in both locations where 'Regina' trees were sampled. Generally, pollen tubes in 'Bing' flowers reached the base of the style in less time and lower GDF accumulation (500-750 GDH) than pollen tubes in 'Regina' (700-1500 GDH) (Table 2).

- *Pollen viability* – Pollen viability varies from year to year and by location (Table 3). Adequate germination percentages to develop pollen tubes in hand-pollinated styles were as low as 7%. Pollen collected from open flowers in the field had higher germination percentages than those

forced inside at room temperature. Pollen viability can be ascertained after two hours of incubation in a liquid sucrose solution.

Materials and methods:

- *In situ ovule longevity*- Ovule longevity of 'Regina' and 'Bing' flowers were determined in three locations, The Dalles, Hood River and the Lewis Brown Farm, over a two week period. A branch from each of four trees was covered with a pollination bag to prevent pollen from pollinating flowers during the sample collection period. Flowers were removed every morning for 11 days. Flowers were placed in a fixative. Ovules are excised after rinsing out the fixative, stained with aniline blue, and observed under a fluorescence microscope. Fluorescence of callose at the chalazal end indicates ovule senescence (Fig. 6A). More than 700 ovules were evaluated in 2007 requiring over 75 hours.

- *Pollen tube growth rates*- 'Regina' and 'Bing' flowers were hand-pollinated with pollen from 2-4 standard pollinizers, alone and in combination. Twelve flowers were collected daily, placed into fixative, stained with aniline blue and observed under a fluorescence microscope. The percent of the style traveled by the pollen tube was recorded for each sampling date. Callose plugs and tubes are observed (Fig. 6B). In 2007, over 5000 pistils were collected and 3030 were evaluated (180 hrs). Seed were collected from mature fruit in 'Bing' and will still be analyzed for s-alleles using molecular markers and PCR technologies. The s-alleles in the seed will indicate the pollen parent. No fruit were obtained for 'Regina' in either location.

- *Pollen viability*- Flowers were collected and brought back in garbage bags to prevent dessication. Bases of twigs were cut and put into water. As flowers opened each day, for 3 days, anthers were cut off, induced to dehisce pollen and pollen collected for observing pollen germination and viability. Pollen was collected and put into vials plugged with cotton then placed into the freezer with dessicant in a plastic container. Pollen collection required 96 hours. A simple liquid sucrose medium was used to induce pollen germination and pollen viability was tested prior to placing it in the freezer (45 hrs).

Results: See details of the findings in the following figures and tables.

Fig. 1. Ovule longevity of ‘Bing’ and ‘Regina’ flowers in 2005 at the Lewis-Brown Farm

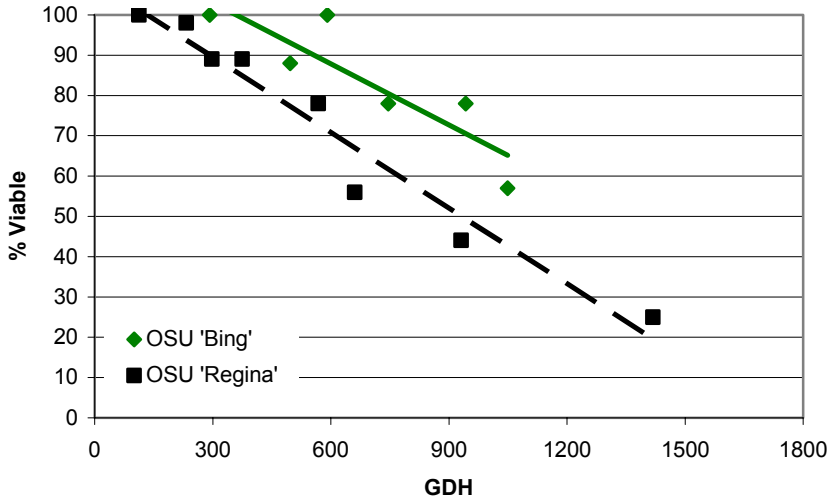

Fig. 2. Ovule longevity of ‘Bing’ and ‘Regina’ flowers in 2006 from 4 different locations

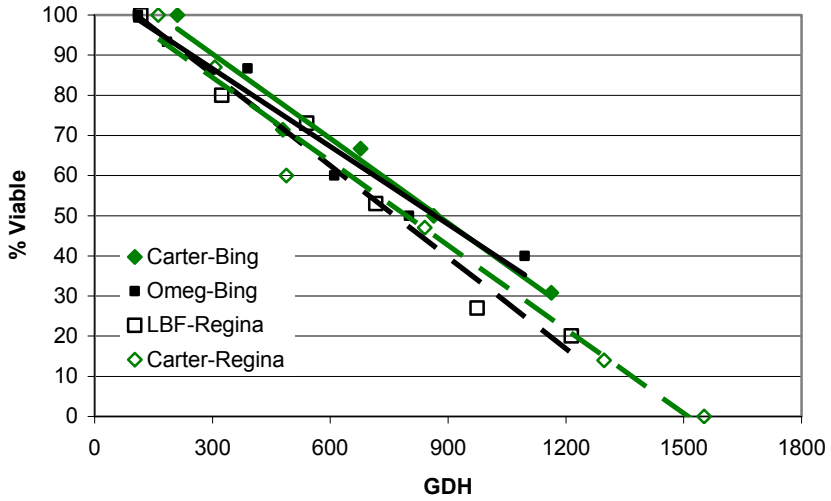

Fig. 3. Ovule longevity of ‘Bing’ and ‘Regina’ flowers in 2007 from 4 different locations

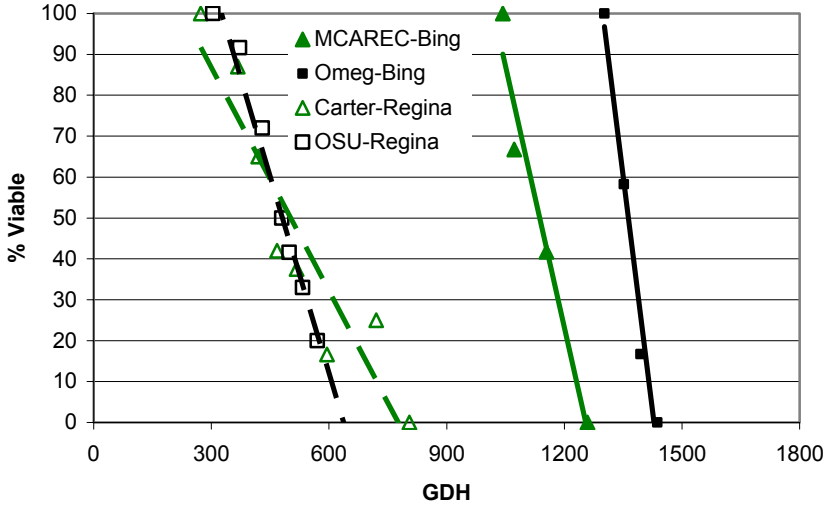

Fig. 4. Growing degree hour accumulation during bloom of ‘Regina’ and ‘Bing’ in 2006 and 2007.

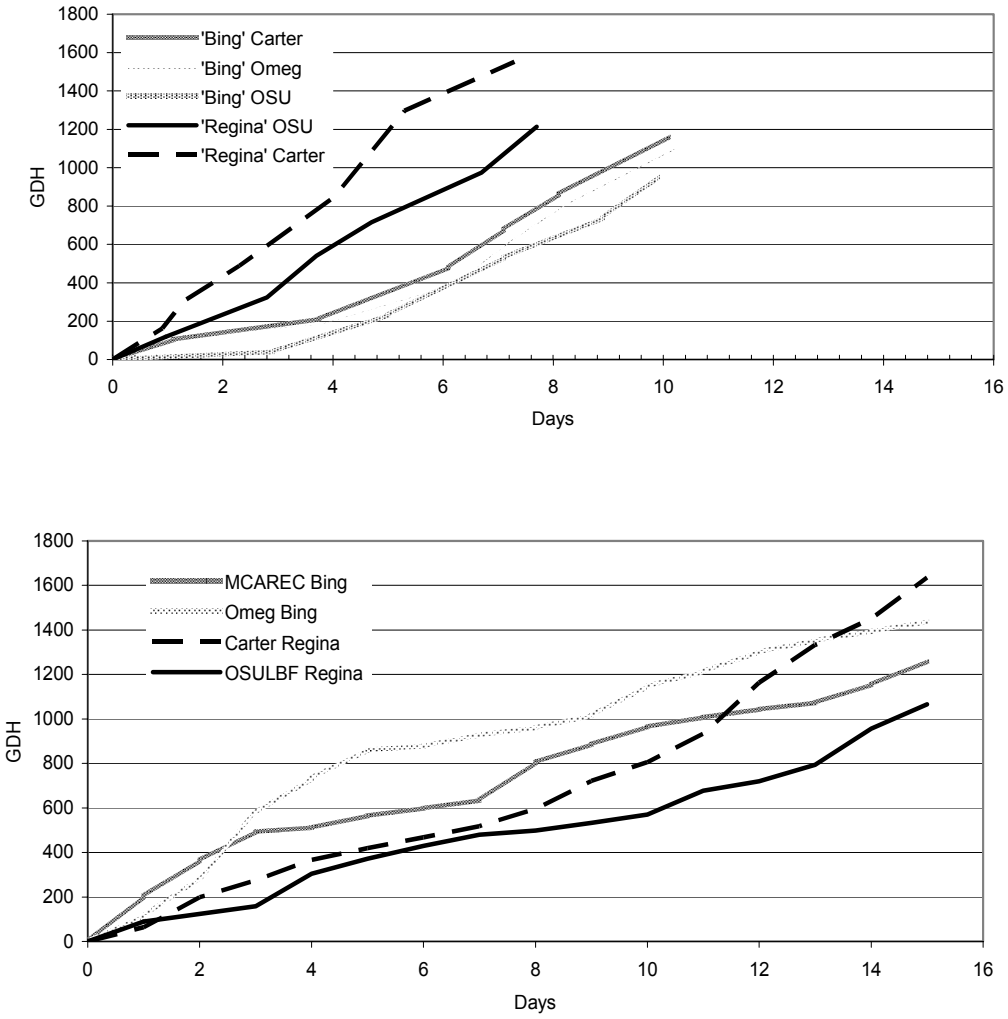

Fig. 5. Pistils of ‘Bing’ flowers (A) and ‘Regina’ flowers (B) in 2007 from The Dalles

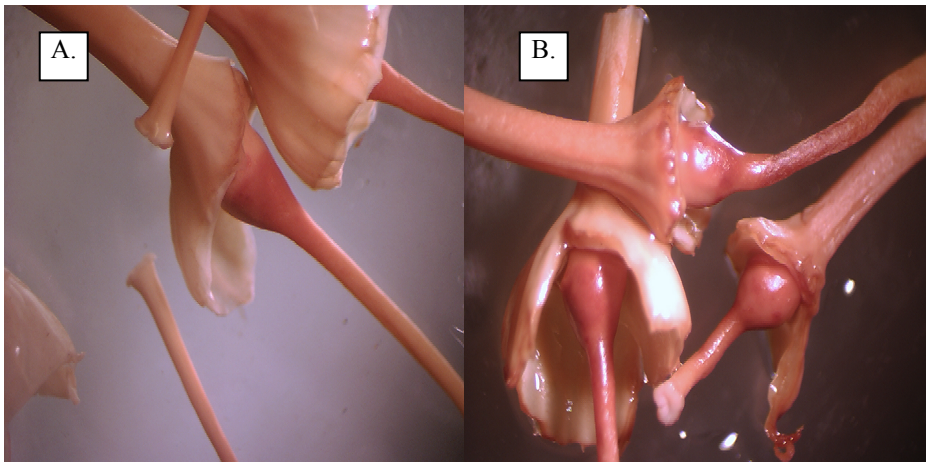

Fig. 6. Senescing ovules (A) and pollen tube growth (B)

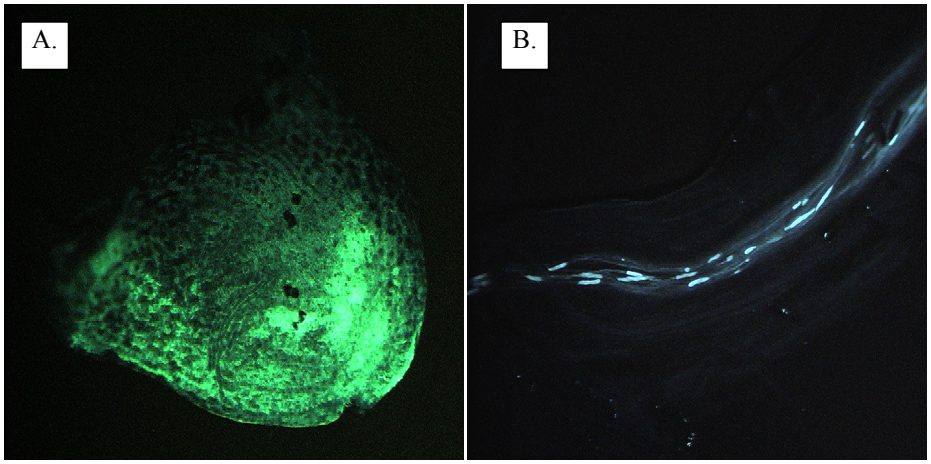

Table 1. Length of style and ovary, and width of ovary of 'Regina' and 'Bing' pistils at time of emasculation.

	Style Length (mm)	SE	Ovary Length (mm)	SE	Ovary Width (mm)	SE
'Regina'	7.3	0.47	2.4	0.07	1.7	0.04
'Bing'	11.0	0.11	2.8	0.06	1.8	0.04

Table 2. Number of growing degree hours (GDH) and maximum observed distance that pollen tubes traveled within the style in 'Bing' flowers pollinated by 'Rainier' and 'Van' in 2007

Pollen source on 'Bing'(s_3s_4)	GDH/Days	Distance of style with pollen tubes (%)
Omeg		
Van (s_1s_3)	734/5	100
Rainier (s_1s_4)	578/4	100
Van + Rainier	734/5	100
MCAREC		
Van	511/5	100
Rainier	493/4	100
Van + Rainier	511/5	100

Table 3. Pollen viability of compatible pollinizers for 'Bing' and 'Regina' in 2005, 2006 and 2007

Pollen genotype	s-alleles	Viability (%)			
		2005	2006	2007 (Range)	2007 (Mean)
Bing	s_3s_4				
Rainier	s_1s_4	69	10	9-33	21
Van	s_1s_3	67	10	25-40	33
Regina	s_1s_3				
Sam	s_2s_4	52	18	6-15	11
Schneider's Späte Knorpel	s_3s_{12}	49	22	8	8
Stark's Gold	s_3s_6		24	20	20
Skeena	$s_1s_{4'}$	28	23	19-28	24
Sandra Rose	$s_3s_{4'}$	11	33	17-33	25
Sylvia	s_1s_4	6	22	21-23	22