

**FINAL PROJECT REPORT****WTFRC Project Number:** CH-05-504**Project Title:** Breeding and Genetics Program for PNW Sweet Cherries

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**Cooperators:** James Olmstead, Amy Iezzoni, Fred Bliss, WTFRC**Other funding Sources****Agency Name:** None**Amount awarded:****Notes:****Total Project Funding:** \$207,285**Budget History:**

<b>Item</b>	<b>Year 1: 2005</b>	<b>Year 2: 2006</b>	<b>Year 3: 2007</b>
<b>Salaries</b>		30,000	30,804
<b>Benefits</b>		11,400	10,781
<b>Wages</b>	3,500	8,500	8,000
<b>Benefits</b>			920
<b>Equipment</b>	500	500	1,500
<b>Supplies</b>	500	1,000	1,500
<b>Travel</b>		1,000	1,500
<b>Plant Material</b>	3,200	2,700	2,900
<b>Virus Indexing</b>	1,000	1,000	1,000
<b>DNA Genotyping</b>	10,000	10,000	15,000
<b>Breeding Consultant</b>	10,000	10,000	10,000
<b>Expenses</b>	3,000	3,000	3,000
<b>Plot Fees</b>		1,200	7,080
<b>Miscellaneous</b>		800	500
<b>Total</b>	31,700	81,100	94,485

## Objectives

New sweet cherry cultivars with production and fruit traits superior to current cultivars are needed to provide differentiated products and production advantages that will allow the Washington and Oregon sweet cherry industries to remain competitive. Currently, there is no breeding program, domestic or foreign, able and/or designed to provide a series of new cultivars that target the specific demands of the Washington and Oregon industries. New cultivars are needed to extend the ripening season, minimize production costs and post-harvest cullage, and that have excellent fruit size, firmness, texture and flavor. Genetically improved cultivars could also reduce the risks and costs from biotic (e.g., powdery mildew) and abiotic (e.g., temperature stress) factors. The breeding goal of this project is to meet this need by developing a full-season series of sweet cherry cultivars that exceed current cultivars for a range of characteristics desired for current and future domestic and foreign market opportunities. The research goals address the need to minimize time between parental crosses for selection population development and commercial release of new cultivars to maximize the efficiency and cost-effectiveness of the breeding program. Therefore, to ensure the success of the breeding program, strategies have been put into place to (1) use an extensive array of unique germplasm, (2) achieve large seedling populations for evaluation, (3) reduce the juvenility period, and (4) utilize genetic and genomic approaches that increase the efficiency and capability of the breeding program.

The specific objectives of this research project were to:

1. Develop a comprehensive framework for a sweet cherry breeding and genetics program.
2. Produce through hybridization and selfing, genetically-variable sweet cherry selection populations that segregate for important target traits.
3. Select outstanding families and elite individual seedlings in multiple target environments for important production and fruit traits.
4. Establish elite selections in replicated multi-site locations for pre-commercial evaluation.
5. Install a database for storage and analysis of critical information that will effectively support the breeding goals.
6. Acquire and develop molecular information and tools that facilitate rapid and efficient marker-assisted selection for production and fruit traits critical for commercial success.

## Significant Findings and Accomplishments

### End of YEAR 1 (2005)

- A comprehensive oversight and communications framework was established and implemented. Oversight of the breeding program is provided by an Advisory Committee (AC) consisting of cherry growers from WA and OR.
- Germination of seeds from YR 2004 crosses in the seed beds at Willow Drive Nursery was extremely poor with only 250 seedlings resulting from the 4,641 seeds (5% germination). Thus, new seed stratification and germination strategies were employed.
- Novel germplasm was successfully imported as pollen and used in crosses by Amy Iezzoni.

- Despite the consistently cold weather conditions, 90 crosses including reciprocal crosses were successfully accomplished at Prosser in spring 2005 resulting in 7,349 hybrid seed.
- Matt Whiting planted a new orchard to evaluate the potential for rootstock and nursery production system to hasten productivity. ‘Bing’ and ‘Tieton’ on Mazzard seedling, Gisela®6, and Maxma14 trees were planted at the Roza farm as sleeping eyes (i.e., fall-budded, spring dug and planted), and standard nursery trees (i.e., fall-budded, nursery-grown, spring dug and planted +1yr).
- It was decided that the Oregon selection site will be located at the Hood River Station.
- Amy Iezzoni received a \$400,000 three year USDA-CSREES-NRI grant entitled “Genomic resources to improve fruit size and quality in sweet cherry”. This grant which covers the period of June 1, 2005 to May 31, 2008 provided funds to obtain the baseline genetics/genomics research required for the breeding program. The grant team members are Drs. Wayne Loescher (fruit biochemistry/physiology), Dechun Wang (quantitative genetics) and Esther van der Knaap (molecular genetics).

#### End of YEAR 2 (2006)

- Jim Olmstead began as manager of the breeding program January 1.
- The existing database system used in the apple breeding program was effectively utilized for the cherry breeding program with minor modifications. The platform used for this database can be user-modified, a function not readily available from many commercial breeding database systems.
- Germination of year 2005 seedlings was significantly better than those in 2004 (20% vs. 5%), with 1,460 seedlings currently available.
- The spring 2006 crosses were extremely successful, with 14,848 seed generated from 109 crosses including reciprocal crosses.
- A seedling evaluation block was established at WSU-Prosser with planting of the initial set of 250 seedlings resulting from the year 2004 crosses.
- Shoot-tip micrografting on to Gisela rootstocks did not increase efficiency over traditional chip budding due to low success rate and slow growth after grafting.
- The project to identify a molecular marker linked to the gene controlling powdery mildew resistance was undertaken. Because of a high level of genetic similarity, no candidate markers linked to the *Pmr-1* gene have been identified. Therefore, in collaboration with Drs. Angela Baldo and Gayle Volk, we initiated an alternate project to identify Resistance Gene Analogs (RGA) using a set of conserved DNA primers that will specifically target resistance genes in the cherry genome. If any RGA are found from the powdery mildew resistant parent that are not in susceptible parents, Amy Iezzoni will screen them on the available segregating progeny to determine if they are linked to *Pmr-1*.
- Greenhouse incidence of powdery mildew was used as a screening method to select seedlings resistant to the disease. Amy Iezzoni’s lab will use molecular markers to screen

those resistant seedlings for self-fertility. Combining the two early screening methods is expected to reduce by three-quarters the number of seedlings sent to field testing.

#### End of YEAR 3 (2007)

- Germination of year 2006 seedlings was similar to that of 2005 (overall 34%), with 5,120 trees generated for planting in 2008.
- Cold-room cycling to produce two growing seasons in one year with potted trees was successfully implemented. One-third of the 2005 seedlings (500) were subjected to two growth cycles during the summer/winter of 2006/2007 and were planted in the field following completion of the second dormancy treatment.
- An agreement was reached with Gisela Inc. to purchase Gisela 5 rootstock liners for use in the breeding program. 750 liners were available in 2007; 200 were used to bud progeny from selected parents thought to transmit varying degrees of precocity and 550 were retained in a nursery bed for propagation and establishment of a parental crossing block at Prosser.
- Existing royalty proceeds from previous WSU-Prosser sweet cherry releases were used to construct four new greenhouses for use by the cherry breeding and physiology program.
- All 1,460 seedlings from year 2005 crosses were planted in the seedling evaluation block at WSU-Prosser. At a density of ~900 trees/acre, the selection blocks currently total 2 acres with 6 acres more to be planted in 2008.
- Spring 2007 crosses resulted in 6,827 seed generated from 49 crosses including reciprocal crosses.
- Washington State University initiated a search for a full-time faculty position in Stone Fruit Breeding and Genetics.

#### **Results and Discussion**

A comprehensive oversight and communications framework was established and implemented in year 2005. Oversight of the breeding program is provided by an Advisory Committee (AC) consisting of cherry growers from WA and OR. Executive summaries of progress were provided to the AC two to three times per year. These summaries can be freely distributed. Matt Whiting established a secure web site to enhance communication with the AC. This web site also contains seedling and pedigree information that must remain confidential to protect IP. After consultation with the AC, more frequent face-to-face meetings were recommended, and will be scheduled to occur during bloom, harvest and in conjunction with the annual Cherry Research Review. Communication with the entire grower community was accomplished through three articles in the Good Fruit Grower and one in the 2006 WSHA Proceedings, presentations at the 2006 Cherry Institute, 2006 Washington State Horticultural Association meeting, and participation in the annual Cherry Field Days at WSU-Prosser.

Upon consultation with the AC and breeding program consultants Amy Iezzoni and Fred Bliss, a set of variety targets were established to guide parental selection for crosses and selection priorities (Table 1).

Table 1. Variety targets for the cherry breeding program.

Current leading variety	Traits desired to improve upon current variety leader
Bing	Mid-season, self-fertile, mahogany, larger fruit size than Bing
Sweetheart	Late-season, self-fertile, mahogany, powdery mildew resistant
none	Late-season, self-fertile, blush, powdery mildew resistant
Chelan	Early-season, self-fertile, mahogany, larger fruit size than Chelan
Early Robin	Early-season, self-fertile, blush
none	Early-mid-late season, self fertile, mahogany, suitable for mechanical harvest

Since the initial crossing year in 2004, 255 different crosses (including reciprocal crosses) have been attempted; seedling trees were generated from 215 of those crosses. Included in this list of crosses are those utilizing imported pollen. To date, the project has seedlings resulting from 25 newly utilized or foreign sweet cherry varieties. These selections were chosen specifically to add the following superior traits to the breeding program: heat tolerance, very late ripening time, very early ripening time, rain cracking tolerance, fruit firmness, large fruit size, suitability for mechanical harvest, a novel source of self-fertility, and different sources of powdery mildew resistance.

Due to poor germination in nursery seed beds, many of the crosses attempted in 2004 were repeated in later years of the project. In 2005, crossing goals included the generation of self-fertile, powdery mildew resistant, late-season selections, use of Regina as a parental source of fruit quality, rain cracking tolerance, and late bloom and maturity, and inter-mating among the various varieties with ‘Van’ in their pedigree in an attempt to concentrate the favorable alleles that have been selected for over the years of cherry breeding. Crosses made in 2006 were those that combined elite parents anticipated to yield potential cultivars in the first generation, used novel germplasm sources to incorporate useful traits, and pyramided potential sources of powdery mildew resistance. Again, some crosses that were lost in 2004 or could not be completed due to frost in 2005 were repeated. In 2007, the primary goal was to enrich the germplasm base for potential early-maturing, self-fertile, powdery mildew resistant blush and dark cherries. Although crosses toward these goals had been made in previous years, a concerted effort was placed on utilizing existing germplasm selections bred by Tom Toyama.

Our target seed germination goal continues to be 50%, although seed germination in each of the three project years did not reach this goal (2004 – 5%; 2005 – 20%; 2006 – 34%). Given the fact that poor germination continues to be a problem and may be limited due to the parental genotypes used in the breeding program, the number of crosses made each year has been increased to more realistically achieve seedling number goals. Improved seed handling techniques (seed cracking, altered storage and germination temperatures) devised based on our experiences with the 2005 seed, have increased germination to ~35% in the last two years of the project (Table 2). Likewise, seedling target goals for each year have been adjusted due to land and resource availability. Rather than the originally proposed 5000 seedling per year, the current goal, arrived at in consultation with the AC, is 2000 seedlings per year to be planted in the field. With this current level of effort we are confident that we are adequately utilizing all the available germplasm and crosses. If our experience with the most recent selection populations holds true, where three variety release candidates were selected from a total of 425 progeny, at least 15 new varieties could be developed from each year of the project. However, crossing levels will be adjusted each year to most effectively utilize as parents the superior selections to be identified in the current seedling populations. Additionally, as work on developing molecular markers for early selection of key traits comes to fruition, more crosses and seed can be generated initially, with marker assisted selection used to reduce the final number of seedling trees planted in the field to 2000.

Table 2. Population development summary for the cherry breeding program.

	Year			
	2004	2005	2006	2007
No. crosses made	61	90	109	49
No. seed	4,466	7,349	14,848	6,827
No. seedling trees	250	1,460	5,120	n.a.
% germination	5%	20%	34%	n.a.
Avg. family size	9	18	50	n.a.



Figure 1. Seed germination, greenhouse growth and field planting of seedlings from 2005 crosses.

Effective methods to accelerate seedling flowering and fruiting are essential to increase efficiency in the breeding program. Over the course of this project, we have experimented with several horticultural-based methods to accelerate seedling flowering in comparison to the traditional own-rooted seedling timeline (up to five years). One obvious method to do this is to propagate seedling trees on a precocious rootstock. Furthermore, research in the sweet cherry physiology program has suggested that sleeping eye trees planted a year prior to traditional nursery-propagated trees in the production cycle show better overall growth, likely due to root system establishment. To test the relative efficiencies and develop a cost analysis of traditional own-rooted seedling establishment vs. propagating all breeding program seedlings on precocious rootstock, we established a test block comparing ‘Bing’ scion grafted on Gisela 6 and Mazzard rootstock, with replicate trees planted as a sleeping eye or traditional nursery produced tree headed at planting to 18 inches. Although no sleeping eye trees were available at the time of establishment, ‘Tieton’ scion grafted on both Gisela 6 and Mazzard and headed similarly were included for comparison. In this trial, ‘Tieton’ represented a non-precocious scion variety, similar to the range in endogenous precocity expected to be present in seedling populations. The trial was planted in the spring of 2005, and the percentage of spurs containing floral buds was evaluated in September 2007, the first year that significant spur flower development was evident (Table 3). Thus, the standard headed trees were fourth leaf trees, while the sleeping eye trees were third leaf at the time of evaluation. Survival of the sleeping eye trees was acceptable (ranging from 76% to 96%) while survival of the standard nursery stock ranged from 92% to 100%. As expected, the use of precocious rootstock resulted in a significantly greater percentage of spur flowers, regardless of the planting system. However, sleeping eye trees had similar floral bud percentages to that of the standard nursery trees, regardless of rootstock, and in spite of the fact they were effectively one year younger than the standard nursery trees. Of note is the fact that rootstock induced accelerated flowering also appears to be strongly genotype dependant, as ‘Tieton’ grafted on precocious rootstock had significantly fewer floral buds than ‘Bing’.

Table 3. Tree survival and percentage of spurs with flower buds for ‘Bing’ and ‘Tieton’ trees propagated on precocious and standard rootstock and established as sleeping eye trees or standard nursery trees.

Variety	Rootstock	Planting system	Survival (%)	Spurs with flower buds (%)
Bing	Gi6	Sleeping eye	76%	47%
		Standard	92%	65%
Bing	Mazzard	Sleeping eye	96%	8%
		Standard	100%	9%
Tieton	Gi6	Standard	100%	8%
Tieton	Mazzard	Standard	96%	2%

For comparison, the initial set of 250 seedling trees generated from crossing in 2004 were in their third leaf in 2007. Unlike the grafted trees, there were very few flower buds found on spurs. However, a significant amount of flowering can occur from single flower buds formed at the basal end of current season growth. Progeny individuals from five families in this initial set of seedlings were randomly selected and examined for the percentage of basal buds containing flowers (Table 4). Overall, the percentage of basal buds containing flowers was near 50% for three of the five families. Again, the seedling precocity appeared to be genotype dependant, with some progeny from each cross having no flowers present.

Table 4. Percentage of progeny and basal one-year buds containing flowers for five families from crosses made in 2004.

Cross	Progeny with flowers (%)	Buds containing flowers (%)
Lapins x Tieton	75%	53%
Lapins x Chelan	80%	48%
Sweetheart x Regina	83%	46%
Sweetheart x Ambrunes	40%	13%
Gold x Dzherlo	75%	28%

Although grafting seedling trees on precocious rootstock has the potential to increase flower density on those seedlings already predisposed to short juvenility periods, flowers are present on a majority of the seedling trees four years after the cross was made, *with no additional expense or horticultural manipulation*. These results agree with our previous experience with sweet cherry selection populations planted at both WSU and MSU that had significant fruit five years after the cross was made.

A cost analysis of the two production methods (Table 5), based on actual labor and plant material costs tracked during the three years of this project indicates minor differences in cost between the two methods. However, if the goal is simply to accelerate the time to first flowering so selection evaluation can proceed, it is evident that there is little advantage, save more rapid development of spur borne fruit, in using precocious rootstock (Table 6). The highly variable survival rate using sleeping eye propagation further reduces the potential for successful implementation of this process. Presumably, single flower buds were present as early as year two after planting on Gisela 6 sleeping eye trees, although this was not evaluated. However, at that stage of development the tree architecture (few lateral branches) would severely limit the flower number compared to ungrafted seedling trees.

Table 5. Cost of producing a single seedling tree in the cherry breeding program under standard seedling management and by grafting to precocious rootstock.

	Seedling tree	Grafted tree
Crossing labor	\$2.04	\$2.04
Harvest labor	\$0.68	\$0.68
Greenhouse labor	\$10.20	\$10.20
Rootstock purchase	n.a.	\$1.56
Budding labor	n.a.	\$0.68
Planting labor	\$0.41	\$0.41
Plot establishment	\$2.75	\$2.75
Plot maintenance	\$2.75	\$2.75
Plot maintenance	\$2.75	\$2.75
<b>Cost per seedling</b>	<b>\$21.58</b>	<b>\$23.82</b>

In contrast, seedling material available for analysis did show significant within-family variability for early flowering – at most, ~80% of the progeny within a family had flowers after four years. Although flowering on ‘Tieton’ was clearly not as abundant as ‘Bing’ when grafted on precocious rootstocks, the fact remains that there were flowers present on all ‘Tieton’ trees sampled. To more thoroughly examine the impact of precocious rootstocks on seedling material with variable precocity, progeny from several families were budded on Gisela 6 rootstock in August 2007. These budded trees are paired with their seedling counterparts in the field (a single bud was removed from each seedling tree for grafting). Time to flowering, flower density, and fruit quality characteristics will be evaluated for each paired genotype in the coming years.

Table 6. Comparison of timelines for first fruit between standard seedling trees and seedling trees grafted to precocious rootstock.

	Seedling tree	Grafted tree
Year 1	Cross made	Cross made
Year 2	Greenhouse growth	Greenhouse growth – fall budding
Year 3	Field planting	Field planting – sleeping eye
Year 4	Field growth	Field growth
Year 5	First fruit	First fruit, spur flowers

These observations suggest that to most effectively accelerate flowering, more fully developed plant material should be generated prior to planting. For example, the relative time advantage of grafting to precocious rootstocks is lost because a new tree must be grown subsequent to the grafting process. Earlier budding in the greenhouse, allowing for a full season of growth prior to dormancy induction would alleviate this problem. In 2006, a subset of seedling trees actively growing in the greenhouse were green shoot tip grafted to Gisela 6 rootstocks. This process has been used by the NRSP5 program during heat therapy to remove virus contamination. However, only 30% of the grafts were successful, and growth following the grafting process was extremely slow. An alternative strategy, using seedling material without grafting was also devised to increase the number of growth cycles prior to field planting. Potted seedling plants were grown to an approximate height of 18 inches in the greenhouse, moved to a cold room for at least three months to complete a dormant cycle, brought back to the greenhouse under supplemental light during the winter months for an additional growth period, and then allowed to go dormant in the cold room again. After the simulated two years of growth and dormancy, the seedling trees were planted in the field selection block. In the coming years, these trees will be compared to sibs from the same family subjected to standard seedling tree management. We speculate that those seedlings which are genetically predisposed to a short



juvility period would respond well to the cycling strategy and would flower profusely in Year 3. However, those seedlings that are genetically predisposed to a longer juvenility period may need to be grafted to a precocious rootstock to ensure early flowering. In order to determine what procedure to use for each seedling, we will explore the possibility of identifying molecular marker(s) linked to juvenility time. The onset of flowering and fruiting will be recorded in the Emperor Francis x New York 54 population and subjected to QTL analysis using the developing sweet cherry linkage map. This initial QTL discovery objective will be funded by a USDA-CSREES Cherry Genomics Project. Results from 2006 were promising as a QTL [LOD ~ 5.0] for early flower abundance was identified on linkage group 3.

In year 2005, we proposed propagation of the first year seedlings on MxM 14 rootstock. However, no MxM14 or Gisela rootstock liners were available commercially during the first two years of the project. An agreement was reached in 2007 with Gisela Inc. for purchase of Gisela rootstock liners for the breeding program.

In addition to the horticultural based methods of increasing efficiency in the breeding program, we are endeavoring to develop and apply molecular marker assisted breeding strategies toward cherry improvement. To this end, Fred Bliss, the genetics consultant to the breeding program organized a cherry trait assessment exercise aimed at identifying key traits for improvement, availability of genetic populations to develop molecular markers, and organize a priority list for developing and implementing these molecular markers in the breeding program. The process was extremely instructive, as it brought together expertise in breeding, physiology, genomics and genetics. Traits identified during the process that were given a high priority for molecular marker development were: tree juvenility period, fruit weight, fruit firmness, total soluble solids, and titratable acidity.

The infrastructure required to perform molecular marker analyses on the scale needed for the breeding program is substantial. Dr. Cameron Peace, tree fruit genomics in Pullman, is collaborating with both the WSU apple and cherry breeding programs to develop high-throughput DNA extraction and genotyping methods. Dr. Peace also collaborates through a WTFRC funded project to identify candidate genes and markers for flavor components in cherry. This is an important collaboration, as these flavor components were given priority status for molecular marker development in our trait assessment exercise. Similarly, Dr. Amy Jezzoni, tart cherry breeding and genetics at Michigan State University, developed a USDA-CSREES project designed to generate the genomic resources required to implement marker-assisted selection for fruit size and quality traits in cherry breeding programs. We plan to accomplish this goal using a QTL strategy focused on fruit size and quality traits, followed by QTL validation and allele mining using a newly-developed pedigree genotyping approach. The research consisted of the following steps: (1) Construct a sweet cherry genetic linkage map for comparative mapping with the *Prunus* reference map and other *Prunus* linkage maps. (2) Identify QTL for fruit size and quality traits. (3) Fine map the major QTL identified and design markers for marker assisted selection. (4) Validate the QTL across genetic backgrounds and identify QTL alleles. This supplemental funding allowed us to greatly exceed our prior expectations for objectives 5 and 6, database capability and acquisition of molecular information, respectively. In addition, one of our team members, Dr. Wayne Loescher, is studying the biochemical basis of the differences in fruit quality using fruit from the varieties used as parents in the breeding program. This information will greatly enhance our selection and QTL discovery capabilities.

Although powdery mildew disease screening can be readily accomplished using traditional lab, greenhouse and field assays, associating molecular markers with the different genes carried in the parents used to confer resistance has the advantage of allowing gene pyramiding in a single improved variety. This concept is similar to strategies already used by PNW grower to manage fungicide resistance – two or more different resistance genes in the same variety makes it much less likely that the powdery mildew organism can overcome the plant resistance. In 2005, leaves were collected and DNA was extracted from the parents and 375 seedlings from crosses between PMR-1 with Van, Bing, and Rainier that had previously been screened for powdery mildew resistance. Also included were four other cultivars that are powdery mildew resistant; Venus, Moreau, Chelan and Hedelfingen.

Resistant and susceptible bulk populations were designed and screened using AFLPs generated from *EcoRI* with *MseI* and *PstI* with *MseI* selective primer pair combinations. Because of a high level of genetic similarity, no candidate markers linked to *Pmr-1* have been identified to date. The limited number of polymorphic fragments identified among the four sweet cherry cultivars (PMR-1, Bing, Van and Rainier) highlights the genetic uniformity present in sweet cherry cultivars. For this reason, an alternate approach using conserved DNA primers to target resistance gene analogs was initiated in collaboration with Drs. Angela Baldo and Gayle Volk at USDA-ARS. Using this approach, 90 RGAs were identified that will be converted to molecular markers, mapped in populations segregating for powdery mildew resistance, and associated with the resistance phenotype.

Two powdery mildew resistant genotypes from the WSU sweet cherry breeding program are being propagated for advanced testing based on performance of second test trees at Prosser (Table 7). Both were identified from crosses between ‘Rainier’ and ‘PMR-1’ and have been given testing names of PC9816-104 ‘DD’ and PC9817-97 ‘GG’. Fruit quality from both have continued to be excellent, and further quality and storage/shipping characteristics will be evaluated in 2008 as larger plantings become available.

Table 7. Summary of key traits for elite powdery mildew resistant selections.

Trait	PC9816-104 ‘DD’	PC9817-97 ‘GG’
Bloom (days +/- Bing)	Early (-2)	Early (-3)
S-alleles	S <sub>1</sub> S <sub>9</sub>	S <sub>1</sub> S <sub>4</sub>
Harvest (days +/- Bing)	Late (+12)	Mid (+5)
Avg. fruit size	15.1 g (8.5 row)	14.4 g (9 row)
Avg. soluble solids	22	23
Avg. firmness (g/mm)	244	208

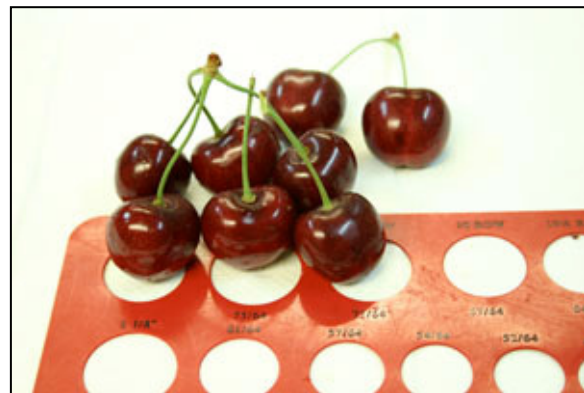
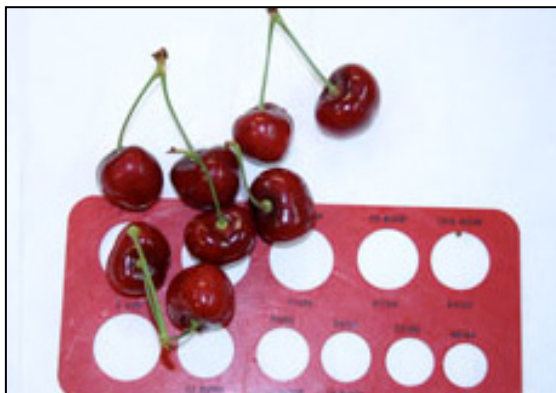


Figure 2. PC9816-104 ‘DD’ (left) and PC9817-97 ‘GG’ (right).