

FINAL PROJECT REPORT**WTFRC Project Number:** CH-05-503**Project Title:** MSU's sweet cherry dwarfing rootstocks

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Other funding Sources**Agency Name:****Amount awarded:****Notes:****Total Project Funding:** \$ 52,010**Budget History:**

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	\$4,725	\$4,867	\$6,343
Benefits	2,183	2,351	3,191
Wages (MSU)	1,500	3,000	1,500
Wages (WSU)	2,000	2,000	2,000
Benefits			
Equipment			
Supplies	400	1,500	600
Travel	1,500	2,500	2,500
Tree & Freight cost	100	500	750
Plot cost at MSU	1,000	1,000	1,000
Greenhouse cost		3,000	
Miscellaneous			
Total	\$13,408	\$20,718	\$17,884

Objective:

Identify MSU rootstock selections that may have commercial potential as dwarfing precocious rootstocks for sweet cherry.

Specific Objectives:

1. Complete the planting of the rootstock candidates at the WSU-Prosser and MSU-Clarksville test sites.
2. Identify the most promising rootstock candidates by evaluating tree health, precocity, trunk cross-sectional area, flower density, crop load, fruiting habit and fruit size.
3. Vegetatively propagate the most promising rootstock selections to provide grafted trees for advanced trials at multiple test locations.

Significant findings:

- The establishment of the first test plots was completed in 2004 with 71 and 41 MSU rootstock candidates planted at MSU-Clarksville and WSU-Prosser, respectively.
- In 2006, 28 of the MSU rootstock selections were chosen for advanced testing based on evaluations of tree health and yield potential.
- Quarantine restrictions were met to permit shipment of these 28 rootstock candidates to nurseries in Washington and California to make trees for the next set of trials.
- In 2006, ~ 4,300 cuttings from these 28 selections were rooted in Michigan. A total of 2,080 rooted cuttings were obtained. These cuttings were shipped to Willow Drive Nursery in March 2007 and planting in one of their nursery rows spring 2007. In August, 1,493 of the cuttings were available for budding.
- In 2006, we reached an agreement with Duarte Nursery for liner increase and eventual tree production. Budwood from each of the 28 rootstock selections was sent to Duarte Nursery and subsequently established in culture.
- Upon transfer to the nurseries, the rootstocks were given Michigan county code names to reduce the likelihood of identity mix ups at the commercial nurseries and throughout the testing process.
- In 2007, the 28 MSU rootstock candidates were narrowed down to 11 selections for advanced testing. See Results and Discussion for a review of these 11 rootstocks.
- DNA markers were identified that can discriminate among the 11 MSU rootstock selections. These diagnostic markers were used to confirm the identity of the rootstock cultures at Duarte Nurseries tissue culture lab (Dry Creek Laboratory) and confirm the 11 MSU rootstock selections in the test plot at MSU-Clarksville.
- Plans for the next phase of testing were developed in collaboration with Matt Whiting and Tom Auvil. This included scion selection and Washington test site selection.

- Tree requests were communicated to Willow Drive Nursery and Duarte Nursery and we are on schedule for planting in spring 2009.

Results and Discussion

Test plot establishment:

The rootstock evaluation plot at MSU-Clarksville has 30 more rootstock selections under test than the WSU-Prosser plot. This occurred for two reasons. For the initial planting in 2001, all the trees were planted in Michigan and not split between MSU and WSU as the leadership for the cherry position at WSU was uncertain at that time. In 2004, we were able to add more trees to the MSU-Clarksville plot as these trees were grown at Hilltop Nurseries in Hartford, Michigan, and we personally participated in plant care. Unfortunately quarantine restrictions did not permit us to ship any of the finished trees to Washington. Therefore, the following six advanced selections have only been tested in Michigan: Lake, Iron, Clinton, Cass, Clare and Crawford. Lake and Iron were planted in 2001 and the other four selections were planted in 2004.

The MSU rootstock selections:

GI6 was planted as a rootstock control in 2001 and 2002. Four of the seven MSU rootstock candidates selected for these advanced trials, and planted in 2001 or 2002, confer to the scion lower tree vigor than GI6 (Figure 1). These four rootstocks are Iron, Lake, King and Garfield, and the reduced vigor is apparent from both a reduction in trunk cross-sectional area and lateral and terminal shoot growth. Of these four rootstock selections, Lake has the highest spur density and will likely over-crop with a resulting decrease in fruit size if not managed properly. However, as size reduction is critical to improve harvest efficiency, we will continue to test this rootstock. This potential pit-fall was taken into consideration in the proposed testing strategy (see below). As Iron, King and Garfield, had less fruiting spurs than GI6, crop load management on these small trees may be less challenging. Lincoln, Glen, and Kent, have vigor comparable or higher than GI6 (e.g. Kent); yet, they also appear to induce fewer spurs compared to GI6. However, due to potential over-cropping challenges with self-fertile cultivars we included them in the next testing phase as they exhibited other desirable attributes such as wider branch angles compared to GI6. Kent in particular appears to produce trees with excellent branch angles.

In 2007, there was a spring freeze in Michigan that effectively thinned the flowers. This gave us an opportunity to evaluate fruit size potential without the confounding influence of high crop loads. All the MSU rootstocks selected from the 2001 and 2002 plantings had mean fruit sizes equivalent to or larger than that for trees on GI6, except for Lincoln. However, as the fruit size for Lincoln was significantly greater than that for GI6 in 2006, this selection was still included but for more limited testing (see below). In particular, the fruit sizes from the trees on Garfield, Kent, and Iron have consistently been larger than the fruit from trees on GI6.

Four of the selected rootstocks were only planted in 2004; therefore, limited data is available on their performance. However, Crawford, Clinton, and Cass clearly impart excellent precocity to the scion. Clare does not promote as early fruiting as the other three selections; however, the trunk cross sectional area is extremely small suggesting that it may be a very dwarfing rootstock. Therefore, we will include Clare in more limited trials (see below). Crawford will also only be included in limited trials (see below) as the mean fruit size was less than that for Clinton and Cass, both of which exhibited excellent scion fruit size.

Liner increase and budding:

To move forward most efficiently and expeditiously with rootstock evaluation and eventual commercialization of any promising selections, it was important to assure that we could generate a sufficient number of uniform rootstock liners. In 2006 we vegetatively propagated the rootstocks in Michigan (Fig. 2); however, the number of liners obtained was still way below that needed for the rootstock trials in the PNW. Therefore, we explored the possibility of having the liners produced by a commercial company. We were able to reach an agreement with Duarte Nursery whereby they would produce liners and eventually trees for the rootstock testing phase. Duarte's Nursery has a tissue culture lab that puts each rootstock into shoot culture and therefore liner number can be ramped up as needed. In addition, they make their trees by budding onto liners that are in pots in the greenhouse, therefore providing very uniform plant material. In addition, having stock plants of the advanced rootstocks at Duarte's Nursery provides a "back-up" mother block to assure that we have access to virus-clean rootstock material that can be sent to any region of the U.S.

In discussions with John Duarte and Javier Castillon (Director of Research, Dry Creek Laboratory), all the 11 selections are doing very well in culture and they anticipate no problems in meeting the liner needs. This result is not surprising as Dry Creek Lab specializes in fruit micropropagation, including cherry, and the MSU selections were pre-selected for their rooting potential.

DNA fingerprinting:

Avoiding clonal mix-ups among different rootstock selections is extremely challenging as the rootstock plant material is below ground. Therefore in 2006 we developed and implemented a DNA fingerprinting strategy to "barcode" all 11 rootstock candidates and use this "barcode" to check clonal identity at critical stages.

We identified four PCR primer pairs that exhibited sufficient polymorphisms in fragment size to discriminate among the 11 rootstock candidates. These four primer pairs are PceGA59, PMS40, PMS67 and the *S*-locus RNase. For example, use of the *S*-locus RNase primer pair is able to distinguish among the majority of the rootstock candidates (Figure 3). With the addition of data from the other three primer pairs, the identity of each of the 11 rootstocks can be easily verified.

As Dry Creek Laboratory is currently increasing the rootstock liners to produce test trees, we requested and received culture tubes from Dry Creek Laboratory so we could do DNA fingerprinting to verify the identity of these cultures. No mix-ups were identified. We propose to continue this quality control fingerprinting at critical stages within the rootstock program, e.g. liner increase, budding, and even verification of planted trees using occasional sucker shoots. In addition, to be 100% certain about identity, we are fingerprinting the test trees of the 11 selections in the MSU-Clarksville plot. This is done using occasional sucker shoots. The MSU-Clarksville plot was purposefully never sprayed with Round-up so that we would have occasional suckers for rootstock verification.

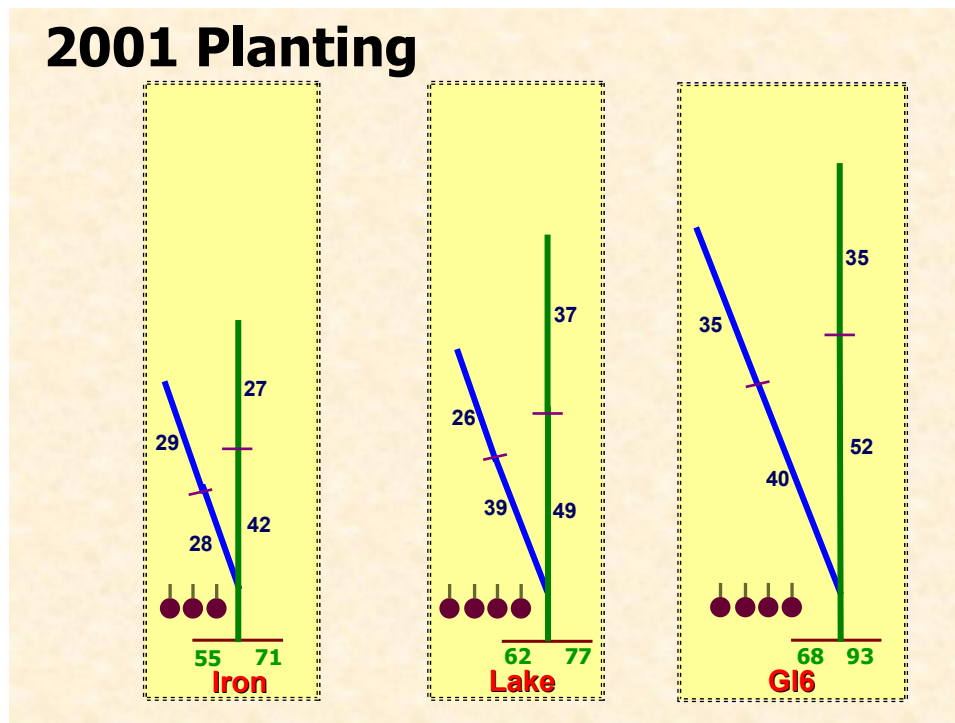
Preparations for the next test sites:

Four test plots are planned for the Pacific North West, one in Oregon and three in Washington. The Oregon plot will be at a grower location that will be determined this winter. The three Washington plots include the WSU Roza Farm in Prosser and sites in Manson and Mattawa to represent other environments within the state. There will be one site in Michigan, at MSU's Clarksville Horticultural Experiment Station that will be a much scaled down version of the sites in the PNW. Bing and Sweetheart were chosen as the two scions for the PNW sites. The growth habit of Bing is so well

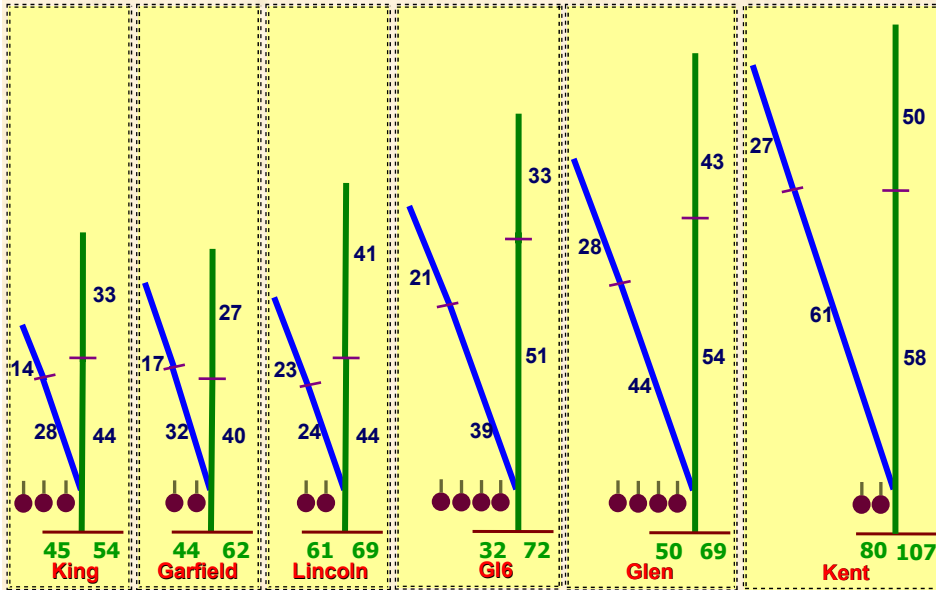
known, that this scion would be a good indicator of rootstock induced scion modifications. Sweetheart was included to represent those self-fertile high fruiting varieties, where reduced stature is required but not a higher spur/flower bud density. Rainier will be the scion for the MSU plot as this cultivar is important in the PNW, yet it is adapted to the Michigan climate.

Of the 11 rootstocks still under test, four are predicted to give very small trees so there is some concern about the fruit size potential, i.e. Clare, Crawford, Lake and Lincoln. These rootstocks may have promise for high density systems, but at this time we are only recommending that these are planted in more limited trials. As a result, these four will only be included in the trials at Prosser and MSU with Bing and Rainier, respectively, whereby the PI and Co-PI can provide the necessary management practices for these rootstocks. The remaining seven selections will be at all sites. Budding requests were communicated to Willow Drive Nursery and Duarte Nursery. In addition, the control trees of GI5, GI6 and mazzard have been ordered from commercial nurseries.

Figure 1. Schematic representation of the 11 MSU rootstocks candidates selected for advanced testing plus Gisela 6 control. The selected rootstocks are grouped by planting year, 2001, 2002 and 2004 and arranged in order of increasing trunk cross-sectional areas. The numbers at the bottom of each frame represent the trunk cross-sectional areas, calculated $\pi(r^2)$, with Fall of 2006 and 2007 values on the left and right, respectively. The numbers of cherries represent the relative predicted cropping potential. The vertical and angled lines represent two years of growth (cm) for terminal and lateral shoots, respectively.



2002 Planting



2004 Planting

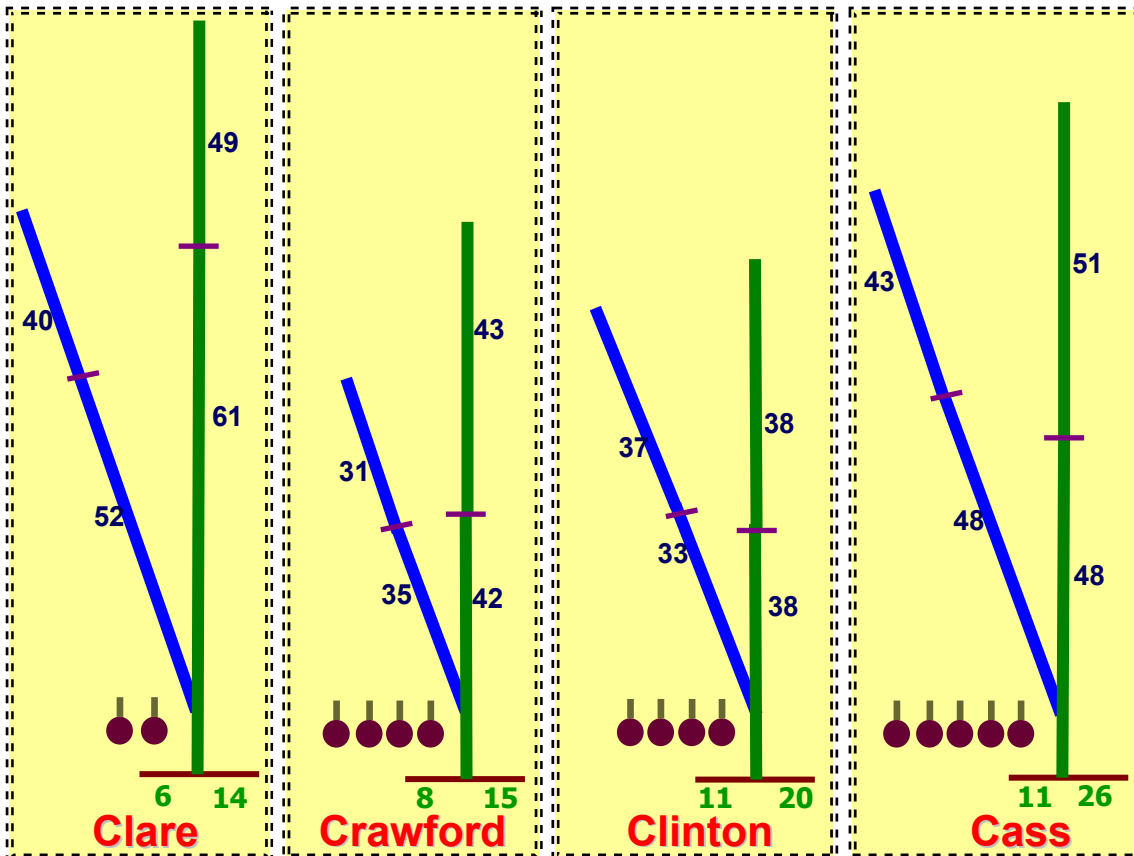


Figure 2. MSU cuttings propagated under mist in June 2006.



Figure 3. An example of one of the rootstock primer pairs that is used to differentiate the MSU rootstock selections. The primer pair used for this figure amplifies the *S-RNase* gene. The samples in the lanes are as follows: ML, size ladder; 1, Cass; 2, Clare; 3, Clinton; 4, Crawford; 5, Garfield; 6, Glen; 7, Iron; 8, Kent; 9, King; 10, Lake; 11, Lincoln; 12, Sherman; 13, GI5; 14, GI6.

