FINAL PROJECT REPORT

Project Title: Establishment of test plots for MSU sweet cherry rootstocks

PI: Amy Iezzoni
Organization: Mich. State Univ.
Telephone: (517) 355-5191 ext 1391
Email: iezzoni@msu.edu
Address: Dept. of Horticulture
Address 2: Mich. State Univ.
City: East Lansing
State/Zip: MI 48824

Co-PI(2): Matt Whiting
Organization: Wash. State Univ.
Telephone: (509) 786-9260
Email: mdwhiting@wsu.edu
Address: IAREC
Address 2: 24106 N. Bunn Rd.
City: Prosser
State/Zip: WA 99350

Co-PI(3): Todd Einhorn
Organization: Oregon State Univ.
Telephone: (541) 386-2030
Email: todd.einhort@oregonstate.edu
Address: OSU Mid-Columbia Expt. Sta.
Address 2: 3005 Experiment Station Dr.
City: Hood River
State/Zip: OR 97031-9512

Cooperators: Tom Auvil**,** Bryce Molesworth

Total project funding: $74,304

Other funding sources: None

WTFRC Collaborative expenses:

Item	2008	2009	2010
Stemilt RCA room rental			
Crew labor			
Shipping			
Supplies			
Travel			
Miscellaneous			
Plot costs	$ 0	$ 5,310[1]	$ 4,420[1]
Tree cost for PNW	$ 0	$ 7,209[2]	$ 14,070[2]
Total	$ 0	$ 12,519	$ 18,490

Footnotes:

[1]Plot cost for the sites in Mosier, OR and Chelan, WA. Plot costs are based on $6.50/tree for plot establishment in 2009 which covers site prep, fumigation and irrigation supplies; $3.50/tree in 2010 for planting, and first year general farming, water, taxes. A portion of the 2009 and 2010 plot expenses may be claimed in 2011 and have not been included in the 2011 budget request.

[2]The budget is based on 973 trees which includes the confirmed tree numbers for the Mosier and Chelan plots plus an additional projected 5% increase in tree numbers from sleeping eye budding done n fall of 2009. These funds will be used to pay the WDN tree cost in Spring 2011 and therefore this budget item is not included in the 2011 budget request.

Budget 1: Amy Iezzoni

Organization Name: Mich. State Univ. **Contract Administrator:** Lorri Busick
Telephone: (517) 355-5191 x 1363 **Email address:** busick@msu.edu

Item	2008	2009	2010
Salaries	$ 5,163	$ 5,317	$ 5,477
Benefits	2,411	2,553	2,689
Wages	500	500	500
Benefits			
Equipment			
Supplies	500	500	500
Travel	1,000	1,000	1,000
Misc. (tree freight)	500[1]		
Plot cost	1,000	1,000[2]	1,000 / 1,500[2]
Gisela liners		$1,200[3]	
Total	$ 11,074	$ 12,070	$ 11,666

Footnotes:
[1] This freight fee has been encumbered to cover the cost of tree delivery in 2010.
[2] The 2009 request is reduced and the 2010 request is increased as tree planting has been delayed until 2010.
[3]Total cost of the 750 Gisela liners @ $1.60 per liner (no royalty fee).

Budget 2: Matt Whiting
Organization Name: WSU - Prosser **Contract Administrator:** Mary Lou Bricker
Telephone: (509) 335-7667 **Email address:** mdeseros@wsu.edu

Item	2008	2009	2010
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot charges	$3,500[1]	0	$2,500[2]
Miscellaneous			
Total	$3,500[1]	0	$2,500[2]

Footnotes:
[1] Due to the delay in planting, no funds were expended in 2008. These funds have been encumbered and year 2 and 3 requests have been reduced by $3,500.
[2] This budget line was increased $1,000 due to increased expenses at the Roza farm that were not anticipated last year when J. Olmstead was the Washington project leader.

Budget 3: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541 737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	2008	2009	2010[1]
Salaries[1]			$1,500
Benefits[2]			$885
Wages			
Benefits			
Equipment			
Supplies			
Travel[3]			$100
Miscellaneous			
Total			$2,485

Footnotes

[1] Salary is calculated for 2 weeks of a Full Time Technician's salary, for oversight of planting, mapping, plant measurements, and data management.

[2] Benefits are calculated according to actual OPE rate of 59 %.

[3] Travel is based on a rate of 50.5 cents/mile, and includes visits to OR orchard site for data collection and grower support.

OBJECTIVES

Overall project objective: Identify dwarfing precocious rootstocks that increase the profitability of sweet cherry production in the PNW through the establishment of test plots.

Specific Objectives:
1. Evaluate the existing trees of the 10 rootstock candidates to determine if they continue to show commercial promise.
2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.
3. Plot establishment to include site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.

SIGNIFICANT FINDINGS AND ACTIVITIES

- Of the 11 MSU candidate rootstocks under evaluation at Clarksville, Mich., one showed graft incompatibility and was discontinued for future testing. All other ten MSU rootstocks that were planted in 2001 – 2004 are currently showing no signs of graft incompatibility.
- In general the MSU rootstocks candidates confer a lower flower bud density than Gi 6 when both spur number and flower buds per spur are considered together.
- In spring 2009 the first second test rootstock plot was planted at WSU-Prosser with trees grown at Willow Drive Nursery (WDN). All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to the large number of liners and excellent percentage bud take for this rootstock. Gi 5 and Gi 6 are the controls.
- TCSA measurements taken in September 2010 indicated that the majority of the MSU candidate rootstocks result in 'Bing' trees that are smaller than trees on Gi 6 with several rootstocks resulting in trees similar in size to trees on Gi 5.
- Based on discussions with the Advisory Committee in January 2009, 'Chelan' was added as a scion for the trials in the PNW, along with 'Bing and 'Sweetheart', because of its incompatibility with mahaleb rootstock.
- Trees for the other second test plots were produced at Duarte Nursery. Because of extreme difficulty with liner production for Iron, this candidate rootstock was not put forward for virus indexing (see below). Unfortunately reduced tree numbers resulting from budding in April 2009 required us to redesign our plots and eliminate a second grower cooperator site in Washington. The three sites to be planted in 2011 are Mosier, Ore., Manson, Wash. and Clarksville (MSU Research Station), Mich.
- Nine rootstock candidates were established at the National Clean Plant Network (formally NRSP-5) providing the source material for virus testing and the generation of certified plant material.
- DNA diagnostics conducted during critical stages of liner and tree production did not identify any clonal mix-ups.

RESULTS AND DISCUSSION

MSU-Clarksville - Rootstock performance of the originally grafted trees: Of the 11 MSU rootstock candidates planted at Clarksville, Mich. from 2001 to 2004, one rootstock (Crawford) showed signs of graft incompatibility and was discontinued from future testing. The other ten rootstocks are showing no signs of graft incompatibility with 'Hedelfingen', and in some cases 'Bing' scion, although continued testing is warranted. The vigor of seven of the MSU rootstocks could be directly compared to the vigor of Gi 6 (Table 1). Of these seven, four were less vigorous (Lake, Iron, Garfield, King), two were similar (Lincoln, Glenn) and one was significantly more vigorous (Kent). Although the vigor of Clare, Clinton and Cass could not be directly compared to that of Gi 6, all three

rootstocks appear to confer vigor less than Gi 6. This observation is supported by TCSA measurements for the Clare, Clinton, Cass and Gi 6 trees planted at WSU-Prosser Roza Experiment Station (Fig. 1).

In general the MSU rootstocks have a lower flower bud density than Gi 6 when both spur number and flower buds per spur are considered together (Table 1). Mean fruit size was variable, however as the trees were minimally pruned so that the natural growing habit could be evaluated, fruit size measurements may not be predictive of trees that are heavily pruned.

Table 1 Trunk cross-sectional area (TCSA), % size, total number of spurs, mean number of flower buds/spur, mean number of fruit/spur, mean fruit size, total shoot length of the Gi 6 control for the 10 MSU rootstock selections planted in Clarksville, MI. The scion is 'Hedelfingen'.

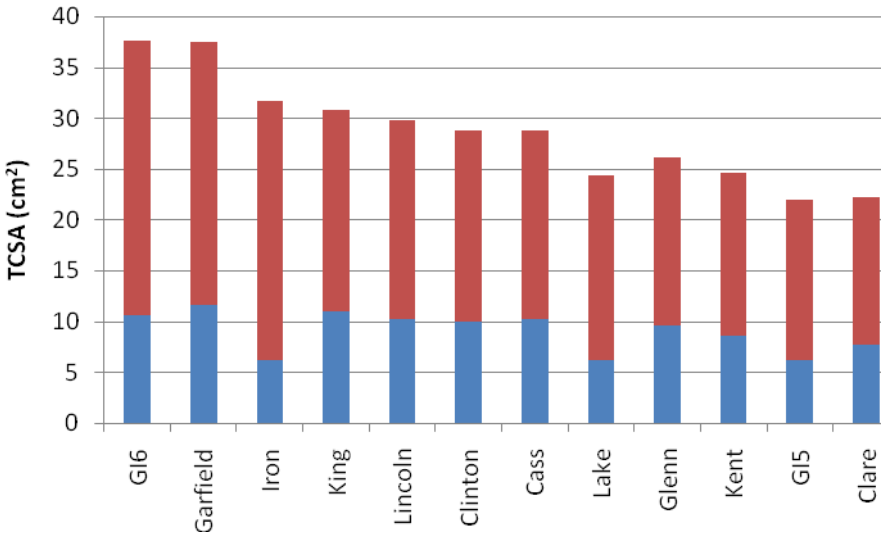
Rootstock	TCSA (cm^2)[1]	Vigor (% of Gi 6)[1]	Total no. of spurs[2,5]	Mean no. of flower buds[2,5]	Mean number of fruit/spur[3]	Mean fruit size (g)[3]	Total shoot length (cm)[1,6]
				2001[7]			
Lake	137	69	5	10	5.5b[4]	6.6	21
Iron	174	88	6	9	4.1a	7.2	24
Gi 6	198	100	7	15	5.4b	7.1	42
				2002			
Garfield	104	61	7	14	4.4a	5.7	35
King	123	71	6	16	5.0ab	6.3	27
Gi 6	171	100	8	15	6.3b	6.1	30
Lincoln	178	104	6	12	4.5a	7.1	26
Glenn	181	106	7	10	5.1ab	6.4	35
Kent	216	126	7	13	5.0ab	7.1	28
				2004			
Clare	58	-	9	16	4.9b	8.0	42
Clinton	47	-	8	23	2.0a	8.0	37
Cass	78	-	8	23	2.8a	7.5	45

[1] Data taken in 2010.
[2] Data taken from 2008 to 2010.
[3] Data taken in 2009.
[4]Means denoted by same letters within the column are not significantly different at $P < 0.05$.
[5]Data represents the number of spurs and flower buds on two branches for second and third year wood.
[6]Data represents the shoot length of two branches for third year wood.
[7]Year in which the rootstock selections were planted.

WSU – Roza Station Plot - Rootstock generation and plant performance: Liners of the MSU rootstock candidates were propagated at MSU in 2006 and planted at Willow Drive Nursery in spring of 2007 (Fig. 2A-C). The 276 trees (including Gi 5 and Gi 6 controls) from these test rootstocks were budded in fall of 2007 and were planted at the WSU-Prosser Roza Station in spring of 2009 (Fig. 2D). Due to unequal liner numbers, the rootstock candidates were represented in this plot by a minimum and maximum of 5 and 46 trees, respectively. All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to a large number of liners and excellent percentage bud take (90%). The trees were trained to 3-5 leaders evenly distributed and tied down if necessary (Fig. 2E, F).

TCSA measurements taken in September 2010 indicated that the majority of the MSU candidate rootstocks result in 'Bing' trees that are smaller than trees on Gi 6 with several rootstocks that are resulting in trees similar in size to trees on Gi 5 (Fig. 1).

Fig 1 Trunk cross-sectional area (TCSA; cm^2) of 'Bing' trees grafted on 10 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU-Prosser Roza Experiment Station. The top of the red bar indicates TCSA recorded on 24 September 2010. The blue bar is the TCSA measurement on 15 March 2010. Therefore the red indicates the TCSA increase in the 2010 growing season.



Duarte Nursery – Tree Production: In 2006, a collaboration was initiated with Duarte Nursery to produce the MSU rootstock liners and trees needed to establish additional second test trials. At the time, it was necessary to increase the liners in tissue culture due to limited plant material. Secondly, having the liners produced at a second location outside of Michigan would assure that any unexpected quarantine on *Prunus* from Michigan would not cripple this project. Plant material of the MSU rootstock selections was sent to Duarte Nursery, established in tissue culture, and liners were grown in their greenhouses (Fig. 3 A-C). Liners of Gi 5, Gi 6, and Gi 12 were purchased from ProTree and sent to Duarte Nursery so that these control trees could be grown using similar horticultural practices as the test trees. Due to unfortunate delays in liner production, budding was delayed until April 2009. In January 2010, A. Iezzoni and T. Auvil visited Duarte Nursery to assess the tree numbers and tree growth. Unfortunately, the trees grown in the Duarte 'pot culture' system were too small to be field planted in spring 2010 (Fig. 3D). Therefore Willow Drive Nursery (WDN) generously agreed to grow out these trees for the 2010 growing season so that the rootstock plots could be established in spring 2011 with trees of excellent quality. In January 2010, the plant material from WDN (including budded trees and extra unbudded liners) were trucked to WDN and stored in their cooler (Fig. 3E). The plant material was planted in the nursery in spring of 2010 (Fig. 3F).

Tree counts made in July 2010 at WDN confirmed that the final tree numbers were only sufficient for the establishment of two large plantings in the PNW (Mosier, Ore. and Manson, Wash.) and one smaller planting in Clarksville, Mich (Table 2). This lower than anticipated tree number was due to poor bud take. A low percentage bud take was also observed for the controls (e.g. mazzard, Gi5, Gi6, and Gi12) indicating that there was not an inherent problem with bud take that was unique to the MSU rootstock candidates.

Table 2. Plant material propagated at Duarte Nursery in April 2009 for the MSU rootstock trials to be planted in spring of 2011.

Location	Scions utilized[1]	Maximum no. of replications	Total Tree No.[2]
Manson WA	'Bing', 'Sweetheart' Chelan', & 'Rainier'	5 reps of 5 trees each	513
Mosier OR	'Bing' & 'Chelan'	5 reps of 5 trees each	371
Clarksville MI	'Rainier'	2 reps of 5 trees each	102

[1]Underline indicates the scion(s) used for comparisons with the other scions included as pollinators and guard trees.

[2]This number includes the addition of the following rootstock controls: Gi 5, Gi 6, Gi 12 and mazzard, plus guard row trees.

Rootstock genetic check & virus indexing: To avoid any future intellectual property and identification issues, fingerprints of the rootstock selections were developed using molecular markers. With the combination of a primer set specific for the self-incompatibility *S-RNase* locus and three SSR markers (PceGA59, PMS40, and PMS67), all ten rootstocks can conclusively be differentiated from each other and Gi5 and Gi6 (Fig. 4A). Fingerprints done on plant material increased at Duarte Nursery did not uncover any clonal mix-ups. Genetic checks were also done on the plant material received from Duarte Nursery and planted in WDN in 2010. As rootstock tissue for DNA diagnostic tests could not be obtained from the grafted trees, leaves were collected in July by A. Iezzoni from a random sample of unbudded liners. I reasoned that if the unbudded liners were labeled correctly, that the liners that were used to produce the grafted trees were also correct. Once again no clonal mix-up was identified (Fig. 4B).

If any of the MSU rootstocks are of sufficient interest to warrant the generation of commercial plant material by nurseries, it would be necessary to have certified plant material available. As this process can frequently be a bottleneck in rootstock commercialization, the initial steps in this certification process were undertaken for nine of the 10 rootstock selections (Fig. 5). Iron was not included in the indexing as it was extremely difficult to generate liners from this selection. For the remaining nine rootstock selections, dormant liners were taken by A. Iezzoni from the plant material in the WDN cooler and planted at the National Clean Plant Network (NCPN, Prosser, Wash.) in April 2010. Some of these liners had sleeping eyes that were removed prior to planting at the NCPN. All 9 rootstocks were tested for four viruses (PDV, PNRSV, CLRV and PPV). Three of the selections tested positive to PDV. We suspect that the inserted sleeping eye bud that we removed prior to planting may have carried this virus. The other six selections tested negative for the four viruses. As these six selections are now poised to enter the testing required for certification, leaf samples were taken in July 2010 and diagnostic DNA tests were conducted. These rootstocks were determined to be true-to-type based on the results from four DNA tests (Fig. 4C). Budwood of the remaining three rootstock candidates will be shipped to the NPCN from the mother block in Clarksville, Mich. in December 2010.

EXECUTIVE SUMMARY

The long term objective of this project is to identify dwarfing precocious rootstocks that increase the profitability of sweet cherry production in the PNW through the establishment of test plots. At the start of this three year period, 11 MSU rootstocks were under consideration; however, one was subsequently eliminated due to graph incompatibility and a second one is of less interest due to extremely poor propagation performance. The remaining nine MSU rootstock candidates confer a range of tree sizes with the smallest trees having TCSA that are ~ 60% the size of trees on Gi 6. In 2009, one rootstock test plot was established at WSU-Prosser Roza Station. The trees are performing well and data on TCSA indicates that the trees will exhibit a range in vigor. Liners and trees for the other test plots were produced at Duarte Nursery. Due to delays in liner production and poor bud take (poor bud take was also exhibited by the control rootstocks), tree planting was unfortunately delayed until 2011. Reduced tree numbers resulting from budding in April 2009 also required us to redesign our plots and eliminate a second grower cooperator site in Washington. The three sites to be planted in 2011 are two large plots with one in Mosier, Ore. and the second in Manson, Wash., and a smaller plot in Clarksville (MSU Research Station), Mich. As problems with the availability of virus certified genetically correct plant material can cause a bottle neck in the potential commercialization of a rootstock candidate, we initiated the process of establishing the MSU rootstock candidates at the National Clean Plant Network (formally NRSP-5) so they can be available for virus screening. DNA diagnostics conducted during critical stages of this project did not identify any clonal mix-ups.

Fig. 2. Flow Diagram of Activities
Plot of MSU Rootstock candidates at the WSU-Prosser Roza Station.

2006 Liners were propagated from softwood cuttings at MSU (Fig. 2A).

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2007 Liners were shipped from Michigan and planted at WDN for August budding (Fig. 2B).

↓

2008 Trees grew at Willow Drive Nursery (Fig 2C).

↓

2009 Trees were planted and trained at WSU – Prosser, Roza Station (Fig. 2D).

↓

2010 Tree Training, spring (Fig. 2E) and tree growth, summer (Fig. 2F).

Fig. 3. Flow Diagram of Activities
Generation of liners and budded trees for the MSU rootstock plots to be planted in Mosier, Ore., Manson, Wash, and Clarksville, Mich. in 2011.

2006 Fall	Budwood of the MSU rootstock liners was sent to Duarte Nursery for budding on mahaleb rootstock.
2007	Duarte Nursery – Meristem cultures were established using shoot tips from the MSU selections (Fig. 3A).
2007 – 2009 May	Duarte Nursery – Generation of MSU rootstock liners (Fig. 3B,C). MSU - DNA diagnostic fingerprinting was carried out at various times during the propagation process (Fig. 4A).
2009 April	Duarte Nursery – The liners were budded
2010 Jan	The trees and extra unbudded liners were prepared for shipment from Duarte Nursery to Willow Drive Nursery (Fig. 3D). The plants were transported from Duarte Nursery to Willow Drive Nursery.
2010 Jan-Apr	Willow Drive Nursery – the plant material was stored in the cooler (Fig. 3E). Willow Drive Nursery – the budded trees and unbudded liners were planted in the nursery (Fig. 3F). DNA diagnostic fingerprinting was conducted for a random sample of unbudded liners planted at Willow Drive Nursery (Fig. 4B).
2010 July	Willow Drive Nursery – final tree counts were taken and used to design plots for 2011 planting.

Fig. 3 (cont.)

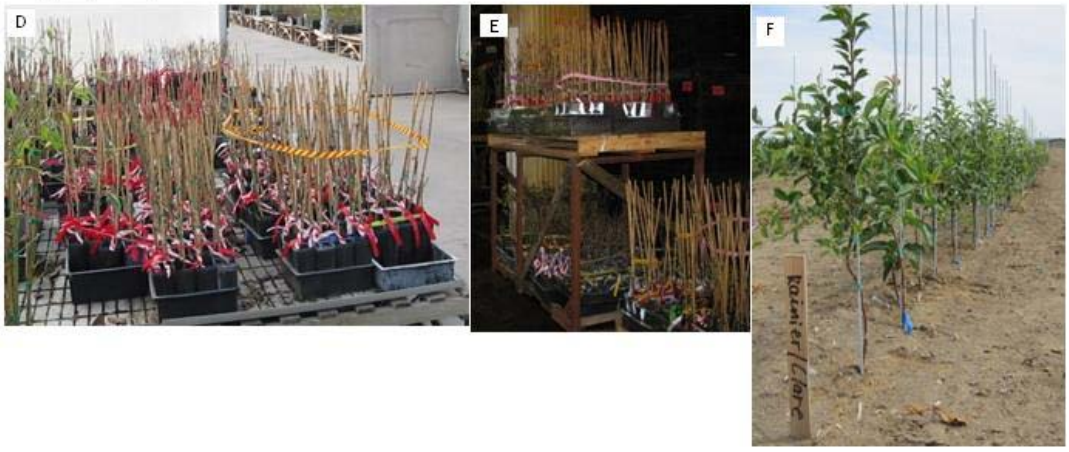

Fig. 4: DNA diagnostics illustrating the use of the S-allele primer pair: (A) Marker polymorphisms for the rootstock selections, (B) Fingerprints from the plant material at WDN, (C) Fingerprinting of the plant material at NCPN.

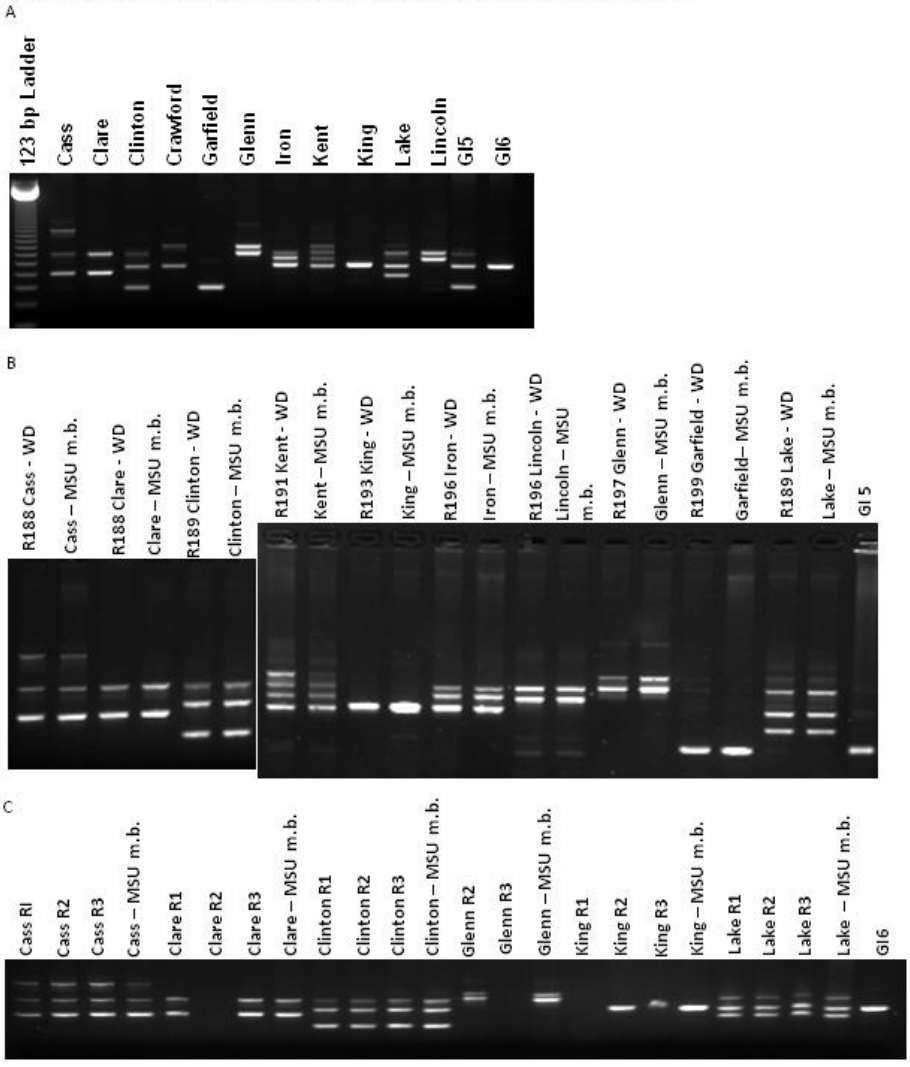

Fig. 5. Flow Diagram of Activities
Virus testing and confirmation

2010 Apr	Three rootstocks per selection were taken from the supply at Willow Drive Nursery and planted at the National Clean Plant Network (NCPN) Greenhouse in collaboration with Bill Howell (see image below).

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2010 Summer	Six of the nine MSU rootstocks tested free of PNV, PNRSV and PPV

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2010 Summer	DNA diagnostic fingerprinting confirmed that these six rootstocks are true to type (Fig. 4B).

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2010 Summer	A Michigan Department of Agriculture Permit was obtained for the shipment of budwood of the three candidate rootstocks that tested virus positive from the Mother Block at MSU's Clarksville Station to the NCPN in Dec. 2010.