Research Funding for 2000 and Research Progress Reports for 1999

The IDFTA Rootstock Research Committee evaluated 21 proposals, requesting a total of $112,550 in funding. Unfortunately, only $60,000 was available for distribution. The research funding comes from three sources: 1) IDFTA rootstock research fund provided from conference proceeds, membership fees and gifts to IDFTA; 2) from tree fruit rootstock nurseries, Research Partners, raised from a one-half cent assessment on each apple rootstock liner sold in North America and 3) from Gisela Inc. The following proposals were funded for 2000.

REGENERATION OF M.9

Project Leader: Herb Aldwinckle and Jay Norelli

1) Development of a regeneration medium for M.9 apple rootstock: M.9 apple rootstock regenerates poorly on media that were developed for the regeneration of other apple scion and rootstock cultivars. Previous research had established that the regeneration of M.9 was greatly improved on a medium containing 1/2 strength Murashige and Skoog (MS) basal salts rather than full strength MS or McCown's Woody Plants basal salts, 3% sorbitol rather than 3% sucrose, and the cytokinin 1-phenyl-3-(1,2,3-thiadiazol-5yl) urea (TDZ) rather than 6-benzylaminopurine (BAP). To determine the optimal growth regulator concentrations for M.9 regeneration TDZ at 0.2, 0.1, and 0.05 mg/L; indole-3-butyric acid (IBA) at 0.3, 0.1, and 0.05 mg/L; and gibberellic acid (GA3) at 0.0 and 1.0 mg/L were tested in factorial combinations. The lesser TDZ concentrations of 0.1 or 0.05 mg/L resulted in a greater number of meristems per leaf piece than 0.2 mg/L. There was a significant interaction between IBA and GA3 concentration with better results obtained when the greatest concentration of IBA was used in combination with GA3 or the lesser concentrations of IBA were used without GA3. The best combination of growth regulators for M.9 regeneration was 0.05 mg TDZ, 0.05 mg IBA, and 0.0 mg GA3/L resulting in 92% of the leaf pieces regenerating and 2.7 meristem initials forming per leaf piece.

RESEARCH FUNDING FOR 2000

Project Leader	Project Title	Funding approved for 2000
Aldwinckle, H and J. Norelli	Regeneration of M.9	$4,000
Barritt, B. and B. Johnson	Early intermediate level testing of new CG. apple rootstocks in the Pacific Northwest	$6,000
Hoying, S.A.,T.L. Robinson and I. Merwin	Assessing the response of G.16 to replant disease and preplant soil amendments	$2,000
Johnson, W.C., J.L. Norelli, H.T. Holleran and H.S. Aldwinckle	Differential virulence of fire blight strains on apple rootstocks	$1,500
Kappel, F.	Sweet cherry rootstock evaluation	$7,000*
Marini, R. (for NC-140 committee)	NC-140 data summarization	$15,000
Neilsen, D.	Nutrient and water management in high-density sweet cherry	$5,000*
Reighard, G.	NC-140 peach rootstock trial for 2001	$2,000* $1,500
Robinson, T. and NC-140 Technical Committee	National evaluation of the new Cornell-Geneva rootstocks and other promising rootstocks from around the world	$10,000
Robinson, T.L., R. Andersen and S. Hoying	High-density orchard planting systems for sweet cherry in the Northeast	$6,000*
	Total	$60,000

*Funded from donation of $20,000 from Gisela®, Inc.

2) Effect of shorter TDZ exposure period on meristem formation and survival: Although addition of TDZ to regeneration medium resulted in significant improvement in meristem formation when compared to media containing BAP, meristem initials formed on TDZ media failed to develop normally and higher rates of survival were observed for meristems derived from BAP containing media. In an attempt to obtain a high rate of regeneration and improve survival of meristem initials, leaf pieces were first placed on TDZ medium to initiate regeneration and then transferred to BAP media after 2 week (meristem initials normally form 4 to 9 week after transfer of leaf pieces to regeneration medium).

Although recovery of meristems on BAP medium was improved after 9 week when leaf pieces were first placed on TDZ medium to initiate regeneration, these meristems failed to develop any more normally than those obtained from leaf pieces that were on TDZ media for the entire 9 week period.

EFFECT OF SHORTER TDZ EXPOSURE

Initial medium	2 wk transfer medium	% leaves with meristem 4 wk	9 wk
TDZ	no transfer	42	39
TDZ	TDZ	37	36
BAP	no transfer	3	2
TQZ	BAP	3	29

3) Effect of BAP concentration, presence of GA3 and presence of casein hydrolysate in "recovery" of M.9 meristems on growth media: Previous research had established that the growth and multiplication of M.9 in tissue culture was superior on a growth medium containing McCown's Woody Plants basal salts, Gamborg's B5 vitamins, 3% sucrose, 2.5 mg/L BAP and 0.3 mg/L IBA than on M.26 medium containing MS salts and vitamins, 3% sucrose, 1.0 mg/L BAP, 0.3 mg/L IBA, and 0.5 mg/L GA3.

However, M.9 meristems obtained from regeneration media containing TDZ died when transferred to M.9 growth medium. The addition of 0.4 g/L casein hydrolysate to M.9 growth media significantly improved the survival of meristems. In contrast, reducing the BAP concentration significantly reduced the survival of meristems (14%, 0%, and 2% survival for all media combinations containing 2.5, 1.0, and 0.5 mg/L BAP, respectively).

The addition of 0.5 mg/L GA3 to the media had no significant effect on survival but resulted in a numerical improvement in % survival. For growth media containing 2.5 mg/L BAP the survival of meristems on media containing both casein and GA3, only casein, or neither casein nor GA3 was 33%, 23% and 0%, respectively.

ASSESSING THE RESPONSE OF G.16 TO REPLANT DISEASE AND PREPLANT SOIL AMENDMENTS

Project Leaders: Stephen A. Hoying, Terence L. Robinson and Ian A. Merwin

Apple Replant Disease (ARD) is an important factor limiting the success of many replanted apple orchards. It can cause slow growth, delayed cropping, and reduced yields through the life of the orchard. Often the negative effects of replant disease are worse with dwarfing rootstocks such as M.9 and M.26 than more vigorous stocks. New dwarfing rootstocks may offer some tolerance to replant disease. A new competitor to M.9 and M.26 from the Cornell/Geneva rootstock breeding program is G.16. This is the smallest of the currently released CG stocks and should allow for tree densities ranging from 500-900 trees/acre. This project is designed to study the response of G.16 and M.9 to replant disease and to a variety of preplant soil treatments to control replant disease.

A replant site (Don Ophardt Farm in Hilton, NY) was prepared in 1998 and planted in 1999. The previous orchard was approximately 20 years old when removed in 1997. The soil is a sandy loam and interpretation of the Cornell soil tests indicated the need for additions of Phosphorous, Potassium, Calcium, Magnesium, Boron, Zinc, Manganese and Copper. In addition, Xiphinema sp. nematodes were present (116 dagger nematodes per 100 cc of soil).

The preplant treatments were: 1) Untreated; 2) Lime and fertilizer amendments based on standard Cornell recommendations from soil test values; 3) Soil fumigation with 100 gallons of Vapam/acre; 4) Soil Fumigation by injection with 40 gallons of Telone C-17/acre; 5) Preplant cover crops of Brassica seeded in June, tilled under and followed by Sudan grass in late July, tilled down in September; 6) Lime/fertilizer amendments plus treatment with 100 gallons of Vapam/acre in September; 7) Lime/fertilizer treatment plus the preplant Brassica/Sudan cover crops; 8) Soil application of DiTerra (biologically derived nematicide).

In April of 1999, Gala trees on G.16 and on M.9 were planted in the treated plots (4 G.16 trees and 4 M.9 trees per plot). Growth was assessed in the first year and growth and productivity will be assessed annually for the next 5 years.

Results from the first year show that growth of trees on M.9 was considerably improved by several of the preplant soil treatments. The least effective treatment was lime and fertilizer amendments alone or the Telone C-17. The most effective treatments were Vapam fumigation, DiTerra, and the Brassica/Sudan treatment. The combination of lime and fertilizer with either the Brassica/Sudan treatment or Vapam was intermediate.

Across all treatments the trees on G.16 grew more than those on M.9. In addition, there was little difference between the untreated trees on G.16 and any of the preplant treatments. Thus, G.16 appears to have some tolerance to replant disease. With G.16 the Telone C-17 treatment gave the best response.

The preliminary results of this project show that G. 16 grows well in unfumigated soil. It may have some tolerance to replant disease. If these results hold up over the next few years, G.16 may allow growers to avoid the expense of fumigation.

NC-140 DATA SUMMARIZATION

Project Leader: Richard Marini

Last year's funds were used by coordinators of 9 rootstock plantings to summarize data from the 1998 growing season. Annual reports for each planting, including means with statistical analyses, were presented to the NC-140 Technical Committee in Biglerville, PA, in November 1999. Additionally, manuscripts were prepared to summarize the first 5 years of the dwarf and semi-dwarf Gala plantings established in 1994. These papers will be submitted for publication in *Fruit Varieties Journal*. Below are a few of the most important results presented in the 1999 summary reports.

1. After 5 years in the 1994 peach rootstock planting, Ishatara and Chui Lum Tao seem least vigorous and in 1998 the most productive stocks were Stark's Redleaf, Montclar and GF.305. In 1998 trees on Chui Lum Tao and Tzim Pee Tao bloomed about 2 days later than trees on Lovell. After 5 years, no peach rootstock is performing better than Lovell.
2. In the 1990 cultivar/rootstock planting there is not a strong interaction between cultivar and rootstock, so results from rootstock trials with one

cultivar can likely be extrapolated to other cultivars not in the trial. Average over all cultivars, tree size was greatest for M.26 EMLA, followed by O.3, M.9 EMLA, B.9 and Mark. Cumulative yield efficiency was greatest for B.9 and Mark and lowest for M.26 EMLA. During the winter, data for this 10-year experiment will be summarized for publication.

3. In the 1990 systems trial, cumulative yields are still greatest for the vertical axe, but during the last two years yields were similar for the central leader and vertical axe trees. It seems that vertical axe has the highest early production but, as central leader trees fill their space, yields are similar for central leader and vertical axe trees. During the next year, data for the 10 years will be summarized for publication.
4. The M.9 clones in the 1994 trial are quite different. RN29 and Pajam 2 are nearly as large as M.26 EMLA. NAKB T337 and Fleuren 56 are the smallest of the M.9 clones. Rootstock significantly influenced yield efficiency at 19 of 25 locations. Yield efficiency was highest for P.16 in the most dwarfing size class, and O.3, MARK and M.9 NAKB T337 in the intermediate size class. M.26 EMLA had lower yield efficiency than V.1, M.9 RN29 and M.9 Pajam 2. Results from the first 5 years have been summarized and the manuscript is being reviewed.
5. In the 1994 semi-dwarf trial, four rootstocks (M.26 EMLA, P.1, V.2 and G.30) are being compared. Tree losses were greatest for G.30 and M.26 EMLA. P.1 produced the largest trees and G.30 produced the smallest trees. Yield and yield efficiency tended to be highest for G.30 and lowest for P.1. Results from the first 5 years have been summarized and the manuscript is being reviewed.

NATIONAL EVALUATION OF THE NEW CORNELL-GENEVA ROOTSTOCKS AND OTHER PROMISING ROOTSTOCKS FROM AROUND THE WORLD

Project Leaders: Terence Robinson and NC-140 Technical Committee

The new series of Cornell-Geneva rootstocks have the potential to replace existing rootstocks because they have resistance to fire blight and *phytophthora* root rot. Four stocks have now been released (G.16, G.30, G.65 and G.11) and are being commercialized. About a dozen more elite selections are in the pipeline. As these new stocks become available to fruit growers, orchard tests in several climatic areas on a variety of soils are needed. We have established a series of trials within NY state and nationally through NC-140 to further evaluate their commercial potential. A new NC-140 trial was planted in 1999 and second trial is planned for 2002 with both new CG stocks and other promising stocks from around the world. Data from these trials will give growers unbiased information about the potential not only of the CG stocks, but also for the Supporter, Morioka and JTE stocks. A short summary of several of our rootstock plots follows:

NY 1990 Gala Rootstock Trial: This trial completed 10 years in 1999. The highest cumulative yield was with O.3 followed by M.9, B.9, M.26, and Mac.39, respectively. The highest cumulative yield efficiencies were with M.27, Mark, M.9, and Mac.39. B.9 had significantly lower cumulative yield efficiency than M.9. M.26 and P.1 showed the lowest yield efficiency. At the conclusion of this trial we conclude that M.9 is the best dwarfing stock in terms of production. However, the excellent performance of B.9 and O.3 coupled with their reported winter hardiness lead us to recommend the latter two stocks especially in the colder climates of NY.

NY 1992/1993 CG-Liberty Rootstock Trial: The 1992 CG rootstock plot had a very heavy crop in 1999 with significant differences among stocks. Among dwarf stocks, CG.6737, CG.3029, and G.11 continued to have the highest cumulative yield efficiency and also had good average fruit size. They all exceeded the performance of M.9. G.65 had significantly lower cumulative yield efficiency than CG.11 and also had significantly smaller fruit size. Among semi-dwarf stocks, G.30, CG.6210, CG.2a, CG.67, CG.222, CG.6143, CG.517 were top performers. They exceeded the performance of M.7. Among vigorous stocks CG.8189 and CG.6239 were top performers. These stocks exceeded the performance of MM.111.

The 1993 plot also had heavy crop this year. Among dwarf stocks, the highest yield efficiencies were with CG.26, CG.4247, CG.3041, CG.3902, CG.4003, CG.38 and CG.5046. All performed significantly better than M.9. Among this group CG.3041 has been tested on several growers' farms where it has been a top performer in the dwarf class. Among the semi-dwarf stocks top performers were G.30, CG.6874, CG.5012 CG.6210 and CG.7760. All performed significantly better than M.7. Among vigorous stocks CG.756, CG.6239, CG.6253, CG.5156, CG.6723, and CG.8189 were top performers. These stocks exceeded the performance of MM.111.

NY 1994 Apple Rootstock Trial: Trees in this trial had an excellent crop in 1999. The greatest cumulative yield efficiency was with B.491 followed by Mark, P.16, P.22 and M.9T337. All of the other M.9 clones had lower efficiency as well as B.9 and O.3. There were significant differences in tree size among M.9 clones.

The smallest clone was M.9 Fleuren 56 followed by M.9T337. The M.9 EMLA clone was intermediate while the Pajam l, Pajam 2 and RN29 clones were the most vigorous. The three most vigorous clones were similar in size to M.26. The lowest yield efficiency was with V.1 and P.2.

In the semi-dwarf plot, G.30 had the highest yield efficiency followed by V.2. M.26 was third while P.1 was lowest. G.30 was the smallest tree and was significantly smaller than V.2.

NY 1998 NC-140 Gala-Jonagold/G.16 Trial: Three plantings of G.16 were established in NY in 1998 at the Experiment Station, in the Hudson Valley and in the Champlain Valley. These trees have grown very well. With both Gala and Jonagold the G.16 trees have grown significantly larger than M.9 trees or CG.3041 in the case of Jonagold.

If this result continues it appears that G.16 may be more similar in tree size to M.26 than M.9. G.16 had the greatest flowering and significantly greater flowering than M.9 in the Gala trial. CG.3041 was very similar in size to M.9.

NC-140 1999 NC-140 Mac-Fuji Rootstock Trial: The 1999 NC-140 trial was planted in 19 states and included G.16, CG.41, CG.13, CG.179, CG.202, CG.935, G.30, CG.814, CG.210, CG.707, CG.8, Supporter 1, Supporter 2, Supporter 3, Supporter 4, M.9, M.26 and M.7.

The colder climate sites (9 sites) used McIntosh as the scion while the warmer climate sites (10 sites) used Fuji as the scion. This is the first national trial of many CG stocks and the 4 Supporter stocks. Three plantings were established in NY at the Geneva Experiment Station, in Wayne County and in the Champlain Valley. These trees have grown very well.

The planting at the Experiment Station will be used for field inoculation with fire blight to test the field tolerance of the Supporter rootstocks to this disease. IDFTA funds paid for the shipping costs of these trees and made possible the production of the trees.

Conclusions

Based on our rootstock and systems work we continue to recommend M.9, B.9, and M.26 as the preferred rootstocks for NY. O.3 rootstock is also recommended but is not readily available. Since O.3 is more vigorous than M.9, it should be especially useful in low vigor sites and in cooler climates. Trees on G.16 are recommended only for trial since long-term trials are not yet complete and its ultimate tree size is unclear at this time. It may be closer to M.26 in size than M.9.

Within M.9 clones the more vigorous clones (Pajam 2 or RN29) which are very similar to M.26 in size should be used in weaker soils or with weak scions while the weaker scions should be used in virgin ground or with vigorous scions. Both M.9 EMLA and M.9T337 are intermediate in size and similar in performance. There does not seem to be any justification for choosing one over the other. B.9 and O.3 are specifically recommended over M.9 for the cold climate areas of New York.

Among semi-dwarf stocks, G.30 which is M.7 size continues to perform much better than M.7 and in some cases better than M.26. Its problems are that it is difficult to produce in the stool bed due to spines and the graft union is more brittle than M.7, especially with Gala. **Thus G.30 will have to be supported with a post and wire system in all orchards.** Despite its problems, G.30's yield performance is spectacular and is recommended for planting in NY.

HIGH-DENSITY ORCHARD PLANTING SYSTEMS FOR SWEET CHERRY IN THE NORTHEAST

Project Leaders: Terence L. Robinson, Robert Andersen and Steve Hoying

Sweet cherries offer an opportunity for diversification for many apple growers in the northeastern U.S. However, the production difficulties of rain cracking, large trees, non-precocious rootstocks and relatively soft small-fruited cultivars have limited the extent of new plantings. The introduction of dwarfing cherry rootstocks and newer varieties has allowed new possibilities for developing high-density cherry orchards with smaller trees that will be more precocious and productive and can either be covered with rain exclusion shelters or treated with $CaCl_2$ to prevent rain cracking. New varieties offer the possibility of firmer larger cherries. This project is designed to help growers successfully make the transition to high-density cherry orchards.

In 1999, we established a replicated cherry systems trial at Geneva, NY, with 3 cultivars (Hedelfingen, Lapins and Sweetheart) and 2 rootstocks (Gi.6 and MXM.2.) The purpose of this trial is to compare high-density training systems that utilize precocious rootstocks and new pruning and training strategies. We chose to compare 6 systems (Modified Central Leader, Vogel Slender Spindle, Spanish Bush, Free Standing V, Zahn Vertical Axis, and Marchant trellis). Spacings for each rootstock and training system are:

All trees were planted on 12 inch high berms to control winter damage associated with excessive soil moisture. In addition, a subsurface tile line was installed in the center of each tractor alley to remove excess moisture in the spring and during heavy rainfall before harvest. We will determine if the raised berms and tile result in less rain cracking than orchards planted on level ground.

First year training points of the Central Leader system were:

- head leader at 36 inches.
- remove large diameter feathers.
- remove buds below the new leader bud along 8 inches of the leader.
- attach clothespins to lateral branches when 4 inches long to improve crotch angle.

First year training points of the Spanish Bush system were:

- head leader at 15 inches.
- attach clothespins to lateral branches when 4 inches long to improve crotch angle.
- head each lateral shoot in early July to multiply number of shoots.
- fertilize trees with nitrogen in early July to force new growth.
- seed cover crop of rye in early August to use up excess nitrogen to prevent winter injury.

First year training points of the Vogel Slender Spindle system were:

- head leader at 36 inches.
- remove all feathers.
- remove buds below the new leader bud along 8 inches of the leader.
- attach clothespins to lateral branches when 4 inches long to improve crotch angle.
- attach weighted clothespins to the ends of lateral branches to maintain horizontal branch angle.

First year training points of the Zahn Vertical Axis system were:

- head leader at 48 inches.
- remove large diameter feathers (larger than 2/3 diameter of leader).
- use bud removal on leader to stimulate remaining buds to grow (remove 2 buds and leave 1 bud along the entire length of the leader).
- attach clothespins to lateral branches when 4 inches long to improve crotch angle.

First year training points of the Free Standing V system were:

- head leader at 12 inches.
- attach clothespins to 2 lateral branches that are oriented toward the tractor alleys when 4 inches long to improve crotch angle.
- keep central leader shoot for first year but suppress growth with pinching in mid-summer.

First year training points of the Marchant system were:

- plant trees at 45˚ angle down the row.
- head leader at 40 inches.
- remove all side branches.
- remove buds on underside of the leader.
- thin remaining buds to an 8-inch spacing.
- train leader to a 60˚ angle along the row using a 4-wire trellis and a bamboo pole at each tree.

HIGH-DENSITY ORCHARD PLANTING SYSTEMS FOR SWEET CHERRY

Rootstock	System	Spacing (ft.)
MXM 2	Modified Central Leader	16 x 20
	Spanish Bush	10 x 16
	Vogel Slender Spindle	8 x 15
	Free Standing V	6 x18
	Zahn Vertical Axis	6 x 15
	Marchant Trellis	8 x 13
Gi.6	Modified Central Leader	16 x 20
	Spanish Bush	10 x 16
	Vogel Slender Spindle	8 x 15
	Free Standing V	6 x18
	Zahn Vertical Axis	6 x 15
	Marchant Trellis	8 x 13

A second objective of this experiment is to study the feasibility and practicality of rain crack control methods. In the year 2000 we will construct a rain exclusion shelter over one of the three replicates. On the second replicate we will establish a $CaCl_2$ sprinkler system and the third replicate will be left unprotected.

RELATIVE TOLERANCE OF APPLE ROOTSTOCK GENOTYPES TO *PHYTOPHTHORA* SPP. ROOT ROTS

Project Leader: W.C. Johnson*, H.T. Holleran, H.S. Aldwinckle, W.F. Wilcox, M.-H. Simard

1. Geneva direct inoculation screens: In late March and again in late April, we planted 20 liners for each of 26 rootstock genotypes in containers with the sand/vermiculite planting mix. Genotypes assayed included G.11, G.16, G.30, G.65, 5046, 5179, 6210, 7707, 5757, 4814, 6874, 5890, 5935, M.26, M.9 EMLA, and MM.106. Plants were grown vigorously for 7 weeks, then transplanted to larger pots containing the same soil mix with (treatment) or without (control) added inoculum (*P. cactorum* in first test, *P. cryptogea* in the second test). Pots were then placed in a newly constructed table designed for controlled flooding. Pots were then flooded to a level even with the soil surface for 72 hours each week for 5 or 6 weeks. In both tests we included an extra replicate of G.16 and M.9 EMLA to determine if similar results could be obtained in a standard potting mixture, which would decrease the level of transplant shock.

Results: Growth of treatment and control plants was disappointingly similar, there were no visually observable differences. We have attributed our lack of results to an unusually hot summer, and the lack of adequate temperature controls in our greenhouses apparently led to inactivation of the inoculum in the soil before infection could be established. As a result, we have retained the plants in the dormant state over winter, and have delayed the planned 2000 screening by 1 year.

We plan to move the plants back to the flood tables in the spring, prepare new inoculum, and resume the experiment in April (*P. cactorum*) and May (*P. cryptogea*) before summer temperatures can thwart the experiment again. We will also check out the virulence of the *Phytophthora* strains we use. The tests originally planned for 2000 will occur in 2001, but we will modify the protocol to allow the inoculation and flooding to occur much earlier in the growing season.

2. Dutch field trials: Forty liners were planted on the infected (20) and uninfected (20) soil sites at Nederweert in spring 1999. Genotypes included AR 86-1-20, AR 628-2, AR 86-1-25, AR 486-1, AR 295-6, P16, P14, PiAu 7-33, JTE-G, JTE-H, B.9, J.9, Pi-80 Select, G.30, G.11, X2765 (INRA selection), 5179, 6210, 3007, 6253, 4013, 3041, and 7707. At each site 10 liners were planted to be trained as hedge trees, and 10 to be layered for stoolbeds. Some damage to the trial occurred from rabbits, but the planting is reportedly developing well. Plants will be harvested and scored for infection ratings in October 2000.
3. Nursery for US orchard trials: Results from tests in 2000 will be used to choose genotypes for nursery plantings in 2001.

RELATIVE TOLERANCE OF APPLE ROOTSTOCK CULTIVARS TO REPLANT DISORDERS

Project Leader: W.C. Johnson*, H.T. Holleran, I. Merwin, M. Mazzola

Geneva greenhouse screens: Orchard soils with severe replant problems were collected in March 1999 from sites in western New York, Champlain Valley, and the Hudson Valley. The soils were mixed and half of the soil was pasteurized to create a negative control. Thirty rootstock liners for each of 19 genotypes were surface sterilized, and half were planted to the control and half to the treatment soils. In early summer, the pots were moved outdoors to avoid extreme high temperatures in the greenhouse and surrounded with straw to prevent solarization of the pot soils. Trees developed normally through the first growing season. Visual differences were observable, and the pasteurized soil plants appeared to have better vigor and overall health. Trees were moved to the nursery cellar in the autumn. Measurements of biomass and shoot growth will be made in early January, and raw data should be available for review at the IDFTA meeting. Genotypes in the 1999 trial include: 3041, 4013, 4814, 5046, 5179, 5890, 5935, 6210, B.118, G.16, G.30, G.65, M.9 EMLA, and MM.106.

For pot trials in 2000 we were forced to modify the protocol because the cost of shipping replant soils from Washington was prohibitive. Instead, 30 rooted liners for 11 genotypes were delivered to Dr. Mazzola in December, and similar trials will be conducted at the USDA lab in Wenatchee, WA, during the coming growing season. Genotypes in the 2000 trial include 3041, 5087, 5179, 5935, 6253, 7707, G.16, G.30, G.65, M.9 EMLA, and G.11.

The results from trials in 1999 and 2000 will be used to determine the genotypes to be planted for orchard trials in 2003.

RELATIVE TOLERANCE OF APPLE ROOTSTOCK GENOTYPES TO FREEZING INJURY

Project Leader: W.C. Johnson*, H.T. Holleran, J.-P. Privé, D. Hebb, C. Embree, M.L. Kaps

1. Canadian programmed freezing trials: 17 rootstock genotypes were examined, but the quality of the liners used was insufficient to obtain good measurements. Poor rooting on liners from Geneva stoolbeds resulted in inadequate numbers of roots to examine, and clear differences were difficult to ascertain. We have changed our protocol to continue the measurements in 2000. Instead of liners from stoolbeds, we will use liners that were grown in greenhouse pots in 1999 to ensure adequate rooting. We will continue our measurements an additional year and will plant liners in our nursery in 2000 to ensure adequate rooting for freezer trials in 2001. Rootstocks evaluated in 1999 include: G.30, M.9 EMLA, O.3, 5179, 3041, 6210, 4013, 5046, 4214, 6874, and 6253. Additional genotypes tested in 1999 were later discarded from the program due to inadequate stoolbed and nursery characteristics. Interestingly, visual damage symptoms on O.3, a rootstock reported to be particularly cold hardy, did not appear to be better than M.9 EMLA. The tentative list of rootstocks to be evaluated in 2000 includes Pi-80 Select, P.16, Naga, MM.111, MM.106, M.9 EMLA, M.7, M.27 EMLA, M.26 EMLA, G.65, G.30, G.16, G.11, B.9, 7707, 6874, 6253, 5935, 5890, 5179, 5046, and 3041 (as mentioned, some of these will be delayed 1 year to obtain better rooting).
2. Geneva greenhouse trials examining the cold hardiness of rootstock genotypes during late winter deacclimation appear to have been quite successful. Clear damage to most

genotypes was observable in the treatment plants, though some appear to have escaped serious injury. Control plants grew and developed normally through the 1999 growing season. Potted plants have been lifted and moved to the nursery cellar, and measurements of biomass and shoot growth will occur in early January 2000. Raw data should be available for distribution at the IDFTA meeting. Seventeen genotypes were evaluated in 1999. They included O.3, Naga, MM.106, M.9 EMLA, M.7 EMLA, G.65, G.30, G.16, 6874, 6253, 6210, 5179, 5046 and 3041. Additional genotypes tested in 1999 were later discarded from the program due to inadequate stoolbed and nursery characteristics. Access to the following genotypes has been secured for continued testing in 2000: Pi-80 Select, P.16, MM.111, MM.106, M.9 EMLA, M.7 EMLA, M.27 EMLA, M.26 EMLA, G.30, G.16, B.9, 7707, 6253, 5179, and Mark. Additional genotypes will be added as they become available.

3. Results from the Canadian and Geneva trials in 1999 and 2000 will be used to choose genotypes for contract nursery production in 2001.

GENETIC FINGERPRINTING OF GISELA ROOTSTOCKS

Project Leader: Dr. Darush Struss; Cooperator: Dr. Amy Iezzoni

Objectives

Distinguish among Gisela rootstocks with a series of molecular markers. Determine if all the GI 5 (148/2), GI 6 (148/1) and GI 12 (195/2) accessions obtained from different sources are identical.

Justification and Background

Three Gisela sweet cherry rootstocks, GI 5 (148/2), GI 6 (148/1) and GI 12 (195/2), are becoming commercially important in the US because they increase precocity and reduce tree size. As a result, numerous US tree fruit nurseries are aggressively propagating these three rootstocks to meet grower demand for the trees. The management of these rootstocks in the nursery is particularly challenging because the plants of the three Gisela rootstocks look very much alike in the nursery row. Therefore the objective of this proposal is to genetically fingerprint the three Gisela rootstocks most prevalent in the US and other potentially commercially important Gisela rootstocks using a series of DNA markers that will be able to distinguish among the clones. Genetic fingerprinting using DNA markers is an accepted technique for distinguishing among closely related biological specimens. The GI 5 (148/2) and GI 6 (148/1) material fingerprinted will be chosen to represent the original sources of these two rootstocks in the US that have formed the stock plants for the entire US Gisela nursery industry. The US plant material will be checked against the original German accessions that are available.

TABLE 1

Gisela genotypes investigated and their origin.

Genotype	Origin			
	Germany	USA1	USA2	USA3
148/1 (GI 6)	+	+	+	+
148/2 (GI 5)	+	+	+	+
148/13	+	-	-	-
148/12/195/2 (GI 12)	-	+	+	+
195/20	+	-	-	-
473/10 (GI 4)	+	-	-	-
148-8	-	-	-	+
148-9	-	-	-	+

Gisela 4 (473/10) is derived from hybridization between *Prunus avium* and *Prunus fruticosa*. 195/20 is a new selection and derived from hybridization between *P. canescens* and *P. cerasus* cultivar "Leitzkauer'.

TABLE 2

Allelic differences between 5 tested Gisela rootstocks based on molecular markers.

Marker-System	Microsatellites					AFLPs				
Genotype	148/1 (GI 6)	148/2 (GI 5)	148/13	195/20	473/10	148/1	148/2	148/13	195/20	473/10
GI 6 (148/1)	—	6	11	19	19	—	13	15	16	19
GI 5 (148/2)	6	—	12	19	29	13	—	15	14	16
148/13	11	12	—	17	24	15	15	—	10	11
195/20	19	19	17	—	28	16	14	10	—	11
473/10	19	29	24	28	—	19	16	11	11	—

TABLE 3

Estimation of genetic distances between different Gisela rootstocks.

	148/1 (GI 6)	148/2 (GI 5)	148/13	195/20	473/10
148/1	0.0000				
148/2	0.517	0.0000			
148/13	0.743	0.643	0.0000		
195/20	0.743	0.759	0.682	0.0000	
473/10	0.814	0.828	0.721	0.778	0.0000

Materials and Methods: Struss Lab

Plant material: In the first set, 5 Gisela rootstocks from Germany were assayed: GI 6 (148/1), GI 5 (148/2), 148/13, 195/20, and 473/10 (Table 1). These rootstocks were provided by the CDB German nursery consortium. The molecular marker data for these 5 rootstocks were used in genetic distance and cluster analyses. Three Gisela rootstocks

TABLE 4

PCR-Products (in bp) of cherry microsatellite markers in Gisela rootstocks from various US locations.

SSR	GI 6 Ger.	GI 6 USA1	GI 6 USA2	GI 6 USA3	GI 5 Ger.	GI 5 USA1	GI 5 USA2	GI 5 USA3	GI 7 USA3	GI 8 USA3	GI 12 USA3	GI 12 USA2	GI 12 USA1
PMS 2	143,150	142,150	143,151	141,147	133,142, 149	133,142, 149	131,143, 149	131,143, 149	127	143,151	127	127,134 143,149	133,142 148
PMS 3	155,160, 187	155,161, 187	n.a.	155,161, 186	155,161, 187	155,161, 187	155,161	n.a.	155,161, 200	155,161, 188	n.a.	161	161
PMS 5	155,160, 175	155,160, 175	155,160, 174	155,160, 175	155,160, 168	155,160, 168	155,160, 168	155,160, 168/155, 160,175	155,173	155,160, 175	160,168	160,168	160,168
PMS 13	145,160, 176	145,160, 176	147,159, 175	147,160, 176	153,159, 174	153,159, 174	153,159, 176	153,159, 176/147, 159,176	153,159 176	147,159, 176	153,159, 170	153,159, 170	153,159, 170
PMS 15	126	126	126	126	133	133	133	133/126	126,133	126	126	126	126
PMS 18	114,126, 150	114,126, 150	114,150	114,151	114,128, 151	114,128, 151	129,150	114,150/ 114,128, 150	114,154	114,150	129,151	129,151	129,151
PMS 30	135,154, 171	135,154, 171	n.a.	135,153, 172	137,155, 173	137,155, 173	135,154, 172	136,153, 172	125,153, 173	135,153, 172	125,153, 172	125,153, 171	125,133, 153,171
PMS 67	148,161	148,161	148,161	148,161	148,160	148,160	148,160	148,161	149,164	149,161	161	160	161
PMS 222	189	189	189	189	189	189	189	189	184	189	182,189	182,189	n.a.

[GI 5 (148/2), GI 6 (148/1), and GI 12 (195/2)] were evaluated from USA sources USA1 and USA2, while 5 Gisela rootstocks were evaluated from USA source USA3: GI 5 (148/2), GI 6 (148/1), GI 12 (195/2), 148-8 and 148-9.

DNA markers: Two DNA molecular marker types, microsatellites (also termed Simple Sequence Repeats, SSRs) and AFLP (Amplified Fragment Length Polymorphism) markers were employed to evaluate the Gisela rootstocks.

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Plant material: Plants of GI 5 (148/2) and GI 6 (148/1) were obtained from three US nurseries for evaluation, Hilltop (H), Meadow Lake (ML), and Willow Drive Nursery (WD). The plants from ML were plantlets sent in tissue culture boxes. In addition leaves of GI 5 (148/2), GI 6 (148/1), and GI 12 (195/2) were sent from IR 2. Plants of GI 12 (195/2) were also provided by Hilltop.

DNA markers: The seven PCR primer pairs used were one chloroplast primer pair that was designed from sequence from sour cherry and 6 microsatellite primer pairs with the following designations: 42/43, 46/47, 48/49, 78/79, 80/81 and 82/83.

Results: Struss Lab

Each marker system could clearly differentiate among the five Gisela genotypes provided by the German nursery. The AFLPs detected 33 polymorphisms in this material whereas microsatellite markers revealed 43 alleles (Table 2). When the fingerprint data were used in a cluster analysis, the smallest genetic distances were observed between GI 6 (148/1) and GI 5 (148/2) and the highest between GI 6 (148/1) and GI 4 (473/10) (Table 3). The cluster analysis showed 3 groups consisting of GI 5 (148/2) and GI 6 (148/1), GI 13 (148/13) and 195/20 and as expected the third group was a single genotype GI 4 (473/10).

Using microsatellite markers, GI 5 (148/2) and GI 6 (148/1) from three US locations were compared with the original German rootstocks. In addition GI 12 from three US locations and GI 7 and G 8 from one USA location were also screened.

The microsatellite markers clearly distinguished between the five different GI genotypes revealing a total of 34 alleles (Table 4). The GI 6 (148/1) from Germany and USA1, USA2 and USA3 were identical and showed the same pattern indicating

that they are correctly labeled. With GI 5 (148/2) the samples from USA2 and USA3 matched the German samples; however, there was some deviation within the GI 5 (148/2) samples from USA3. Out of the three GI 5 (148/2) samples from USA3, two showed the pattern of GI 6 (148/1), indicating that there is a mix-up in this material. To exclude possible PCR artifacts and verify the results, the test was repeated 3 times. In all the three repetitions, the same results were obtained (Table 4).

The GI 12 (195/2) from the three US locations were identical indicating that these selections are correctly identified. No variations were observed between different samples of GI 6, GI 7, GI 8 and GI 12 (195/2) from USA3. Therefore the only error identified was the incorrect labeling of one set of GI 5 (148/2) from USA3 which is really GI 6 (148/1).

To verify the results obtained by microsatellite markers, AFLPs were used to differentiate among the Gisela rootstocks. AFLP markers identified polymorphic bands among the Gisela rootstocks. The AFLP fingerprints verified the results of the microsatellite markers. The microsatellite marker PMZ 15 is able to distinguish between GI 6 and GI 5 by producing a specific band at 126 bp in GI 6 and at 133 bp in GI 5. This marker can be easily used in an automated detecting system for high resolution and high throughput in a short time.

Iezzoni Lab

GI 5 (148/2), GI 6 (148/1) and GI 12 (195/2) samples from Hilltop were used as controls since they had been shown to be correctly identified by data from the Struss lab. In addition, we were able to get DNA suitable for PCR amplification only from the IR-2 sample of GI 5 (148/2).

Only one primer pair (46/47) failed to identify a band difference among GI 12 (195/2), GI 5 (148/2), and GI 6 (148/1) (Table 5). GI 12 (195/2) could be distinguished from GI 5 (148/2) and GI 6 (148/1) using the 5 remaining microsatellite primer pairs and the one chloroplast primer pair. GI 5 (148/2) and GI 6 (148/1) could be distinguished from each other using 3 of the 6 primer pairs [48/49, 78/79 and 82/83].

Based on these results, we recommend that the chloroplast primer pair be used to routinely distinguish GI 5 (148/2) and GI 6 (148/1) from GI 12 (195/2) since it amplifies one fragment in each sample that differs by 11 base pairs and is therefore very easy to score. To differentiate between GI 6 (148/1) and GI 5 (148/2) we would recommend the use of primer pair 78/79. This primer pair clearly revealed a total of 5 bands and GI 6 (148/1) and GI 5 (148/2) have only one of the 5 bands in common.

GI 5 (148/2) and GI 6 (148/1) from Hilltop Nursery (H) and GI 6 (148/1) from Meadow Lake (ML) and Willow Drive (WD) were similar to the appropriate selections indicating that they are labeled correctly. However, the GI 5 (148/2) selections from Meadow Lake and Willow Drive exhibited the banding pattern of GI 6 (148/1) indicating that they are incorrectly labeled. This mix-up is the same as that identified in one of the Meadow Lake samples run by the Struss lab.

Conclusion

These results demonstrate the potential of molecular markers in genotype identification and underline the necessity of routine application of molecular markers in the nursery to avoid complications such as material mix-up and the detection of possible mutations. We successfully established and developed two very efficient marker systems for genome analysis in cherry which can be used for characterization and identification of cherry genotypes, product safety, pursuing patent protection, and insuring the proper genetic material for cherry growers.

TABLE 5

Band size (bp) for 7 primer pairs tested on samples of GI 5 (148/2), GI 6 (148/1) and GI 12 (195/2) from 4 US sources.

Primers	Hilltop GI 5	Hilltop GI 6	Hilltop GI 12	ML GI 5	ML GI 6	ML GI 12	WD GI 5	WD GI 6	IR-2 GI 5
cp primers	249	249	238	249	249	238	249	249	
42-43	187	187	187						
	181	181	181						
	174	174	-						
46-47	165	165	165						
	159	159	159						
48-49	174	174	-	174	174	-	174	174	174
	-	169	-	169	169	-	169	169	-
	-	-	166	-	-	166	-	-	-
	156	156	156	156	156	156	156	156	156
78-79	-	226	226	226	226	226	226	226	-
	194	-	-	-	-	-	-	-	194
	-	189	-	189	189	-	189	189	-
	186	-	186	-	-	186	-	-	186
	182	182	182	182	182	182	182	182	182
80-81	-	-	175						
	170	170	-						
82-83	-	132	-				132	132	