FINAL PROJECT REPORT

Project title: Replant disease tolerance of Geneva rootstocks.
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Introduction

Apple Replant Disease (ARD) is one of the major problems that Washington State growers face as virgin land optimal for orchards becomes less available. Growers planting orchards on "replant" sites have few options to avoid losses due to this disease complex which include fumigation (Chloropicrin, Telone, Metam sodium, etc.), fallow, or treatment with bio-derived agents – all these options however can be expensive and give spotty results. Geneva apple rootstocks were bred using intensive disease screening methodology and diverse germplasm as the source of resistance. Preliminary studies indicate that some Geneva rootstocks show tolerance or resistance to ARD in New Zealand and NE United States. These rootstocks (including advanced selections) have not yet been tested in replant soils in Washington State. Knowledge of their performance in WA replant soils could give growers another viable option to maintain productivity in combination with available soil fumigation treatments. Preliminary results also indicate that there may be differences among rootstocks in the way they interact with beneficial organisms such as fluorescent pseudomonads which have been shown to antagonize the replant disease complex. Knowledge about the genetic components of these plant-microbe interactions may yield new methodology for selection of improved rootstocks and more viable options to combat the ARD complex.

Objectives

1. Test Geneva™ rootstocks in three or four grower cooperator replant sites where ARD has been known to occur. Compare these rootstocks to M9 standards with commercial scion varieties such as Gala, Pink Lady™ and Fuji.
2. Investigate genetic interactions between beneficial microorganisms that antagonize ARD and rootstock selections.

Significant Findings:

- **We have been able to detect tolerance to ARD among rootstocks. We have established that M.26 is very susceptible to ARD. We are still establishing long term performance of rootstocks in ARD soils.**
- **The effect of fumigation on relative tree growth sharply decreases with time.**
- **Relationships between apple rootstocks and beneficial pseudomonads are too complex to be unraveled with current resources.**

Methods: 2004 planting

Three new trials were planted in the spring of 2004. The location of these trials was picked on the basis of existing replant problems. The locations were Wapato (WA), Chelan (CH) and Naches (NA). The rootstock trials in WA and CH were planted in a split plot fashion where one half of the orchard was fumigated with Telone C-17 and the other half not fumigated. The trial in NA was also planted as a split plot however the orchard was split into three main plots treated with Telone C-17, Metam Sodium and unfumigated. The variety used for the WA and CH locations was Brookfield Gala and the variety used for the NA location was Honeycrisp. Table 1 shows the rootstock

genotypes and locations where they are being tested. During winter 2005 trees were pruned in to uniform scaffolds at the CH location whereas trees at the WA and NA locations were pruned according to individual tree needs. Trunk circumference data was taken at planting and in October 2004 and 2005and trunk cross sectional areas (TCSA) were derived. The mean amount of TCSA growth was calculated for the rootstocks using a mixed model approach adapted to a split-plot experimental design. Another method was used to measure the site variation of each location: a mixed model analysis with the rootstock genotype as the main effect was used to calculate the residual for each sampling unit. That residual was then plotted in a contour plot based on the orchard map which shows the effectiveness of fumigation as well as the effectiveness of the tolerance of certain rootstocks.

Table 1. Locations and rootstocks planted in 2004.

Rootstock	Location*	Scion Varieties
G.16	WA, CH, NA	Brookfied Gala, Honeycrisp
G.11	WA, CH	Brookfied Gala
G 3041	WA, CH	Brookfied Gala
G 5935	WA, CH, NA	Brookfied Gala, Honeycrisp
PiAU-56-83	WA, CH	Brookfied Gala
Pajam 2	WA, CH	Brookfied Gala
M.26 EMLA	WA, CH, NA	Brookfied Gala
Bud 9	WA, CH, NA	Brookfied Gala
Supporter 1	WA, CH	Brookfied Gala
Supporter 2	WA, CH	Brookfied Gala
Supporter 3	WA, CH	Brookfied Gala
4214	WA, NA	Brookfied Gala
4003	NA	Honeycrisp
4814	NA	Honeycrisp
4210	NA	Honeycrisp
G.30	NA	Honeycrisp
5087	NA	Honeycrisp
G 4202	NA	Honeycrisp
4013	NA	Honeycrisp
4213	NA	Honeycrisp
M.9 EMLA	NA	Honeycrisp

*** WA=Wapato, CH=Chelan, NA=Naches**

General Results

The tree growth data that was collected at all three locations showed that there was a definite positive effect of fumigation for the first year of trunk measurements. The relative effect of fumigation was less at the WA and CH locations during the second year of the experiment (Figure 1). This is may indicate that either the effect of fumigation is temporary (i.e. the disease pressure is returning to pre-fumigation levels) or that the rootstocks are generally being affected less by the soilborne diseases as they grow larger. At planting time the mean size of the trees for each rootstock genotype was different and that difference had a significant effect on the relative growth potential of that tree. We tried to account for those differences in the statistical analysis and corrected this effect where possible. There were significant rootstock by treatment interactions at every test location – this means that some rootstocks show at least a partial tolerance to the biological components of replant disease. The rootstocks that were affected the most at all locations by replant were M.26 and PiAU56-83. Overall clones of M.9 did not do as bad as M.26. The most interesting results will come in the future, where we will be able to quantify the increase in production (and profit) due to

fumigation and rootstocks and make some inferences on the long term effects of pre-plant decisions. It will be useful to have a field day in the near future at each location to discuss the findings.
The relationship between ARD microbial antagonists (such as fluorescent pseudomonads) and tolerant apple rootstocks was investigated in Geneva. We isolated fluorescent pseudomonads from stoolbeds in Geneva and collaborators (Rumberger et al.) performed tests on stool bed root samples and found no significant difference in the composition of the general bacterial community. We are continuing to investigate the possibility of strain specific relationships that would not have been detected by the methods used given current resources.

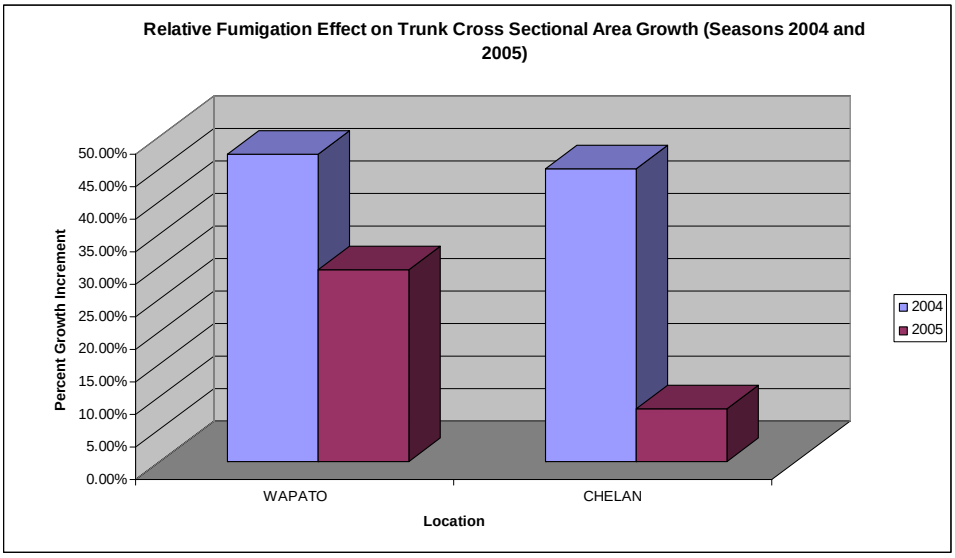

Figure 1. Relative TCSA increase of Brookfield Gala trees due to fumigation over two growing seasons. Although fumigation had the effect of increasing tree growth 45-50% in the first season of growth its effect has decreased considerably in the second season.

Wapato Location Findings

A decision was made to let all trees crop in 2005 and the results are shown in Figure 3. The next few years will tell whether each rootstock is able to overcome the effects of replant and still maintain productivity. As a general rule the trees that had a crop this year grew less – the correlation between fruit number per tree and TCSA growth was negative (-0.24, $p < 0.001$). One rootstock stands out as very vigorous and non precocious (PiAU56-83) being almost twice the size as B.9. At planting PiAU56-83 had the largest initial caliper of all trees. The vigor of this rootstock may give an opportunity to test the hypothesis that more vigorous trees perform better in weak replant sites than more dwarfing rootstocks. Although rootstocks G.11, G3041, G4214, Supporter 2 and M.9 Pajam 2 did not show a significant difference in growth during the first season between the fumigated and unfumigated treatments, during the second season the trends were not the same and only Supporter 2 showed no significant differences. Rootstocks M.26, M.9 Nic 29 and PiAU56-83 exhibited the largest differences in growth between fumigated and unfumigated treatments during both years. Trees on Bud.9 and G.11 were on average the smallest in the group. One major finding that has to be noted is that this site had been fumigated once before prior to the fumigation treatment that was performed for this experiment. The fact that we witnessed a positive effect of a second fumigation on tree growth shows how temporary the fumigation effect is and that only a proper choice of rootstock genotypes may give a long term solution to the replant problem.

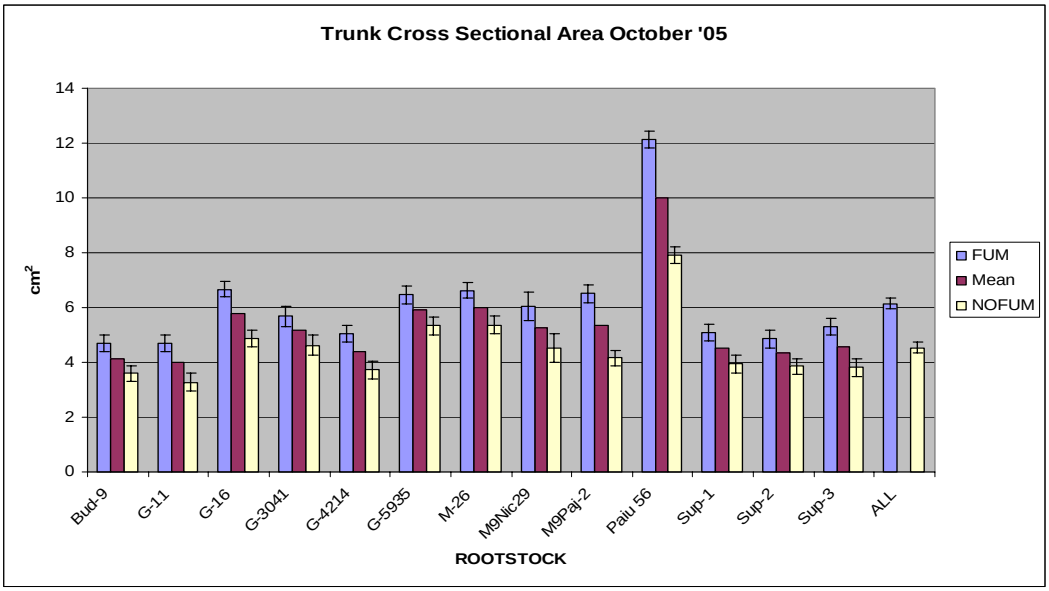

Figure 2. Mean TCSA of Brookfield Gala trees on 13 different rootstocks in fumigated and non fumigated soils at the Wapato (WA) location. Trees on fumigated land were on average larger than trees on unfumigated. For some rootstocks (Pi AU 56-83) the relative difference was greater than others (Supporter 2 and G3041).

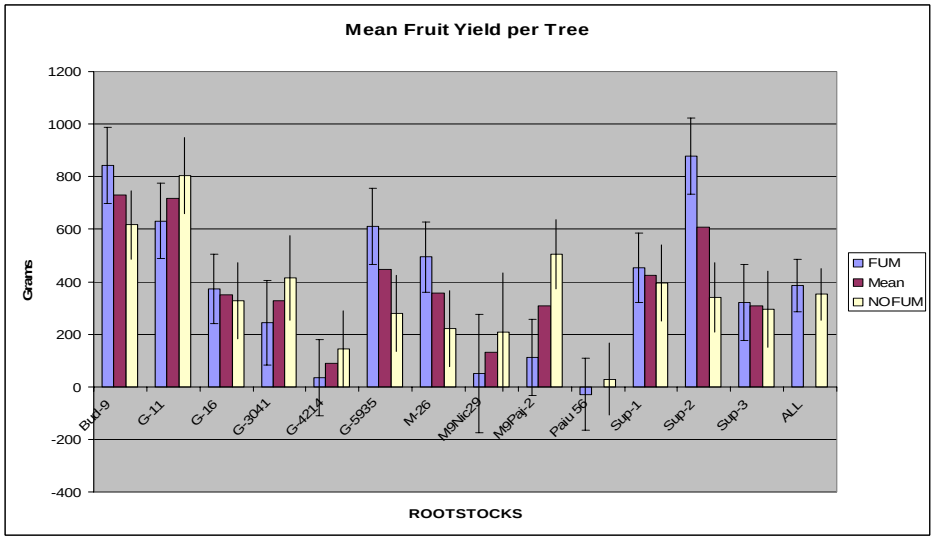

Figure 3. Mean fruit yield per tree (Brookfield Gala) in the first bearing year (2005) at the WA location. The error bars are quite large showing that there was a lot of inconsistence in fruit bearing within each plot. Yield differences among rootstocks give a measure of their precocity potential. There was a significant negative interaction between fruit yield and tree growth (trees with the most yield grew less – this may have an effect on return bloom in 06'.

Naches Location Findings

The experimental design at this location (Figures 3,4,and 5) is different than WA or CH as it was set up as single trees for each replication. There was a positive overall effect of fumigation (Telone C-17 and Metam Sodium). There were some surprising effects seen in some of the Geneva rootstocks where Metam Sodium was able to impact growth more positively than Telone C-17. Whereas M.26, Bud.9 and G.935 where impacted equally by both fumigations. This effect may be an indication that Metam Sodium was more effective at eliminating a specific component of replant disease that affects these rootstocks differentially. Although B.9 rootstock did not seem to differ significantly between

the fumigated and unfumigated treatments there were significant tree losses of B.9 rootstock at this location. Geneva 4210 was the only rootstock that came close to not showing statistical differences among treatments in the first season. This site was also characterized by significant root lesion nematode populations (as indicated in the report from Mark Mazzola) for which no plant disease resistance is known. This factor may have impacted the genetic resistance potential to the other components of the replant disease complex.

Another significant finding measured in a separate experiment at the same location was that organic pre-plant treatment did not have a significantly different impact on growth of Honeycrisp/M.9 sleeping eyes when compared to the no-dig treatment (Figure 6). It is possible (but unlikely) that by random chance all the no-dig treatments in this experiments landed on better plots. All treatments however grew much better than the control dig plots. We are puzzled by the fact that the control dig treatment exhibited the worst growth of all treatments.

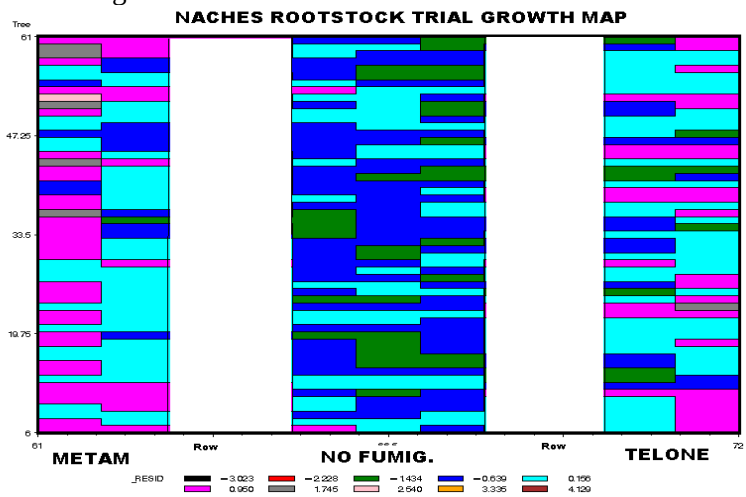

Figure 4. Contour plot of circumference growth of Honeycrisp trees at the Naches location. Unfumigated rows are sandwiched between rows treated with Telone C17 and Metam Sodium. Although it is not clearly demarcated we can see the effect of the fumigation treatment (lighter colors) on both sides of this map. Trees treated with Metam Sodium grew better on average.

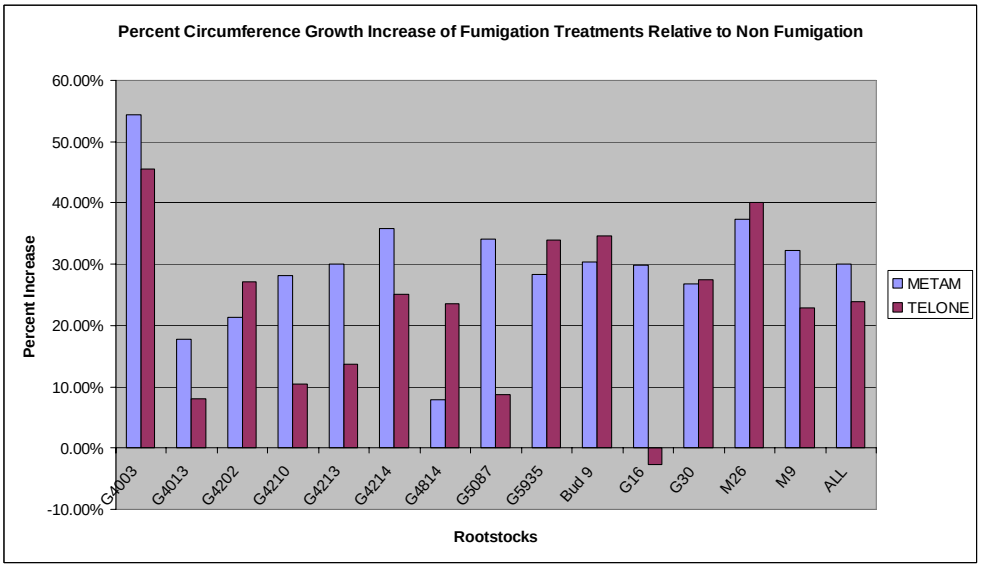

Figure 5. Percent increase of circumference growth (two seasons) of a variety of rootstocks at the Naches replant location. Overall the Metam Sodium treatment worked better than Telone C17 treatment. Some rootstocks like G4013 and G4814 seem to be more tolerant to the replant problem at this location since they did

not experience the same relative growth increase of susceptible M.26 and G.4003. It is also evident that some rootstocks perform much better with the addition of Metam Sodium relative to Telone.

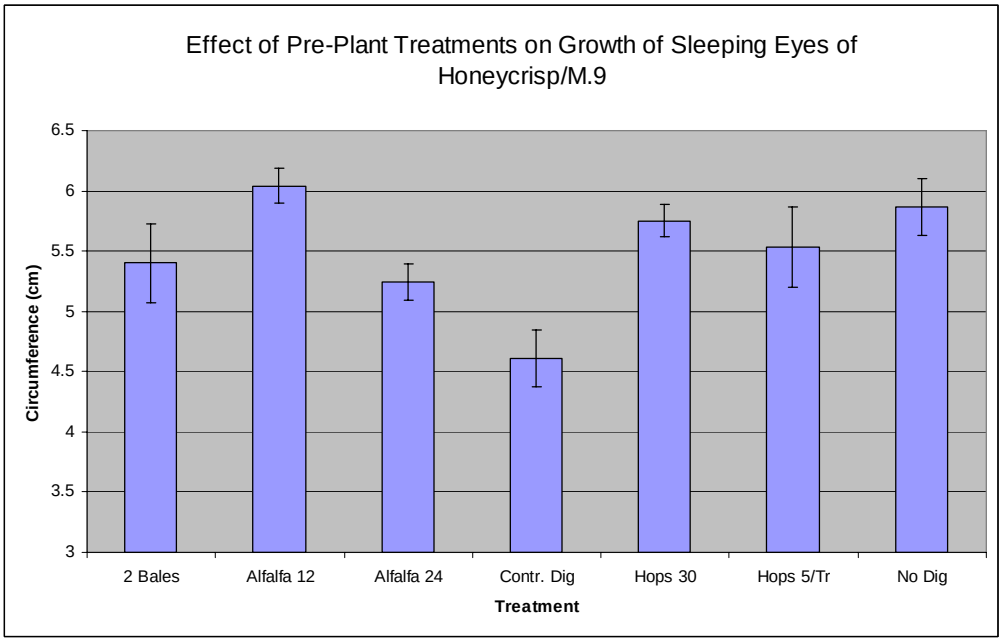

Figure 6. This experiment had the goal of evaluating alternatives to fumigation in the same replant plot as the rootstock trials in NA. The soil was amended with hops waste, alfalfa, and straw and compared to No-Dig and Control-Dig treatments. The plots were planted with sleeping eyes (Honeycrisp/M.9) and circumference measurements were taken at the end of the first leaf. The measurements show that trees in the Control-Dig plots exhibited the least growth and trees with Alfalfa 12 grew the best.

Chelan Location Findings

The positive effect of fumigation on tree growth sharply decreased to 8% in the 2005 growing season from 45% in the 2004 growing season (Figure1). The planting in CH was pruned to a uniform scaffold after the first year (2004) and therefore unlike the WA location there was no fruit production in 2005. Bud.9 was the weakest rootstock overall. G.935, Pajam 2 and Supp. 1 showed no significant differences in tree size between the fumigated and the unfumigated plots. M.9T337 and M.26 had the largest differences in tree growth between fumigation treatments. The most vigorous rootstock in this trial was PiAU56-83 (almost double the size of Bud.9). This may be a good opportunity to test whether the practice of using more vigorous rootstocks on replant sites is a viable solution to the replant problem. This site is also unique as it is under organic management. This site may provide useful information on relative rootstock performance given increased weed competition because of the absence of an herbicide strip and the somewhat different composition of nutrients applied in the form of organic fertilizers.

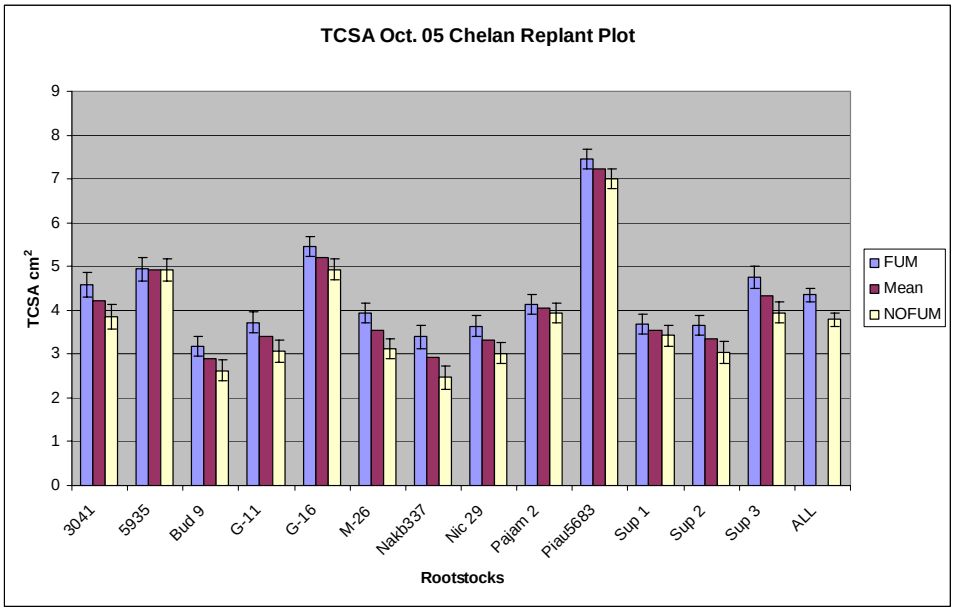

Figure 7. Trunk cross sectional area of Brookfield Gala trees on 13 different rootstocks at the CH replant site. This site is under organic management.

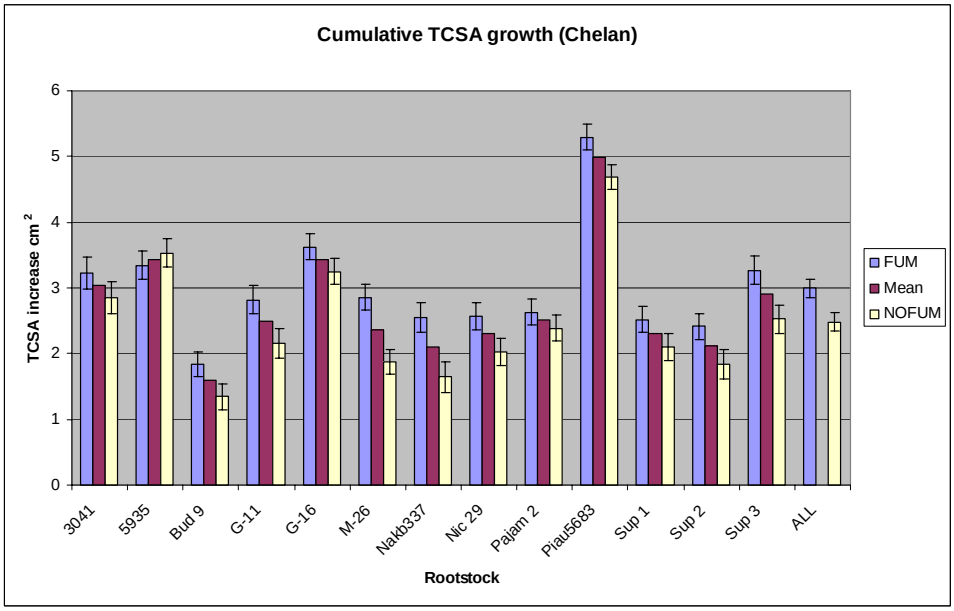

Figure 8. TCSA increase over the life of the orchard at the CH location.

Other activities supported by this grant not included in initial proposal:
Frenchmen Hill Honeycrisp rootstock trial

In the spring of 2003 24 rootstock genotypes with the scion Honeycrisp were planted in two rows at the Frenchmen Hill location. This grower-cooperator trial is managed under the auspices of the WFTRC. Over 500 trees and 25 rootstock genotypes were planted. These trees were propagated and grown in Geneva's apple rootstock nursery. Trunk circumferences have been recorded for Spring 2003, Fall 2003 and Fall 2004, Fall 2005. Bloom, yield and fruit size data was collected in the fall of 2005. Data was analyzed with the SAS 8.2 statistical package and PROC MIXED. Some of the results are summarized in figures 8 and 9. Although we detected significant yield differences, there

was a definite effect of fruit load on fruit size and some of the yields (G.16 and B.9) exhibited smaller fruit than normal (Figure 11). The trees on M.9 were not significantly different in size than M.26 – M.26 probably does not grow that well at this location. This site was plagued by two major irrigation accidents that have compromised two to four replications.

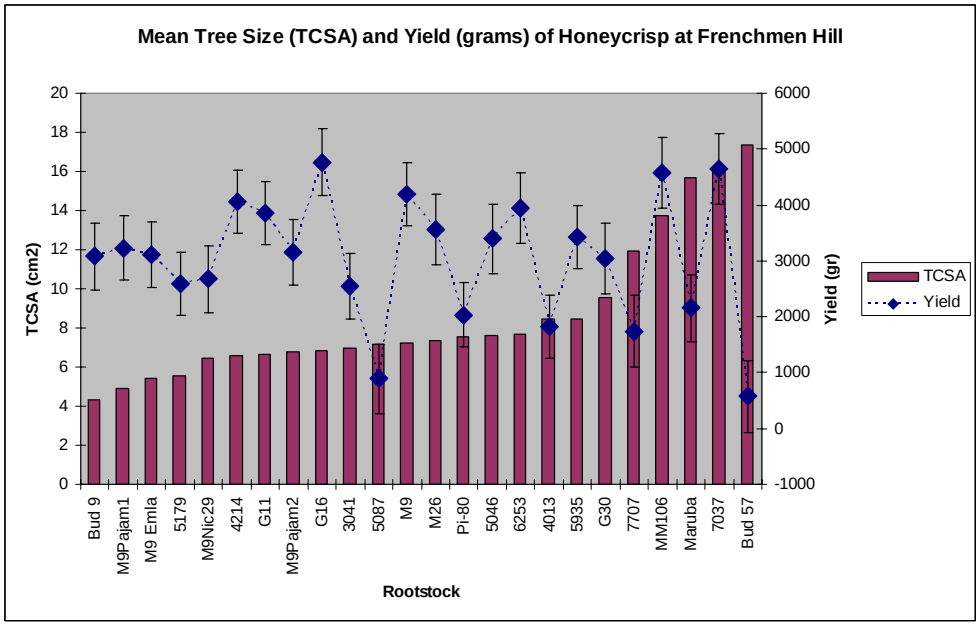

Figure 9. Mean yield per tree and mean tree size measured in the fall of 2005. Several classes of rootstock vigor are represented in this experiment (Dwarf: G.3041, G.11 B.9, M.9 clones. Semi-Dwarf: M.26, G.5935, G.6253, G.30, G.4013. Vigourous: G.7037, G.7707, MM.106 and Marubakaido).

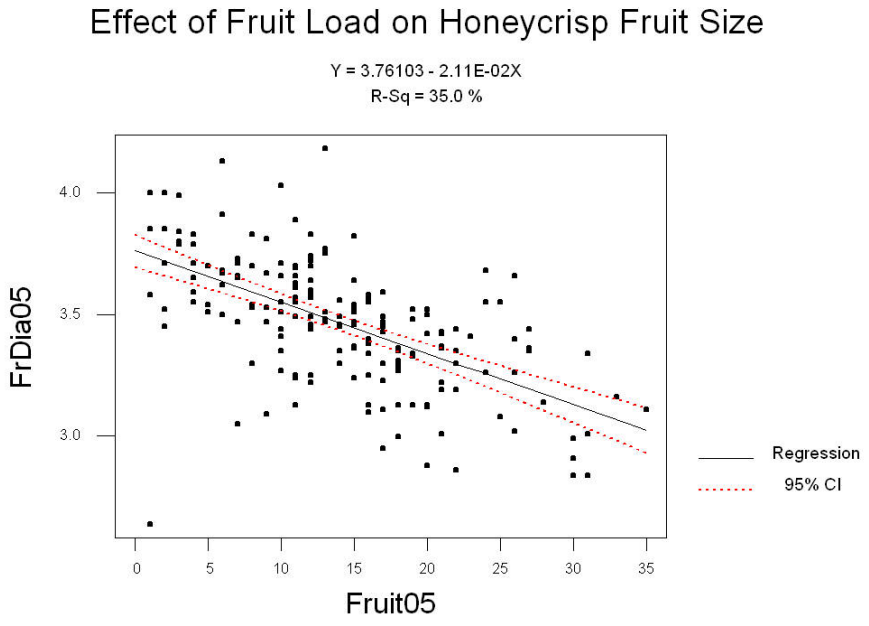

Figure 10. Fruit size was affected by the fruit number per tree at the Frenchmen Hill location. In an ideal situation where fruit are thinned to a uniform cropload the fruit should have a more uniform size.

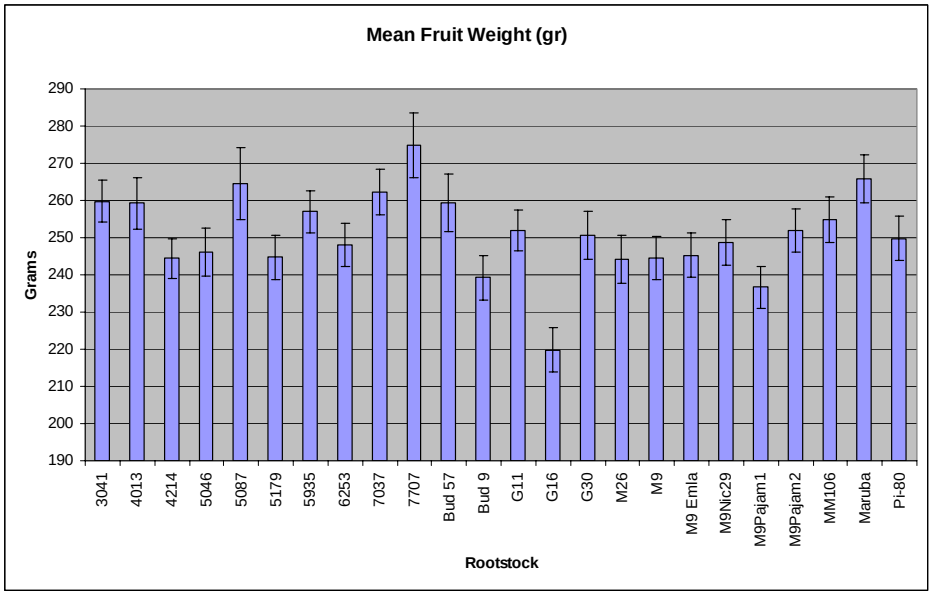

Figure 11. Average fruit weight of Honeycrisp at the Frenchmen Hill location.

Stormy Mountain (Chelan) Honeycrisp Rootstock Trial

This trial mirrors the trial on Frenchmen Hill. This trial was planted on virgin ground and is under organic management. Several trees of have been lost to rodents, however the statistical capacity of the trial is still very good. Rootstocks G.5087 and G.4214 had the highest yield followed by G.5935, G.16 and G.30. Fruit size was not significantly affected by crop load at this location. On average the trees at this location grew less than Frenchmen Hill – this is probably because of different light and season conditions. When compared to Frenchmen Hill we are surprised by the differential performance of rootstock G.5087 at the two sites. G.4214 ranked high in production at both locations.

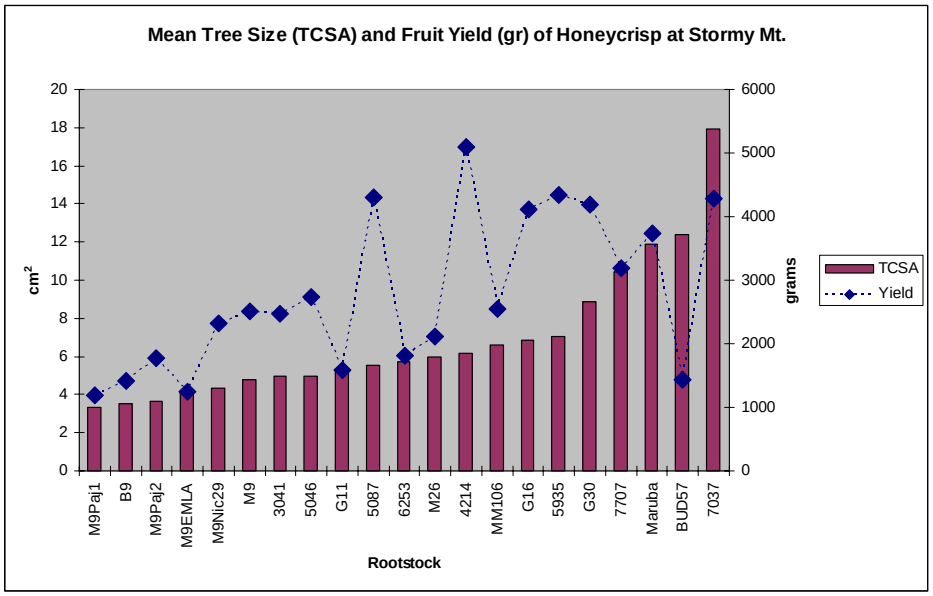

Figure 12. Rootstocks have been arranged by tree size. A surprising find in this trial when compared to Frenchmen Hill is that trees on MM.106 are smaller than semi-dwarf rootstocks

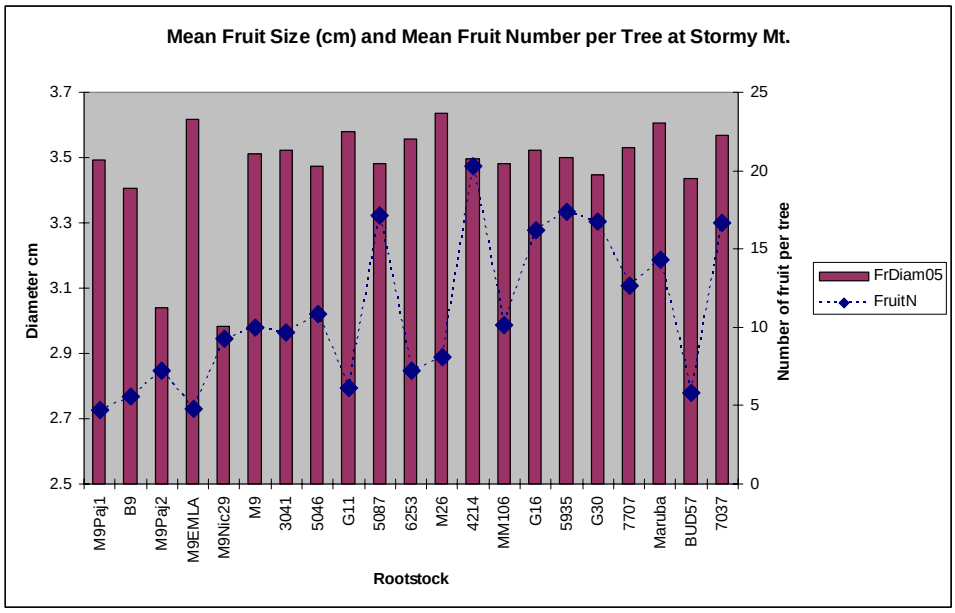

Figure 13. Mean fruit size (Diameter) and fruit number per tree. There was no detectable effect of crop load on fruit size at this location.

Budget:
Project title: Replant disease tolerance of Geneva rootstocks
PI: Dr. Gennaro Fazio
Project duration: 2003-2010 (8 years) first three years shown.
Current year: 2005
Project total (3years):

Item	Year 1 (2003**)**	Year 2 (2004)	**Year 3 (2005)**
Salaries[1]	3,000	3,000	1,500
Benefits (38.31%)	1,149.3	1,149.3	574.65
Wages[2]	8,000	8,000	4,000
Benefits (38.31%)	3,064.80	3,064.80	1,532.40
Equipment[3]	2,000	0	0
Supplies[4]	17,085.90	11,085.90	4092.95
Travel[5]	3,000	3,000	3,000
Miscellaneous[6]	700	700	300
Total	38,000	30,000	15,000

[1]Technician salary for part-time assistance in propagation budding and maintenance of stoolbeds.
[2]Wages for assistance in trial design and establishment and for laboratory technician part time help.
[3]Includes digital calipers for measuring TCSA etc.
[4]Includes cost for rootstock liners, trees, support system, laboratory supplies etc.
[5]Travel to and from trials.
[6]Includes shipping expenses, communication costs etc.