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Screening and Evaluation of New Rootstocks with Resistance to *Phytophthora cinnamomi* 2005

Continuing Project: Year 15 of 20

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Benefits to the Industry

Ultimately, the control of Avocado root rot will be accomplished with a resistant rootstock. This project has already provided the industry with several new tolerant rootstocks, which are greatly improving the yields of avocado on land infested with *Phytophthora cinnamomi*. The goal is to find a rootstock that will eliminate *Phytophthora cinnamomi* as a serious pathogen on avocado. Our ability to find such a rootstock has been enhanced as a result of our breeding blocks where we focus on crossing already resistant rootstocks.

Objectives

To collect, select, breed and develop avocado germplasm that exhibits resistance to Phytophthora root rot of avocado.

As of July 1, 2005, I have replaced Dr. John Menge as project leader. My objectives over this last year have been to: i) evaluate the overall progress of the program, ii) critically evaluate the analytical methodologies currently used in the program, iii) evaluate areas in which additional technologies may improve the breeding program, and iv) to gain knowledge of the UC patenting process so that valuable root stocks can get delivered to the growers as soon as possible, including varieties that Dr. Menge has been trying to get patented over the past couple of years.

Since 1989, over 45,000 seedlings have been screened for root rot resistance and around 2,500 have begun to be screened in 2006. Over 60 rootstocks have made it through the initial root stock greenhouse screening. There are currently 22 rootstock varieties that have been developed from this project that are currently being tested under field conditions throughout the northern and southern avocado growing regions of California (Table 1). Seven varieties have been terminated from the program due to poor performance as well as 2 VC lines. We are also testing additional rootstocks that were not developed in this current research project (Table 1). Three new field plots were set up this year; two in the northern growing region and one in the southern growing region. Three new rootstocks (PP56, PP58, & PP63) were added to the field plots this year. For next year, this will be increased to around 6 new rootstocks developed from this program and 4 from 'escape' trees growing in *Phytophthora* infested soil. However, we still have approximately forty untested UCR rootstocks that showed resistance to *P. cinnamomi* in the initial two-year greenhouse screening process.

Table 1. Field distribution of the rootstocks being tested for root rot resistance. PP numbers indicate rootstocks developed from this project. Dates indicate year of planting.

Southern CA

Rootstocks	1 (2002)	2 1(2003)	3 2(2005)	4 (2005)	5 (2006)	6 (2005)	7 (2000)	8 (2003)	9 (2002)	10 (2002)	11 (2004)	12 (2003)
Thomas	X	X				X	X	X	X	X	X	
Merensky II (Dusa)			X	X	X		X					
Merensky I (Latas)		X					X					
Duke 7							X					X
Parida	X											X
Topara												
Toto Canyon		X						X				
VC44		X										X
VC207		X		X							X	X
VC218	X		X									X
VC225		X		X						X		
VC241		X		X			X					
VC801	X		X	X		X				X		X
VC256						X				X		
Zentmyer PP4	X						X		X			
Berg PP5			X	X								X
PP14 Uzi		X		X			X	X		X	X	
PP15 Guillemet							X					
PP16 Rio Frio			X	X			X				X	X
PP18 Afek		X		X						X	X	
PP19 McKee												
PP21 Erin												
PP22 Medina												
PP24 Steddom		X					X	X			X	X
PP26 Martin												
PP28 Elinor								X				
PP29 Pond	X								X			
PP33 Margy	X								X			
PP34 Crowley	X								X			
PP35 Anita	X							X	X			
PP36 Dirac			X	X				X				
PP37 Frolic									X			
PP40 Eddie					X	X		X				
PP41 Witney			X	X		X			X			
PP42 Johnson								X				
PP43 Campbell											X	
PP44 Fred	X							X	X		X	
PP45 Brandon					X							
PP47 CI #2			X	X							X	
PP52 Downer			X	X		X						
PP56 Gabor												
PP58 Lovatt												
PP63 O'Connell					X							
SA-1 Lansfield					X							
Spencer											X	
UC2035											X	
Duke 9									X			
Number of trees	200	200	180	260	100	120	300	200	200	100	200	200
Disease Pressure	1	2	2	1	?	2	3	1	1	2	3	2

Northern CA

Rootstocks	13 (2004)	14 (2004)	15 (2004)	16 (2005)	17 (2003)	18 (2003)	19 (2003)	20 (2006)	21 (2003)	22 (2001)	23 (2006)	24 (2002)	25 (2005)	26 (2005)
Thomas		X	X		X	X	X	X	X	X		X		
Merensky II (Dusa)							X	X		X	X		X	X
Merensky I (Latas)				X	X									
Duke 7	X			X										
Parida														
Topara											X			
Toro Canyon														
VC44														
VC207							X							
VC218														X
VC225														
VC241							X							
VC801														
VC256														
Zentmyer PP4		X		X	X	X	X		X	X	X	X		
Berg PP5														
PP14 Uzi					X	X	X	X	X		X			X
PP15 Guillemet										X				
PP16 Rio Frio							X	X			X			
PP18 Afek							X	X			X		X	
PP19 McKee							X			X				
PP21 Erin		X			X			X			X			
PP22 Medina								X			X			
PP24 Steddom					X	X			X	X				
PP26 Martin	X		X		X	X								
PP28 Elinor	X		X											
PP29 Pond	X	X					X			X		X		
PP33 Margy	X	X				X						X		
PP34 Crowley		X								X		X		
PP35 Anita	X	X	X		X	X			X			X	X	
PP36 Dirac	X	X	X				X							
PP37 Frolic	X	X	X						X			X	X	
PP40 Eddie			X					X			X		X	
PP41 Witney	X	X	X				X		X			X	X	
PP42 Johnson								X			X		X	
PP43 Campbell									X		X			
PP44 Fred												X		
PP45 Brandon								X			X		X	
PP47 CI #2							X							
PP52 Downer							X							
PP56 Gabor								X			X			
PP58 Lovatt								X			X			
PP63 O'Connell								X						
SA-1 Lansfield								X						
Spencer													X	
UC2035														
Duke 9					X		X			X		X		
Number of trees	200	200	180	180	180	140	200	300	160	180	280	200	160	80
Disease Pressure	2	2	1	1	2	1	2	3	1	1	3	1	3	3

1 = low
2 = medium
3 = high

bold = not being further tested

Total of 4900 trees planted

2006 Yield Data

Four field trials were harvested this year. Only one field plot was considered to be heavily infested with *Phytophthora cinnamomi* (Table 2). Under these conditions, our resistant rootstocks Zentmyer, Uzi and Steddom produced 51.75, 43.66, and 41.00 kg/tree, respectively (Table 2). In the previous two years, the Thomas control trees only produced approximately 15 kg/tree. The Thomas trees were not harvested in 2006 due to a communication error but it is obvious that our rootstocks are doing much better under these harsh conditions than Thomas. The South African varieties, Dusa and Latas, also produced significantly more than the Thomas controls (also based on 2004/2005 yield data). However, on field plots with little to moderate disease, the differences between rootstocks were not as obvious. Therefore in the future, it will be important to have field plots with as much disease pressure as possible so that valid comparisons can be made.

Thus far, Dr. Menge's program has been very successful with new varieties currently in the patenting process. However, the 'in house' UC committee that the Dean's office formed did not approve his proposal for releasing the material. As I mentioned in the 6-month report, I am in the process of trying to get a copy of the original document and the comments made by the committee members. I have spoken with the Dean's office several times and am waiting to receive this document. I can then resubmit once the concerns have been addressed. Also, I have spoken to the people at the Office of Research and we will likely not go through the formal patenting process because it is too costly and will take 2-3 years once the 'in house' committee has approved the new varieties release. The Office of Research will simply write up some type of agreement that who ever uses the rootstocks must pay a certain amount of money to the UC just as if it were a patent.

The ways that I have seen to improve the breeding program are as follows. First, we need to drop out varieties quicker that do not appear to do well. Secondly, all new plots that are selected need to have a heavy inoculum load. Many of our current trials do not have root rot in the soils, which defeats the purpose of screening for resistance. Once we find potential rootstocks that show promise, then we can plant them in soil that is free of the disease to evaluate yield effects. This will be done, even if it means having fewer field trials. We need good trials, not just as many as possible.

We also plan on using molecular markers to learn something about the parentage of our resistant varieties because previous research has shown that some pollen donors are better than others. For example, Sulaiman, *et al.* (2004) found that a minimum of 46% and a maximum of 85% of embryos from the variety 'Gwen' were pollinated by the variety 'Ryan'. This was true even in cases where a 'Ryan' donor was up to 50 m away from a 'Gwen' tree that was surrounded by other varieties. Thus, it will be important for us to know if one or more of our resistant rootstocks were preferentially the pollen donor(s) so breeding blocks could be set up to maximize genetic exchange among all the best resistant rootstock varieties. Preliminary data suggests that our resistant rootstocks are genetically diverse, which is good news (Figure 1). We can also use the molecular markers to choose which rootstocks to test instead of picking them at random. It seems logical to me to choose the most genetically diverse rootstocks to test first. Moreover, we have also set up two new breeding blocks this year with our most resistant rootstocks.

I have also been working on developing a molecular based soil assay to quantify *Phytophthora cinnamomi*. This will be an important tool to evaluate the disease pressure found in and throughout a field plot. The key is to find a DNA region that is specific to *P. cinnamomi*. The first region I have tried is a gypsy-like retrotransposon (a genetic element that could be thought of as a molecular parasite). This region is attractive because it has been estimated to exist in thousands of copies in the genome of *P. cinnamomi* (Judelson 2002), which makes it an ideal target for polymerase chain reaction (PCR). This is important since I will try to develop the assay directly from DNA extracted from soil samples.

Lastly, we will also eliminate greenhouse testing of the resistant rootstock lines after the initial two-year screening. After looking at the data over many years, there is no correlation between how well a variety does in the greenhouse compared to the field. This is possibly due to the fact that the Hass scion was not grafted onto the rootstock when testing under greenhouse conditions. This will also free up Brandon's time so he can do other work such as screen more germplasm.

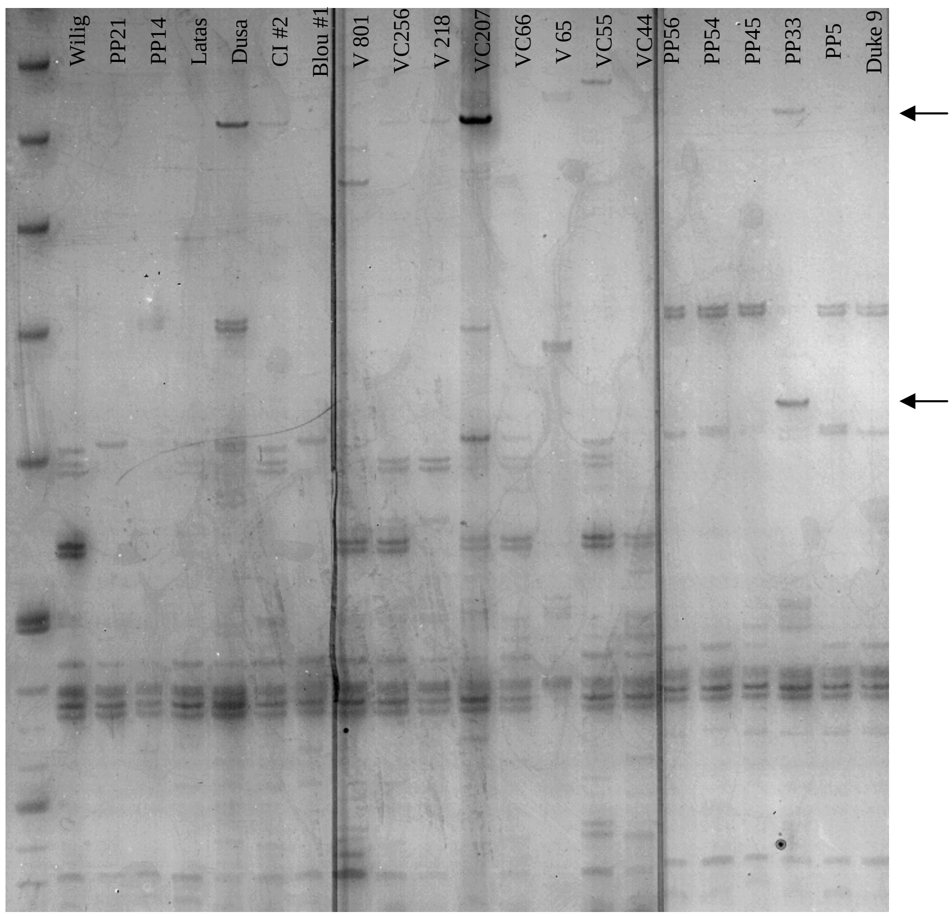

Figure 1. Amplified fragment length polymorphism gel image with various rootstock varieties: A) South African varieties, some of our PP lines, and one escape tree. B) Putative salt tolerant Israeli VC lines. C) Duke9 and 5 lines derived from the parent Duke 9 tree. Polymorphism within PP33 indicates out crossing from some other variety. Shared polymorphic band (upper arrow) with VC207 and Dusa indicates they are potential pollen donors but more data would be needed to statistically verify this due to the small sample size. Lower polymorphic band indicates a potential rootstock specific marker that could be used in parentage analysis. However, significantly more data would be needed to verify this. This gel is just an example of how DNA polymorphisms can be used to understand overall genetic variation and parentage relationships among rootstocks.

Table 2. 2006 yield data, disease pressure and plot age of four harvested rootstock trials.

Rancho Ca Harvest, April 2006	**Moderate disease pressure**	**4 year old plot**
Rootstock	Total fruit weight per tree (kg)	Individual fruit weight (kg)
VC801	27.55a	0.173abc
Afek	21.56ab	0.194a
VC256	16.49b	0.157c
Thomas	13.19b	0.189ab
VC225	11.41b	0.159bc

Escondido Harvest, April 2006	**Moderate disease**	**3 year old plot**
Rootstock	Total fruit weight per tree (kg)	Individual fruit weight (kg)
Latas	26.34a	0.233a
Steddom	23.76ab	0.233a
Toro Canyon	22.90ab	0.241a
VC207	20.95ab	0.232a
Uzi	18.86b	0.229a
VC225	12.93c	0.234a
Afek	9.52cd	0.229a
VC241	6.33de	0.224a
Thomas	4.33de	0.242a

Fallbrook Harvest, April 2006	**Little disease**	**4 year old plot**
Rootstock	Total fruit weight (kg)	Individual fruit weight (kg)
Witney	36.62a	0.229a
Crowley [1]	35.43ab	0.221a
Anita	34.51ab	0.231a
Thomas	30.66abc	0.232a
Pond	30.48abc	0.234a
Zentmyer	29.74abc	0.223a
Margy	29.05abc	0.237a
Duke 9	28.45bc	0.241a
Fred	27.79bc	0.233a
Frolic	23.28c	0.237a
VC44	2.85e	0.242a

[1] Only 10 blks. All other rootstocks have 20 blks.

Escondido Harvest, May 2006	**Heavy disease pressure**	**6 year old plot**
Rootstock	Fruit weight per tree (kg)	Individual fruit weight (kg)
Merensky II (Dusa)	53.24 a	0.18 a
Zentmyer	51.75 a	0.22 a
Merensky I (Latas)	50.46 a	0.23 a
Uzi	43.66 a	0.19 a
Steddom	41.00 a	0.20 a
VC241	27.96 a	0.20 a

Mean values in each column followed by identical letter are not statistically different according to Waller's k-ratio t test.

References

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