

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

## **Continuing Project: Year 11 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 which exhibits resistance to *Phytophthora* root rot of avocado.

## **Summary**

### **Collection and Selection of Germplasm**

We have obtained 40 genetically engineered avocado rootstocks from Richard Litz, University of Florida. These avocados have had the plant resistance genes, which produce gluconase and chitinase, inserted into their genome. The avocados were received in test tubes and were conditioned to greenhouse conditions, transplanted to greenhouse mist chambers and eventually moved into the greenhouse. Of the 40 avocados received, 32 are still alive and 10 are growing very well and will be budded to field avocado stumps to increase budwood for experimental testing. While there is little evidence gluconase and chitinase genes are directly involved in resistance to *Phytophthora cinnamomi* root rot, they are known to function in resistance to other fungal diseases.

### **Breeding Program**

We have screened 2016 seeds from the breeding blocks for resistance to *Phytophthora cinnamomi* in 2002. We have retained 22, which showed a high degree of resistance. Most of these varieties had maternal parents of Toro Canyon, Duke 9 or Duke 7. While we can handle up to 12,000 seeds per year, we have begun to revamp one of the 9 breeding blocks every year.

Resistant trees will be planted in the blocks instead of grafting resistant buds into existing trees. This will allow more uniform plantings, the establishment of replicated trees and prevent shading and suppression of slower growing germ plasm. We now have 51 seedlings from the breeding blocks, which have shown exceptional resistance to *Phytophthora cinnamomi* after extensive testing. Twenty-two of these are being field-tested and one more is being grafted for field tests in 2004. Twenty-seven more are ready for field-testing. Two of seedlings from the breeding block, the Guillement and the Mckee have been judged not commercial and will not be tested further. Three varieties, Zentmyer, Uzi and Steddom will be released to the growers as soon as possible.

We are now covering trees with a mesh screen and are using beehives in an attempt to get more crosses between varieties. Results from Dr. Clegg's lab indicate that 79% of our seeds from covered trees containing beehives are outcrossed. However, 68% of our seeds are outcrossed from uncovered trees without beehives. These values are not significantly different. We now intend to harvest pollen from selected varieties and allow the bees to spread it to the maternal parent. This will allow us to manage and create the crosses we are seeking.

In 2003 a new breeding block was initiated which contains many of the new *Phytophthora* resistant varieties. The breeding blocks are now made up of Merensky I, Merensky II, VC 256, G755A, Thomas, G810, Toro Canyon, Spencer, Barr Duke, UC2001, CRI-71, Duke 7, G6, D9, UC2011, Zentmyer, *Persea steyermarkii* *Persea nubigena*, Agucate de Anis, Agucate de mico, Berg, Uzi, Guillemet, Rio Frio, Afeck, Mckee, Erin, Medina, Steddom, Martin, Elinor, Pond, Dirac, Eddie, Witney, Johnson, Faber, Bender, Mauk, Downer, Turney, Janice, Gabor, Mary Lou, Lovatt, VC 207 and VC 218.

In 2003 a new breeding block for salt resistance was established at Agricultural Operations in Riverside. Varieties in this salt block will include Merensky I, Merensky II, Toro Canyon, VC 207, VC 208 and VC 801. Seeds from this block will be harvested and planted on to a strip of land donated by Harlan Beck in Escondito, CA. He will water these trees with extremely salty water and after two years, salt resistant varieties will be harvested and returned to Riverside for cloning further testing.

### **Screening and Greenhouse Evaluation of Rootstocks**

Intensive greenhouse experiments involving the root rot resistance of Elinor (PP28, maternal parent D9), Eddie (PP40, maternal parent Toro Canyon), Anita (PP35, maternal parent UC2001), Dirac (PP36, maternal parent UC 2001) and Thomas are currently being carried out in the greenhouse. Data from these experiments are not yet available. Plants being grafted for intensive greenhouse studies in 2003 include Martin (PP26, maternal parent D9), Margy (PP 33, maternal parent D9), Frolic (PP 37, maternal parent D9), Campbell (PP43, maternal parent UC 2001) and Thomas.

### **Field Evaluation**

We now have 29 field trials (6,500+ trees) testing 52 clonal root rot tolerant rootstocks throughout Southern California. The following are brief summaries of the older trials.

In a 7-year-old trial in Camarillo, CA under heavy root rot pressure, trees yielded as follows from the greatest to least: Merensky IV (W-14 South Africa), VC 256 (Israel), Halma Duke, Spencer (Pauma Valley), Merensky III (Evstro -South Africa), Thomas, Gordon (South Africa), UC 2023 (G755 C seedling) and Borchard. All of the varieties seem to be doing well except for Borchard. Cumulative yields for the past four years show nearly the same relative yields.

A 5-year old trial established in Carpinteria CA in salty soil under heavy root rot pressure was rated as follows from healthiest to the poorest: Merensky II (Dusa- South Africa), VC 256 (West Indian-Israel), Zentmyer (PP4- maternal parent Barr Duke), and Thomas. Tree sizes from largest to smallest were: Merensky II (Dusa- South Africa), Thomas, Zentmyer (PP4- maternal parent Barr Duke), and VC 256 (West Indian-Israel). Fruit set ratings from heaviest to lightest are: Merensky II (Dusa- South Africa), Thomas, VC 256 (West Indian-Israel) and Zentmyer (PP4-maternal parent Barr Duke). A few VC 207 (Day-West Indian-Israel) were planted at this site and they have virtually no salt damage. Salt damage for the rest of the varieties, from least to worst were VC 256 (West Indian-Israel), Merensky II, ( Dusa-South Africa), Thomas and Zentmyer (PP4-maternal parent Barr Duke). Thomas and Zentmyer are far more susceptible to salt damage than the other varieties. Only Merensky II is performing adequately in this difficult plot.

A 4-year-old trial established in Temecula CA on root rot infested soil. It rated as follows from healthiest to poorest: Thomas, Zentmyer (PP4-maternal parent Barr Duke), Toro Canyon, Merensky III (Evstro-South Africa) and Duke 7. Tree sizes from largest to smallest were: Thomas, Zentmyer (PP4-maternal parent Barr Duke), Toro Canyon, Merensky III (Evstro-South Africa) and Duke 7. Fruit set ratings from heaviest to lightest are: Thomas, Zentmyer (PP4-maternal parent Barr Duke), Merensky III (Evstro-South Africa), Duke 7 and Toro Canyon. All yields were light. Duke 7 and Merensky III are beginning to fail on this plot, while the other varieties are performing adequately.

A 4-year-old trial established in Escondido, CA on root rot infested soil. It rated as follows from healthiest to poorest (Table 1): Merensky I (Latas-South Africa), VC 241 (Israel), Rio Frio (Guatemala), Zentmyer (PP4- maternal parent Thomas), Merensky II (Dusa-South Africa), Spencer seedling (Pauma Valley), Uzi (PP14-maternal parent G6), Steddom (PP24-maternal parent Toro Canyon), Thomas, Leo (Brokaw selection), Guillemet (PP15-maternal parent Thomas), Duke 7, Spencer (Pauma Valley), G755A (*P. schiedeana* x *P. americana* seedling), and Poly N (polyploid, UCLA). Tree sizes from largest to smallest were: Uzi (PP14-maternal parent G6), Merensky I (Latas-South Africa), Merensky II (Dusa-South Africa), Steddom (PP24-maternal parent Toro Canyon), Zentmyer (PP4-maternal parent Barr Duke), Thomas, Rio Frio (Guatemala), G755A (*P. schiedeana* x *P. americana* seedling), VC 241 (Israel), Leo (Brokaw selection), Spencer seedling (Pauma Valley), Spencer (Pauma Valley), Guillemet (PP15-maternal parent Thomas), Duke 7 and Poly N (polyploid UCLA). Only Poly N and G755A are performing poorly in this trial. VC 241 may be a dwarfing rootstock. Rio Frio is a surprise and is performing very well. Merensky I and II, Uzi, and Steddom are all excellent rootstocks.

A 4-year-old trial established in Carpinteria, CA on root rot infested soil experienced some water stress in the past year. It rated as follows from healthiest to poorest (Table 2): Uzi (PP 14, maternal parent G6), Zentmyer (PP4-maternal parent Barr Duke), Merensky II (Dusa-South Africa), Merensky III (Evstro-South Africa), Merensky I (Latas-South Africa), Thomas, Mckee

(PP19-maternal parent UC 2001), Merensky IV (South Africa), Aguacate de Mico (Mexico) and Poly N (polyploid UCLA). Tree sizes from largest to smallest were: Uzi (PP14-maternal parent G6), Zentmyer (PP4-maternal parent Barr Duke), Merensky II (Dusa-South Africa), Thomas, Merensky III (Evstro-South Africa), Merensky I (Latas-South Africa), Mckee (PP19-maternal parent UC 2001), Merensky IV (South Africa), Aguacate de Mico (Mexico), and Poly N (polyploid-UCLA). Fruit set rating from heaviest to lightest is as follows: Zentmyer (PP4-maternal parent Barr Duke), Uzi (PP 14, maternal parent G6), Merensky I (Latas-South Africa), Merensky II (Dusa-South Africa), Thomas, Mckee (PP19-maternal parent UC 2001), Merensky III (Evstro-South Africa), Merensky IV (South Africa), Aguacate de Mico (Mexico), and Poly N (polyploid-UCLA). Only Uzi, Zentmyer, and Merensky II are now performing well in this trial. It appears that Uzi and Zentmyer are able to thrive even when stressed for water.

A 4-year-old trial in Escondido, CA on root rot infested soil was rated as follows from healthiest to poorest: Zentmyer (PP4-maternal parent Barr Duke), Thomas, Aguacate de Mico (Guatemala). Tree size ranked from largest to smallest was: Zentmyer (PP4-maternal parent Barr Duke), Thomas, Aguacate de Mico (Guatemala). Yield from heaviest to lightest was: Thomas, Zentmyer (PP4-maternal parent Barr Duke), Aguacate de Mico (Guatemala). Fruit yield was extremely light due to wind induced fruit drop. None of the varieties in this trial are performing adequately. Salt stress is impacting both the Zentmyer and Thomas.

A 3-year-old trial in Escondido, CA on root rot infested soil was rated as follows from healthiest to poorest: Thomas, Mckee (PP19, maternal parent UC2001), VC 256 (Israel). Tree size ranked from largest to smallest was: Mckee (PP19, maternal parent UC2001), Thomas, VC 256 (Israel). Yield from greatest to least was: VC 256 (Israel), Mckee (PP19, maternal parent UC2001), Thomas. Only the Thomas is performing adequately in this trial.

A 3-year-old trial established in Carpinteria CA in salty soil under heavy root rot pressure was rated as follows from healthiest to the poorest: Toro Canyon (Brokaw selection), Merensky II (Dusa, South Africa), Mckee (PP19, maternal parent UC2001), Zentmyer (PP4-maternal parent Barr Duke). Tree size was rated from largest to smallest was: Zentmyer (PP4-maternal parent Barr Duke), Toro Canyon (Brokaw selection), Merensky II (Dusa, South Africa), Mckee (PP19, maternal parent UC2001). Fruit set rating from largest to smallest was: Toro Canyon (Brokaw selection), Merensky II (Dusa, South Africa), Mckee (PP19, maternal parent UC2001), Zentmyer (PP4-maternal parent Barr Duke). None of the trees exhibited a heavy fruit set. Only Toro Canyon and Merensky II were performing adequately. Zentmyer was severely damaged by the salt.

A 2-year-old trial in Ventura, CA on root rot infested soil was rated as follow from healthiest to poorest: Zentmyer (PP4, maternal parent Barr Duke), Thomas, Pond (PP29, maternal parent G6), Mckee (PP19, maternal parent UC2001), D9. Tree size was rated from largest to smallest was: Thomas, Pond (PP29, maternal parent G6), Zentmyer (PP4, maternal parent Barr Duke), Mckee (PP19, maternal parent UC2001), D9. This is a severe site and only the Zentmyer is performing adequately.

A 2-year-old trial in Escondido, CA on root rot infested soil was rated as follows from healthiest to poorest: Uzi (PP 14, maternal parent G6), Berg (PP5, maternal parent D9), Zentmyer (PP4,

maternal parent Barr Duke), Merensky II (Dusa, South Africa), Steddom (PP24, maternal parent Toro Canyon), Pond (PP29, maternal parent G6), Spenser seedlings (Fuerte, Pauma Valley), Crowley (PP34, maternal parent UC2001), Thomas, Guillemet (PP15, maternal parent Thomas), Elinor (PP28, maternal parent D9), G755A (*P. schiedeana* x *P. americana* seedling), Duke 9 (irradiated Duke seedling). Tree size from largest to smallest was: Uzi (PP14, maternal parent G6), Merensky II (Dusa, South Africa), Elinor (PP28, maternal parent D9), Steddom (PP24, maternal parent Toro Canyon), Pond (PP29, maternal parent G6), Zentmyer (PP4, maternal parent Barr Duke), Spenser seedlings (Fuerte, Pauma Valley), Thomas, G755A (*P. schiedeana* x *P. americana* seedling), Berg (PP5, maternal parent D9), Guillemet (PP15, maternal parent Thomas), Crowley (PP34, maternal parent UC2001), Duke 9 (irradiated Duke seedling). All varieties except for D9 were performing adequately. Zentmyer, Uzi, Thomas and G755A were damaged by the salt.

Five new field trials were established in 2002, two in San Louis Obispo County., one in Santa Barbara County and two in San Diego County. These trials included: Merensky I (Latas, South Africa), Steddom (PP 24, breeding block, maternal parent Toro Canyon), Afeck (PP18, breeding block, maternal parent Thomas), Uzi (PP14, breeding block, maternal parent G6), VC 44 (West Indian, Israel), VC 225 (West Indian, Israel), VC 241 (West Indian, Israel), VC 207 (Day, Israel), Martin (PP26, breeding block material, maternal parent D9), Zentmyer (PP4, maternal parent Barr Duke), Anita (PP35, maternal parent UC 2001), Campbell (PP43, maternal parent UC 2001), Witney (PP41, maternal parent D9), Frolic (PP 37, maternal parent D9), Johnson (PP42, maternal parent D9), Fred (PP44, maternal parent UC2001), Eddie (PP40, maternal parent Toro Canyon), Dirac (PP36, maternal parent UC2001), Elinor (PP28, maternal parent D9), Margy (PP33, maternal parent D9), Toro Canyon (Brokaw) and Thomas.

Trees being propagated for 2004 include: Erin (PP21,maternal parent Duke 9), Martin (PP26, maternal parent Duke 9), Elinor (PP28, maternal parent Duke 9), Pond (PP29, maternal parent G6), Margy (PP33, maternal parent Duke 9), Crowley (PP34, maternal parent UC 2001), Anita (PP35, maternal parent UC 2001), Dirac (PP36, maternal parent UC 2001), Frolic (PP37, maternal parent Duke 9), Eddie (PP40, maternal parent Toro Canyon), Witney (PP41, maternal parent Duke 9), Berg (PP5, maternal parent Duke 9), Uzi (PP14, maternal parent G6), Rio Frio (Guatemala), Medina (PP22, maternal parent, Thomas), Merensky I (Latas, South Africa) VC207 (Day, Israel), VC 218 (West Indian, Israel), VC 225 (West Indian, Israel), CI #2 (West Indian, Canary Islands) and Downer (PP52, Spencer seedling).

### **Conclusions**

It appears that we have several rootstocks that are consistently performing better than our standard resistant variety, Thomas under root rot conditions. These are Uzi (PP14-maternal parent G6), Merensky I (Latas –South Africa), and Steddom (PP24-maternal parent Toro Canyon). Zentmyer (PP4- maternal parent Barr Duke) is also growing well but shows some saltburn and has consistently low yields. We are preparing to release these 4 rootstocks to growers. New rootstocks which appear to be showing promise are Rio Frio (Guatemala), Medina (PP22, maternal parent Thomas) and Afeck (PP18, maternal parent Thomas). We have established a new salt resistance-breeding block where, with the help of David Crowley, we hope to select rootstocks resistant to salt.

Table 1. Four-year-old field plot in Phytophthora-infested soil in Escondido CA, 2003<sup>1</sup>

Rootstock	Tree rating (0-5; 5=dead)	Canopy volume (cu ft)	Trunk diam. (cm)	Salt (0-5;5=heavy)	Cankers (05;5=heavy)	Dead trees %
Merensky I	0.00d	551ab	10.7a	0.08cd	0a	0
VC241	0.06d	281efgh	8.0abc	0.03cd	0a	0
RioFrio	0.07d	362efcd	8.7abc	0.00d	0a	0
Zentmyer	0.07d	410bcde	9.2ab	0.32bc	0a	0
Merensky II	0.18d	532abc	9.4ab	0.21dc	0.1a	0
Spenser sdlg.	0.36d	263efgh	6.9bc	0.00d	0a	7
Uzi	0.38d	669a	10.6a	0.68a	0a	6
Steddom	0.39d	478bcd	8.6 abc	0.32bc	0a	7
Thomas	0.47cd	367cdef	8.4abc	0.62ab	0a	6
Leo	0.77cbd	274efgh	7.3abc	0.13cd	0a	13
Guillemet	0.83cbd	190ghi	6.2bc	0.13cd	0a	13
Duke7	1.34cb	127hi	8.8abc	0.16cd	0a	19
Spenser cl.	1.44b	211fghi	5.3c	0.12cd	0a	23
G755A	1.69b	322defg	7.0bc	0.25cd	0a	25
PolyN	4.15a	77i	1.5d	0.06cd	0a	82

<sup>1</sup>Mean values in each column followed by identical letters are not statistically different according to Waller's k-ratio t test

Table 2. Four-year-old drought-stressed field plot in Phytophthora-infested soil in Carpinteria CA, 2003 <sup>1</sup>

Rootstock	Tree rating (0-5; 5=dead)	Canopy vol (cu ft)	Trunk diam (cm)	Fruit set	Canker	Salt	Dead trees (%)
				(0-5; 5=heavy)			
Uzi	0.72 f	167.5 a	6.51 a	3.25 a	0.85 c	2.15 a	6
Zentmyer	1.06 ef	140.0 ab	6.31 a	3.28 a	0.58 c	1.44 ab	0
Merensky II	1.50 def	104.5 bc	5.36 ab	2.63 abc	0.76 c	0.85 b	11
Merensky III	1.71 de	74.4 cde	4.86 bc	1.53 c	1.27 c	0.63 b	11
Merensky I	2.13 cd	72.5 cde	4.83 bc	2.71 ab	1.72 bc	0.63 b	16
Thomas	2.63 bc	77.7 cd	4.12 bcd	2.37 abc	1.12 c	2.12 a	32
McKee	3.29 b	50.2 de	2.85 d	1.61 bc	1.56 bc	1.78 a	53
Merensky IV	3.42 b	36.8 ef	3.47 cd	1.53 c	1.46 bc	0.58 b	32
Aquacate	4.92 a	1.6 f	0.52 e	0.00 d	3.00 ab	0.67 b	84
PolyN	4.95 a	0.7 f	0.34 e	0.00 d	4.50 a	2.00 a	95

<sup>1</sup> Mean values in each column followed by identical letters are not statistically different according to Waller's k-ratio t test.