

Avocado Rootstock Research Program at UCR

G. W. Douhan
Department of Plant Pathology
and Microbiology
University of California, Riverside, USA

Overview

Host-pathogen system

Breeding program

Rootstock AFLP analysis

Pathogen population biology

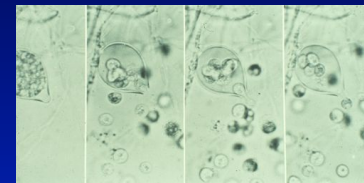
Avocado Root Rot

- “Avocado tree decline” noted in the 1920’ s in California



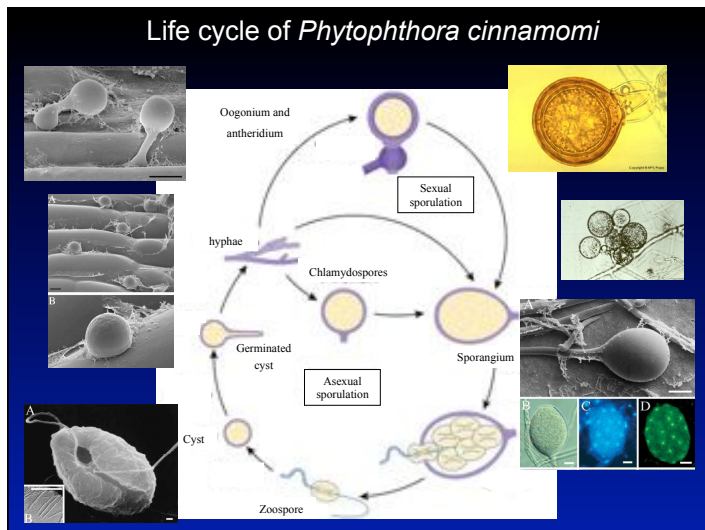
Avocado Root Rot

- “Avocado tree decline” noted in the 1920’ s in California
- *P. cinnamomi* identified in the early 1940’ s as the causal agent



Root Rot Survey in 1969
CAS yearbook 1969, Platt


	San Diego	Orange	Los Angeles	Riverside	San Bernardino	Ventura	Santa Barbara	Tulare-Fresno	State Total
No. properties reporting	886	102	83	12	8	138	189	12	1430
Total acres reporting	6603	549	253	40	41	1821	1438	87	10832
% of county total acres	51	42	20	8	34	57	58	72	50
No. properties w/o root rot	463	44	41	8	8	116	107	9	796
% of total reporting	52	43	49	67	100	84	57	75	56
No. properties with root rot	423	58	42	4	0	22	82	3	634
% of total reporting	48	57	51	33	0	16	43	25	44
No. trees known infected	17306	2562	4257	42	0	786	6125	41	31119
No. trees suspect infection	9965	2273	1544	91	0	1963	2195	0	18031
No. trees removed	31335	3985	3114	139	0	678	5681	36	44968



Host: Avocado (*Persea sp.*)

California production: ~ 50,000 acres
California value 2010/11: \$445,902,671

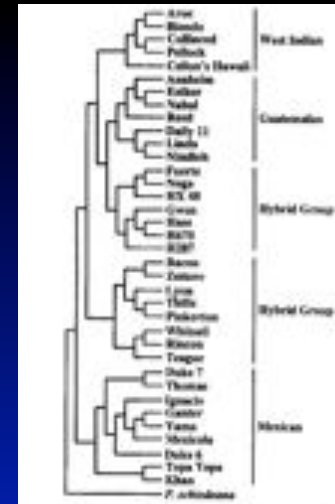
Source: www.avocado.org



- San Diego (34%)
- Riverside (13%)
- Ventura (12%)
- Santa Barbara (12%)
- San Luis Obispo (8%)
- Minor Counties (9%)

Avocado: *Persea americana*

Type	Leaves	Bloom/Harvest Season	Fruit Skin	Hardiness (cold tolerance)
West Indian	no anise scent	Blooms in the spring, and the fruit ripens in the summer of the same year	thin skin and unusually smooth	poor
Guatemala n	no anise scent	Blooms in the spring, and ripens in the spring & summer of the following year	thick skin, often rough	medium
Mexica n	usually has an anise scent	Blooms in the winter, and the fruit ripens the following summer & fall	thin skin and usually smooth	good



Davis *et al.* 1998
Heredity 89:319-323

Avocado production: scion versus rootstock

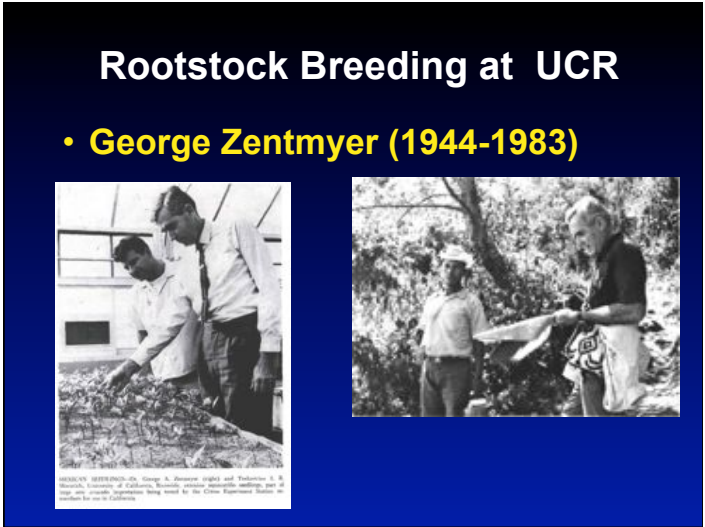
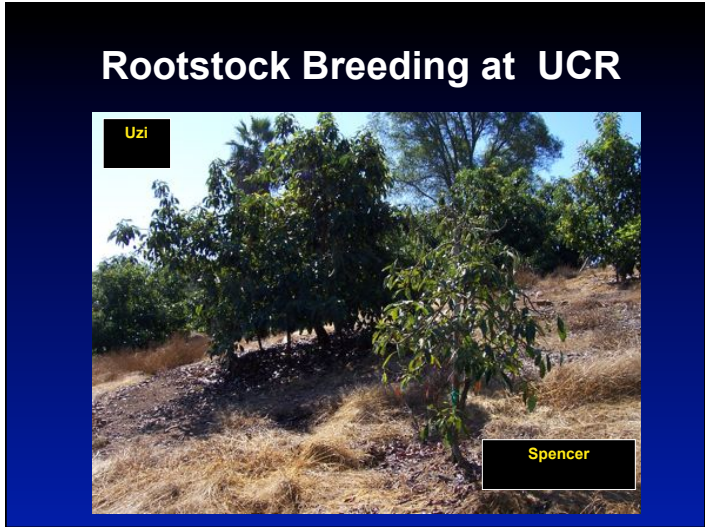
- Scion (Hass) grafted to random seedling rootstock
- Rootstock cloning; rootstock (Uzi) grafted to random seedling then selected scion grafted to selected rootstock



Control

- Nursery practices

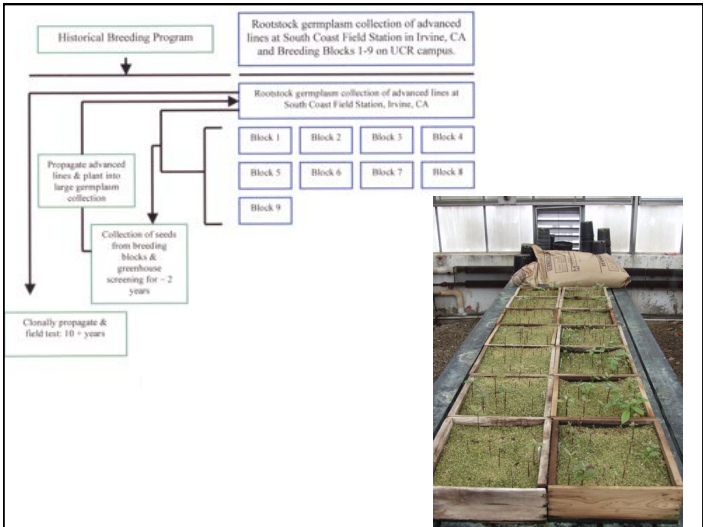




- **George Zentmyer**

“It wasn’t so much that I chose to focus on this, but that research was needed in this area.” “When I came here, avocado root rot was rather an obscure disease. The fungus had just been isolated the year before, and scientists thought at the time that it was mainly water-related. It was very difficult to control, and damage it caused was just terrific.”

George A. Zentmyer, Retired but never quite finished, by: Lisa Dunlap (June 2000) UCR Flat Flux



Rootstock Breeding at UCR Greenhouse screening selection

Year	No. seeds screened	No. of seedlings saved for further screening	No. of final advanced selections
1989	49	0	0
1990	2410	44	0
1991	1260	11	0
1992	6207	46	4 = .06%
1993	5752	24	5 = .09%
1994	4278	15	3 = .13%
1995	2337	7	3 = .13%
1996	3869	18	3 = .08%
1997	2535	16	12 = .47%
1998	1985	18	9 = .45%
1999	5180	66	8 = .15%
2000	3801	21	3 = .08%
2001	2304	16	3 = .13%
2002	1566	58	3 = .19%
2003	1728	81	2 = .12%
2004	612	20	3 = .49%
2005	3425	227	10 = .29%
2006	1584	126	6 = .38%
2007	3893	185	8 = .21%
2008	1118	60	4 = .36%
2009	2607	122	5 = .19%
Total	58500	1181	101

Maternal avocado varieties and advanced selections in the UC program

Duke 7	Thomas	G6	Spencer	Duke 9	UC 2001	Harr Duke	Toro Canyon	PP 4	PP 29	PP 36	PP 40	PP 52	PP 57	PP 80	PP 81	VC 7	VC 66	VC 256	VC 801	
PP 62	PP 4	PP 14	PP 48	PP 5	PP 19	PP 157	PP 24	GD 18	GD 2	GD 8	PP 65	GD 27	GD 5	GD 16	GD 22	GD 24	GD 25	GD 11	GD 26	
PP 96	PP 157	PP 22	PP 49	PP 21	PP 34		PP 40				GD 1	GD 28								
GD 7	PP 18	PP 25	PP 50	PP 26	PP 35						GD 3									
GD 12	PP 55	PP 29	PP 51	PP 28	PP 36						GD 4									
GD 13	PP 68	PP 60	PP 52	PP 33	PP 43						GD 10									
GD 14	PP 69	PP 83	PP 88	PP 37	PP 44															
GD 23	GD 9	PP 89	PP 80	PP 41	PP 57															
	GD 17	PP 94		PP 42	PP 57															
				PP 43	PP 56															
				PP 53	PP 61															
				PP 54	PP 63															
				PP 56	PP 70															
				PP 59	PP 71															
				PP 62	PP 72															
				PP 67	PP 73															
				PP 79	PP 80															
				PP 85	PP 81															
				GD 15	PP 82															
				GD 16	PP 84															
					PP 85															
					PP 87															
					PP 90															
					PP 91															
					GD 6															

Maternal avocado varieties and advanced selections in the UC program

Duke 7	Thomas	G6	Spencer	Duke 9	UC 2001	Harr Duke	Toro Canyon	PP 4	PP 29	PP 36	PP 40	PP 52	PP 57	PP 80	PP 81	VC 7	VC 66	VC 256	VC 801	
PP 62	PP 4	PP 14	PP 48	PP 5	PP 19	PP 157	PP 24	GD 18	GD 2	GD 8	PP 65	GD 27	GD 5	GD 16	GD 22	GD 24	GD 25	GD 11	GD 26	
PP 96	PP 157	PP 22	PP 49	PP 21	PP 34		PP 40				GD 1	GD 28								
GD 7	PP 18	PP 25	PP 50	PP 26	PP 35						GD 3									
GD 12	PP 55	PP 29	PP 51	PP 28	PP 36						GD 4									
GD 13	PP 68	PP 60	PP 52	PP 33	PP 43						GD 10									
GD 14	PP 69	PP 83	PP 88	PP 37	PP 44															
GD 23	GD 9	PP 89	PP 80	PP 41	PP 57															
	GD 17	PP 94		PP 42	PP 57															
				PP 43	PP 56															
				PP 53	PP 61															
				PP 54	PP 63															
				PP 56	PP 70															
				PP 59	PP 71															
				PP 62	PP 72															
				PP 67	PP 73															
				PP 79	PP 80															
				PP 85	PP 81															
				GD 15	PP 82															
				GD 16	PP 84															
					PP 85															
					PP 87															
					PP 90															
					PP 91															
					GD 6															

Rootstock Breeding at UCR

- Since 1989, 58,500 seedlings screened
- 42 advanced selections are currently being tested under field conditions (6 SA & 2 Chile)



Low Disease Pressure

Fallbrook, harvest April 2006

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

Moderate to Heavy Disease Pressure

Escondido CA, Harvest, April 2006

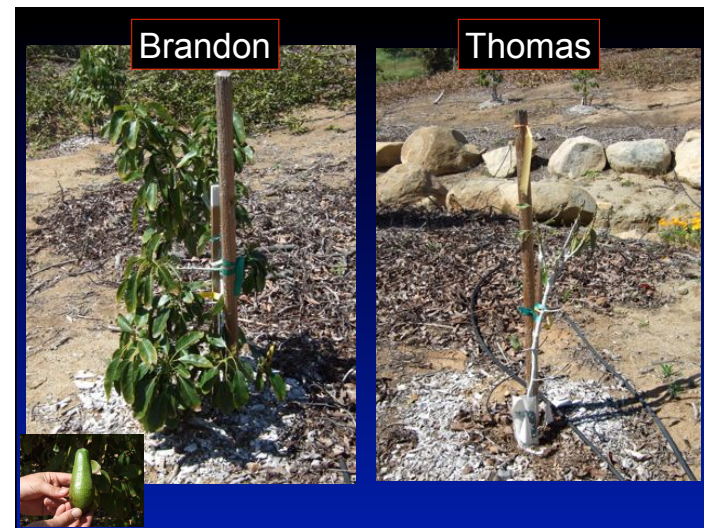
Rootstock	Total fruit weight per tree (kg)	Individual fruit weight (kg)
Latas	26.34a	0.233a
Steddom	23.76ab	0.233a
Toro Canyon	22.90ab	0.241a
VC207	20.95ab	0.232a
Uzi	18.86b	0.229a
VC225	12.93c	0.234a
Afek	9.52cd	0.229a
VC241	6.33de	0.224a
Thomas	4.33de	0.242a
VC44	2.85e	0.242a

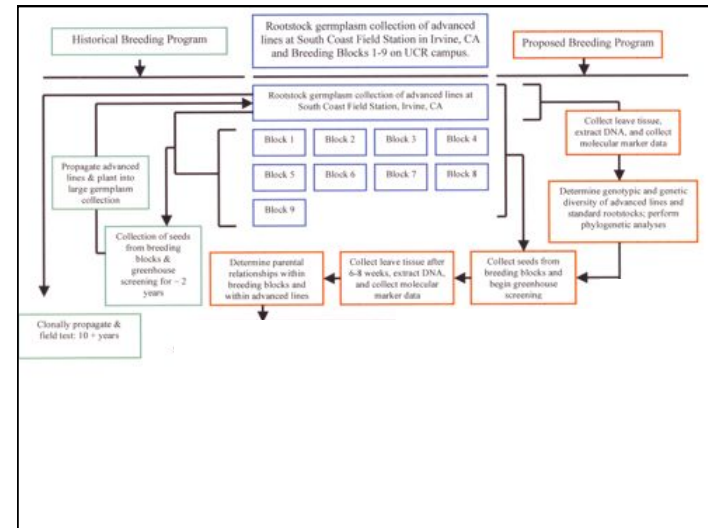
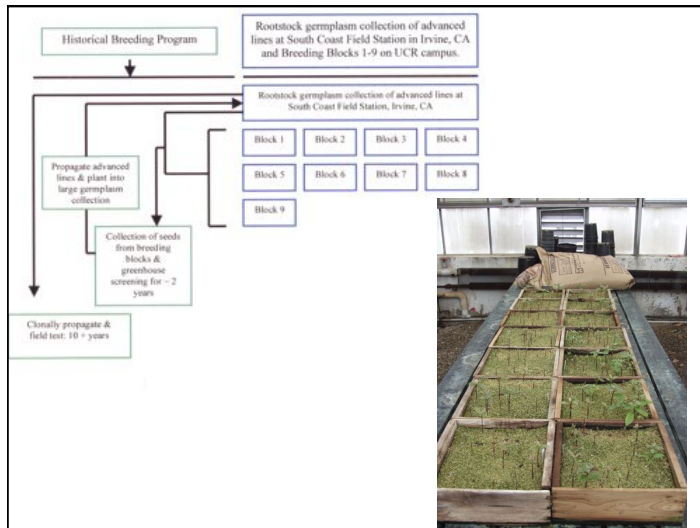
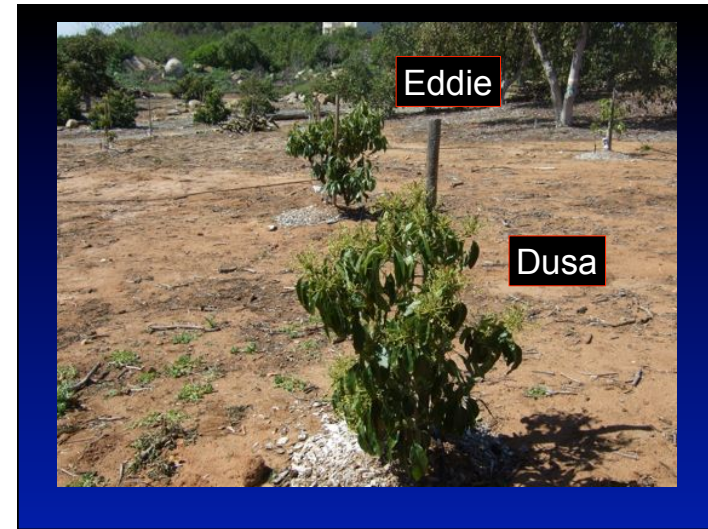
Recently Released Rootstocks

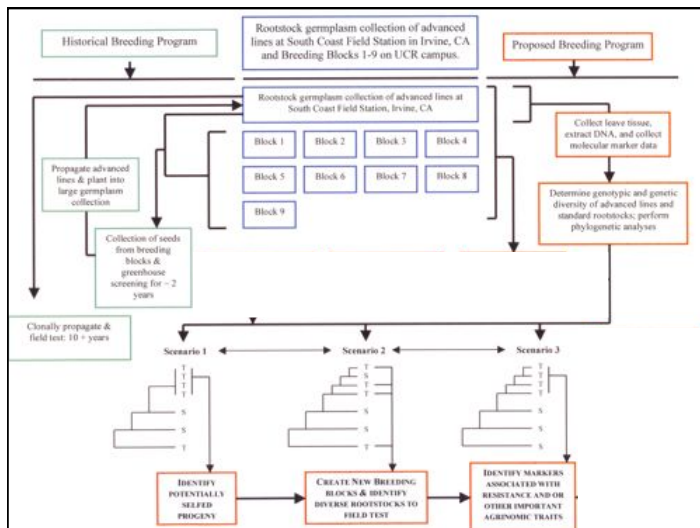
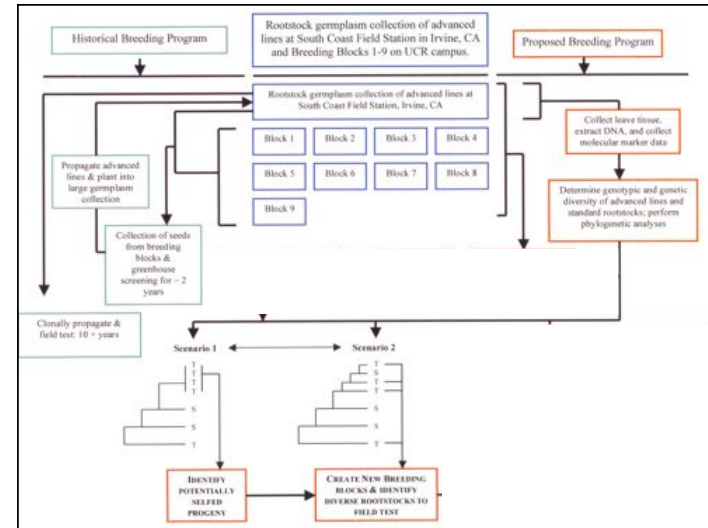
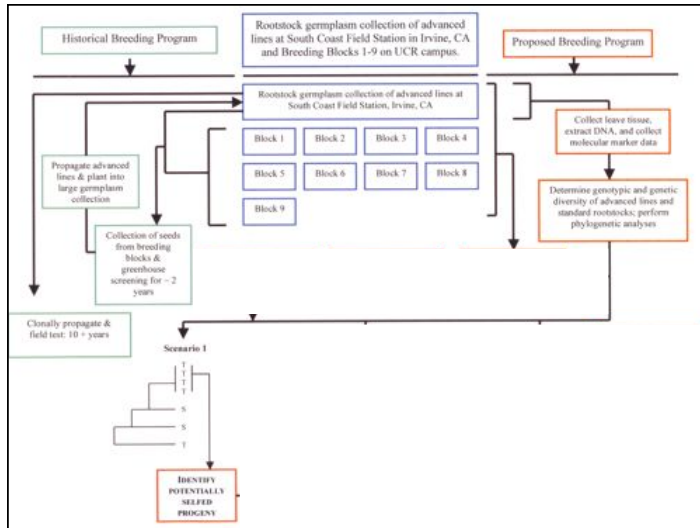
'Zentmyer' UC 1999-082

'Uzi' UC 1999-088

'Steddom' UC 1999-098







Genetic diversity analysis of Avocado (*Persea americana* Miller) rootstocks selected under greenhouse conditions for tolerance to Phytophthora Root Rot (PRR) caused by *Phytophthora cinnamomi*

- **Objectives:**
 - Determine genetic diversity of selected rootstocks
 - Determine if any trends could be in seen in the clustering of rootstocks

Diversity of avocado rootstocks

• Methods

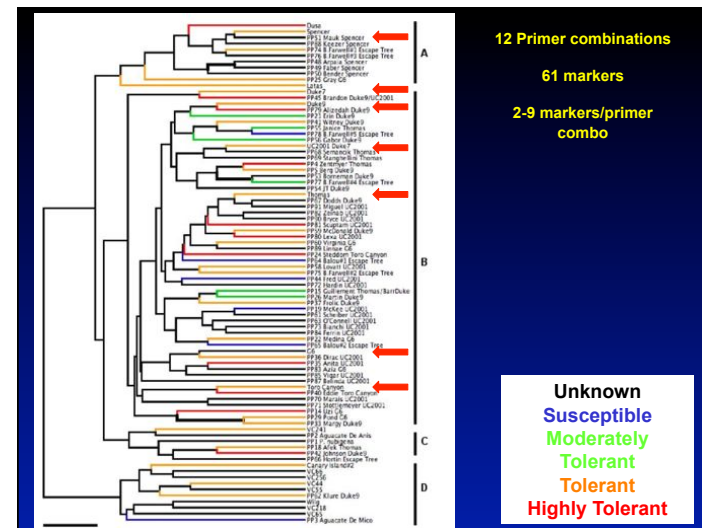
- Collection plant material
- Extract DNA
- Use Amplified fragment length polymorphisms (AFLP) to detect date
- UPGMA

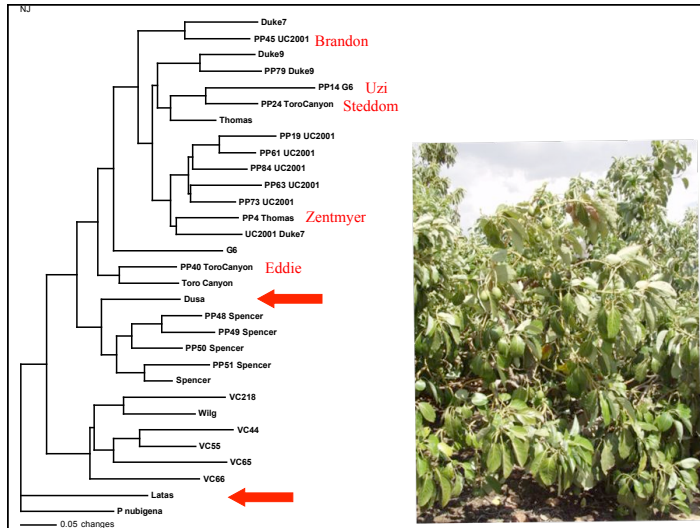
Table 1. Information regarding the avocado rootstock selections used in this study.

Rootstock No.	Rootstock variety	Maternal Parent	PRR ^a tolerance	Salt tolerance	Origin	Tree stature ^b	Years tested	No. of locations
PP1	<i>P. nubiligena</i>	ND	ND	ND	ND	ND	ND	ND
PP2	Aguate De Anis	ND	ND	ND	Honduras	ND	ND	ND
PP3	Aguate De Mico	ND	S	NS	Guatemala	small	5	2
PP4	Zentmyer	Thomas	HT	MT	UCR	large	12	29
PP5	Berg	Duke 9	T	T	UCR	medium	10	8
PP14	Uzi	G6	HT	S	UCR	large	11	21
PP15	Guillemet	Thomas or Barr Duke	MT	T	UCR	medium	8	8
PP18	Afek	Thomas	T	S	UCR	large	9	12
PP19	McKee	UC2001	S	T	UCR	medium	11	10
PP21	Erin	Duke 9	MT	T	UCR	small	9	7
PP22	Medina	G6	T	T	UCR	medium	10	7
PP24	Steddum	Toro Canyon	HT	MT	UCR	large	11	17
PP25	Gray	G6	T	HT	UCR	small	3	2
PP26	Martin	Duke 9	T	T	UCR	large	7	6
PP29	Pond	G6	MT	T	UCR	large	6	10
PP33	Margy	Duke 9	MT	T	UCR	large	4	6
PP35	Anita	UC 2001	HT	T	UCR	large	8	17
PP36	Dirac	UC 2001	T	MT	UCR	large	7	7
PP37	Frolic	Duke 9	T	T	UCR	small	8	9
PP40	Eddie	Toro Canyon	HT	T	UCR	large	6	9
PP41	Witney	Duke 9	T	T	UCR	medium	8	13
PP42	Johnson	Duke 9	HT	T	UCR	large	7	7
PP43	Campbell	UC2001	S	T	UCR	small	7	5
PP44	Fred	UC2001	S	S	UCR	large	3	6
PP45	Brandon	D9 or UC2001	HT	S	UCR	large	5	10
PP47	Canary Isl. #2	ND	T	HT	ND	large	6	5

Rootstock No.	Rootstock variety	Maternal Parent	PRR ^a tolerance	Salt tolerance	Origin	Tree stature ^b	Years tested	No. of locations
PP48	Apsia	Spencer	ND	ND	UCR	ND	0	0
PP49	Faber	Spencer	ND	ND	UCR	ND	1	4
PP50	Bender	Spencer	ND	ND	UCR	ND	1	3
PP51	Mauk	Spencer	ND	ND	UCR	ND	0	0
PP52	Downer	Spencer	MT	MT	UCR	large	6	4
PP53	Borneman	Duke 9	ND	ND	UCR	ND	0	0
PP54	JT	Duke 9	ND	ND	UCR	ND	1	3
PP55	Janice	Thomas	MT	T	UCR	large	2	8
PP56	Gabor	Duke 9	NRR	NS	UCR	large	4	2
PP58	Lovatt	UC2001	NRR	NS	UCR	small	4	3
PP59	McDonald	Duke 9	ND	ND	UCR	medium	1	3
PP60	Virginia	G6	T	T	UCR	medium	2	6
PP61	Schieber	UC2001	ND	ND	UCR	medium	1	3
PP62	Klure	Duke 9	ND	ND	UCR	medium	1	4
PP63	OConnell	UC2001	NRR	NS	UCR	large	4	1
PP64	Balou #1	Escape Tree	S	T	Fallbrook	small	3	5
PP65	Balou #2	Escape Tree	S	T	Fallbrook	small	3	5
PP66	Hortin	Escape Tree	ND	ND	Escondido	ND	0	0
PP67	Dodds	Duke 9	ND	ND	UCR	ND	1	2
PP68	Semancik	Thomas	ND	ND	UCR	ND	1	2
PP69	Stanghellini	Thomas	ND	ND	UCR	ND	0	0
PP70	Maras	UC2001	ND	ND	UCR	ND	0	0
PP71	Stottlemeyer	UC2001	ND	ND	UCR	ND	0	0
PP72	Hardin	UC2001	ND	ND	UCR	ND	0	0
PP73	Bianchi	UC2001	ND	ND	UCR	ND	0	0
PP74	B Farwell#1	Escape Tree	T	MT	Fallbrook	medium	3	3
PP75	B Farwell#2	Escape Tree	T	T	Fallbrook	medium	3	5
PP76	B Farwell#3	Escape Tree	ND	ND	Fallbrook	ND	0	0

...ect





Conclusions

- Advanced selections are genotypically diverse
- No obvious trends were evident based on the cluster analysis
- Some insights were gained about potential parentage/inbreeding
- Results will help in creating new breeding blocks

Population genetic structure of *Phytophthora cinnamomi* Rands associated with Phytophthora root rot of Avocado (*Persea americana* Miller) within California

Diagram illustrating the life cycle of *Phytophthora cinnamomi*, showing sporangia and avocado fruit. The University of California Riverside logo is present at the bottom left.

Objectives of this study:


- To **characterize isolates** of *P. cinnamomi* collected from both the Northern and Southern avocado growing regions within California using mating-type and molecular markers (AFLP);
- To determine the **genotypic diversity** of the pathogen population;
- To test for **population structure** based on growing region to make inferences on potential **pathogen movement, gene-genotype flow.**

Material & Methods

Isolate Collection

15 locations sampled- separated by 0.5 Km to 300 Km – 138 samples;

- 7 locations from the northern avocado growing region (N1-N7);
- 8 locations from the southern avocado growing region (S1-S8)



Isolate /Population	No. of isolates	Country /State	County	Host /origin	Date
N1	2	CA	Ventura	Avocado	2010
N2	2	CA	Ventura	Avocado	2009
N3	15	CA	Ventura	Avocado	2009/10
N4	16	CA	Ventura	Avocado	2009/10
N5	1	CA	Ventura	Avocado	2010
N6	13	CA	S. Barbara	Avocado	2010
N7	8	CA	S. Barbara	Avocado	2010
S1	18	CA	Riverside	Avocado	2009
S2	13	CA	Riverside	Avocado	2009
S3	14	CA	Riverside	Avocado	2009
S4	5	CA	San Diego	Avocado	2009
S5	6	CA	San Diego	Avocado	2009
S6	11	CA	San Diego	Avocado	2009
S7	6	CA	San Diego	Avocado	2009
S8	8	CA	San Diego	Avocado	2009


Isolate /Population	No. of isolates	Country /State	County	Host /origin	Date	Mating-type*
M253	1	CA	San Diego	Avocado	1993	?
M254	1	CA	San Diego	Avocado	1993	?
M262	1	CA	San Diego	Avocado	1994	?
M280	1	CA	Ventura	Avocado	1995	?
M281	1	CA	Ventura	Avocado	1995	?
M282	1	CA	Ventura	Avocado	1995	?
M293	1	CA	Ventura	Avocado	1996	?
M295	1	CA	Ventura	Avocado	1996	A2
M381	1	CA	Riverside	Avocado	2006	?
M382	1	CA	San Diego	Avocado	2007	?
M385	1	CA	S. Barbara	Avocado	2007	?
M386	1	CA	S. Barbara	Avocado	2007	?
A1495	1	CA	Los Angeles	Pine	1975	A2
A1496	1	CA	Riverside	Azalea	1976	A2
A1498	1	FI	ND ^a	Avocado	1989	A2
A1574	1	CA	Riverside	Rhododendron	1976	A2
A1494	1	China	ND	Cameallia	1986	A1
A1497	1	CA	S. Barbara	Avocado	1970	A1
A1575	1	Madagascar	ND	Avocado	1966	A1
A1576	1	Papa New Guinea	ND	Soil	ND	A1
A1579	1	China	ND	Rhododendron	1989	A1
A1580	1	South Africa	ND	Grape	ND	A1
A1581	1	Taiwan	ND	Pineapple	1989	A1

Material & Methods

Mating Type

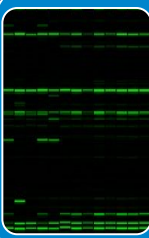
Each isolate was crossed with

- 1) a known A1 mating type isolate (M416 from *Camellia*);
- 2) a known A2 isolate (M295 A2 from Avocado);
- 3) and crossed with itself.



AFLP

- Fluorescently labeled primers FAM and HEX were used to resolve the markers via a capillary sequencer
- 12 primer sets tested on 8 tester isolates – chose the best 4
- SCORING - Genographer 2.0 fragment analysis software



RESULTS

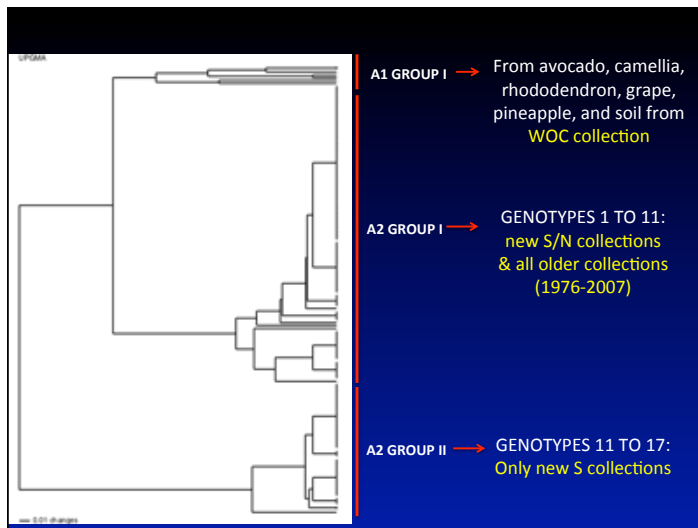
Mating Type

All isolates collected from the 15 locations belong to the A2 mating type.

Isolate /Population	No. of isolates	Country /State	County	Host /origin	Date	Mating-type*
N1	2	CA	Ventura	Avocado	2010	A2
N2	2	CA	Ventura	Avocado	2009	A2
N3	15	CA	Ventura	Avocado	2009/10	A2
N4	16	CA	Ventura	Avocado	2009/10	A2
N5	1	CA	Ventura	Avocado	2010	A2
N6	13	CA	S. Barbara	Avocado	2010	A2
N7	8	CA	S. Barbara	Avocado	2010	A2
S1	18	CA	Riverside	Avocado	2009	A2
S2	13	CA	Riverside	Avocado	2009	A2
S3	14	CA	Riverside	Avocado	2009	A2
S4	5	CA	San Diego	Avocado	2009	A2
S5	6	CA	San Diego	Avocado	2009	A2
S6	11	CA	San Diego	Avocado	2009	A2
S7	6	CA	San Diego	Avocado	2009	A2
S8	8	CA	San Diego	Avocado	2009	A2

Isolate /Population	No. of isolates	Country /State	County	Host /origin	Date	Mating-type*
M253	1	CA	San Diego	Avocado	1993	A2
M254	1	CA	San Diego	Avocado	1993	A2
M262	1	CA	San Diego	Avocado	1994	A2
M280	1	CA	Ventura	Avocado	1995	A2
M281	1	CA	Ventura	Avocado	1995	A2
M282	1	CA	Ventura	Avocado	1995	A2
M293	1	CA	Ventura	Avocado	1996	A2
M295	1	CA	Ventura	Avocado	1996	A2
M381	1	CA	Riverside	Avocado	2006	A2
M382	1	CA	San Diego	Avocado	2007	A2
M385	1	CA	S. Barbara	Avocado	2007	A2
M386	1	CA	S. Barbara	Avocado	2007	A2
A1495	1	CA	Los Angeles	Pine	1975	A2
A1496	1	CA	Riverside	Azalea	1976	A2
A1498	1	FI	ND*	Avocado	1989	A2
A1574	1	CA	Riverside	Rhododendron	1976	A2
A1494	1	China	ND	Cameallia	1986	A1
A1497	1	CA	S. Barbara	Avocado	1970	A1
A1575	1	Madagascar	ND	Avocado	1986	A1
A1576	1	Papa New Guinea	ND	Soil	ND	A1
A1579	1	China	ND	Rhododendron	1989	A1
A1580	1	South Africa	ND	Grape	ND	A1
A1581	1	Taiwan	ND	Pineapple	1989	A1

Distribution of genotypes																		
Genotype	Multilocus data	N1	N2	N3	N4	N5	N6	N7	S1	S2	S3	S4	S5	S6	S7	S8	No. of times found	No. of populations observed in
1	1000011000001100001010		1	1	10	1	1	8	15			3			4	1	45	10
2	1000011000001100001000	2						12								5	19	3
3	1000011000001100001011		1						2							2	5	3
4	1000001000001100001010								1								1	1
5	1011011000001100001010					4											4	1
6	0000011000001100000010				1												1	1
7	1000011000001011001010														1		1	1
8	1000010010000100001110		7														7	1
9	10000100100001100001110					7											7	1
10	10110100100001100001110			1													1	1
11	0011010011110011000100									13	11						24	2
12	0011010011110011000101										3						3	1
13	0011010011010011000100											2	6	6			14	3
14	0011010011010011000101													3			3	1
15	0011010001010011000100														2		2	1
16	0011010011110100000100															1	1	1



Conclusions

- Population genetic structure exists in CA
- Low genotypic diversity, one mating type and no evidence of sexual reproduction
- Potential new introduction into Southern CA?
- Will this impact disease control?

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