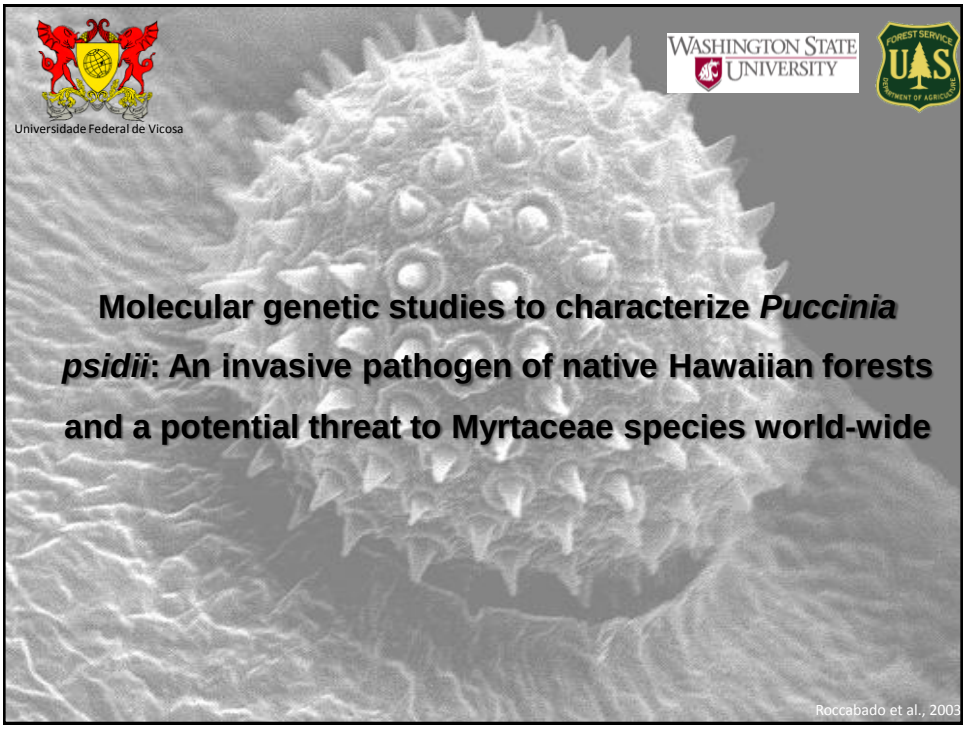

Rodrigo Neves Graça (Federal University of Vicosa - UFV, Brazil)
Acelino Couto Alfenas (Federal University of Vicosa - UFV, Brazil)
Cristina Pierrot Aun (Federal University of Vicosa - UFV, Brazil)
Tobin L. Peever (Washington State University – WSU)
Ned Klopfenstein (USDA – Forest Service, Moscow - ID)
Amy Ross-Davis (USDA – Forest Service, Moscow - ID)
Phil Cannon (USDA – Forest Service, Vallejo - CA)
Mee-Sook Kim (Kookmin University - Korea)

Collaborators:
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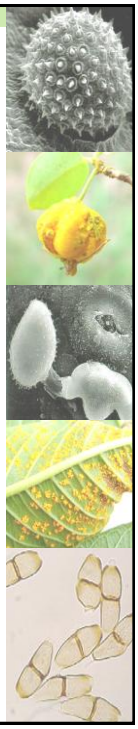
Chris Y. Kadooka ((University of Hawai‘i at Mänoa, Hawai’i)
Anne Marie LaRosa (USDA Forest Service, Hawaii)
Robert Hauff (Hawai'i Department of Land and Natural Resources)
Sofia Cimento (National Institute for Agriculture Investigation – Uruguay)
Morag Glen (Australian Centre for International Agricultural Research-Australia)

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Rio Grande do Norte
Paraíba
Pernambuco
Alagoas
Sergipe
Piauí
Brasil
Brazil
Perú
Acre
Rondônia
Tocantins
Bahia
Mato Grosso
Goiás
Bolivia
Minas Gerais
Espírito Santo
Rio de Janeiro
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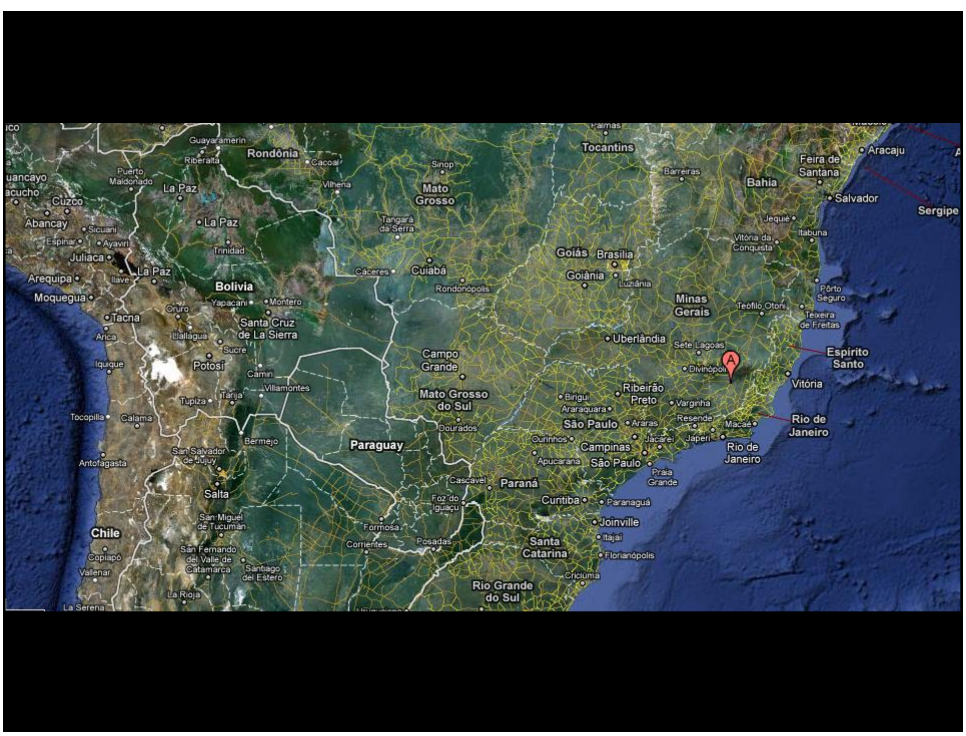
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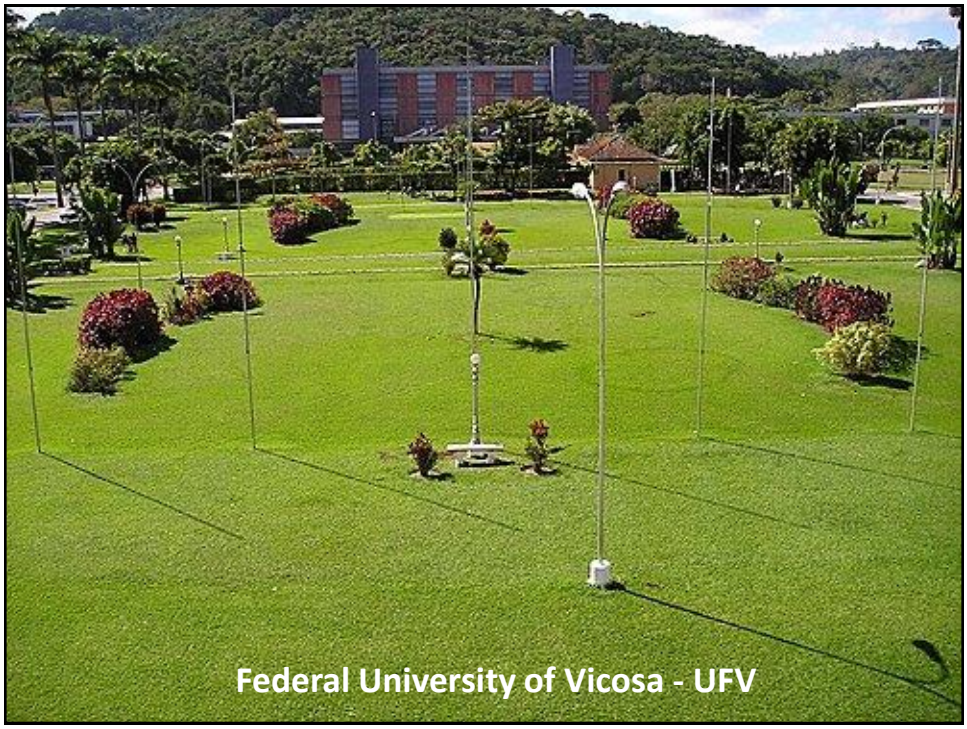
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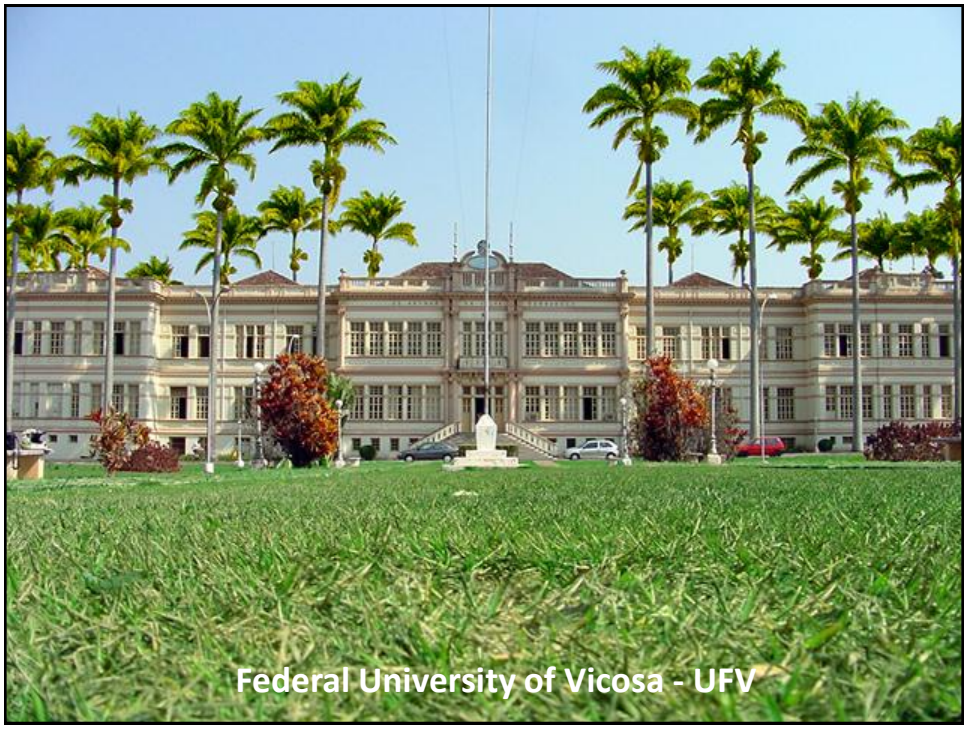
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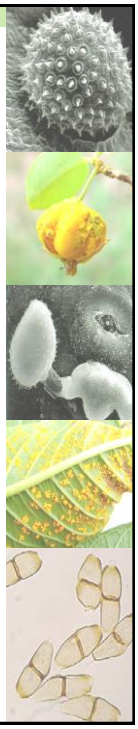
OVERVIEW

<u>BACKGROUND</u>

- Pathogen characteristics
- Resistance mechanism
- Disease control
- Host range
- Physiologic variability
- Resistance gene breakdown
- *Puccinia psidii* in Hawai'i

<u>GENETIC STUDIES</u>

- Molecular studies to assess *P. psidii* genetic diversity



Guava, Eucalypt, Ohi'a or Myrtaceae rust (*Puccinia psidii*)

Psidium guajava (Guava)

(G. Winter, 1884)

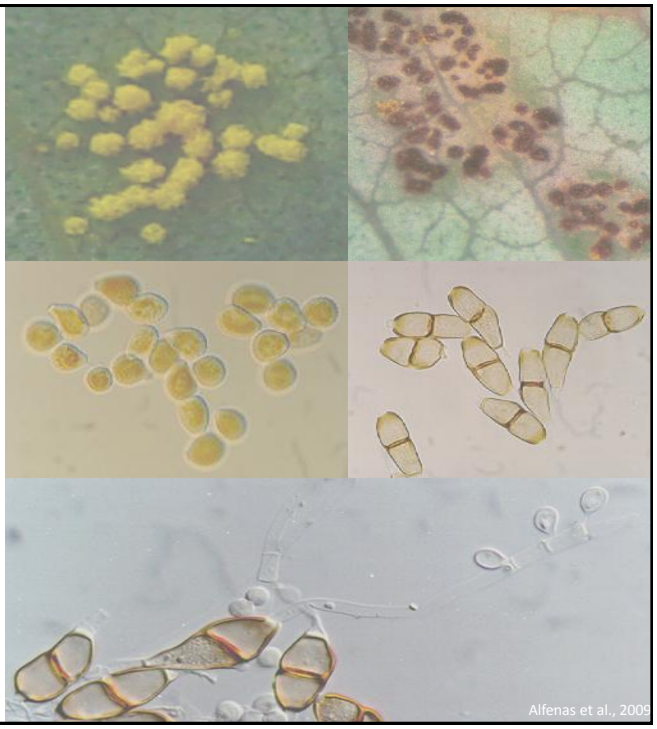
Corymbia citriodora

(=*Eucalyptus citridodora*) (1912)

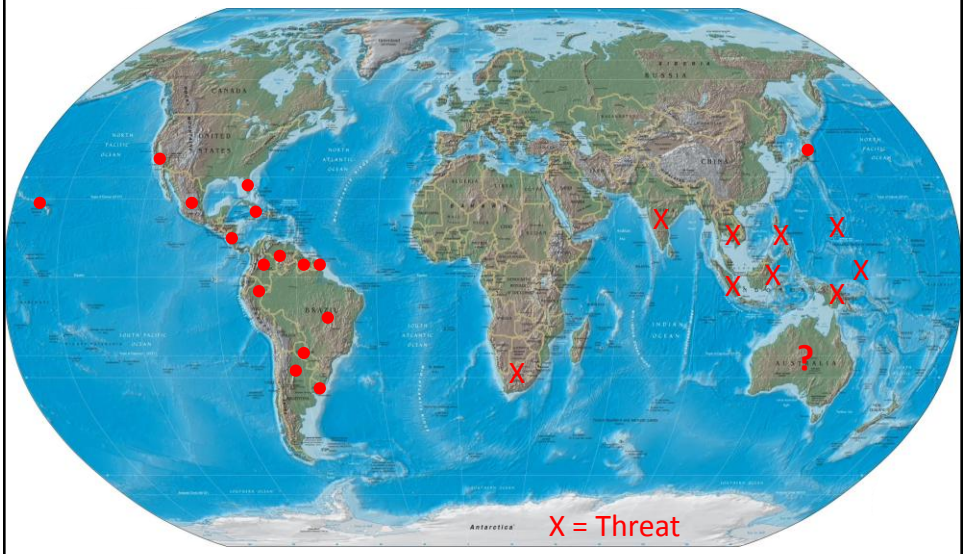
(Joffily , 1944)

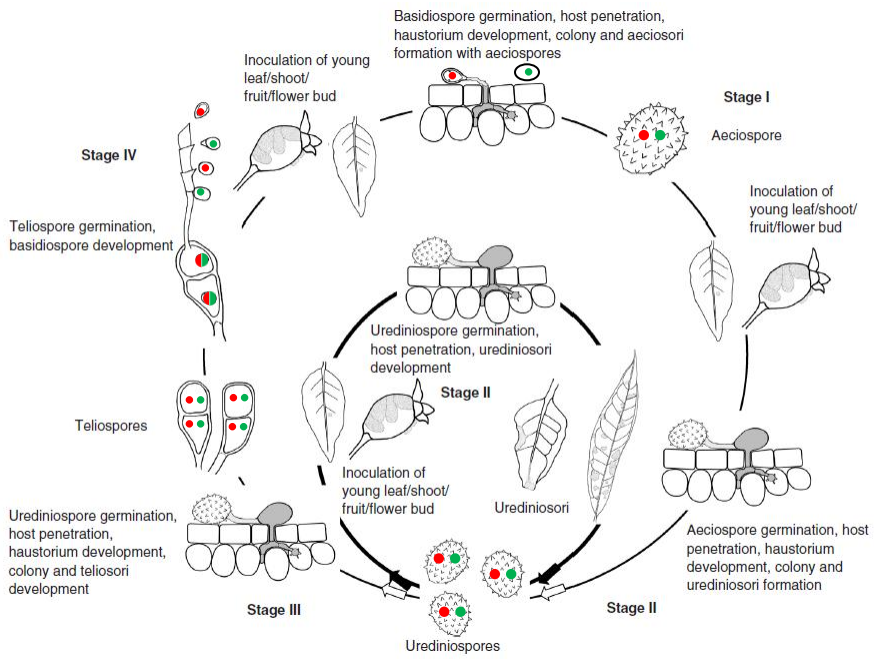
Eucalyptus grandis (1973)

(Ferreira, 1983)



Currently geographic distribution





Glen et al., 2007

Symptoms and sign

Eucalyptus spp.

Alfenas et al. 2009

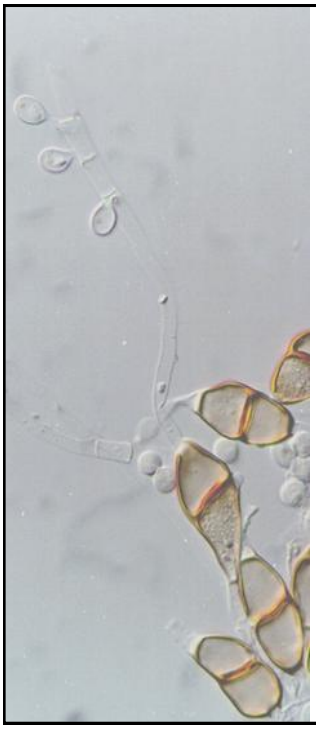
Symptoms

Eucalyptus spp.

Alfenas et al. 2009

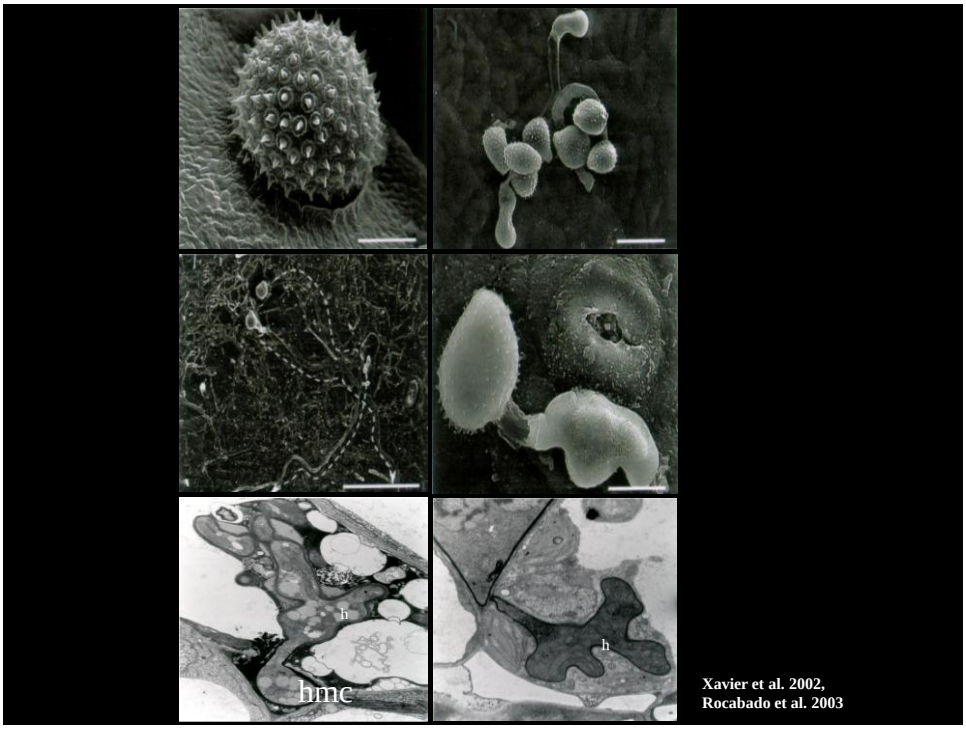
Eucalyptus coppice (stump sprouts)

Alfenas et al. 2009

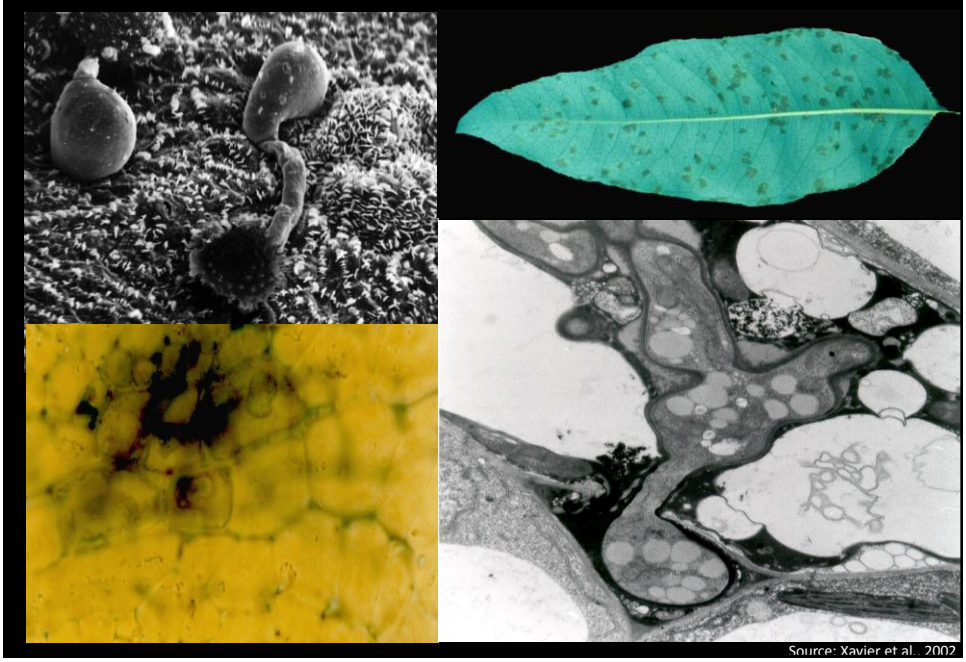


PATHOGENESIS

How does the fungus infect the host?



Hypersensitive Response - HR



Theor Appl Genet (2003) 108:175–180
DOI 10.1007/s00122-003-1415-9

ORIGINAL PAPER

D. T. Junghans · A. C. Alfenas · S. H. Brommonschenkel · S. Oda · E. J. Mello · D. Grattapaglia

Resistance to rust (*Puccinia psidii* Winter) in *Eucalyptus*: mode of inheritance and mapping of a major gene with RAPD markers

Received: 19 December 2002 / Accepted: 2 June 2003 / Published online: 19 September 2003

Table 2 Segregation of resistance to rust in three families of *E. grandis* seedlings inoculated with a single pustule isolate of *P. psidii* (UFV-2) under controlled conditions and evaluated at three times after inoculation

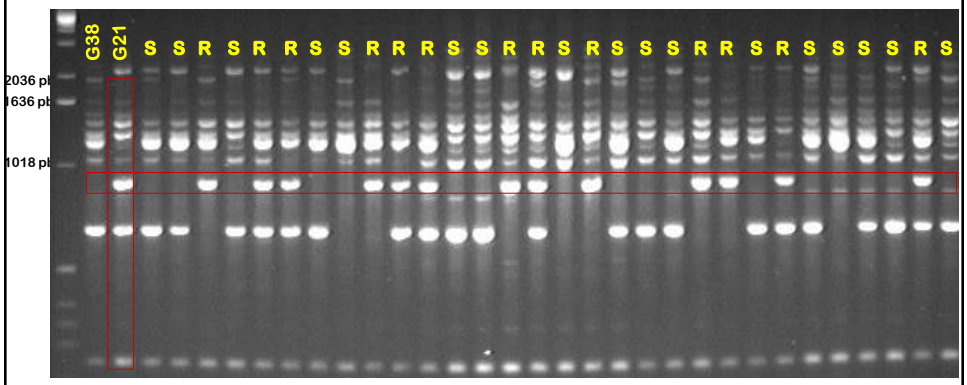
Family		Days after inoculation	Plants/Severity class (SO:SI:S2:S3)	R:S	Expected ratio	χ^2	*P* value
8	(G9×G35)	12	50:22:35:9	72:44	1:1	6.76	0.009
		16	43:20:19:34	63:53	1:1	0.86	0.353
		24	50:12:11:43	62:54	1:1	0.55	0.458
9	(G38×G21)	12	46:13:25:34	59:59	1:1	0.0	1.000
		14	32:23:23:40	55:63	1:1	0.54	0.461
		24	37:24:19:38	61:57	1:1	0.13	0.713
10	(G38ss×G21)	12	25:21:27:46	46:73	1:1	6.13	0.013
		15	22:24:24:49	46:73	1:1	6.13	0.013
		24	23:23:21:52	46:73	1:1	6.13	0.013

Table 3 Segregation of rust resistance in family G38×G21 of *E. grandis* seedlings inoculated with a single pustule isolate of *P. psidii* (UFV-2) and evaluated 12 days and confirmed 24 days after inoculation

Family		Plants/Severity class (SO:SI:S2:S3)	R:S	Expected proportion	χ^2	*P* value
9	(G38×G21)	269:212:165:354	481:519	1:1	1.44	0.229

Dominant monogenic resistance

Resistance gene (*Ppr-1*) on *Eucalyptus grandis*



Junghans et al., 2003

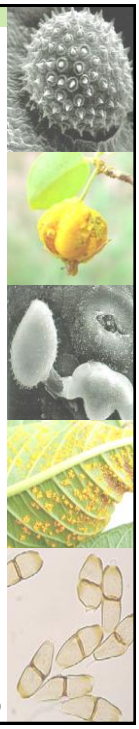
Response of *E. grandis* resistant G21 clone to different single pustule isolates of *Puccinia psidii* obtained from different hosts and geographic areas in Brazil

Isolates	Host	Origin (Brazilian state)	Reaction
1	eucalypt	Guaíba, RS (Riocell)	"fleck"
UFV-2	eucalypt	Itapetininga, SP (Suzano)	"fleck"
16	eucalypt	Guaíba, RS (Riocell)	"fleck"
21	eucalypt	Aracruz, ES (Aracruz)	"fleck"
36	eucalypt	Ipatinga, MG (Cenibra)	"fleck"
38	eucalypt	Ipatinga, MG (Cenibra)	"fleck"
39	eucalypt	Ipatinga, MG (Cenibra)	"fleck"
7116	eucalypt	Ipatinga, MG (Cenibra)	"fleck"
8	guava	Luís Antônio, SP (Votorantin)	"fleck"
32	guava	Santa Maria de Jetibá, RS	"fleck"
40	guava	Guanhães, MG (Cenibra)	"fleck"
42	guava	Belo Oriente, MG (Cenibra)	"fleck"
15	guava	Passo Fundo, RS	"fleck"
3	jaboticaba	Lençóis Paulista, SP (Duratex)	"fleck"
5	Rose apple	Viçosa, MG	"fleck"
7	Rose apple	Mogi-Guaçú, SP (Champion)	immune
17	Rose apple	Porto Alegre, RS	immune
31	Rose Apple	Brasília, DF	"fleck"
13	jambolão	Guaíba, RS (Riocell)	"fleck"
19	pitanga	Passo Fundo, RS	"fleck"
20	cereza	Porto Alegre, RS	"fleck"

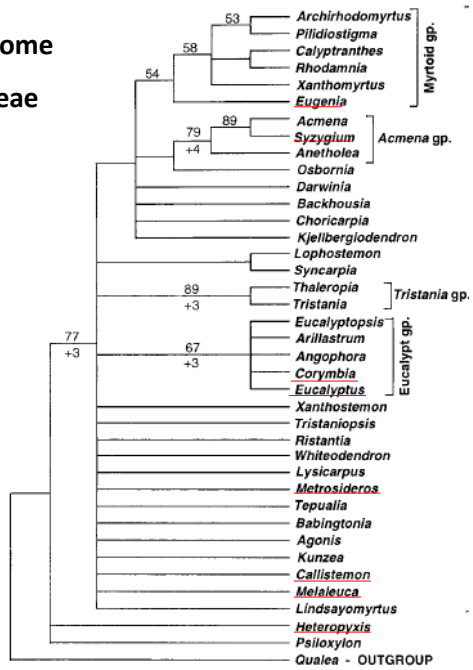
Junghans et al., 2003

Why are some *Eucalyptus* genotypes resistant to *P. psidii* if they did not coevolved?

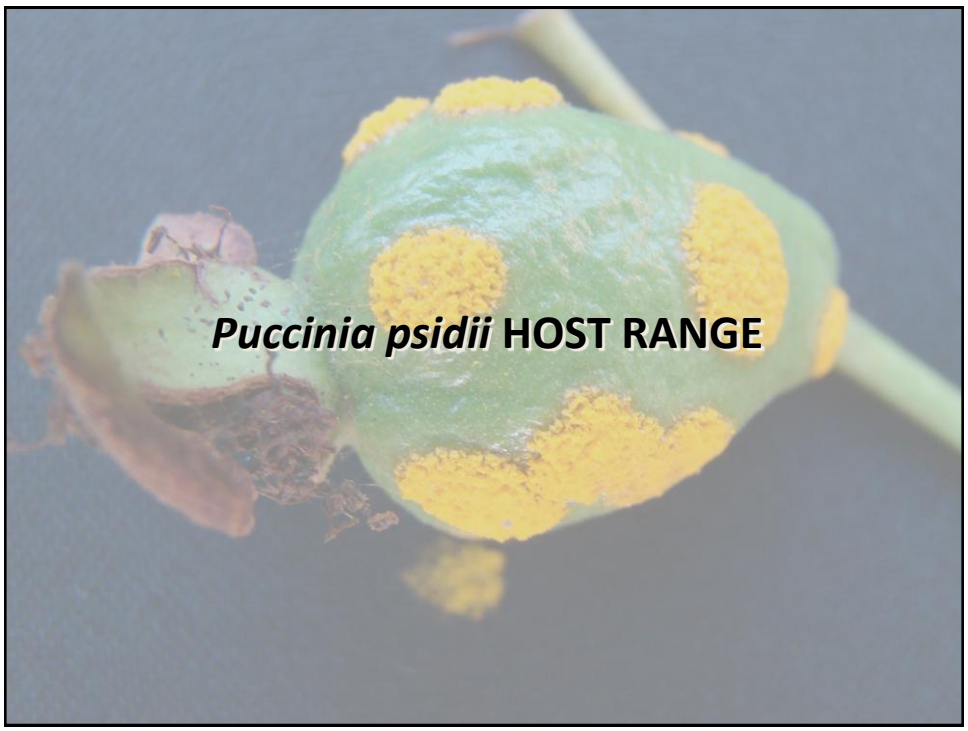
Source: Alfenas et al., 2009



Phylogenetic tree for some genera in the Myrtaceae



Wilson et al., 2001

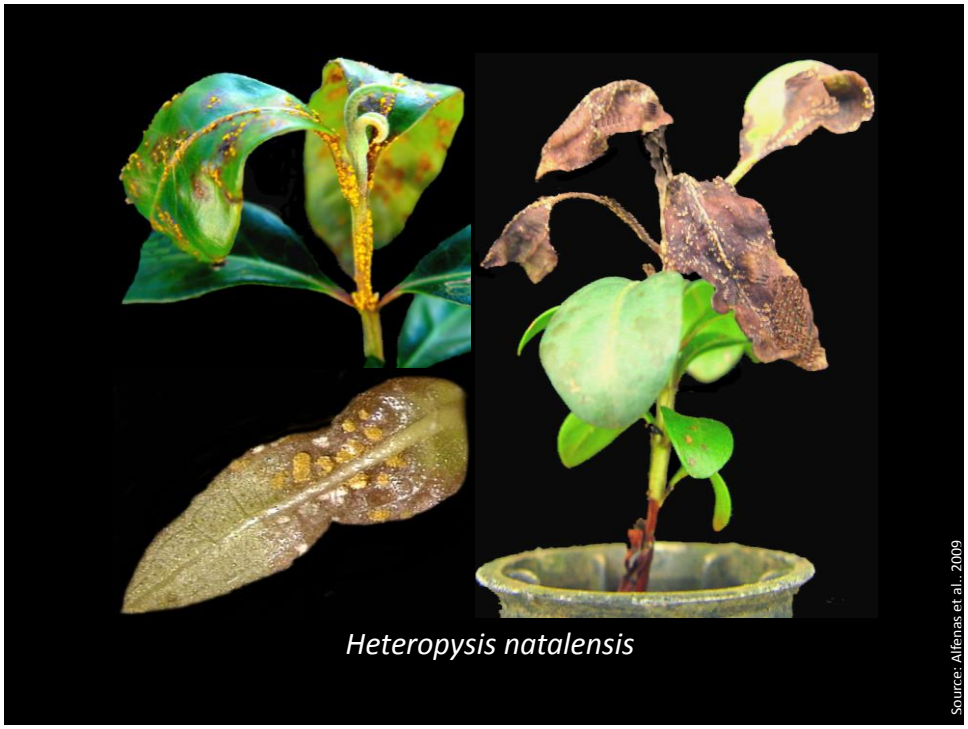



Pitanga (*Eugenia uniflora*)
Rose Apple (*Syzigium jambos*)
Source: Alfenas et al., 2009


Guava (*Psidium guajava*)
Source: Alfenas et al., 2009


Jaboticaba (*Myrciaria cauliflora*)
Source: Alfenas et al., 2009


Callistemon viminaliis
Source: Alfenas et al., 2009


Heteropysis natalensis
Source: Alfenas et al., 2009


Araçá (Psidium araca)
Source: Alfenas et al., 2009

Ohia (*Metrosideros polymorpha*)

Source: http://www.hear.org

Eugenia koolauensis

Source: Chris Kadooka

Susceptibility of different provenances of selected Australian and South Africa Myrtaceae species to *P. psidii*

Australian Centre for International Agricultural Research (ACIAR), Australia
CSIRO Forestry and Forest Products, Australia,
Forestry and Agricultural Biotechnology Institute, South Africa
Federal University of Vicosa, Brazil

- 58 Australian and South Africa species tested, 52 had some degree of susceptibility
- In most species, the degree of susceptibility varied among provenances
- 39 of the species tested were species of *Eucalyptus,* and *37* were susceptible.

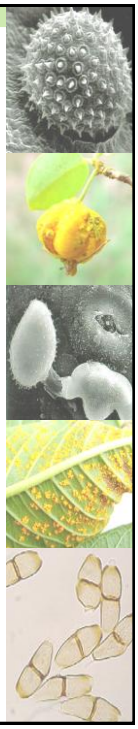
Alfenas and Zauza (unpublish. Data)

Physiological variability – *Puccinia psidii*

- Maclachlan (1938)
- Marlatt and Kimbrough (1979)
- Ferreira (1983)
- Castro et al.; 1983
- Coutinho & Figueiredo (1984)
- Coelho (2001)
- Rayachhetry et al., 2001
- Xavier et al., 2002

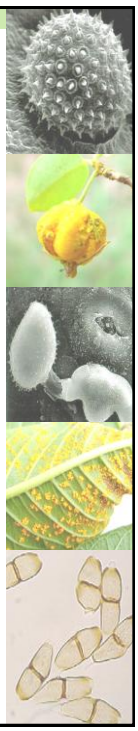
Maclachlan, 1938

Isolate	Host	Reaction
S. jambos	*S. jambos*	S
	P. dioica	R
	P. guajava	R
P. dioica	*S. jambos*	R
	P. dioica	S
	P. guajava	R



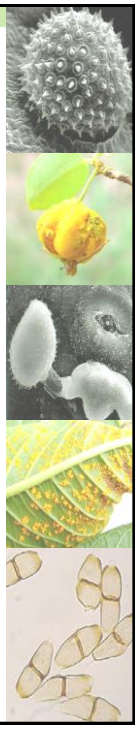
Ferreira (1983)

Isolate	Infects
S. jambos	*S. jambos* + *Eucalyptus*
Eucalyptus spp.	*S. jambos* + *Eucalyptus*
P. guajava	*P. guajava*
M. cauliflora	*S. jambos* + *Eucalyptus*
C. viminallis	*S. jambos* + *Eucalyptus*



Coutinho & Figueiredo, 1984

Isolate	Host	Reaction
S. jambos	*S. jambos*	S
	P. guajava	R
P. guajava	*S. jambos*	R
	P. guajava	S



Identification of *Puccinia psidii* races

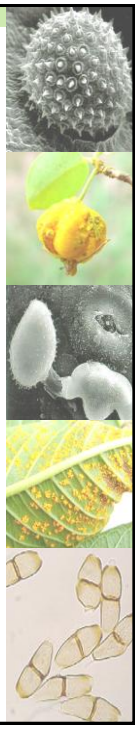
Isolate	Differential set (clones)								Race
	D UFV - 1 (3918)	D UFV - 2 (1205)	D UFV - 3 (847)	D UFV - 4 (1501)	D UFV - 5 (GU530)	D UFV - 6 (GU315)	D UFV - 7 (GU511)	D UFV - 8 (GU24)	
E UFV - 01	1	0	0	0	1	1	1	1	1
E UFV - 02	1	0	0	0	1	1	1	1	1
E UF V - 03	1	0	0	0	1	1	1	1	1
E UFV - 04	1	0	0	0	1	1	1	1	1
E UFV - 05	1	0	0	0	1	1	1	1	1
E UFV - 06	1	0	1	0	1	1	1	1	3
E UFV - 08	1	0	0	0	1	1	1	1	1
E UFV - 09	1	0	0	0	1	1	1	1	1
E UFV - 10	1	0	0	0	1	1	1	1	1
E UFV - 11	1	0	0	0	1	1	1	1	1
E UFV - 13	1	0	0	0	1	1	1	1	1
G UFV - 01	1	0	0	0	1	1	1	1	1
G UFV - 02	1	0	0	0	1	1	1	1	1
G UFV - 04	1	0	0	0	1	1	1	1	1
G UFV - 07	1	1	0	0	1	1	1	1	2
G UFV - 08	1	0	0	0	1	1	1	1	1
J UFV - 03	1	0	0	0	1	1	1	1	1
J UFV - 05	1	0	0	0	1	1	1	1	1
J UFV - 06	1	0	0	0	1	1	1	1	1
C UFV - 01	1	0	0	0	1	1	1	1	1
C UFV - 02	1	0	0	0	1	1	1	1	1

Xavier et al., 2001

Rust on clone 6021?

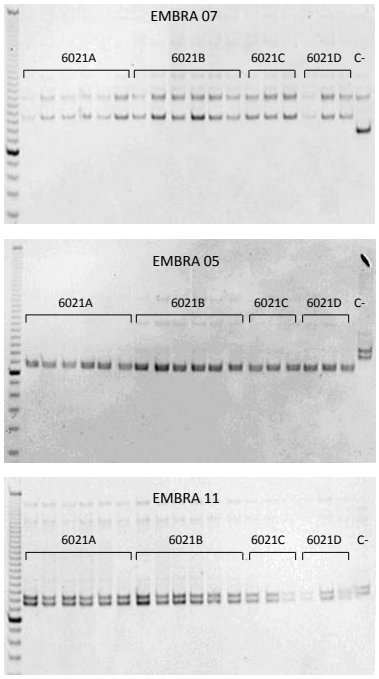
Hypothesis 1 – Clone was mislabeled

Hypothesis 2 – A new race emerged in the pathogen population that can infect clone 6021



Graca et al., 2010 (in preparation)

Microsatellite markers confirm that the clone 6021 was labeled correctly



Graca et al., 2010 (in preparation)

Difference in virulence among isolates on the "resistant" clone 6021

Clone 6021(A)

EUBA-1
(New race)

UFV – 2
(Race 1)

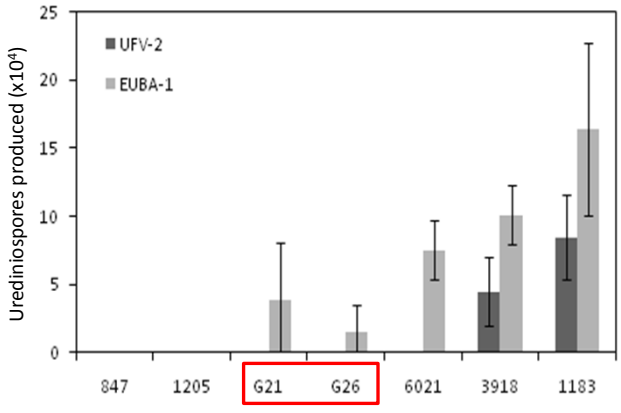
Clone 6021(B)

EUBA-1
(New race)

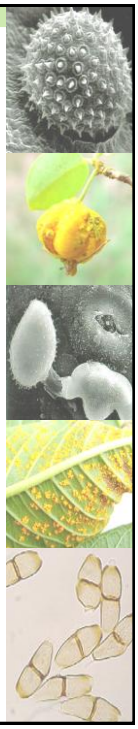
UFV – 2
(Race 1)

Graca et al., 2010 (in preparation)

- Resistance gene overcome



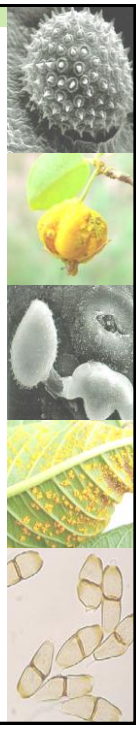
Graca et al., 2010 (in preparation)



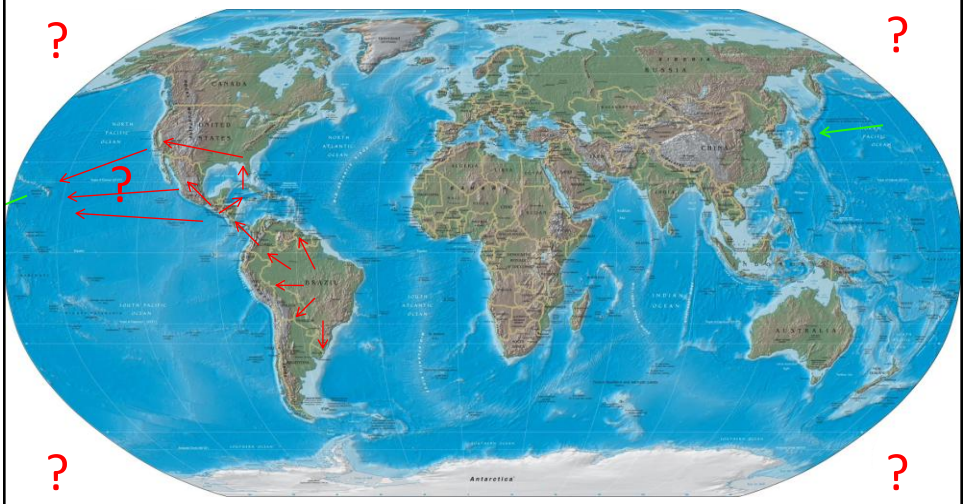
Puccinia psidii in Hawaii

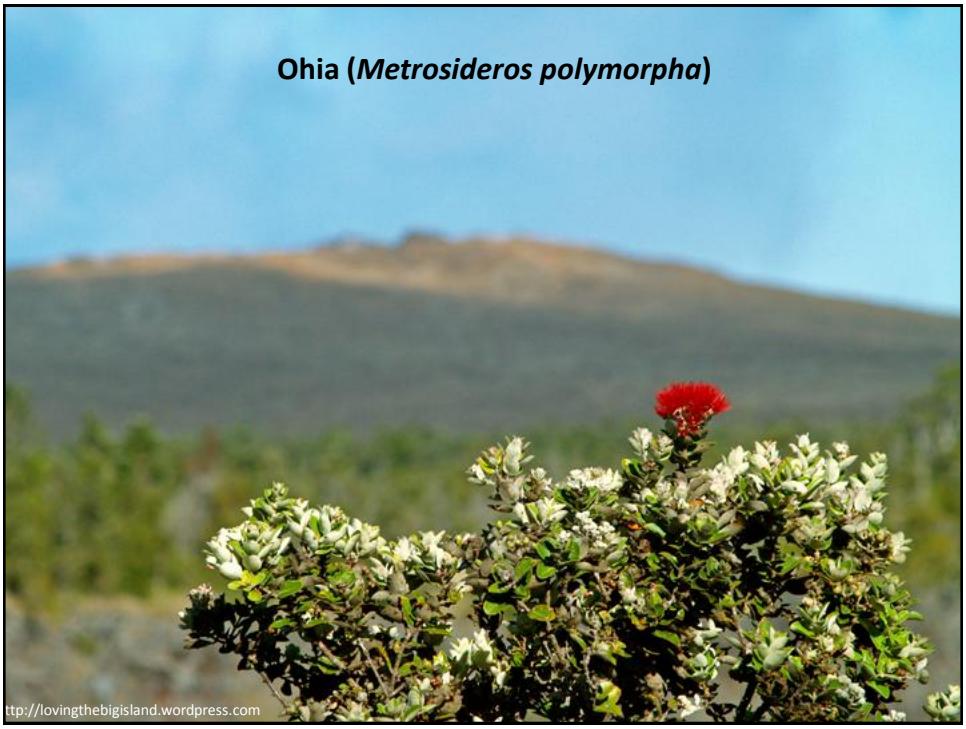
Why we are concerned about the introduction of new *P. psidii* races to Hawaii

- Potential for more severe damage on its current hosts;
- Potential risk of sexual reproduction with existing races;
- New races may infect different hosts in Hawaii;
- Previous history of overcoming the resistance gene.



Potential dispersal pathway




Ohia (*Metrosideros polymorpha*)
ttp://lovingthebigisland.wordpress.com


Ohia rust
Fonte: http://www.hear.org

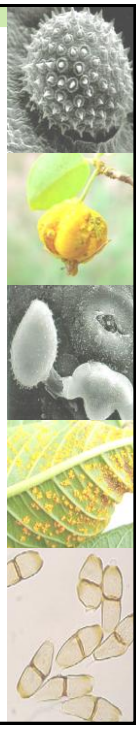
Puccinia psidii in *Eugenia koolauensis*

Source: Chris Kadooka

Source: http://www.hear.org

Interesting aspect

Despite the existence of guava and eucalypt in Hawaii, *P. psidii* has not been reported on these hosts so far.

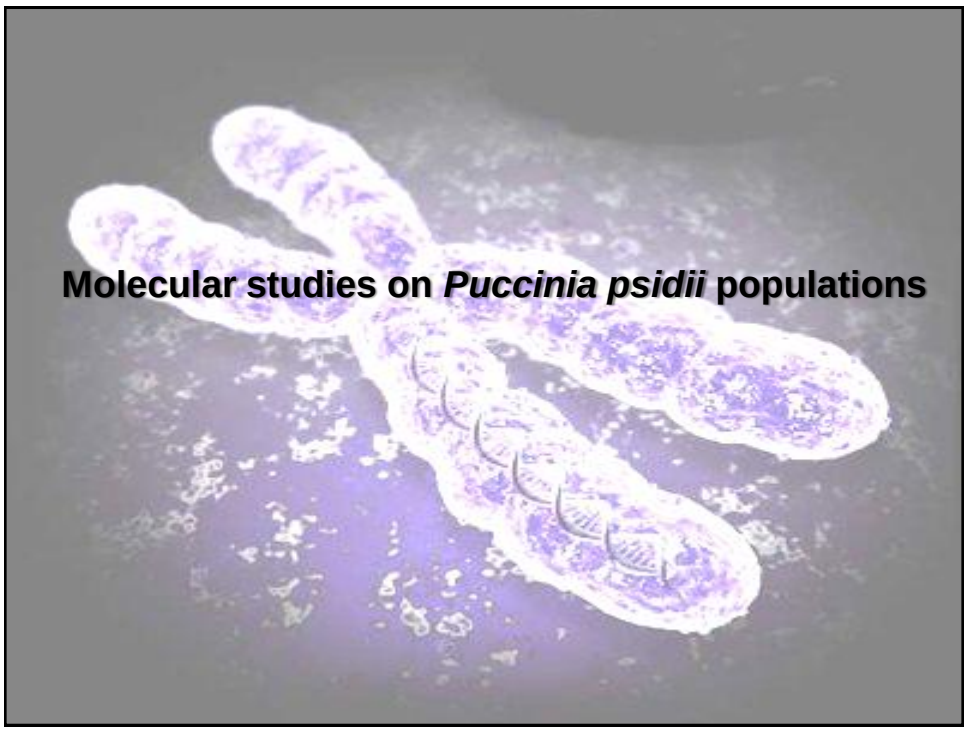


Why?

1) Lack of *P. psidii* races capable to infect those hosts (so far)

2) Very few infections make it difficult to be observed

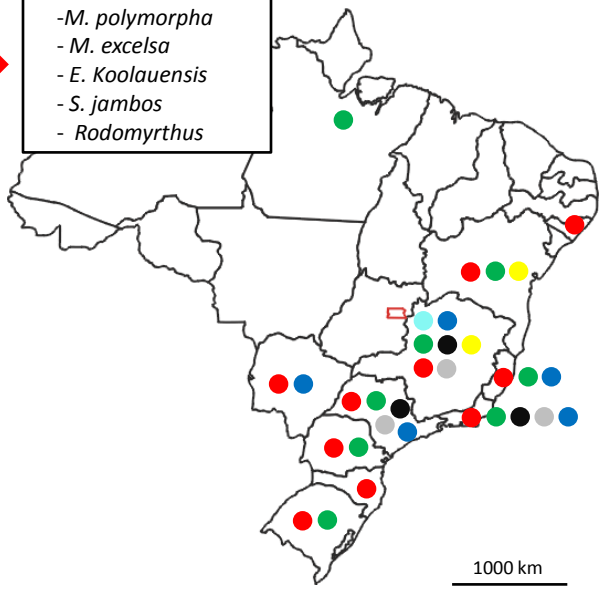
3) Lack of young susceptible tissue during the period of favorable environment conditions for rust infection



Puccinia psidii sampling

- Brazil (165) – 7 hosts
-Hawaii (25) – 5 hosts
- Paraguay (2) – 2 hosts
- Uruguay (5) – 2 hosts

-*M. polymorpha*
- *M. excelsa*
- *E. Koolauensis*
- *S. jambos*
- *Rodomyrthus*

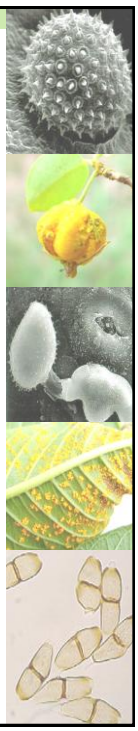


- *Eucalyptus* spp.
- *P. guajava*
- *P. araca*
- *S. Jambos*
- *S. jambolanum*
- *M. cauliflora*
- *E. uniflora*

DNA extractions



DNA



MOLECULAR ANALYSIS

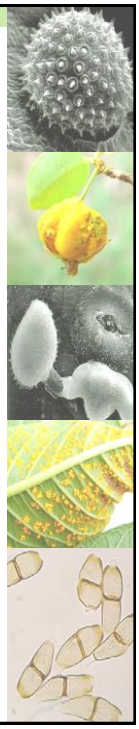
• 15 microsatellites markers

Locus	GenBank Accession no.	Primer sequence (5′– 3′)	T_a (°C)	Repeat motif	No. of alleles	Size range	H_O	H_E
PpSSR010	EF523500	F: AGAAGTATGGGTTGAAAGGG	49	$C_{12}(CA)_9$	5	143–148	0.80	0.73
		R: CTGATGGACATGAAGTTTGAC	49					
PpSSR012	EF523501	F: TTCAATCCCCATAAGGCTTTC	54	$(AG)_9$	5	235–258	1.00	0.70
		R: AAATCCTGAGTCTTCTTCCCC	53					
PpSSR014	EF523502	F: TTCGACATCCAACGCTCTCAT	57	$(AG)_{13}$	3	213–226	0.95	0.57
		R: AAAGGCTAAGTGAATGGGCA	53					
PpSSR018	EF523503	F: AGCCTTCTCTCTCCTCCGTTA	53	$(AG)_{10}$	2	161–167	0.80	0.48
		R: TCAGGAAGGACAAGACCAAGT	52					
PpSSR022	EF523504	F: TCCCTTCATGTCCTTTAGGCT	54	$(AG)_{18}$	8	151–166	1.00	0.67
		R: CCCACTCTGTCAAGAGGAAAT	52					
PpSSR078	EF523505	F: TTCACCTTGTTCCTTTGG	47	$(AG)_{13}$	2	162–165	0.75	0.48
		R: CCATATAAACAGATGCAATCA	47					
PpSSR080	EF523506	F: CGATCATCCACAGGTTAGTAA	49	$(AG)_{13}$	3	86–99	0.05	0.31*
		R: AAGTGAATGGGCACTAGAGA	48					
PpSSR087	EF523507	F: GGGAATGATGACGTCACGTTA	54	$(AG)_{14} + C_6 + A_9$	5	143–154	0.70	0.70
		R: CCAGACGAAGGGTAAGTCATT	52					
PpSSR102	EF523508	F: GGCTTTGGGTGAGGTTTTTT	54	$T_7 + (AG)_{22} + T_7$	3	255–295	0.70	0.39
		R: TCCCCTTCCTTCATCATTGA	54					
PpSSR136	EF523509	F: CAGAACCATTTCTTACAAGAT	45	$(AG)_{14}$	6	132–144	0.90	0.66
		R: CTCGTGAATGAACTCCATACA	50					
PpSSR146	EF523510	F: AGATTGGTAAAGAGGAGGGGA	53	$(AG)_{73}$	4	64–84	0.80	0.62
		R: TCAGCACCAACCATTACCTT	52					
PpSSR161	EF523511	F: TCGAGGGGTCTCAGTTTTCA	54	$(AG)_{25}$	5	276–287	1.00	0.68
		R: GAGATCTATCGGACCAACGAA	53					
PpSSR178	EF523512	F: TTCGTGTGCATGTGTGTATCG	55	$(AG)_{62}$	6	271–290	0.75	0.56
		R: TAGCCTTGGGTGCACACTTTA	54					
PpSSR195	EF523513	F: TCACCGGTTATCCACTCATGT	54	$(AG)_{18}$	6	134–145	0.95	0.70
		R: GACGAGGAAAGTGATGGTTT	50					
PpSSR208	EF523514	F: CGATCATCCACAGGTTAGTA	47	$(AG)_{12}$	8	85–96	0.05	0.31*
		R: TAAGTGAATGGGCACTAGAGA	49					

T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity. *Indicates the loci deviated from Hardy–Weinberg equilibrium.

Zhong et al., 2007

Microsatellites



• <u>Isolate 1</u>

• (Allele 1) ------CACACACACACACACACA------- = (CA)9

• (Allele 2) ------CACACACACACACACACA------- = (CA)9

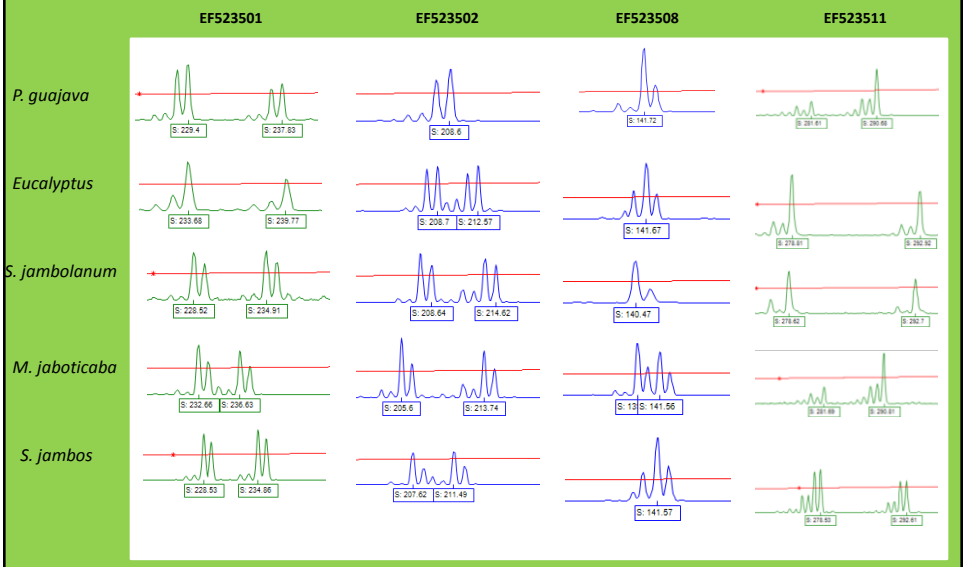
• <u>Isolate 2</u>

X

• (Allele 1)------CACACACACACACACACA------- = (CA)9

• (Allele 2)------CACACACACACACACA------- = (CA)8

Examples of microsatellites chromatograms for *Puccinia psidii* isolates



Puccinia psidii populations are host specific in Brazil, Uruguay and Paraguay regardless of geographic location (10 loci analyzed)

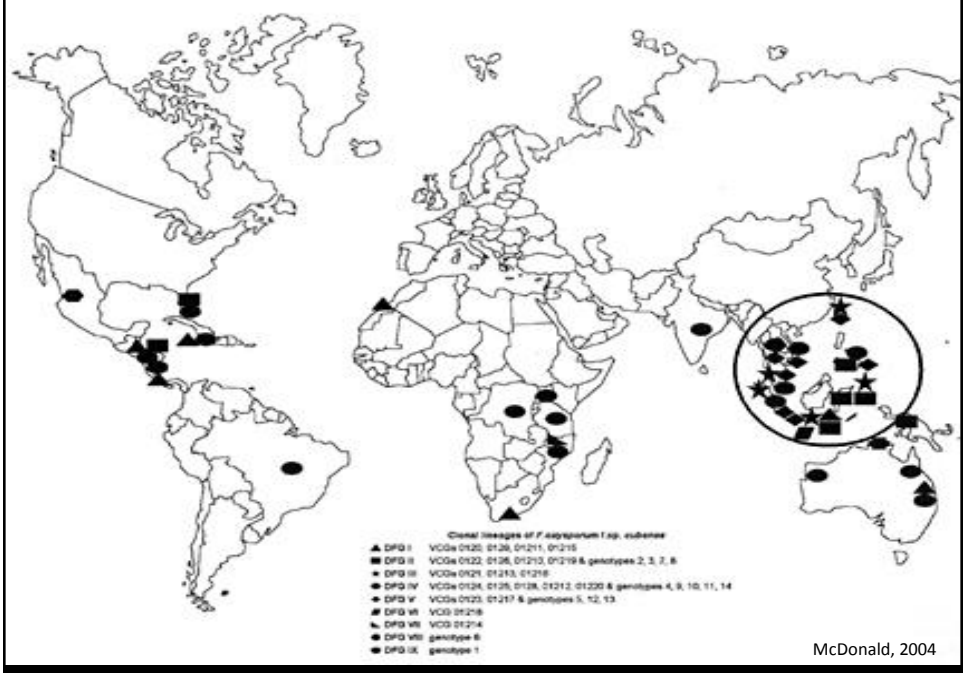
	501		502		503		504		507		508	
Eucalypts												
SUZ5_EU	233	239	208	212	168	168	153	157	154	160	140	142
RoseApple												
RJ6A_RA	233	239	208	212	168	168	153	157	154	160	142	142
Guava												
SUZ1_GA	229	237	208	208	168	170	149	149	160	160	142	142
Araca												
V1_AR	229	237	208	208	168	170	149	149	160	160	142	142
Jaboticaba												
JBMG1_JBT	233	237	206	214	162	172	149	153	172	178	140	142
Pitanga												
PTMG1_PT	235	239	212	212	168	168	147	147	162	164	142	142
Jambolao												
SP9_JAO	229	235	208	214	168	172	155	159	160	160	140	140

Preliminary results for *Puccinia psidii* populations from Hawaii (6 loci analyzed)

	501		502		503		504		507		508	
Host - 1												
Rose apple	229	235	208	212	168	170	157	159	160	170	172	178
Host - 2												
E. Koolauensis	229	235	208	212	168	170	157	159	160	170	172	178
Host - 3												
M. polymorpha	229	235	208	212	168	170	157	159	160	170	172	178
Host - 4												
M. excelsa	229	235	208	212	168	170	157	159	160	170	172	178
Host - 5												
Rhodomyrtus sp.	229	235	208	212	168	170	157	159	160	170	172	178

"All isolates from Hawaii have the same multilocus genotype"

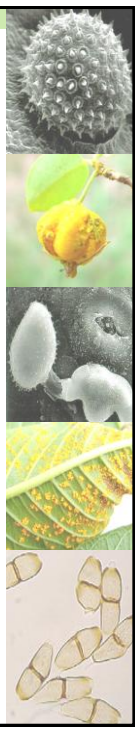
Distribution of clonal lineages of *F. oxysporum* f. sp. *cubense*



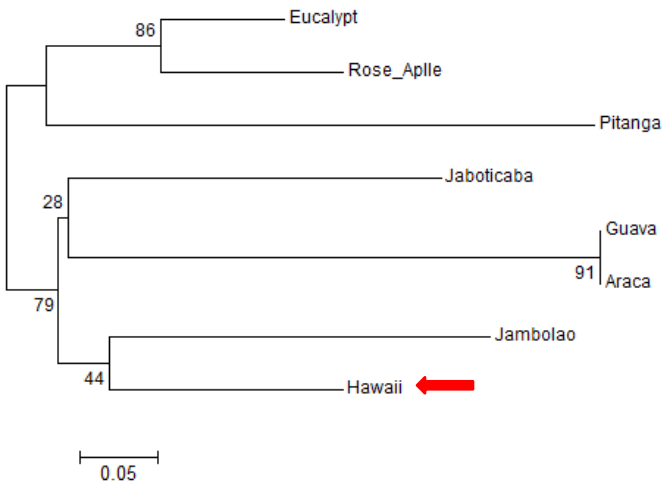
McDonald, 2004

Relationship of rust multilocus genotypes from Hawaii, Brazil, Uruguay and Paraguay

	501		502		503		504		507		508	
Eucalypts												
SUZ5_EU	233	239	208	212	168	168	153	157	154	160	140	142
RoseApple												
RJ6A_RA	233	239	208	212	168	168	153	157	154	160	142	142
Guava												
SUZ1_GA	229	237	208	208	168	170	149	149	160	160	142	142
Araca												
V1_AR	229	237	208	208	168	170	149	149	160	160	142	142
Jaboticaba												
JBMG1_JBT	233	237	206	214	162	172	149	153	172	178	140	142
Pitanga												
PTMG1_PT	235	239	212	212	168	168	147	147	162	164	142	142
Jambolao												
SP9_JAO	229	235	208	214	168	172	155	159	160	160	140	140
Hawaii												
1HW-RA	229	235	208	212	168	170	157	159	160	170	**172**	**178**



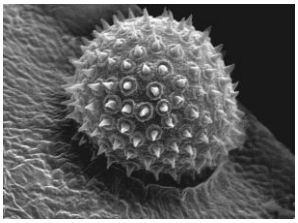
- **NJ Tree (using 6 loci) showing the relationship among *P. psidii* isolates**



Susceptibility of different families of *Metrosideors polymorpha* from Hawaii to *P. psidii isolates*

United States Department of Agriculture (USDA – Forest Service)
Federal University of Vicosa, Brazil

X



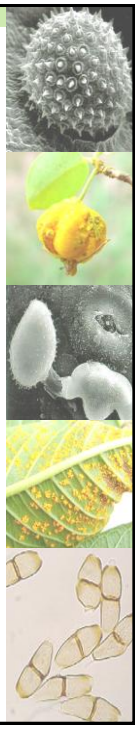
28 Ohia families

Different *P. psidii* isolates (about 8)

- This test will be a well replicated experiment conducted at Federal University of Vicosa

Overall conclusions

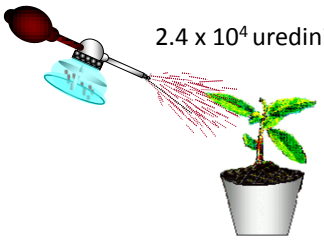
- *Puccinia psidii* is increasing its geographic distribution, and its host range;
- Strong selection by host occurs in *P. psidii* populations from Brazil;
- *P. psidii* populations from Brazil are not structured by location,
- In Brazil *P. psidii* seems to be in the way to speciation by host (eg. *f.sp.*);
- Hawaiian isolates are genetically all the same, and distinct from all Brazilian isolates studied so far;
- It seems that the Hawaiian isolates didn't came directly from Brazil, but the Hawaiian isolates have several alleles in common with Brazilian isolates.



Thank you very much

"MAHALO"

Screening of plants for rust resistance



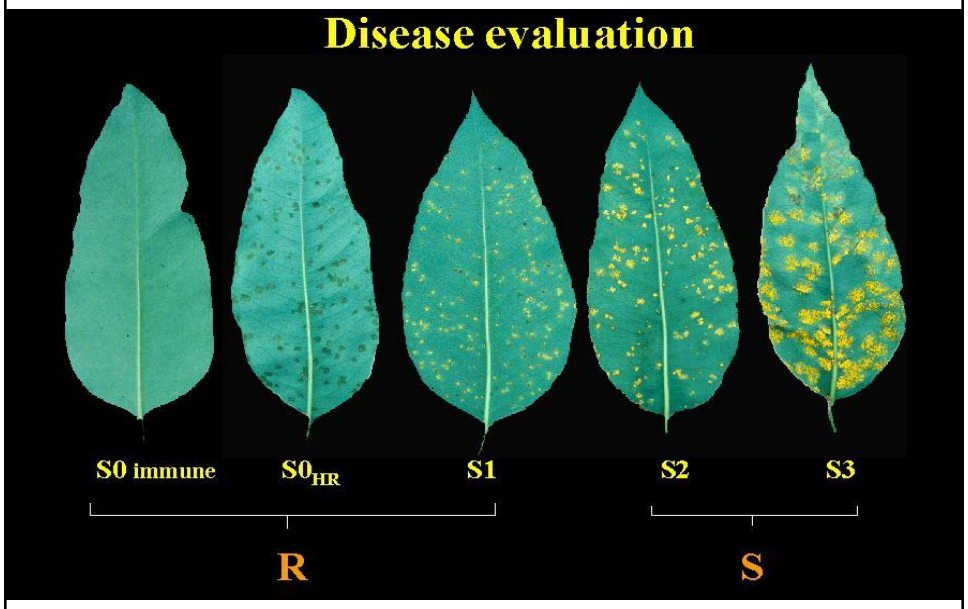
2.4×10^4 urediniospores/mL

24h dark: humid chamber

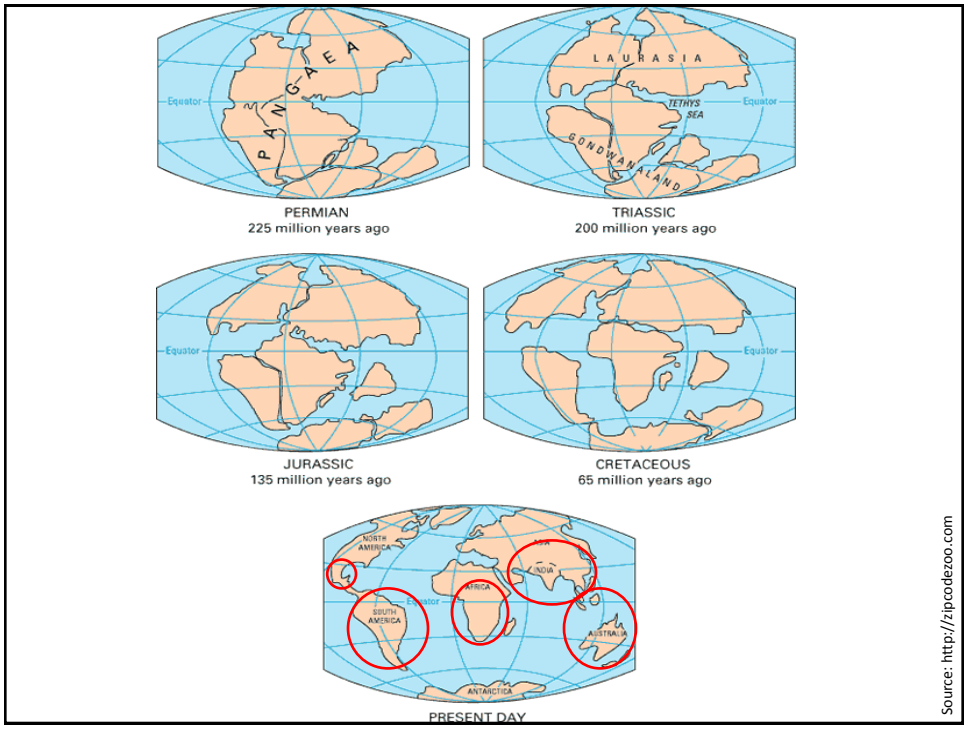
Growth chamber: (22 ºC, 12 Photoperiod)

Ruiz et al., 1989

Screening of plants for rust resistance



Junghans et al., 2003


PANGAEA
Equator
PERMIAN
225 million years ago
LAURASIA
TETHYS SEA
GONDWANALAND
Equator
TRIASSIC
200 million years ago
Equator
JURASSIC
135 million years ago
Equator
CRETACEOUS
65 million years ago
NORTH AMERICA
INDIA
AFRICA
SOUTH AMERICA
AUSTRALIA
ANTARCTICA
PRESENT DAY
Source: http://zipcodezoo.com