MECHANISMS OF RESISTANCE TO PESTS AND PATHOGENS

Moderator: John Russell

Genetic basis of resistance in *Eucalyptus* spp. - pathosystems

by

Acelino Couto Alfenas
Lúcio Mauro da Silva Guimarães
Marcos Deon Vilela de Resende

Eucalypts in tropical sites have a very high growth rate

Current mean annual increment (MAI) in industrialized managed Euc forest in Brazil is about 45 m^3/ha/y

Productivity at rotation age 7 years – 315 m^3/ha

ABRAF (2010)

Eucalyptus x several crops x cattle x horse

Fazenda Aroeira – Candiota RS – segundo ano

Homogenous clonal plantation:

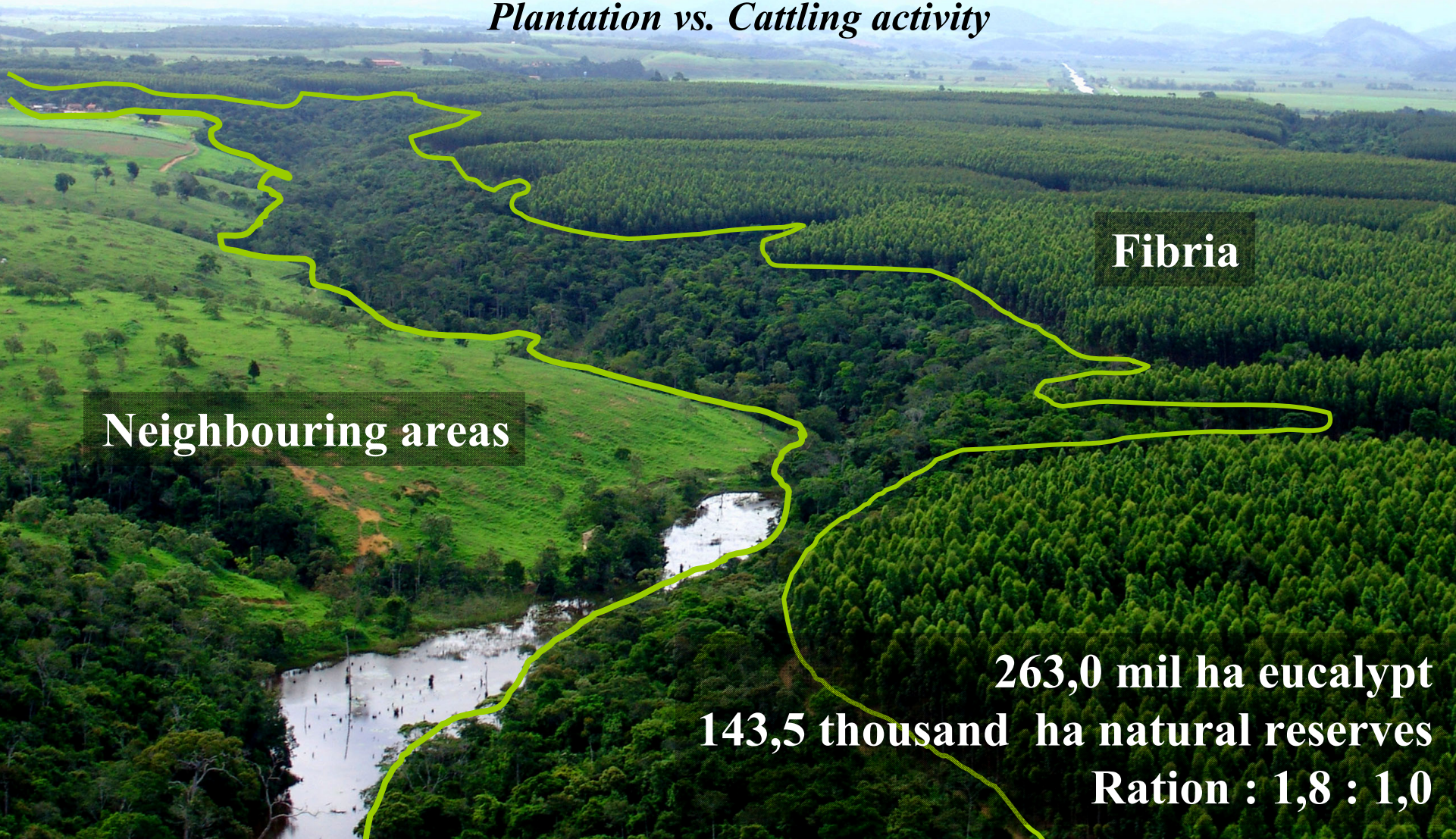
High growth, disease resistant and uniform raw material

Suzano Papel e Celulose

The applied model by the forest companies in Brazil

Conservation and preservation of "Legal Reserves" and "Permanent Preservation Areas "

Plantation vs. Cattling activity

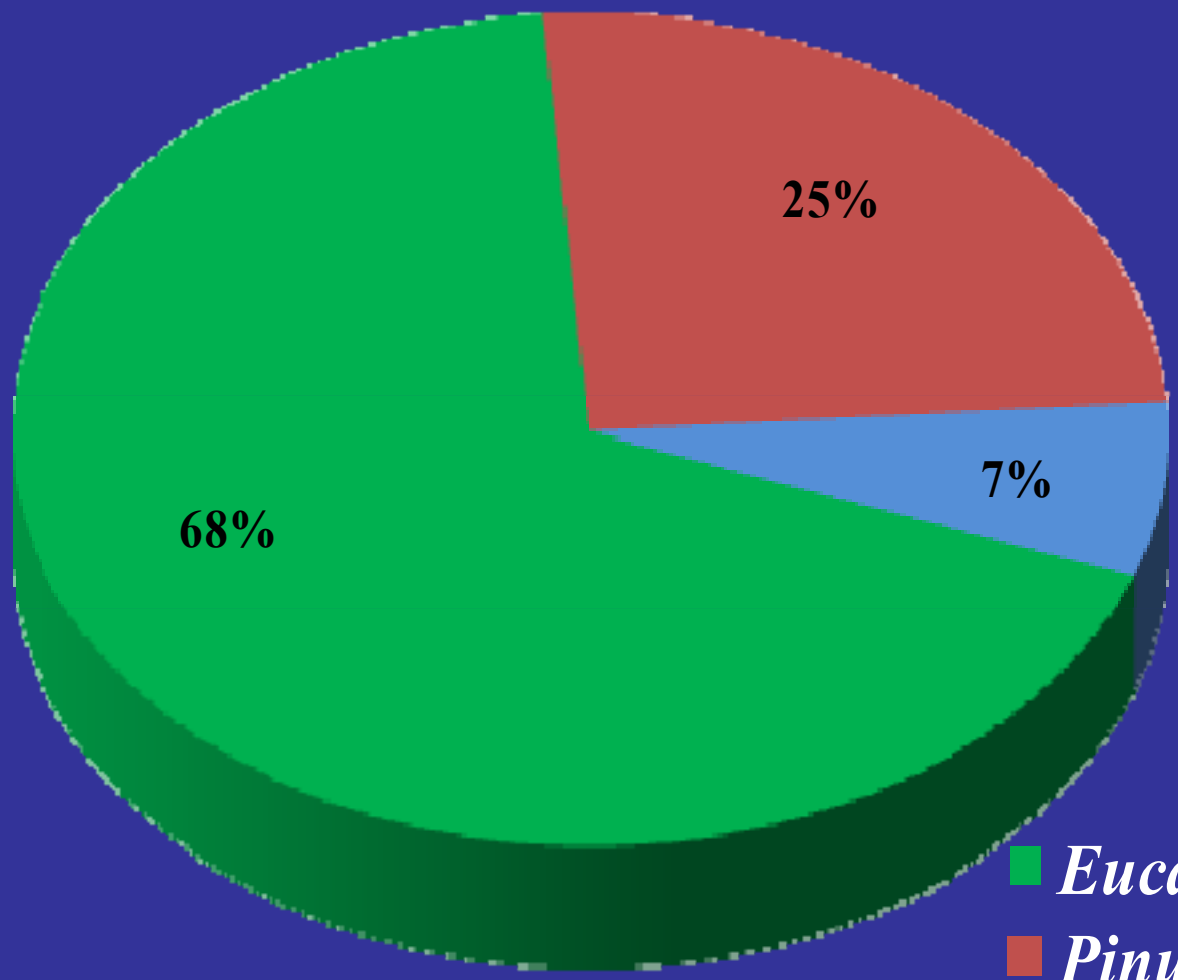


263,0 mil ha eucalypt

143,5 thousand ha natural reserves

Ration : 1,8 : 1,0

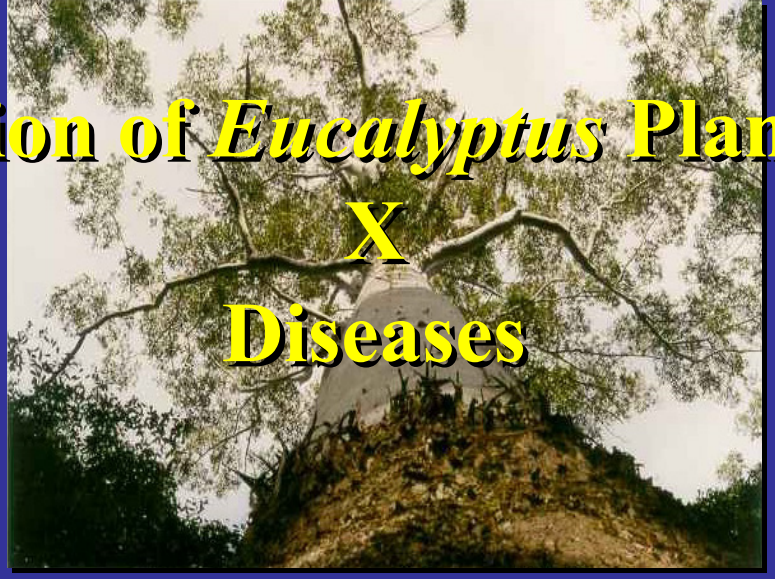
Forest plantations in Brazil
6,973,083 ha



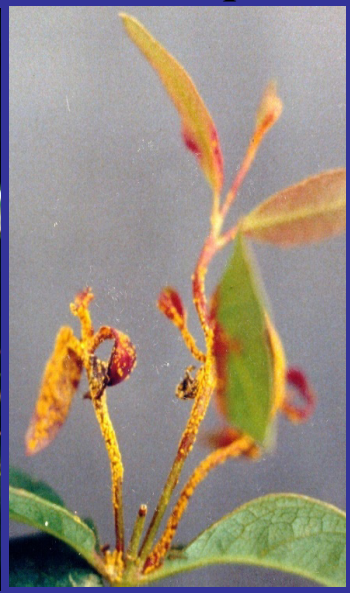
Eucalyptus	4,754,334 ha
Pinus	1,756,359 ha
Others	462,390 ha

Abraf (2011)

Expansion of *Eucalyptus* Plantations X Diseases



☞ *Chrysoporthe* ☞ *Erytricium* ☞ Ceratocystis ☞ Ralstonia ☞ Leaf diseases ☞ *Puccinia psidii*



Alfenas et al. (2009)

Control of diseases in the field

Genetic Resistance

Alfenas et al. (2009)

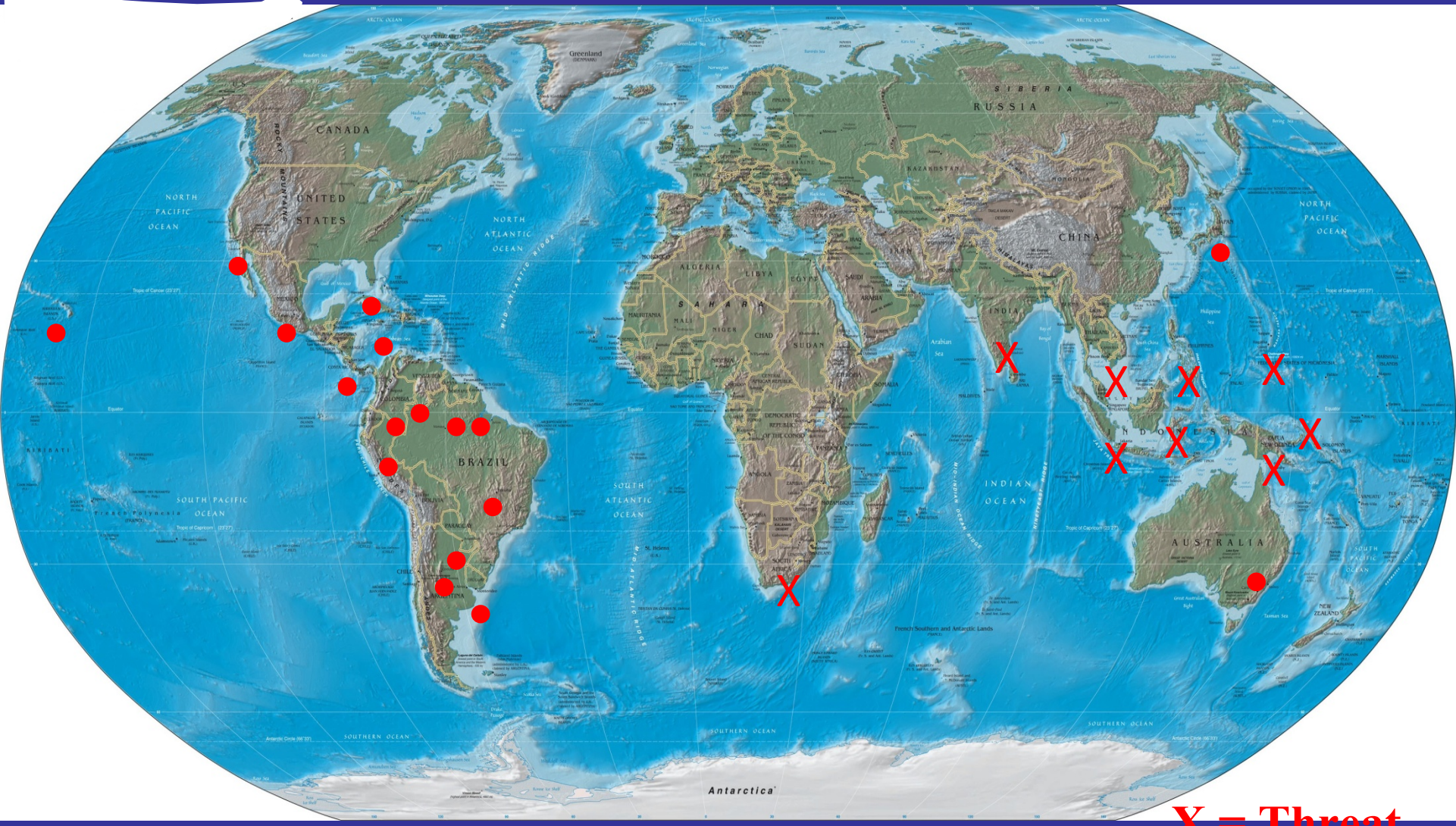
Pathosystems studied

- *Puccinia psidii – Eucalyptus*
- *Ceratocystis fimbriata - Eucalyptus*
- *Chrysoporthe cubensis - Eucalyptus*
- *Cylindrocladium pteridis - Eucalyptus*
- *Teratosphaeria – Eucalyptus*
- *Ralstonia solanacearum – Eucalyptus*
- *Xanthomonas - Eucalyptus*

Rust (*Puccinia psidii*): as model system

Alfenas et al. (2009)

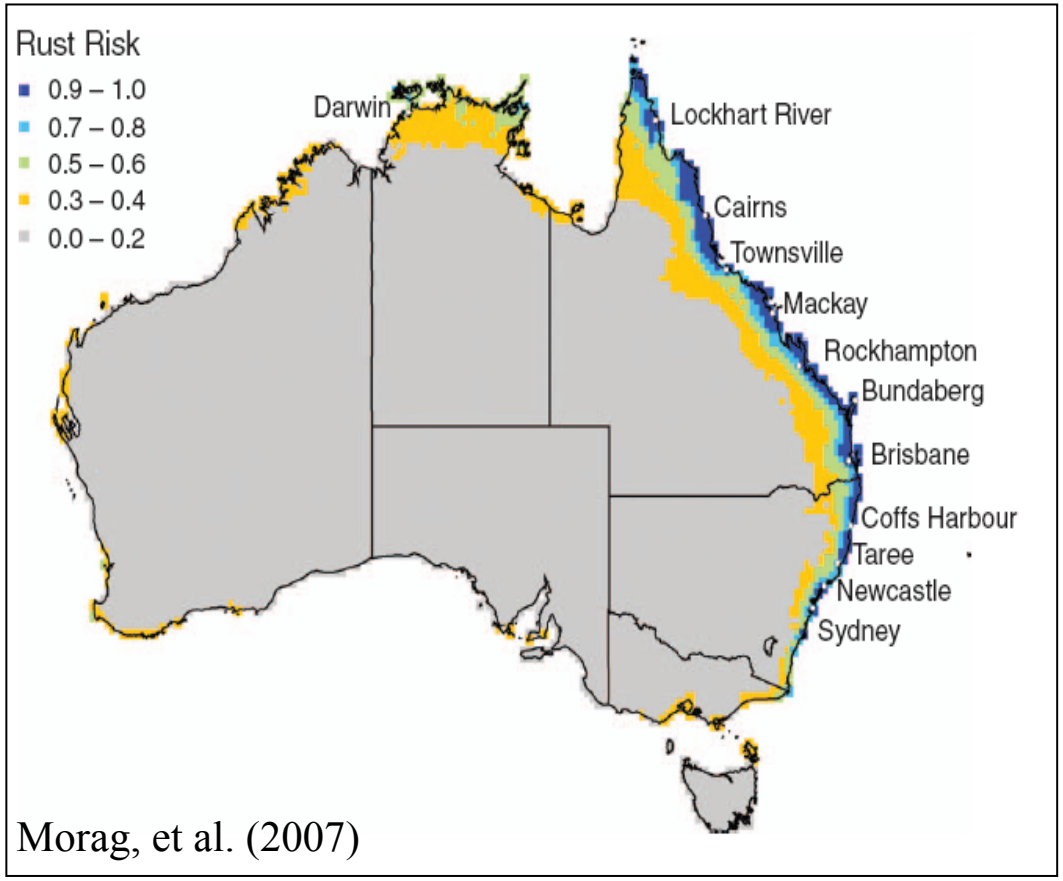
Current global geographic distribution and regions of potencial rust spread



X = Threat

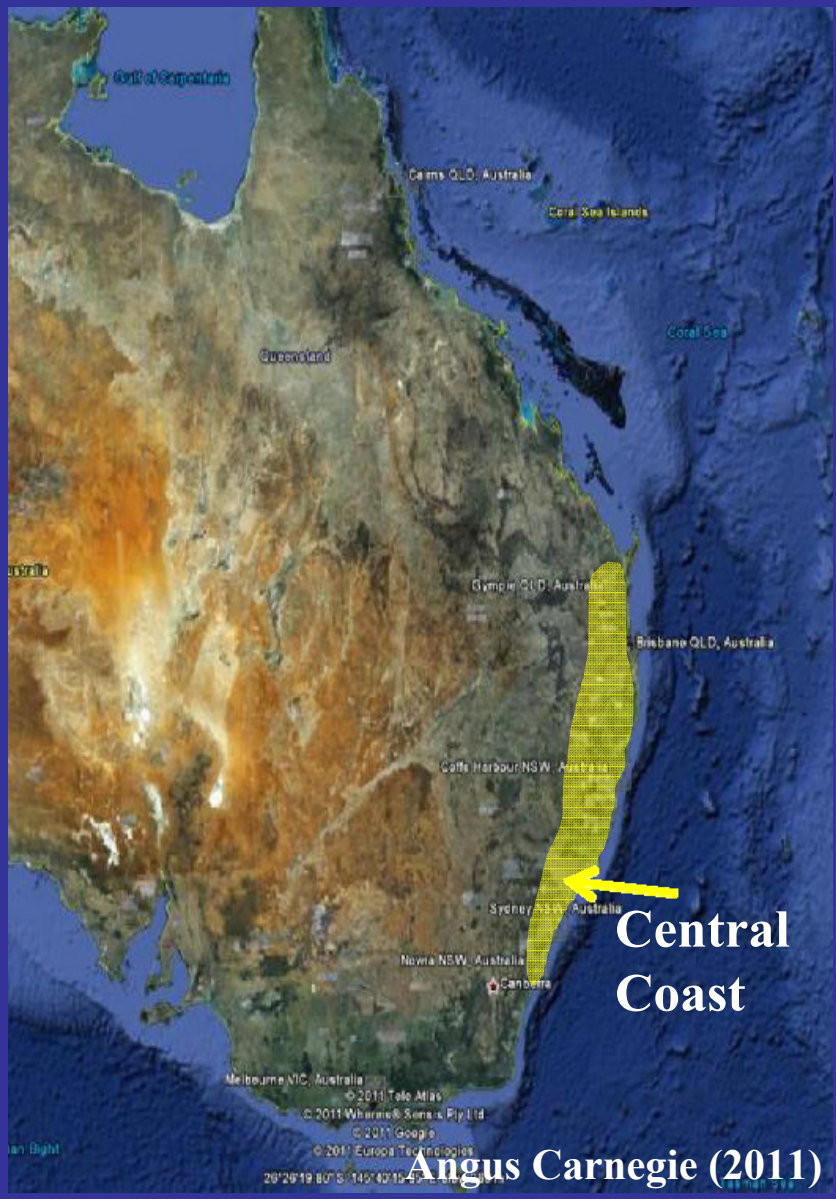
Glen et al. (2007)

Revised rust risk areas for *Puccinia psidii in Australia (T. H. Booth* and T. Jovanovic, pers. comm.). Dark blue areas represent highest risk, light blue, light green and orange areas show decreasing levels of risk. Grey areas signify lowest risk.



Morag, et al. (2007)

Current rust distribution in AU



Angus Carnegie (2011)

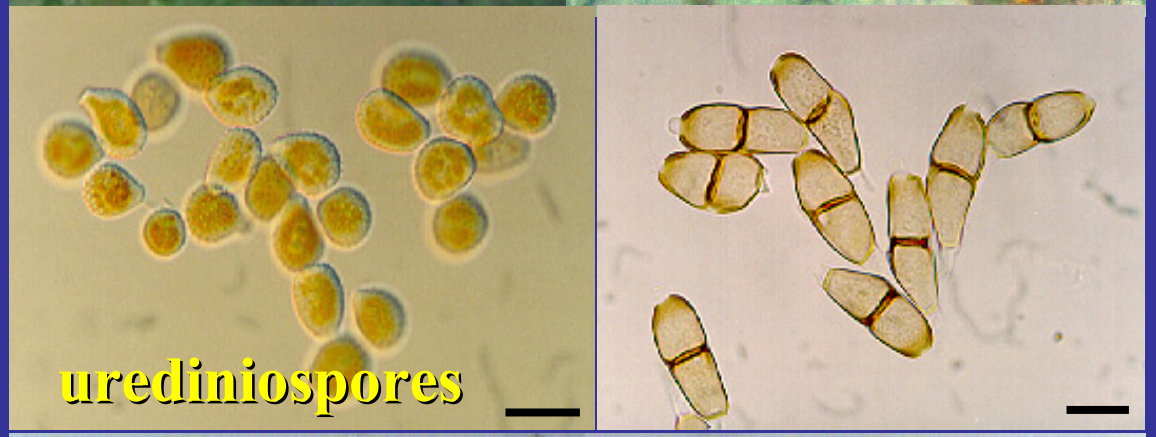
Host Range

Angophora
Callistemon
Corymbia
Eucalyptus
Eugenia
Heteropixis
Marlierea
Melaleuca
Metrosideros
Myrcia
Myrciaria
Pimenta
Psidium
Syzigium

Alfenas et al. (2009)

Alfenas et al. (2009)

Alfenas et al. (2009)



Alfenas et al. (2009)

Races of *Puccinia psidii*

Isolate	Host	Diferencial set (*E. grandis* clones)						Race
		G45	1205	847	6021	G21	G26	
UFV02	*E. grandis*	C	I	I	I	I	I	1
UFV06	*E. grandis*	C	I	C	I	I	I	2
UFV07	*P. guajava*	C	C	I	I	I	I	3
EUBA1	*E. grandis.*	C	I	I	C	C	I	4

C = compatible

I = incompatible

Adapted Xavier et al. (2002); Graça et al. (2011)

Control of rust in the field on *Eucalyptus* is currently done mainly by planting resistant genotypes

How to screen for resistance, under controlled conditions?

Inoculation of *Puccinia psidii* in *Eucalyptus* plants

Inoculation: 2.0 x 10^4 urediniospores/mL

Evaluatation of the phenotype

Growth chamber: 22 °C 12 h photoperiod, 20 d

Mist irrigation chamber 24 h, in the dark

Alfenas et al. (2011)

Disease severity scale

S0 imune | $S0_{HR}$ | S1 | S2 | S3

R

S

Junghans et al. (2003)

Plants in pots

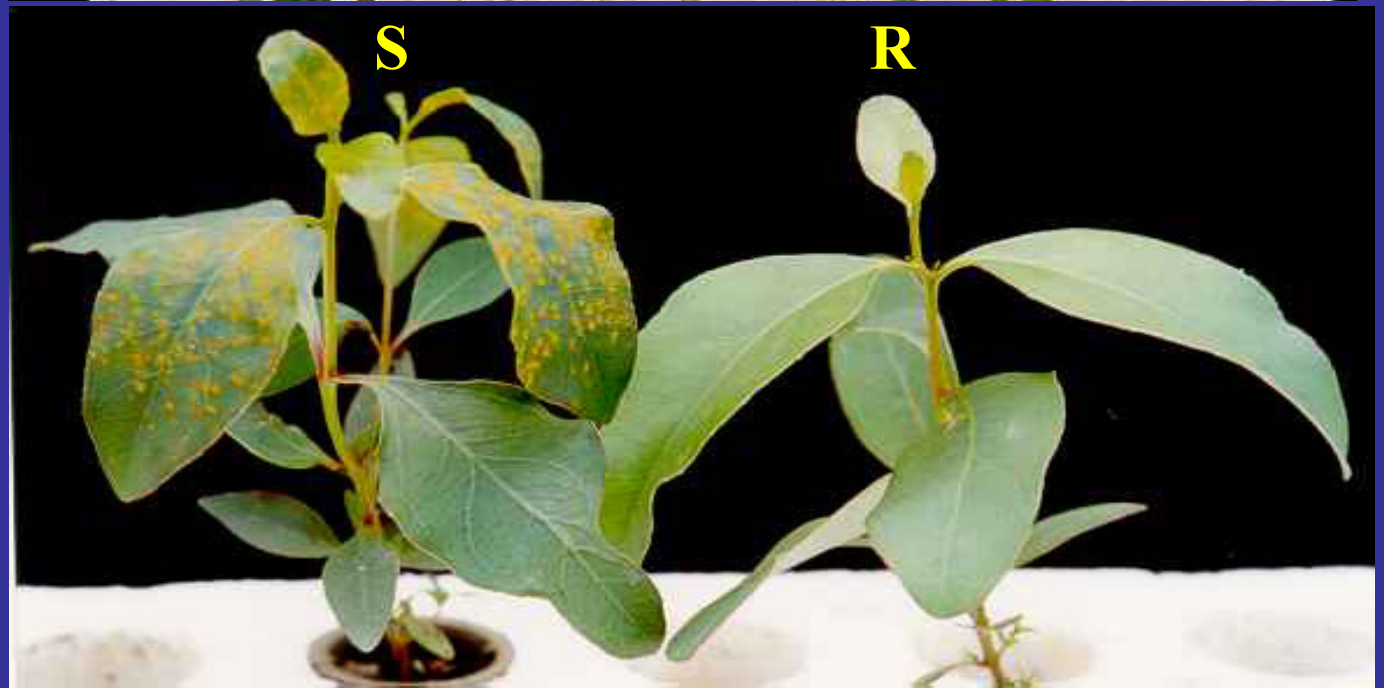
S R S R

Alfenas et al. (2010)

Plants in tubes

Alfenas et al. (2009)

Segregation for resistance



Alfenas et al. (2009)

Myrtaceae species resistance to rust caused by *Puccinia psidii*

E. A. V. Zauza *et al.*

Australasian Plant Pathology, 2010, 39, 406–411

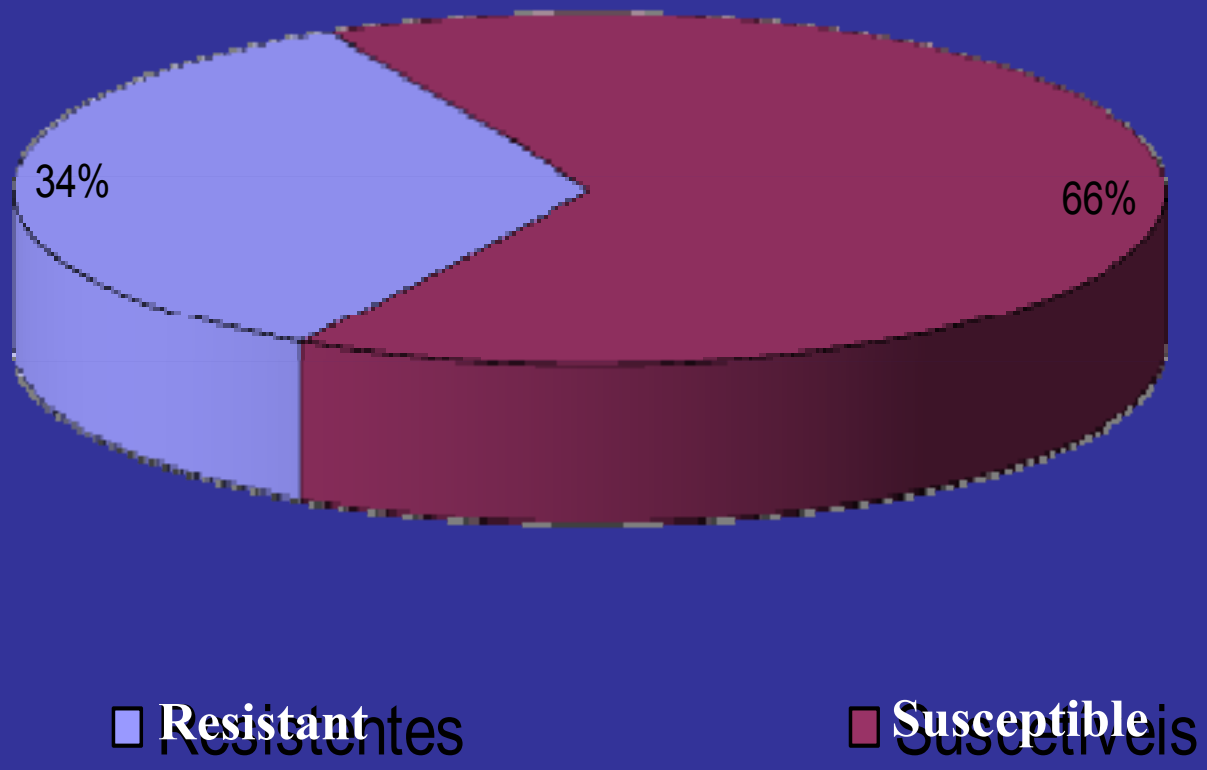
Species	Resistant (%)	Class
Corymbia tessellaris	100,00	AR
Eucalypus tereticornis	98,75	AR
Eucalypus resinifera	95,88	AR
Eucalypus scias ssp. Scias	95,50	AR
Eucalypus paniculata	91,38	AR
Eucalypus pellita	90,25	AR
Corymbia intermedia	90,00	AR
Angophora costata	85,00	R
Eucalyptus brassiana	84,82	R
Eucalypus camaldulensis var. simulata	84,75	R
Eucalypus melanophloia	84,75	R
Eucalypus alba	72,00	R
Eucalypus camaldulenseis var. obtusa	77,89	R
Corymbia maculata	76,38	R
Eucalyptus elata	74,00	R
Eucalyptus acmenoides	73,00	R
Eucalypus urophylla	72,33	R
Corymbia citriodora	72,13	R
Eucalyptus obliqua	71,88	R
Eucalyptus nitens	70,00	R
Eucalyptus microcorys	67,50	R
Eucalyptus globulus	67,06	R
Eucalyptus pilularis	65,99	R
Eucalyptus saligna	62,63	R
Eucalyptus robusta	60,88	R
Eucalyptus dunnii	54,50	R
Eucalyptus deglupta	51,00	R
Eucalyptus grandis	49,20	S
Eucalyptus regnans	46,25	S
Eucalyptus cloeziana	40,75	S

Best sources of R (+ 90% R genotypes)

- *Corymbia calophylla* "*rosea*" (WA)
- *Corymbia intermedia* (QLD)
- *Corymbia tesselaris* (QLD)
- *Eucalyptus alba* (QLD)
- *Eucalyptus amplifolia* var. *amplifolia*(NSW)
- *Eucalyptus brassiana* (PNG)
- *Eucalyptus camaldulensis var.* obtusa (QLD)
- *Eucalyptus diversicolor* (QLD)
- *Eucalyptus paniculata* (NSW
- *Eucalyptus pellita* (QLD)
- *Eucalyptus resinifera* (NSW)
- *Eucalyptus scias* sub *scias* (QLD)
- *Eucalyptus tereticornis* (QLD)
- *Melaleuca ericifolia*

Zauza et al. (2010)

Resistance of *Eucalyptus* clones ("*E. grandis* and "urograndis") to the rust (*Puccinia psidii*) (351 clones)



Eucalyptus pellita as a source of resistance to rust, ceratocystis wilt and leaf blight

LMS Guimarães et al.

Crop Breeding and Applied Biotechnology 10: 124-131, 2010

Inheritance studies and genetic mapping

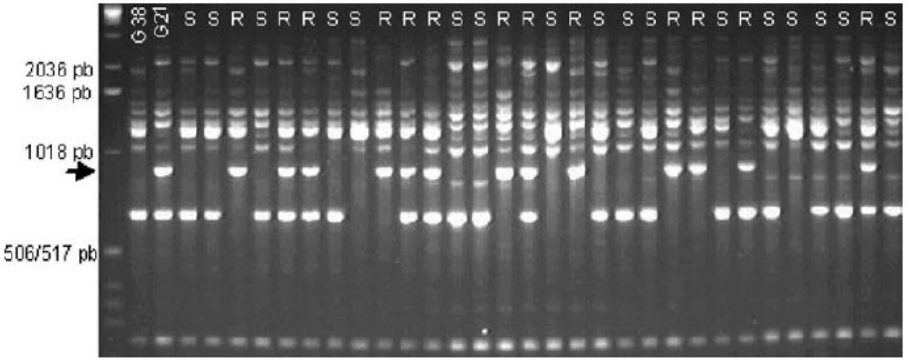
Resistance to rust (*Puccinia psidii* Winter) in *Eucalyptus*: mode of inheritance and mapping of a major gene with RAPD markers

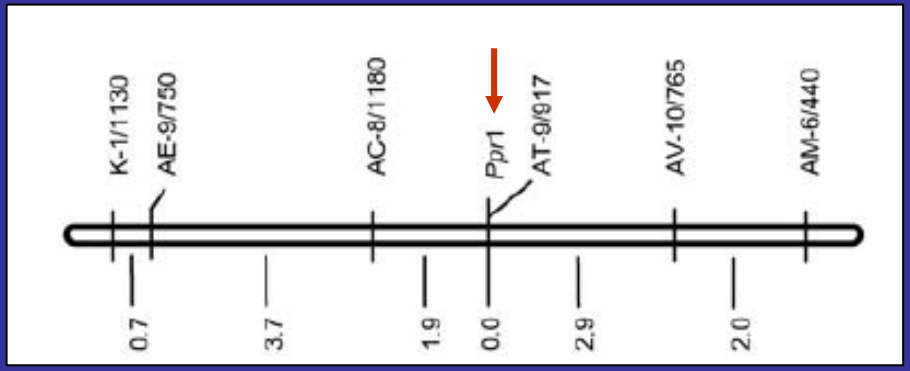
D. T. Junghans · A. C. Alfenas · S. H. Brommonschenkel · S. Oda · E. J. Mello · D. Grattapaglia

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

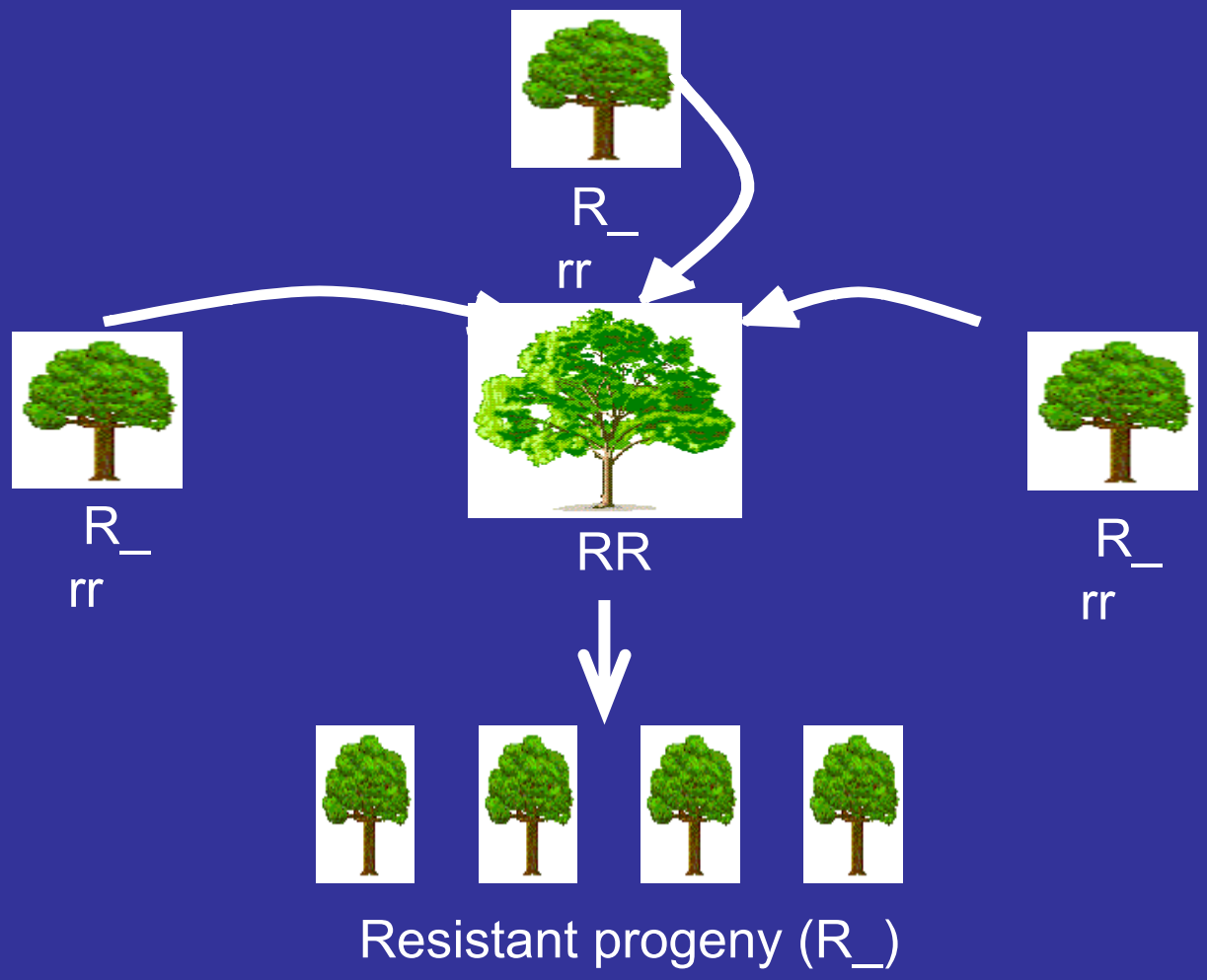
Family		Number of plants/severity class (S0:S1:S2:S3)	R:S[a]	Expected ratio[b]	χ^2	*P* value
1	(G38×G15)	0:0:34:81	0:115	0:1		
2	(G38ss×G15)	0:0:34:85	0:119	0:1		
3	(G38×G33)	0:4:44:71	4:115	0:1		
4	(G38×G45)	0:6:38:73	6:111	0:1		
5	(G9×G26)	108:11:1:0	119:1	1:0		
6	(G38×G26)	96:20:3:0	116:3	1:0		
7	(G38ss×G26)	98:12:7:1	110:8	1:0		
8	(G9×G35)	50:22:35:9	72:44	1:1	6.76	0.009
9	(G38×G21)	46:13:25:34	59:59	1:1	0.0	1.000
10	(G38ss×G21)	25:21:27:46	46:73	1:1	6.13	0.013
11	(G21×G21)	11:1:2:2	12:4	3:1	0.0	1.000

[a] The Resistant:Susceptible (R:S) ratio is based on R=S0+S1 and S=S2+S3
[b] The resistance was hypothesized to be controlled by a major dominant gene





Ppr1 – 0,043cM
AT9/917



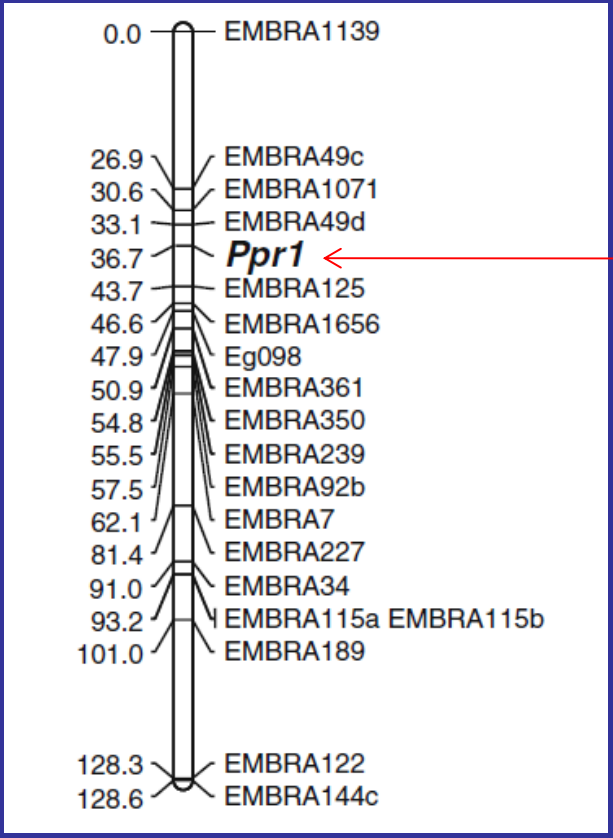
Clonal orchard for rust R seed production at Suzano

Genetic Mapping

Positioning of the major locus for *Puccinia psidii* rust resistance (*Ppr1*) on the *Eucalyptus* reference map and its validation across unrelated pedigrees

Eva M. C. Mamani • Nathalia W. Bueno • Danielle A. Faria • Lucio M. S. Guimarães • Douglas Lau • Acelino C. Alfenas • Dario Grattapaglia

Tree Genetics & Genomes
DOI 10.1007/s11295-010-0304-z



Euphytica
DOI 10.1007/s10681-011-0455-5

Genetic mapping provides evidence for the role of additive and non-additive QTLs in the response of inter-specific hybrids of *Eucalyptus* to *Puccinia psidii* rust infection

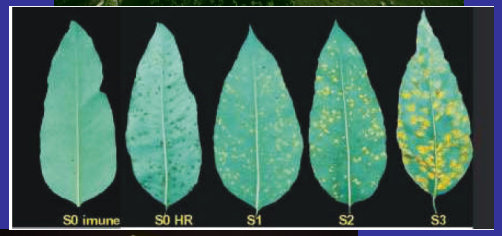
Alexandre Alonso Alves · Carla Cristina Gonçalves Rosado · Danielle Assis Faria · Lúcio Mauro da Silva Guimarães · Douglas Lau · Sérgio Hermínio Brommonschenkel · Dario Grattapaglia · Acelino Couto Alfenas

Segregation patterns in inter-specific pedigrees, has shown:

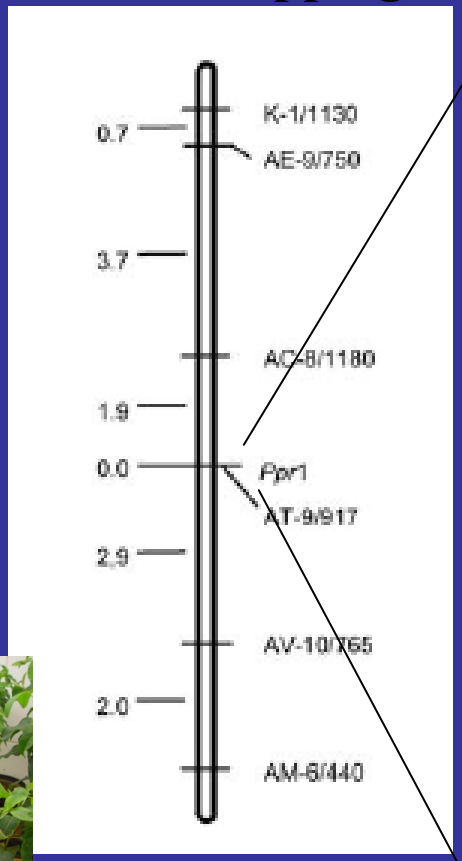
1. **A distinct segregation pattern of that obtained in *E. grandis* (one major gene *Ppr*-1).**
2. **A QTL of major effect on linkage group 3 as previous the found by Mamani et al.**
3. **The role of additive and non-aditive (epistasis) QTL´s in rust resistance of inter-specific hybrids**

Map based cloning of *Ppr*-1

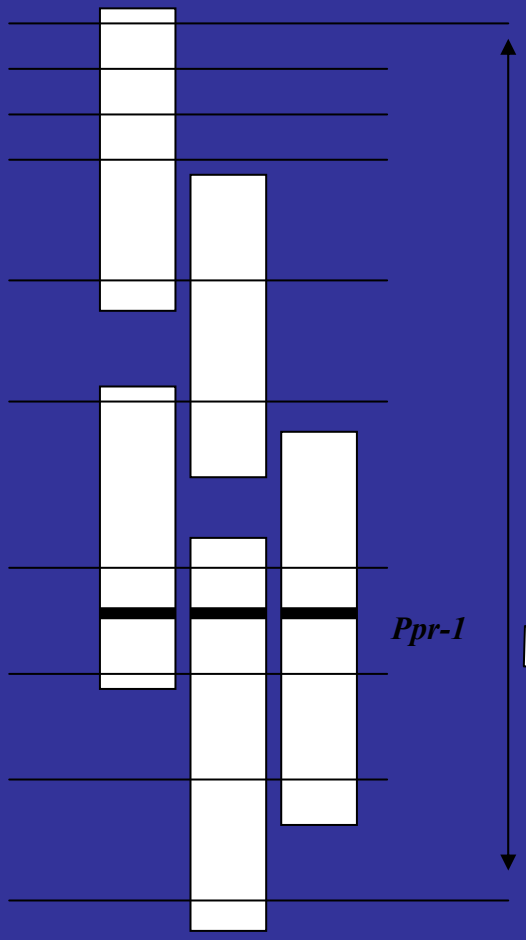
Phenotyping of segregating population



Genetic Mapping



Physical Mapping (BAC Fingerpritting)

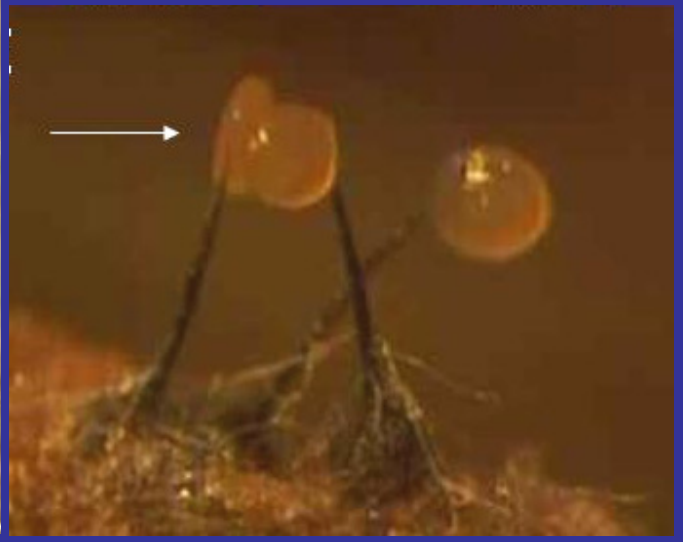


Clonning and functional assays

Forward genetics: from phenotypes to genes

S. Brommonschenkel

Ceratocystis fimbriata wilt



Alfenas et al. (2009)

Ceratocystis fimbriata wilt in *Eucalyptus*

Alfenas et al. (2009)

Ceratocystis fimbriata infection in other hosts

Alfenas et al. (2009)

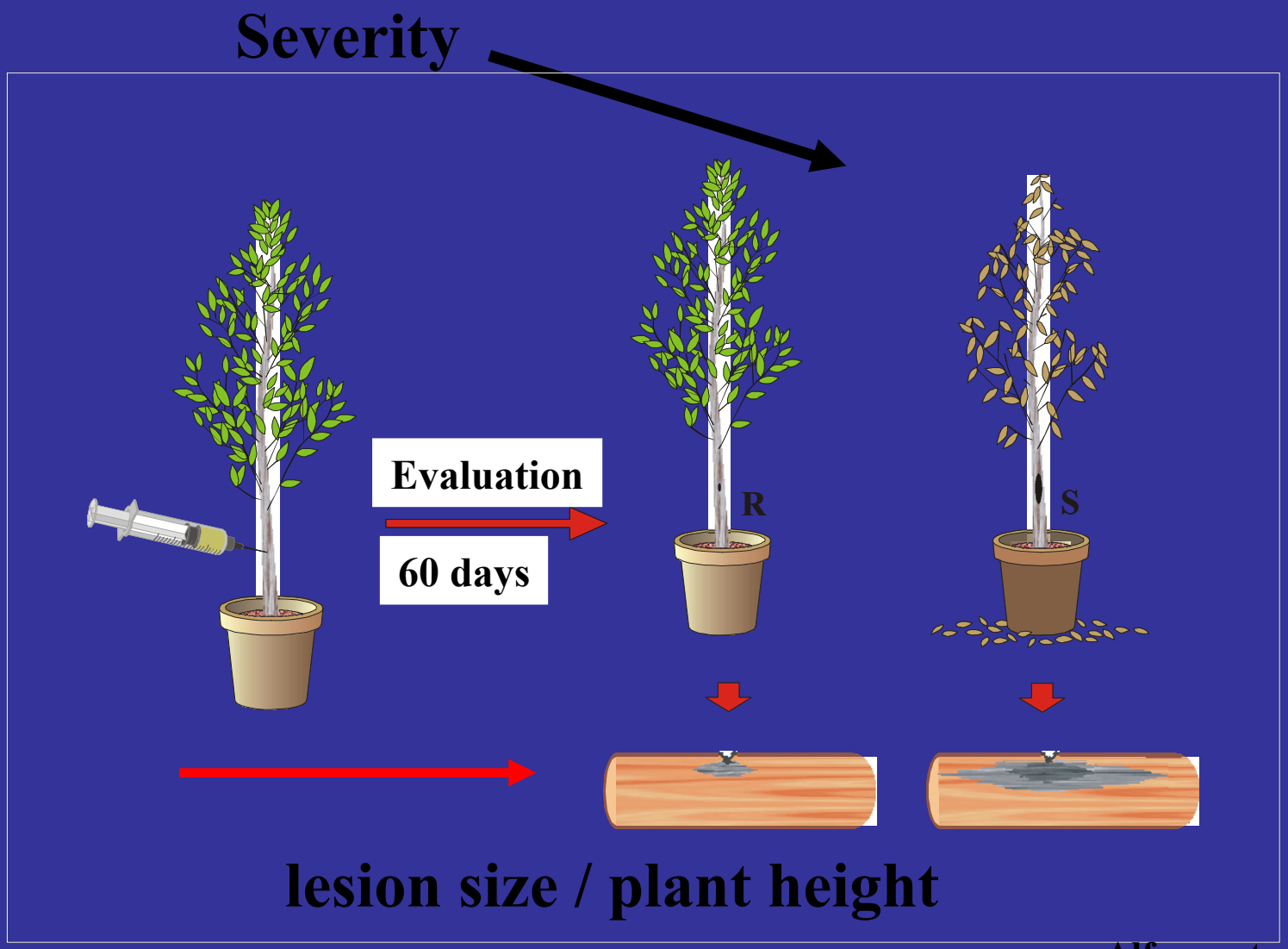
Resistant clone

Susceptible clone

Inoculation

Laia et al. (1999)

How to evaluate resistance?

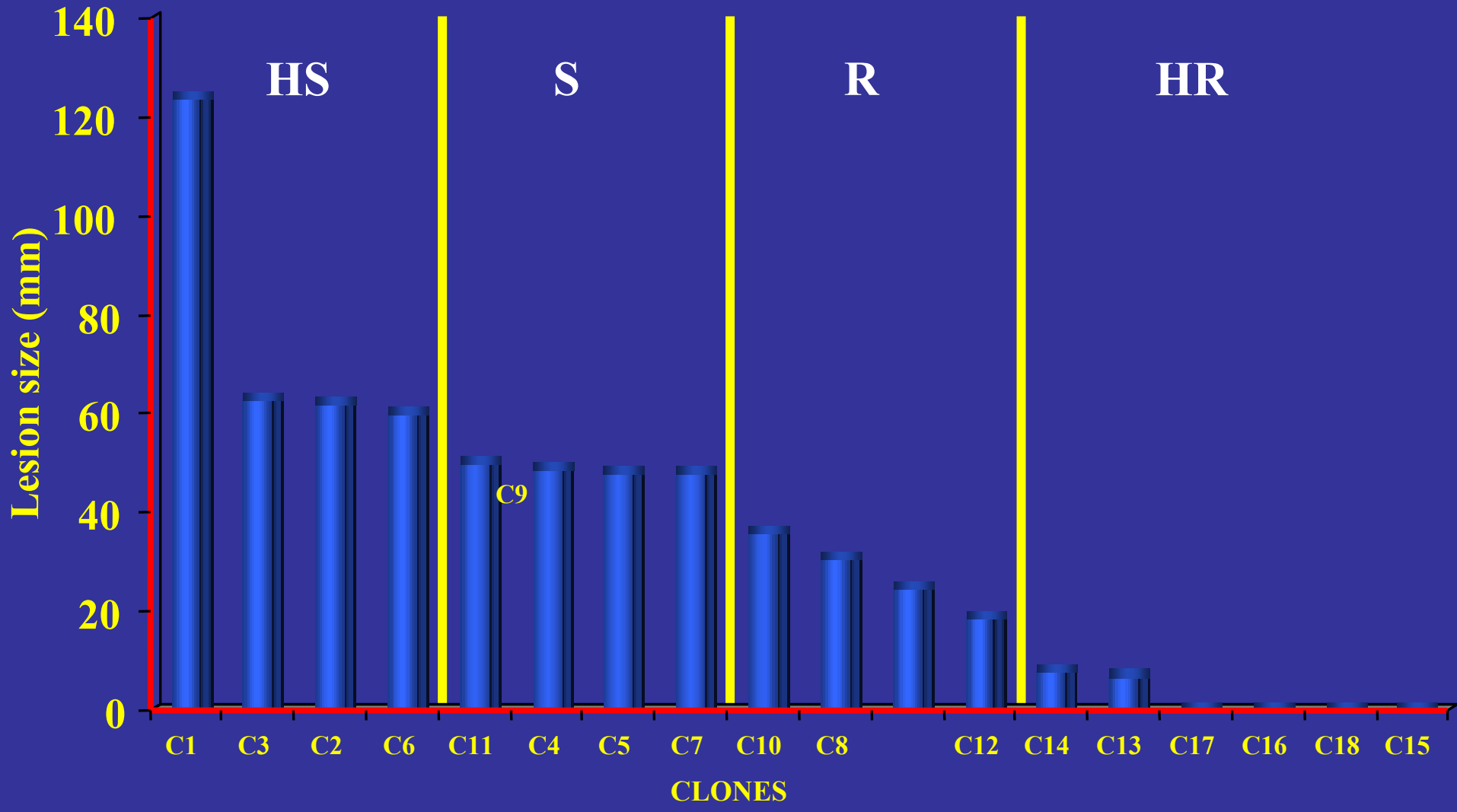


Alfenas et al. (2009)

Alfenas et al. (2011)

Evaluation of resistance

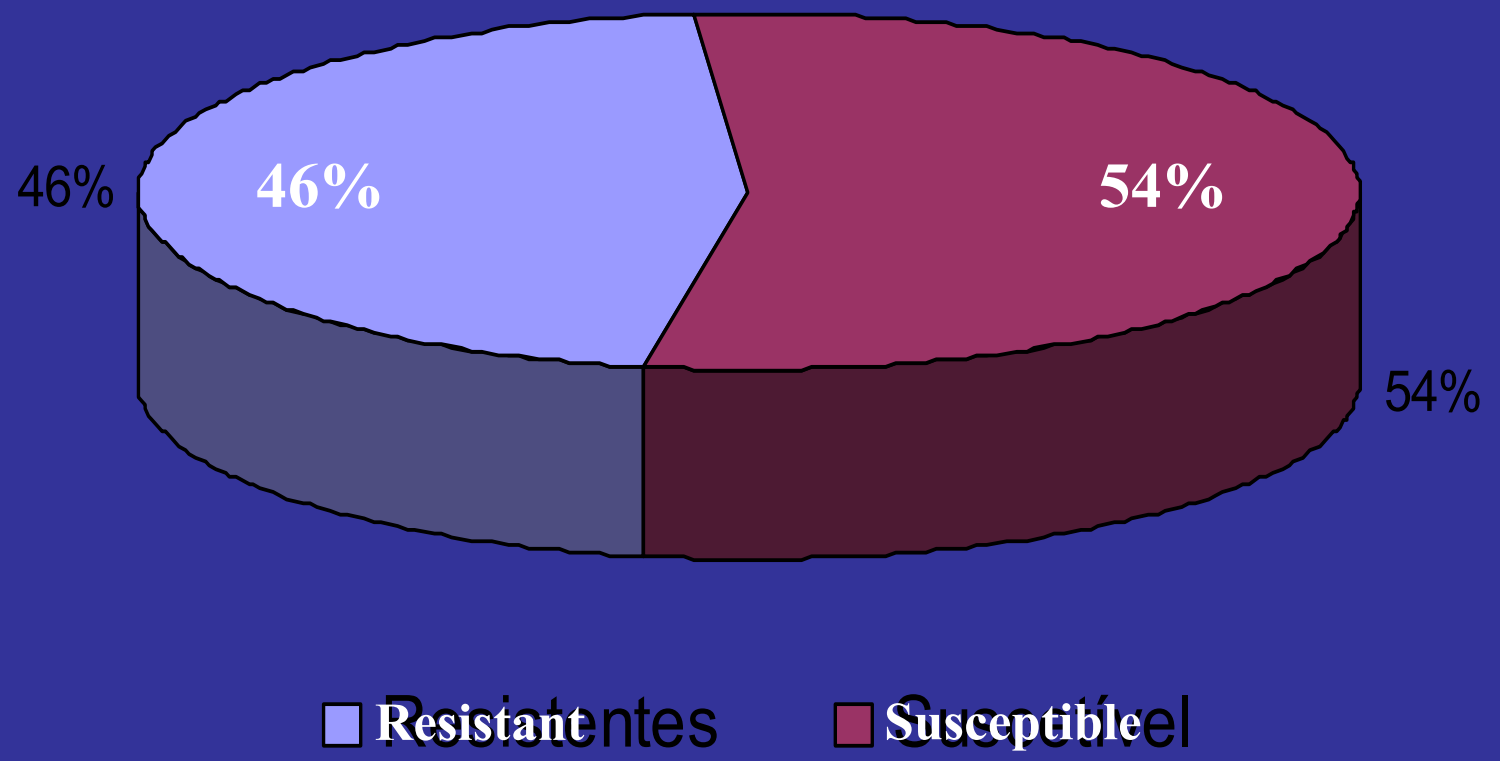
Resistance of *Eucalyptus* spp. clones to *Ceratocystis fimbriata* wilt



HS - Higly susceptible
S - Susceptible
R - Resistant
AR - Highly resistant

Zauza et al. (2004)

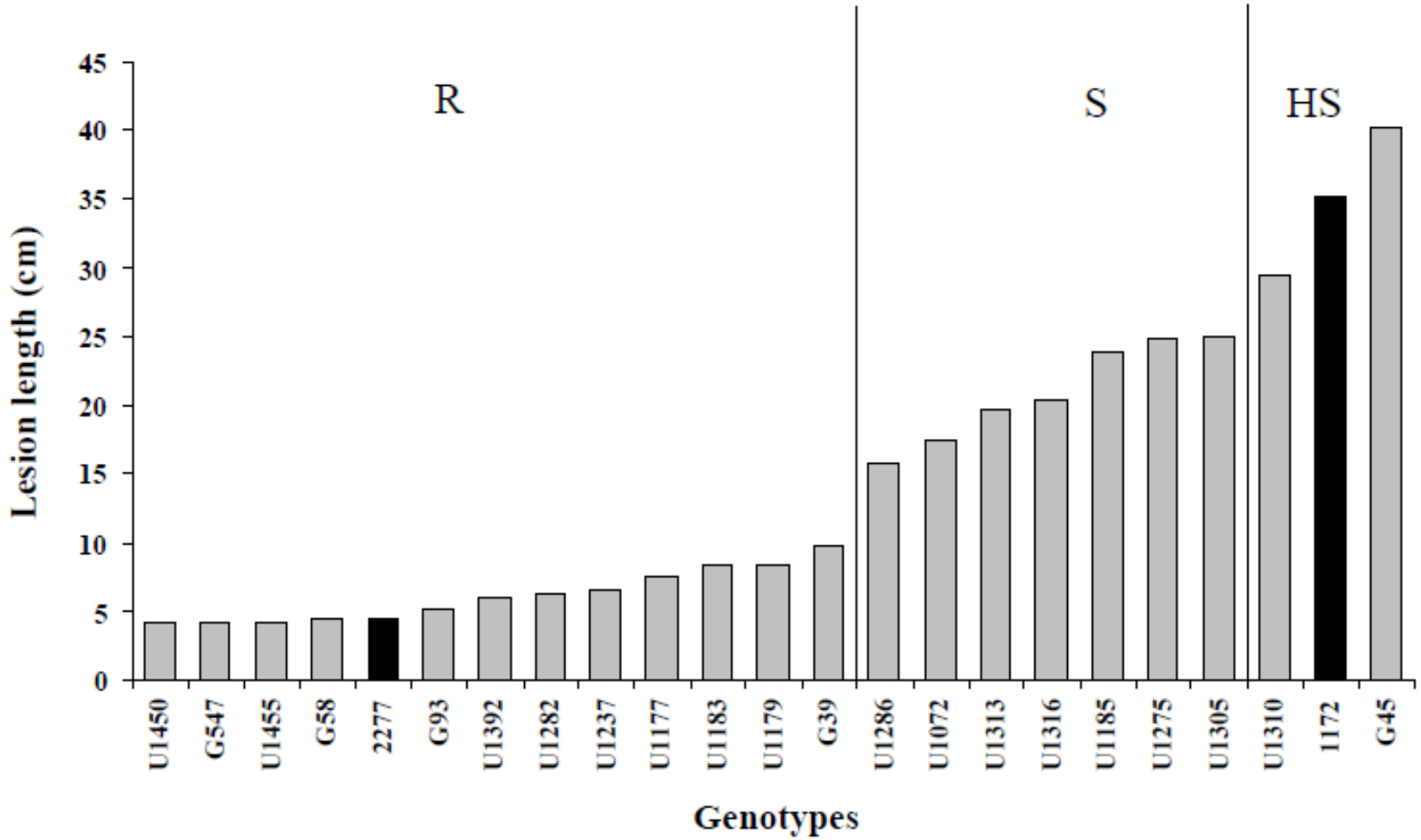
Resistance of *Eucalyptus* to *Ceratocystis fimbriata* wilt (181 clones)



Resistance to ceratocystis wilt (*Ceratocystis fimbriata*) in parents and progenies of *Eucalyptus grandis x E. urophylla*

By Carla Cristina Gonçalves Rosado[1], Lúcio Mauro da Silva Guimarães[1], Miranda Titon[1], Douglas Lau[2], Leonardo Rosse[3], Marcos Deon Vilela de Resende[4] and Acelino Couto Alfenas[1]*

Silvae Genetica 59, 2 (2010)



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Silvae Genetica 59, 2 (2010)

Segregation of resistance in hybrid progenies

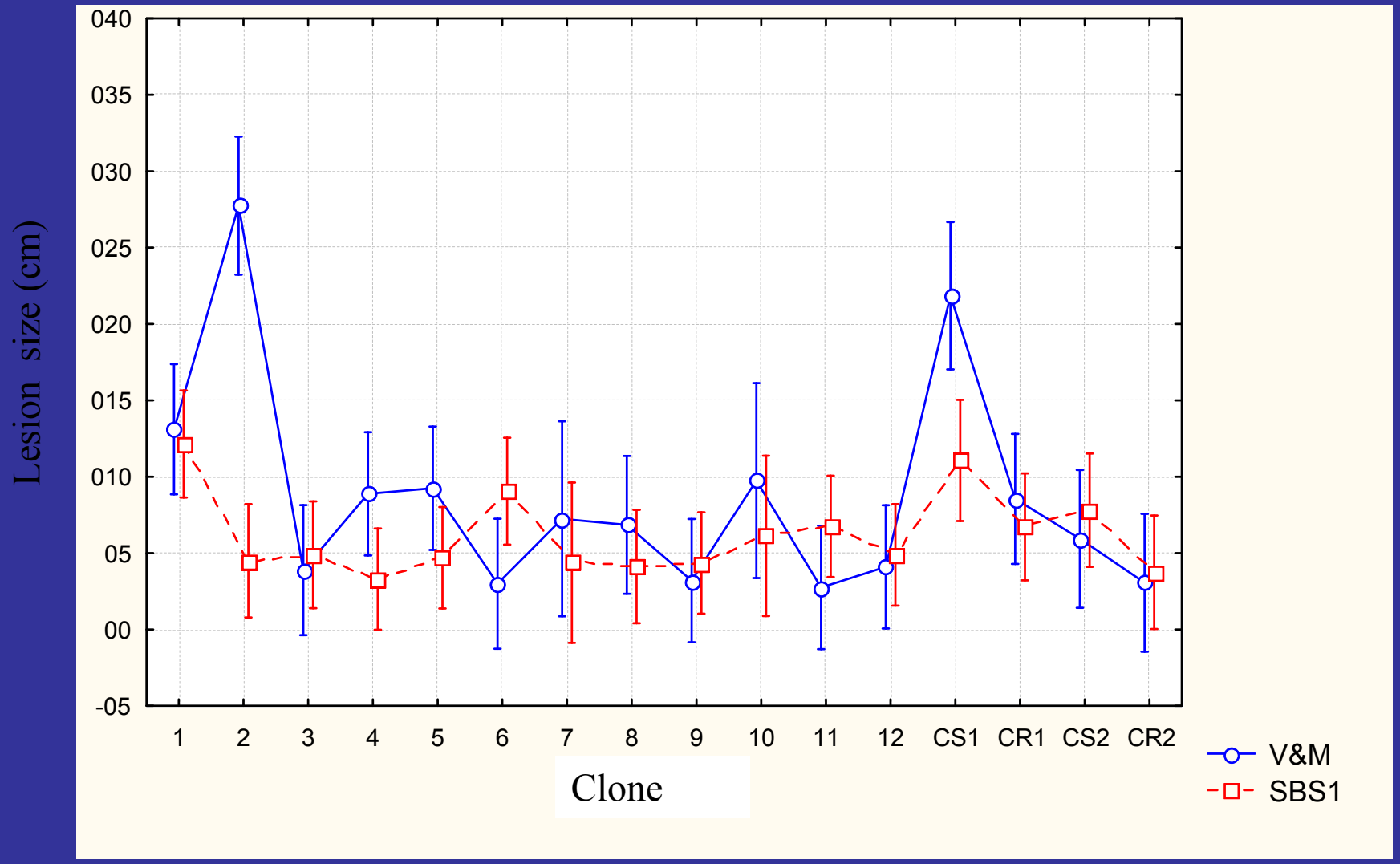
Genetic parameters analysis for the full-sib experiment	Values
Narrow-sense individual heritability	0.50 ± 0.11
Broad-sense individual heritability from the joint analysis	0.58 ± 0.

1. **Due to the relatively high inheritability (50 and 58% in the narrow and broad sense, respectively) the degree of genetic control of the trait is high and probably controlled by a small number of genes;**

2. **The allelic dominance of the trait is low, since the two values of heritability are very close.**

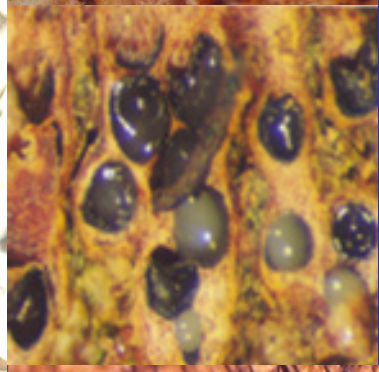
3. **The determination coefficient of additive genetic resistance is therefore 50% and the determination degree of genetic dominance is only 8%.**

Variability in pathogenicy of *Ceratocystis fimbriata*

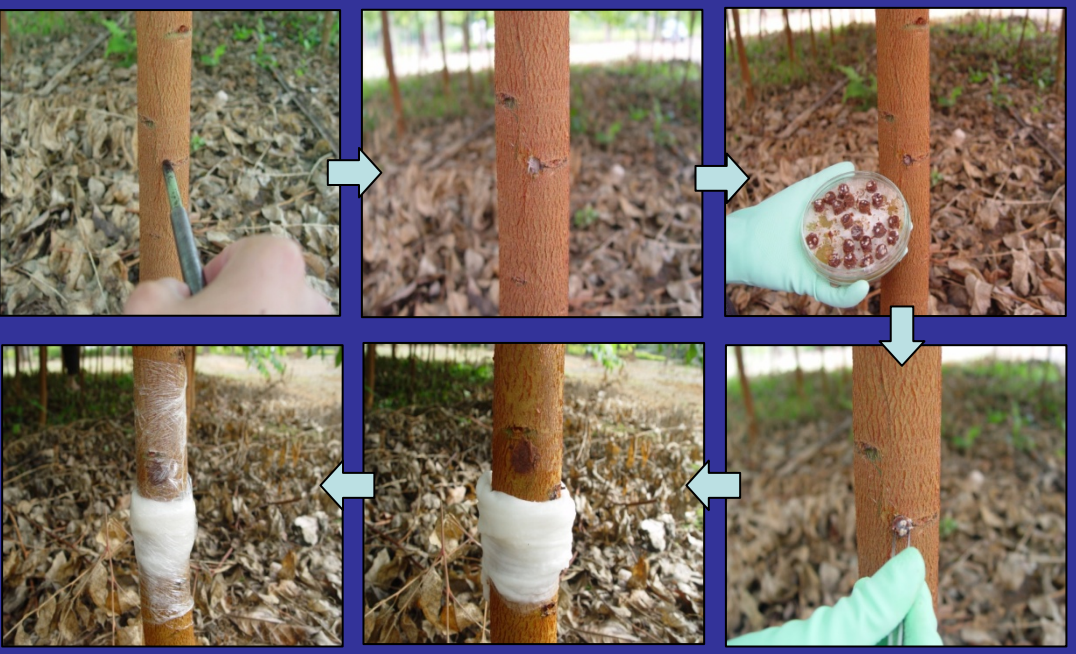


Ferreira et al. (2009)

Chrysoporthe canker (*Chrysoporthe cubensis*)



Inoculation *Chrysoporthe cubensis* in *Eucalyptus* spp.



Hodges et al. (1976)

Evaluation of resistance

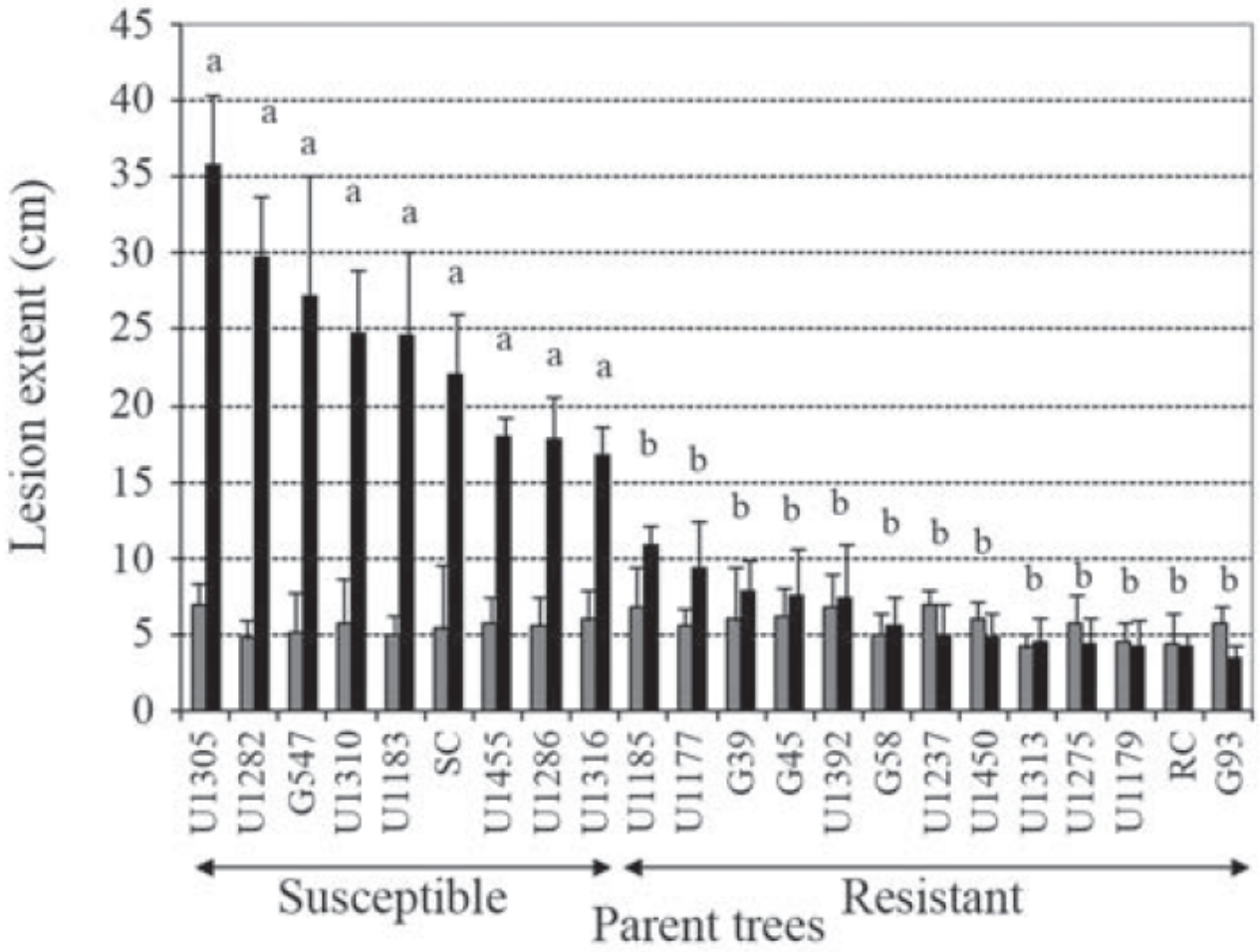
Size of bark lesion

Size of sapwood lesion

Guimarães et al. (2010)

Genetic control of *Eucalyptus urophylla* and *E. grandis* resistance to canker caused by *Chrysoporthe cubensis*

Guimarães *et al.* *Genetics and Molecular Biology*



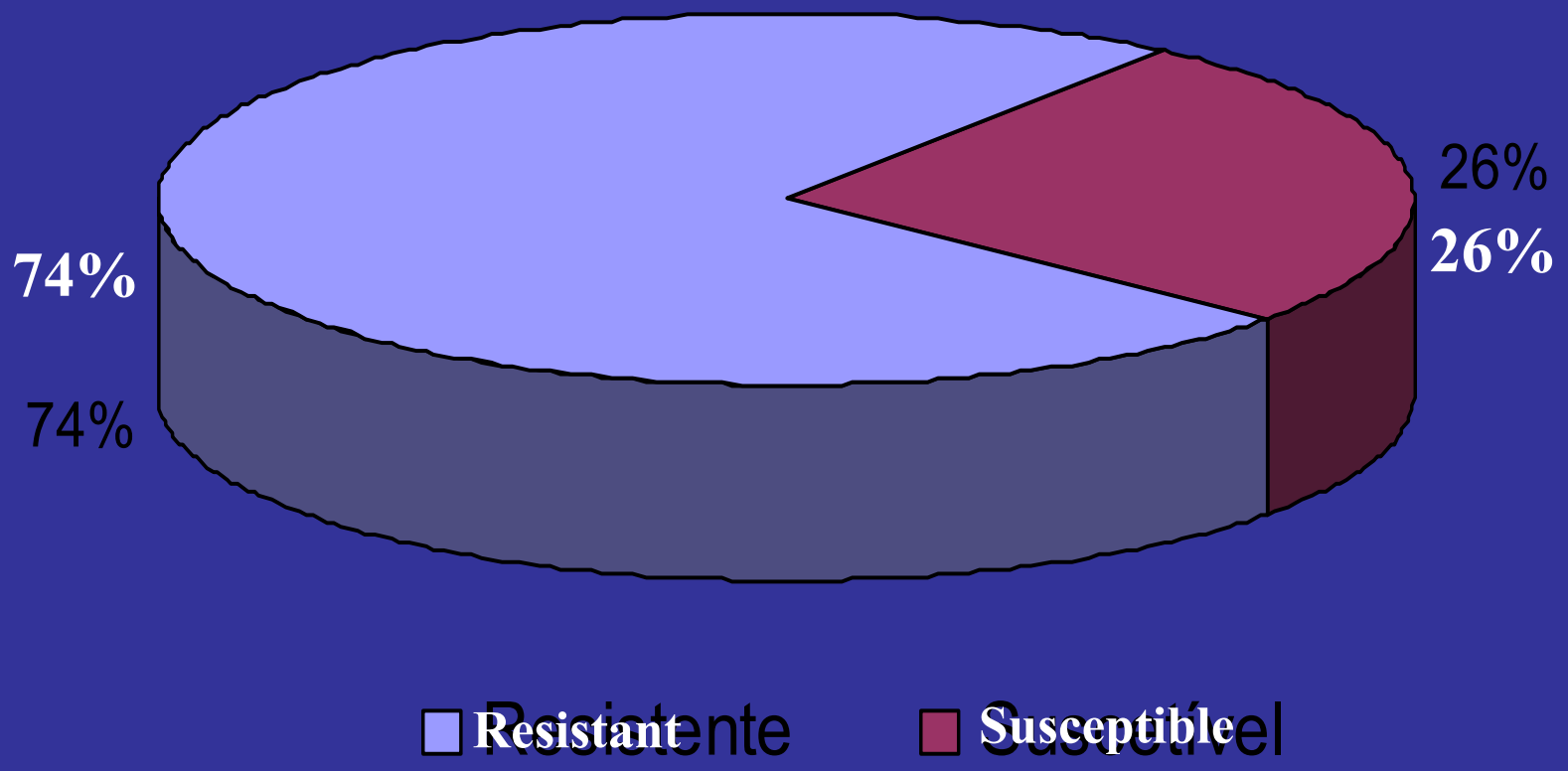
+ S species

- ☞ *Eucalyptus saligna*
- ☞ *Eucalyptus grandis*

+ R species

- ☞ *Eucalyptus urophylla*
- ☞ *Eucalyptus pellita*
- ☞ *Eucalyptus microcorys*
- ☞ *Corymbia torelliana*

Resistance of clones to *Chrysoporthe cubensis* canker (66 clones)



Leaf blight and defoliation caused by *Cylindrocladium pteridis*

Graça et al.(2005)

Inoculation of *Cylindrocladium* on *Eucalyptus pteridis*

4 month-old potted plants

1 x 10^4 conida/mL

Mist irrigation 26º.C/48h

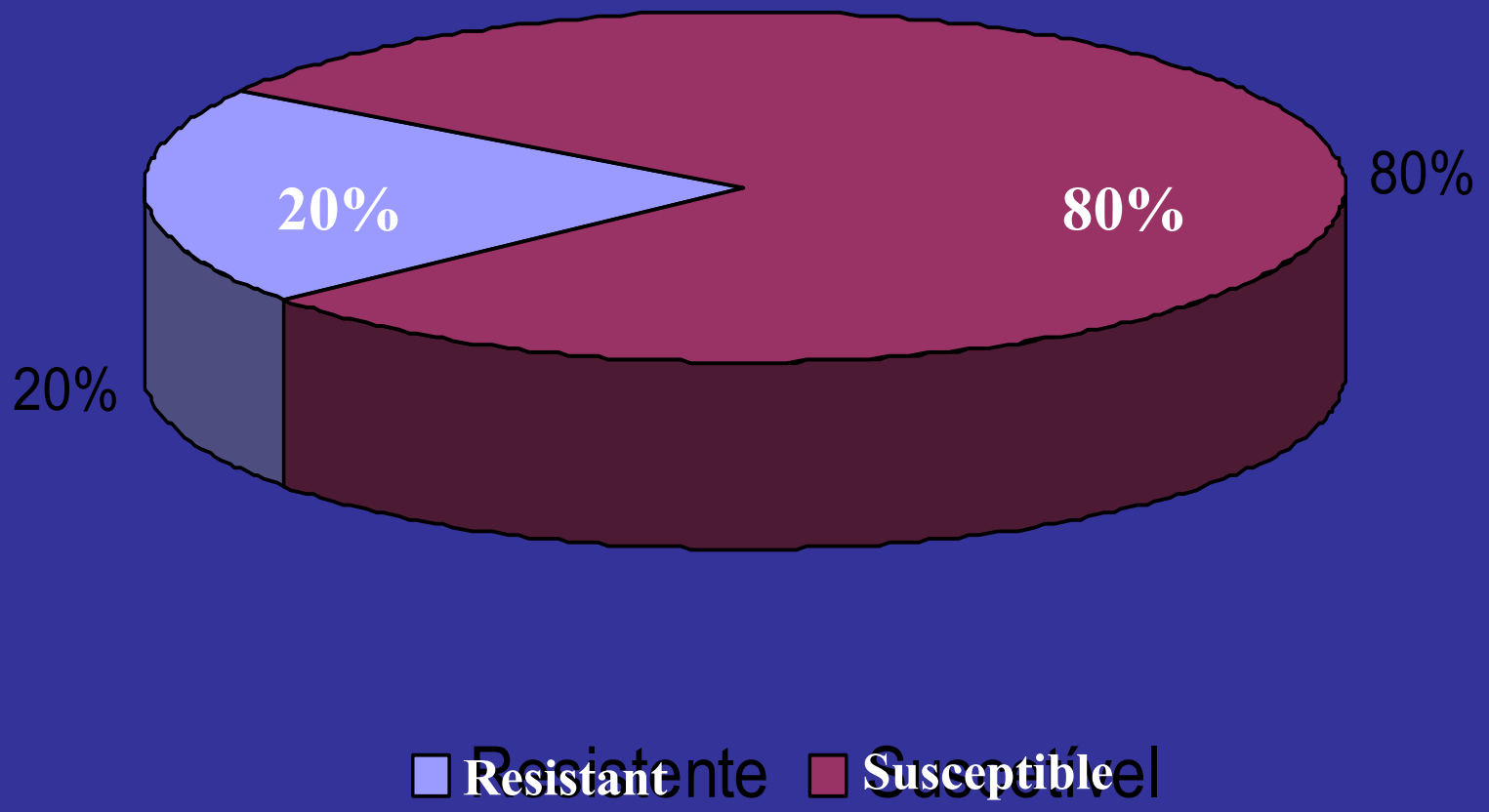
Defoliation: 30/50 d

Distribution of symptoms in the plant

Resistant clone

Susceptible clone

Resistance of *Eucalyptus* clones to defoliation caused by *Cylindrocladium pteridis* (89 clones)



How to minimize the risks of diseases in eucalyptus plantations?

1. Monitoring the incidence of diseases in plantations
2. Avoid planting large areas with a same clone
3. Planting of resistant clones selected by inoculation under controlled conditions
4. Determination of the variability in the pathogen population

What are the stages of a breeding program of *Eucalyptus* spp. for disease resistance?

1. Survey of the main diseases in each region.

2. Determination of possible sources of resistance.

3. Inheritance studies.

4. Implementation of the program based on results of the inheritance studies.

Conclusions

1. Wide inter and intra-speficific variabiliy for resistance in *Eucalyptus*

Possibility for new R genes

Conclusions

2. Cloning of resistant genotypes is the best strategy for disease control in *Eucalypus* spp.

Healthy and higly productive clonal plantations

Current Research Team

Acelino
P.hD.
Orientador

Lúcio
Eng. Agrônomo
Pós-Doutorando

Talyta
Eng. Florestal
Pós-Doutorando

Carla
Eng. Florestal
Estudante DS

Rafael
Eng. Florestal
Estudante DS

Graziela
Eng. Florestal
Estudante DS

Leonardo
Eng. Florestal
Estudante DS

Patrícia
Eng. Florestal
Estudante DS

Camila
Bióloga
Estudante MS

Daniele
Eng. Florestal
Estudante MS

Denise
Eng. Florestal
Estudante MS

Natália
Eng. Florestal
Estudante MS

Marcela
Bióloga
Aperfeiçoamento

Marcelo
Eng. Agrônomo
Aperfeiçoamento

Camila
Eng. Florestal
Bolsista IC

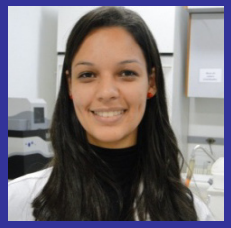
Cristina
Eng. Florestal
Bolsista IC

Nicolle
Eng. Florestal
Bolsista IC

Pedro
Agronomia
Bolsista IC

Ramon
Eng. Florestal
Bolsista IC

Rodrigo
Eng. Florestal
Bolsista IC



Thaísa
Eng. Florestal
Bolsista IC

Márcia
Assistente
Técnica

Thank you!

Acelino Couto Alfenas
aalfenas@ufv.br
Departament of Plant Pathology
Federal Universisty of Viçosa
Viçosa – Minas Gerais
Brazil

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