

Genetic Basis of Resistance in *Eucalyptus* spp. Pathosystems

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Introduction

Eucalyptus is the most widely planted hardwood crop in world-wide tropical and subtropical regions because of its high growth rate, broad adaptability, and multipurpose wood properties. Until the 1970s, the *Eucalyptus* plantations in Brazil were practically disease free. However, plantations have continued to expand into warmer and more humid areas that are more favorable to pathogen infection. Furthermore, the use of high-yielding genotypes with unknown disease resistance, the implementation of clonal forestry, and the introduction of new management techniques have favored the emergence of disease epidemics in recent decades. Presently, prominent diseases such as rust (*Puccinia psidii*), Ceratocystis wilt (*Ceratocystis fimbriata*), and canker (*Chrysosporthe cubensis*) are the most damaging diseases in *Eucalyptus* plantations. During recent years, we have conducted a series of inoculation experiments with controlled-crossed progenies to better understand the genetic basis of resistance for the prominent *Eucalyptus* pathosystems.

Eucalyptus x *Puccinia psidii* Pathosystem

Under favorable conditions, *Puccinia psidii* infects juvenile organs of the plant, either in the nursery or field. Rust infection can reduce tree growth and lead to loss of apical dominance. In highly susceptible genotypes, rust can also induce malformations of the affected organs, necrosis, hypertrophy, mini-cankers, and death of growing tips (Alfenas et al. 2009, Coutinho et al. 1998, Glen et al. 2007). The wide inter- and intra-specific genetic variability for rust resistance allows selection of resistant clones, progenies, or species for planting (Carvalho et al. 1998, Xavier et al. 2007). *Eucalyptus grandis* (Hill) Maiden is one of the most planted eucalypt species, but it is also among the most susceptible species. Thus, rust is currently the most damaging disease to eucalypt plantations in tropical regions because of the wide use of *E. grandis* (Zauza et al. 2010). In contrast, species such as *E. pellita* F. Muell. can be considered an important reservoir of genes for rust resistance (Guimarães et al. 2010b). Resistance to rust is predominately expressed by a hypersensitive reaction (HR) (Xavier 2002), which is a common type of response triggered when plants that contain resistance genes (R genes) are challenged by pathogens that contain avirulence genes (Avr genes).

In 2003, a major gene for rust resistance, *Ppr-1*, was identified in a control pollinated family of *E. grandis* and mapped near the AT9₉₁₇ RAPD marker (Junghans et al. 2003). The locus *Ppr-1* was positioned on a microsatellite reference genetic map for *Eucalyptus* on the linkage group 3 (Mamani et al. 2010). Additionally, its position was validated by association genetics in one related and two unrelated background families. These results are consistent with the hypothesis that *Ppr-1* controls a large proportion of the variation for rust resistance in *E. grandis*. However, in recent studies, analyses of several inter-specific families, displaying different segregation patterns for rust resistance, provided additional evidence that the genetic control of rust resistance in *Eucalyptus* is more complex

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and includes quantitative resistance traits (Alves et al. 2012). The recognition that both additive and non-additive genetic variation (epistasis) are important contributors to rust resistance in eucalypts reveals the complexity of this host-pathogen interaction and helps explain the success that breeding has achieved in selecting rust-resistant clones, where the additive and non-additive effects are readily captured.

***Eucalyptus x Ceratocystis fimbriata* Pathosystem**

Due to its lethal nature, broad host range, and wide geographical distribution, Ceratocystis wilt caused by *Ceratocystis fimbriata* is currently one of the most important diseases of eucalyptus plantations in Brazil (Alfenas et al. 2009). Genetic variability for resistance to Ceratocystis wilt in *Eucalyptus* genotypes was confirmed by Zauza et al. (2004). Subsequently, Rosado et al. (2010) studied Ceratocystis wilt resistance by stem inoculations of *E. grandis* and *E. urophylla* S. T. Blake genotypes, and estimated the heritability and gains of selection in families derived from controlled interspecific crosses. In both species, some genotypes were highly resistant and others highly susceptible to Ceratocystis wilt. Estimates of individual narrow (50 percent) and broad (59 percent) sense heritability suggested a high degree of genetic control and low allelic dominance of the trait. A genetic gain in lesion size up to -74.4 percent was obtained from selection of the 50 best clones in the evaluated families.

Continuing the studies with this pathosystem, Rosado (2009) built a microsatellite genetic map for the inter-specific, full-sibling family DGxUGL [*(E. grandis x E. dunnii* Maiden) x (*E. urophylla x E. globulus*)] (127 individuals) and detected five QTLs for Ceratocystis wilt resistance. Marker analysis showed that five markers located in the linkage groups 1, 3, 5, 8, and 10 were associated with significant quantitative effects on resistance with heritabilities ranging from 9.6 to 34.2.

***Eucalyptus x Chrysoporthe cubensis* Pathosystem**

The existence of inter- and intra-specific genetic variability for canker resistance in eucalypts (Alfenas et al. 1983, Ferreira et al. 1978), together with the development of large-scale cloning in the 1980s, has led to the control of the disease through the selection and cloning of resistant genotypes (Alfenas et al. 2009). Recently, Guimarães et al. (2010a) evaluated resistance in *E. grandis* and *E. urophylla* trees, as well as in individuals from progenies derived from controlled crosses between the trees. Six-month-old plants were inoculated, and xylem and bark lesions were measured at 8 months post-inoculation. The results demonstrated that xylem lesions are strong indicators for the selection of resistant clones. The phenotypic analyses indicated the existence of high genetic variability for resistance in both species. Individual narrow and broad sense heritability estimates were 17 percent and 81 percent, respectively, suggesting that canker resistance is quantitative and highly dependent on dominance and epistasis.

Due to the wide genetic variation for canker resistance in *E. grandis* and *E. urophylla*, the introduction of resistant parent trees into ongoing breeding programs may increase the chances of obtaining disease-resistant clones at the end of the selection program. The results of our study also reinforce the need for using artificial inoculation with *C. cubensis*, when selecting pathogen-resistant parent trees and progenies of *Eucalyptus* spp. Furthermore, these studies also demonstrate the importance of cloning resistant genotypes for disease control.

Breeding for Disease Resistance

Plantations of clones or elite-resistant varieties represent the most efficient strategy for disease control. Currently, most Brazilian commercial eucalyptus clones are based on inter-specific hybrids (Assis and Resende 2011). However, artificial inoculations have shown that 80 percent of these

clones are susceptible to at least one important disease. In contrast to canker resistance, most eucalypt breeding programs have not targeted rust and *Ceratocystis* wilt resistance until recently. Rust and *Ceratocystis* wilt resistance has usually been assessed in the final stages of clonal trials, and susceptible clones are recommended for areas with no history of epidemics or simply discarded. With the expansion of clonal plantations, epidemics have become more frequent. As a result, several tropical breeding programs now specifically include rust and *Ceratocystis* wilt resistance as a selection criterion.

Since forestry breeding programs are usually medium to long term in duration, it is essential to have disease-resistant parents. Thus, before the formation of a breeding orchard, it is recommended that the resistant parental materials are thoroughly evaluated and selected. Moreover, one should also evaluate the resistance clones selected in the breeding program. This evaluation should be performed after the selection of promising clones prior to implementation of expanded clonal trials.

In reciprocal recurrent selection (RRS), evaluation of eucalyptus rust resistance should be made in the hybrid progeny before selection of parents for recombination, submitting all the families to mass inoculation of the pathogen under controlled conditions. Only the parents of families with resistant plants must be recombined and thus participate in the RRS. A new screening test must be performed in pure progeny test, selecting only plants that are resistant to rust (fig. 1). Evaluation of resistance to *Ceratocystis* wilt and canker, which require clonal replicates, should be performed in progeny tests of pure species. Resistant plants should be selected for the new breeding orchard (fig. 1).

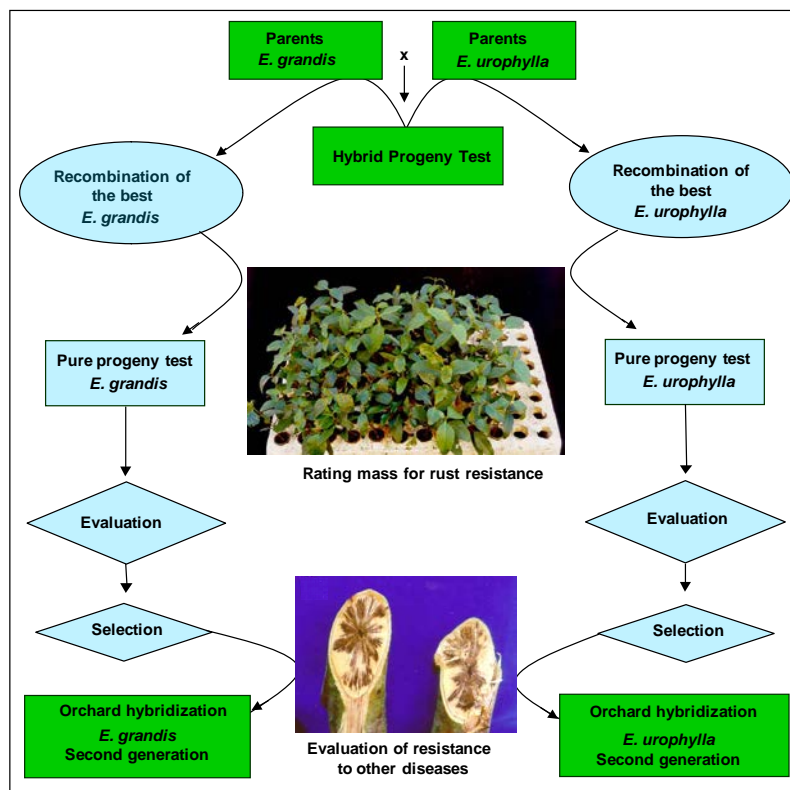


Figure 1— Stages of the breeding program in the reciprocal recurrent selection, in which assessments should be performed for disease resistance.

In addition to determining when disease resistance of the clones will be evaluated during the breeding program, other issues should be considered: (i) reliable inoculation protocols are needed for disease-resistance evaluations; (ii) determining the genetic basis and the mode of inheritance of disease resistance is an essential step to obtaining disease-resistant plant material for the breeding program; (iii) the introgression of genes from different *Eucalyptus* species has been suggested to broaden the genetic basis of commercial clones for resistance to the main diseases; and (iv) a better understanding of the population genetic structures of the pathogens is needed to determine the role of pathogen variability in disease resistance that is targeted by the breeding strategies.

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Literature Cited

- Alfenas, A.C.; Jeng, R.; Hubbes, M. 1983.** Virulence of *Cryphonectria cubensis* on *Eucalyptus* species differing in resistance. *European Journal of Forest Pathology*. 13: 197–205.
- Alfenas, A.C.; Zauza, E.A.V.; Maffia, R.G.; Assis, T.F. 2009.** Clonagem e doenças do Eucalipto. Viçosa. Editora UFV. 2ª edição. 500p.
- Alves, A.A.; Rosado, C.C.G.; Faria, D.A.; Guimarães, L.M.G.; Lau, D.; Brommonschenkel, S.H.; Grattapaglia, D.; Alfenas, A.C. 2012.** 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. *Euphytica*. 183: 27–38.
- Assis, T.F.; Resende, M.D.V. 2011.** Genetic improvement of forest tree species. *Crop Breeding and Applied Biotechnology*. S1: 44–49.
- Carvalho, A.D.O.; Alfenas, A.C.; Maffia, L.A.; Carmo, M.G.F. 1998.** Resistance of *Eucalyptus* species, progenies and provenances to *Puccinia psidii*. *Pesquisa Agropecuária Brasileira*. 33: 139–147.
- Coutinho, T.A.; Wingfield, M.J.; Alfenas, A.C.; Crous, P.W. 1998.** Eucalyptus rust: a disease with the potential for serious international implications. *Plant Disease*. 82: 819–825.
- Ferreira, F.A.; Alfenas, A.C.; Freitas, A.L. 1978.** Determinação da resistência de 16 procedências de *Eucalyptus* ao cancro do eucalipto causado por *Diaporthe cubensis* Bruner, no Vale do Rio Doce, MG. *Revista Árvore*. 2: 110–129.
- Glen, M.; Alfenas, A.C.; Zauza, E.A.V.; Wingfield, M.J.; Mohammed, C. 2007.** *Puccinia psidii*: a threat to the Australian environment and economy - a review. *Australasian Plant Pathology*. 36: 1–16.
- Guimarães, L.M.S.; Resende, M.D.V.; Lau, D.; Rosse, L.N.; Alves, A.A.; Alfenas, A.C. 2010a.** Genetic control of *Eucalyptus urophylla* and *E. grandis* resistance to canker caused by *Chrysosporthe cubensis*. *Genetics and Molecular Biology*. 33: 525–531.
- Guimarães, L.M.S.; Titon, M.; Lau, D.; Rosse, L.; Oliveira, L.S.S.; Rosado, C.C.G.; Christo, G.G.O.; Alfenas, A.C. 2010b.** *Eucalyptus pellita* as a source of resistance to rust, Ceratocystis wilt and leaf blight. *Crop Breeding and Applied Biotechnology*. 10: 124–131.
- Junghans, D.T.; Alfenas, A.C.; Brommonschenkel, S.H.; Oda, S.; Mello, E.J.; Grattapaglia, D. 2003.** Resistance to rust (*Puccinia psidii* Winter) in *Eucalyptus*: mode of inheritance and mapping of a major gene with RAPD markers. *Theoretical and Applied Genetics*. 108: 175–180.
- Mamani, E.M.C.; Bueno, N.W.; Faria, D.A.; Guimarães, L.M.S.; Lau, D.; Alfenas, A.C.; Grattapaglia, D. 2010.** Positioning of the major locus for *Puccinia psidii* rust resistance (Ppr1) on the *Eucalyptus* reference map and its validation across unrelated pedigrees. *Tree Genetics & Genomes* 6: 953–962.

- Rosado, C.G.C. 2009.** Genética da resistência à murcha de ceratocystis (*Ceratocystis fimbriata*) em *Eucalyptus* spp. Viçosa, MG: UFV. Dissertação de Mestrado em Genética e Melhoramento.
- Rosado, C.C.G.; Guimarães, L.M.S.; Titon, M.; Lau, D.; Rosse, L.; Resende, M.D.V.; Alfenas, A.C. 2010.** Resistance to ceratocystis wilt (*Ceratocystis fimbriata*) in parents and progenies of *Eucalyptus grandis* x *E. urophylla*. *Silvae Genetica*. 59: 99–106.
- Xavier, A.A. 2002.** Histopatologia da interação *Puccinia psidii* e virulência de isolados do patógeno em espécies de Myrtaceae. Viçosa, MG: UFV. Tese de Doutorado em Fitopatologia.
- Xavier, A.A.; Sanfuentes, E.V.; Junghans, D.T.; Alfenas, A.C. 2007.** Resistência de *Eucalyptus globulus* e *Eucalyptus nitens* à ferrugem (*Puccinia psidii*). *Revista Árvore*. 31(4): 7317–7335.
- Zauza, E.A.V.; Alfenas, A.C.; Harrington, T.C.; Mizubuti, E.S.; Silva, J.F. 2004.** Resistance of *Eucalyptus* clones to *Ceratocystis fimbriata*. *Plant Disease*. 88: 758–760.
- Zauza, E.A.V.; Alfenas, A.C.; Old, K.; Couto, M.M.F.; Graça, R.N.; Maffia, L.A. 2010.** Myrtaceae species resistance to rust caused by *Puccinia psidii*. *Australasian Plant Pathology*. 39: 406–411.