

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

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ABSTRACT - *Rust* (*Puccinia psidii*), *ceratocystis wilt* (*Ceratocystis fimbriata*) and *cyndrocladium leaf blight* (*Cylindrocladium pteridis*) are important diseases of *eucalyptus*. Planting of resistant genotypes is the most suitable control strategy of forest diseases under field condition. Resistance level of 23 *Eucalyptus pellita* clones was evaluated by artificial inoculations. Among the inoculated clones, 12 were resistant to rust, 16 to *ceratocystis wilt* and 12 to *cyndrocladium leaf blight*, and three of them were resistant to all three diseases. The high intra-specific variability found in this study demonstrates the importance of *E. pellita* as a disease resistance source to be employed for introgression of novel resistance genes in *eucalyptus* genetic breeding programs.

Key words: *Eucalyptus*, *Puccinia psidii*, *Ceratocystis fimbriata*, *Cylindrocladium pteridis*, disease.

INTRODUCTION

The main objectives of forest genetic breeding are to reduce damage caused by disease and pests and produce trees adapted to grow in adverse environments (Sobrosa and Martins-Coder 2001). Until the 1970s, a relatively small area was planted with *eucalyptus* in Brazil and the plantations, concentrated in the states of Minas Gerais, São Paulo and Rio Grande do Sul, were considered practically disease-free. However, with the expansion of plantations, the use of high-yielding genotypes with no previous knowledge of its resistance level to many diseases, the implementation of clonal forestry and the introduction of new management techniques have favored the

emergence of epidemics, caused by endemic or accidentally introduced pathogens (Alfenas et al. 2004). Rust (*Puccinia psidii* Winter), *ceratocystis wilt* (*Ceratocystis fimbriata* Ellis and Halsted) and *cyndrocladium leaf blight* (*Cylindrocladium pteridis* Wolf) are currently among the most damaging diseases in *eucalyptus* plantations (Alfenas and Zauza 2007).

Under favorable conditions, *P. psidii* infects juvenile organs of the plant either in nursery or in the field (Coutinho et al. 1998). Rust infection may 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, minicankers and death of growing tips (Glen et al. 2007). The wide inter

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and intra-specific genetic variability for rust resistance allows selection of resistant clones, progenies or species for planting (Dianese et al. 1984b, Carvalho et al. 1998, Xavier et al. 2007). More recently the genetic control of rust resistance has been studied. Junghans et al. (2003a) found that the phenotypic variation of rust resistance in *E. grandis* W. Hill ex Maiden controlled pollinated families is determined by a dominant “major” gene, denominated *Ppr-1*, with incomplete penetrance and variable expression, according to the genetic background. Although selection of resistant genotypes has been practiced and the existence of inter and intra-specific variability is well documented; only a limited number of *Eucalyptus* species or hybrids have been studied in an attempt to assess its resistance level. Presently, most commercial clones in Brazil are hybrids of *E. grandis* x *E. urophylla* (called “urograndis”). Artificial inoculations conducted in our laboratory have shown that about 75% of these clones are susceptible to *P. psidii* (Santos et al. 2008).

Ceratocystis fimbriata was first reported in *Eucalyptus* in Brazil (Ferreira et al. 1999) and subsequently in Congo (Roux et al. 2000), Uganda (Roux et al. 2001), Uruguay (Barnes et al. 2003) and in South Africa (Roux et al. 2004). The pathogen penetrates the plant through fresh lesions on the stem and roots and induces wilting, die-back, wood darkening, canker, and tree death (Ferreira et al. 2006). The pathogen spreads into the host parenchyma tissue causing dark stripes at different heights of the stem and killing a portion of the vascular cambium, phloem and pheloderm. This results in longitudinal, continuous or interrupted lesions on the outer part of the trunk, causing tree wilting and death (Ferreira et al. 2006). The existence of inter and intra-specific genetic variability for ceratocystis wilt resistance allows the use of resistant genotypes as the most efficient and economic method for the control of the disease (Zauza et al. 2004).

Cylindrocladium leaf blight, caused by *C. pteridis*, may also be a growth-limiting factor of highly susceptible *Eucalyptus* genotypes in warm and humid regions, which are highly favorable to its infection (Alfenas et al. 2004). The disease is initially characterized by small, round and light grey leaf lesions that progress to light brown coalesced spots that can occupy the entire leaf, resulting in intense defoliation in susceptible genotypes (Ferreira et al. 1995). Observations of natural infection in the field and artificial inoculations under controlled conditions conducted in our laboratory indicate the existence of variability for resistance in *Eucalyptus* spp. making

possible the identification and selection of resistant genotypes for planting and crossing (Santos et al. 2008). In a recent study, a screening technique was developed to select eucalypts genotypes resistant to cylindrocladium leaf blight (Graça et al. 2009).

Although about 700 species of the genus *Eucalyptus* have been described, only a limited number of species is planted in Brazil, mainly *E. grandis*, *E. urophylla* S.T. Blake and their hybrids “urograndis”. To reduce the impact of the major diseases, it is important to broaden the genetic basis of commercial clones by introgressing resistance genes from other eucalypts species. Among these, *E. pellita* F. Muell. has been considered as a promising source of resistance (Alfenas et al. 2004). Thus, the objective of this study was to evaluate the resistance of 23 *E. pellita* clones to rust, ceratocystis wilt and cylindrocladium leaf blight, by artificial inoculations.

MATERIAL AND METHODS

Plant material

In the experiment 23 clones of *E. pellita* were evaluated, five from Atherton (Queensland, Australia), two from Kuranda (Queensland, Australia), one from Cardwell (Queensland, Australia), ten from Melville (island from Australia), one from Goe (Papua New Guinea) and four from Kiriwo (Papua New Guinea). Sixty-day old cuttings were transplanted to 2 L (for *P. psidii* and *C. fimbriata*) or 6 L (for *C. pteridis*) pots containing a mixture of soil: sand:manure (3:1:1) supplemented with superphosphate (18% P₂O₅) at 1 g dm⁻³. The plants were grown in greenhouse until the adequate age for inoculation: 30 days for *P. psidii*, 60 days for *C. fimbriata* and 90 days for *C. pteridis*. Each plant was weekly fertilized with 100 mL of Ouro Verde solution (15-15-20 NPK) at 7.5 g L⁻¹. Ten plants of each clone were inoculated for rust and ceratocystis wilt and five for cylindrocladium leaf blight. The experiments were conducted from January 2007 to December 2008 in a nursey at the Federal University of Viçosa, Minas Gerais, Brazil.

Evaluation of rust resistance

An inoculum suspension at 2 x 10⁴ urediniospores mL⁻¹ of a single pustule isolate (UFV-2) of *P. psidii* was homogeneously sprayed on both leaf surfaces of the plants by using a DeVilbiss no. 15 atomizer, linked to an electric compressor (0.6-0.8 kgf cm⁻²). The inoculated plants

were incubated in a mist chamber at 25 ± 2 °C in the dark for 24 h, and then maintained in a growth chamber (12 h photoperiod, $40 \text{ mmol s}^{-1} \text{ m}^{-2}$ light intensity, at 22 ± 2 °C) for 20 days (Ruiz et al. 1989), when disease severity was evaluated using the diagrammatic scale developed by Junghans et al. (2003b). This scale comprises four severity degrees, based on the pustule size: S0 = immunity or hypersensitive reaction (HR), with necrosis or fleck; S1 = small pustules, < 0.8 mm diameter; S2 = medium sized pustules, from 0.8 to 1.6 mm diameter and S3 = large pustules, > 1.6 mm diameter. Plants of classes S0 or S1 were considered resistant, while plants of classes S2 and S3 susceptible following Junghans et al. (2003b). A completely randomized design, containing ten replicates per treatment (clone from *E. pellita* evaluation) and one-plant plot, was employed. Clones C1179 and C1183, both *E. grandis* x *E. urophylla* hybrids, were used as resistant and susceptible controls, respectively.

Evaluation of ceratocystis wilt resistance

Ceratocystis fimbriata isolate SBS-1, obtained from an infected tree of a hybrid clone *E. grandis* x *E. urophylla* was grown on MYEA medium (2% malt extract, 0.2% yeast extract and 2% agar) in Petri dishes (9 cm diameter) at 28 ± 1 °C, 12 h photoperiod under $20 \mu\text{mol photons s}^{-1} \text{ m}^{-2}$ of light intensity. Eight days after incubation, sterilized distilled water was added to the colony and the shores were scrapped off with a Drigalski spatula. Spore suspension was filtered through double layer sterilized gauze and the inoculum concentration was adjusted at 2.5×10^6 spores mL^{-1} . At 60 days after transplanting, plant stem was superficially cut lengthwise (about 2 cm) and 0.5 mL of the spore suspension was added on the surface of the cut. Subsequently, the inoculated area was covered with plastic film and the plants were maintained under greenhouse conditions (26 ± 5 °C). Xylem discoloration was measured 45 days after inoculation. Clones C2277 (*E. grandis* x *E. urophylla*) and C1172 (*E. grandis* x *E. urophylla*) were used as resistant and susceptible controls, respectively. A completely randomized design, containing ten replicates per treatment was employed. Each experimental unit consisted of a single plant. The data was subjected to an analysis of variance (ANOVA), followed by a mean comparison test (Dunnett, $P = 0.05$), to identify resistant and susceptible plants. The clones that did not differ statistically from the resistant control (C2277) were considered resistant.

Evaluation of cylindrocladium leaf blight resistance

A monosporic culture of *C. pteridis* (PF-1) isolated from diseased plants in a commercial eucalyptus plantation in Lençóis Paulista, São Paulo, Brazil was used in the experiment. This isolate was identified as *C. pteridis* based on morphological features as previously described (Crous 2002). Mycelium plugs of the *C. pteridis* were transferred to Petri dishes (9 cm diameter) containing a PDA (potato-dextrose-agar), following incubation at 25 ± 1 °C, 12 h photoperiod, under $20 \mu\text{mol photons s}^{-1} \text{ m}^{-2}$ of light intensity until the mycelium covered the surface of the dishes. Autoclaved distilled water (20 mL) was added on the culture and all aerial mycelium was sapped off from the medium surface with a soft brush and the water excess was poured off. Subsequently, 10 mL of sterilized distilled water was added to the dish and the colony was kept immersed for 48 h, when the water excess was discarded and the culture was incubated at 25 ± 1 °C, 12 h photoperiod for 48 h to obtain abundant spore production. A volume of 20 mL distilled water containing 0.05% Tween 20 was added to the surface of the culture and the spores were scraped with an autoclaved soft brush. After filtration, through double layer sterilized gauze, the suspension was adjusted to 1×10^4 conidia mL^{-1} and sprayed on both leaf surfaces of 90 day-old eucalyptus plants (Graça et al. 2009). After incubation in a mist chamber for 24 h (photoperiod 12 h, $40 \text{ mmol s}^{-1} \text{ m}^{-2}$ light intensity, at 25 ± 2 °C), the inoculated plants were kept in a greenhouse (26 ± 5 °C). A completely randomized design, containing five replicates (plants) per treatment was employed. Each experimental unit consisted of a single plant. Defoliation was evaluated 30 days after inoculation in three branches of the basal third. The clones were compared considering the total mean of defoliation of the trees. Clone CA06 of *E. grandis* was used as a resistant control, based on the information of disease incidence under natural infection in the field. The data was subjected to an ANOVA, followed by a mean comparison test (Dunnett, $P = 0.05$), to identify resistant and susceptible plants. The clones that did not differ statistically of the resistant control (A06) were considered resistant.

RESULTS AND DISCUSSION

Among the 23 *E. pellita* clones tested to rust resistance, 12 were resistant and 11 were susceptible (Table 1). A wide spectrum of rust reaction was found among resistant genotypes, as some of them showed

immunity (Figure 1A), hypersensitivity reaction (HR) (Figure 1B), or HR followed by puntiform pustules with sporulation (Figure 1C). In some clones, typical HR was characterized by necrosis in the leaf center and chlorotic margins.

The clones PE10, PE29, PE40, PE52 and PE114 were the most resistant, showing immunity reaction (Table 1). Hypersensitive reaction with eventual presence of puntiform pustules were observed in the clones PE18, PE41, PE71, PE73, PE78, PE143, and PE151. Leaf curling was observed for PE18, PE41 and PE78, probably due to a large number of infection points and to necrotic lesion size as a result of HR. Defoliation was observed for PE78, mainly in the lateral branches, due to several HR lesions. Atypical HR was observed in plants of clone PE151, without a clear differentiation of chlorotic and necrosed areas. Clones PE01, PE11, PE22, PE47, PE76, PE89, PE103, PE110, PE117, PE129, and PE160 were susceptible (Figure 1D). Plants of clone PE22 showed HR followed by a profuse sporulation.

The wide spectrums of reactions found in different provenances of the resistant clones indicate the possible role of different genes for resistance. Among the rust resistant clones, five were immune (two from Melville, one from Atherton, one from Goe and one from Kiriwo), four showed hypersensitive reaction (HR) (one from Melville, one from Cardwell, one from Kuranda and one from Kiriwo) and three presented HR followed by puntiform pustule formations (two from Melville and one from Kuranda). This wide variation in rust intensity was also observed by Junghans et al. (2003a) in full-sib progenies of *E. grandis*, in which resistance is controlled by a dominant major effect gene, denominated *Ppr-1*, with incomplete penetrance and a variable expression, depending on the genetic background. The incapacity of the HR in preventing the spreading of the pathogen indicates incomplete penetrance of the gene(s) for resistance. Under field conditions, it is expected that clones with HR followed by puntiform pustule would be resistant, due to the lower progress rate of the disease and also because of the low spores production. Among the susceptible clones, PE22 showed HR followed by abundant uredinial sporulation, typical of susceptible plants, which indicates low efficiency and slow activation of the host defense responses. It is likely that the resistance gene present in PE22, by itself, does not guarantee the resistance, since the action of secondary effect genes should be equally important (Junghans et al. 2003a).

The use of resistant plants has been used successfully for the control of ceratocystis wilt in several crops as *Mangifera* (Ribeiro et al. 1995, Rossetto et al. 1997), *Coffea* (Castilla 1982) and *Crotalaria* (Ribeiro et al. 1977). In *Eucalyptus* spp., the genetic variability for resistance to ceratocystis wilt was previously demonstrated by Zauza et al. (2004) for hybrid clones *E. grandis* x *E. urophylla*. Significant variability for ceratocystis wilt resistance was found among *E. pellita* clones this study. Seven clones were susceptible (PE73, PE151, PE11, PE143, PE89, PE71 and PE47) and 16 clones (PE52, PE117, PE41, PE103, PE22, PE114, PE1, PE110, PE76, PE160, PE40, PE10, PE129, PE78, PE29 and PE18) which do not differ statistically from the resistant control (C2277) were considered resistant. This unequivocally demonstrates that *E. pellita* is a promising source of resistance to *C. fimbriata* (Figure 2 and Table 1). Of the clones considered susceptible, just PE47 presented symptoms of wilt and lesion length comparable to the susceptible control (C1172). However, it must be emphasized that the results of this study are based on the inoculation of one single *C. fimbriata* isolate, obtained from infected plants in the south of Bahia (Brazil). Possible variations in the pathogen population for virulence may exist and therefore can originate differentiated plant-pathogen interactions, as observed by Zauza et al. (2004).

Significant variation was observed for resistance to the defoliation caused by cylindrocladium leaf blight (Figure 3). Clones PE10 and PE47 were the most susceptible (50% defoliation) while PE151 and PE40 were the most resistant ($\leq 30\%$ defoliation) (Figure 3 and Table 1). Clone CA06 (resistant control) had the lowest percentage of defoliation (23.9%). Clones PE47, PE10, PE41, PE18, PE11, PE1, PE143, PE114, PE76, PE52, PE73 and PE110 were classified as susceptible, while PE71, PE22, PE103, PE117, PE78, PE160, PE129, PE89, PE29, PE151 and PE40, which do not statistically differ from the resistant control (CA06) were considered resistant (Figure 3).

As found in other *Eucalyptus* species, the cylindrocladium leaf blight in *E. pellita* was most severe in expanded leaves and the highest percentage of defoliation occurred in the basal branches of the lower third of the canopy. These results corroborate those found by Graça et al. (2009) in inoculations of *E. grandis* x *E. urophylla* hybrids. Although, the clone CA06 (resistant control) is considered resistant to leaf blight under natural infection, 24% defoliation was found in the basal third, probably due to the highly favorable conditions. Defoliation in the basal third of *E. pellita* clones varied

Table 1. Origin of *Eucalyptus pellita* clones and phenotype for rust (*Puccinia psidii*), ceratocystis wilt (*Ceratocystis fimbriata*) and cylindrocladium leaf blight (*Cylindrocladium pteridis*) resistance

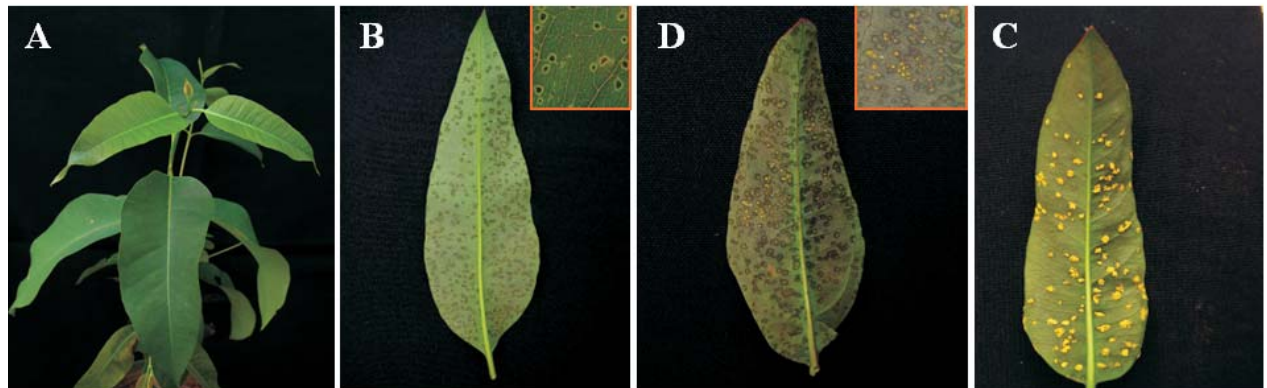
Clone	Provenance	Rust		Ceratocystis wilt		Cylindrocladium leaf blight	
		Class ¹	Phenotype	Lesion (cm) ²	Phenotype	Defoliation (%) ³	Phenotype
PE1	Atherton	S3	S	3.2	R	48.2	S
PE10	Melville	S0	R	4.0	R	53.0	S
PE11	Melville	S3	S	5.7	S	48.4	S
PE18	Melville	HR-S1	R	4.6	R	48.5	S
PE22	Melville	HR-S2	S	3.1	R	41.3	R
PE29	Goe	S0	R	4.4	R	31.0	R
PE40	Melville	S0	R	3.8	R	26.1	R
PE41	Melville	HR-S0	R	3.0	R	48.9	S
PE47	Melville	S3	S	10.1	S	56.7	S
PE52	Kiriwo	S0	R	2.3	R	44.5	S
PE71	Kuranda	HR-S0	R	7.0	S	41.5	R
PE73	Kuranda	HR-S1	R	5.0	S	43.5	S
PE76	Atherton	S3	S	3.3	R	45.4	S
PE78	Melville	HR-S1	R	4.3	R	35.4	R
PE89	Kiriwo	S2	S	6.9	S	31.7	R
PE103	Melville	S3	S	3.0	R	40.5	S
PE110	Atherton	S3	S	3.3	R	39.1	R
PE114	Atherton	S0	R	3.2	R	46.8	S
PE117	Kiriwo	S3	S	2.4	R	40.3	R
PE129	Melville	S3	S	4.2	R	34.4	R
PE143	Kiriwo	HR-S0	R	6.0	S	48.0	S
PE151	Cardwell	HR-S0	R	5.5	S	29.2	R
PE160	Atherton	S3	S	3.5	R	34.6	R

¹ Classification based on the diagrammatic scale proposed by Junghans et al. (2003b): S0 = immunity or hypersensitive reaction (HR), with necrosis or fleck; S1 = small pustules, < 0.8 mm diameter; S2 = medium sized pustules, from 0.8 to 1.6 mm diameter and S3 = large pustules, > 1.6 mm diameter. R = Resistance, S = susceptible.

² Mean lesion size on xylem 45 days after inoculation.

³ Mean from percentage of defoliation in the basal third.

Resistant clones to all three diseases are highlighted in bold.

**Figure 1.** Variation in the phenotype of *Eucalyptus pellita* clones inoculated with *Puccinia psidii*. (A) Immune reaction; (B) hypersensitivity reaction; (C) hypersensitivity reaction and slight sporulation; and (D) susceptible reaction.

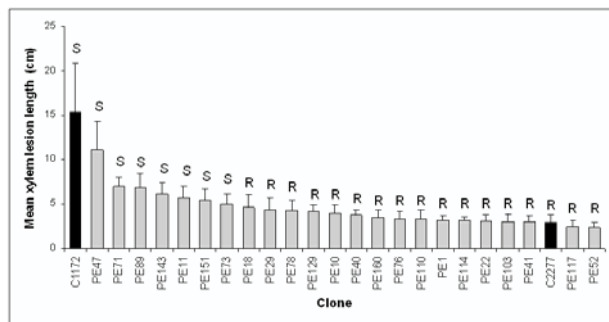


Figure 2. Mean xylem lesion length observed in *Eucalyptus pellita* clones (PE) and in the resistant (C2277) and susceptible controls (C1172) (black bars), inoculated with *Ceratocystis fimbriata*. Different letters indicate statistically significant differences for resistance between clones (ANOVA - Dunnett test, P = 0.05). R = resistant and S = susceptible clone. CV = 33%.

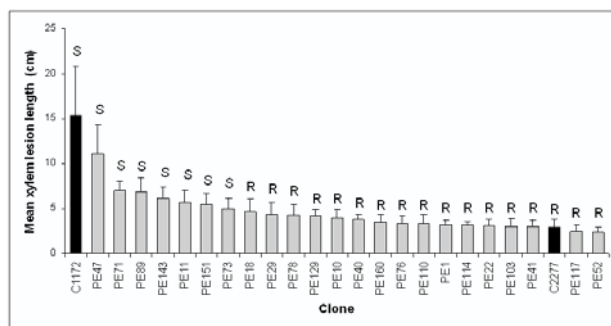


Figure 3. Mean percentage of defoliation in the basal third of 23 *Eucalyptus pellita* clones and the resistant control A06 (black bar), 30 days after inoculation with *Cylindrocladium pteridis*. Different letters indicate statistically significant differences for resistance between clones (ANOVA - Dunnett test, P = 0.05). R = resistant and S = susceptible clone. CV = 20%.

from 26.1% to 56.7%. Among these, 12 did not differ from the resistant control (CA06) and were classified as resistant. Additional information on disease incidence in *E. pellita* and the knowledge on the genetic basis of resistance are fundamental for the use of cylindrocladium leaf blight resistant genotypes.

Currently, most commercial clones of eucalyptus are based on *E. grandis* x *E. urophylla* crosses. Artificial inoculations have shown that 80% of these clones are susceptible to at least one of the evaluated diseases (Santos et al. 2008). Therefore, 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 (Alfenas et al. 2004). Under natural infection conditions and under artificial inoculations, *E. pellita* has been shown as a potential source of resistance to many diseases (Dianese et al. 1984a, Dianese et al. 1984b, Carvalho

et al. 1998, Alfenas et al. 2004). Thus, in this study, we tested 23 clones of *E. pellita* to resistance to rust, caused by *P. psidii*, ceratocystis wilt, caused by *C. fimbriata* and cylindrocladium leaf blight and defoliation, caused by *C. pteridis*. Of the evaluate clones, 12 were resistant to rust, 16 to ceratocystis wilt and 12 to cylindrocladium leaf blight, corroborating previous reports that indicated this species as a potential resistance source.

Out of the 23 *E. pellita* clones tested, three (PE40 and PE78 from Melville, and P29 from Goe) were resistant to all three diseases, representing important resistance sources to be exploited in genetic breeding programs. Although two clones (Melville provenance) performed particularly well, most were resistant or susceptible to the three diseases evaluated, independent of its origin. These results suggest that, basically, resistance sources to rust, ceratocystis wilt and cylindrocladium leaf blight can be found independently on the provenance.

Eucalyptus pellita has considerable potential for plantation forestry in the tropics, both as a pure species and as a parent in interspecific hybrid combinations (Harwood 1998). In Brazil it has been planted for charcoal and firewood production on account of the relatively high basic density of its wood and the good quality of its charcoal (Harwood 1998). This study demonstrated the intraspecific variability of *E. pellita* for resistance to three of the most important diseases and this species can be a base for selection of resistant genotypes to be introduced in genetic breeding programs. However, the efficiency of these resistance sources requires studies on inheritance and determination of resistance inheritability in interspecific crosses, involving mainly *E. pellita* in ongoing genetic breeding programs. Specifically for rust, where studies on genetics of resistance are more advanced, it is possible to investigate if the resistance gene(s) in *E. pellita* are homologous of *Ppr-1*, described in *E. grandis* by Junghans et al. (2003b). This information is important not only to expand the genetic basis, but also to understand how the loci that confer rust resistance are distributed in different *Eucalyptus* species.

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***Eucalyptus pellita* como fonte de resist ncia   ferrugem,   murcha-de-ceratocystis e   mancha-de-pteridis**

RESUMO - A ferrugem, causada por *Puccinia psidii*, a murcha-de-ceratocystis, causada por *Ceratocystis fimbriata*, e a mancha foliar e desfolha, causadas por *Cylindrocladium pteridis* est o entre as mais importantes doenas do eucalipto. O plantio de gen tipos resistentes   a melhor estrat gia de controle das doenas florestais em condi es de campo. Visando buscar novas fontes de resist ncia a essas doenas, avaliaram-se, neste trabalho, 23 clones de *Eucalyptus pellita* atrav s de inocula es artificiais. Dentre os clones avaliados, 12 foram resistentes   ferrugem, 16   murcha-de-ceratocystis e 12   mancha foliar de *cylindrocladium*, sendo tr s deles resistentes  s tr s doenas. A alta variabilidade intraespec fica encontrada neste estudo demonstra a import ncia de *E. pellita* como fonte de resist ncia a doenas, cujos gen tipos selecionados poder o ser empregados na introgress o de novos genes de resist ncia em programas de melhoramento gen tico do eucalipto.

Palavras-chave: Eucalipto, *Puccinia psidii*; *Ceratocystis fimbriata*; *Cylindrocladium pteridis*; resist ncia gen tica.

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