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Screening of rust and gall wasp in eucalypts species and provenances

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Abstract - The present study investigated myrtle rust and gall wasp from non-commercial eucalypt taxa with potential to hybridization. We selected 38 genotypes from 18 species of *Corymbia* and *Eucalyptus* (*Symphyomyrtus*) genus. Two rust inoculations were evaluated using inoculums from rose apple and eucalypt, and natural gall occurrence in a high pest infestation condition. *Corymbia* was more susceptible to rust than *Eucalyptus* genus, and it was observed severity variation due to the sources of rust inoculum. Species that belong to *Exsertaria* and *Transversaria* (*Latoangulatae)* sections were more susceptible to gall wasp, and *Corymbia* species showed no development of gall.

Avaliação da ferrugem e da vespa da galha em espécies e procedências de eucalipto

Resumo - Foi avaliada a ferrugem e a vespa da galha em táxons de eucalipto não comerciais com potencial para hibridização. Nós selecionamos 38 genótipos de 18 espécies dos gêneros *Corymbia* e *Eucalyptus* (*Symphyomyrtus*). Foram avaliadas duas inoculações de ferrugem com esporos provenientes de jambo e eucalipto e a ocorrência natural da vespa da galha em condições de alta infestação. Observou-se maior suscetibilidade à ferrugem em *Corymbia* do que em *Eucalyptus* e respostas diferentes às fontes de inóculo. Espécies das seções *Latoangulatae* e *Exsertaria* foram mais suscetíveis à vespa, e as espécies que pertencem ao gênero *Corymbia* não apresentaram desenvolvimento de galha.

Hundreds of eucalypt species are endemic in Australia and nearby islands; however, commercial eucalypt plantations have great importance worldwide. Nevertheless, just a few species and their hybrids are used in world plantations, especially those of the *Symphyomyrtus* subgenus (Potts & Dungey, 2004; Harwood, 2011).

To obtain high productivity, genotypes have been selected to adapt to the environmental conditions, and plantations management features have been improved throughout the years (Gonçalves et al., 2012). However, pests and diseases may decrease the yield of commercial plantations. In Brazil and in other countries, myrtle rust represents an economic risk and threatens the biodiversity, damaging plants of *Myrtaceae* family (Silva et al., 2013). Another cause of damage on eucalypt stands in Brazil is gall wasp, which has caused significant damage to commercial stands and eucalypt nurseries since 2008.

Puccinia psidii (myrtle rust) is native to South America (Argentina, Brazil, Colombia, Ecuador, Paraguay, Uruguay, and Venezuela) and Central America (Alfenas et al., 2005), showing a wide distribution. This fungus represents a high risk due to its great potential to cause damage to plants of *Myrtaceae* family (Tommerup et al., 2003; Grgurinovic et al., 2006; Glen et al., 2007; Booth & Jovanovic, 2012; Miranda et al., 2012). Although native to Brazil, this fungus started to affect the commercialization of Brazilian eucalypt stands in the 1980s.

Leptocybe invasa (blue gum chalcid), a pest insect of young eucalypt trees and seedlings, is native to Australia. A relatively narrow range of eucalypt hosts species was already described (Mendel et al., 2004; Thua et al., 2009), but the range of host species may be greater than already described, and it should be verified. This pest causes bump-shaped galls on petioles, stems, and leaves of young eucalypt trees. Severely affected trees show leaf fall, gnarled appearance and loss of growth. This insect ao invés de Gall is a global pest in eucalypts stands. In Brazil it has spread to several states, causing significant damages in nursery and field conditions.

Hybridization in *Eucalyptus* genus is commonly used to obtain a superior genotype in genetic improvement programs. There is a great potential for intra and interspecific hybridized eucalypts that could be cloned for commercial plantations, after an appropriate selection process. Gaining knowledge about behavior of potential species and provenances is important component of this selection process for commercial genotypes.

This study aimed to carry out an initial screening of taxa from genus *Corymbia* and *Eucalyptus* to investigate susceptibility to *Puccinia psidii*, using inoculum from two hosts (rose apple and eucalypt), and to verify galls formation (*L. invasa*).

Thirty eight taxa from 18 species of *Corymbia* and *Eucalyptus* were chosen, all of *Eucalyptus* species belonged to *Symphyomyrtus subgenus* (Table 1). Some seeds were imported from Australia in 2012 and others were collected in Brazil.

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Table 1. Description of species and number of provenances.

Number	Genus	Species	Subgenus and section	Provenances
1	*Corymbia*	*citriodora citriodora*	Ochraria	2
2	*Corymbia*	*citriodora variegata*	Ochraria	10
3	*Corymbia*	*henryi*	Ochraria	2
4	*Corymbia*	*torelliana*	Ochraria	1
5	*Eucalyptus*	*amplifolia*	Symphyomyrtus Exsertaria	2
6	*Eucalyptus*	*argophloia*	Symphyomyrtus Adnataria	1
7	*Eucalyptus*	*brassiana*	Symphyomyrtus Exsertaria	1
8	*Eucalyptus*	*brookeriana*	Symphyomyrtus Maidenaria	1
9	*Eucalyptus*	*camaldulensis*	Symphyomyrtus Exsertaria	1
10	*Eucalyptus*	*cladocalyx*	Symphyomyrtus Aenigmataria	1
11	*Eucalyptus*	*crebra*	Symphyomyrtus Adnataria	1
12	*Eucalyptus*	*longirostrata*	Symphyomyrtus Transversaria	4
13	*Eucalyptus*	*macarthurii*	Symphyomyrtus Maidenaria	1
14	*Eucalyptus*	*major*	Symphyomyrtus Transversaria	4
15	*Eucalyptus*	*moluccana*	Symphyomyrtus Adnataria	3
16	*Eucalyptus*	*occidentalis*	Symphyomyrtus Bisectaria	1
17	*Eucalyptus*	*thozetiana*	Symphyomyrtus Adnataria	1
18	*Eucalyptus*	*urophylla*	Symphyomyrtus Transversaria	1
			Total	38

The seedlings were produced in the municipality of Piracicaba, São Paulo State, Brazil, in IPEF Nursery, from September 2012 to January 2013. Commercial production protocol was used. Seeds were sowed directly in the containers (polypropylene tubes of 50 cm^3), with a mixture: rice hulls, coconut fiber, and vermiculite. The fertilization was performed four times a week with a solution of Calcium nitrate 450 g; Ammonium nitrate 300 g; Mono Ammonium Phosphate (MAP) 250 g; Potassium nitrate 300 g; Magnesium

sulfate 250 g; Ammonium sulfate 250 g; Tenso iron 2.5 g; Manganese sulfate 0.85 g; Boric Acid 0.75 g; Zinc sulfate 0.325 g; Copper sulfate 0.1 g; and Sodium molybdate 0.005 g in 1000 L of water.

In January 2013, 10 seedlings (≈90 days old), per taxa, were inoculated with rust spores. This experiment was conducted in duplicate, one with inoculums from *Syzygium jambos* (7.2 x 10^4 spores mL^{-1}) and another from *Eucalyptus urophylla* (7.6 x 10^4 spores mL^{-1}). Urediniospores were collected with a brush (Tiger ®) number 6, soft bristle and placed in Petri dishes. After collection, they were separately suspended in distilled water containing 0.05% of Tween 80 and were sprayed at the leaves. After inoculation, the plants were kept in control conditions with temperature of 21 °C ± 1 ºC and 80% air moisture, with nebulization every 30 min and photoperiod of 12 h.

Latent period, incidence and disease severity were evaluated (Castro et al., 1983; Ruiz et al., 1989), according to Coutinho & Figueiredo's (1984) scale, adapted by Pieri (2012).

Natural infestation of gall wasp was also evaluated, and no control was applied until the occurrence observation was carried out in the nursery during January/February, 2013. We evaluated 20 seedlings per genotype and classified a genotype as "susceptible" when at least one seedling exhibited galls caused by *Leptocybe invasa*.

It was observed more susceptibility to rust in *Corymbia* genus than in *Eucalyptus*, different responses in eucalypt taxa to the sources of rust inoculum (rose apple and eucalypt sources), and differences among provenances within the species for the same rust inoculum. Signs of pathogen occurrence (latent period) were visible 12 days after inoculation in all susceptible taxa for both inoculums. There was a genetic interaction of disease and eucalypt species and provenance. *Corymbia citriodora variegata* from Brooyar was the most susceptible taxa to rust collected from the eucalypt source but it did not show susceptibility to rust from rose apple. *Corymbia citriodora citriodora* from Kirrima showed no symptom to rust from eucalypts, however it was the most susceptible to rust from rose apple, showing high sporulation of pustules in all plants. All *Eucalyptus* genus seedlings exhibited high resistance, with hypersensitivity reactions observed only in *E. brookeriana* and *E. crebra* under inoculation of rust from apple rose. *E. crebra* showed hypersensitivity reaction in 20% of the plants, whereas *E. brookeriana* showed it in all plants (Table 2).

Taxa significantly differ in terms of tolerance (Van Heerden & Wingfield, 2002). Thus, rust genotypic interaction with eucalypt taxa is an important knowledge to drive eucalypt conservation or strategies in breeding program, and biological research is important to manage rust in eucalypt stands (Yamaoka, 2014). Rust shows rapid development that overcome resistance in short time (Samils et al., 2011). Resistance breakdown could quickly occur where there is high genetic variability of fungus, leading to a greater recombination potential among pathogens (Graça et al., 2011). Genetic variability is commonly low in sites where *Puccinia psidii* was recently introduced (Zhong et al., 2011). However, in Brazil, a considerable genetic rust diversity is observed, which is strongly related to host genotypes (Graça et al., 2011).

Another important fact for breeding programs is the ecological zoning of eucalypt rust that may provide useful information by indicating risk classes of disease and occurrence in the field (Masson et al., 2007; Booth & Jovanovic, 2012; Silva et al., 2013).

Many taxa were susceptible to gall wasp – more than 50% of studied eucalypts showed gall development. In *Eucalyptus* genus it was observed that 2/3 of the species were susceptible with higher levels of *Leptocybe invasa* susceptibility for species's that belong to *Exsertaria* and *Transversaria* (*Latoangulatae*) sections while no development of gall was found in the *Corymbia* species tested. Only four species of *Eucalyptus* showed no gall wasp development: *E. argophloia*, *E. brookeriana*, *E. crebra* and *E. urophylla*. Provenances also influence specie susceptibility to gall wasp. *E. moluccana* showed one susceptible provenance of the three evaluated (Table 2).

The results observed in seedling nursery is an indicative, but field infestation can be different. Thua et al. (2009) studied 18 species of eucalypts and observed gall occurrence in 13 species from six sections of *Eucalyptus* and one species of *Corymbia.* However, five species that showed susceptibility in the nursery were not damaged in the field as even the most susceptible species may have variation in susceptibility among provenances.

In Brazil, E. camaldulenis is an important species for dry areas as a parent of hybrids with *E. grandis* and/or E. urophylla. Gall wasp caused severe damage to commercial stands of E. camaldulensis when planted as pure species or hybridin the parentage of a hybrid. Therefore, study of species with drought tolerance should encompass susceptibility to gall as well.

Table 2. Rust and gall susceptibility in eucalypts genotypes.

Cod	Species	Section	Provenance	Rust (rate)		Gall
				Rose apple	*Eucalypt*	
1	*C. citriodora citriodora*	Ochraria	Yeppoon	1	4 (40%)	NE
2	*C. citriodora citriodora*	Ochraria	Kirrima	5 (100%)	1	NE
3	*C. citriodora variegata*	Ochraria	Barakula S.F.	4 (40%)	4 (40%)	NO
4	*C. citriodora variegata*	Ochraria	Saddler Springs	4(40%)	4 (20%)	NE
5	*C. citriodora variegata*	Ochraria	Richmond Range	1	1	NO
6	*C. citriodora variegata*	Ochraria	Barclays Deniliquin	1	4 (20%)	NO
7	*C. citriodora variegata*	Ochraria	Wolvi	1	4 (40%)	NE
8	*C. citriodora variegata*	Ochraria	Brooyar	1	5 (80%)	NE
9	*C. citriodora variegata*	Ochraria	Woondum	1	4 (20%)	NO
10	*C. citriodora variegata*	Ochraria	Cherry Tree	1	4 (20%)	NE
11	*C. citriodora variegata*	Ochraria	Woondum	1	4 (20%)	NO
12	*C. citriodora variegata*	Ochraria	Anhembi, SP – BR	1	4 (40%)	NO
13	*C. henryi*	Ochraria	Lockyer	1	4 (40%)	NO
14	*C. henryi*	Ochraria	Myrtle	1	1	NE
15	*C. torelliana*	Ochraria	Anhembi, SP – BR	1	1	NO
16	*E. amplifolia*	Exsertaria	Nerong S.F.	1	1	OB
17	*E. amplifolia*	Exsertaria	Clouds Ck Sf & Tsr	1	1	OB
18	*E. argophloia*	Adnataria		1	1	NO
19	*E. brookeriana*	Maidenaria	Otways	2 (100%)	1	NO
20	*E. cladocalyx*	Aenigmataria		1	1	NE
21	*E. crebra*	Adnataria	Nw Baradine Po	2 (20%)	1	NO
22	*E. longirostrata*	Transversaria	Starkvale Creek	1	1	OB
23	*E. longirostrata*	Transversaria		1	1	OB
24	*E. longirostrata*	Transversaria	Diamondy	1	1	OB
25	*E. longirostrata*	Transversaria	Coominglah	1	1	OB
26	*E. macarthurii*	Maidenaria	Paddys River, Nsw	1	1	OB
27	*E. major*	Transversaria	Se Gympie	1	1	OB
28	*E. major*	Transversaria	Blackdown Tableland	1	1	OB
29	*E. major*	Transversaria		1	1	OB
30	*E. major*	Transversaria	Brooweena	1	1	OB
31	*E. moluccana*	Adnataria	Crediton S.F.	1	1	NO
32	*E. moluccana*	Adnataria	Mt Garnet	1	1	NO
33	*E. moluccana*	Adnataria	Ravenshoe	1	1	OB
34	*E. occidentalis*	Bisectaria		1	1	NE
35	*E. thozetiana*	Adnataria	Clermont	1	1	NE
36	*E. camaldulensis*	Exsertaria	Selviria, MS – BR	1	1	OB
37	*E. brassiana*	Exsertaria	Urbano Santos, MA – BR	1	1	OB
38	*E. urophylla*	Transversaria	Avaré, SP – BR	1	1	NO

NE – No evaluation; NO – Not observed; OB – Observed; Rust classification 1 to 5. (1) Total absence of symptoms or hypersensitivity reaction; (2) Reaction to hypersensitivity and/or bronzing of leaves; (3) Injury not showing pustules; (4) injury with little spore pustules; (5) Injury presenting highly sporulating pustules.

Conclusions

C. torelliana, *E. argophloia*, *E. crebra*, two provenance of *E. moluccana* and one provenance of *C. citriodora* variegata showed good results for rust and gall wasp resistance, thus, these species could be a good choice to start new hybrids combinations.

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