Detection, transmission and pathogenicity of fungi on *Blepharocalyx salicifolius* (H.B.K.) Berg. seeds[1]

Suelen Santos Rego[2]*, Álvaro Figueredo dos Santos[3], Antônio Carlos Nogueira[2], Yoshiko Saito Kuniyoshi[2]

ABSTRACT - The objective of this study was to identify the fungi associated with the fruit and seeds of *Blepharocalyx salicifolius* and verify their transmission and pathogenicity to seeds and seedlings. Fungal identification on seeds was made using the blotter test and potato-dextrose-agar but only the blotter test was used for fruit. Fungal transmission to seedlings was evaluated using four replications of 50 seeds planted in vermiculite. The pathogenicity of the fungi, *Colletotrichum* sp., *Curvularia* sp., *Cladosporium* sp. *Pestalotia* sp. and *Macrophomina* sp. was tested. Potentially pathogenic and saprophytic fungi were found on the fruits and seeds. The transmission of *Cladosporium* sp. from seeds to seedlings was verified, and *Cladosporium* sp. *Pestalotia* sp. and *Macrophomina* sp. were found to be pathogenic to *B. salicifolius* seedlings.

Index terms: seed pathology, forest seeds, diseases.

Detecção, transmissão e patogenicidade de fungos em sementes de *Blepharocalyx salicifolius* (H.B.K.) Berg.

RESUMO – Objetivou-se nesse trabalho identificar os fungos associados aos frutos e sementes de *Blepharocalyx salicifolius* e sua transmissão e patogenicidade às sementes e plântulas. A identificação dos fungos nas sementes foi realizada pelos métodos de ”blotter test” e batata-dextrose-ágar e para os frutos foi utilizado somente o ”blotter test”. Para verificar a transmissão dos fungos para as plântulas foram semeadas quatro repetições de 50 sementes em vermiculita. Testou-se a patogenicidade dos fungos *Colletotrichum* sp., *Curvularia* sp., *Cladosporium* sp., *Pestalotia* sp. e *Macrophomina* sp. Nos frutos e sementes foram encontrados fungos potencialmente patogênicos e saprófitas. Verifica-se transmissão do fungo *Cladosporium* sp. das sementes para as plântulas, e os fungos *Cladosporium* sp., *Pestalotia* sp. e *Macrophomina* sp. foram patogênicos às plântulas de *B. salicifolius*.

Termos para indexação: patologia de sementes, sementes florestais, doenças.

Introduction

Blepharocalyx salicifolius, commonly known as “murta” in Portuguese, is common in riparian forests (Lorenzi, 1998), and is one of the most common species of the Myrtaceae to be found in mixed ombrophilous forest (Legrand and Klein, 1978). This species may be used for landscaping (Lorenzi, 1998) and for planting along river margins since it occurs naturally in these situations and attracts seed-dispersing birds (Carvalho, 2006). Tree size varies widely (4 to 30 m), with a generally straight trunk and a DBH (diameter at breast height) which can reach 40 cm (Carvalho, 2006; Lorenzi, 1998; Marchiori and Sobral, 1997; Landrum, 1986).

The need to expand forestry areas has led to increased interest in native Brazilian tree species. However, there are problems in seedling production due to a lack of information since healthy seedling production depends on knowing the health and quality of the seed used (Carneiro, 1987; Mendes et al., 2005).

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[2]Departamento de Floresta, UFPR, 80210-170 - Curitiba, PR, Brasil.

[3]Embrapa Floresta, Caixa Postal 319, 83411-000 - Colombo, PR, Brasil.

*Corresponding author < suelen_srego@yahoo.com.br>

The study of fungi associated with the seeds of forestry species is important for developing a basis for epidemiological models, for seed storage and for seedling production (Santos et al., 2000). Fungal attack on seeds can cause various kinds of diseases or damage, including: seed wrinkling size reduction, seed rotting, necrosis, discoloration and a reduction in viability and germination (Araújo and Rossetto, 1987). Among the diseases transmitted by forestry seeds are damping-off, which is one of the most common diseases observed in seedlings (Smith, 1970), and caused by various fungi, including: *Fusarium* sp., *Rhizoctonia* sp., *Pythium* sp., *Phytophthora* sp., *Cylindrocladium* sp., *Sclerotium* sp., *Botrytis* sp., *Curvularia* sp., *Pestalotia* sp. and *Diplodia pinea*. This disease affects seeds at germination and also recently-emerged seedlings (Carneiro, 1987; Catie, 1991; Smith, 1970; Boyce, 1961).

Other descriptions of pathogens transmitted by seeds can be found in the literature, and cause diseases in native forestry species, including, *Fusarium solani* and *Pestalotiopsis* sp. on *Mimosa caesalpiniaefolia* (Mendes et al., 2005) and *Fusarium oxysporum* and *Rhizoctonia solani* on *Bixa orellana* (Santos et al., 1992). Pathogens causing damage at germination, include *Phoma* sp. and *Phomopsis* sp. on seeds of *Tabebuia serratifolia* and *Anadenanthera perigrina* (Coêlho et al., 1996); *Phomopsis* sp. on *Dipteryx alata* (Santos et al., 1997); *Fusarium* sp., *Phomopsis* sp. and *Colletotrichum* sp. on *Anadenanthera colubrina* and *Piptadenia paniculata* (Rego et al., 2006).

Netto and Faiad (1995) observed a high incidence of pathogenic and saprophytic fungi on seeds of *Didymopanax morototoni* and *Virola sebifera*, which was probably one of the factors responsible for the seed deterioration and the low percentage of germination.

Fungi on seeds can cause serious damage during storage, especially when conditions are unsuitable. The growth of storage fungi, such as *Aspergillus* sp. and *Penicillium* sp., cause a loss of germinative power, an increase in the quantity of fatty acids provoked by the oxidation of oils, and also seed warming due to an increase in the respiration rate, resulting in faster seed deterioration (Machado, 1988).

Due to the above and the importance of evaluating the association of fungi with seeds, the objective of this study was to identify the fungi associated with the fruits and seeds of *B. salicifolius* and verify their transmission and pathogenicity to seeds and seedlings.

Material and Methods

B. salicifolius fruits were collected from 12 donor trees at Colombo, Paraná state, in 2007. Seeds were extracted by macerating and washing the fruits in running water and they were then dried on paper towels under laboratory conditions for 24 hours (mean maximum temperature of 23- 24 ºC, minimum of 12-13 ºC and mean of 17-18 ºC).

Detection of fungi on fruits and seeds: two methods were used to detect fungi on seeds: incubation on a paper substrate ('blotter test') and plating on an agar medium (PDA). For the blotter test, four repetitions of 100 seeds were distributed on two sheets of sterilized filter paper, moistened with sterilized distilled water and placed in plastic boxes ('gerbox'), previously disinfected with 1% sodium hypochlorite. In the PDA test, four repetitions of 50 seeds were disinfected with 70% alcohol and 1% sodium hypochlorite for 30 seconds, washed in sterilized distilled water and distributed in sterilized Petri dishes containing PDA culture medium. Fungi associated with the fruits were detected by using the blotter test with four repetitions of 50 fruits.

The seeds and fruits were incubated for seven days under a 12 hour black light/ 12 hour no light photoperiod at a temperature of 20 ± 1 °C. Fungal structures were observed under a stereoscopic and optical microscope and identified with the help of an identification key (Barnett and Hunter, 1982).

Transmission of seed fungi to seedlings: Four repetitions of fifty seeds were sown on isopor seed trays containing a vermiculite medium and kept in a greenhouse with daily irrigation. The percentage emergence and number of seedlings with symptoms were evaluated. The seedlings with symptoms and non-germinated seeds were disinfected with 70% alcohol and 1% sodium hypochlorite for 30 seconds before washing in sterilized distilled water. They were then placed on two sheets of sterilized filter paper, moistened with sterilized distilled water in a gerbox previously disinfected with 1% sodium hypochlorite, and maintained for seven days to detect pathogens.

Pathogenecity test: the potentially phytopathogenic fungi, *Colletotrichum* sp., *Curvularia* sp., *Cladosporium* sp., *Pestalotia* sp. and *Macrophomina* sp., detected by the blotter test and PDA, were isolated on an agar medium and then replicated on other plates to purify them. The fungal cultures were incubated in a BOD chamber at 20 ºC for ten days. After the incubation period, the fungi were inoculated on the seeds with the fungal culture using the contact method. The seeds were previously disinfected with 70% alcohol and 1% sodium hypochlorite for 30 seconds and washed in sterilized distilled water. Four repetitions of 25 seeds were then placed in contact with the fungal cultures and the control only in contact with the PDA medium, and

maintained for 24 hours at 20 ºC. The inoculated seeds were sown on isopor seed trays containing a vermiculite medium and kept in a greenhouse with daily irrigation.

The percentage of dead seedlings, percentage emergence, mean emergence time (MT) (Laboriau, 1983) and the emergence velocity index (EVI) were evaluated (Maguire, 1962). Those seedlings with symptoms and non-emerged seeds were collected and incubated in a wet chamber for seven days to verify the presence of the inoculated fungi.

The experimental design was completely random. The means of the percentage fungal incidence were submitted to Bartlet's test and analysis of variance, and the means compared with Tukey's test at the 5% probability level. The data on percentage emergence, mean time, emergence velocity index and percentage of dead seedlings were submitted to the Kruskal-Wallis test and the means compared by non-parametric multiple comparisons at the 5% probability level.

Results and Discussion

Detection of fungi on fruits and seeds: the following potentially pathogenic fungi were found: *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp., *Macrophomina* sp. and *Pestalotia* sp., and also the saprophytic fungi: *Rhizopus* sp. and *Trichoderma* sp. However, *Trichoderma* sp. only occurred on the fruits and *Curvularia* sp., *Rhizopus* sp. and *Macrophomina* sp. only occurred on the seeds (Table 1). The percentage occurrence of these fungi was low for both fruits and seeds. Only *Cladosporium* sp. had a higher incidence on the fruits (7%) compared to the seeds (2.5%) (Table 1).

Table 1. Incidence of fungi on fruits and seeds of *Blepharocalyx salicifolius*.

Fungi	Incidence (%)		
	Fruits	Seeds	
	Blotter test	Blotter test	PDA
Rhizopus sp.	0.0	0.0	2.5
Trichoderma sp.	1.0	0.0	0.0
Cladosporium sp.	7.0 a	2.5 b	2.5 b
Colletotrichum sp.	4.5 a	0.0	0.5 a
Curvularia sp.	0.0	0.5	0.0
Macrophomina sp.	0.0	0.0	1.0
Pestalotia sp.	2.5 a	3.8 a	5.0 a

Row means followed by distinct letters differ between themselves according to the Tukey test at the 5% probability level.

The fungi, *Cladosporium* sp. and *Pestalotia* sp. were found on both fruits and seeds, demonstrating a possible transmission of these fungi from fruits to seeds since the interior of the fruits acts as a wet chamber, resulting in the establishment and development of fungi inside the fruits, consequently affecting the seeds. The fruit wall may even serve as a nutritional base for the invasion of the seeds by fungi (Menten and Bueno, 1987).

Transmission of seed fungi to seedlings: the seeds of *B. salicifolius* showed 97.5% emergence with only three dead seedlings. Only one of the three dead seedlings placed in the wet chamber showed growth of *Cladosporium* sp., demonstrating a low transmission of this fungus from the seed to the *B. salicifolius* seedling. No fungal growth was observed on ungerminated seeds.

According to Toledo and Marcos-Filho (1977) and Machado (1988), there are several factors limiting the efficiency of pathogen transmission by seeds. Each pathogen has specific requirements of temperature, humidity, oxygen, quantity of nutrients, mechanical injury, seed age and the presence of antagonistic microorganisms on the seed. This may have occurred with the pathogens present on the *B. salicifolius* seeds, which were not transmitted to the seedlings because of a lack of favorable conditions of temperature, humidity, oxygen, nutrients and/or the presence of antagonistic microorganisms.

Although Mendes et al. (2005), Nascimento et al. (2006) and Ruiz Filho et al. (2004) observed potentially pathogenic fungi associated with seeds, they did not observe any fungal transmission from the seeds to the seedlings of *Mimosa caesalpiniaefolia*, *Pterogyne nitens* and *Cedrela fissilis*. According to the last author, even though fungal transmission from the seeds to the seedlings was not observed, it is known that fungi can cause damage both in storage, at germination and during subsequent growth stages. Rego et al. (2006) verified the transmission of *Fusarium* sp. from the seeds to the seedlings of *Piptadenia paniculata* and *Anadenanthera colubrina*.

Pathogenicity test: there was a significant difference for the percentage seedling emergence between seeds inoculated with *Colletotrichum* sp. (81%) and *Curvularia* sp. (98%) (Table 2). It was observed that *Colletotrichum* sp. reduced seed emergence. The fungus, *Colletotrichum* sp., has been observed to reduce germination of *Anadenanthera colubrina* and *Piptadenia paniculata* seeds (Rego et al., 2006).

Table 2. Percentage emergence, emergence velocity index (EVI), mean time (MT), and percentage of dead seedlings of *Blepharocalyx salicifolius* seeds inoculated with different fungus species.

Fungi	Emergence (%)	EVI	MT	Dead Seedlings (%)
Control	92.0 AB	0.27 A	88.8 A	0.0
Colletotrichum sp.	81.0 B	0.24 A	85.6 A	0.0
Cladosporium sp.	97.0 AB	0.29 A	90.4 A	4.0
Pestalotia sp.	97.0 AB	0.29 A	88.1 A	3.0
Curvularia sp.	98.0 A	0.27 A	92.9 A	0.0
Macrophomina sp.	96.0 AB	0.28 A	90.2 A	9.0

Means followed by distinct letters differ between themselves according to the non-parametric multiple comparison test at the 5% probability level.

No significant difference was observed for any of the treatments for emergence velocity index and mean time data and, therefore, none of the fungi inoculated had any influence on these factors in seedlings.

The fungi *Cladosporium* sp., *Pestalotia* sp. and *Macrophomina* sp. caused leaf burning and seedling death to seedlings originating from inoculated seeds. *Pestalotia* sp. was also observed to cause wilting and leaf spots on *Mimosa caesalpiniaefolia* seedlings (Mendes et al., 2005).

Conclusions

Potentially pathogenic fungi were found on the fruits and seeds of *B. salicifolius*, including: *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp., *Macrophomina* sp. and *Pestalotia* sp., and the saprophytic fungi: *Rhizopus* sp. and *Trichoderma* sp.

The transmission of *Cladosporium* sp. from seeds to seedling was observed and the fungi, *Cladosporium* sp., *Pestalotia* sp. and *Macrophomina* sp. are pathogenic to *B. salicifolius* seedlings.

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