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Detection of fungi in rubber tree (*Hevea brasiliensis*) seeds harvested in northeast of Mato Grosso do Sul, Brazil

Detecção de fungos em sementes de seringueira (Hevea brasiliensis) colhidas na região Nordeste de Mato Grosso do Sul, Brasil

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Resumo: O cultivo da seringueira na região nordeste de Mato Grosso do Sul está se expandindo vertiginosamente e acredita-se que contribuirá para que o Estado se torne um grande produtor de látex nos próximos anos. O objetivo deste trabalho foi identificar o gênero e estimar a incidência de fungos encontrados em sementes de seringueira coletadas nos municípios de Cassilândia (CA) e Chapadão do Sul (CS), MS. Foram avaliadas 400 sementes oriundas de cada local e, para este fim, oito sementes foram dispostas em cada caixa de germinação (11 x 11 x 3,5 cm) com duas folhas de papel de filtro embebidas em solução salina a - 1,0 Mpa. Após incubação a 25^{o}C, por sete dias, em fotoperíodo de 12 h, os fungos foram identificados por meio de sua morfologia e a média de incidência dos gêneros, em cada município, foi comparada por meio do teste de Tukey, a 5% de probabilidade. O delineamento experimental empregado foi o inteiramente casualizado, com 25 repetições, e cada parcela experimental foi representada por duas caixas de germinação, totalizando 16 sementes. Foram detectados 11 gêneros de fungos nas sementes coletadas em Cassilândia e apenas quatro nas obtidas em Chapadão do Sul. O gênero *Fusarium*, fitopatogênico, esteve em 63,5% e 49,8% das sementes de CA e CS, respectivamente . Já *Rhizoctonia* incidiu em 11,3% em CA e em 36,6% das sementes de CS. Foram identificados outros gêneros, que tiveram maior incidência significativa em Cassilândia, conforme segue: *Aspergillus*, *Curvularia*, *elminthosporium*,*Penicillium*, *Rhizopus* e *Trichoderma*.

Palavras-chave: patologia de sementes; patógenos; doença.

Rubber trees (*Hevea brasiliensis*) are explored in Brazil since century XIX, when they were important for national economy. However, the South American Leaf Blight or SAFB (*Microciclus ulei*), an important fungal disease has prevented Brazil from becoming a major world rubber producer mainly when seeds were sent to Europe to be cultivated in free pathogen zone in Asian countries (Onokpise, 2004). Nowadays this specie is cultivated in areas with the adoption of high technology in some Brazilian states as Mato Grosso do Sul (MS). In these areas there is not favorable environment factors for SAFB and the rubber trees are used for wood, pulp and natural rubber (Anuário Brasileiro da Silvicultura, 2012).

There are some diseases, in addition to SAFB, that can cause several losses to rubber tree plantations in all Brazilian fields. The most common symptoms are canker, blight and abnormal leaf drop, dark lesions, apical death, damage to the panel of sap extraction and root rot, caused by a wide variety of fungi that can be associated with seeds (Gasparotto et al., 1997).

A low physiological quality of rubber tree seeds is usually detected because of the high water content which is favorable for microorganisms that can reduce its viability. This is a common characteristic in recalcitrant seeds, which becomes worst because they reach physiological maturity and are harvested in a rainy period (Fonseca & Freire, 2003). There is still a lack of information about microflora present in rubber tree seeds produced in Brazil, even it had been commented for several decades ago by Tanaka (1985).

There are some cities in the northeast of MS, as Chapadão do Sul and Cassilândia, with large areas cultivated with rubber trees and some seedling producers. It is common the use of seeds from other

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states instead of MS and there are seedling producers that want to use seeds harvested in MS but cannot do it because there is a lack of information about the sanity and physiological profile of them. The objective of this paper was identify the genera and incidence of fungi present in seeds of rubber trees cultivated in two cities located in the Northeast region of MS, Brazil.

The analyses were done in the Laboratory of Phytopathology, at the Federal University of Mato Grosso do Sul, Chapadão do Sul, Mato Grosso do Sul, Brazil. In April 2012 seeds were collected in commercial rubber trees orchards with 20-year-old located in the cities of Cassilândia (Latitude: S 19° 6′ 48″; Longitude: W 51° 44′ 03″; Altitude: 470 m) and Chapadão do Sul (Latitude: S 18° 47′ 39″; Longitude: W 52° 37′ 22″; Altitude: 790 m), without grass mulching in the soil. The method of harvest was done collecting seeds from the ground near the trees, as described by Medeiros et al. (2007). A total of 400 seeds from each city were obtained near the trees and were put in paper bags.

Subsequently, eight seeds were deposited on two pieces of filter paper placed in a germination box (11 x 11 x 3.5 cm), and a saturated salt (NaCl) solution (- 1.0 MPa) was used to inhibit germination (Machado et al., 2012). The seeds were incubated at 25 °C for 7 days under a photoperiod of 12 hours to promote fungal growth. The genera of fungi growing on seeds were identified by morphological structures using optical and stereoscopic microscopes (Barnett & Hunter, 1998). The experimental design was completely randomized with two treatments represented by cities with 25 replications. It was considered each replication two boxes with a total of 16 seeds. The mean incidences for each fungus identified in the treatments were compared using the Tukey Test at 5% of probability.

The genera and incidence of fungi growing from incubated seeds are shown in Table 1. Most of the genera detected were found in higher incidence in Cassilândia, probably because of the climatic conditions besides other factors. We believe that this was the main cause of the observed data and as example, the average temperature during the seed formation is superior than that exist in Chapadão do Sul (data not showed). *Fusarium* was the genera that was observed with the major seed incidence in both cities and its incidence in rubber trees seeds collected in Cassilândia was significant superior (P<0,05) than that from Chapadão do Sul. Various species of *Fusarium* are known as stem and root pathogen (Liyanage & Dantanarayana, 1983) and Jayasinghe (1999) cited that *Fusarium* spp. is one economically important pathogen that was only founded in Asia and absent in Africa and Tropical America. Probably the present work is the first report of an important pathogen present in *Hevea* seed in Brazil.

Table 1. Genera and incidence (% of seeds infected) of fungi associated with rubber tree seeds harvested in Cassilândia and Chapadão do Sul, Mato Grosso do Sul, Brazil .

	City		
Fungi	Cassilândia*	Chapadão do Sul*	Prob. > F
Alternaria spp.	0.3	0.0	0.3222
Aspergillus spp.	15.3 a	3.8 b	< 0.010
Cercospora spp.	0.5	0.0	> 0.050
Chaetomium spp.	0.8	0.0	0.1490
Curvularia spp.	3.3 a	0.0 b	0.0196
Fusarium spp.	63.5 a	49.8 b	0.0193
Helminthosporium spp.	3.3 a	0.0 b	0.0129
Penicillium spp.	9.3 a	0.0 b	< 0.010
Rhizoctonia spp.	11.3 b	36.3 a	< 0.010
Rhizopus spp.	6.5 a	0.0 b	< 0.010
Trichoderma spp.	05 b	3.3 a	0.0129
Outros	0.5	1.2	0.2972

* 400 seeds were analyzed in each city. Means without the same letter are significantly different by Tukey test at 1 or 5% of probability.

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Other relevant genera that we detected in this work was *Rhizoctonia*, which had more incidence in seeds evaluated from Chapadão do Sul than Cassilândia. We hypothesized this has relation with the prior use of the land in Chapadão do Sul that was with annual crops. The rubber tree orchard in this city was surrounded by cereal crops and the fungal dispersal was made through soil particles carried by winds.

It can be considered a high incidence of *Rhizoctonia* once it can cause an important foliar disease in rubber trees and it is associated with root damages in other vegetal species cultivated in Brazil (Kimati et al., 2005). *Thanatephorus cucumeris* is the teleomorphic phase of *Rhizoctonia* and when there are favorable climatic conditions it causes the target leaf spot disease, which incites necrosis in the hypocotyls of germinated seeds and cause damping off and foot rot of seedling of *Hevea* in nurseries in the countries that produce rubber around the world and must be controlled to prevent damages (Jayasinghe et al., 1997). Either *Fusarium* or *Rhizoctonia* species are necrotrophic microorganisms and can survive by specialized structures for many years in some environments as the substrate and the recipients used in nurseries.

Curvularia, *Helminthosporium* and *Alternaria* were detected only in Cassilândia and these fungi were related causing damages in mini-grafted seedlings of rubber trees. According to Lemos Filho et al. (1994) hyphaes of these identified fungi were infecting the remaining leaves and in the local where graft was done, causing die-back of all mini-grafted seedlings. *Cercospora* was also detected only Cassilândia and Gasparotto et al. (1997) related that this fungi is the causal agent of a disease that occurs in leaves of rubber seedlings in nurseries and growers have to control it to avoid seriously losses.

The fungi observed in the seeds evaluated in both cities can be found in leaves of rubber trees causing diseases or as member of saprophytic flora as *Chaetomium*, which has a potential use for biological control of plant diseases (Sales Júnior et al., 2007). Aiming identify the phylloplane mycoflora of both healthy and infected leaves of *Hevea* trees in Sri Lanka, Jayasinghe & Fernando (2000) detected some genera of fungi in healthy leaves, as follow: *Aspergillus*, *Botryodiplodea*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Mucor*, *Penicillium*, *Phomopsis*, *Pestalotiopsis* and *Trichoderma*. In diseased leaves, with the surface sterilized, the authors frequently observed *Botryodiplodia*, *Cladosporim*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Periconia*, *Pestalotiopsis* and *Phomopsis*.

Storage fungi as those from the genera *Aspergillus*, *Penicillium* and *Rhizopus* were detected probably because they are from soil where the seeds were collected and the recalcitrant characteristic of the rubber tree seeds, that have high water content (Fonseca & Freire, 2003). Igeleke & Omorusi (1997) detected some agents that causes seed deterioration of rubber seeds and the genera of storage fungi identified were *Aspergillus*, *Penicillium* and *Rhizopus*. The authors also observed some microorganisms that cause pre-harvested infection including *Helminthosporium*, *Colletotrichum* and *Fusarium*.

Some authors recommend a chemical treatment of the rubber tree seeds because of the common incidence of storage fungi (Vieira et al., 1995). However, actually there are not registered chemical products to treat rubber seed in Brazil and the data that were observed in this work suggest that there are important pathogens associated with these seeds from Cassilândia and Chapadão do Sul. The use of rubber seeds harvested in the northeast region of MS must be done using alternative or biological control methods to avoid problems with pathogenic fungi either in nurseries or when there is direct sowing (Pereira & Pereira, 1998).

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