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Seed Sanity of *Diospyros Inconstans* Jacq

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ABSTRACT

One of the factors of influence in germination are the temperature and the substrate are the most relevant for the emergence of seeds and initial development of the seedlings, and the temperature and the substrate can have a direct effect on the proliferation of pathogens during the germination tests, which can compromise the final results of the tests. Stands out the sector of seed production of native forest species in Brazil has little information about the occurrence of pathogens in seeds. Thus, the objective of this study is to evaluate the effect of the substrate, temperature and seed treatment in the incidence of pathogens in seeds of *Diospyros inconstans* Jacq. The factorial scheme was 4x2x being tested four substrates: paper, sand, vermiculite and paper roll, at two temperatures 25 and 30°C, with and without the application of Captan fungicide. After the treatments the seeds were stored in B.O.D. the end of the experiment was performed to identify the fungi present, and the final germination and Germination Speed Index have been determined. The substrate temperature and the fungicide did not cause significant differences in germination, though the paper substrate showed higher incidence of attack seeds and fungicide reduced the incidence of pathogens. The captan treatment did not significantly influence the final germination, however, the Ivg was higher and the number of seeds attacked less when the fungicide was applied. Twelve species of fungi were found being the most common *Aspergillus* and *Fusarium*, and fungicide was responsible for reducing the occurrence of all fungi found except *Rhizopus* and *Trichoderma* genera.

INTRODUCTION

The growing demand for forest seedlings of native species in Brazil, whether for restoration or commercial plantations, emphasizes the importance of studies with seed technology, since for the production of good quality seedlings we need seeds with superior characteristics, and stored in appropriate manner, and free of pathogens.

Several native species are still poorly studied and, therefore, there is a lack of information on seed quality and evaluation of Brazilian native forest species. Among these species is the *Diospyros inconstans* Jacq., belonging to the family Ebenaceae, popularly known as marmeleiro tree (Lorenzi, 2002).

The species has an occurrence in all regions of the Amazon, being recommended according to Lorenzi (2002) for urban afforestation and composition of heterogeneous reforestation with ecological purposes, because it presents attractiveness to the local fauna.

Guedes *et al.* (2010) emphasize that among the factors of influence in germination the temperature and the substrate are the most relevant for the emergence of seeds and initial development of the seedlings. It should also be considered that the temperature and the substrate can have a direct effect on the proliferation of pathogens during the germination tests, which can compromise the final results of the tests.

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The sector of seed production of native forest species of Brazil according to Souza *et al*. (2013) has little information about the occurrence of pathogens in seeds. Several pathogenic microorganisms may be associated with the seeds of forest species, with the most frequent damage occurring in the form of necrotic spots, deformations, rotting and, consequently, a decrease in germination, vigor and power, problems in seedling formation (Oliveira *et al*., 2011b).

The objective of this study was to evaluate the effect of substrate, temperature and seed treatment on the incidence of pathogens and the germination of seeds of *Diospyros inconstans* Jacq.

MATERIAL AND METHODS

This study was conducted in the Cytogenetic and Seeds Laboratory of Campus II of the State University of Mato Grosso - Campus Alta Floresta, in July and August of 2015. The *Diospyros inconstans* seeds were supplied by Instituto Ouro Verde and were collected in Alta Floresta and Paranaíta (MT), Southern Amazon Region, between April and June 2015. After collection, these were stored in a Kraft paper bag at a temperature of 20 ºC.

This experiment was a factorial consisting of the analysis of four substrates (Factor 1), application of fungicide (Factor 2) and two temperatures (Factor 3). The substrates evaluated were: Paper, Sand, Vermiculite, and Paper Roll, associated to the application of fungicide (dicarboximide) or not , and two temperatures of 25 and 30 ºC. After the study, final germination, germination speed index, and seed sanity were evaluated.

The germination test was performed with 100 seeds per treatment divided into four replicates of 25 seeds. For the germination test on paper and roll of paper, two sheets of germitest paper (sterilized) were used, and the results were 2.5 times their weight. When the vermiculite was used (medium texture, sterilized for 24 h at 120 ° C), it was moistened with twice its weight, and 70% retention moisture was used for sand (fine and sieved, sterilized and autoclaved).

It is noteworthy that all the procedures performed in the germination test followed the recommendations for the substrates of the RAS (Rules for Seed Analysis) (Brazil, 2009) and Instructions for analysis of seeds of forest species (Brazil, 2013).

The fungicide (chemical group dicarboximide: active ingredient Captan or Captana) was applied dry, mixing the product with the seeds. It is a dry powder, and the dosage of 240 g for 100 kg of seeds was used.

Before the tests were installed the seeds were disinfested with sodium hypochlorite (1%) for 10 min. And later they were taken to germination chambers type B.O.D. (Biochemical Oxigen Demand), at constant temperatures of 25 and 30 ° C, and 12 h photoperiod of fluorescent white light / 12 h dark for 46 days.

For the sanity test the seeds after going through the treatments described above were evaluated eight days after the installation of the experiment. The fungi that developed on the seeds were identified at the genus and species level, based on their morphological characteristics visualized under a stereoscopic and optical microscope with the aid of the phytopathogenic fungi (Menezes and Oliveira, 1993).

The Germination Speed Index was calculated according to Equation presented by Maguire (1962):

$$IVG= \sum \frac{G1}{N1} + \frac{G2}{N2} \dots\dots \frac{Gn}{Nn}, \quad (1)$$

G= Number of germinated seeds;

N= Days of test installation.

Data were analyzed for homogeneity and normality test (Barlett and Shapiro Wilk, respectively), where the data were homogeneous, and only germination showed normality, the other data were transformed into bow sine (X / 100)), however in the tables the actual data were presented. Subsequently, the Variance Analysis was performed, and the Tukey's test (P <0.05%) was used to compare the means. The statistical analyzes performed by the statistical package R.

RESULTS AND DISCUSSIONS

Analyzing germination and germination rate index and number of infested seeds, no significant interaction was observed between the evaluated factors, so in the sequence the individual analyzes for each factor are presented in Table 1.

Table 1: Means of germination and sanity parameters of *Diospyros inconstans* seeds submitted to different substrates, temperature and fungicide application..

Factor 1			
Substrates	Germination (%)	IVG	Infested seeds (%)
Paper	34,00 a	0,88 b	50,00 a
Sand	43,25 a	2,71 a	40,25 ab
Vermiculite	47,75 a	2,88 a	32,25 b
Paper roll	37,00 a	2,05 ab	39,25 ab
Factor 2			
Fungicide	Germination	IVG	Infested seeds (%)
With application	43,62 a	2,76 a	12,37 b
No application	37,37 a	1,50b	68,50 a
Fator 3			
Temperature	Germination	IVG	Infested seeds (%)
25ºC	45,75 a	1,97 a	40,75 a
30ºC	35,25 a	2,30 a	40,12 a
CV%	38,50	66,24	38,61

Means followed by the same letter in the column within each factor did not differ statistically by the Tukey's test (P <0.05%). Unprocessed data.

According to the data in Table 1, it was noted that for the analysis of the substrates (Factor 1) there was no significant statistical difference for the final germination, and for the Ivg, only the paper substrate differed from the others with lower value. Lone *et al.* (2010) also did not observe statistical difference for final germination and Ivg in *Schlumbergera truncata* seeds when they evaluated different substrates for germination test.

With regard to the percentage of infested seeds, it was again observed that the paper presented a difference, but this time due to the higher number of seeds attacked, however, it only differed from the vermiculite that presented lower value. As for the temperature (Factor 3), it was verified that it had no influence on any of the evaluated parameters.

Santos *et al.* (2015) analyzing different substrates (sand, paper and vermiculite) and temperatures (25 and 20-30 ºC) in guarita seeds (*Astronium graveolens*), cedre (*Cedrela fissilis)* and pau-viola (*Cytharexyllum myrianthum*) found that there was no difference for the germination and Ivg parameters for the species analyzed.

The application of captan (Factor 2) did not influence the germination, however, it provided higher Ivg and a significantly lower number of infested seeds. Lazarotto *et al.* (2013) using captan for treatment of cedre (*Cedrella fissilis*) seeds noticed increased percentage of germinated seeds.

Fantinel *et al.* (2015) emphasize that captan is a protective fungicide, not being translocated by plant tissues. In this way, it does not affect the vascular system of the seeds or the seedlings produced, without influencing the physiological processes of the plants.

It can be observed that the germination did not present significant values not exceeding 50%, and that it requires significant time (45 days), being these more susceptible to the attack of pathogens. Costa *et al.* (2010), evaluating the germination of *Diospyros ebenaster* Retz. seeds, found that it started at 45 days and stabilized at 110 dias, evidencing that species of the genus *Diospyros* need a long period for germination.

During the study were found 12 pathogenic fungi species distributed in seven genera, as presented in Table 2.

Table 2: Mean percentage of attack of pathogenic fungi associated with *Diospyros inconstans* seeds on the different factors analyzed (substrates, temperature and fungicide application).

Factores	*A*sp.	*Af*	*An*	*Ac*	*P*sp.	*F*sp.	*Fm*	*Fo*	*L*sp.	*R*sp.	*C*sp.	*T*sp.
Substrate												
Paper	8,2^{a}	14,0^{a}	12,0^{a}	0,2^{b}	28,0^{a}	11,0^{ab}	0,0^{b}	0,2^{a}	5,7^{a}	0,2^{a}	2,7^{a}	3,5^{a}
Sand	0,5^{a}	4,0^{b}	5,5^{a}	0,2^{b}	5,7^{b}	19,7^{a}	0,0^{b}	0,2^{a}	7,2^{a}	0,5^{a}	1,0^{a}	0,2^{a}
Vermiculite	6,2^{a}	4,7^{b}	12,5^{a}	0,0^{b}	7,2^{b}	6,7^{b}	2,0^{a}	0,2^{a}	1,7^{b}	2,5^{a}	2,7^{a}	1,5^{a}
Paper roll	0,0^{a}	2,2^{b}	5,4^{a}	4,0^{a}	22,2^{a}	15,7^{ab}	0,0^{b}	0,0^{a}	7,0^{a}	1,0^{a}	1,7^{a}	0,0^{a}
Fungicide												
Whith	0,2^{b}	1,7^{b}	0,9^{b}	2,2^{a}	31,1^{a}	3,7^{b}	0,1^{b}	0,0^{a}	9,2^{a}	1,2^{a}	4,0^{a}	3,8^{a}
No	7,2^{a}	11,0^{a}	16,7^{a}	0,0^{b}	0,5^{b}	22,8^{a}	0,8^{a}	0,3^{a}	1,6^{b}	0,8^{a}	0,1^{b}	0,2^{a}
Temperature												
25ºC	4,0^{a}	3,7^{b}	9,2^{a}	0,1^{b}	14,7^{a}	12,1^{a}	1,0^{a}	0,3^{a}	10,5^{a}	1,1^{a}	2,0^{a}	0,8^{a}
30ºC	3,5^{a}	9,0^{a}	8,4^{a}	2,1^{a}	16,8^{a}	14,5^{a}	0,0^{b}	0,0^{a}	0,3^{b}	1,0^{a}	2,1^{a}	3,2^{a}

Means followed by the same letter in the column within each factor did not differ statistically by the Tukey's test (P <0.05%). Unprocessed data.Asp=*Aspergilus* sp., Af=*A. flavus*, An=*A. niger*, Ac=*A. carbonarius*, Psp=*Penicilium* sp., Fsp=*Fusarium* sp., Fm=*F. moniliforme*, Fo=*F. oysporum*, Lsp=*Lasiodiplodia* sp., Rsp.=*Rhizopus* sp., Csp=*Curvularia*sp., Tsp=*Trichoderma* sp.

From the data in Table 2, it was observed that the genus *Aspergilus* and *Fusarium* were the most frequent in the evaluation, and that the factors analyzed had an influence on the incidence of the identified pathogens. Regarding the substrates, it can be observed that for most of the identified fungi these did not present difference.

Evaluating *Aspergilus* sp. and *A. flavus*, it was observed that the paper substrate presented higher incidence, and for *A. carbonarius* the paper roll had a higher number of infested seeds. *Penicilium* sp. was more frequent in paper and paper roll, while *Fusarium* sp. and *Lasiodiplodia* sp. Only presented statistical difference for vermiculite having the lowest occurrence.

Note that for vermiculite only *F. moniliforme* presented higher incidence. The temperature did not influence much, only *A. flavus* and *A. carbonarius* were more frequent in the temperature of 30 ºC, and *A. carbonari*us and *F. moniliforme* obtained higher values of seeds infested at 25 ºC.

Sousa *et al.* (2012) and Oliveira et al. (2012) also found a high incidence of *Aspergillus* spp., *Penicillium* sp., and *Fusarium* sp. in *Tabebuia impetiginosa, Tabebuia ochracea* and *Schizolobium amazonicum* seeds. And Souza *et al*. (2013) found *Aspergillus* sp. and *Penicillium* sp as major pathogens associated with *Copaifera langsdorffii* seeds.

Aspergillus flavus, Aspergillus niger, Penicillium sp., and *Fusarium spp.* were observed associated with *Acca sellowiana* by Fantinel *et al.* (2015). *Fusarium sp., Cladosporium sp., Rhizophus sp., Curvularia sp.* and *Alternaria sp.* were detected by Medeiros *et al*. (2013) in *Pterogyne nitens seeds.* Thus, it is noted that the pathogens identified in this study are commonly found associated with seeds of forest species.

Only *Lasiodiplodia* sp. is less reported in seeds of forest species, however Oliveira *et al*. (2013) emphasizes that this is a genus associated with *Jatropha curcas* L, causing high infection capacity and resistant to methods of disinfestation with sodium hypochlorite, being considered one of the most efficient pathogens disseminated through seeds.

Ruiz Filho *et al*. (2004) and Goldfarb *et al*. (2010) point out that *Penicillium* sp. and *Aspergillus* spp., are typical storage pathogens, and *Fusarium* species cause loss of germination and vigor, as well as being associated with seedling tipping. The application of captan decreases the incidence of all the pathogens found, only there was no statistical difference for *Rhizopus* sp. and *Trichoderma* sp. Lazarotto *et al*. (2010) cite that captan was efficient in reducing the incidence of *Fusarium* sp. in *Ceiba speciosa* seeds, and Oliveira *et al.* (2011a) observed that the application of captan fungicides associated with carbendazin + thiram was efficient in decreasing the incidence of *Penicillium* sp., *Cladosporium* sp., *Fusarium* sp., *Pestalotiopsis* sp. and *Alternaria* sp. in stored seeds of *Eugenia* spp.

Fantinel *et al.* (2015) observed in their study that captan was efficient in reducing the incidence of *A. flavus*, *A. niger, Penicillium* sp., and *Fusarium* spp. in *Acca sellowiana* seeds. It is noted that the use of captan is efficient to reduce the incidence of pathogens associated with forest seeds.

According to Silva *et al.* (2011) chemical seed treatment is an efficient control measure aimed at reducing future diseases in forest species, since there are many species of economic value that are still scarce information about the health of their seeds.

Analyzing the interaction of the factors and the species of fungi identified, it is possible to note the positive interaction for some species, with seven interacting with the factors analyzed as shown in Table 3.

Table 3: Interaction between substrate, fungicide application and temperature for pathogenic fungi species found in *Diospyros inconstans* seeds.

Substrate x Fungicide Interaction		
Aspergilus flavus		
Substrate	Fungicide	
	No	With
Paper	26,0aA	3,00 aB
Sand	5,00 bA	3,00 aA
Vermiculite	4,50 bA	0,00 aA
Paper roll	8,50 bA	Fungicide
Aspergilus carbonarius		
Substrate	Fungicide	
	No	With
Paper	0,50 bA	0,00 aA
Sand	0,50 bA	0,00 aA
Vermiculite	0,00bA	0,00 aA
Paper roll	8,00aA	0,00 aB
Penicilium sp,		
Substrate	Fungicide	
	No	With
Paper	55,50 aA	0,50 aB
Sand	11,00 bA	0,50 aA
Vermiculite	14,00bA	0,50 aA
Paper roll	44,00 aA	0,50 aB
Fusarium sp,		

Fungicide x Temperature Interaction		
Aspergilus carbonarius		
Fungicide	Temperature	
	25ºC	30ºC
No	4,25 aA	0,25 aB
With	0,00 bA	0,00 aA
Aspergilus sp,		
Fungicide	Temperature	
	25ºC	30ºC
No	No	7,00 aA
With	0,50 aA	0,00 aA
Fusarium sp,		
Fungicide	Temperature	
	25ºC	30ºC
No	17,25 aB	28,50 aA
With	7,00 bA	0,50 bA
Fusarium moniliforme		
Fungicide	Temperature	
	25ºC	*30ºC*
No	1,75aA	0,00 aA
With	0,25 bA	0,00 aB
Lasiodiplodia sp,		
Fungicide	Temperature	
	25ºC	*30ºC*
No	17,75 aA	0,75 aB
With	3,25 bA	0,00 aA
Substrate x Temperature Interaction		

Substrate	Fungicide	
	No	With
Paper	17,00bcA	5,00 aB
Sand	34,50 aA	5,00 aB
Vermiculite	9,50 cA	0,00 aB
Paper roll	8,50 cA	5,00 aA

Aspergilus flavus		
Substrate	Temperature	
	25ºC	30ºC
Paper	7,00 aB	22,00 aA
Sand	2,0000 aA	6,0000 bA
Vermiculite	2,0000 aA	2,5000 bA
Paper roll	4,0000 aA	5,5000 bA

Fusarium moniliforme		
Substrate	Fungicide	
	No	With
Paper	0,00 aA	0,00 bA
Sand	0,00 aA	3,50 aA
Vermiculite	0,50 aB	0,00 bA
Paper roll	0,00 aA	0,00 bA

Aspergilus carbonarius		
Substrate	Temperature	
	25ºC	30ºC
Paper	0,50 bA	0,00 aA
Sand	0,50 bA	0,00 aA
Vermiculite	0,00 bA	0,00 aA
Paper roll	7,50 aA	0,00 aB

Means followed by the same lowercase letter in the column, and upper case in the row did not differ statistically by the Tukey test (P <0.05%), for each interaction combination.

Analyzing the data in Table 3, it can be seen that for *A. flavus* and *Penicilum* sp. the association between substrate paper and without captan application favored its development. *A. carbonarius* was observed in a larger number of seeds in paper roll substrates without application of captan and *Fusarium* sp and *F. moniliforme* were more frequent in sand and vermiculite respectively without application of fungicide.

For substrate interaction and temperature *A. flavus* presented higher frequency in paper substrates at 30 ° C, and *A. carbonarius* in paper roll at 25 ° C. In the interaction between fungicide and temperature it is noted that the temperature of 25 ºC and the non-application of captan had a higher number of seeds attacked for *A. carbonarius*, *F. moniliforme*, and *Lasiodiplodia* sp. already *Fusarium* sp. Obtained higher incidence without application of fungicide at 30 ºC, and *Aspergilus* sp. was found in higher number of infested seeds without application of captan without differing between the evaluated temperatures or fungicide.

Conclusions:

At the end of this study it was observed 12 pathogenic fungi species distributed in seven genera:*Aspergilus* sp., *Aspergilus flavus*, *Aspergilus niger*, *Aspergilus carbonarius*, *Penicilium* sp., *Fusarium* sp., *Fusarium. moniliforme*, *Fusarium oysporum*, *Lasiodiplodia* sp., *Rhizopus* sp., *Curvularia* sp., and *Trichoderma* sp. The genus *Aspergilus* and *Fusarium* were the most frequent in the evaluation.

For the evaluated factors, substrate, temperature and application of fungicide it was observed, that t the four substrates tested (paper, sand,Vermiculite and paper roll) did not influence the final germination, however the Ivg presented its lowest value in paper and higher values in vermiculite. The highest index of seeds attacked occurred in the paper substrate, with great occurrence of the genera *Aspergilus* and *Penicilium*.Note that for vermiculite the lowest values of infested seeds were observed.

The temperature (25 and 30Cº) did not influence the final germination, the Ivg, and the number of infested seeds. However the temperature of 30 degrees favored the development of the species *Aspergilus flavus* and *Aspergilus carbonarius* important seed pathogens.

The captan treatment did not significantly influence the final germination, however, the Ivg was higher and the number of seeds attacked less when the fungicide was applied. Being that the presence of captan decreased the incidence of all genera foundexcept *Penicilium* sp, *Lasiodiplodia sp., Rhizopus sp., Curvularia sp.*, and *Trichoderma.* However it proved to be efficient for the genres *Aspergilus* and *Fusarium*,pathogens of higher occurrence in forest seeds mainly associated with storage.

Analyzing the interaction of the factors and the species of fungi identified, it is possible to note the positive interaction for some species, *Aspergilus flavus*, *Aspergilus carbonarius* and *Penicilium* sp, obtained higher incidence on paper roll without application of fungicide, while *Fusarium* sp. was more frequent on paper without captan application.*Aspergilus carbonarius* and *Lasiodiplodia* sp.occurred at higher values associated with temperature of 25 degrees without application of fungicide, and*Fusarium* sp. was more frequent in 30 degrees without application of fungicide.*Aspergilus flavus*interaction with a temperature of 30 degrees on paper, and*Aspergilus carbonarius* on paper roll at 25 degrees.

In general, based on the observed results, the germination test with *Diospyros inconstans* seeds can be conducted in vermiculite, under a temperature of 25 degrees with captan application, because the combination of these factors provided greater final germination, higher IVG, and lower index of seed infested by pathogens.The application of captan can also be indicated for the production of seedlings of the species in forest nurseries, since the species presents a long period for beginning of the germination.

This study is the beginning of a series of works developed with seeds of native forest species, which lack information about germination, dormancy, sanity and vigor tests, mainly forest species from southern Amazonia that have few species with studies related to seed technology. Considering the growing demand for seedlings of native species mainly for the recovery of degraded areas will be necessary the knowledge of protocols with the

objective of evaluating the seed quality used in the production of seedlings, since the quality of the seed directly interferes with the quality of seedlings produced.

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