

## Influence Of Pelleting On The Susceptibility Of *E. Benthamii* Seeds To *Fusarium Asiaticum*

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### Abstract:

This study aimed to evaluate the pathogenicity of two isolates of *Fusarium asiaticum* on *Eucalyptus benthamii* seeds, both in their natural and pelleted forms. The pelleting technique, developed to facilitate precision sowing, involves coating the seeds with materials that improve handling and distribution. For this purpose, bare and pelleted seeds of *E. benthamii* were immersed in a *Fusarium* suspension for one hour and transferred to test tubes. The control consisted of disinfested seeds not inoculated with the pathogens. After 15 days, the disease incidence was assessed based on the number of diseased seedlings. The results indicated that the pathogenicity of *Fusarium asiaticum* affected the *E. benthamii* seeds regardless of pelleting, with no significant difference in germination rate or pathogen incidence between pelleted and bare seeds. This suggests that pelleting does not offer an effective physical barrier against these pathogens. These findings are relevant for seed management and the improvement of *Eucalyptus* seedling production, highlighting the need for seed protection strategies against pathogens.

**Keywords:** forest pathology, seed treatment, seeds, phytosanitary

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### I. Introduction

The seed coating process is a practice that evolved from the 1990s mainly due to precision sowing. This technique was initially developed to improve and facilitate seed handling, thereby avoiding waste and irregular distribution in seedbeds and planting furrows. The process involves coating seeds with cementing materials that dissolve in water for the application of the coating material, applying layers alternately and successively (Almeida, 2004; Silva, 1997; Silva & Nakagawa, 1998). Depending on the material used in pelleting and the amount of water in the soil, the seeds exhibit different behavior according to their hydrophobic and hydrophilic properties (Roos & Moore, 1975).

Subsequently, the coating has also been used to incorporate substances that can act to protect against pathogens and improve the germination capacity of seeds through the use of phytosanitary products, nutrients and growth regulators (Delouche et al., 1995).

The success achieved in sowing using pelleted seeds has allowed significant improvements in planting, thus, this technique has been used in seeds of leguminous, forage, ornamental, vegetable, and forestry species (Oliveira et al., 2003). Among forestry species, those of the *Eucalyptus* genus have great economic interest for the forestry sector. However, the production of seedlings from unpelleted seeds becomes an activity that requires a lot of attention and care, since species of this genus have very small seeds which complicates their individualization and distribution at the time of sowing.

Pelleting of *Eucalyptus* spp. seeds is a satisfactory alternative for carrying out sowing more efficiently, this technique already being used for species of this genus (Kanashiro et al., 1978). This process consists of covering seeds with a solid and dry coating, which is essentially made of an inert and adhesive material, aimed at increasing the seed size, as well as modifying its shape and texture (Nascimento et al., 2009).

The advantages of seeds covered with pellets, as considered by Roos & Moore (1975) cited by Coraspe et al., (1993), are several, including: easier handling during hand sowing; lower thinning costs; reduced stress from thinning; more uniform seed microenvironment; possibility of including chemical products; use of a smaller amount of seed; precision sowing in small and irregular seeds.

However, some difficulties are encountered when working with pelleted seeds compared to those without coating, such as lower emergence of seedlings, as well as seedlings with atypical aspects and also a delay in germination time (Millier and Sooter, cited by Coraspe, Idiarte, and Minami, 1993). On the other hand, Antonov et al., (1978) stated that despite the delay in germination time, seed coating provides the achievement of more precise sowing, in addition to eliminating costs with thinning or transplants.

Although the coating technique is carried out using an inert material, it is not known whether this material can act as a physical barrier against pathogens. Therefore, the objective of this work was to evaluate the pathogenicity of two isolates of *Fusarium asiaticum* (given the lack of research on the species and its damage) on bare and pelleted seeds of *Eucalyptus benthamii* Maiden & Cambage.

## II. Material And Methods

The pathogenicity of *F. asiaticum* on *E. benthamii* seeds was evaluated through an in vitro test. For the assays, two isolates of *F. asiaticum* belonging to the microbial collection of the Forest Pathology laboratory at UNICENTRO were used. The pathogen was isolated from *Eucalyptus* spp. and Bracatinga plants that showed symptoms of damping-off and canker in the UNICENTRO Forest Nursery.

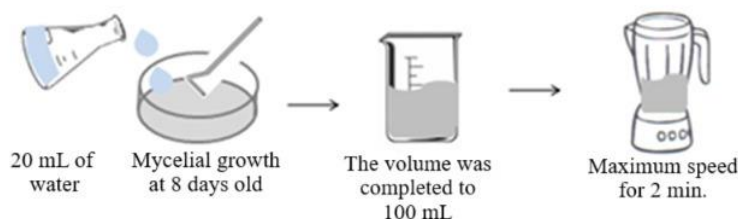
The pathogen's sequence was compared with other sequences available in "GenBank" using BLASTn to confirm the pathogen's identity (Access No. MF120225).

Initially, before inoculation, the inoculum suspension was prepared. For the preparation of the suspension, two Petri dishes containing BDA culture medium with eight-day-old fungal mycelial growth were used. The mycelial growth of the fungi was scraped from the plates with the help of a Drigalski loop and triturated at maximum speed in a mixer for two minutes, adding 100 ml of sterilized distilled water, to obtain the inoculum suspension (Figure 1).

In this assay, *E. benthamii* seeds with and without pellets were disinfested with 1% sodium hypochlorite (NaCl) for two minutes and washed twice with sterilized distilled water (Figure 2). Afterward, the seeds were placed on filter paper to dry, at room temperature, for 10 minutes.

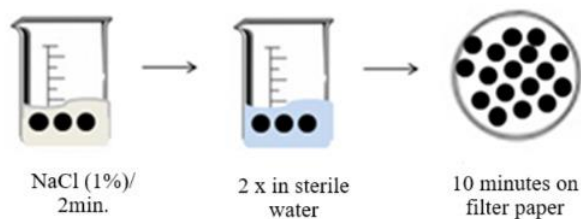
Then, the seeds were immersed in the *F. asiaticum* suspension for one hour. After being treated and dried, the seeds were transferred to test tubes containing 10 mL of Agar-Water culture medium, sterilized in an autoclave at 121 °C for 20 minutes, using four seeds per tube. The tubes were incubated for fifteen days in BOD, at a temperature of 25 °C ± 1 °C, in a 12-hour photoperiod (Figure 3). The control consisted of disinfested seeds not inoculated with *Fusarium* isolates.

**Figure 1:** 1st step of the procedure, preparation of the suspension.



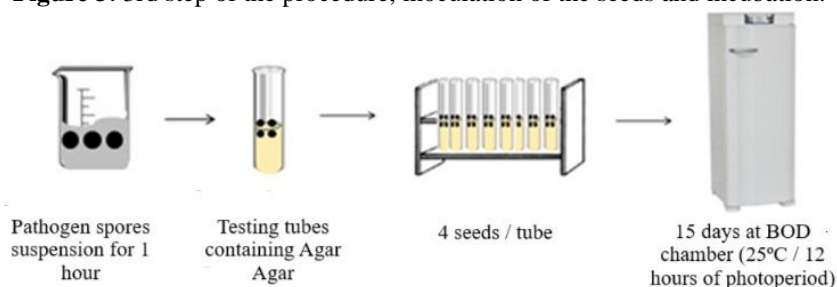
Source: Authors

**Figure 2:** 2nd step of the procedure, seed disinfection.



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**Figure 3:** 3rd step of the procedure, inoculation of the seeds and incubation.



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The experiment was conducted in a completely randomized design, in a 2 x 2 factorial scheme (two isolates of *F. asiaticum* and two forms of seed coating) with 5 repetitions, the experimental unit being a test tube with four seeds. At 15 days, the incidence of pre- and post-emergence damping-off was assessed by counting the number of diseased seedlings. Statistical analyses were performed using the ASSISTAT 7.7 Beta statistical software.

### III. Results And Discussion

The study found that the isolates of *F. asiaticum* are pathogenic to *E. benthamii* seeds. The seeds inoculated with the fungi differed significantly from the control in terms of the percentage of germinated seeds and seedlings with pathogen incidence. The highest percentage of germinated seeds was obtained in the control with 95% germination, while in the seeds inoculated with the *F. asiaticum* fungus, germination was 70% for the eucalyptus isolate and 72.5% for the bracatinga isolate, respectively (Table 1).

After 15 days of inoculation, the seeds inoculated with the bracatinga isolate showed 60% colonization, while the seeds inoculated with the eucalyptus isolate showed 37% pathogen colonization on the seedlings and/or ungerminated seeds. The uninoculated seeds (control) differed from the two treatments tested, where 10% of seeds showed contamination by another pathogen. Even with the disinfection procedure, it was found that the control showed contamination, indicating that the observed fungus is possibly present inside the seed, with disinfection not being sufficient to inhibit its growth (Table 1).

From the results, no significant difference was observed between pelleted and non-pelleted seeds in the presence of the inoculum, confirming that pelleting did not act as a physical barrier against the development of *F. asiaticum* isolates.

**Table 1:** Average values of germination and incidence of *F. asiaticum* in *E. benthamii* seeds.

Treatment	Germination Rate (%)	Incidence (%)
Testemunha	95 a	10 a
<i>Fusarium</i> (Eucalipto)	70 b	37,5 b
<i>Fusarium</i> (Bracatinga)	72,5 b	60 b

\*Averages followed by the same letter do not differ statistically from each other by the Tukey test at a 5% error probability.

Source: Authors

The *Fusarium* genus is one of the most important groups of Ascomycete fungi. They are well-distributed soil inhabitants capable of being parasites or saprophytes. Most survive on plant debris and live close to the soil surface (Nwanma & Nelson, 1993). The genus is considered an important pathogen for various forest and agricultural crops, with various symptoms caused by the fungus, such as stunting, base and crown rot, root rot (Trigiano et al., 2010), however, there are not many reports in the literature about the damage of the *Fusarium asiaticum* pathogen and the genus on seeds of forest species.

In the present study, even with the use of pellets, it was found that the fungus was able to colonize the seed and the emerged seedlings, indicating that the pellets did not act as a barrier against the tested pathogens.

In germination tests conducted in laboratories on vegetable seeds, a higher percentage of germination was observed in non-pelleted seeds, but after the removal of the pellets, no damage effect was observed (Millier & Sooter, 1967). For the seeds of the forest species *Guazuma ulmifolia* Lam., Nascimento (2011) found through physiological conditioning and pelleting that bare seeds showed higher germination and emergence speed than pelleted seeds and that regardless of the conditioning, a decrease in the germination percentage was due to the pellets, another fact was that the germination percentage also decreased due to the action of fertilizer.

Problems in seeds involved by pellet, as described by Roos & Moore (1975), still need solutions, such as: improvement of management; the choice of the right material for a given soil condition; storage conditions; development of standard germination procedures aimed at each type of seed and the development of special seed drills.

Studies related to the incorporation of chemical and/or biological substances in seed coating may be an alternative for the reduction or control of pathogens.

#### IV. Conclusion

The two isolates of *F. asiaticum* are pathogenic to *E. benthamii* seeds. The pelleting of seeds does not constitute a physical barrier against the pathogens.

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