# Transmission of *Colletotrichum truncatum* and *Macrophomina phaseolina* by lima bean seeds

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## ABSTRACT

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The fungi *Colletotrichum truncatum* and *Macrophomina phaseolina* are among the main pathogens associated with lima bean seeds in Brazil, and they are the causal agents of anthracnose and charcoal rot, respectively. The aim of this study was to verify the seed-seedling transmission of *C. truncatum* and *M. phaseolina* in artificially inoculated lima bean seeds. Potato-dextrose-agar (PDA) media with different hydric potentials were obtained after the addition of mannitol. Isolates of *C. truncatum* and *M. phaseolina* were peaked to these media. Forty grams of seeds were distributed over the fungal mycelium, remaining there for different exposure periods, as a function of each treatment: T1: 0.0 MPa without inoculum for 48 h; T2: 0.0 MPa for 48 h; T3: -0.2 MPa for 48 h; T4: -0.4 MPa for 48 h; T5: -0.6 MPa for 72 h; T6: -0.8 MPa for 96 h and T7: -1.0 MPa for 120 h. The seeds were incubated at 25 ºC under a 12-hour photoperiod. Germination in a paper roll, emergence of seedlings in sand and seed health in PDA were evaluated. T4 provided the best conditions to temporarily prevent seed germination, promoting a greater incidence of seeds infected by *C. truncatum* and a greater percentage of diseased plants. *M. phaseolina* reduced seed germination, independently of the inoculated treatment. Both fungi were transmitted from the seeds to the seedlings of lima bean, causing symptoms in various aerial parts. *C. truncatum* and *M. phaseolina* transmission rate was 83.1% and 76.46%, respectively.

**Keywords**: Germination, *Phaseolus lunatus*, seed health, transmission by seeds.

## RESUMO

Mota, J.M., Melo, M.P., García, M.F.M.; Sousa, E.S.; Sousa, E.M.J.; Barguil, B.M.; Beserra Jr, J.E.A. Transmissão de *Colletotrichum truncatum* e *Macrophomina phaseolina* por sementes de feijão-fava. ***Summa Phytopathologica,*** v.45, n.1, p.33-37, 2019.

Os fungos *Colletotrichum truncatum* e *Macrophomina phaseolina* estão entre os principais patógenos associados a sementes de feijão-fava no Brasil, sendo os agentes causais da antracnose e da podridão de carvão, respectivamente. Este trabalho teve como objetivo verificar a transmissão semente-plântula de *C. truncatum* e *M. phaseolina* em sementes de feijão-fava inoculadas artificialmente. Meios de cultura batata-dextrose-agar (BDA) com diferentes potenciais hídricos foram obtidos após a adição de manitol. Isolados de *C. truncatum* e *M. phaseolina* foram repicados para esses meios. Quarenta gramas de sementes foram distribuídas sobre o micélio fúngico, permanecendo por diferentes períodos de exposição, em função do tratamento: T1: 0,0 MPa sem inóculo por 48 h; T2: 0,0 MPa por 48 h; T3: - 0,2 MPa por 48 h; T4: -0,4 MPa por 48 h; T5: -0,6 MPa por 72 h; T6: - 0,8 MPa por 96 h e T7: -1,0 MPa por 120 h. As sementes foram incubadas a 25 ºC, com fotoperíodo de 12 horas. Foram avaliadas a germinação em rolo de papel, a emergência de plântulas em areia e a sanidade de sementes em BDA. O T4 proporcionou melhores condições para impedir temporariamente a germinação das sementes, promovendo maior incidência de sementes infectadas por *C. truncatum* e maior porcentagem de plântulas doentes. *M. phaseolina* reduziu a germinação das sementes, independente do tratamento inoculado. Ambos os fungos foram transmitidos das sementes para as plântulas de feijão-fava, causando sintomas em diferentes órgãos aéreos. A taxa de transmissão de *C. truncatum* e *M. phaseolina* foi de 83,1% e 76.46%, respectivamente.

**Palavras-chave**: Germinação, *Phaseolus lunatus*, sanidade de sementes, transmissão por sementes.

Seeds can efficiently harbor and transport microorganisms of all taxonomic groups, pathogenic or not, and are thus excellent dispersion vehicles and initial inoculum sources in the field, being capable of starting epidemics. Low seed quality is considered one of the main causes of low yield in plantations because the association of pathogens with seeds can have negative effects on the germination process and seedling development (7).

In lima bean (*Phaseolus lunatus* L.) plantations in Brazil, fungi are among the main causal agents of diseases. Anthracnose caused by *Colletotrichum truncatum* (Schw.) Andrus & Moore frequently occurs and is considered one of the main diseases of lima bean, especially in the Northeastern region, where the weather conditions favor its development (14). Another fungus that has a deleterious action on leguminous seeds is *Macrophomina phaseolina* (Tassi) Goid., which can cause failure before and after seedling emergence (1, 8). Development of the fungus is stimulated by high temperatures and low humidity (9).

Transmission of *Colletotrichum* spp., the cause of various diseases in legumes, is known to occur in seeds, contributing to the large-scale spread of these pathogens (10). One example is *Colletotrichum lindemuthianum* (Sacc. Magnus) Cavara, which is transmitted efficiently with seed-seedling transmission rates of up to 80% in common bean (*Phaseolus vulgaris* L.) (15).

Studies related to the effect of seed health often need seeds to be infected by pathogens in quantity and quality sufficient for research. The inoculation of fungi using the hydric restriction method has been used in investigation involving germination, pathogen transmission and seed health (11). The efficiency of the method was demonstrated in studies on the transmission of *C. truncatum, Phomopsis sojae* Lehman and *Sclerotinia sclerotiorum* (Lib.) de Bary in soybean (*Glycine max* (L.) Merrill) seeds (12) and of *Fusarium oxysporum* f. sp. *phaseoli* Kendrick et Snyder in common bean (6).

As there is the need for information about seed-seedling transmission of *C. truncatum* and *M. phaseolina*, which will subsidize the implementation of appropriate management measures, this study proposed to verify the transmission of these two pathogens from artificially infected lima bean seeds.

## MATERIALS AND METHODS

Lima bean seeds var. Boca de Moça were disinfected in a sodium hypochlorite solution (NaOCl) 1%, for three minutes, followed by two washes with sterile distilled water. Then, they were subjected to tests of germination in a paper roll, seedling emergence in sand, and health tests (4; 5). The fungal isolates COUFPI29 (*Colletotrichum truncatum*) and COUFPI37 (*Macrophomina phaseolina*) used in this study were obtained from lima bean seeds collected in March 2015 from the county of Esperantina and Teresina, Piauí State, respectively.

The culture medium used in this study was Potato-dextrose-agar (PDA) added of mannitol, obtaining the hydric potentials of -0.2; -0.4; -0.6; -0.8; -1.0 MPa. The concentrations of solutes for medium preparation, at each hydric potential, were obtained by means of the Van't Hoff formula (17), namely: yos = RTC, in which: yos = osmotic potential (atm); R = ideal gas constant (8.32 J $mol^{-1}$ $K^{-1}$); T = temperature (K); C = concentration (mol $L^{-1}$) and T (K) = 273 + T (°C). The culture media were autoclaved at 121 °C for 20 min and distributed in Petri dishes of 15cm diameter. Each plate was added of three PDA discs containing structures of the isolate (*C. truncatum* or *M. phaseolina*). The plates were incubated under a 12h photoperiod and temperature of 25 ± 1 °C until total fungal growth; then, 40 grams of seeds were deposited on the fungal mycelia, remaining there for different exposure periods, depending on the treatment.

The treatments consisted of different binomials of hydric potential / exposure time of seeds to the fungus, being: T1. 0.0 MPa without inoculum for 48 h (control); T2. 0.0 MPa for 48 h; T3. -0.2 MPa for 48 h; T4. -0.4 MPa for 48 h; T5. -0.6 MPa for 72 h; T6. -0.8 MPa for 96 h and T7. -1.0 MPa for 120 h. Evaluation of germination was in paper roll, that of seedling emergence was in sand and that of seed health was in PDA.

**Germination test in paper roll:** the seeds were placed on sterile filter paper sheets, totaling 25 seeds per treatment and 16 replicates. The conditions of the experiment followed the rules of seed analysis (5). Germinated seeds and dead seeds were recorded.

**Emergence tests in sand:** these were carried out in plastic trays of 47 x 27 x 08 cm containing sterile autoclaved sand (121 °C for two hours). All 100 seeds used per treatment were sown at a depth of 1.5 cm. The trays were randomly distributed in a greenhouse. The evaluations were carried out at seven, 14 and 21 days after sowing (DAS) for the seeds inoculated with *C. truncatum* and at seven and 14 DAS for the seeds inoculated with *M. phaseolina*. Evaluation included the number of emerged seedlings and the incidence of seedlings with symptoms. The non-germinated seeds and seedlings were pulled up and evaluated, and fragments of organs were plated in PDA medium to check the presence of the fungus. Data on fungal transmission from the seed to the seedling were expressed as percentages for each plant organ, evaluated as a function of the incidence of fungus.

**Health test:** six seeds per 15cm-diameter Petri dish were evaluated, totaling 120 seeds per treatment. The seeds were plated in PDA medium and maintained in an incubator at 25 ± 1 °C, in a regime alternating 12 hours of light. The evaluation occurred at up to seven days after sowing, based on visual analysis and using a stereoscopic microscope (19). Total transmission rate (T.R.) was determined for each treatment using the formula described (20): TR (%) = [IR (%) / IS (%)] * 100, where: IR = infection rate of *C. truncatum* and *M. phaseolina* determined by analyzing the data (pre-emergence death and asymptomatic transmission rate), IS = Incidence of *C. truncatum* and *M. phaseolina* determined by the health test in inoculated seeds.

The assays were carried out in completely randomized design, and means were compared according to Tukey's test at 5%. Statistical analysis was carried out by using ANOVA in the ASSISTAT program, version 7.7 beta (18).

## RESULTS AND DISCUSSION

The increase in hydric potential / exposure time promoted a gradual reduction in the percentage of germinated seeds and a consequent increase in the number of dead seeds (Figure 1A). There were high germination rates in treatments T2, T3, T4 and T5 ($P<0.05$), inoculated with *C. truncatum,* as observed in the non-inoculated control (Figure 1A). In these treatments, the presence of the fungus only slightly affected the germination power of seeds. T6 and T7 were the treatments that promoted the highest seed mortality in germination test roll. Osmotic potentials of -0.8 MPa or higher and times from 96 hours onwards normally inhibit the germination of seeds of other plant species, as is the case for cotton seeds (*Gossypium hirsutum* L.) inoculated with *C. gossypii* var. *cephalosporioides* (2) and soybean seeds inoculated with *C. truncatum* (11).

When the seeds were inoculated with *M. phaseolina*, independently of the time of exposure and of the presence (T3, T4, T5, T6 and T7) or absence of hydric potential, there was a sharp reduction in germination (Figure 1A). Seed germination fell from 73.5% in the non-inoculated treatment (T1) to 21.2% in the inoculated treatment (T2); in other words, the presence of the fungus under the evaluated conditions caused widespread death of the seeds, possibly due to the long exposure period and the high aggressiveness of the fungus. In cotton seeds, at 48 hours onwards of exposure to the fungus *Botryodiplodia theobromae*, there was a reduction of 80% in germination (13).

There was a reduction in the number of seedlings emerging in sand as the level of hydric restriction and the time of exposure increased (Figure 1B), and this was similar to the result of the germination test in the paper roll (Figure 1A).

For *C. truncatum,* treatments T2, T3 and T4 did not differ ($P<0.05$) from the non-inoculated control treatment (T1), with an mean emergence percentage of 88.5%, demonstrating that the rise in the hydric potential did not interfere with seedling development. T6 presented the lowest germination percentage, with only 36% seedlings emerging, not differing from T7 (Figure 1B).

Symptoms were observed (spots on cotyledons, stems and leaves) in the seedlings that came from seeds inoculated with *C. truncatum*

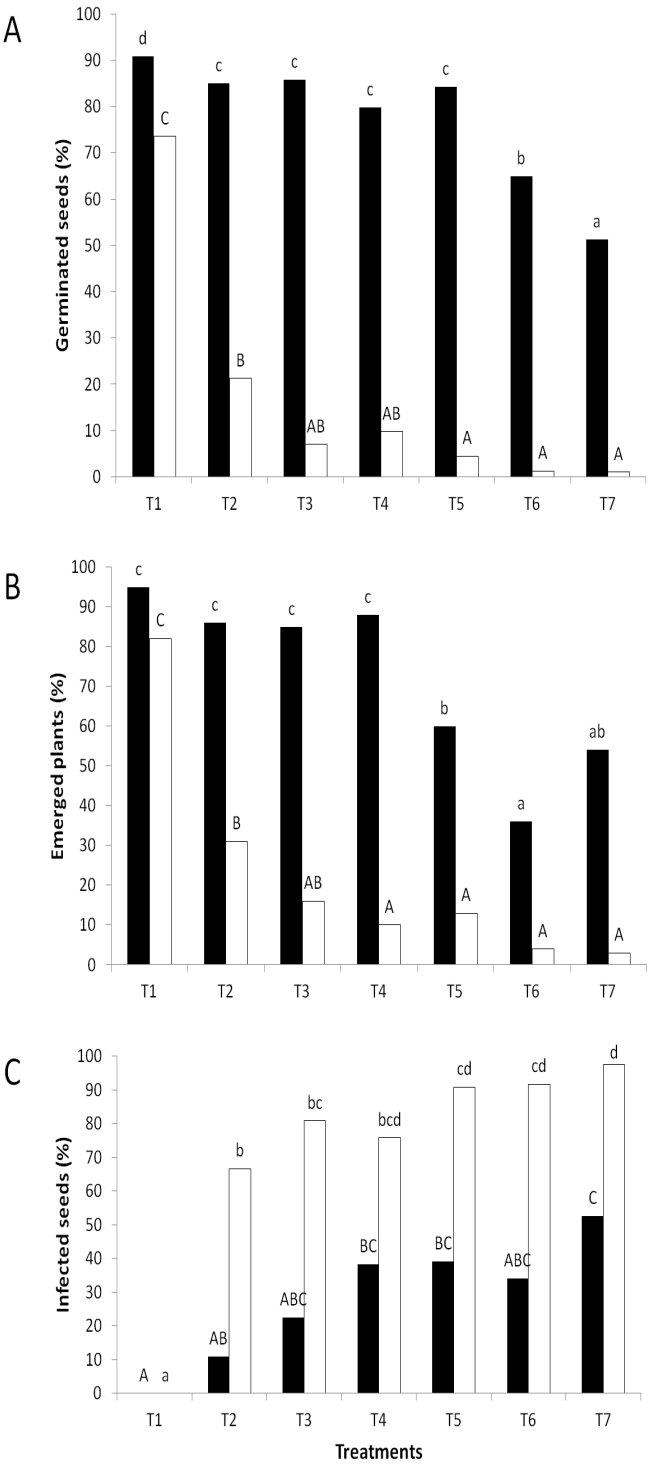


**Figure 1.** Germination in a paper roll (A), emergence in sand (B) and health test in potato-dextrose-agar medium (C) for seeds of lima bean (*Phaseolus lunatus* L.) inoculated with *Colletotrichum truncatum* and *Macrophomina phaseolina* in different treatments (hydric potentials and time of exposure to the fungus). Means followed by the same letter do not differ statistically according to Tukey's test at 5%. T1. 0.0 MPa without inoculum for 48 h; T2. 0.0 MPa with inoculum for 48 h; T3. -0.2 MPa for 48 h; T4. -0.4 MPa for 48 h; T5. -0.6 MPa for 72 h; T6. -0.8 MPa for 96 h and T7. -1.0 MPa for 120 h. Black column (■): *C. truncatum*; White column (□): *M. phaseolina*.

(Figure 2A), from 7 DAS onwards, and these became more severe at 14 and 21 DAS. At 7 DAS the incidence of symptoms was more frequent in treatments T4 and T5 ($P<0.05$), dropping in treatments T6 and T7. At 14 DAS there was no difference among the inoculated treatments ($P<0.05$). At 21 DAS there was, in general, an increase in the incidence of symptoms and there was no statistically significant difference among treatments T4, T6 and T7. These results, added to those regarding emergence in sand, indicate T4 (-0.4 MPa and 48 h exposure) as the best treatment for inoculation of lima bean seeds with *C. truncatum* because it did not interfere in seed germination and provided a higher incidence of symptoms in the seedling (Figure 1B).

Similar results were found for cotton seeds inoculated with *C. gossypii* var. *cephalosporioides*, which presented a reduction in the number of seedlings emerging at 7 DAS in the treatments in which the hydric restriction was -0.8 and -1.0 MPa. At 21 DAS no significant differences were found among treatments (13).

For the seeds inoculated with *M. phaseolina* there was a reduction in the percentage of emerged seedlings in all inoculated treatments (Figure 1B). There was 82% seedling emergence in treatment T1, only 31% in treatment T2, and lower values in the following treatments, demonstrating the high aggressiveness of the fungus, which is capable of penetrating and killing the seed in the absence of the restrictor. Furthermore, the exposure period of 48 hours seems to be enough to establish the fungal infection, which corroborates the results of the germination test (Figure 1A).

At 21 DAS symptoms could not be evaluated for *M. phaseolina* due to the high incidence and severity of the symptoms, which led to the death of the seedlings (Figure 2B). At 7 DAS the health of the seedlings was already compromised, independent of the treatment, with 79.8% plants showing symptoms even in the treatment with no hydric restrictor added and exposed to the fungus for 48 hours (T2). Treatments T5, T6 and T7 presented 100% diseased plants. This result was also maintained in the second evaluation at 14 DAS.

These results, together with those from emergence in sand, revealed that the methodology was efficient in obtaining lima bean seeds infected with *M. phaseolina*, but due to the high aggressiveness of the pathogen, which seems to make the seed deteriorate rapidly, the used parameters may not have been appropriate. New experiments with exposure times below 48 hours will be carried out. The high aggressiveness of *M. phaseolina* was proven after the inoculation of common bean seeds with 96 isolates of the pathogen obtained in various countries, among which was Brazil (16).

With the aim of guaranteeing that the symptoms observed in the seedlings were indeed caused by *C. truncatum* and *M. phaseolina*, different organs were collected from 10% seedlings in each treatment after the last evaluation. For the seedlings emerged from seeds inoculated with *C. truncatum*, the fungus was re-isolated from cotyledons, stems and leaves, with mean percentages of 58.2, 33.2 and 56.4%, respectively (Table 1A), which attests not only to the efficiency of the hydric restriction method in the inoculation of lima bean seeds, but also to the fact that the pathogen is transmitted from the seed to the seedlings. Mean percentages of 76.7, 55.3 and 30.5% were obtained from seedlings that emerged from seeds inoculated with *M. phaseolina* in the cotyledons, stems and leaves, respectively (Table 1B).

Lower percentages of diseased plants were found in a study (3) which sought to confirm the transmission of *Bipolaris sorokiniana* from seeds to seedlings of barley (*Hordeum vulgare* L.).. They found aerial and root organs infected by the fungus in coleoptiles (40%), apical extremity (38.6%), below-ground region (48.1%), sheaths (31.6%),

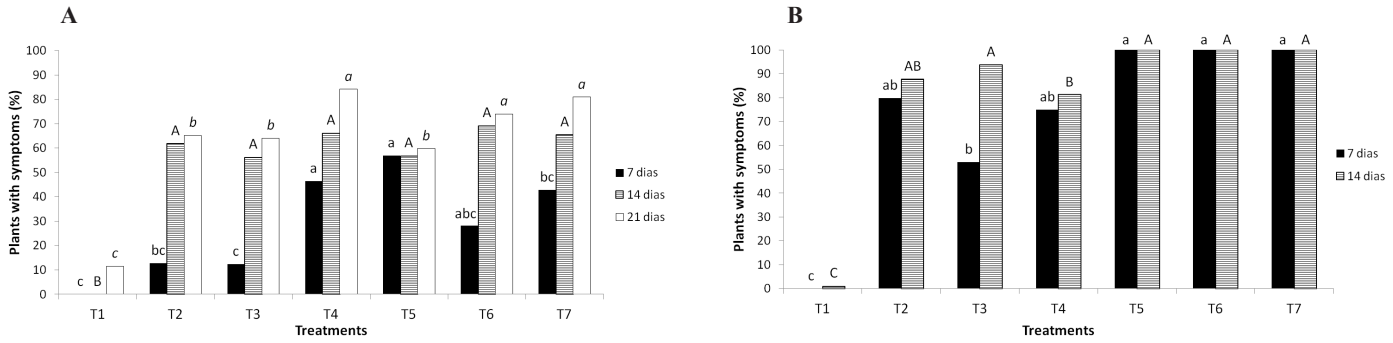


**Figure 2.** Lima bean seedlings (*Phaseolus lunatus* L.) with symptoms caused by infection with *Colletotrichum truncatum* (A) and *Macrophomina phaseolina* (B) in different treatments (hydric potentials and time of exposure to the fungus). Means followed by the same letter do not differ statistically according to Tukey's test at 5%. Column 7 days: lowercase letters, column 14 days: capital letters, column 21 days: lowercase letters in italics. T1. 0.0 MPa without inoculum for 48 h; T2. 0.0 MPa with inoculum for 48 h; T3. -0.2 MPa for 48 h; T4. -0.4 MPa for 48 h; T5. -0.6 MPa for 72 h; T6. -0.8 MPa for 96 h and T7. -1.0 MPa for 120 h.

**Table 1.** Percentage of *Colletotrichum truncatum* (A) and *Macrophomina phaseolina* (B) recovered from organs of lima bean (*Phaseolus lunatus* L.) seedlings that emerged after inoculation of seeds subjected to different treatments.

**(A)**

| Treatment | Cotyledons | Stem | Leaf |
|---|---|---|---|
| T1* | 0.0 | 18.0 | 18.1 |
| T2 | 26.3 | 20.0 | 31.4 |
| T3 | 48.1 | 15.0 | 40.0 |
| T4 | 71.8 | 30.0 | 57.1 |
| T5 | 86.1 | 60.0 | 74.2 |
| T6 | 96.8 | 45.0 | 80.0 |
| T7 | 78.5 | 45.0 | 94.2 |
| Mean (%) | 58.2 | 33.2 | 56.4 |

**(B)**

| | | | |
|---|---|---|---|
| T1 | 0.0 | 0.0 | 0.0 |
| T2 | 62.5 | 40.0 | 10.0 |
| T3 | 100.0 | 70.0 | 10.0 |
| T4 | 90.0 | 83.3 | 50.0 |
| T5 | 84.6 | 100.0 | 76.9 |
| T6 | 100.0 | 44.4 | 66.6 |
| T7 | 100.0 | 50.0 | 0.0 |
| Mean (%) | 76.7 | 55.3 | 30.5 |

*T1. 0.0 MPa without inoculum for 48 h; T2. 0.0 MPa with inoculum for 48 h; T3. -0.2 MPa for 48 h; T4. -0.4 MPa for 48 h; T5. -0.6 MPa for 72 h; T6. -0.8 MPa for 96 h and T7. -1.0 MPa for 120 h.

plumules (8.1%) and mesocotyls (28.1%).

From the results of the health test in PDA medium for the two evaluated pathogens, it was noted that the inoculum of both penetrated the seeds in all binomials (hydric potential / time), causing infection (Figure 1C). Seeds inoculated with *C. truncatum,* in T7, presented higher incidence (52.5%). In this treatment, however, there was a high incidence of ungerminated seeds, making them unviable for obtaining seeds infected by the pathogen. In treatments T4 and T5 there was fungal incidence of about 38.7%; in addition to this result, these treatments barely affected seed germination and seedling emergence (Figures 1A and 1B). Seeds inoculated with *M. phaseolina* presented high incidences, reaching 97.5% in T7 (Figure 1C). The lowest incidence among the inoculated treatments was 66.7% in T2.

Considering the pre-emergence death and asymptomatic transmission rate, as well as the incidence of fungi on seeds, the infection (IR) and transmission (TR) rates for *C. truncatum* and *M. phaseolina* were calculated considering treatment 2. For *C. truncatum* IR was 9% and TR was 83.1%. For *M. phaseolina* IR was 51% and TR reached 76.46%.

The use of hydric restriction can be efficient in obtaining lima bean seeds infected with *C. truncatum* in order to conduct research that needs infected seeds, such as in studies of chemical treatment of seeds, epidemiology and development of methods to detect pathogens. However, appropriate times need to be established, apparently less than 48 hours of exposure to *M. phaseolina*, due to the death rate in seeds when exposed for long periods.

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