

# Influence of previous crops and fungicide seed treatment in the incidence and control of damping-off caused by *Rhizoctonia solani* in cotton seedlings under greenhouse conditions

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## ABSTRACT

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The aim of this study was to verify the influence of previous crops and fungicide seed treatment in the incidence and control of damping-off caused by *Rhizoctonia solani* in cotton seedlings under greenhouse conditions. This experiment was carried out during two years at Embrapa Western Agriculture, in Dourados, Mato Grosso do Sul State, Brazil. In addition to cotton (treated and untreated seeds) and fallow, the following cover crops were tested as previous crops: black oats, millet, corn, forage sorghum, soybean, common beans, crotalaria (*Crotalaria juncea*), brachiaria (*Urochloa ruziziensis*) and brachiaria (*Urochloa ruziziensis*) + crotalaria (*Crotalaria juncea*). The fungicide mixture used as treatment to cotton seeds was triadimenol + penicuron + tolylfluanid (50 + 50 + 30 g a.i./100 kg seeds). Seeds from cotton and previous crops were sown in soil contained in plastic trays and pots; the seeds were placed in individual and equidistant 3cm-deep wells. Inoculation of *R. solani* was obtained by homogeneously distributing the fungal inoculum onto the substrate surface (2.5g/tray and 0.34g/pot). The fungus was grown for 35 days on autoclaved black oat seeds subsequently ground to powder using a mill (1mm). Damping-off was

daily evaluated from the seventh day after sowing. There was a significant effect of the interaction previous crops x fungicide treatment ( $P < 0.05$ ). The fungicide seed treatment was efficient in controlling seedling damping-off caused by *R. solani* and its effect was potentiated when grasses were the previous crops. Use of grasses such as brachiaria (*Urochloa ruziziensis*), black oats, millet, corn and forage sorghum as previous crops, besides fallow, significantly contributed to a smaller *R. solani* population in the soil, which resulted in lower rates of cotton seedling damping-off. On the other hand, using cotton continuously, as well as the legumes soybeans, beans, crotalaria (*Crotalaria juncea*), and brachiaria (*Urochloa ruziziensis*) + crotalaria (*Crotalaria juncea*) as previous crops to cotton, was consistently associated with higher rates of seedling damping-off, contributing to the increase or at least the maintenance of *R. solani* inoculum in the soil. The highest damping-off percentages were observed in plots under continuous cotton cultivation without fungicide seed treatment. The present results reinforce the need of improving damping-off control in cotton seedlings by adopting integrated management programs in areas infested with *R. solani*.

**Keywords:** seedling emergence, *Gossypium hirsutum*, injured seedlings, crop rotation, soilborne pathogen management.

## RESUMO

Goulart, A.C.P. Influência da cultura antecessora e do tratamento de sementes com fungicidas na incidência e controle do tombamento de plântulas de algodoeiro causado por *Rhizoctonia solani*, sob condições de casa de vegetação. *Summa Phytopathologica*, v.47, n.3, p.157-161, 2021.

O objetivo desse trabalho foi verificar a influência da cultura antecessora e do tratamento de sementes com fungicidas na incidência e no controle do tombamento de plântulas de algodoeiro causado por *R. solani*, sob condições de casa de vegetação. O experimento foi conduzido por dois anos na casa de vegetação da Embrapa Agropecuária Oeste, em Dourados, MS. Além do algodão (sementes tratadas e não tratadas) e do pousio, as seguintes coberturas vegetais foram testadas como culturas antecessoras ao algodoeiro: aveia preta, milheto, milho, sorgo forrageiro, soja, feijão, crotalária (*Crotalaria juncea*), braquiária (*Urochloa ruziziensis*) e braquiária (*Urochloa ruziziensis*) + crotalária (*Crotalaria juncea*). A mistura fungicida utilizada no tratamento de sementes de algodão foi triadimenol+penicuron+tolylfluanid (50+50+30g do i.a./100kg de sementes). Sementes de algodoeiro e das culturas antecessoras foram semeadas em solo contido em bandejas plásticas e em vasos, dispostas em orifícios individuais, equidistantes e a 3cm de profundidade. A inoculação com *R. solani* foi feita pela distribuição homogênea do inóculo do fungo na superfície do substrato (2,5g/bandeja e 0,34g/vaso). O fungo foi cultivado por 35 dias em sementes de aveia preta autoclavadas e trituradas em moinho (1mm). A avaliação de tombamento foi realizada diariamente, a partir dos 7

dias após a semeadura. Foi observado efeito significativo da interação culturas antecessoras x tratamento com fungicidas ( $P < 0.05$ ). O tratamento de sementes de algodoeiro com fungicidas foi eficiente no controle do tombamento de plântulas causado por *R. solani* e teve seu efeito potencializado quando se usou gramíneas como culturas antecessoras ao algodoeiro. Cultivos prévios de gramíneas, como braquiária (*Urochloa ruziziensis*), aveia preta, milheto, milho e sorgo forrageiro, além do pousio, contribuíram de forma significativa na redução da população de *R. solani* do solo, o que resultou em menores índices de tombamento de plântulas de algodoeiro. Por outro lado, além do uso contínuo do algodão, as leguminosas soja, feijão, crotalária (*Crotalaria juncea*) além de braquiária (*Urochloa ruziziensis*) + crotalária (*Crotalaria juncea*) usadas como culturas antecessoras ao algodoeiro, consistentemente estiveram associados aos maiores índices de tombamento de plântulas, contribuindo para o aumento ou pelo menos para a manutenção do inóculo de *R. solani* no solo. As maiores percentagens de tombamento foram observadas nas parcelas onde foi adotado o cultivo contínuo do algodão sem o tratamento de sementes com fungicidas. Os nossos resultados reforçam a necessidade de melhorar o controle do tombamento de plântulas de algodoeiro, pela adoção de programas de manejo integrado em áreas infestadas com *R. solani*.

**Palavras-chave:** emergência de plântulas, *Gossypium hirsutum*, plântulas lesionadas, rotação de culturas, manejo de patógenos de solo.

Diseases caused by soilborne fungi can have a significant impact on almost all crops. Those occurring in the early stage of seedling development have been a major constraint on cotton production worldwide (12, 20, 36). In general, damage at this crop stage is related to decreased seedling population, seed rotting before germination (pre-emergence damping-off) or seedling death (post-emergence damping-off), as stated by Dorrance et al. (10), Goulart et al. (13), Goulart (15), Malvick (25), Rizvi & Yang (30).

*Rhizoctonia solani* Kühn, anastomosis group AG-4 [teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk], is among the most important soilborne pathogens affecting cotton crops in Brazil. The importance of this pathogen is especially related to its occurrence frequency, its predominance on most soils and under different environmental conditions, and its consequent damage in the early stage of crop development (16, 17). This fungus is the major cause of seedling damping-off and is widely distributed in Brazil (19, 20).

Economic losses caused by root pathogens have been reported in the Brazilian and global agriculture. According to the Cotton Disease Council, in the last 50 years, significant losses in the cotton crop yield due to incidence of damping-off caused by *R. solani* ranged from 2% to 3% (31).

Control of cotton seedling damping-off caused by *R. solani* can be achieved with the establishment of integrated management programs, which have fungicide seed treatment as the major tool (15, 18, 24). According to Garber et al. (11), Davis et al. (8) and Goulart & Melo Filho (14), this control method is the most efficient and economical option to minimize the negative effects of this disease. Goulart (17) found that treating seeds with fungicides is less efficient in the presence of high pathogen inoculum densities. Thus, the treatment performance often depends on the strategies adopted to reduce *R. solani* population in the soil. Among these strategies, using suppressive crops previous to cotton may enhance the benefits of seed treatment with fungicides.

Integrated strategies, including crop rotation, can favor the management of cotton damping-off caused by *R. solani* for decreasing the inoculum amount in the subsequent crop. Thus, using previous crops that minimize the occurrence of this disease, based on the initial inoculum reduction, is recommended. However, due to the ecological versatility of this fungus, this may not occur at all. Therefore, the beneficial effects of these previous crops have been attributed to their capability of changing the soil microbial community, stimulating populations of antagonists to *R. solani*, thereby inducing suppressiveness (7, 26, 32). Since crop rotation, in most cases, does not eradicate this fungus from the soil, its effect on reducing the number of pathogen propagules must be considered. In the scientific literature, there are few studies showing such an effect. Results obtained by Costa (6), under greenhouse conditions, indicated that *Brachiaria plantaginea* contributed to the fall in *R. solani* population in a common bean crop. Corrêa et al. (5) pointed out that soils cultivated with sugarcane and pasture showed *R. solani* suppressive characteristics. Similar results were obtained by Toledo-Souza et al. (33) by growing grasses (brachiaria alone or intercropped with corn) previously to common beans. On the other hand, those researchers stated that some legumes such as crotalaria and pigeon pea should be avoided as previous crops to common beans, since they increase the soil population of this pathogen. Berni et al. (1) also found that the rotation rice/calopogon-common beans contributes to an increase in the incidence and severity of *R. solani* root rot in common bean plants. Differently, Costamilan et al. (7) found no influence of diverse cover crops such as wheat, corn, oats and vetch on decreasing the soil population of *R. solani* under temperate

climate conditions.

The aim of the present study was to verify the influence of previous crops and fungicide seed treatment in the incidence and control of damping-off caused by *R. solani* in cotton seedlings under greenhouse conditions.

## MATERIAL AND METHODS

The trials were conducted under greenhouse conditions during two years (2013 and 2014) at Embrapa Western Agriculture, Dourados, Mato Grosso do Sul State, Brazil.

The pathogen inoculum was composed of black oat seeds colonized by *R. solani* AG-4 isolated from infected cotton seedlings. The fungus was cultivated in potato-dextrose-agar medium (PDA) for 10 days at 25°C. After this period, 5mm-diameter mycelial-agar disks were transferred from the margin of growing colonies to Erlenmeyer flasks containing autoclaved substrate composed of 2kg black oat seeds and ½ L water. After 35-day incubation in a BOD chamber at 22°C and 12-h period alternating darkness and fluorescent white light, the oat seeds colonized by *R. solani* were allowed to dry on trays for 10 days and then ground to powder using a mill (1mm).

Besides cotton (treated and untreated seeds) and fallow, the following cover crops were tested as previous crops to cotton: black oats, millet, corn, forage sorghum, soybean, common beans, crotalaria (*Crotalaria juncea*), brachiaria (*Urochloa ruziziensis*) and brachiaria (*Urochloa ruziziensis*) + crotalaria (*Crotalaria juncea*).

Seeds from both acid-delinted cotton and previous crops were sown in soil contained in plastic trays and in ceramic pots; they were placed in equidistant 3cm-deep wells. A pre-established amount of pathogen inoculum was mixed in 1kg soil for the tests on plastic trays (56x35x10cm) and 0.5kg soil for the tests in ceramic pots of 5kg capacity. Before closing the wells, *R. solani* was inoculated by homogeneous distribution onto the substrate. Inoculum densities were adjusted to 2.5g/tray and 0.34g/pot, based on the pathogenicity. These inoculum quantities were adjusted to provide maximum 50% cotton seedling damping-off, according to the methodology proposed by Weinhold (35) and Wang & Davis (34). The trays and pots were kept in a greenhouse and watered daily.

Cotton sowing density was 200 seeds/tray and 25 seeds/pot. For the previous crops, the sowing density was based on the technical recommendations for each crop. Cotton damping-off was daily evaluated, from seven to 30 days after sowing for the tests using plastic trays and from seven to 45 days after sowing for the tests using ceramic pots.

The experiments on plastic trays were conducted for 30 days and those in the pots were carried out during 45 days. Initially, for both experiments, cotton seeds of the cultivar DeltaOpal, without fungicide treatment, were planted and *R. solani* was inoculated (2.5g/tray and 0.34g/pot) according to the previously mentioned methodology to establish the fungal population on the substrate. At this stage, post-emergence damping-off was recorded for comparison in subsequent evaluations. Then, the following treatments were introduced: cotton (treated and untreated seeds), fallow, black oats, millet, corn, forage sorghum, soybean, common beans, crotalaria (*Crotalaria juncea*), brachiaria (*Urochloa ruziziensis*) and brachiaria (*Urochloa ruziziensis*) + crotalaria (*Crotalaria juncea*).

At 30 days after sowing for plastic trays and at 45 days for ceramic pots, the cover plants were cut and kept on the substrate surface

during 45 days. After this period, treated and untreated cotton seeds were replanted and seedling damping-off was evaluated in each plot. The fungicide mixture used to treat cotton seeds was triadimenol + pencycuron + tolylfluaniid (50 + 50 + 30 g a.i./100 kg seeds).

A completely randomized block design was adopted with four replicates and 2 (treated and untreated cotton seeds) x 11 (previous crops) factorial arrangement. The data obtained as percentage were transformed in arc sen  $\sqrt{x}/100$ . Means were compared according to Scott Knott test (P=0.05). All analyses were conducted using ASSISTAT. The experimental data obtained over the two years underwent a joint

analysis, allowing the comparison of means of treatments belonging to different experiments.

## RESULTS AND DISCUSSION

The results obtained over the two years are presented in Table 1 (experiment using plastic trays) and Table 2 (experiment using ceramic pots). As the results obtained in both experiments, trays and pots, were consistent and very similar (Tables 1 and 2), they will be

**Table 1.** Incidence of cotton seedling damping-off (*R. solani*) according to previous crop and fungicide seed treatment – experiment conducted on plastic trays under greenhouse conditions. Embrapa Western Agriculture, Dourados, MS, Brazil.

Damping-off incidence before cover crop treatment (%)	Treatments	Damping-off incidence after cover crop treatment – treated cotton seeds (%)	Damping-off incidence after cover crop treatment – untreated cotton seeds (%)
48.7	Cotton without ST	20.2 a B	80.4 a A
45.4	Fallow	11.1 e B	32.6 e A
45.6	Black oats	13.3 d B	29.4 f A
45.7	Millet	12.8 d B	25.4 g A
45.4	Corn	15.1 e B	25.4 g A
47.9	Forage sorghum	13.1 d B	26.0 g A
48.3	Soybean	21.1 a B	63.3 c A
47.7	Common beans	21.5 a B	64.5 c A
47.4	Crotalaria <sup>3</sup>	23.2 a B	74.0 b A
45.2	Brachiaria <sup>4</sup>	13.7 d B	26.5 g A
45.9	Bachiaria+Crotalaria	19.8 b B	46.0 d A
Mean	-	16.81	44.83
C. V. (%)	-	8.89	10.29

Means followed by the same lowercase letter in the column and uppercase letter on the line do not differ statistically according to Scott Knott test (P=0.05). ST=seed treatment. <sup>3</sup>Crotalaria=(*Crotalaria juncea*). <sup>4</sup>Brachiaria=(*Urochloa ruziziensis*).

**Table 2.** Incidence of cotton seedling damping-off (*R. solani*) according to previous crop and fungicide seed treatment – experiment conducted in pots under greenhouse conditions. Embrapa Western Agriculture, Dourados, MS, Brazil.

Damping-off incidence before cover crop treatment (%)	Treatments	Damping-off incidence after cover crop treatment – treated cotton seeds (%)	Damping-off incidence after cover crop treatment – untreated cotton seeds (%)
36.0	Cotton without ST	16.4 b B	76.9 a A
36.8	Fallow	8.6 d B	28.2 d A
34.6	Black oats	10.1 d B	22.4 e A
31.9	Millet	9.3 d B	20.3 e A
31.3	Corn	12.6 c B	23.0 e A
32.3	Forage sorghum	10.7 d B	22.8 e A
35.8	Soybean	20.8 a B	60.0 b A
34.2	Common beans	19.6 a B	62.6 b A
32.4	Crotalaria <sup>3</sup>	23.2 a B	70.4 a A
31.4	Urochloa <sup>4</sup>	10.8 d B	21.9 e A
36.2	Urochloa+Crotalaria	16.8 b B	37.2 c A
Mean	-	14.14	40.51
C. V. (%)	-	8.28	6.06

Means followed by the same lowercase letter in the column and uppercase letter on the line do not differ statistically according to Scott Knott test (P=0.05). ST=seed treatment. <sup>3</sup>Crotalaria=(*Crotalaria juncea*). <sup>4</sup>Brachiaria=(*Urochloa ruziziensis*).

discussed jointly.

There was a significant effect of the interaction between previous crops and treated and untreated cotton seeds ( $P < 0.05$ ).

Regardless of the tested previous crops, the incidence of post-emergence damping-off among seedlings was lowest when cotton seeds received the fungicide mixture tolylfluanid + pencycuron + triadimenol, compared to the results for untreated seeds (Tables 1 and 2). This finding confirms the importance of treating the seeds with fungicides for the integrated management of diseases, significantly contributing to the control of post-emergence damping-off in cotton seedlings, which corroborates the results obtained by Davis et al. (8), Chitarra et al. (4) and Goulart (19, 20). The present study evidenced that the effectiveness of this fungicide mixture improved when grasses or fallow was adopted before cotton planting (Tables 1 and 2). Similarly, Reis et al. (28) pointed out that control strategies should focus on the development of suppressive soils, integrating cultural practices with other control methods, such as fungicide seed treatment. The present findings also confirm this concept.

Using grasses such as brachiaria (*Urochloa ruziziensis*), black oats, millet, corn and forage sorghum as previous crops, as well as fallow, significantly contributed to a fall in the soil population of *R. solani* in the present study, which led to lower rates of cotton seedling damping-off. It is worth mentioning that these results were obtained for both treated and untreated cotton seeds (Tables 1 and 2) and corroborate the findings by Correa et al. (5), Toledo-Souza et al. (33), Reis et al. (28), Derpsch & Calegari (9) and Berni et al. (1), who also recommended the use of grasses such as corn, black oats, rice, brachiaria and mombaça grass as previous crops in order to reduce *R. solani* inoculum densities in the subsequent cotton crop. A possible disease suppressive mechanism of grasses is stimulation of microbial activity by specific antagonists that minimize the disease (22). In the rhizosphere, microorganisms are stimulated by exudates and root tissues. Although grasses have exudates showing a higher C/N ratio, their rhizosphere effect is greater than that of legumes for presenting denser root system and more intense renovation (2). According to Rios (29), in diverse situations, the absence of a host (fallow) decreases the pathogen population due to natural mortality, lowering the incidence of damping-off, as observed in the current study.

On the other hand, the present results showed that, besides the continuous use of cotton, the use of legumes as previous crops, including soybeans, beans, crotalaria (*Crotalaria juncea*), as well as brachiaria (*Urochloa ruziziensis*) + crotalaria (*Crotalaria juncea*), was consistently associated with higher rates of damping-off in cotton seedlings, contributing to an increase or the maintenance of *R. solani* inoculum in the soil (Tables 1 and 2). This was also reported by Berni et al. (1), Toledo-Souza et al. (33) and Paula Júnior et al. (27), who found that legume crops may have serious epidemiological implications for increasing the soil populations of *R. solani*; those authors recommended avoiding the use of legumes as previous crops to cotton.

Higher damping-off percentages were observed in plots where cotton was continuously employed without fungicide seed treatment (Tables 1 and 2), confirming the results obtained by Café Filho & Lobo Júnior (3), who stated that monoculture practices significantly contribute to the increase in *R. solani* population in the soil, leading to high damping-off levels.

*R. solani* was isolated from all plants showing symptoms of damping-off, and this pathogen was identified based on morphological characteristics of cultures on PDA.

In the current cotton production system in Brazil, there is

predominance of crop succession, especially with *R. solani* non-suppressive previous crops, such as soybeans. However, the continuous use of this model has resulted in a series of problems linked to the increase in *R. solani* population in the soil, which significantly contribute to a greater vulnerability of the system. As a strategy to reduce *R. solani* inoculum in infested areas, the present results suggest the use of *R. solani* suppressive species like grasses as previous crop to cotton or fallow, which makes the system more productive and self-sustainable over time, since rotations with non-host crops have been effective in controlling this pathogen. Crop rotations with grasses can suppress diseases by allowing inoculum levels to decline in the absence of a host, directly inhibiting the pathogen by producing a toxic compound, favoring specific antagonists, or increasing general microbial populations that compete with the pathogen (21, 23). On the other hand, legumes should be avoided as previous crops to cotton. Treating the seeds with fungicides was also confirmed as efficient in controlling post-emergence damping-off caused by *R. solani* in cotton seedlings and its effect can be potentiated when grasses are chosen as previous crops. The present results reinforce the need to improve the control of cotton seedling damping-off by adopting an integrated management approach in areas infested with *R. solani*.

The fungicide seed treatment was efficient in controlling damping-off in cotton seedlings and its effect was potentiated when grasses were adopted as previous crops.

Using *R. solani* suppressive species like grasses as previous crops to cotton or fallow significantly contributed to decreasing *R. solani* population in the soil.

On the other hand, using cotton continuously, as well as legumes as previous crops to cotton, should be avoided since they contribute to the increase or the maintenance of *R. solani* inoculum in the soil.

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