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Original Article

Substrates and cover crop residues on the suppression of sclerotia and carpogenic germination of Sclerotinia sclerotiorum

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Abstract

Cover crops influence populations of microorganisms, such as a pathogen. In this work the objective was to evaluate the influence of different cover crops on the sclerotia of *Sclerotinia sclerotiorum* recovering rate in soil as well as the mycelial growth rate for recovered sclerotia. The influence of different substrates for carpogenic germination was also evaluated. The cover crops used in the experiment were *Crotalaria juncea* L., *Urochloa ruziziensis* R. Germ. & Evrard, *Panicum maximum* cv. Mombaça Jacq., *Pennisetum glaucum* (L.) R. Brown, *Cajanus cajan* (L.) Millsp.) and *Stylosantes capitata* Vog.; *Stylosanthes macrocephala* Ferr. Et Costa). The substrates used for carpogenic germination were agar-water, agar- water + filter paper, sand and mixed sand and soil. The cover crops showed efficiency in the suppression of *S. sclerotiorum*, especially for *Stylosanthes* spp. and *Panicum maximum* cv. Mombaça in the rate recovering of sclerotia and for *Stylosanthes* spp. in the rate of mycelial growth by providing a suitable environment for growth of antagonistic microorganisms. As for the number of carpogenic germinated sclerotia and for the number of apothecia formed on different substrates, the sand; agar-water and soil + sand mixture stood out, respectively.

Key-Words: white mold, cultural management, biological control; apothecia; biomass

Substratos e resíduos de plantas de cobertura na supressão dos escleródios e germinação carpogênica de *Sclerotinia sclerotiorum*

Resumo

As plantas de cobertura influenciam as populações de microrganismos, tal como o fitopatógeno. Neste trabalho o objetivo foi avaliar a influência de diferentes plantas de cobertura sobre a taxa de recuperação dos escleródios de *Sclerotinia sclerotiorum* no solo, bem como o índice da velocidade de crescimento micelial para os escleródios recuperados. Avaliou-se também a influência de diferentes substratos para germinação carpogênica. As plantas de cobertura utilizadas no experimento foram *Crotalaria juncea* L., *Urochloa ruziziensis* R. Germ. & Evrard, *Panicum maximum* cv. Mombaça Jacq., *Pennisetum glaucum* (L.) R. Brown, *Cajanus cajan* (L.) Millsp.) e *Stylosantes capitata* Vog.; *Stylosanthes macrocephala* Ferr. Et Costa). Os substratos utilizados para a germinação carpogênica foram ágar-água, ágar-água+papel filtro, areia e mistura areia e solo. As plantas de cobertura apresentaram eficiência na supressão de *S. sclerotiorum*, com destaque, para *Stylosantes* spp. e *Panicum maximum* cv. Mombaça quanto à taxa de recuperação de escleródios e para o *Stylosantes* spp. quanto ao índice da velocidade de crescimento micelial, por proporcionar ambiente adequado ao crescimento de organismos antagonistas. Quanto ao número de escleródios germinados carpogenicamente e ao número de apotécios formados em diferentes substratos, destacaram-se a areia; e o ágar e mistura solo+areia, respectivamente.

Palavras-chave: mofo branco, manejo cultural, controle biológico, apotécio, fitomassa

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Introduction

The fungus *S. sclerotiorum* (Lib.) De Bary survive in soil for long time periods, due to a structure of resistance called sclerotia, which in suitable environments can germinate in a mycelial or carpogenic way. Carpogenic germination forms a fruiting body called apothecia, which houses the ascospores of the fungus and is considered crucial for the expression of the disease and the epidemic process (Kohn, 1979).

Several plants have been reported as hosts of this fungus, including, soybean, bean, sunflower and cotton (Nasser et al., 1999). In addition to that, some weeds are considered multipliers of the fungus (Valarini & Spadotto, 1995), what makes it difficult to manage the properties that do not eradicate these plants from the area. Another aggravating fact is the absence of plants resistant to this disease. A study developed by Garg et al. (2010) first demonstrated, at the cellular level, that the resistance of *Brassica napus* L. for the *S. sclerotiorum* is a result of delayed development of the pathogen.

Soil cover crops have the potential to promote improvements in the chemical, physical and biological properties of soil (Toledo-Souza et al., 2008; Machado & Assis, 2010), what can make the soil suppressive to pathogens. According to Costa & Rava (2003), there is a reduction of the inoculum source of some necrotrophic pathogens when *Brachiaria* spp. is used as a source of mulch. These soils commonly have the presence of a predominant population (Hornby, 1983).

The soil microbial population has great importance in phytopathological studies, since soil management may alter the relationship between those deemed beneficial and detrimental to plant growth, according to the stimulus received (Marschner et al., 2001). Each plant species behaves differently to this stimulus, and it was possible due to the release of nutrients and chemicals that promote or may be deleterious to the growth of certain organisms groups (Rocha et al., 2004).

In an attempt to check the progress of diseases caused by *S. sclerotiorum* in Brazil, several control measures have been employed, such as the fungicides (Oliveira, 2005), application of antagonistic organisms (Moretini & Melo, 2007) and crop rotation (Kluthcouski et al., 2003). The use of cover crops in crop rotation may be an alternative to the management of this pathogen by promoting the release of substances, such as fungicides, nutrients, and especially allow the antagonistic microorganisms growth (Asmus et al., 2005). Pereira Neto & Blum (2010), in a study with the addition of *P. glaucum* biomass to the soil, have observed that stem rot caused by *Sclerotium rolfsii* Sacc. was reduced in beans. Guini & Zaroni (2001) observed that soils with the presence of sugar cane, corn and coffee were conducive to *Rhizoctonia solani* Kuhn.

Although there are some studies elucidating the characteristics of the fungus, some doubts still remain about the interference of cover crops on disease development. This work was carried out in order to know the influence of different cover crops on sclerotia of *S. sclerotiorum* allocated in the soil recovering rate, recovered sclerotia mycelial growth rate and the influence of different substrates in the formation of apothecia, elucidating the requirement of nutrients as for this type of germination.

Material and Methods

The experiment was conducted in Lavras, MG, located at coordinates 21° 14' 43" south latitude 44° 59' 59" west longitude and an altitude of 919 m, from January to July, 2010.

The sclerotia production of *S. sclerotiorum* for use in the experiment was artificially induced, with all production from a single sclerotia collected in soybean crops in the region of Rio Verde county, Goiás state, Brazil. Sclerotias were placed on the center of each Petri dish with Potato Dextrose Agar (PDA) medium and then placed in a growth chamber at 20°C with a photoperiod of 12 hours for 20 days. After producing the first sclerotia, mycelial disks were transferred to other plates containing the same culture medium to induce the production of more sclerotia; this procedure was repeated over and over until it was possible to reach the required amount of sclerotia.

The experiment consisted of seven treatments in a completely randomized design with four replications. The treatments consisted of the cover crops species *Crotalaria juncea* L., *Urochloa ruziziensis* R. Germ. & Evrard, *Panicum maximum* Jacq. Cv. Mombaça, *Pennisetum glaucum* (L.) R. Br., *Cajanus cajan* (L.) Millsp. and *Stylosanthes capitata* Vogel x *Stylosanthes macrocephala* MB Ferr. et S. Costa, and a control without the presence of cover crops. The soil used was a Red-Dark oxisol, sieved and sterilized according to the method developed by Menezes & Silva-Hanlin (1997).

The experimental unit used was a plastic Gerbox box filled with soil, in which the cover plants were sown. The control was only added to the ground to simulate the absence of the species studied. Then the treatments (plastic Gerbox boxes containing soil with the cover crops sown and the control were placed in growth chambers at a temperature of 20°C and a photoperiod of 12 hours. After 40 days, plants were harvested and 10 g of biomass of each species were placed on the soil surface for each plastic box. At this time, nine sclerotia were added at a depth of 0.5 cm, equally distributed and placed in a growth chamber for 40 days. Throughout the experiment, the soil was irrigated periodically to keep the moisture close to field capacity through the installation of nebulizers to maintain relative humidity above 60% in order to promote the germination of sclerotia.

After sclerotia were stored for forty days, the rate of recovery of sclerotia was evaluated,

showing a percentage relationship between the sclerotia recovered in each treatment, based on the nine sclerotia initially allocated in each plastic box. In this procedure, only the sclerotia which had rigid consistency were recovered.

Using the sclerotia recovered in the previous experiment it has measured the daily radial mycelial growth and then, it have calculated the growth rate index of mycelial (IVCM), according to the formula adapted by Salgado et al. (2003): IVCM = Σ (D-Da)/N, in which IVCM = mycelial growth rate (cm day^{-1}), D = current average diameter (cm), Da= the average diameter of the previous day (cm), and N = number of days after inoculation. In this procedure, the sclerotia that were in contact with the cover crops were sterilized using standard procedures (submerged for 30 seconds in 70% alcohol, then immersed in sodium hypochlorite 2% for 2 minutes, followed by three successive washes in sterile distilled water). As for sclerotia without a layer of melanization, we have employed a procedure with triple the time and concentrations used in the standard procedure in an attempt to eradicate the microorganisms associated with these sclerotia.

After this operation, the sterilized sclerotia were individually placed in Petri dishes containing PDA medium and were placed in a growth chamber with a temperature of 20 ° C and photoperiod of 12 hours. In addition to the control, sclerotia from soil without cover crops, stored at room temperature for a period of 8 months in Falcon type tubes, were also assessed for mycelial growth.

As for the study related to carpogenic germination on different substrates, we have evaluated the germination of sclerotia in Petri dishes containing agar-water (2%), agar-water (2%) + filter paper, sand and plastic gerbox boxes containing soil and sand in a 1:1 ratio in a completely randomized design with observations on the type of germination that occurred in different substrates. All treatments were watered only once, and the amount of water in the Petri dishes was 15 mL and for the soil 50 mL was added, what simulates the field capacity. The treatments were stored in a growth chamber with a temperature of 20°C and a photoperiod of 12 hours. The evaluation of the number of germinated sclerotia and apothecia formed was done after 32 days.

The results were submitted to analysis of variance and when significant by F test, means were compared by Tukey and Scott-Knott statistical test, using Sisvar software (Ferreira, 2003).

Results and Discussion

By analyzing the rate of recovery of sclerotia kept in soil, fitomass of *Stylosantes* spp. and *P. maximum* cv. Mombaça stands out, showing the lowest number of sclerotia recovered (Table 1). Sutherst et al. (1982) reported that *Stylosantes* spp. plants are covered with trichomes or by secreting a viscous fluid that acts as a repellent against insects that feed on plants. It also has a reputation against ticks, reducing the population where these plants are found (Zimmerman et al., 1984). Thus, it is likely that the *Stylosantes* spp. plants release substances that could harm the integrity of the sclerotia. The release of nutrients by the fitomass and the capacity to retain soil moisture may have provided conditions for microbial growth (Zambolim et al., 2005) in the treatments with cover crops.

The difference between the responses obtained can be explained by the action of fungi and bacteria, and also by the presence of a Diptera from the Sciridae family known as *Bradysia coprophila* (Lintner), which took advantage of decaying organic matter and high soil moisture for oviposition, especially for treatments with *Stylosantes spp.* and *Panicum maximum* cv. Mombaça. Anas & Reeleder (1988) observed that larvae of *B. coprophila* cause damage by feeding on sclerotia, affecting the survival of the resistance structure and increasing their susceptibility to mycoparasites organisms. So, the sclerotia damaged by the larvae have a higher degree of parasitism than those which were not damaged. In this experiment, a high degree of parasitism was found in sclerotia that had the layer of melanization completely consumed by the larvae, and it was impossible to eradicate these antagonistic organisms, even by submitting the sclerotia to a more concentrated disinfection process. Gracia-Garza et al. (1997) pointed out

Table 1. Recovery rate of sclerotia after 40 days of being kept in soil in contact with the debris of the cover crops.

Cover crop	Recovery rate of sclerotia
	%
Stylosantes spp.	5,55a
Panicum maximum cv. Mombaça	16,65a
Pennisetum glaucum	19,44bc
Cajanus cajan	19,44bc
Crotalaria juncea	30,55c
Urochloa ruziziensis	94,45d
Control	86,11d
V.C.	15,27

Means followed by same letter do not differ by Tukey test at 5% probability. V.C. = variation coefficient.

that those salivary secretions of the larvae reduce the ability of the sclerotia to germinate. This author also reports that few sclerotia survived when *B. coprophila* and *Trichoderma* spp. were applied either separately or in combination.

For treatments with the species of *Pennisetum glaucum*, *Crotalaria juncea* and *Cajanus cajan,* the rate of recovered sclerotia was intermediate if compared to other treatments. This result is attributed to the insufficient amount of antagonistic compounds of the sclerotia, which are released by these plants as well as their inefficiency in promoting the growth of antagonistic organisms.

The highest rate of recovery was obtained in treatment with the fitomass of *Urochloa ruziziensis*, which was statistically equal to control. These results do not agree with those obtained by Görgen et al. (2009), who have reported that the fitomass of *Urochloa ruziziensis* contributed to the increase of the parasitism of sclerotia. However, it should be noted that the phenological stage of the culture exerts significant influence on the amount of biomass produced, organic matter accumulation and biological activity in the soil. In this experiment, it was observed that the fitomass of this specie was not effective in promoting antagonistic organisms growth, what can be attested by the lower population of larvae of *B. coprophila* that has been observed while carrying out this work. Pacheco et al. (2008) reported the high persistence of the fitomass *B. ruzizienzis* during the offseason, when seeded in soybeans. This result highlights the difficulty of microorganisms to use this fitomass as a substrate in a quickly and efficiently way.

As for the mycelial growth rate index, the results showed that there was statistical difference between the sclerotia kept under the studied cover crops (Table 2). The sclerotia stored in Falcon type tube had the highest growth rate index of mycelial, what can be explained by the integrity of the layer of melanin, having been previously stored in a favorable environment for preservation. This result indicates that the standard procedure for disinfection was effective for the eradication of agents associated with these antagonists in a latent form.

Table 2. Growth rate index of mycelial sclerotia kept in soil for 40 days in the presence of crop residues of different cover crops.

Cover crops	Growth rate index of mycelial
	Cm/day
Stylosantes spp.	1,37a
Pennisetum glaucum	2,95b
Urochloa ruziziensis	3,03b
Panicum maximum cv. Mombaça	3,13b
Crotalaria juncea	3,37b
Cajanus cajan	3,19b
Control soil	3,24b
Control tube	3,71c
V.C.	7,95

Means followed by same letter do not differ by Scott-Knott test at 5% probability.
V.C. = variation coefficient.

The sclerotia allocated under the *Stylosanthes* spp. fitomass have shown the lowest growth rate, due to significant damage caused by the presence of larvae of *B. coprophila*. In these resistant structures, the melanization layer was not complete, allowing rapid mycelial germination when placed in PDA culture medium (Figure 1). However, after 12 hours, there was an increased reduction in growth rate. According to Huang (1985), mycelial germination of sclerotia is linked to the melanization degree of the cell wall surface on these resistant structures. He also reports that an incomplete melanization promotes immediate mycelial germination. It is important to mention that some of these structures did not present any layer of melanization (Figure 1) and it was found, in these sclerotia, the parasitism of antagonistic microorganisms, making it impossible to use them at this stage of the experiment, due to ineffectiveness of the disinfestation procedure.

Anas & Reeleder (1988) have reported that damage to sclerotia by an antagonist population was higher when the organic matter content increased from 7% to 80%. This explains the increased action of organisms (fungi and insects) in treatments with vegetation cover.

Treatment with sand had the lowest value for both the number of carpogenic germinated sclerotia as well as the number of apothecia formed (Figure 2). The observations allow us to infer that the main factor affecting germination was lack of direct contact of sclerotia with water, since it was observed that their infiltration through the substrate occurred immediately after application, being deposited on the bottom of the Petri dish. On the other hand, the behavior of sclerotia in dealing with water-agar + filter paper can be explained by the high loss of water from the paper, which leaves the sclerotia in a dry surface after two weeks of the experiment, limiting the formation of apothecia.

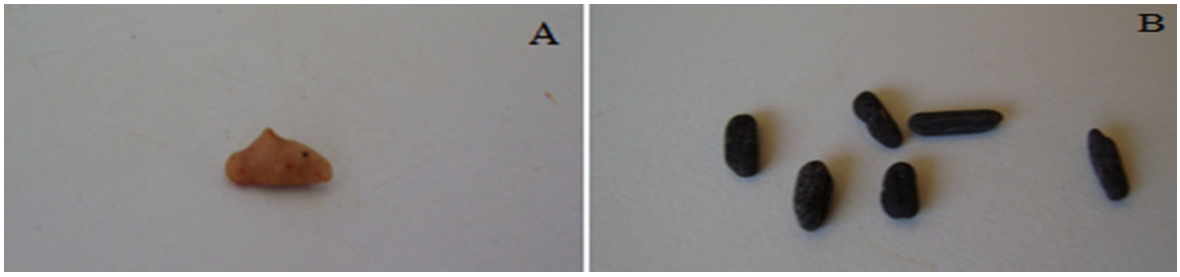

Figure 1. Sclerotia recovered from soil after 45 days in contact with cover crops, A) sclerotia without melanization layer; B) sclerotia with complete layer of melanization stored in Falcon type tube.

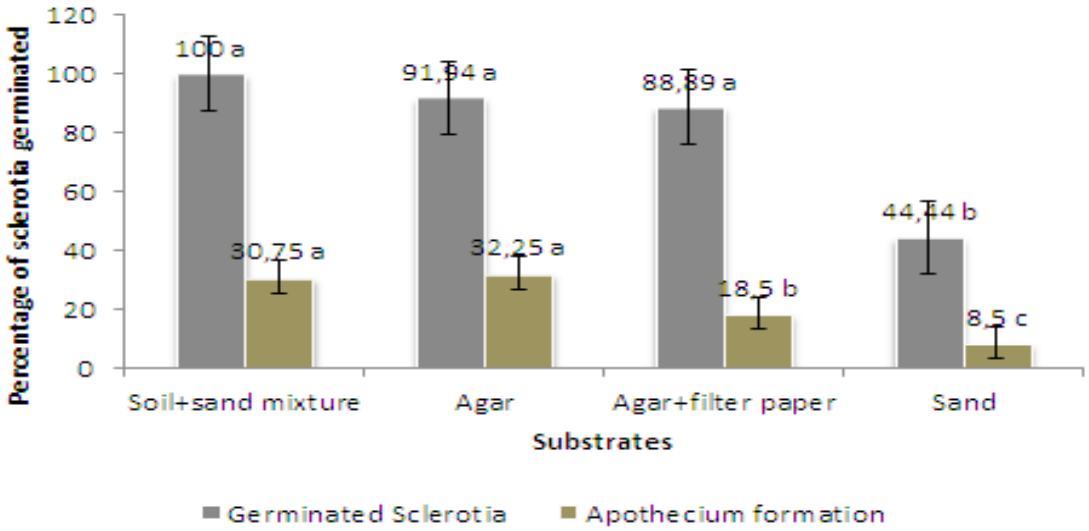

Figure 2. Comparison of percentage germinated sclerotia and number of apothecia formed in different substrates. Means followed by same letters in germinated apothecia and sclerotia separately; do not differ at 5% probability by Scott-knott test.

The treatments with the mixture sand+soil and water-agar showed the highest values for both the germination of sclerotia and the number of apothecia formed. The water-agar treatment is a substrate with a few amounts of nutrients, so it can be deduced that for carpogenic germination their presence is not vital. These results differ from those presented by Purdy & Grogan (1954), who have reported the dependence of macro and micronutrients for induction of carpogenic germination in liquid medium and agar culture. A similar trend is reported by Vega & Tourneau (1974), who have claimed the dependency of zinc in this type of germination.

Other authors have reported that the type of soil, like substrate, influences carpogenic germination (Costa & Costa, 2006). Although not accounted for, differences were observed in mycelial germination.As for the substrate with soil + sand, it was possible to see the production of mycelium by sclerotia in the first week after beginning the experiment, what was not observed for water + agar substrate. Thus, based on the comparison of these substrates it is possible to deduce that the availability of nutrients influences mycelial germination. A similar result was found by Gonçalves et al. (2004), concerning *Peronospora destructor* (Berck. Casp.) and onion pathosystem.

Conclusions

The differences in the recovery rate of sclerotia and the growth rate index of mycelial are due to differences in the integrity of the structures of resistance influenced by antagonistic microorganisms.

For sclerotia stored in a tube, the highest growth rate index of mycelia was seen. The sclerotia kept under the *Stylosantes* spp. plants presented the lowest value.

The highest carpogenic germination of sclerotia and apothecia formation was observed in agar-water and soil+sand mixture substrates.

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References

Anas, O., Reeleder, R.D. 1988. Consumption of sclerotia of *Sclerotinia sclerotiorum* by larvae of *Bradysia coprophila*: Influence of soil factors and interaction between larvae and *Trichoderma viride*. *Soil Biology and Biochemistry* 20: 619-624.

Asmus, G.L., Inamoto, M.M., Sazaki, C.S.S., Ferraz, M.A. 2005. Reação de algumas culturas de coberturas utilizadas no sistema plantio direto a *Meloidogyne incognita*. *Nematologia Brasileira*

29: 47-52.

Costa, G.R., Costa J.L.S. 2006. Influência do solo e de substratos para produção de escleródios na germinação carpogênica de *Sclerotinia sclerotiorum*. *Pesquisa Agropecuária Tropical* 36: 83-87.

Costa, J.L.S., Rava, C.A. 2003. Influência da braquiária no manejo de doenças do feijoeiro com origem no solo. In: Kluthcouski, J., Stone, L.F., Aidar, H. (ed.) *Integração lavoura-pecuária*. EMBRAPA Arroz e Feijão, Santo Antônio de Goiás, Brazil. p. 523-533.

Ferreira, D.F. 2003. *Sisvar versão 4.2*. DEX/UFLA, Lavras, Brazil. CD-ROM.

Garg, H.Li, Sivasithamparam, K., Kuo, J., Barbetti, J. 2010. The infection processes of *Sclerotinia sclerotiorum* in cotyledon tissue of a resistant and a suceptible genotype of *Brassica napus*. *Annals of Botany* 1: 1-12.

Gonçalves, P.A.S., Silva, C.R.S. Boff, P. 2004. Incidência do míldio em cebola sob adubação mineral e orgânica. *Horticultura Brasileira* 22: 538-542.

Görgen, C.A., Silveira Neto, A.N., Carneiro, L.C., Ragagnin, V., Lobo Junior, M. 2009. Controle do mofo-branco com palhada e *Thichoderma harzianum* 1306 em soja. *Pesquisa Agropecuária Brasileira* 44: 1583-1590.

Gracia-Garza, J.A., Reeleder, R.D., Paulitz, T.C. 1997. Degradation of sclerotia of *Sclerotinia sclerotiorum* by fungus gnats (*Bradysia coprophila*) and the biocontrol fungi *Trichoderma* spp. *Soil Biology and Biochemistry* 29: 123-129.

Guini, R., Zaroni, M.M.H. 2001. Relação entre coberturas vegetais e supressividade de solos a *Rhizoctonia solani*. *Fitopatologia Brasileira* 26: 10-15.

Hornby, D. 1983. Suppressive soils. *Annual Review of Phytopathology* 21: 65-85.

Huang, H.C. 1985. Factors affecting myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. *Phytopathology* 75: 433-437.

Kluthcouski, J., Cobucci, T., Aidar, H., Costa, J.L.S., Portela, C. 2003. *Cultivo do feijoeiro em palhada de braquiária*. EMBRAPA Arroz e Feijão, Santo Antônio de Goiás, Brazil. 28 p.

Kohn, L. 1979. Delimitation of the economically important plant pathogenic *Sclerotinia sclerotiorum* species. *Phytopathology* 69: 881-886.

Machado, L.A.Z., Assis, P.G.G. 2010. Produção de palha e forragem por espécies anuais e perenes em sucessão à soja. *Pesquisa Agropecuária Brasileira* 45: 415-422.

Marschner, P., Yang, C.H., Lieberei, R., Crowley, D.E. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biology and Biochemistry* 33: 1437-1446.

Menezes, M., Silva-Hanlin, D.M.W. 1997. *Guia prático para fungos fitopatogênicos*. UFRPE, Recife, Brazil. 106 p.

Moretini, A., Melo, I.S. 2007. Formulação do fungo *Coniothyrium minitans* para controle do mofo branco causado por *Sclerotinia sclerotiorum*. *Pesquisa Agropecuária Brasileira* 42: 155-161.

Nasser, L.C.B., Cafe Filho, A.C., Azevedo, J.A., Gomes, A.C., Vivaldi, L.J., Albretch, J.C., Freitas, M.A., Karl, A.C., Ferraz, L.L.C., Medeiros, R.G., Arancibia C.R., Napoleao, R.L., Junqueira, N.T.V. 1999. Método alternativo para o manejo do cancro-da-haste da soja (Diaphorte phaseolorum f. sp. Meridionalis) e mofo branco (Sclerotinia sclerotiorum) em sistemas de produção de grãos do Cerrado. *Comunicado Técnico* 11: 4.

Oliveira, S. H. F. 2005. Manejo do mofo branco. *DBO Agrotecnologia* 2: 1-4.

Pacheco L.P., Pires, F.R., Monteiro, F.P., Procópio, S.O., Assis, R.L., Carmo, M.L., Petter, F.A. 2008. Desempenho de plantas de cobertura em sobressemeadura na cultura da soja. *Pesquisa Agropecuária Brasileira* 43: 815-823.

Pereira Neto, J.V., Blum, L.E.B. 2010. Adição de palha de milheto ao solo para redução da podridão do colo em feijoeiro. *Pesquisa Agropecuária Tropical* 40: 354-361.

Purdy, L.H., Grogan, R.G. 1954. Physiological studies of *Sclerotinia sclerotiorum* in liquid and agar culture. *Phytopathology* 44: 36–38.

Rocha, F.S., Campos, V.P., Souza, R.M. 2004. Efeito de exsudatos radiculares em endósporos de *Pausteria penetrans* e em juvenis do Segundo estádio de *Meloidogyne incognita*. *Fitopatologia Brasileira* 29: 644-650.

Salgado, A.P.S.P., Cardoso, M.G., Souza, P.E., Souza, J.A., Abreu, C.M., Pinto, J.E.B.P. 2003. Avaliação da atividade fungitóxica de óleos essenciais de folhas de *Eucalyptus* sobre *Fusarium oxysporum, Botrytis cinerea* e *Bipolaris sorokiniana*. *Ciência e Agrotecnologia* 27: 249-254.

Sutherst, R.W., Jones, R.J., Schnitzerling, H.J. 1982. Tropical legumes of the genus Stylosanthes immobilize and kill cattle ticks. *Nature* 295: 320-321.

Toledo-Souza, E.D., Silveira, P.M., Lobo Junior, M., Café Filho, A.C. 2008. Sistemas de cultivo, sucessões de culturas, densidade do solo e sobrevivência de patógenos de solo. *Pesquisa Agropecuária Brasileira* 43: 971-978.

Valarini, P.J., Spadotto, C.A. 1995. Identificação de nichos de sobrevivência de fitopatógenos em áreas irrigadas de Guaíra-SP. *Pesquisa Agropecuária Brasileira* 30: 1239-1243.

Vega, R.R., Tourneau, L.D. 1974. The effect of zinc on growth and sclerotial formantion in Whetzelinia sclerotiorum. *Mycologia* 66: 256-264.

Zambolim, L., Costa, H., Vale, F.X.R. 2005. Nutrição mineral e patógenos radiculares. In: SAMI, J. M. et al. *Ecologia e Manejo de Patógenos Radiculares em Solos Tropicais*. UFRPE, Recife, Brazil. 398 p.

Zimmerman, R.H., Garris, G.I., Beaver, J.S. 1984. Potential of Stylosanthes plants as a component in an integrated pest management approach to tick control. *Preventive Veterinary Medicine* 2: 579-588.