

## ANTAGONISM OF *ASPERGILLUS TERREUS* TO *SCLEROTINIA SCLEROTIUM*

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### ABSTRACT

An *Aspergillus terreus* strain showed in vitro antagonistic activity against the plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary. The interaction between *A. terreus* and sclerotia revealed that the mycoparasite sporulated abundantly on the sclerotial surface. Cell breakdown due to host cell wall disruption was observed in inner rind cells, by a scanning electron microscopy.

**Key words:** biological control, mycoparasitism, morphology

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### INTRODUCTION

In Brazil, sclerotinia wilt is a serious disease of many important crops, including tomato, dry beans, soybeans, sunflower, lettuce and many others, caused by infection of roots by hyphae from myceliogenic germination of *Sclerotinia sclerotiorum*.

Several microorganisms including bacteria and fungi have been reported to be good antagonists to *Sclerotinia sclerotiorum*. The most prominent and extensively studied antagonists are several species of *Trichoderma* (11,13), *Coniothyrium minitans* (15), *Sporidesmium sclerotivorum* (1) and *Talaromyces flavus* (10). The success of these antagonists is based, in general, on their ability to parasitize sclerotia, the primary source of inoculum. Because they prove to be effective agents of biological control for pathogenic fungi, mycoparasites have attracted widespread attention in recent years with many commercial biofungicides available worldwide (3,5,8,12).

During a survey of soils for antagonists of *S. sclerotiorum*, many isolates belonging to the genus *Aspergillus* were obtained besides others antagonistic organisms, such as *Trichoderma* and *Penicillium*. This mould produces substances which are able to inhibit growth of different bacteria and of *Trichophyton mentagrophytes* and *Sporothrix schenckii* (4). Antifungal activity of *A. terreus* was observed by Slagg and

Fellows (14), who reported complete inhibition of *Ophiobolus graminis*, the causal organism of take-all of wheat. A similar effect on *Fusarium udum*, the cause of wilt of pigeon peas, was noted by Vasudeva and Roy (16). Inhibition of nematode-trapping fungi by *A. terreus* was reported by Mankau (9). Therefore, the present study was conducted to investigate, using scanning electron microscopy, the mode of hyperparasitism of *S. sclerotiorum* by an isolate of *A. terreus*, isolated from soil, using the sclerotial bait technique.

*S. sclerotiorum* sclerotia were produced on oat-agar (50g rolled oat, 18g agar, 1000 mL water esterilized at 121°C for 30 min.) by growing at 26°C for three weeks in the dark. Soil samples, obtained from rhizosphere of tomato, were air-dried and placed on the sterile petri dishes. Twenty 2-3 mm diameter sclerotia were placed on the soil surface. The soil was moistened with 10 mL of sterile distilled water. After 3 weeks of incubation, sclerotia was recovered, washed in sterile distilled water, surface sterilized by immersion in 10% sodium hypochlorite for 10 minutes, transferred to potato, dextrose, agar medium (PDA) + aureomycin (0.003%) and incubated at 26°C. Unsterilized sclerotia were also incubated as above. After 48 hours, fungal colonies were purified and transferred to (PDA) slopes.

Survival of *S. sclerotiorum* sclerotia when paired with conidia of *A. terreus* was tested by immersing sclerotia in a conidial

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suspensions ( $10^8$  conidia  $\text{mL}^{-1}$ ) from 14 - day-old *Aspergillus* cultures for 10 minutes. Sclerotia were blotted on sterile filter paper, placed on to sterile sand in Petri dishes, and incubated at 28°C for 30 days. Sclerotia, washed in sterile distilled water were treated similarly to act as controls. After this period, sclerotia were recovered, washed, surface sterilized and placed on PDA containing aureomycin (0.003% w/v). Viability was determined after 7 days of incubation, when sclerotia were score for germination, when the antagonist could be observed growing on non-viable sclerotia.

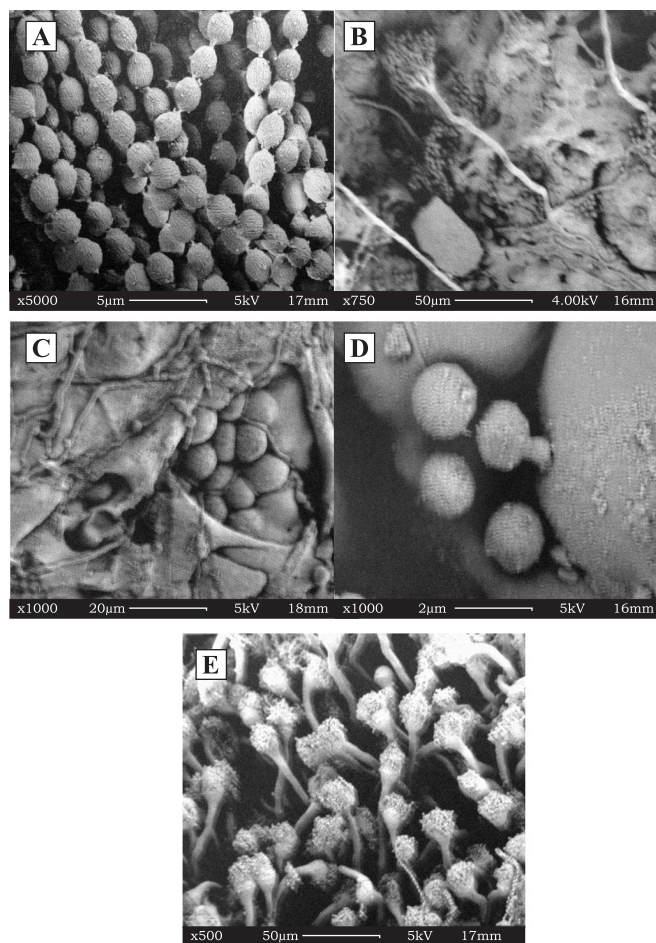
For scanning electron microscopy SEM, parasitized sclerotia were fixed in 2% glutaraldehyde. Following buffer – rinsing, the specimens were post-fixed in 2% osmium tetroxide at 8°C for 2h, rinsed in buffer, and dehydrated in an ethanol series. Following ethanol dehydration, the material was critical – point dried, mounted on SEM stubs, sputter–coated with gold and examined on an ultra – high performance SEM (Leo 982-Zeiss and Leica) at Embrapa Environment.

The sclerotial bait technique was successful in isolating different fungal strains. In dual culture, an *Aspergillus* isolate showed a high degree of antagonism by overgrowing *S. sclerotiorum* and was readily distinguishable from its host by fine hyphae it produced in contrast to the coarse hyphae of *S. sclerotiorum*. This antagonistic fungus was identified by its morphological characteristics as *A. terreus* (Fig. 1A). Conidia are globose smooth and connected each other.

*A. terreus* occurs in tropical and subtropical zones and has a worldwide distribution on different soil. One very common habitat is the rhizosphere of plants (4). This species was found by White *et al.*, (17) to be the most strongly cellulolytic species of the genus. This fungus produces a large number of specific metabolites, including the nephrotoxin citrinin, the neurotoxins citroviridin, patulin, terrain, terreic acid and geodin. (4) and several other compounds.

*A. terreus* (EQ) was found to affect sclerotial viability when sclerotia of the pathogen were treated with conidia of the hyperparasite. The fungus caused 100% mortality of sclerotia (Data not shown). This strain of *A. terreus* was able to colonize sclerotia of *S. sclerotiorum* and produce small hyphal branches that penetrated host cell wall without forming appressoria.

Plasmolysis was evident in some of the host cells infected by *A. terreus*. A close examination of these parasitized sclerotia revealed that hyphae of the antagonist grew abundantly on the sclerotial surface, forming dense forest of conidiophores (Fig. 1 E). At a more advanced stage of parasitism, *A. terreus* completely destroyed the sclerotial cells and penetrated and collapsed the medullary tissue (Fig. 1 C, D), with internal growth, and subsequent sporulation outside the host. This mode of parasitism is similar to previously described necrotrophic mycoparasites of *S. sclerotiorum*, such as *Trichoderma harzianum* (7,13), *Talaromyces flavus* (10) and *Coniothyrium minitans* (6).



**Figure 1.** Scanning electron micrograph of conidia *A. terreus* and interactions with sclerotia of *S. sclerotiorum*. (A) Conidial heads long, compactly columnar; (B) Note the presence of a conidiophore and a mass of conidia on the surface of the sclerotia; (C) The cell wall has been disintegrated and hyphae of the microparasite has penetrated and colonized the modular tissue. The breakage of the host cell wall is disintegrated; (D) A conidium of *A. terreus* germination towards to medullary tissue of a disintegrated sclerotia; (E) A forest of conidiophores with conidia of *A. terreus* on the surface of a *S. sclerotiorum* sclerotia.

*A. terreus* has proved to be a destructive necrotrophic parasite of *S. sclerotiorum*, where this agent destroys the host cell after or slightly before invasion.

For a mycoparasite to be considered a successful biocontrol agent, it should be effective against resistant survival structures of plant pathogens (2). These results herein obtained showed that *A. terreus* was able to exploit sclerotial tissue of *S. sclerotiorum*.

## RESUMO

**Antagonismo de *Aspergillus terreus* contra *Sclerotinia sclerotiorum***

Uma linhagem de *Aspergillus terreus* mostrou forte atividade parasítica contra *Sclerotinia sclerotiorum*. Interações entre o patógeno e o antagonista revelaram que *A. terreus* esporulou profusamente sobre os escleródios. Quando visto em microscopia eletrônica de varredura, o antagonista mostra-se rompendo e lisando a parede celular e penetrando o interior do escleródio, onde se estabelece no tecido medular.

**Palavras-chaves:** controle biológico, micoparasitismo, morfologia

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