

Effect of temperature on mycelial growth of *Trichoderma*, *Sclerotinia minor* and *S. sclerotiorum*, as well as on mycoparasitism

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ABSTRACT

Domingues, M.V.P.F.; Moura, K.E.; Salomão, D.; Elias, L.M.; Patrício, F.R.A. Effect of temperature on mycelial growth of *Trichoderma*, *Sclerotinia minor* and *S. sclerotiorum*, as well as on mycoparasitism. *Summa Phytopathologica*, v.42, n.3, p.222-227, 2016.

Environmental conditions are very important for the biological control of plant diseases. In a previous study, isolates of *Trichoderma asperellum* (IBLF 897, IBLF 904 and IBLF 914) and *T. asperelloides* (IBLF 908) were selected as antagonists of *S. minor* and *S. sclerotiorum*, causal agents of lettuce drop, one of the most relevant diseases affecting the lettuce crop. In this subsequent study, the mycelial growth of these isolates and pathogens, as well as the mycoparasitism of isolate IBLF 914, was evaluated at different temperatures. The mycelial growth of the isolates of *T. asperellum* and *T. asperelloides*, as well as of *S. minor* and *S. sclerotiorum*, was evaluated at temperatures ranging from 7 to 42°C. The parasitism of propagules of *S. minor* and *S. sclerotiorum* by the isolate IBLF 914, as well as the number of lettuce seedlings surviving drop, was evaluated at 12, 17, 22, 27 and

32°C, in gerboxes containing substrate. *S. minor* and *S. sclerotiorum* showed mycelial growth at temperatures ranging from 7 to 27°C, but no growth occurred at 32 °C, and both pathogens had greater mycelial growth at 22°C. The isolates of *Trichoderma* grew at temperatures ranging from 12 to 37°C, with maximum growth at 27°C. The isolate IBLF 914 had mycoparasitism and reduced the disease in lettuce seedlings at temperatures ranging from 22 to 32°C. Since lettuce drop occurs when mild temperatures and high humidity prevail and the antagonist was more effective at higher temperatures, it is recommended that *Trichoderma* is applied in lettuce fields in Brazil also during warmer months of the year to reduce the inoculum remaining in the soil before planting the winter crop, which is more affected by the disease.

Keywords: lettuce drop, *Lactuca sativa*, *Trichoderma asperellum*, *Trichoderma asperelloides*, biological control.

RESUMO

Domingues, M.V.P.F.; Moura, K.E.; Salomão, D.; Elias, L.M.; Patrício, F.R.A. Efeito da temperatura sobre o crescimento micelial de *Trichoderma*, *Sclerotinia minor* e *S. sclerotiorum* e sobre o micoparasitismo. *Summa Phytopathologica*, v.42, n.3, p.222-227, 2016.

As condições ambientais são muito importantes para o controle biológico de doenças de plantas. Em um estudo prévio, isolados de *Trichoderma asperellum* (IBLF 897, IBLF 904 e IBLF 914) e *T. asperelloides* (IBLF 908) foram selecionados como antagonistas a *Sclerotinia minor* e *S. sclerotiorum*, agentes causais da murcha de esclerotínia, uma das mais importantes doenças da cultura da alface. Neste estudo subsequente o crescimento micelial destes isolados e dos patógenos foi avaliado em diferentes temperaturas, assim como o micoparasitismo do isolado IBLF 914. O crescimento micelial dos isolados de *T. asperellum* e *T. asperelloides*, bem como de *S. minor* e *S. sclerotiorum*, foi avaliado em temperaturas variando de 7 a 42 °C. O parasitismo de propágulos de *S. minor* e *S. sclerotiorum* pelo isolado IBLF 914, assim como o número de plântulas de alface sobreviventes ao tombamento, foram avaliados aos 12, 17,

22, 27 e 32 °C em caixas gerbox contendo substrato. *S. minor* e *S. sclerotiorum* apresentaram crescimento micelial nas temperaturas de 7 a 27 °C, mas não cresceram a 32 °C e ambos os patógenos apresentaram maior crescimento micelial a 22 °C. Os isolados de *Trichoderma* cresceram em temperaturas entre 12 e 37°C, com um máximo a 27°C. O isolado IBLF 914 exibiu micoparasitismo e reduziu a doença nas plântulas de alface em temperaturas entre 22 e 32°C. Como a murcha de esclerotínia ocorre quando predominam temperaturas amenas e elevada umidade e o antagonista foi mais efetivo em temperaturas médias a elevadas, sugere-se que *Trichoderma* seja aplicado em lavouras de alface no Brasil também nos meses mais quentes do ano visando a reduzir o inóculo presente no solo antes da instalação da cultura de inverno, mais afetada pela doença.

Palavras-chave: murcha de esclerotínia, *Lactuca sativa*, *Trichoderma asperellum*, *Trichoderma asperelloides*, controle biológico.

One of the most important diseases affecting lettuce is lettuce drop, caused by *Sclerotinia minor* and *S. sclerotiorum* (8, 9). This disease is favored by mild temperatures and high humidity (11). Managing lettuce drop is complex and involves integrating fungicide applications with several other control methods such as deep plowing, roughing, crop rotation and subsurface-drip irrigation (12).

Biological control is very important in lettuce crops because

lettuce has a very short cycle and is consumed fresh. Previous studies have reported diverse results: Knudsen et al. (4) and Chitrampalam et al. (1) controlled lettuce drop caused by *S. sclerotiorum* with isolates of *Trichoderma* spp. or *Coniocytrium minitans*, and Rabeendran et al. (9) controlled *S. minor* with *Trichoderma*. On the other hand, Chitrampalam et al. (1) were unable to control *S. minor* with antagonists. In a previous study carried out in Brazil, three isolates of

T. asperellum and one of *T. asperelloides* showed positive control of lettuce drop caused by *S. sclerotiorum* and *S. minor* under greenhouse conditions (2).

Environmental factors such as soil humidity and temperature can influence the mycoparasitic ability and biocontrol provided by antagonists (4, 7, 10). Partridge et al. (7) showed that the mycoparasitism of sclerotia of *S. minor* by *C. minitans* occurred at temperatures ranging from 14 to 22 °C but was suppressed at temperatures above 28 °C. On the other hand, *Trichoderma* tend to be favored by higher temperatures (3, 10). In the study of Santamarina and Rosselló (10), *T. harzianum* showed higher mycelial growth at 25 than at 15 °C. Hjeljord et al. (3) found that conidia of commercial products formulated with *T. harzianum* germinated in 40 to 62 hours at 25 °C but needed 129 to 182 hours to germinate at 12 °C. Similarly, the radial growth of these isolates was higher at 25 °C than at 12 °C.

Considering that biological control is influenced by environmental conditions, this study was carried out to evaluate the effect of temperature on the mycelial growth of three isolates of *T. asperellum* and one of *T. asperelloides* obtained in a previous study as antagonists of *S. minor* and *S. sclerotiorum*, causal agents of lettuce drop (2). The effect of temperature on the pathogens *S. minor* and *S. sclerotiorum* was also evaluated. The mycoparasitism of propagules of the pathogens by the isolate IBLF 914, as well as the disease reduction in lettuce seedlings, was evaluated at different temperatures. These studies were carried out to assess the environmental conditions most favorable for biocontrol of lettuce drop with *Trichoderma*.

MATERIALS AND METHODS

Isolates of *S. minor*, *S. sclerotiorum* and *Trichoderma*

The isolates of *S. minor* and *S. sclerotiorum* used in this study were obtained from lettuce plants from the municipality of Mogi das Cruzes, São Paulo State, Brazil. The isolates of *T. asperellum* (IBLF 897, IBLF 904 and IBLF 914) and *T. asperelloides* (IBLF 908) were selected as antagonists to *S. minor* and *S. sclerotiorum* in a previous study (2). The isolates of *S. minor* and *S. sclerotiorum* were grown in wheat grains and the isolates of *T. asperellum* and *T. asperelloides* in rice grains, using the methodology described by Elias et al. (2).

Mycelial growth of *S. sclerotiorum* and *S. minor* and isolates of *Trichoderma* at different temperatures

The rate of mycelial growth was measured in colonies grown in Petri dishes containing PDA at the temperatures of 7, 12, 17, 22, 27 and 32 °C for the isolates of *S. sclerotiorum* and *S. minor*, and at the same temperatures plus 37 and 42 °C for *Trichoderma*. A mycelial disc removed from the margin of the colonies of the pathogens and the antagonist was placed in the center of each Petri dish (9 cm diameter) containing PDA. Two days after the onset of the experiments, the average diameter of each colony was measured daily, until the colony reached the edge of the Petri dish.

Mycoparasitic activity of *Trichoderma* at different temperatures

The experiments of mycoparasitic activity were carried out by using a method adapted from Partridge et al. (7). Gerboxes were filled with 100 g of the commercial substrate Plantmax (Eucatex™) previously humidified with 10 mL of sterile distilled water. Before being used, the substrate was autoclaved for 60 minutes at 121 °C in two consecutive days. Twenty baits colonized with *S. minor* or *S. sclerotiorum*, prepared as previously described by Elias et al.

(2), were placed on the surface of the substrate. Twenty sclerotia of *S. minor* and 10 sclerotia of *S. sclerotiorum* were also placed over strips of sterile filter paper on the borders of each gerbox (Figures 4). The gerboxes were sprayed with a spore suspension of each isolate of *Trichoderma* containing 10^6 conidia.mL⁻¹ and placed in BODs with the temperature adjusted to 12, 17, 22, 27 and 32 °C. After ten days, *S. minor* or *S. sclerotiorum* baits were examined under a stereomicroscope and the baits colonized with *Trichoderma* were counted, as well as the baits containing mycelial growth of the pathogens, which were counted as viable. The sclerotia were removed from the gerboxes, washed in water, superficially sterilized with a 10% NaClO solution for 30 seconds, washed again in sterile distilled water, and placed in Petri dishes containing water-agar medium with 0.2% of a veterinary antibiotic (benzylpenicillin benzathine 350,000 UI g⁻¹, benzylpenicillin procaine 174,000 UI g⁻¹, benzylpenicillin potassium 174,000 UI g⁻¹, dihydrostreptomycin base 145 mg g⁻¹ and streptomycin base 145 mg g⁻¹). After the sclerotia were removed, 25 pre-germinated lettuce seedlings were placed in the gerboxes. The seeds were pre-germinated in Petri dishes containing two humidified filter papers, which were maintained for 48 hours in a BOD at 20 °C.

The Petri dishes containing the sclerotia were maintained for seven days in BODs at 20 °C. The sclerotia were examined using a stereoscopic microscope, and the sclerotia colonized with *Trichoderma* spp. were counted, as well as the sclerotia that germinated, which were counted as viable. The gerboxes that contained the lettuce seeds and the baits were maintained in BODs at 12, 17, 22, 27 and 32 °C, during four days, and the number of surviving seedlings was counted.

Experimental design and statistical analysis

The experiments of mycelial growth of *Trichoderma* isolates and the pathogens were carried out in a completely randomized design with four replicates per temperature, and each replicate was represented by a Petri dish. ANOVA of the data was performed and the means were compared according to Tukey's test at 5% probability.

The experiments of mycoparasitism were carried out in a completely randomized design with four replicates, each replicate represented by a gerbox. The data were subjected to ANOVA and the means were compared according to Tukey's test at 5% probability. A factorial analysis of variance was performed for the data of the surviving lettuce seedlings with *S. minor* or *S. sclerotiorum*. The media of the treatments were compared according to Tukey's test at 5% probability.

RESULTS

Rate of mycelial growth of the isolates of *Trichoderma*, *S. minor* and *S. sclerotiorum* at different temperatures

The mycelial growth of all isolates of *Trichoderma* was inhibited at the temperature of 7 °C, was proportional to the increase in the temperatures ranging from 12 to 27 °C, and decreased until 37 °C, being inhibited at 42 °C (Figure 1). For the isolates of *T. asperellum* (IBLF 897, IBLF 904 and IBLF 914) the maximum growth rate occurred at 27 °C, but for the isolate of *T. asperelloides* (IBLF 908) the maximum growth rate occurred at 32 °C (Figure 1).

The pathogens were able to grow at temperatures ranging from 7 to 32 °C for *S. sclerotiorum* or 27 °C for *S. minor* and showed maximum growth rate at 22 °C (Figure 2).

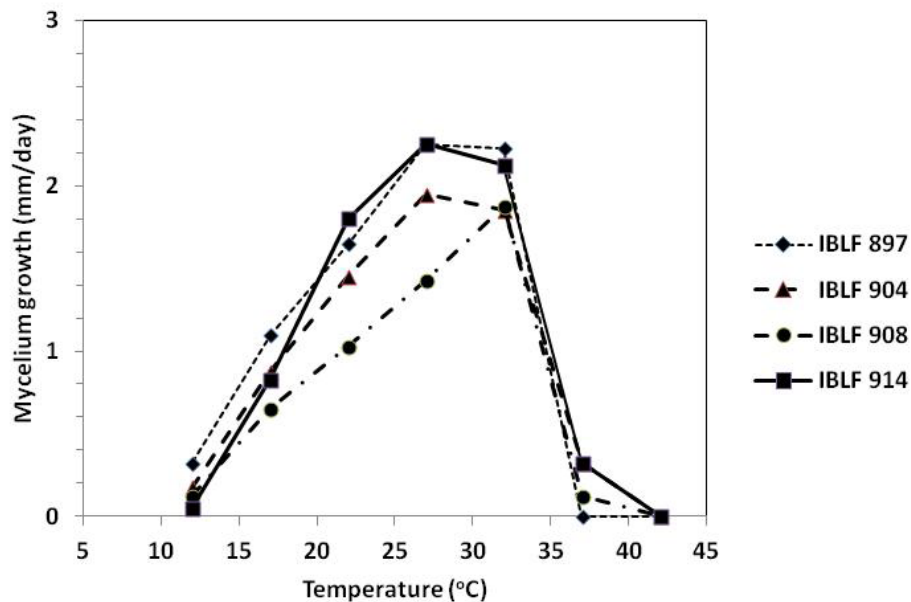


Figure 1. Rate of mycelial growth of isolates of *Trichoderma asperellum* (IBLF 897, IBLF 904, IBLF 914) and *T. asperelloides* (IBLF 908), at temperatures of 12, 17, 22, 27, 32, 37 and 42 °C.

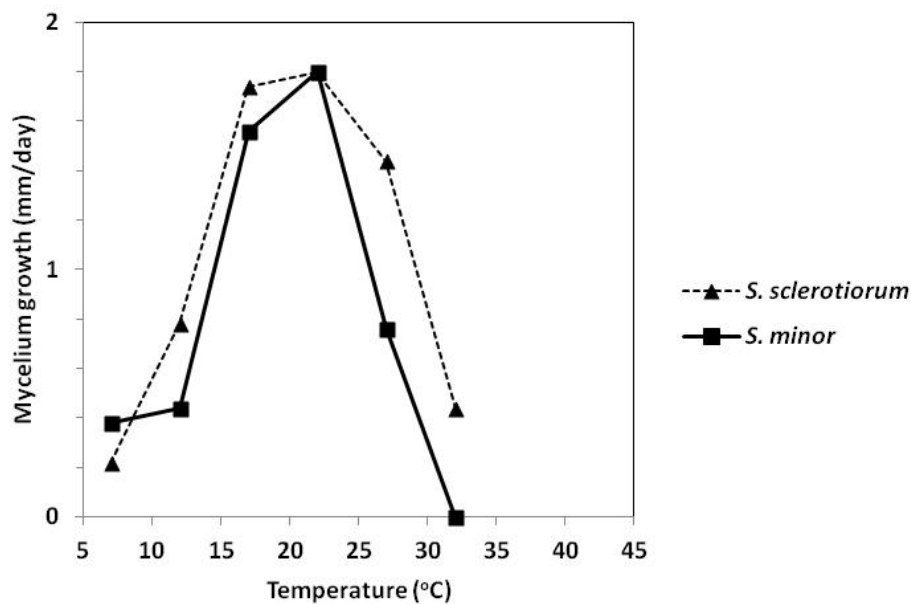


Figure 2. Rate of mycelial growth of *S. minor* and *S. sclerotiorum* at temperatures of 7, 12, 17, 22, 27 and 32 °C.

Mycoparasitic activity at different temperatures

The baits and sclerotia of *S. minor* were not mycoparasitized by the isolate IBLF 914 at the temperature of 12°C, but they were colonized by the antagonist and lost their viability at temperatures from 17 to 32°C. For the treatment without the antagonist, the baits were viable at all temperatures, except at 32°C (Table 1, Figure 3).

At 12°C the *T. asperellum* isolate did not parasitize the baits and sclerotia of *S. sclerotiorum*, but at 17°C, 61.2 % of the baits were mycoparasitized. At temperatures from 22 to 32°C, all baits and sclerotia were colonized by the antagonist and these temperatures are probably most suitable for biological control with this antagonist. For the control treatment, baits and sclerotia of *S. sclerotiorum* were viable at temperatures ranging from 12 to 27 °C, but no bait showed mycelial

growth at 32 °C. The sclerotia removed from the gerboxes kept at all different temperatures exhibited mycelial growth after being removed from the gerboxes and maintained at 20 °C (Table 2, Figure 4).

Survival of lettuce seedlings at different temperatures

At the temperature of 12 °C, parasitism of the baits of *S. minor* by *T. asperellum* isolate was not evident (Figure 4), but the viability of lettuce seedlings was higher for the *Trichoderma*-inoculated treatment than in the non-inoculated one at the same temperature (Table 3). At 32 °C, both inoculated and non-inoculated treatments showed the same number of lettuce seedlings (Table 3). Although the antagonist colonized the pathogen, *S. minor* was unable to reduce the viability of the lettuce seedlings because its mycelial growth was inhibited at

Table 1. Mycelial growth of *Sclerotinia minor* baits and sclerotia inoculated or not with the isolate IBLF 914 of *Trichoderma asperellum* and maintained at different temperatures.

Temperature (°C)	Parasitism of <i>S. minor</i> baits		<i>S. minor</i> baits with mycelial growth		<i>S. minor</i> sclerotia with mycelial growth	
	Inoculated with isolate IBLF 914	Inoculated with isolate IBLF 914	Non inoculated	Inoculated with isolate IBLF 914	Non inoculated	
12	0.0 b	100.0 a ¹	100.0 ²	100.0 a ¹	100.0 ²	
17	95.0 a	5.0 b	100.0	70.0 b	100.0	
22	96.2 a	3.8 b	100.0	12.5 c	100.0	
27	85.0 a	5.0 b	100.0	0.0 c	100.0	
32	100.0 a	0.0 b	0.0	0.0 c	100.0	

¹ Means followed by the same letter do not differ according to Tukey's test at 5% level.

² No variance.

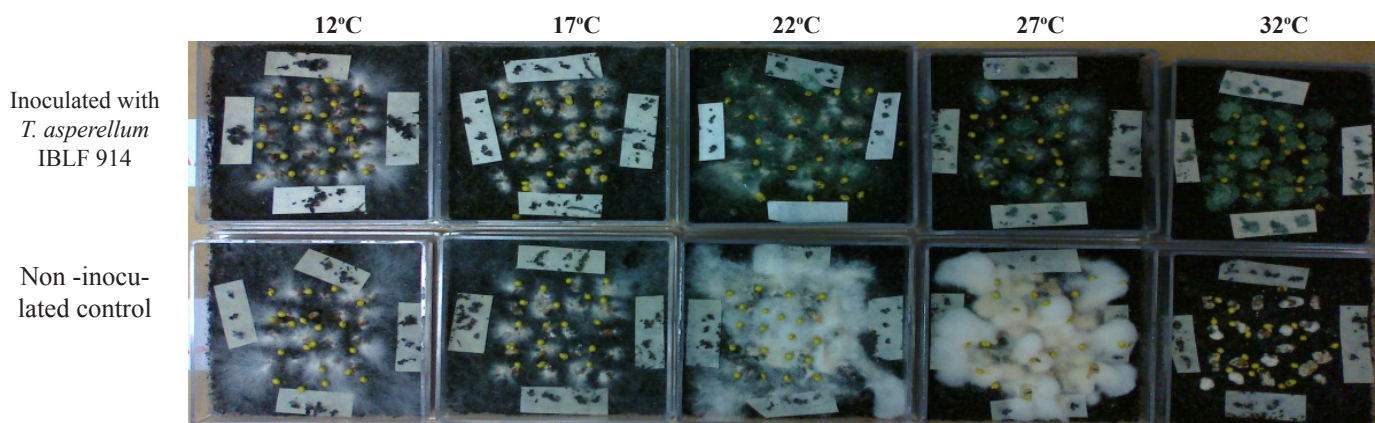


Figure 3. Viability of baits of *S. minor* inoculated or not with *T. asperellum* (IBLF 914), ten days after inoculation.

this temperature (Figure 3).

The same tendency was observed for treatments carried out with *S. sclerotiorum*, except at the temperatures of 27 and 32 °C, when this pathogen was unable to reduce the viability of seedlings (Table 3). The isolate of *T. asperellum* significantly reduced the disease in seedlings maintained at 17 and 22 °C (Table 3).

DISCUSSION

The mycelial growth of *S. minor* and *S. sclerotiorum* occurred at temperatures ranging from 12 to 27 °C, and the pathogens reduced the viability of lettuce seedlings at the same temperatures. Similarly, Imolehin et al. (5) showed that sclerotia of *S. minor* germinated and exhibited mycelial growth at temperatures ranging from 6 to 30 °C with optimum growth at 18 °C and infection of lettuce plants occurred

at temperatures ranging from 6 to 24 °C. For *S. sclerotiorum*, Young et al. (14) verified that ascospores could cause bottom rot in lettuce at temperatures ranging from 8 to 27 °C, but the disease occurred more rapidly and was more severe at temperatures ranging from 16 to 27°C, peaking at 22 °C.

The mycelial growth shown herein by the isolates of *Trichoderma* was proportional to the increase in temperature when the latter ranged from 12 to 27 °C for *T. asperellum* isolates and from 12 to 32 °C for *T. asperelloides* isolate. Jackson et al. (6) observed similar results using isolates of *T. viride* and *T. pseudokoningii*, which showed mycelial growth at temperatures ranging from 10 to 30 °C, with maximum growth at 25 °C. In another study, Santamarina & Roselló (10) found that the mycelial growth of a *T. harzianum* strain was higher at 25 than at 15 °C.

The results of this study showed that the *T. asperellum* isolate was not effective at 12°C but was favored by temperatures varying

Table 2. Viability of *S. sclerotiorum* baits and sclerotia inoculated or not with the isolate of *Trichoderma asperellum* (IBLF 914) and maintained at different temperatures.

Temperature (°C)	Parasitism of <i>S. sclerotiorum</i> baits		<i>S. sclerotiorum</i> baits with mycelial growth		<i>S. sclerotiorum</i> sclerotia with mycelial growth	
	Inoculated with isolate IBLF 914	Inoculated with isolate IBLF 914	Non inoculated	Inoculated with isolate IBLF 914	Non inoculated	
12	0.0 b	100.0 a ¹	100.0 ²	17.5 a ¹	100.0 ²	
17	61.2 a	43.7 b	100.0	0.0 b	100.0	
22	97.5 a	6.2 c	100.0	5.0 b	100.0	
27	92.5 a	2.5 c	100.0	0.0 b	100.0	
32	100.0 a	0.0 c	0.0	0.0 b	100.0	

¹ Means followed by the same letter do not differ at 5% level according to Tukey's test

² No variance

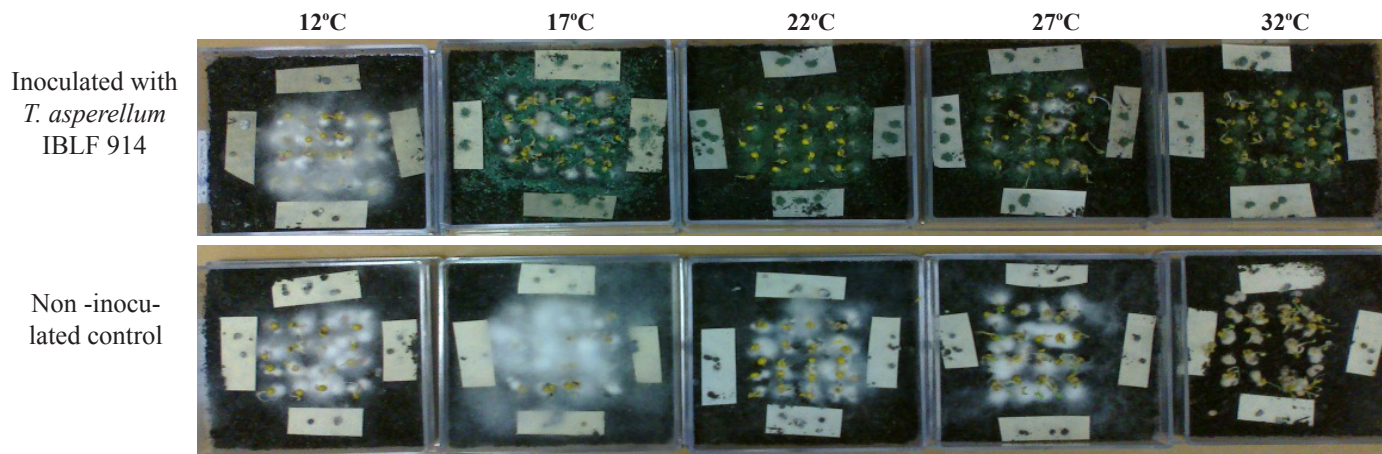


Figure 4. Viability of baits of *Sclerotinia sclerotiorum* inoculated or not with *T. asperellum* (IBLF 914), ten days after inoculation

Table 3. Percentage of surviving lettuce seedlings in gerboxes containing baits of *Sclerotinia minor* or *S. sclerotiorum* inoculated or not with *T. asperellum* (IBLF 914).

Temperature (°C)	Percentage of surviving lettuce seedlings			
	<i>Sclerotinia minor</i>		<i>S. sclerotiorum</i>	
	<i>T. asperellum</i> (IBLF 914)	Control	<i>T. asperellum</i> (IBLF 914)	Control
12	46.2 c A ¹	5.0 c B	20.0 c A	27.5 b A
17	68.8 b A	31.2 b B	55.0 b A	26.3 b B
22	63.8 bc A	23.8 bc B	76.2 ab A	30.0 b B
27	91.3 a A	15.0 bc B	77.5 ab A	82.5 a A
32	93.8 a A	83.8 a A	90.0 a A	83.8 a A

¹ Means followed by the same letter do not differ according to Tukey's test at 5% probability.

² Capital letters compare means between lines and lowercase letters compare means in the rows.

from 17 to 32°C. Similarly, Trutmann and Keane (13) verified that conidia of an isolate of *T. konigii* germinated and were able to mycoparasitize sclerotia of *S. sclerotiorum* at temperatures ranging from 7 to 35°C, but the optimum temperature varied from 15 and 30°C for germination and from 20 to 35 °C for sclerotial infection. On the other hand, Partridge et al. (7) observed that the mycoparasitism of sclerotia of *S. minor* by *C. minitans* occurred at temperatures ranging from 14 to 22 °C and that only a small percentage of sclerotia were parasitized by the antagonist at temperatures above 28 °C.

The present study and that of Trutmann and Keane (13) with an isolate of *T. konigii* showed that species of *Trichoderma* tend to grow and parasitize sclerotia at temperatures higher than those favorable for the occurrence of lettuce drop. In Brazil, commercial products formulated with *Trichoderma* have been used in combination with other methods for controlling white mold, caused by *S. sclerotiorum*, in soybeans and beans, but producers usually apply the isolates during warmer months of the year, generally before planting, when conditions are more favorable to the mycoparasitism of sclerotia, and this system could be adapted for the management of lettuce drop. In light of these findings, the antagonist could be applied in lettuce crop during warmer months of the year, aiming to reduce the viability of the sclerotia present in the soil or in lettuce debris before the winter crop, which is most affected by lettuce drop in Brazil, although the disease may occur whenever favorable conditions prevail.

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