Compatibility of *Trichoderma* isolates with pesticides used in lettuce crop

Micaele Aparecida Franco da Silva[1], Karina Elaine de Moura[1], Kamila Ellen de Moura[1], Denise Salomão[1] and Flávia Rodrigues Alves Patricio[1]

[1]Centro Experimental Central do Instituto Biológico, Instituto Biológico, Caixa Postal 70, CEP 13012-970, Campinas, SP, Brazil.
Autor para correspondência: Flávia Rodrigues Alves Patrício e-mail: flavia@biologico.sp.gov.br
Data de chegada: 10/03/2017. Aceito para publicação em: 07/06/2017.

10.1590/0100-5405/176873

ABSTRACT

Silva, M.A.F; Moura, K.E.; Moura, K.E.; Salomão, D.; Patricio, F.R.A. Compatibility of *Trichoderma* isolates with pesticides used in lettuce crop. ***Summa Phytopathologica,*** v.44, n.2, p.137-142, 2018.

Lettuce drop, caused by *Sclerotinia minor* and *S. sclerotiorum,* is one of the most important diseases that affect lettuce crop in Brazil. In previous studies, isolates of *Trichoderma asperellum* (IBLF 897, IBLF 904 and IBLF 914) and *T. asperelloides* (IBLF 908) were selected for the biocontrol of this disease. In this subsequent study, the compatibility of these isolates with pesticides used in lettuce crop in Brazil was evaluated. Initially, the mycelial growth of isolates was evaluated in culture medium plus pesticides. Then, the effect of pesticides on the parasitism of *T. asperelloides* isolate IBLF 914 in baits and sclerotia of *S. minor* and *S. sclerotiorum,* as well as on the survival of lettuce seedlings, was evaluated in gerboxes after application on baits and sclerotia of the antagonist with pesticides at their respective commercial doses. The fungicides pencycuron and mandipropamid and the insecticide imidacloprid did not affect the mycelial growth of *Trichoderma* isolates. The fungicide iprodione did not affect the mycelial growth of *T. asperellum* isolates, but the isolate of *T. asperelloides* was sensitive from the concentration of 10 $\mu g.L^{-1}$ fungicide. Procymidone reduced the mycelial growth of *Trichoderma* isolates from the concentration of 10 $\mu g.L^{-1}$ fungicide, and azoxystrobin reduced the conidial germination of the isolates of the antagonist, showing LD_{50} between 0.36 and 0.42 $\mu g.L^{-1}$ fungicide. On the other hand, in the experiment carried out in "gerboxes", none of the pesticides reduced the parasitism of baits and sclerotia or reduced the control of *S. minor* and *S. sclerotiorum* in lettuce seedlings. Results indicate that the biological control of lettuce drop with *T. asperelum* isolate IBLF 914 can be compatible with the remaining phytosanitary treatments used in lettuce crop.

Keywords: *Sclerotinia minor* , *Sclerotinia sclerotiorum, Trichoderma asperellum, Trichoderma asperelloides*, *Lactuca sativa,* controle biológico, controle químico.

RESUMO

Silva, M.A.F; Moura, K.E.; Moura, K.E.; Salomão, D.; Patricio, F.R.A. Compatibilidade de isolados de *Trichoderma* com pesticidas utilizados na cultura da alface. ***Summa Phytopathologica,*** v.44, n.2, p.137-142, 2018.

A murcha de esclerotinia, causada por *Sclerotinia minor* e *S. sclerotiorum,* é uma das mais importantes doenças que afetam a cultura da alface no Brasil. Em estudos anteriores os isolados de *Trichoderma asperellum* (IBLF 897, IBLF 904 and IBLF 914) e de *T. asperelloides* (IBLF 908), foram selecionados para o controle biológico dessa doença. Neste estudo subsequente foi avaliada a compatibilidade desses isolados com pesticidas utilizados na cultura da alface no Brasil. Inicialmente o crescimento micelial dos isolados foi avaliado em meio de cultura acrescido com os pesticidas. Em seguida, o efeito dos pesticidas sobre o parasitismo do isolado de *T. asperellum* IBLF 914 em iscas e escleródios de *S. minor* e *S. sclerotiorum* e a sobrevivência de plântulas de alface foi avaliado em caixas tipo gerbox, após aplicação sobre a iscas e escleródios do antagonista e dos pesticidas em suas respectivas doses comerciais. Os fungicidas pencicuron, mandipropamid e o insecticida imidacloprida não afetaram o crescimento micelial dos isolados de *Trichoderma.* O fungicida iprodione não afetou o crescimento micelial dos isolados de *T. asperellum,* mas o isolado de *T. asperelloides* foi sensível a partir da concentração de 10 $\mu g.L^{-1}$ do fungicida. Procimidone reduziu o crescimento micelial dos isolados de *Trichoderma* a partir da concentração de 10 $\mu g.L^{-1}$ de fungicida, e axozistrobina reduziu a germinação dos conídios dos isolados do antagonista, apresentando DLs 50 entre 0,36 e 0,42 $\mu g.L^{-1}$ de fungicida. Por outro lado, no experimento realizado em caixas tipo gerbox, nenhum pesticida reduziu o parasitismo de escleródios e iscas, bem como o controle de *S. minor* e *S. sclerotiorum* em plântulas de alface. Os resultados indicam que o controle biológico da murcha de esclerotínia com o isolado de *T. asperelum* IBLF 914 pode ser compatível com os demais tratamentos fitossanitários realizados na cultura da alface.

Palavras-chave: *Sclerotinia minor*, *Sclerotinia sclerotiorum, Trichoderma asperellum, Trichoderma asperelloides*, *Lactuca sativa,* biological control, chemical control.

Lettuce drop, caused by *Sclerotinia sclerotiorum* and *S. minor,* is considered one of the most important diseases that affect this crop in Brazil (21); it is also widespread worldwide, causing considerable losses in lettuce crops (22).

This disease causes wilting and collapse of lettuce heads in infested fields where weather conditions favorable for its development, such as mild temperatures and high humidity, prevail (13, 28). The main difference between *S. sclerotiorum* and *S. minor* is that the latter forms a white mycelium with several small black sclerotia (0.5 to 3 mm diameter), while *S. sclerotiorum* forms fewer and larger sclerotia, irregular in size, on the lettuce heads (28).

The management of lettuce drop is usually obtained by integrating

fungicide applications with other control methods such as deep plowing, roughing, crop rotation and subsurface-drip irrigation (28). Bioproduct formulations containing *Trichoderma* or *Coniothyrium* have shown potential to control lettuce drop (16, 22) and, in a recent study, isolates of *Trichoderma asperellum* and *T. asperelloides* were selected for the biocontrol of *S. sclerotiorum* and *S. minor* (11).

Although Brazilian lettuce producers are willing to reduce the use of pesticides in their crops, fungicides and insecticides cannot be discarded from the conventional cultivation of lettuce due to several pests and diseases that affect this culture, for which biological alternatives are not available or not totally effective yet. Diseases such as lettuce bottom rot, caused by *Rhizoctonia solani;* mildew, caused by *Bremia lactucae,* and septoria leaf spot*,* caused by *Septoria lactucae,* as well as pests such as aphids and whitefly, can reduce the production or quality of the harvested lettuce (8, 21).

Therefore, the antagonist developed to be used in lettuce crops should be compatible with pesticides sometimes adopted to reduce damages caused by other phytosanitary problems in the lettuce crop. Previous studies obtained diverse results concerning the effect of pesticides on the viability and efficacy of biological control agents. Saxena et al. (24) observed in *in vitro* experiments that an isolate of *T. harzianum* was very sensitive to the fungicides benomyl, thiophanate methyl, triadimefon and iprodione, less sensitive to copper hydroxide and mancozeb, and quite insensitive to the insecticides monocrotophos, dichlorvos, profenophos and triazophos. While studying the biological control of lettuce drop, Chitrampalam et al. (7) verified, under field conditions, that isolates of *T. harzianum* were tolerant to the fungicides iprodione, dicloran and vinclozolin, although the antagonists were not effective. Aguiar et al. (3) did not observe any interference of the fungicides fluazinam and procymidone in the performance of *Trichoderma* sp. applied through the irrigation system for the control of white mold, caused by *S. sclerotiorum,* in a tomato crop.

In a study carried out with a similar antagonist, *C. minitans,* Partridge et al. (19) observed that conidia and mycelia were sensitive to some pesticides used in peanut crop, such as azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole and diclosulfan, and the antagonist showed lower mycoparasitic activity in the presence of these fungicides. On the other hand, in the study conducted by Budge & Whipps (6), three isolates of *C. minitans*, antagonist of *S. sclerotiorum* in lettuce, were very sensitive to the fungicide iprodione, moderately sensitive to thiram, less sensitive to metalaxyl + thiram, tolclofos-methyl and zineb, and not sensitive to four insecticides, but these pesticides did not interfere in the parasitism of the antagonist when tested in the soil of a lettuce crop inside a greenhouse.

The effective use of biological control agents in conventional lettuce producing areas requires knowing their compatibility with the most common pesticides applied in this crop. Therefore, this study was carried out to evaluate the effect of fungicides used to control lettuce drop, bottom rot, mildew and septoria leaf spot, as well as the effect of the insecticide imidacloprid, used for the management of aphids and whitefly, on the mycelial growth and mycoparasitism of *Trichoderma* isolates previously selected for the biocontrol of lettuce drop.

MATERIAL AND METHODS

The experiments were conducted from 2011 to 2014 in the "Laboratório de Fitopatologia" of "Instituto Biológico", in Campinas, São Paulo State (SP), Brazil.

Isolates of *Trichoderma, S. minor* and *S. sclerotiorum:* the isolates of *T. asperellum* (IBLF897, IBLF904 and IBLF914) and *T. asperelloides* (IBLF908) used in this study were previously selected for the biocontrol of lettuce drop (11). Before being used in all experiments, *Trichoderma* isolates were multiplied in rice grains that were previously sterilized (200 ml rice grains plus 100 ml distilled water placed in plastic bags and autoclaved at 121°C for 60 minutes on two consecutive days). Twenty mycelial discs from the edge of colonies of the isolates of *Trichoderma* spp. were added to one bag each, and bags were maintained for 10 days at room temperature.

The isolates of *S. minor* and *S. sclerotiorum* were obtained from lettuce plants cultivated in the region of Mogi das Cruzes, SP. Before being used in the experiments, the pathogens were multiplied in wheat grains according to the methodology described by Elias et al. (11).

Fungicides and insecticide used in the experiment: the tested fungicides were registered for lettuce crop by the Brazilian Ministry of Agriculture - AGROFIT (2). The fungicides Rovral SC ™ FMC QUÍMICA DO BRASIL LTDA, formulated with 50% iprodione, and Sialex 500™ SUMITOMO CHEMICAL DO BRASIL REPRES. LTDA, formulated with 50% procymidone, are registered for the control of lettuce drop. Monceren PM ™ BAYER S.A., formulated with 50% pencycuron, is used to control bottom rot, caused by *Rhizoctonia solani,* Amistar 500 WG ™ SYNGENTA PROTEÇÃO DE CULTIVOS LTDA., formulated with 50% azoxystrobin, is employed to control septoria leaf spot, caused by *Septoria lactucae*, and Revus™ SYNGENTA PROTEÇÃO DE CULTIVOS LTDA., formulated with 25% mandipropamid, is used to control downy mildew of lettuce, caused by *Bremia lactucae.* The insecticide Evidence 700 WG™ BAYER S.A., formulated with 48% imidacloprid, is usually applied to control whitefly (*Bemisia tabaci* race B) and lettuce aphid (*Dactinochus sonchi*).

Mycelial growth and conidial germination: suspensions of the fungicides and insecticide were prepared in sterile distilled water and added to PDA at concentrations adjusted to 0.1, 1, 10 and 100 µg active ingredient per mL medium. The media added of the fungicides and insecticide were placed in 9-cm Petri dishes and each Petri dish received a 7-mm mycelial disc collected from the borders of 7-day-old colonies of each *Trichoderma* isolate. After four days of incubation at 25°C the radial growth of colonies was measured in two perpendicular axes.

The sensitivity of *Trichoderma* isolates to the fungicide azoxystrobin was evaluated based on conidial germination. Once the stock solutions were prepared, azoxystrobin was added to water agar medium at the concentrations of 0.01, 0.1, 1 and 10 µg mL^{-1} active ingredient. The medium was placed onto microplates containing 24 cells. Four cells were filled with each concentration of the fungicide. Suspensions containing 5 x 10^5 conidia of each *Trichoderma* isolate mL^{-1} were prepared and 30 µL were distributed onto the surface of each cell of the microplates. The latter were maintained for 24 hours at 25ºC in the dark. Conidial germination was assessed by counting the number of germinated conidia under a compound microscope. Conidia were considered germinated when the length of the germ tube was equal to or greater than that of conidia.

Effect of pesticides on mycoparasitic activity of *T. asperelloides* IBLF914: the experiments in this step were carried out according to a method adapted from Partridge et al. (19) and Domingues et al. (9). Gerboxes were filled with 100 g commercial substrate Plantmax (Eucatex™), autoclaved for two consecutive days (60 minutes at 121ºC) and humidified with 10 ml sterile distilled water. Twenty wheat grains colonized with *S. minor* or *S. sclerotiorum* were placed onto the surface

of the substrate. Twenty sclerotia of *S. minor* and 10 sclerotia of *S. sclerotiorum* were also placed onto strips of sterile filter paper at the borders of each gerbox. The gerboxes were placed on a surface and sprayed with a spore suspension of each *Trichoderma* isolate containing 10^6 conidia ml^{-1}. Four hours later, the gerboxes were sprayed with the fungicides and the insecticide. The pesticides were prepared at doses equivalent to those used under field conditions: iprodione - 1.5ml commercial product (c.p.) per liter of water; procymidone - 1.0 g c.p. per liter of water, pencycuron - 4.0 ml c.p. per liter of water, mandipropamid - 4.0 ml c.p. per liter of water, azoxystrobin - 1.26 g c.p. per liter of water and imidacloprid - 0.6 ml c.p. per liter of water. The gerboxes remained for 6 days in BODs where the temperature was adjusted to 27°C; then, *S. minor* or *S. sclerotiorum* baits were examined under a stereomicroscope and the baits colonized with the antagonist were counted as parasitized. On the same day, all sclerotia were taken from each Petri dish, washed in sterilized 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 IU g^{-1}, benzylpenicillin procaine 174,000 IU g^{-1}, benzylpenicillin potassium 174,000 IU g^{-1}, dihydrostreptomycin base 145 mg g^{-1} and streptomycin base 145 mg g^{-1}). On the same day, 25 pre-germinated lettuce seedlings were evenly scattered over the bait-added substrate of each recipient. The seeds were pre-germinated after being placed in Petri dishes containing two humidified filter papers that were kept for 48 hours in BODs at 20ºC. The gerboxes remained in BODs adjusted to 27°C during four days, when the number of viable seedlings was counted. After one week in BODs at 20ºC, the Petri dishes containing the sclerotia were examined under a stereoscopic microscope, and the sclerotia colonized with the isolate of *T. asperelloides* were counted as parasitized.

Data analysis: experiments of the mycelial growth of the antagonists in media added of pesticide were carried out in a completely randomized design, with four replicates per isolate, and each replicate was represented by a Petri dish. Data were subjected to analysis of variance and the media were compared according to Tukey's test at 5% probability. The experiment of conidial germination in the presence of azoxystrobin was carried out in a completely randomized design, with four replicates, and each replicate was represented by a cell in the microplate. The experiments in gerboxes were carried out in a completely randomized design with four replicates, and each replicate was represented by a gerbox. The experiment with sclerotia was carried out in a completely randomized design with four replicates, and each replicate was represented by a Petri dish, which contained the sclerotia from each gerbox. Data of all experiments were subjected to analysis of variance and the media were compared according to Tukey's test at 5% probability.

RESULTS

Mycelial growth and conidial germination of *Trichoderma* isolates*:* the mycelial growth of the isolates of *T. asperellum* (IBLF897, IBLF904 and IBLF914) and *T. viridae* (IBLF908) was not reduced in the presence of the fungicides pencycuron and mandipropamid and the insecticide imidacloprid at the concentrations of 0.1, 1, 10 and 100 µg active ingredient per liter of medium (Table 1). The mycelial growth of the isolates of *T. asperellum* was not inhibited in PDA added of the fungicide iprodione (Table 1), but the isolate of *T. asperelloides*

Table 1. Mycelial growth of four isolates of *Trichoderma* spp. in PDA added of fungicides at different concentrations

Isolates	Fungicides	Colony diameter for fungicide concentration				
		0	0.1	1	10	100
IBLF897	Pencycuron	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Mandipropamid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Iprodione	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Procymidone	8.5 a[1]	8.5 a	8.0 a	0.9 b	0.8 b
	Imidacroprid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
IBLF904	Pencycuron	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Mandipropamid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Iprodione	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Procymidone	8.5 a	8.5 a	5.6 b	1.2 c	1.05 c
	Imidacroprid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
IBLF908	Pencycuron	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Mandipropamid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Iprodione	8.5 a	8.5 a	8.4 a	1.4 b	0.0 c
	Procymidone	8.5 a	8.5 a	6.0 b	1.3 c	1.0 c
	Imidacroprid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
IBLF914	Pencycuron	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Mandipropamid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Iprodione	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a
	Procymidone	8.5 a	8.5 a	7.9 a	1.3 b	0.9 b
	Imidacloprid	8.5 a	8.5 a	8.5 a	8.5 a	8.5 a

(IBLF908) was sensitive to this fungicide at the concentration of 10 µg.L^{-1} and its growth was completely inhibited at 100 µg.L^{-1} iprodione in the medium (Table 1).

The conidia of isolates IBLF897, IBLF904, IBLF908 and IBLF91 were sensitive to azoxystrobin and no germination was observed at the concentration of 10 µg.L^{-1} fungicide (Table 2). The isolates showed similar LD_{50} values, varying from 0.42 to 0.36 µg.L^{-1} azoxystrobin (Table 3).

Effect of pesticides on mycoparasitic activity of *T. asperelloides* IBLF914: In the experiment carried out in "gerboxes", the baits

Table 2. Conidial germination of four *Trichoderma* isolates at different concentrations of the fungicide azoxystrobin.

Axozystrobin	***Trichoderma* isolates**			
Doses µg. L^{-1}	**IBLF897**	**IBLF904**	**IBLF908**	**IBLF914**
0	78.58 a[1]	70.39 a[1]	86.99 a[1]	93.17 a[1]
0.01	73.91 a	67.85 a	88.26 a	81.82 a
0.10	77.61 a	60.05 a	78.99 a	84.36 a
1.00	17.82 b	19.49 b	23.65 b	31.34 b
10.00	0.00 b	0.00 b	0.00 c	0.00 c

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

Table 3. Lethal doses for 50% (LD 50%) reduction in conidial germination of *Trichoderma* in the presence of the fungicide azoxystrobin; equation used to calculate the LD 50% and the R^2 of the equation.

Isolates	**LD 50**	**Equation**	**R^2**
IBLF897	0.405787	y=-15.56ln(x) + 35.966	0.8550
IBLF904	0.369569	y=-15.06ln(x) + 35.009	0.9433
IBLF908	0.420448	y=-15.79ln(x)+36.319	0.9306
IBLF914	0.392384	y=-13.91ln(x)+36.987	0.8839

Table 4. Percentage of *Sclerotinia sclerotiorum* baits and sclerotia parasitized with *T. asperellum* IBLF914 isolate, and percentage of surviving lettuce seedlings.

Treatments	**Baits parasitized with *T. asperellum* (%)**	**Sclerotia parasitized with *T. asperellum* (%)**	**Surviving lettuce seedlings (%)**
Sclerotinia sclerotiorum	0.0 d[1]	0.0 c[1]	12.5 c
S. sclerotiorum + *T. asperellum* (IBLF 914)	38.7 c	92.5 a	60,0 b
S. sclerotiorum + Pencycuron + *T. asperellum* (IBLF 914)	45.0 c	75.0 b	82.5 b
S. sclerotiorum + Mandipropamid + *T. asperellum* (IBLF 914)	70.0 b	90.0 a	72.5 b
S. sclerotiorum + Azoxystrobin + *T. asperellum* (IBLF 914)	70.0 b	85.0 a	75.0 b
S. sclerotiorum + Iprodione + *T. asperellum* (IBLF 914)	88.7 a	50.0 b	97.5 a
S. sclerotiorum + Procymidone + *T. asperellum* (IBLF 914)	88.7 a	100.0 a	100.0 a
S. sclerotiorum + Imidacloprid + *T. asperellum* (IBLF 914)	91.2 a	100.0 a	70.0 b

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

Table 5. Percentage of *Sclerotinia minor* baits and sclerotia parasitized with *T. asperellum* IBLF914 isolate, and percentage of surviving lettuce seedlings.

Treatments	**Baits parasitized with *T. asperellum* (%)**	**Sclerotia parasitized with *T. asperellum* (%)**	**Surviving lettuce seedlings (%)**
Sclerotinia minor	0.0 b[1]	0.0 d[1]	67.5 b
S. minor + *T. asperellum* (IBLF 914)	63.7 a	25.9 c	82.5 a
S. minor + Pencycuron + *T. asperellum* (IBLF 914)	100.0 a	58.6 b	89.4 a
S. minor + Mandipropamid + *T. asperellum* (IBLF 914)	82.5 a	79.2 a	92.5 a
S. minor + Azoxistrobin + *T. asperellum* (IBLF 914)	82.5 a	65.9 b	85.0 a
S. minor + Iprodione + *T. asperellum* (IBLF 914)	70.0 a	12.8 d	90.0 a
S. minor + Procymidone + *T. asperellum* (IBLF 914)	81.2 a	92.5 a	90.0 a
S. minor + Imidacloprid + *T. asperellum* (IBLF 914)	93.7 a	39.8 c	95.7 a

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

colonized with *S. sclerotiorum* were parasitized by *T. asperellum* isolate IBLF914 at levels varying from 38.7 to 91.2%. Treatments with the pesticides did not reduce the number of parasitized baits, and treatments with iprodione or procymidone showed a larger number of parasitized baits than the other treatments. The pesticides did not reduce the parasitism of sclerotia, but treatments with iprodione or pencycuron showed a smaller number of parasitized sclerotia than the remaining treatments (Table 4). All treatments showed a larger number of lettuce seedlings than *S. sclerotiorum* control, but treatments with iprodione or procymidone showed a greater number of lettuce seedlings than the remaining treatments (Table 5).

All treatments showed a larger number of *S. minor* baits parasitized with *T. asperellum* IBLF914 than the control and did not differ among themselves. Treatments with procymidone or mandipropamid + *T. asperellum* IBLF914 showed a larger number of parasitized sclerotia, but treatments with the antagonist only or imidacloprid + *T. asperellum* IBLF914 showed a smaller number of parasitized sclerotia. All treatments showed a larger number of surviving lettuce seedlings than the control and did not differ among themselves (Table 5).

DISCUSSION

The isolates of *T. asperellum* and *T. asperelloides* tested in this study showed no reduction in their mycelial growth in medium added of pencycuron. Treatments with this fungicide and the antagonist resulted in a number of seedlings similar to that of the treatment with *Trichoderma* alone, which was larger than that of *S. sclerotiorum* or *S. minor* controls, showing that the fungicide did not reduce the effect of the antagonist. In a previous study, pencycuron did not inhibit the conidial germination of wild type and mutant isolates of *Trichoderma* (15). Pencycuron has been described as a nonsystemic phenyl urea fungicide with specific action against diseases caused by *R. solani* and *Pellicularia* spp. in several horticulture plants (12); it is used for the control of lettuce bottom rot, caused by *R. solani* in Brazil (2). Herein we showed that pencycuron may not affect the biological control of lettuce drop with *T. asperellum* IBLF914.

The fungicide mandipropamid showed no effect on the mycelial growth of *Trichoderma* isolates and did not affect the parasitism of baits and sclerotia of both pathogens with *T. asperellum* isolate IBLF914, as well as the control of lettuce bottom rot in lettuce seedlings. Mandipropamid belongs to the FRAC group H5, cellulose synthase, from the group of carboxylic acid amides (12), and has specific antioomycete activity. Therefore, it is expected not to affect fungi like *Trichoderma,* as observed in this study. Mandipropamid is used to control lettuce downy mildew, caused by *Bremia lactucae* (2). This disease can occur under field conditions simultaneously with lettuce drop since *B. lactucae* sporulation is optimum at 15ºC and requires 80-100% RH to occur (27). Therefore, the control of both diseases is sometimes necessary, and this study showed that mandipropamid is compatible with *T. asperellum* IBLF914.

The fungicide azoxystrobin had no effect on the mycelial growth (data not shown) but reduced the conidial germination of the studied *Trichoderma* isolates. Similarly, Ranganathswamy et al. (23) observed that the mycelial growth of two isolates of *T. harzianum* and *T. viridae* was not affected by azoxystrobin, but Achana et al. (1) detected a small reduction in the mycelial growth of an isolate of *T. viride* in PDA added of 20 and 25 ppm of the fungicide. Azoxystrobin is a QoI (Quinone outside inhibitor) fungicide, toxic to several plant pathogens of the phylum of ascomycetes, basidiomycetes, deuteromycetes and oomycetes (4). Considering that this group of fungicides interferes in the respiration of fungal cells and that conidial germination is a high energy demanding process (4), azoxystrobin reduced the germination of *Trichoderma* conidia in this study, as expected. On the other hand, in the experiments carried out in gerboxes, azoxystrobin did not interfere in the parasitism by *T. asperellum* (IBLF914) of baits and sclerotia of both pathogens and did not reduce the number of surviving lettuce seedlings. Results showed that azoxystrobin could be used simultaneously with *T. asperellum* (IBLF914) in lettuce crop if applied for the control of septoria leaf spot caused by *Septoria lactucae* (2). This disease may occur at a wide range of temperatures, from 10 to 28ºC, optimum at 24 ºC, and is favored by high humidity (21), which may also be favorable conditions for lettuce drop.

Iprodione only affected the mycelial growth of the isolate of *T. asperelloides* (IBLF908), showing no effect on the parasitism of *S. minor* and *S. sclerotiorum* baits and increasing the number of lettuce seedlings in the treatment that combined *T. asperellum* and iprodione. In a previous study, Harmann et al. (14) showed that the efficiency of two *T. hamatum* isolates for the control of *Botrytis cinerea* in grapes was not reduced by the tank mixture of iprodione with the isolates, although they were sensitive to the fungicide in PDA plates added of iprodione. Similar results were obtained by Elad et al. (10) for the biological control of *B. cinerea* in grapes with a *T. harzianum* isolate that was compatible with iprodione and other fungicides, as well as by Budge & Whipps (6) with another antagonist, *Coniothyrium minitans*. In the study carried out by Budge & Whipps (6), although three isolates of *C. minitans* were sensitive to iprodione in agar plate tests, the fungicide did not interfere in the parasitism of sclerotia of *S. sclerotiorum* in trays containing soil or in trials carried out with lettuce in a glasshouse infested with the same pathogen.

Although in this study procymidone reduced the mycelial growth of *Trichoderma* isolates, Paula Jr. et al. (20) did not observe inhibition of the mycelial growth of isolates of *T. stromaticum* and *T. harzianum* in PDA added of procymidone and McLean et al. (17) did not observe spore germination inhibition of a *T. harzianum* isolate in the presence of procymidone. In the present study, there was no negative effect of procymidone on the parasitism of baits and sclerotia of *S. minor* and *S. sclerotiorum* or biocontrol of the pathogens in lettuce seedlings. Similarly, McLean et al. (18) did not observe any negative effect of procymidone on soil colonization by an isolate of *T. atroviride* and there was no interference of procymidone in the efficacy of a mixture of *T. harzianum* + *T. viride* in controlling *S. sclerotiorum* in a commercial production of tomato (3). Aguiar et al. (3) also observed that the combination of fungicides with biocontrol agents provided a higher level of control than the biological or the chemical treatments alone.

Based on this study, the fungicides iprodione and procymidone, used for the control of lettuce drop in Brazil (2), can be combined with *T. asperellum* IBLF914 for the management of this disease. These fungicides belong to the dicarboximides FRAC code group E3, and their main mode of action is in the osmotic signal transduction, specially MAP/Histidine-Kinase of the fungal cell (12). The loss of efficacy of dicarboximides, especially iprodione, by soil microorganisms is a well-documented phenomenon (26), and the combination of fungicides and biocontrol agents could reduce these side effects.

The insecticide imidacloprid did not inhibit the mycelial growth of *Trichoderma* isolates and did not interfere either in the parasitism of baits and sclerotia or in the control of both pathogens in lettuce seedlings. Singh et al. (25) did not observe any interference of imidacloprid in the mycelial growth of an isolate of *T. harzianum,* and Bindu Madhavi et al. (5) found that an isolate of *T. viridae* was not

affected by imidacloprid added to PDA medium.

Although some fungicides tested in this study, such as procymidone and azoxystrobin, showed some toxicity to the mycelial growth or conidial germination of the studied isolates of *Trichoderma,* all pesticides practically did not interfere in the parasitism of baits and sclerotia of both pathogens. *Trichoderma* species are extremely well adapted to soil or substrate conditions, can produce lytic enzymes, antibiotics and/or secondary metabolites and rapidly colonize the soil, roots and rhizosphere (29); these features may enhance their ability to tolerate pesticides. The isolates tested in this study showed a high capacity to colonize baits and sclerotia, despite the presence of pesticides, indicating that biological control with *T. asperellum* isolate IBLF 914 is compatible with pesticides used for other relevant phytosanitary problems of the lettuce crop.

REFERENCES

1. Achana, S.; Hubballi, M.T.; Ranjitham, T.P.; Prabakar, K.; Raguchander, T. Compatibility of Azoxystrobin 23SC with biocontrol agents and insecticides. **Madras Agricultura Journal,** Madras, v.99, n. 4/6, p.372-377, 2012.
2. Agrofit – Sistema de agrotóxicos fitossanitários. **Ministério da Agricultura, Pecuária e Abastecimento**. Brasília, 2016. Available at: <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Access in: 10 Jan. 2017.
3. Aguiar, R.A.; Cunha, M.G.; Lobo Junior, M. Management of white mold in processing tomatoes by *Trichoderma* spp. and chemical fungicides applied by drip irrigation. **Biological Control**, Atlanta, v.74, p.1-5, 2014.
4. Bartlett, D.W.; Clough, J.M.; Godwin, J.R.; Hall, A.A.; Hamer, M.; Parr-Dobrzanski, B. Review The strobilurin fungicides. **Pest Management Science**, Sussex, v.59, p.649-662, 2002.
5. Bindu Madhavi, G.; Bhattiprolu, S.L.; Bali Reddy, V. Compatibility of biocontrol agent *Trichoderma viride* with various pesticides **Journal of Horticultural Sciences**, **Hessaraghatta**, v.6, n.1, p.71-73, 2011.
6. Budge, S.P.; Whipps, J.M. Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. **Phytopathology,** St. Paul, v.91, p.221-227, 2001.
7. Chitrampalam, P.; Figuli, P.J., Matheron, M.E.; Subbrao, K.V., Pryor, B.M. Biocontrol of lettuce drop caused by *S. sclerotiorum* and *S. minor* in desert agroecossystems. **Plant Disease,** St. Paul, v.92, p.1625-1634, 2008.
8. Davis, R.; Subbarao, K.V.; Raid, R.N.; Kurtz, E.A. (Ed.). **Compendium of lettuce diseases.** St. Paul: American Phytopathological Society, 1997. 79p.
9. Domingues, M.V.P.F.; Moura, K.E.; Salomão, D.; Elias, L.M.; Patricio, F.R.A. Effect of temperature on mycelial growth of *Trichoderma, Sclerotinia minor* and *S. sclerotiorum,* as well as on mycoparasitism. **Summa Phytopathologica**, Botucatu, v.42, n.3, p.222-227, 2016.
10. Elad, Y. Biological control of grape grey mould by *Trichoderma harzianum.* **Crop Protection,** Guildford, v.13, n.1, p. 35-38, 1994.
11. Elias, L.M.; Domingues, M.V.P.F.; Moura, K.E.; Harakava, R.; Patricio, F.R.A. Selection of *Trichoderma* isolates for biological control of *Sclerotinia minor* and *S. sclerotiorum* in lettuce. **Summa Phytopathologica,** Botucatu**,** v.42, p.216-221, 2016.
12. FRAC Code List 2016: fungicides sorted by mode of action. **Fungicide Resistance Action Committee**. Brussels, 2016. Available at: <http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2016.pdf?sfvrsn=2>. Access in: 12 Jan. 2017.
13. Hao, J.J.; Subbarao, K.V. Comparative analyses of lettuce drop epidemics caused by *Sclerotinia minor* and *S. sclerotiorum*. **Plant Disease,** St. Paul, v.89 n.7, p.717-725, 2005.
14. Harmann, G.E.; Latorre, B.; Agosin, E.; San Martin, R.; Riegel, D.G.; Nielsen, P.A.; Tronsmo, A.; Pearson, R.C. Biological and integrated control of botrytis bunch rot of grape using Tichoderma spp. **Biological Control,** Atlanta, v.7, p.259-266, 1996.
15. Herrera, R.; Núñez, D.; Besoain, X.; Pérez, L.M.; Montealegre, J. Sensitivity of wild-type and mutant *Trichoderma harzianum* strains to fungicides. **Ciencia e Investigacion Agraria**, Santiago, v.39, n.3, p.569-576, 2012.
16. Knudsen, G.R.; Eschen, D.J. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum.* **Plant Disease,** St. Paul, v.75, n.5, p.466-470, 1991.
17. McLean, K.L.; Hunt, J.; Stewart, A.; Compatibility of the biocontrol agent *Trichoderma haarzianum* C52 with selected fungicides. **New Zealand Plant Protection**, Lincoln, v.54, p.84-88, 2001.
18. McLean, K.L.; Hunt, J.S.; Stewart, A.; Wite, Porter, I.J.D.; Villalta, O. Compatibility of a *Trichoderma atroviride* biocontrol agent with management practices of *Allium* crops. **Crop Protection**, Guildford, v.33, p.94-100, 2012.
19. Partridge, D.E.; Sutton, T.B.; Jordan, D.L. Effect of environmental factors and pesticides on mycoparasitism of *Sclerotinia minor* by *Coniothyrium minitans.* **Plant Disease,** St. Paul, v.90, p.1407-1412, 2006.
20. Paula Júnior, T.J.; Vieira, R.F.; Rocha, P.R.R.; Bernardes, A.; Costa, E.L.; Carneiro, J.E.S.; Vale, F.X.R.; Zambolim, L. White mold intensity on common bean in response to plant density, irrigation frequency, grass mulching, *Trichoderma* spp., and fungicide. **Summa Phytopathologica***,* Botucatu, v.35, n.1, p.44-48, 2009.
21. Pavan, M.A.; Krause-Sakate, R.; Kurosawa, C. Doenças da alface. In: Kimati, H.; Amorim, L.; Rezende, J.A.M.; Bergamin Filho, A.; Camargo, L.E.A. (Ed.). **Manual de fitopatologia:** doenças das plantas cultivadas. 4.ed. São Paulo: Ceres, 2005. v.2, p.27-33.
22. Rabeendran, N.; Jones, E.E.; Moot, D.J.; Stewart, A. Biocontrol of *Sclerotinia* lettuce drop by *Coniothyrium minitrans* and *Tricoderma hamatum.* **Biological Control,** Atlanta, v.39, p.352-362, 2006.
23. Ranganathswamy, M.; Patibanda, A.K.; Rao, G.N. Evaluation of toxicity of agrochemicals on *Trichoderma* isolates *in vitro***. Journal of Micropathological Research**, Calcuta, v.51, n.2, p.289-293, 2013.
24. Saxena, D.; Tewari, A.K.; Rai, D. The *in vitro* effect of some commonly used fungicides, insecticides and herbicides for their compatibility with *Trichoderma harzianum* PBT23. **World Applied Sciences Journal**, Dubai, v.31, n.4, p.444-448, 2014.
25. Singh, V.P.; Srivstata, S.; Shrivastata, S.K.; Singh, H.B. Compatibility of different inseticides with *Trichoderma harzianum* under *in vitro* condition. **Plant Pathology Journal,** Seoul, v.11, p.73-76, 2012.
26. Slade, E.A.; Fullerton, R.A.; Stewart, A.; Young, H. Degradation of the Dicarboximide Fungicides Iprodione, Vinclozolin and Procymidone in Patungmahoe Clay Loam Soil, New Zealand. **Journal of Pesticide Science**, Tokyo, v.35, p.95-100, 1992.
27. Su, H.; van Bruggen, A.H.C.; Subbarao, K.V.; Scherm, H. Sporulation of *Bremia lactucae* affected by temperature, relative humidity, and wind in controlled conditions. **Phytopathology**, St. Paul, v.94, p.396-401, 2004.
28. Subbarao, K.V. Progress toward integrated management of lettuce drop. **Plant Disease,** St. Paul, v.82, n.10, p.1068-1078, 1998.
29. Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Marra, R.; Woo, S.L.; Lorito, M. *Trichoderma*-plant-pathogen interactions. **Soil Biology & Biochemistry***,* Amsterdam, v.40, p.1-10, 2008.