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Effect of *Trichoderma* spp. isolates for biological control of tan spot of wheat caused by *Pyrenophora tritici-repentis* under field conditions in Argentina

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Abstract *Trichoderma* spp. have been used as biocontrol agents to protect plants against foliar diseases in several crops, but information from field assays is scarce. In the present work, experiments were carried out to determine the effect of six isolates of *Trichoderma harzianum* and one isolate of *T. koningii* on the incidence and severity of tan spot, caused by *Pyrenophora tritici-repentis* (anamorph: *Drechslera tritici-repentis*) under field conditions. Significant differences between years, wheat cultivars and treatments were found. In 2003, two of the isolates assayed (T5, T7) showed the best performance against the disease applied as seed treatments or sprayed onto wheat leaves at different stages. The application of six of the treatments on wheat plants significantly reduced disease severity by 16 to 35% in comparison with the control. Disease control provided by isolate T7 was similar to that provided by the fungicide treatment (56% reduction). This is the first report on the efficacy of *Trichoderma* spp. against tan spot under field conditions in Argentina.

Keywords Wheat · Biocontrol · *Trichoderma* · Tan spot · *Drechslera tritici-repentis* · *Pyrenophora tritici-repentis*

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Introduction

Tan spot of wheat caused by the necrotrophic pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. (PTR) (anamorph *Drechslera tritici-repentis*) (Died.) Shoem., an air and seed-borne fungus, is one of the most destructive foliar diseases of wheat worldwide, including Argentina (Annone 1997, 2005). Breeding, fungicide treatments, and appropriate cultural practices currently are used for disease control. Biological control using antagonistic microbes alone or as supplements has been investigated in recent years to minimize the use of chemicals (Annone 2005). Antagonistic fungi are present in substantial quantity in nearly all agricultural environments, and their use is now being recognized world-over as an alternative in plant disease control (Harman et al. 2004; Sutton 2005). Biological control agents of tan spot of wheat foliage have been tested (Lee and Sutton 1995; Pfender 1988; Pfender et al. 1989), and different species of *Trichoderma* have been used successfully as biocontrol agents for biocontrol of pathogens on plant surfaces (Monte 2001; Papavizas 1985; Tronsmo 1986; Windels and Lindows 1985). Based on our laboratory experiments, the biocontrol potential of *Trichoderma* isolates to suppress tan spot was previously reported (Perelló et al. 1997; Perelló et al. 2003), but further evaluation is necessary to determine their performance outside the optimized environments of laboratory or greenhouse research. Therefore, the aim of this research was to evaluate the effectiveness of biocontrol of *Trichoderma* spp. isolates on tan spot expression in two application systems: foliar spray suspensions and seed treatments, on wheat plants under field conditions.

Materials and methods

Pyrenophora tritici-repentis

A culture of *P. tritici-repentis* strain HO119 was used for all inoculum production. It was isolated from naturally infected leaves of wheat in the field at the Estación Experimental J. Hirschhorn, belonging to the Facultad de Ciencias Agrarias y Forestales de la Universidad Nacional de La Plata, Los Hornos, Buenos Aires Province, Argentina, and stored on potato dextrose agar (PDA) at 4°C until used for inoculations. Conidia for application in suspension were prepared as described by Raymond and Bockus (1982). Petri dishes with V8 medium (20 ml V8® juice, 3 g of $CaCO_3$, 20 g of agar and 800 ml distilled water) were used. Mycelial plugs were taken from the advancing margin of a 6-day-old culture of *P. tritici-repentis* grown on PDA. These plugs were then placed on Petri dishes with V8 agar and incubated for 5 days at 20 ± 2°C with alternating 490 μmol m^{-2} s^{-1}dark cycles of 12 h plus the addition of near UV light (365 nm). On the 6th day of incubation, aerial mycelia were knocked down with a sterile, bent glass rod. The dishes were then incubated in continuous light for 24 h. Finally, the dishes were incubated in darkness for another 24 h. The light cycle stimulated formation of conidiophores and reduced mycelial fragments, while the dark cycle stimulated the completion of sporulation (Platt et al. 1977). The conidial harvest was completed by flooding the plate with 5 ml of sterile distilled water and dislodging the conidia with a bent glass rod. The resulting suspension was filtered through cheesecloth, and the concentration of inoculum suspension was adjusted to 2×10^4 conidia ml^{-1}. Following the methods of Raymond and Bockus (1982), oat grains (100 g) were autoclaved with 80 ml distilled water and 20 ml V8 juice in Erlenmeyer

flasks for 30 min at 120°C and 1 atm pressure. Then, in each flask an aliquot of 5 ml of the pathogen suspension was added. This combination was incubated at 23°C ± 2°C in darkness and shaken daily to allow pathogen colonization. After 15–20 days of incubation, the development of mycelia and conidia was confirmed. Grains were spread in trays and dried under laboratory conditions. They were weighed and stored in nylon bags at 5°C prior to their application in the field.

Trichoderma spp.

Five isolates (T4, T5, T6, T7, and T8) of *Trichoderma* spp. used in this study were collected from 2001 to 2004 in Buenos Aires Province (Table 1). They were cultured on PDA medium in Erlenmeyer flasks and incubated for 7–15 days at 20 ± 2°C in a growth chamber under a 12-h fluorescent plus near ultraviolet photoperiod. Conidia of each isolate were harvested by flooding the cultures with sterile distilled water and then rubbing the culture surfaces with a sterile glass rod. After filtering the suspensions through two layers of cheesecloth, concentrations of propagules in suspensions were standarized with the aid of a haemocytometer to 1×10^8 conidia ml^{-1} for each *Trichoderma* isolate tested. Suspensions were amended with one drop of 0.05% Tween 20 surfactant in distilled water immediately before plant inoculation by spray application onto leaves. Plants were sprayed with the inoculum suspension until runoff. After inoculations, plants were kept moist by spraying with water several times per day with sprinklers for a period of 3 days.

Production of *Trichoderma*-coated seed: For the seed treatment technique, a 10-ml suspension of each *Trichoderma* isolate (1×10^8 conidia ml^{-1}) was mixed with 90 ml of 0.25% agar (in water) which served as an adhesive. The mixture was shaken using a magnetic stirring bar until a homogeneous suspension was obtained. Wheat seeds of B. Biguá and B. Brasil cultivars were added to the water-agar-fungal biomass mixture for pelletizing. Seeds uniformly coated with *Trichoderma* formulations were held for air drying overnight in darkness at room temperature. Treated seeds were stored in sealed glass flasks at 5°C in a refrigerator and incorporated 24–48 h later.

Table 1 Identification number and origin of antagonistic isolates of *Trichoderma* spp.

Trichoderma strains	Nomenclature	Origin (wheat cultivar, Argentinian wheat ecological area and site and year of collection)
T. koningii	T4	Wheat phylloplane, Cv. Klein Escorpión, Wheat ecological area: II South (Bragado), Year: 2002
T. harzianum	T5	Wheat phylloplane, Cv. Buck Mataco, Wheat ecological area: II North (Arrecifes), Year: 2003
T. koningii	T6	Hyperparasite of *Sclerotinia sclerotiorum*, lectuce, Procedence: Estación Experimental Agropecuaria J. Hirschhorn, Los Hornos, Year: 2002
T. harzianum	T7	Hyperparasite of *Sclerotinia sclerotiorum*, lectuce, Procedence: Estación Experimental Agropecuaria J. Hirschhorn, Los Hornos, Year: 2002
T. harzianum	T8	Wheat phylloplane, Cv. Klein Don Enrique, Wheat ecological area: II North (Pergamino), Year: 2002

Field assays

Field experiments were carried out at the Estación Experimental J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata (35°S latitude), during 2003 and 2004. The trials were sown on 15 July and 2 August, respectively, under conventional tillage. The soil was a Typic Argiudoll. Analysis of soil samples (top 0.20 m) indicated the following values by weight: organic matter = 3%; $N = 0.17\%$; $P = 7$ mg kg^{-1}, and pH = 5.9. Average temperatures and humidity for the entire period after inoculations were 16.8°C and 78% for 2003 and 16.0°C and 77.5% for 2004.

The experimental design was a factorial randomized block with three replications and a row-to-row distance of 15 cm, in both years. Factors were two bread wheat (*Triticum aestivum* L.) cultivars Buck Biguá and B. Brasil tested at ZGS 23 (tillering) and ZGS 58 (heading) (Zadoks et al. 1974) and twelve treatments: eight *Trichoderma* spp. isolates, each applied in a spray spore of suspensions (SST4, SST5, SST6, SST7 and SST8) or by seed treatment (CST4, CST5, CST6, CST7 and CST8), a fungicide application (F) and a control spread with water (C). The fungicide used was Folicur® {tebuconazole; alpha-[2-(4-chlorophenyl) ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazol-1-ethanol; Bayer Corp., Leverkusen, Germany} at a rate of 1 l ha^{-1}. Between plots, strips of oats were sown to avoid the spread of inoculum between treatments. Similar strips of oats were sown between replications. Plots were 6.3 m^2 (4.50-m long by 1.4-m. wide). The entire experiment was fertilized with 50 kg P_2O_5 ha^{-1} as calcium triple superphosphate at the time of sowing.

Infected oat grains colonized with PTR were applied at the 4-leaf stage (ZGS 14) (Zadoks et al. 1974) by spreading them onto the soil surface on each row at a rate of 300 g m^{-2}. The incidence (number of infected leaves/total leaves) and the severity (percentage leaf area covered by lesions on the upper two leaves) of disease were evaluated on 20 plants of each plot for each experiment 14 days after inoculation.

Statistical analysis

Experimental data were subjected to arcsine-square-root transformation before ANOVA to normalize distributions and stabilize the residual variance. Data were analyzed by ANOVA for a factorial randomized block design in a combined analysis for both years with SPSS statistical package. Because of some significant interactions between years, a separate analysis for each year was also performed. Contrasts were constructed between the water control treatment and the rest of the treatments; between the fungicide treatment and all treatments with *Trichoderma* spp.; between all seed treated and all suspension applied treatments; between the individual isolates and the fungicide control and between the individual isolates and the water control.

Results

Incidence values were only considered at tillering because at heading all treatments had 100%.

The combined ANOVA for both years showed significant differences between years ($P < 0.001$), cultivars ($P = 0.008$), and treatments ($P = 0.003$) for incidence and between years ($P < 0.001$), growth stages ($P < 0.001$), ($P < 0.001$) and cultivars ($P < 0.001$) for

severity. Both incidence and severity values were higher in 2004 (57.68% and 67.21% respectively) than in 2003 (32.99% and 32.75% respectively). The interaction of year × growth stage was significant for severity ($P < 0.001$). Although the severity increased in 2004 at both growth stages, this increase was higher for the adult stage. There were not significant interaction for incidence.

Considering these significant year effects and year interactions, both years were analyzed separately.

In 2003 the ANOVA of the incidence of tan spot at tillering showed significant differences between treatments ($P < 0.001$) and for the interaction of cultivar × treatment ($P = 0.027$). The analysis of contrasts showed that there were not significant differences for the contrast water control vs. the rest of the treatments or fungicides vs. the rest of the treatments. However coat seed treatments had a higher control effect than spore pulverization treatments ($P < 0.001$). When individual isolates were compared to the water control and fungicide treatment, treatments CST6 and CST5 produced a better control than the water control treatment and also than the fungicide treatment (Table 2).

Table 2 Means of incidence (%) and severity (%) of *Drechslera tritici-repentis* for twelve treatments (control, fungicide and *Trichoderma*-application technique combination) at tillering and heading in 2003

	Incidence tillering	$P > F$	Severity tillering	Severity heading	Mean severity tillering-heading	$P > F$
Coated seed (CST6)	22.77	($P = 0.010$)[a]	22.76	37.54	30.15	($P < 0.001$)
		($P = 0.009$)				($P = 0.076$)
Coated seed (CST7)	30.07	($P = 0.291$)	24.10	39.21	31.66	($P < 0.001$)
		($P = 0.262$)				($P = 0.026$)
Coated seed (CST8)	31.21	($P = 0.417$)	22.71	43.54	33.13	($P < 0.001$)
		($P = 0.382$)				($P = 0.008$)
Coated seed (CST4)	29.46	($P = 0.235$)	22.89	52.33	37.62	($P = 0.033$)
		($P = 0.211$)				($P < 0.001$)
Coated seed (CST5)	25.11	($P = 0.036$)	21.12	44.98	33.05	($P < 0.001$)
		($P = 0.031$)				($P = 0.009$)
Spore suspension (SST6)	40.04	($P = 0.271$)	32.57	44.53	38.55	($P = 0.063$)
		($P = 0.298$)				($P < 0.001$)
Spore suspension (SST7)	43.84	($P = 0.058$)	23.14	29.00	26.07	($P < 0.001$)
		($P = 0.066$)				($P = 0.579$)
Spore suspension (SST8)	31.99	($P = 0.520$)	27.59	40.86	34.23	($P = 0.002$)
		($P = 0.479$)				($P = 0.003$)
Spore suspension (SST4)	30.61	($P = 0.345$)	25.72	37.37	31.54	($P < 0.001$)
		($P = 0.313$)				($P = 0.029$)
Spore suspension (SST5)	40.63	($P = 0.219$)	22.39	33.67	28.03	($P < 0.001$)
		($P = 0.244$)				($P = 0.252$)
Water control	34.95		37.23	52.16	44.69	
Fungicide treatment	35.24		19.15	29.32	24.24	

[a] The uppermost P values indicates the probability value (F test) for the contrast between individual treatment vs. water control and the lowest P values indicates the probability for the contrast between individual treatments vs. the fungicide treatment

When the interactions between the contrasts and cultivars were performed, the only significant interaction was caused by the treatment CST7 which was better than the water control in cultivar B. Biguá but similar in cultivar B. Brasil ($P = 0.049$).

For the severity in 2003, there were significant differences between growth stages ($P < 0.001$), treatments ($P < 0.001$) and cultivars ($P < 0.001$). The interaction growth stage × treatments ($P = 0.041$) and the triple interaction ($P = 0.020$) were also significant.

The analysis of contrasts showed that the group of control treatments (fungicide and *Trichoderma* spp. treatments) caused a reduction in the severity compared to the water control ($P < 0.001$). Furthermore, the fungicide produced a better control effect than the antagonists treatments ($P < 0001$). No significant differences were found for coat seed treatments vs. spore suspension treatments. When individual isolates were contrasted with the water control, all of them except treatment SST6 caused a control effect compared to the water control (Table 2). Three *Trichoderma* spp treatments (CST6, SST7 and SST5) were similar to the fungicide treatment. When individual isolates were considered, treatment CST4 showed a significant interaction with growth stage because it caused a reduction in the severity compared to the water control at seedling but not in the adult stage ($P = 0.029$). Also treatments CST4 ($P = 0.004$) and CST5 ($P = 0.040$) had a similar effect to fungicide treatment at seedlings but not at the adult stage. Furthermore coat seed was better than the suspension of spores at seedlings whereas the opposite was found in adult stage ($P < 0.001$).

In 2004, there were significant differences between treatments ($P = 0.003$) and cultivars ($P = 0.006$) for incidence, and for growth stages ($P < 0.001$), treatments ($P = 0.017$) and cultivars ($P = 0.019$) for the severity. No interactions were significant.

For incidence, the analysis of contrasts showed that the control effect caused by fungicides was better than that of the *Trichoderma* spp. treatments ($P = 0.003$) and that coat seed treatments had a higher control effect than spore suspension treatments ($P = 0.021$). When individual isolates were compared, treatments CST6 and CST5 produced a better control than the water control and were also similar to the fungicide treatment (Table 3). No significant interactions were found between any of the contrast and cultivars.

For the severity in 2004 fungicide treatment also caused a better control effect than the *Trichoderma* spp. treatments ($P < 0.001$). Coat seed and spore suspension had similar control effects. When individual isolates were contrasted with water control and fungicide treatments, no treatments caused better control than the water control and all of them produced higher severity values than the fungicide treatment (Table 3). No interactions between this contrast and the growth stage were found.

Discussion

In both years and cultivars tested, the coated seeds application system was better than spore suspension application in reducing the incidence of tan spot. Two of the treatments (CST6 and CST5) tested in 2003 and 2004 reduced significantly the incidence of tan spot in comparison with the water control and the fungicide treatment. The isolates T5 (*Trichoderma harzianum*) and T6 (*T. koningii*) were originally obtained from wheat phylloplane and parasited sclerotia from *Sclerotinia sclerotiorum* respectively. *T. harzianum* has previously been reported as a biocontrol agent in the aerial environment on different crops including other wheat diseases (Cordo et al. 2007; Elad 2000; Hermosa et al. 2000; Mónaco et al. 2004; Perello et al. 1997, 2003). Previous assays in Argentina have demonstrated that some *T. harzianum* isolates were capable of suppressing growth and

Table 3 Means of incidence at tillering (%) and severity at tillering and heading (%) of *Drechslera tritici-repentis* for twelve treatments (control, fungicide and *Trichoderma*-application technique combination) in 2004

Treatments	Incidence tillering	$P > F$	Severity tillering	Severity heading	Mean severity (tillering-Heading)	$P > F$
Coated seed (CST6)	50.38	($P = 0.042$)[a]	33.24	98.86	66.05	($P = 0.433$)
		($P = 0.291$)				($P = 0.008$)
Coated seed (CST7)	60.95	($P = 0.657$)	43.17	98.05	70.61	($P = 0.906$)
		($P = 0.009$)				($P < 0.001$)
Coated seed (CST8)	58.62	($P = 0.421$)	34.31	96.75	65.53	($P = 0.376$)
		($P = 0.023$)				($P = 0.010$)
Coated seed (CST4)	56.72	($P = 0.279$)	36.98	94.30	65.64	($P = 0.389$)
		($P = 0.046$)				($P = 0.010$)
Coated seed (CST5)	48.66	($P = 0.023$)	40.85	92.54	66.69	($P = 0.509$)
		($P = 0.428$)				($P = 0.006$)
Spore suspension (CST6)	57.68	($P = 0.345$)	46.01	97.66	71.83	($P = 0.719$)
		($P = 0.033$)				($P < 0.001$)
Spore suspension (SST7)	63.24	($P = 0.931$)	43.47	99.12	71.29	($P = 0.802$)
		($P = 0.004$)				($P < 0.001$)
Spore suspension (SST8)	62.13	($P = 0.797$)	39.15	99.72	69.43	($P = 0.906$)
		($P = 0.006$)				($P = 0.001$)
Spore suspension (SST4)	64.35	($P = 0.932$)	47.75	96.32	72.03	($P = 0.690$)
		($P = 0.002$)				($P < 0.001$)
Spore suspension (SST5)	62.11	($P = 0.794$)	41.00	88.96	64.98	($P = 0.321$)
		($P = 0.006$)				($P = 0.014$)
Water control	63.80		45.54	94.51	0.02	
Fungicide treatment	43.54		26.89	77.78	52.33	

[a] The uppermost P values indicates the probability value (F test) for the contrast between individual treatment vs. water control and the lowest P values indicates the probability for the contrast between individual treatments vs. the fungicide treatment

mycelial development of PTR and the severity of disease on wheat plants (Perelló et al. 2003, 2006). Isolate T6 that showed significant behavior in reducing the PTR incidence in the present paper, was previously reported in Argentina as an effective control agent of the lettuce wilt caused by the fungal pathogen *S. sclerotiorum* (Mónaco 1989; Mónaco et al. 1998).

The control of foliar pathogens of wheat relies on an integrated combination of cultural practices, fungicides, and the use of partially resistant or tolerant cultivars. Progress in breeding has been slow because of different factors, including a great variability in the pathogen, a certain degree of specificity, and the fact that breeding has concentrated on monogenic resistance, which implies specificity to some isolates is readily broken down (Lee and Gough 1984; Perelló et al. 1991). A complementary strategy within integrated management is biological control (Cook and Baker 1974; Cook and Veseth 1991). Problems of environmental contamination which have adversely affected the biodiversity in agroecosystems, as well as health and public safety problems inherent to the production and inadequate use of agrochemicals, have led to the search for and implementation of ecological alternatives. The goal remains to integrate all available methods for disease

control in a way to optimize their benefits and minimize their risks for producers, consumers and the environment in a sustainable crop production system (Powell 1993). Considerable research on biocontrol of pathogens of wheat in Argentina has focused on specific antagonists as tools to control foliar diseases (Alippi et al. 2000; Dal Bello et al. 2002; Perelló et al. 1997, 2001a, b, 2002, 2003). However, workable strategies for consistent biological control have not yet been developed and implemented under field conditions. There are many reasons for this, but most of the difficulties in developing functional biological control are related to the complexities of the system to be managed. Members of the fungal genus *Trichoderma* have been studied extensively, particularly because of their ability to act as biocontrol agents (Harman 2000; Melo 1991; Monte 2001; Papavizas 1985; Tronsmo 1986). *Trichoderma*-based biofungicides are a reality in agriculture, with more than 50 formulations currently available as registered products worldwide (Lorito and Woo 2004). *Trichoderma* spp. long has been known to interact with other microorganisms especially fungi, through antibiosis, mycoparasitism, competition of various types, and other mechanisms (Elad 2000). More recently, it has been shown that induced systemic resistance (ISR) caused by various microorganisms can protect plants against soil or foliar pathogens (Paulitz and Matta 2000). ISR has been demonstrated in both monocots and dicots, resulting in control of multiple plant pathogens (Harman 2004). In associated studies (unpublished data) to evaluate *Trichoderma* population levels, some isolates tested remained stable, but most of them decreased in density after a month of observation. In our previous greenhouse tests, some of the *Trichoderma* isolates that showed the strongest antagonistic effect on tan spot declined in population density in the final evaluation or consistently occurred at a low density. Conversely, strains that always maintained a high population level did not provide any reduction in disease. Therefore, no correlation was established between the survival of a *Trichoderma* population on the wheat phylloplane and effectiveness in tan spot control (Perelló et al. 2003). Competition for nutrients and space might have played a major role in the control of tan spot, because pathogen conidia need exogenous nutrients for germination and germ-tube elongation. However, if *Trichoderma* did not persist on the leaves but disease control still occurred, then an indirect mechanism such as systemic induced resistance may be presumed to be occurring as part of the mode of action might be involved.

The modes of action by which *Trichoderma* reduces tan spot of wheat are still not clear because of the difficulties encountered during the study of the complex interactions between host, pathogen, and antagonists under field conditions. Further study is required to better elucidate these modes of action. Two of the strains of *Trichoderma* assayed in our study (T4 and T5) originated from the wheat phylloplane were the ones that most consistently reduced disease. However, strains from other origin like T6 and T7, showed a good control effect also in comparison to the fungicide treatment. In general, the isolates of *T. harzianum* and *T. koningii* used in this work have proven to be suitable biocontrol agents of tan spot of wheat under field conditions, either by application as a foliar spray or as a seed treatment. However, their performance differed according the cultivar tested, the application technique, and the crop growth stage at which disease was assessed. Differences in the performance of the *Trichoderma* isolates used in this study may be explained in part by differences in the tolerance to microclimatic conditions on of wheat leaves during the test period. In general, most of the isolates reduced seedling infection of both diseases at an early stage. For field crops such as wheat, seed treatment with biocontrol agents is one of the most suitable application methods for biocontrol of soil-borne pathogens in the rhizosphere (Harman 1992). Appropriate formulation of biological control agents is generally accepted as critical for efficacy in the field. From our results, the seed

treatment preparations were the most effective in decreasing the severity of tan spot, suggesting that part of the mode of action of *Trichoderma* isolates tested against tan spot of wheat might involve induce systemic resistance in the leaves. Similarly, control of tan spot by biological seed treatments was previously reported in Brazil (Luz 1992). It may be assumed that further work on formulation and biological seed treatments is required to improve the control of tan spot with *Trichoderma* isolates in Argentina. Moreover, work on application techniques is required.

The results presented here show that *Trichoderma* isolates can be used as a non-chemical alternative treatment against tan spot of wheat caused by PTR. Future research will be aimed at developing the technology to be used under large-scale operations and investigating the mode of action of *Trichoderma* to control tan spot on wheat plants.

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References

Alippi AM, Perelló AE, Sisterna MN, Greco N, Cordo C (2000) Potential of spore-forming bacteria as biocontrol agents of wheat foliar diseases under laboratory and greenhouse conditions. Z Pflanzankr Pflanzanschutz 107:155–169

Annone JG (1997) Tan spot of wheat in Argentina: importance and disease management strategies. *Helminthosporium* blights of wheat. Spot Blotch and Tan Spot. Proceedings of an International Workshop Held at CIMMYT. El Batán, México, pp 339

Annone JG (2005) El desafío del manejo integrado de enfermedades de los cultivos en sistemas agrícolas conservacionistas: El caso de la sanidad del trigo en siembra directa. In: Proceedings of the 13th Congreso Latinoamericano de Fitopatología, Villa Carlos Paz, Córdoba. 19–22 de abril de 2005, pp 161–164 (640 pp)

Cook RJ, Baker J (1974) In: Cook RJ 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu Rev Phytopathol 31:53–80

Cook RJ, Veseth J (1991) Wheat health management. Plant Health Series. American Phytopathology Society Press, 152 pp

Cordo C, Mónaco C, Segarra C, Simón MR, Bayo D, Mansilla A, Kripels N, Conde R, Perello A (2007) *Trichoderma* spp. as elicitor of wheat plant defense responses against *Septoria tritici*. Biocontrol Sci Technol (in press)

Dal Bello G, Mónaco C, Simón MR (2002) Biological control of seedling blight of wheat caused by *Fusarium graminearum* with beneficial rhizosphere microorganisms. World J Microbiol Biotechnol 18:627–636

Elad Y (2000) Biological control of foliar pathogens by means of *T. harzianum* and potential modes of action. Crop Prot 19:709:714

Harman G (2000) Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis 84:377–393

Harman GE (2004) Overview of new insights into mechanisms and uses of *Trichoderma* based products. Phytopathology 94:S138

Hermosa MR, Grondona I, Iturriaga EA, Díaz-Miguez JM, Castro C, Monte E, Garcia-Acha I (2000) Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. Appl Environ Microbiol 66:1890–1898

Lee S, Gough J (1984) Inheritance of Septoria leaf blotch (*Septoria tritici*) and Pyrenophora tan spot (*Pyrenophora tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis 68:848–851

Li B, Sutton J (1995) Evaluation of leaf associated microorganisms for biocontrol of tan spot in wheat foliage. Fitopatol Brasil 20:545–552

Lorito M, Woo SL (2004) The molecular biology of the interaction between *Trichoderma*, phytopathogenic fungi and plants. Phytopathology 94:S139

Luz WC (1992) Seed microbiolization of wheat to control *Drechslera tritici-repentis*. Proceedings of the Second International Tan Spot Workshop Fargo, North Dakota State University, pp 67

Melo Y (1991) Potencialidades de utilizacao de *Trichoderma* spp. no controle biológico de doencas de plantas. In: Controle biológico de doencas de plantas, Chapter 9. EMBRAPA, Brasilia, DF, pp 135–156 (388 p)

Mónaco C (1989) Ensayos in-vitro para evaluar la eficiencia de microparásitos sobre esclerocios de *Sclerotinia sclerotiorum* (Lib) de Bary con miras a su control. Rev Facult Agron 65:76–73

Mónaco C, Rollán C, Nico A (1998) Efecto de micoparásitos sobre la capacidad reproductiva de *Sclerotinia sclerotiorum*. Rev Iberoam Micol 15:84–87

Mónaco C, Sisterna MN, Perelló A, DalBello G (2004) Preliminary studies on biological control of the blackpoint complex of wheat in Argentina. World J Microbiol Biotechnol 20:285–290

Monte E (2001) Underestanding *Trichoderma*: between biotechnology and microbial ecology. Int Microbiol 4:1–4

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: biology, ecology and potencial for biocontrol. Annu Rev Phytopathol 23:23–54

Paulitz TC, Matta A (2000) IV (B) Biological Control of Diseases. 28. The role of the host in biological control of diseases, pp 394–410. In: Albajes R, Gullino ML, van Lenteren JC, Elad Y (eds) Integrated pest and disease management in greenhouse crops. Series: Developments in plant Pathology, vol 14. Kluwer Academic Publisher, Wageningen, The Netherlands, 568 p

Perelló AE, Cordo CA, Arriaga HO, Alippi HE (1991) Variation in virulence of *Septoria tritici* Rob. Ex Desm isolates in wheat. Agronomie 11:571–579

Perelló A, Mónaco C, Cordo C (1997) Evaluation of *Trichoderma harzianum* and *Gliocladium roseum* in controlling leaf blotch of wheat (*Septoria tritici*) under in vitro and greenhouse conditions. Z Pflanzenkr Pflanzenschutz 104:588–598

Perelló A, Simón MR, Arambarri AM, Cordo C (2001a) Greenhouse screening of the saprophytic resident microflora for control of leaf spots of wheat (*Triticum aestivum* L). Phytoparasitica 29:341–351

Perelló A, Simón MR, Sisterna M, Cordo C, Arambarri AM (2001b) Microflora of wheat (*Triticum aestivum* L.) in Buenos Aires Province (Argentina) and its possible significance in biological control of foliar pathogens. Z Pflanzankr Pflanzanschutz 108:459–471

Perelló A, Simón MR, Arambarri AM (2002) Interactions between foliar pathogens and the saprophytic mycoflora of the wheat (*Triticum aestivum* L.) phylloplane. J Phytopathol 150:232–243

Perelló A, Mónaco C, Sisterna M, Dal Bello G (2003) Biocontrol efficacy of *Trichoderma* isolates for Tan Spot of wheat in Argentina. Crop Prot 22:1099–1106

Perelló A, Mónaco C, Moreno MV, Cordo C, Simón MR (2006) The effect of *Trichoderma harzianum* and *T. koningii* on the control of tan spot (*Pyrenophora tritici-repentis*) and leaf blotch (*Mycosphaerella graminicola*) of wheat under field conditions in Argentina. Biocontrol Sci Technol 16(8):803–813

Pfender WF (1988) Supression os ascocarp formation in *Pyrenophora tritici-repentis* by *Lymonomyces roseipellis*, a basidiomycte from reduce-tillage wheat straw. Phytopathology 78:1254–1258

Pfender WF, Zhang W, Nus A (1989) Field perfomance and greenhouse assay of fungi for biocontrol of residue borne *Pyrenophora tritici-repentis*. Phytopathology 79:1152

Platt H, Morral RA, Gruen HE (1977) The effects of substrate, temperature, and photoperiod on conidiation of *Pyrenophora tritici-repentis*. Can J Bot 55:254–259

Powell K (1993) Is biocontrol the answer for sustainable agriculture? Mycologist 7:75–78

Raymond PJ, Bockus WW (1982) An in vitro technique for profuse sporulation of *Drechslera tritici-repentis*. Phytopathology 72:934

Sutton JC (2005) Present and future perspectives of biological disease control in crops. Proceedings of the 13th Congreso Latinoamericano de Fitopatología; 2005 April 19–22. Villa Carlos Paz, Córdoba, Argentina, pp 640

Tronsmo A (1986) Use of *Trichoderma* spp. in biological control of necrotrophic pathogens. In: Fokkema N, Der Heuvel J (eds) Microbiology of the phyllosphere. Cambridge University Press, Cambridge

Windels CE, Lindows SE (1985) Biological control on the phylloplane. American Phytopathology Society Press, St. Paul, MN, pp 169

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421