BioControl (2009) 54:113–122
DOI 10.1007/s10526-008-9159-8

Biological control of *Septoria tritici* blotch on wheat by *Trichoderma* spp. under field conditions in Argentina

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Received: 16 March 2006 / Accepted: 27 March 2008 / Published online: 26 July 2008

Abstract Biological control is an additional tool available for the design of more sustainable control strategies of wheat diseases. *Trichoderma* spp. have previously been used as biocontrol agents to protect wheat plants against leaf spots diseases in Argentina, but the information from field assays is scarce. The effectiveness of four *Trichoderma harzianum* strains and one *T. koningii* strain in reducing the incidence and severity of the leaf blotching of wheat caused by *Septoria tritici* blotch (STB) under two formulation conditions, spore suspension and the coated-seed technique, was studied under field conditions. Significant differences between wheat cultivars, formulation types and growth stages were found. In 2003, at the tillering stage, all of the treatments tested (except SST1 for incidence) effectively reduced the incidence or the severity of the disease compared to the control. Similarly, in 2004, ten of the treatments reduced the severity at tillering. At the heading stage, none of the treatments tested caused a significant decrease of the disease. These results indicated, therefore, that the antagonism was effective at an early stage of the disease only. Comparing both formulations, spraying spore suspension onto leaves and the coated-seed application technique, both were effective in decreasing the disease. Some isolates, such as CST4 and CST2, reduced the incidence value of STB to 40% and the severity value to 70% of the control values applied as coated-seed formulation. On the other hand, isolates T4 and T2 showed the greatest effectiveness for controlling STB, with similar reduction values to that shown by the fungicide (Folicur®) application treatment. The results of this study indicated that, although the immediate impact of *Trichoderma* isolates may be seen as reduced incidence and severity on the first stages of STB, in the long term, the same disease levels as found in untreated sites may be attained. This study also demonstrated that the incorporation of *Trichoderma* as a biocontrol preparation may be a promising step towards reducing STB disease in the field and the levels of fungicide residues in the context of a more integrated approach to the problem.

Handling editor: Reijo Karjalainen.

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Keywords Wheat diseases · Biological control

Introduction

Septoria tritici Rob. ex Desm. (teleomorph: *Mycosphaerella graminicola* (Fuckel) Schroeter) causes *Septoria tritici* blotch (STB; syn. *Septoria* leaf blotch) of wheat. STB is currently among the most important disease of wheat worldwide (Brown 2001). The disease attacks wheat in all stages of development and, under favourable conditions, may cause serious yield losses reported to range from 31 to 54% (Eyal et al. 1987; Babadoost and Herbert 1984; Polley and Thomas 1991). In Argentina, yield losses from 21 to 37% (Kraan and Nisi 1993) and from 20 to 50% (Annone et al. 1991) in high-yielding cultivars have been found. STB is controlled as part of an integrated crop management system using resistant cultivars, cultural practices and chemical control. Breeding for resistance is the best and most reliable option. However, wheat cultivars reported to be resistant in one country may sometimes succumb to attack by *Septoria* populations in another country (Eyal et al. 1981, 1987). Fungicides give effective control of STB and are widely used in Europe.

Although normally not economical, foliar fungicides can be used to control STB outbreaks in Argentina. Applications should be made between tillering and heading, with the objective being to protect the flag leaf. Depending on the weather conditions from tillering to the early dough stage, one or more applications may be needed. A combination of these practices and biological control need to be considered among the strategies for the management of STB (Annone 2005). The use of biocontrol agents in conjunction with plant resistance may provide an equivalent level of control, with less adverse impact upon the environment than the use of chemicals. Previous studies in Argentina indicate that innocuous epiphytic microorganisms on the wheat leaf surface play important roles in the suppression of STB (Perelló et al. 1998a, b, 2001a, b, 2002).

Trichoderma spp. are fungi that are present in substantial numbers in nearly all agricultural soils and in other environments (Harman 1996). They are not normally regarded as resident organisms on foliar surfaces. Nevertheless, *Trichoderma* spp. were found on wheat on the phylloplane (Biles and Hill 1983; Mangiarotti et al. 1987) and as an endophyte (Larrán et al. 2002). It was demonstrated that *Trichoderma* stains can survive in the aerial part of the plants for long periods (Tronsmo 1986; Vitti and Ghini 1990; Melo 1991; Perelló et al. 2003) and can be used as antagonists of foliar fungal pathogens in a wide range of crops (Dubos and Bulit 1981; Tronsmo 1986; Elad 2000; Elad and Kirschner 1992; Sutton and Peng 1993; Michereff et al. 1995; Cook 1993; Lo et al. 1996). However, little information is available on the utility of *Trichoderma* for controlling wheat pathogens (Biles and Hill 1983; Homechin 1986; Simon and Sivasithamparam 1989; Dawson and Bateman 2001; Dal Bello et al. 2006).

In our early work (Perelló et al. 1994, 1997), we evaluated the inhibitory activity of *Trichoderma* to the in vitro growth of *S. tritici* and the effect on wheat plants under greenhouse conditions. Coiling of the hyphae of *T. harzianum* around the pathogen hyphae and the inhibition of the growth and reproduction of *S. tritici* was observed. Moreover, the pre-treatment of wheat leaves with *Trichoderma* resulted in a significant reduction of the disease compared to the control plants pre-treated with water only. This demonstrated that biological disease control is a promising strategy for managing STB (Perelló et al. 2006). It was also found that *T. harzianum* generated a systemic-induced response, increasing the proteolitic activity in the treated wheat plants (Segarra et al. 2004). Little information is available in the utility of foliar sprays of *Trichoderma* spp. for controlling STB under field conditions. Moreover, the application of isolates of *Trichoderma* spp. by methods of pelletisation of inoculum need to be further examined. In this sense, the contribution of this paper is significant. If *Trichoderma* strains which suppress the development of STB were sustained on wheat leaves in the field during periods favourable for blotch development, they would enhance the value of partial plant resistance by slowing the rate of infection, and they would serve as a second line of defense if plant resistance were to become ineffective due to virulence shifts in *S. tritici*.

As a part of ongoing research aimed at testing the efficacy of the control of *Trichoderma* spp. on *S. tritici* expression, the objective of this study was to test their ability to protect wheat plants against STB under field conditions. The specific objectives were to: (1) determine the effectiveness of *Trichoderma* isolates on wheat plants at different growth stages and

(2) assess the impact of the formulation on the effectiveness of the antagonist.

Materials and methods

Production of inoculum of *Septoria tritici*

A virulent culture of *S. tritici* (SN19) was used for all inoculum production. Culture SN19 was isolated in 2004 from naturally infected wheat leaves, which presented typical lesions with pycnidia at field conditions of the Estación Experimental J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires Province, Argentina. The fungus was stored on potato-dextrose agar (PDA; Difco Laboratories) at 4°C until it was used for inoculations at the Centro de Investigaciones de Fitopatología (CIDEFI) laboratory. Pycnidia developed spore cirrhi. From these, single-spore isolates were cultivated on water agar. On day six, they were replicated in slant tubes onto modified malt agar (30 g malt extract, 5 g mycological peptone, 2 g yeast extract and 1,000 ml distilled water) for sporulation. On day nine, a mucous mass of conidia developed. The conidial harvest was made 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 1×10^7 spores per ml concentration. Oat grains (100 g) with 80 ml of distilled water in Erlenmeyer flasks were autoclaved for 30 s at 120°C and 1 atm pressure. An aliquot of 5 ml from the pathogen suspension was added to each flask, incubated at 23 ± 2°C in darkness and daily shaken to favour good pathogen colonisation. After 15–20 days of incubation, the development of the mycelium and conidia was confirmed. Grains were spread in trays and were dried under laboratory conditions. They were weighed and stored in nylon bags at 5°C before their application in the field.

Production of inoculum of *Trichoderma* spp.

Four *Trichoderma harzianum* isolates (strains T1, T2, T3 and T5) and one *T. koningii* isolate (strain T4) collected from 2001 to 2003 in the Buenos Aires Province were used for the inoculum production (Table 1). Isolates T1 and T2 were obtained with the technique described by Elad et al. (1981) and modified by Mónaco et al. (1989, 1998). Isolates T3, T4 and T5 were isolated following routine phytopathological techniques. The isolates were stored at PDA 2% and maintained under refrigerator conditions (5°C) until they were used. For inoculum production, the strains were cultured on PDA medium in Erlenmeyer flasks and incubated for 7–15 days at 20 ± 2°C in a growth chamber under 12-h fluorescent plus near-ultraviolet photoperiod. The 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, the concentrations of propagules in suspensions were standardised with the aid of a haemocytometer to 1×10^8 conidia per ml for each *Trichoderma* isolate tested. The suspensions were amended with one drop of 0.05% Tween surfactant in distilled water immediately before plant inoculation.

Field assays

The assays of this work were carried out at the Estación Experimental J. Hirschhorn (Facultad de Ciencias Agrarias y Forestales de la Universidad Nacional de La Plata), Provincia de Buenos Aires, Argentina, during July–November 2003 and 2004.

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

Trichoderma	Isolation (code)	Origin (cultivar precedence and location in the Buenos Aires Province, Argentina)	Year of collection
T. harzianum	T1	Carnation soil, commercial greenhouse, Abasto	2001
T. harzianum	T2	Hyperparasite of *Sclerotinia rolfsii*, lettuce, Los Hornos	2001
T. harzianum	T3	Tomato phylloplane, commercial greenhouse, Colonia Urquiza	2001
T. koningii	T4	Wheat phylloplane, Bragado	2002
T. harzianum	T5	Wheat phylloplane, Arrecifes	2003

Bread wheat (*Triticum aestivum* L.) of the cultivars Buck Biguá and B. Brasil were tested at two growth stages of plant development according to Zadoks et al. (1974): ZGS 23 (tillering) and ZGS 58 (heading). A randomised block design with three replications was used. Infected oat grain colonised with *S. tritici* were applied by spreading onto the soil surface on each row of the plots at a rate of 300 g m^{-2} at ZGS 14 (four leaves).

The treatments assessed were the following: isolates T1, T2, T3, T4 and T5 applied as spore suspension (SST1, SST2, SST3, SST4 and SST5) and applied with the coated-seed technique (CST1, CST2, CST3, CST4 and CST5), fungicide application (F) and control (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}.

The incidence (number of infected leaves/total leaves) and the severity (percentage of leaf area covered by lesions) were evaluated 28 days after the inoculation of *S. tritici*.

Statistical analysis

The data of severity percentage and incidence percentage were arcsine square-root transformed before analysis. The experiment was analysed by a combined analysis of variance (ANOVA) for both years according to a factorial design with three replications. The factors were years, treatments and cultivars. Because of some significant year × year interactions, a separate analysis for each year was also performed. The ANOVA and means were compared by an LSD test ($P = 0.05$) for the two growth stages assessed.

Results

The incidence of STB was significantly different for years, treatments and cultivars. The incidence was higher in 2003 (18.75%) than in 2004 (8.29%). Year × treatment, year × cultivar, treatment × cultivar and the triple interaction were also significant (Table 2). The interaction can be explained by the fact that, in 2003, several treatments reduced the incidence of the disease compared to the control at tillering, whereas in 2004, only the fungicide application reduced the incidence of the disease (Fig. 1). The year × cultivar interaction was due to a similar incidence in both cultivars in 2004, whereas in 2003, Buck Brasil showed higher values (Fig. 2).

For the disease severity, the combined ANOVA for both years showed significant differences between years and treatments at tillering. The interactions year × treatment, treatment × cultivar and the triple interaction were also significant (Table 2). The severity at the tillering stage was higher in 2003 (28.32%) than in 2004 (1.70%). The interaction year x treatment was due to similar values for all treatments in 2004, whereas in 2003, several treatments caused a reduction in the severity of the control (Fig. 3). At heading, the differences among years, treatments and cultivars were shown. The interaction year × cultivar was also significant (Table 2). The severity at heading was higher in 2003 (55.15%) compared with 2004 (23.48%). The interaction year x cultivar was due to an increase in the severity of STB on Buck Biguá in 2004 (Fig. 4).

For the separated ANOVA for each year, the incidence of the disease at tillering in 2003 showed significant differences ($P = 0.05$) between treatments and for the interaction treatment × cultivar. Seven

Table 2 Mean squares of the incidence of *Septoria* leaf blotch at tillering and the severity at tillering and heading for two cultivars; 12 treatments in two years

Source	df	Incidence	Severity	
		Tillering	Tillering	Heading
Year	1	3,070.81***	25,513.6***	36,120.6***
Treatment	11	41.44***	334.07***	271.83**
Cultivar	1	74.12***	38.49 ns	626.01**
Year × treatment	11	54.79***	309.65***	66.75 ns
Year × cultivar	1	295.64***	48.93 ns	545.08*
Treatment × cultivar	11	48.28***	48.39**	111.23 ns
Year × treatment × cultivar	11	15.81***	51.14***	70.49 ns
Error	97	4.38	15.86	117.21

ns = non-significant

** = significant at $P = 0.01$

*** = significant at $P = 0.05$

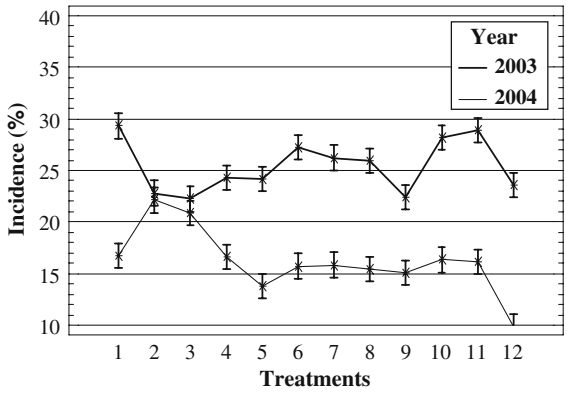

Fig. 1 Interaction year × treatment for the incidence of *Septoria tritici* blotch (STB) caused by *Mycosphaerella graminicola* on two wheat cultivars in 2003–2004 at the tillering stage. The bars indicate the standard errors for the mean values. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11 = control; 12 = fungicide

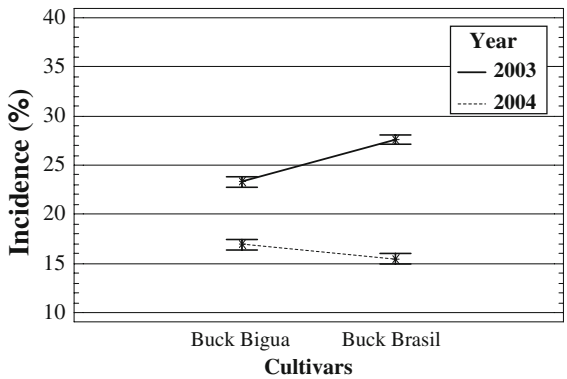

Fig. 2 Interaction year × cultivar for the incidence of STB caused by *M. graminicola* on two wheat cultivars in 2003–2004 at the tillering stage. The bars indicate the standard errors for the mean values

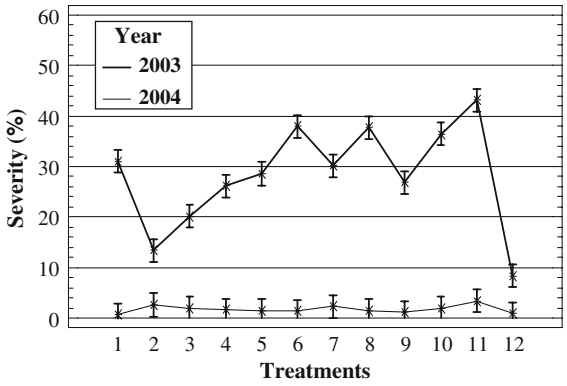

Fig. 3 Interaction year × treatment for the severity of STB caused by *M. graminicola* on two wheat cultivars in 2003–2004 at the tillering stage. The bars indicate the standard errors for the mean values. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11 = control; 12 = fungicide

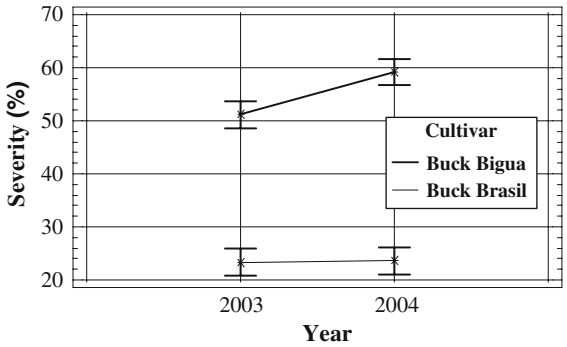

Fig. 4 Interaction year × cultivar for the severity of STB caused by *M. graminicola* on two wheat cultivars in 2003–2004 at the heading stage. The bars indicate the standard errors for the mean values. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11 = control; 12 = fungicide

Trichoderma treatments effectively reduced the leaf blotching compared to the untreated leaves.

All of the treatments tested effectively reduced the disease severity compared with the control at tillering. Isolate T2 applied as a seed-coating (CST2) performed significantly better than other *Trichoderma* treatment combinations and caused the highest level of control, similar to fungicide application. At heading, none of the treatments tested reduced the severity of disease (Table 3). There were significant differences between the two cultivars used. At heading stage, B. Brasil showed the highest severity values (59.2%). Treatments SST1 and SST2 caused a higher reduction in the incidence and severity of STB at tillering in B. Bigua than in B. Brasil (Figs. 5, 6).

In 2004, there were significant differences in the incidence of STB between cultivars, treatments and the interaction cultivar × treatment. The cultivar B. Biguá showed higher values (9.11%) than B. Brasil (7.47%). Only the fungicide treatment had a lower incidence of STB compared to the untreated leaves. The interaction cultivar × treatment can be explained because of the reduction caused by treatment CST5 and SST4 in cultivar B. Brasil only (Fig. 7).

Analysing the severity at tillering, there were significant differences for treatments and for the

Table 3 Effect of seed-coating and foliar spray of *T. harzianum* and *T. koningii* on disease incidence and severity (%) at tillering and heading on two wheat cultivars in 2003

Formulation method	Incidence	Severity	
	Tillering	Tillering	Heading
Fungicide (F) (Tebuconazole)	16.56 ab	8.35 a	45.45 a
Seed-coating T2 (CST2)	20.03 bc	13.38 a	58.44 b
Seed-coating T3 (CST3)	19.25 bc	20.15 b	57.07 b
Seed-coating T4 (CST4)	14.59 a	26.15 bc	56.59 ab
Spore suspension T4 (SST4)	16.95 ab	26.82 c	56.03 ab
Seed-coating T5 (CST5)	22.37 cd	28.61 c	50.16 ab
Spore suspension T2 (SST2)	15.11 a	30.15 cd	60.59 b
Seed-coating T1 (CST1)	21.26 bcd	30.99 cd	58.64 b
Spore suspension T5 (SST5)	16.95 ab	36.45 de	53.63 ab
Spore suspension T3 (SST3)	14.43 a	37.72 ef	59.50 b
Spore suspension T1 (SST1)	24.09 d	37.92 ef	50.68 ab
Control	23.42 d	43.17 g	55.04 ab

Means followed by the same letter are not significantly different (LSD test, $P = 0.05$)

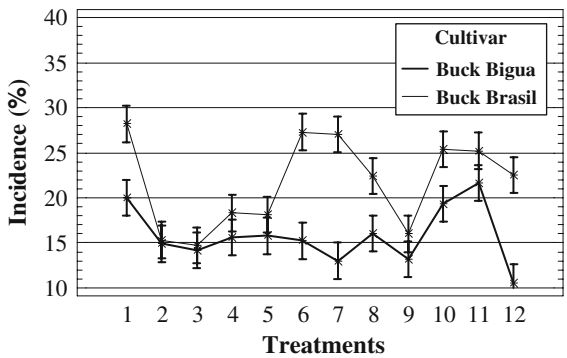

Fig. 5 Effect of seed-coating and foliar spray of *T. harzianum* and *T. koningii* on disease incidence (%) caused by *M. graminicola* on two wheat cultivars at tillering in 2003. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11control; 12 = fungicide. The bars indicate the standard errors for the mean values

interaction cultivar × treatment. All of the *Trichoderma* treatments significantly reduced the disease severity caused by *S. tritici* compared to the control. The formulation CST1 applied as a seed-coating formulation caused the highest reduction of the severity of STB, with values similar to the fungicide application. At the heading stage, none of the *Trichoderma* treatments tested caused a significant decrease in the disease (Table 4). The interaction

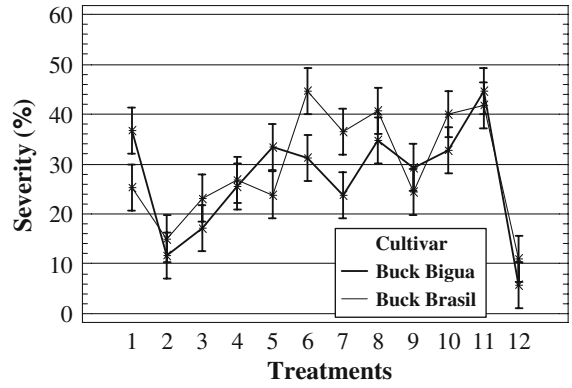

Fig. 6 Effect of seed-coating and foliar spray of *T. harzianum* and *T. koningii* on disease severity (%) caused by *M. graminicola* on two wheat cultivars at tillering in 2003. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11 = control; 12 = fungicide. The bars indicate the standard errors for the mean values

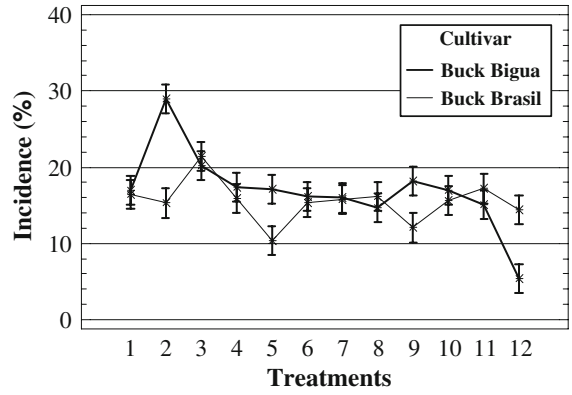

Fig. 7 Effect of seed-coating and foliar spray of *T. harzianum* and *T. koningii* on disease incidence (%) caused by *M. graminicola* on two wheat cultivars at tillering in 2004. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11 = control; 12 = fungicide. The bars indicate the standard errors for the mean values

cultivar × treatment was because all of the treatments caused a significant reduction on the severity of STB in B. Brasil, but only a few in B. Bigua (Fig. 8).

Discussion

Considerable research on the biocontrol of STB has focussed on specific antagonists as tools to control the disease (Fokkema and van der Meulen 1976;

Table 4 Effect of seed-coating and foliar spray of *T. harzianum* and *T. koningii* on disease incidence and severity (%) at tillering and heading on two wheat cultivars in 2004

Formulation method	Incidence	Severity	
	Tillering	Tillering	Heading
Fungicide (F) (Tebuconazole)	3.55 a	0.83 ab	8.79 a
Seed-coating T2 (CST2)	15.20 d	2.57 e	23.03 bc
Seed-coating T3 (CST3)	12.77 c	1.95 cde	26.82 bc
Seed-coating T4 (CST4)	8.20 b	1.55 bcd	23.75 bc
Spore suspension T4 (SST4)	7.33 b	1.09 abc	22.96 bc
Seed-coating T5 (CST5)	6.55 b	1.39 abc	33.64 c
Spore suspension T2 (SST2)	7.47 b	2.30 de	23.66 bc
Seed-coating T1 (CST1)	8.30 b	0.64 a	22.96 bc
Spore suspension T5 (SST5)	7.91 b	1.91 cde	19.30 ab
Spore suspension T3 (SST3)	7.10 b	1.46 abcd	23.64 bc
Spore suspension T1 (SST1)	7.35 b	1.31 abc	30.66 bc
Control	7.77 b	3.37 f	22.51 abc

Means followed by the same letter are not significantly different (LSD test, $P = 0.05$)

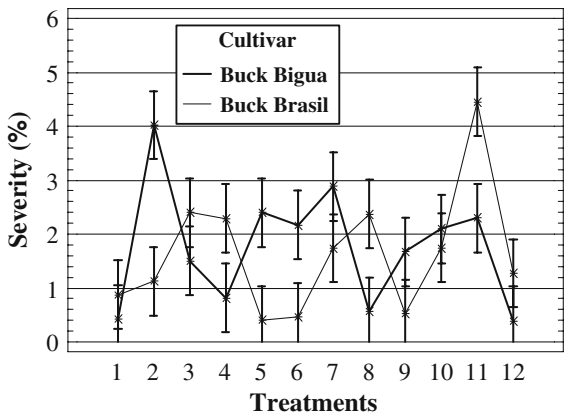

Fig. 8 Effect of seed-coating and foliar spray of *T. harzianum* and *T. koningii* on disease severity (%) caused by *M. graminicola* on two wheat cultivars at tillering in 2004. Treatments: 1 = CST1; 2 = CST2; 3 = CST3; 4 = CST4; 5 = CST5; 6 = SST1; 7 = SST2; 8 = SST3; 9 = SST4; 10 = SST5; 11 = control; 12 = fungicide. The bars indicate the standard errors for the mean values

Fokkema et al. 1979; Biles and Hill 1983; Windels and Lindow 1985; Luz 1982, 1991; Flaishman et al. 1990; Perelló et al. 1997; 1998; 2001a, b; 2002; 2003; Alippi et al. 2000; Nolan and Cooke 2000). However, workable strategies for consistent biological control have only rarely been developed and implemented under field conditions or are in stages of commercial development (McSpadden Gardener and Fravel 2002; http://www.agr.gc.ca/env/pdf.cat-e.pdf). *Trichoderma* species have been extensively studied, particularly due to their ability to act as biocontrol agents (Papavizas 1985; Melo 1991; Tronsmo 1986; Haran et al. 1996a, b; Harman 2000; Monte 2001). *Trichoderma* spp. have long been known to interact with other microorganisms, especially fungi, through antibiosis, mycoparasitism and competition of various types and other mechanisms. More recently, they have been shown also to have strong effects on plants. Induced systemic resistance has been demonstrated with many strains on both monocots and dicots, resulting in the control of bacterial, fungal, oomycete and viral plant pathogens (Harman 2004).

Trichoderma-based biofungicides are a reality in agriculture, with more than 50 formulations available today as registered products worldwide (Lorito and Woo 2004). The levels of control can approach that achieved by those of fungicides applied at similar frequencies to *Trichoderma* spore applications (Blakeman et al. 1992).

In diseases like STB, where no durable resistance currently exists in wheat cultivars, the control relies on an integrated combination of cultural management, fungicides and the use of partially resistant or tolerant cultivars. In a sustainable crop production system, a complementary strategy within the integrated management is the possibility of biological control (Cook and Veseth 1991; Lewis and Papavizas 1991; Powell 1993). This approach has relevance in developing countries where the cost of fungicides is prohibitive, or in low-input, reduced-chemical or organic systems. An effective method of use in wheat diseases would be to use *Trichoderma* strains in conjunction with chemical fungicides. The chemicals provide good short-term seed protection and the biocontrol fungus provides long-term root protection. As a consequence, yields are frequently increased over the use of the chemical alone. *Trichoderma* colonises roots, increases root mass and health, and, consequently, frequently provides yield increases, which chemical fungicides applied at reasonable rates cannot do (Björkman et al. 1994; Harman 1996). This research should progress investigations in this direction and is receiving further study in our laboratory.

Our results indicated that, for the control of STB in this study, greater control was obtained at the tillering stage in the 2003 trials. Similarly, in 2004, most of the

Trichoderma strains tested reduced the severity of STB at tillering. At heading, the results were not as promising. This indicates that most of the isolates tested were effective in controlling the disease during the initial stage of disease development. However, the STB severity increased over the course of the season. It is apparent, therefore, that antagonist applications alone cannot effectively control this disease with either spray or seed applications. This may be explained, in part, by differences in the tolerance of *Trichoderma* conidia to the microclimate conditions of wheat leaves during the testing period, such as temperature, ultraviolet light and wetness. It is important to note that the weather conditions in winter 2003 and 2004 were cool and wet at the time of *Trichoderma* application (tillering), conditions that are likely to be conducive to the antagonists' growth. However, in both years, the weather conditions were hot and dry at the time of the application of *Trichoderma* strains at heading. Another possible explanation for this was the way that the treatments persisted through the year following application. It is likely that the foliar spraying application of *Trichoderma* strains cannot cover the entire plant or have a sufficient quality of inoculum to control the disease caused by this pathogen. The amount of inoculum used could be below the optimum to reduce pathogen inoculum levels under field conditions and the STB level increased at heading. Alternatively, the pathogen inoculum may have been reintroduced into the plots by secondary infections from pycnidial cirri during the spring of 2003 and 2004. This could reduce the antagonist potential efficiency. In either case, one strategy may be to apply the promising antagonists for several years in a row or in combination with reduced levels or application frequencies of chemical fungicides. It is evident from this study that the impact of *Trichoderma* isolates on disease level cannot be easily predicted, and factors such as soil characteristics, microclimate conditions, season of application and probably numerous others influence the ultimate effect of the antagonist populations or their activities. Our conclusion, however, is that, in general, *Trichoderma* spp. isolates showed promise as microbial agents to control the first stages of *S. tritici* disease under field conditions, either by applying it as a foliar spray or as a protecting seed coat. The present study showed that some *Trichoderma* isolates, such as T2, reduced the incidence value of STB at tillering to 40% and the severity value to 70% of that of the control. On the other hand, isolates T2 and T4 had similar behaviour to that showed by the fungicide application treatment and reduced the severity and the incidence of the disease, respectively. The results of this study also indicated that, although the immediate impact of *Trichoderma* isolates may be seen as reduced STB level on the first stage of the disease, in the long term, the same disease levels found in untreated sites may be attained.

However, this study demonstrated that the incorporation of *Trichoderma* as a biocontrol preparation might be a promising step towards reducing the disease in the field and the levels of fungicide residues in the context of a more integrated approach to the problem. In several wheat-growing areas, STB appears at the beginning of the growing season, mainly in early sowings. In those cases, *Trichoderma* spp. isolates may be useful as microbial agents to control the disease in its early stages. For late infections, they should be applied in conjunction with compatible chemical fungicides.

Some private companies in Argentina are currently testing some solid and liquid formulations of *Trichoderma* spp. in wheat experiments in the field (Fálico and Varaschin 2007).

Acknowledgements Financial support was given from the Universidad Nacional de La Plata, Facultad de Ciencias Agrarias y Forestales, Project 11 A142. The authors thank the personnel of the Estación Experimental J. Hirschhorn, Los Hornos, Daniela Bayo, scholarships Silvia Alonso, Virginia Nolasco, Patricio Gannon and Sebastián Rapetti for their technical assistance.

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