Greenhouse Screening of the Saprophytic Resident Microflora for Control of Leaf Spots of Wheat (*Triticum aestivum*)

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Ten microorganisms of the epiphytic microflora of wheat leaves in Buenos Aires Province, Argentina, were evaluated under greenhouse conditions as potential biocontrol agents of the pathogens *Alternaria triticimaculans, Bipolaris sorokiniana, Drechslera tritici-repentis* and *Septoria tritici* in two application sequences (prior to or together with the pathogens). The antagonists significantly reduced the expression of the diseases on wheat plants compared with control plants not inoculated with the antagonists. Maximum percentage of reduction of the necrotic lesion area (NLA) (40–55%) of *S. tritici* resulted when *Cryptococcus* sp., *Rhodotorula rubra* and *Penicillium lilacinum* were sprayed on leaves prior to inoculations with the pathogen. *Bacillus* sp., *Cryptococcus* sp., *Fusarium moniliforme* var. *anthophylium*, *P. lilacinum* and *R. rubra* reduced significantly (34–52%) the NLA of *B. sorokiniana* in both of the application sequences. The best antagonistic effect against *A. triticimaculans* was shown by *Aspergillus niger, Bacillus* sp., *Chaetomium globosum, F. moniliforme* var. *anthophylium* and *Nigrospora sphaerica*, with a NLA reduction from 21% to 35% in the co-inoculation or in the sequential application. All microorganisms except *N. sphaerica* performed better than the control against *D. tritici-repentis*. The area under disease progress curve (AUDPC) of the pathogens appeared to progress similarly, but at lower values, in treated plants than in untreated controls. The two yeasts and the bacteria decreased AUDPC to 50–55% of *S. tritici* and *B. sorokiniana* compared with the control in both application sequences, whereas the maximum efficacy against *A. triticimaculans* was reached by *N. sphaerica* and *A. niger* for the sequential application and by *F. moniliforme* var. *anthophylium* for the co-inoculation. If the parasitism occurs also in nature, application of antagonists for biological control might provide the opportunity to compete with the pathogens and regulate their colonization in wheat leaves.

KEY WORDS: Biological control; wheat; foliar pathogens; phylloplane.

INTRODUCTION

Mycosphaerella graminicola (Fuckel) Schröt. (*Septoria tritici* Rob. ex Desm.), *Pyrenophora tritici-repentis* (Died.) Drechsler (*Drechslera tritici-repentis* (Died.) Shoem.), and *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (*Bipolaris sorokiniana* (Sacc.) Shoem.) are the most important and widespread necrotrophic pathogens of wheat in

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Argentina. They often appear together, causing leaf spots with losses of 50% or more, depending on the environmental conditions (19). *Alternaria triticimaculans* Simmons & Perelló is another pathogen of the complex of foliar diseases of wheat, reported only in Argentina (29); the dried holotype was deposited at the BPI Herbarium, U.S. National Fungus Collection, EGS 41-050, wheat being the only recorded host (41).

Breeding, chemical treatments and appropiate cultural practices are the main ways to control wheat foliar pathogens, but it is well known that no single control method can solve the problem, and that integrated management is required (9,18). The possibility of biological control using antagonistic microorganisms, is a complementary strategy within the integrated management of these diseases.

In Argentina, biological control is considered an attractive option for management of some plant diseases. Introducing microorganisms that possess characteristics antagonistic to pathogens could be a valuable method for control of foliar diseases. Research on this topic was initiated recently at our Research Centre (1,28,30-34). *Nigrospora sphaerica, Aspergillus niger* and *Bacillus* sp. (Bt/97) strongly inhibited most species tested *in vitro* and appear to have potential as biological control agents against Septoria leaf blotch, tan spot and spot blotch of wheat (34). In this study we examined the possibility of treating wheat foliage with ten microorganisms of the resident microflora as biocontrol agents of leaf spots caused by *S. tritici, B. sorokiniana, D. tritici-repentis* and *A. triticimaculans* under greenhouse conditions.

MATERIALS AND METHODS

Antagonists *Stemphylium* sp., *Epicoccum nigrum* Link., *Nigrospora sphaerica* (Sacc.) Mason, *Aspergillus niger* van Tiegh., *Fusarium moniliforme* Sheldon var. *anthophylium* (A. Braun) Wollen, *Penicillium lilacinum* Thom, *Chaetomium globosum*, two yeasts: *Cryptococcus* sp. and *Rhodotorula rubra* Harrison, and a strain of one unidentified *Bacillus* (Bt/97) representing common microorganism colonizers of the wheat phyllosphere in Buenos Aires Province (Argentina) (30,31), were tested for possible antagonistic behavior. All microbial antagonists were isolated from wheat leaves, transferred by the standard pure culture methods (streaking, single-sporing or hyphal-tipping) and maintained at 5°C either on slants of potato dextrose agar (PDA) (yeasts and filamentous fungi) or nutrient agar (NA) (bacteria).

Fungal pathogens *Septoria tritici, B. sorokiniana, D. tritici-repentis* and *A. triticimaculans* were utilized as plant pathogens. They were isolated from lesions of naturally infected wheat leaves grown at the Estación Experimental J. Hirschhorn, Los Hornos, Buenos Aires, Argentina, and maintained in PDA at 4°C until use.

Greenhouse experiments To determine the antagonistic capacities of the saprophytes against the pathogens, wheat leaves were inoculated with a mixture of propagules of the pathogens and saprophytes. Wheat (*Triticum aestivum* L.) plants of the susceptible cultivar 'Buck Poncho' were sown in the greenhouse at 25°C maximum day temperature and 15°C minimum night temperature, with a 14 h photoperiod. A complete randomized design with five replications was used. Each experimental unit consisted of a plastic pot (12 cm diam × 15 cm depth) with five plants.

Inoculum was increased by streaking antagonists on the appropiate medium in Erlenmeyer flasks and incubating the flasks for 1 day at 28°C (yeasts and bacteria) or for 7–15

days at 20±2°C (filamentous fungi) in a growth chamber under a 12-h photoperiod of fluorescent light plus near ultraviolet (NUV).

Inoculum of *B. sorokiniana* and *A. triticimaculans* was produced by growing cultures for 8 days on PDA. *S. tritici* was grown for 5 days on malt agar (MA) in Erlenmeyer flasks at 20±2°C under fluorescent plus NUV for a 12-h photoperiod. In the case of *D. tritici-repentis*, profuse sporulation used for inoculations was obtained by transferring mycelial plugs from slants to V8 agar plates, which were kept for conidiation using the technique reported by Rodriguez and Bockus (38).

Inoculum was harvested by flooding the cultures with 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 standardized with the aid of a hemacytometer to 6×10^6 ml^{-1} conidia for *S. tritici*; to 2×10^5 ml^{-1} for *B. sorokiniana* and *A. triticimaculans*; and to 3×10^5 ml^{-1} for *D. tritici-repentis*. The concentration of *P. lilacinum, Alternaria alternata, Cryptococcus* sp., *R. rubra, E. nigrum, Ch. globosum, F. moniliforme* var. *anthophylium, A. niger* and *N. sphaerica* varied from 4 to 6×10^6 spores ml^{-1} for each. The bacterial strain was adjusted to 1×10^8 cells ml^{-1} (A_{620nm}=0.700).

All suspensions were amended with 0.05% Tween 80 in distilled water.

Inoculations Wheat plants at stage 15 (43) were sprayed with suspensions of *S. tritici, B. sorokiniana, D. tritici-repentis* and *A. triticimaculans* until run-off using a manually operated sprayer. The saprophytes were applied on the leaves 3 days before inoculation with the pathogen (sequential application) or simultaneously with the pathogen (co-inoculation). Plants having received each pathogen alone, served as controls. After inoculation or application of the saprophytes, the pots were covered with plastic bags for 2 days to maintain a high level of humidity.

For evaluation purposes, the 4th leaf of each plant per treatment was selected and the percentage of necrotic area was estimated using specific scales for each disease: *S. tritici:* 0 = no visible infection, 1 = 1–15%, 2 = 15–30%, 3 = 30–50%, 4 = 50–60% and 5 = >60% affected foliar area; *B. sorokiniana*: 0 = no visible infection, 1 = 1–20%, 2 = 20–40%, 3 = 40–60% and 4 = >60% of affected foliar area; *A. triticimaculans*: 0 = no visible infection, 1 = 1–5%, 2 = 5–25% and 3 = 25–50% of affected foliar area; *D. tritici-repentis*: 0 = no visible infection, 1 = 1–10%, 2 = 10–20%, 3 = 20–40%, 4 = 40–60% and 5 = >60% affected foliar area.

The level of infection was compared with that of the leaves inoculated with the pathogens alone. The percentage of necrotic area was recorded 7 days following inoculation and continued at 7-day intervals for 21 days (referred to hereafter as date of scoring). The antagonistic effect in a particular experiment was recorded as reduction percentage of the necrotic area (NAR). This was calculated according to the following formula: NAR (%) = (NAC – NAT/NAC) × 100, where NAC is necrotic area (%) in the control and NAT is necrotic area (%) in the treatment. Area under disease progress curve (AUDPC) (39) was calculated using the three dates of evaluation. Data from each of the experiments were analyzed by an analysis of variance (ANOVA) and means were compared by the Tukey test.

RESULTS

Reduction of the necrotic area The combined analysis (not shown) for both application sequences (sequential and co-inoculation) showed interaction between application sequences and microorganisms. For a clearer interpretation, a separate ANOVA was performed. Table 1 shows that the microorganisms had an antagonistic effect on the

TABLE 1. Mean squares for the separate analysis of two application types for the effect of ten antagonists on the reduction of necrotic area of *Septoria tritici, Bipolaris sorokiniana, Alternaria triticimaculans* and *Drechslera tritici-repentis* at three dates of scoring

Variable	*S. tritici*	*B. sorokiniana*	*A. triticimaculans*	*D. tritici-repentis*
		Sequential application		
Microorganisms	2836(5.0)[z] ***	3059(41.7)***	3201(13.2)***	1652(77.1)***
Date of scoring	6414(11.3)***	315(4.3)**	1378(5.7)**	1794(83.7)***
Microorganisms × date of scoring	826(1.5)ns	170(2.3)**	337(1.4)ns	22(1.0)ns
Error	567	73.3	242	21.4
		Co-inoculation		
Microorganisms	2681(4.1)***	2213(53.6)***	1944(13.6)***	2670(15.2)***
Date of scoring	1513(2.3)ns	137(3.3)*	784(5.5)**	54.3(0.4)ns
Microorganisms × date of scoring	1707(2.6)***	236(5.7)***	493(3.5)***	116.6(0.8)ns
Error	650		143	156

[z] F values are given in parentheses.

*$P = 0.05$; **$P = 0.01$; ***$P = 0.001$; ns = non-significant.

pathogens. The date of scoring was also significant for all the pathogens in the sequential application and was significant only for *B. sorokiniana* and *A. triticimaculans* in the co-inoculation. Interactions between the microorganisms and the date of scoring were also significant for *B. sorokiniana* in the sequential application, and for *S. tritici, B. sorokiniana* and *A. triticimaculans* in the co-inoculation. For *S. tritici,* the two yeasts were the most effective as compared with the control (without reduction) for the sequential application; for the co-inoculation, also *P. lilacinum* was effective, with a NAR up to 55.33% (Table 2). The best control effect against *B. sorokiniana* was shown by *P. lilacinum, F. moniliforme* var. *anthophylium,* the two yeasts and the bacterium for both application sequences, with a reduction of up to 52.89% in the NAR (Table 2). *Bacillus* sp., *N. sphaerica, A. niger* and *F. moniliforme* var. *anthophylium* had a higher antagonistic effect than the rest of the microorganisms tested against *A. triticimaculans* when applied 3 days before the pathogen. However, the NAR was not higher than 33.33%. When they were applied concurrently with the pathogen, *Ch. globosum* can be included (Table 2). Of the ten microorganisms tested against *D. tritici-repentis*, all of them except *N. sphaerica* performed better than the control in both application sequences (Table 2). In this case, the NAR reached values of 60.24% for the sequential application.

When the microorganisms were applied 3 days before *S. tritici*, the NAR was greater at the first and second dates of scoring than at the third date. However, no significant differences were observed among the three evaluations when the antagonists were applied concurrently with the pathogen (Table 3). The antagonistic effect was similar for both types of application on *B. sorokiniana* at the different evaluation dates. Date 1 was better

TABLE 2. Ranking of ten microbial antagonists based on the reduction of necrotic area (NAR) in respect to the necrotic area on wheat seedlings inoculated with *Septoria tritici*, *Bipolaris sorokiniana*, *Alternaria triticimaculans* and *Drechslera tritici-repentis* without the antagonists

	NAR (%)	
	Sequential application	Co-inoculation
	S. tritici	
Aspergillus niger	25.62 ab	4.82 a
Chaetomium globosum	24.43 ab	15.19 ab
Nigrospora sphaerica	13.71 a	16.42 ab
Alternaria alternata	18.86 a	17.89 ab
Fusarium moniliforme var. *anthophylium*	26.35 ab	23.70 ab
Epicoccum nigrum	41.24 ab	27.63 ab
Bacillus sp.	41.29 ab	30.30 ab
Rhodotorula rubra	50.68 b	40.64 b
Penicillium lilacinun	36.33 ab	43.70 b
Cryptococcus sp.	55.33 b	44.28 b
	B. sorokiniana	
Epicoccum nigrum	18.36 b	6.20 a
Alternaria alternata	14.44 ab	8.61 a
Aspergillus niger	8.52 a	8.95 a
Chaetomium globosum	8.56 a	11.02 a
Nigrospora sp.	15.57 b	13.87 a
Fusarium moniliforme var. *anthophylium*	35.60 c	36.92 b
Penicillium lilacinum	34.97 c	37.45 b
Cryptococcus sp.	45.49 cd	44.14 b
Bacillus sp.	52.89 d	48.03 b
Rhodotorula rubra	42.73 cd	49.12 b
	A. triticimaculans	
Epicoccum nigrum	-1.11 a	2.63 a
Alternaria alternata	-3.93 a	5.78 a
Penicillium lilacinun	2.08 ab	6.98 a
Rhodotorula rubra	-1.47 a	7.80 ab
Cryptococcus sp.	6.71 ab	14.39 abc
Bacillus sp.	22.67 c	22.10 bcd
Chaetomium globosum	2.57 ab	27.41 cd
Nigrospora sphaerica	33.33 c	28.61 cd
Aspergillus niger	32.36 c	30.05 d
Fusarium moniliforme var. *anthophylium*	21.45 c	35.63 d
	D. tritici-repentis	
Nigrospora sphaerica	10.19 a	0.27 a
Alternaria alternata	19.93 b	13.73 b
Aspergillus niger	27.50 bc	14.04 b
Penicillium lilacinun	31.39 cd	24.95 bc
Rhodotorula rubra	35.98 d	28.66 bc
Fusarium moniliforme var. *anthophylium*	38.51 d	30.12 bc
Cryptococcus sp.	39.09 d	32.54 bc
Epicoccum nigrum	53.78 d e	41.86 c
Chaetomium globosum	54.94 e	45.89 c
Bacillus sp.	60.24 e	52.32 c

[z] Within columns, for each pathogen, means followed by a common letter do not differ significantly (P=0.05) according to the Tukey test.

than the two others evaluated in both inoculation sequences of the antagonists against *A. triticimaculans*.

Comparisons of scorings one, two and three against *D. tritici-repentis* were statistically

TABLE 3. Percent reduction of the necrotic area (NAR) of *Septoria tritici, Bipolaris sorokiniana, Drechslera tritici-repentis* and *Alternaria triticimaculans* at three dates of scoring

Pathogen	Date of scoring[z]	NAR (%)	
		Sequential application	Co-inoculation
S. tritici	1	44.32 b[y]	32.70 a
	2	34.11 b	22.32 a
	3	21.72 a	24.35 a
B. sorokiniana	1	25.87 ab	28.25 ab
	2	27.96 b	29.03 b
	3	25.47 a	25.86 a
A. triticimaculans	1	19.28 b	25.11 b
	2	6.91 a	17.57 ab
	3	8.20 a	11.73 a
D. tritici-repentis	1	46.01 c	29.65 a
	2	37.76 b	28.57 a
	3	27.68 a	27.10 a

[z] Readings were taken at 7-day intervals following inoculation.
[y] Within columns, for each pathogen, means followed by a common letter do not differ significantly (P=0.05) according to the Tukey test.

TABLE 4. Mean squares for the source of variance of the effect of ten antagonists on the area under disease progress curve of *Septoria tritici, Bipolaris sorokiniana, Alternaria triticimaculans* and *Drechslera tritici-repentis*

Variable	*S. tritici*	*B. sorokiniana*	*A. triticimaculans*	*D. tritici-repentis*
Microorganisms	357.5 (59.49)[z]***	562.6 (95.38)***	126.7 (20.49)**	912.4 (82.61)***
Application sequence	43.22 (7.19)**	3.82 (0.65)ns	0.04 (0.01) ns	26.07 (2.36)ns
Microorganisms × Application sequence	4.73 (0.79)ns	9.77 (1.65)ns	16.15 (2.61)**	29.44 (2.67)**
Error	6.01	5.90	6.18	11.05

[z] F values are given in parentheses.
** P = 0.01; *** P = 0.001; ns = non-significant.

different for the sequential application. However, there were no significant differences when the application was concurrent.

In general, for all the pathogens, there was a more marked antagonistic behavior at the first date and a tendency to a lesser effect of the competitive action at later dates of scoring. Some interaction effects were also detected between the microorganism and the date of scoring, especially due to the low antagonistic effect of some of the microorganisms at the first date of evaluation (not shown).

Effect of the antagonists on the AUDPC There were significant differences among the saprophyte microorganisms in the control of the four pathogens evaluated. The application sequence of the antagonists was significant only for the control of *S. tritici*. The interaction microorganism × application sequence showed differences for the control of *A. triticimaculans* and *D. tritici-repentis* (Table 4). The means of the AUDPC of the four pathogens are presented in Table 5. Wheat leaves sprayed with the antagonists were consistently protected from lesions produced by the four pathogenic fungi. This control was

TABLE 5. Ranking of ten microbial antagonists based on the reduction of area under disease progress curve (AUDPC) compared with inoculation of wheat seedlings with pathogens alone

	AUDPC		
	Sequential application	Co-inoculation	Mean
	Septoria tritici		
Rhodotorula rubra	21.00 ab	21.28 a	21.14 a
Bacillus sp.	20.09 a	23.24 ab	21.66 a
Cryptococcus sp.	21.00 ab	24.50 ab	22.75 ab
Epicoccum nigrum	25.06 bc	25.76 ab	25.41 bc
Penicillium lilacinun	26.74 cd	26.04 ab	26.39 bc
Fusarium moniliforme var. *anthophylium*	27.02 cd	27.86 bc	27.44 c
Chaetomium globosum	31.08 de	32.90 cd	31.99 d
Aspergillus niger	33.60 ef	34.30 d	33.74 d
Nigrospora sp.	33.04 e	34.44 d	33.95 d
Alternaria alternata	34.16 ef	36.68 d	35.42 de
S. tritici (control)	38.36 f	37.94 d	38.15 e
Application sequences (means)	28.29	29.54	
	Bipolaris sorokiniana		
Bacillus sp.	21.84 ab	20.30 a	21.07 a
Cryptococcus sp.	25.06 ab	23.38 a	24.22 a
Rhodotorula rubra	21.42 a	24.39 ab	22.90 a
Penicillium lilacinun	27.44 b	28.56 b	28.00 b
Fusarium moniliforme var. *anthophylium*	27.30 ab	28.84 b	28.07 b
Epicoccum nigrum	39.62 c	35.42 c	37.52 c
Chaetomium globosum	37.10 c	35.56 c	36.76 c
Nigrospora sp.	37.41 c	36.12 c	36.24 c
Alternaria alternata	38.64 c	37.66 c	38.15 c
Aspergillus niger	38.08 c	38.78 cd	38.43 c
B. sorokiniana (control)	42.70 c	43.26 d	42.98 d
Application sequences (mean)	32.33	32.03	
	Alternaria triticimaculans		
Fusarium moniliforme var. *anthophylium*	26.18 bc	23.24 a	24.71 ab
Nigrospora sp.	22.26 a	24.64 ab	23.45 a
Chaetomium globosum	30.80 bc	24.92 ab	27.86 bcd
Aspergillus niger	22.40 a	25.06 ab	23.73 a
Bacillus sp.	25.06 ab	26.46 abc	25.76 abc
Cryptococcus sp.	29.68 bc	29.12 cd	29.40 cde
Alternaria alternata	31.78 c	31.08 cd	31.43 de
Penicillium lilacinun	30.94 c	31.22 cd	31.08 de
Epicoccum nigrum	31.50 c	31.78 d	31.64 e
Rhodotorula rubra	31.92 c	32.20 d	32.06 e
A. triticimaculans (control)	31.50 c	33.88 d	32.69 e
Application sequences (means)	28.54	28.51	
	Drechslera tritici-repentis		
Bacillus sp.	23.52 a	23.10 a	23.31 a
Chaetomium globosum	26.32 a	27.30 b	26.81 a
Epicoccum nigrum	26.46 a	29.40 ab	27.90 ab
Fusarium moniliforme var. *anthophylium*	35.28 b	34.86 c	35.07 c
Penicillium lilacinum	39.20 bc	35.28 cd	37.24 cd
Rhodotorula rubra	35.14 b	30.24 ab	32.69 bc
Cryptococcus sp.	35.84 b	37.38 cd	36.61 cd
Aspergillus niger	40.74 c	41.72 de	41.23 de
Alternaria alternata	15.29 d	44.80 e	45.04 e
Nigrospora sp.	50.54 e	52.08 f	51.31 f
D. tritici-repentis (control)	56.42 f	47.88 f	52.15 f
Application sequences (means)	37.70	36.73	

[z] Within columns, for each pathogen, means followed by a common letter do not differ significantly (P=0.05) according to the Tukey test.

evident in a significant reduction in AUDPC. Application of the two yeasts and the bacteria on wheat leaves with *S. tritici* and *B. sorokiniana* reduced the AUDPC to 50–55% of that in control leaves in both application sequences, whereas the maximum efficacy against *A. triticimaculans* was reached by *N. sphaerica* and *A. niger* for the sequential application

and by *F. moniliforme* var. *anthophylium* for the co-inoculation. For both application sequences, *Bacillus* sp., *Ch. globosum* and *E. nigrum* were significantly effective against *D. tritici-repentis.*

The interaction microorganism × application sequence for *A. triticimaculans* and *D. tritici-repentis* can be explained by the fact that although the ranking of the microorganisms for *D. tritici-repentis* is similar in both sequences, the antagonistic effect is higher for the sequential application, considering that the control had a higher AUDPC. For *A. triticimaculans*, the ranking of the microorganisms is different for the two application sequences. Furthermore, although three microorganisms *(Bacillus* sp., *A. niger* and *Nigrospora* sp.) showed a strong antagonistic effect in both application sequences, for the co-inoculation, *F. moniliforme* var. *anthophylium* and *Ch. globosum* performed better than the control only for the co-inoculation.

DISCUSSION

Current interest in leaf-surface microbiology has increased due to the fact that the epiphytic microflora play an important role, acting as competitor and reducing the incidence of several foliar diseases (2-8,11,13,42). In Argentina, the microflora of wheat leaves and the activity *in vitro* against foliar diseases have already been studied (28,31-34). Necrotrophic pathogens, like *S. tritici, B. sorokiniana, D. tritici-repentis* and *A. triticimaculans,* which use exogenous nutrients, can be antagonized mainly *via* nutrient competition by almost all microorganisms which are able to colonize the phyllosphere (13,25). Results of the present work indicated that most of the ten saprophytes tested reduced significantly the severity and the progress of the diseases caused by the four pathogens, suggesting that – as in other reports – the mechanisms involved in the interactions could be competition for nutrients or nutrient impoverishment (12). Other mechanisms have been postulated to explain such interactions, including nutrient leakage from spores following extensive germ tube growth (13), pH changes in the substrate (10,26), mechanical obstruction and antibiosis (10,17,36). The first date of scoring showed better control of the pathogens, especially for the sequential inoculation. This would indicate that early control is important to minimize the effect of the pathogens. However, as shown by a smaller reduction in necrotic area at the subsequent date of scoring, there would be a partial recovery of some pathogens (*S. tritici, A. triticimaculans* and *D. tritici-repentis*), indicating that more than one application of the antagonists may be necessary. In the case of *B. sorokiniana,* these data showed a long lasting effect of the antagonists, which indicated a lower level of recovery of this pathogen.

Differences in the behavior of the pathogens during the pre-penetration phase of their development could determine the way in which those pathogens react to the presence of other microorganisms on the leaf surface. *Septoria* normally develops only a limited surface mycelium within a few hours after inoculation on a suitable leaf (40). Therefore, the period during which the antagonists could compete with the fungi could be more limited in the co-inoculation than in the sequential application. Data presented in our work suggest that *S. tritici* is more adversely affected by the commonly occurring microorganisms when they arrive before the pathogen colonizes the wheat leaves. This would explain why in this case the application of antagonists prior to pathogen inoculation was a more effective strategy than co-inoculation for enhanced success in controlling disease. Occupancy and pre-emption of the infection court may be enough by itself in the case of *S. tritici.* In our *in vitro* studies (unpublished), antibiosis seems to be the prevalent mechanism for *A.*

triticimaculans, D. tritici-repentis and *B. sorokiniana*. On the other hand, for *S. tritici* the prevalent mechanism seems to be competition for space. When the competition for space is the mode of action, it is almost always necessary for the biocontrol agents to occupy the plant surface prior to invasion by the pathogen, in order for them to be effective.

Some of the microorganisms tested, like *A. niger, E. nigrum, F. moniliforme* var. *anthophylium, Penicillium* sp. and *Bacillus* sp., were previously recognized as antagonists with great potential against pathogenic fungi of cereals in our tests conducted *in vitro*, in which different types of interactions were observed. The effect on the pathogens includes various degrees of growth inhibition, coiling of hyphae, vacuolation, granulation and plasmolysis of hyphae. Antagonists also interfered by causing abnormal elongation of germ tubes, lysis and swelling that affected the germination of conidia of *B. sorokiniana, A. triticimaculans* and *S. tritici* (Perelló, Simón and Arambarri, unpublished).

Despite certain variability observed among individual pathogens in the present study, the most effective microorganisms tested were the yeasts (particularly against *S. tritici* and *B. sorokiniana*), and the bacterium Bt/97. In accordance with the findings in our research, there are several reports in which phyllosphere yeasts interfere with spore germination, superficial hyphal growth and penetration of necrotrophic pathogens, resulting in reduction of infection by 50% or more (7,14,15,23). On the other hand, the use of bacteria as biocontrol agents of foliar diseases of cereals has been reported to be an alternative of great potential against fungal diseases of wheat phylloplane (6,20-22,37). *Bacillus* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* are some of the most effective bacteria used in biocontrol studies of wheat foliar pathogens, with control levels similar to those achieved by the application of chemical products (16,20,24). The ability of bacteria to inhibit fungal plant pathogens by secreting antibiotics with antifungal properties and cell-wall-degrading enzymes has been well studied, particularly within the genus *Bacillus* (27,35). *Bacillus* spp. are attractive candidates as biocontrol agents because of their ability to produce drought-resistant endospores which ensure a long survival and resistance against physical stress (27,35). The results of this study and our previous reports in Argentina on the behavior of *Bacillus* and other spore-forming bacteria as biocontrol agents of wheat foliar diseases, are in agreement with this (28,34).

Based on our observations, disease reduction was interpreted to be the result of antagonists affecting early stages of the disease cycle, perhaps by affecting the pathogens directly or by preventing pathogen colonization of host leaves prior to infection.

The integration of biological, cultural and chemical controls into crop protection strategies for modern agricultural systems will occur because several factors are forcing disease management strategies to change. In Argentina, a sustained long-term programme of integrated control could benefit from the incorporation of biological control as a suitable method for reducing both the incidence and the severity of several foliar diseases of wheat. Field experiments are in progress.

REFERENCES

1. Alippi, A.M., Perelló, A.E., Sisterna, M.N., Greco, N.M. and Cordo, C. (2000) Potential of spore-forming bacteria as biocontrol agents of wheat foliar diseases under laboratory and greenhouse conditions. *Z. Pflanzenkr. Pflanzenschutz* 107:155-169.
2. Andrews, J.H. (1985) Strategies for selecting antagonistic microorganisms from the phylloplane. pp. 31-45. *in:* Windels, C.E. and Lindow, S.E. [Eds.] Biological Control on the Phylloplane. APS Press, St. Paul, MN, USA.

3. Andrews J.H. (1992) Biological control in the phyllosphere. *Annu. Rev. Phytopathol.* 30:603-635.
4. Andrews, J.H. (1992) The screening of microorganisms antagonistic to phytopathogens. *Pes. Agropec. Bras.* 27:165-174.
5. Baker, K.F. and Cook, R.J. (1974) Biological Control of Plant Pathogens. W.H. Freeman and Co., San Francisco, CA, USA. pp. 43-64.
6. Bettiol, W. (1991) Seleçao de microrganismos antagonicos a fitopatógenos. pp. 223-236. *in:* Bettiol, W. [Ed.] Controle Biológico de Doencas de Plantas. EMBRAPA-CNPDA, Jaguariúna, Brasil.
7. Blakeman, J.P. and Fokkema, N.J. (1982) Potential for biological control of plant diseases on the phylloplane. *Annu. Rev. Phytopathol.* 20:167-192.
8. Cook, R.J. and Baker, K.F. (1983) The Nature and Practice of Biological Control of Plant Pathogens. APS Press, St. Paul, MN, USA. pp. 1-29.
9. Cook, R.J. and Veseth, J. (1991) Wheat health management. Plant Health Series. APS Press, St. Paul, MN, USA.
10. Diem, H.G. (1969) Micro-organismes de la surface des feuilles. 3. Effet de la compétition nutritive sur la germination des spores d'une souche d'*Helminthosporium. Bull. Ec. Natl. Super. Agron. Nancy* 11:18-25.
11. Dubos, B. and Bulit, J. (1981) Filamentous fungi as biocontrol agents on aerial plant surfaces. pp. 353-356. *in:* Blakeman, J.P. [Ed.] Microbial Ecology of the Phylloplane. Academic Press, London, UK.
12. Elad, Y. and Zimand, G. (1992) Integrated biological chemical control of grey mould. pp. 272-276. *in:* Verhoeff, R., Williamsen, B. and Malathrakis, N.E. [Eds.] Recent Advances in *Botrytis* Research. Proc. Xth *Botrytis* Symposium. Pudoc Scientific Publishers, Wageningen, the Netherlands.
13. Fokkema, N.J. (1993) Opportunities and problems of control of foliar pathogens with microorganisms. *Pestic. Sci.* 37:411-416.
14. Fokkema, N.J., Den Houter, J.G., Kosterman, Y.J.C. and Nelis, A.L. (1979) Manipulation of yeasts grown on wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum. Trans. Br. Mycol. Soc.* 72:19-29.
15. Fokkema, N.J. and Meulen, F. van der (1976) Antagonisms of yeast-like phyllosphere fungi against *Septoria nodorum* on wheat leaves. *Neth. J. Plant Pathol.* 82:13-16.
16. Hemming, B.C. (1990) Bacteria as antagonists in biological control of plant pathogens. pp. 223-242. *in:* Baker, R. and Dunn, P. [Eds.] New Directions in Biological Control. Alan R. Liss, Inc. New York, NY.
17. Heuvel, J. van den (1970) Antagonistic effects of epiphytic microorganisms on infection of dwarf bean leaves by *Alternaria zinniae.* Diss., Willie Commelin Scholten Phytopathological Lab., Baarn, the Netherlands.
18. Jacobsen, B. and Blackman, A. (1993) Biological and cultural plant disease control: alternatives and supplements to chemicals in IPM systems. *Plant Dis.* 7:311-315.
19. Kholi, M.M., Annone, J.G. and Garcia, R. [Eds.] (1996) Las enfermedades del trigo en el Cono Sur. Curso de Manejo de Enfermedades del Trigo. Pergamino, Argentina, 1995.
20. Levy, E., Eyal, Z., Carmely, S., Kashman, Y. and Chet, I. (1989) Suppression of *Septoria tritici* and *Puccinia recondita* of wheat by an antibiotic-producing fluorescent pseudomonad. *Plant Pathol.* 38:564-570.
21. Levy, E., Eyal, Z. and Chet, I. (1992) Inhibition of *Septoria tritici* and other phytopathogenic fungi by *Pseudomonas fluorescens* and its antibiotics. *Plant Pathol.* 41:335-341.
22. Li, B. and Sutton, J.C. (1995) Evaluation of leaf-associated microorganisms for biocontrol of tan spot in wheat foliage. *Fitopatol. Bras.* 20:545-552.
23. Luz, W.C. da (1982) Controle biologico das manchas foliares do trigo a traves de microorganismos da filosfera. *Fitopatol. Bras.* 7:530-531.
24. Luz, W.C. da (1991) Controle biologico das doencas foliares do trigo. pp. 383-388. *in:* Bettiol, W. [Ed.] Controle Biologico de Doencas de Plantas. EMBRAPA, Brasil.
25. McBride, R.P. (1971) Micro-organisms interactions in the phyllosphere of larch. pp. 545-555. *in:* Preece, T.F. and Dickinson, C.H. [Eds.] Ecology of leaf surface micro-organisms. Academic Press, London, UK.
26. Newhook, F.J. (1951) Microbial control of *Botrytis cinerea* Pers. 1. The role of pH changes and bacterial antagonism. *Ann. Appl. Biol.* 38:169-184.
27. Nielsen, P. and Sorensen, J. (1997) Multi-target and medium independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. *FEMS Microbiol. Ecol.* 22:183-192.
28. Perelló, A. (1998) Interacciones entre la microflora saprotrofa y patógenos foliares del filoplano del trigo. Tesis Doctoral. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Argentina. pp. 70-117.
29. Perelló, A., Cordo, C. and Simón, M.R. (1996) A new disease of wheat caused by *Alternaria triticimaculans* in Argentina. *Agronomie* 16:107-112.

30. Perelló, A., Mónaco, C. and 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.
31. Perelló, A., Simón, M.R., Cordo, C. and Arambarri, A.M. (1998) Use of phylloplane fungi to control leaf blotch of wheat caused by *Septoria tritici*. *Proc. Sixth Int. Mycol. Congress* (Jerusalem, Israel), p. 71.
32. Perelló, A., Simón, M.R., Cordo, C. and Arambarri, A.M. (1998) Microscopic interactions between foliar pathogens and the mycoflora of wheat phylloplane. *Proc. Sixth Int. Mycol. Congress* (Jerusalem, Israel), p. 72.
33. Perelló, A., Simón, M.R., Sisterna, M., Cordo, C. and Arambarri, A.M. (1998) Efecto de microorganismos saprótrofos del filoplano del trigo en el control biológico de patógenos foliares. *I° Congr. Argentino de Control Biológico de Enfermedades de las Plantas (INTA, UBA)* (Buenos Aires, Argentina), p. 22.
34. Perelló, M.R., Simón, A.M, Sisterna, M., Cordo, C. and Arambarri, A.M. (2001) Microflora of wheat (*Triticum aestivum* L.) in Buenos Aires Province (Argentina) and its possible significance in biological control of foliar pathogens. *Z. Pflanzenkr. Pflanzenschutz* 108(5): in press.
35. Podile, A. and Prekash, A. (1996) Lysis and biological control of *Aspergillus niger* by *Bacillus subtilis*. *Can. J. Microbiol.* 42:533-538.
36. Porter, C.L. (1924) Concerning the characters of certain fungi as exhibited by the growth in the presence of other fungi. *Am. J. Bot.* 11:168-188.
37. Robbs, C. (1991) Bacterias como agentes de controle biológico de fitopatógenos. p. 49. *in:* Bettiol, W. [Ed.] Controle Biologico da Doencas en Plantas. EMBRAPA, Brasil.
38. Rodriguez, R. and Bockus, W. (1996) Differences among isolates of *Pyrenophora tritici-repentis* in production of conidia on wheat leaves. *Plant Dis.* 80:686-694.
39. Shanner, G. and Finney, R.E. (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in wheat. *Phytopathology* 72:154-158.
40. Shipton, W.A., Boyd, W.R.J., Rosielle, A.A. and Shearer, B.I. (1971) The common *Septoria* diseases of wheat, *Septoria nodorum, Septoria tritici*. *Bot. Rev.* 37:231-262.
41. Simmons, E. (1994) *Alternaria* themes and variations. *Mycotaxon* 50:409-427.
42. Windels, C.E. and Lindow, S.W. [Eds.] (1985) Biological Control on the Phylloplane. APS Press, St. Paul, MN, USA. pp. 6-30.
43. Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974) A decimal code for growth stages of cereals. *Weed Res.* 14:415-421.