Microflora of wheat (*Triticum aestivum* L.) in Buenos Aires Province (Argentina) and its possible significance in biological control of foliar pathogens

Mikroflora auf Weizen (*Triticum aestivum* L.) in der Provinz Buenos Aires (Argentinien) und ihre potentielle Bedeutung für die biologische Bekämpfung der Erreger von Blattkrankheiten

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Summary

Micro-organisms on the leaf surface of *Triticum aestivum* L. were examined. The wheat leaves were collected from experimental fields in six localities of Buenos Aires Province (Argentina). Thirteen mycelial fungi, two yeasts and a bacterium belonging to the genus *Bacillus* (Bw/97) were isolated from wheat foliage and evaluated for effectiveness in suppressing *Alternaria triticimaculans, Bipolaris sorokiniana, Drechslera tritici-repentis* and *Septoria tritici* under controlled conditions. Antagonistic activity was examined with the dual cultures method on potato dextrose agar media among 10 of these micro-organisms against the four foliar pathogens. Mycelial growth inhibition and colony interactions in all possible paired combinations were evaluated. The results are discussed in relation to the biological control of these cereal pathogens. In this work, the importance of indigenous antagonists in wheat disease suppression and the possibility of managing ecosystem conditions in order to enhance natural biological control is suggested. In our assays, *Aspergillus niger,* Bw/97 and *Nigrospora sphaerica* showed a strong inhibitory effect *in vitro* against the average of the four necrotrophic fungi tested and justify further evaluation for biocontrol of wheat foliar pathogens. The others antagonists ranked variably among the assays. Greenhouse bioassays and field evaluations using these isolates are currently under investigation.

Key words: wheat; biological control; phylloplane; antagonism; foliar pathogens

Zusammenfassung

Die auf den Blattoberflächen von *Triticum aestivum* L. vorkommenden Mikroorganismen wurden untersucht. Die Weizenblätter wurden von Versuchsflächen an sechs verschiedenen Standorten in der Provinz Buenos Aires (Argentinien) entnommen. Dreizehn Pilz- und zwei Hefearten sowie eine Bakterienart, die der Gattung *Bacillus* (Bw/97) angehörte, konnten von den Weizenblättern isoliert und auf ihre Hemmwirkung gegen *Alternaria triticimaculans, Bipolaris sorokiniana, Drechslera tritici-repentis* und *Septoria tritici* unter kontrollierten Bedingungen getestet werden. Die Überprüfung der antagonistischen Wirkung erfolgte mit der Doppelkultur-Methode auf Kartoffel-Dextrose-Agar zwi-

schen 10 dieser Mikroorganismen gegen vier Erreger von Blattkrankheiten. Die Hemmung des Myzelwachstums und die Wechselwirkungen zwischen den Kolonien in allen möglichen Paarungen wurden erfasst. Die Ergebnisse werden in Beziehung zur biologischen Bekämpfung dieser Getreidepathogene diskutiert. Die Bedeutung der lokalen Antagonisten für die Verminderung der Weizenkrankheiten und die Möglichkeit, den Zustand des Ökosystems zu beeinflussen, um die natürliche biologische Bekämpfung zu fördern, werden hervorgehoben. In den vorliegenden Untersuchungen ließen *Aspergillus niger*, Bw/97 und *Nigrospora sphaerica in vitro* eine stark hemmende Wirkung gegen den Durchschnitt der vier untersuchten nekrotrophen Pilze erkennen, so dass weitere Untersuchungen hinsichtlich der biologischen Bekämpfung von Pathogenen auf Weizenblättern gerechtfertigt sind. Die übrigen Antagonisten hatten eine unterschiedliche Wirkung. Gegenwärtig werden mit diesen Isolaten Biotests im Gewächshaus und unter Freilandbedingungen durchgeführt.

Stichwörter: Weizen; biologische Bekämpfung; Phylloplane; Antagonismus; Blattpathogene

1 Introduction

Leaves of wheat under natural conditions constitute a reservoir of native micro-organisms which, potentially, can be used in biological control programmes of foliar diseases. A rich microflora represented by yeasts, filamentous fungi and bacteria inhabits field-grown wheat leaves surfaces and plays an important role in decreasing incidence and severity of several diseases by exerting antagonist effects (Blakeman and Fokkema 1982; Dickinson 1976; Dickinson and Wallace 1976; Fokkema 1988, 1993; Fokkema et al. 1975, 1979; Fokkema and Naaij 1981; Fokkema and van der Meulen 1976; Luz 1982a, b, 1984, 1991; Mangiarotti et al. 1987; Robbinge et al. 1984). Use of micro-organisms in controlling wheat foliar diseases is possible, but the knowledge in this area is scantly. In Argentina, the biocontrol strategies of necrotrophic foliar pathogens of wheat has been scarcely studied (Alippi et al. 2000; Perelló et al. 1998a, b). The purpose of this paper was to identify indigenous microbial species of wheat and provides a perspective of the micro-organisms that occur in different ecological areas in Buenos Aires Province, Argentina. It is presented as a source of potential candidates to continue studies on biocontrol.

2 Material and methods

2.1 Isolation of saprophytic micro-organisms

Micro-organisms for evaluation as biocontrol candidates were isolated from non infected and infected leaves of wheat (*Triticum aestivum* L). Twenty leaves per field at growth stage 50 (Zadoks et al. 1974) were randomly collected from commercial fields at six localities of Buenos Aires Province (three wheat ecological subregions: North II, South II and IV) (Fig. 1). To remove micro-organisms from leaf surfaces, five pieces from each leaf were aseptically cut out and shaken for 30 min in 100 ml of sterile distilled water plus 0.1 % of Tween 80. The suspension was diluted at 1 : 10 in duplicate and 0.1 ml was spread on Petri dishes containing potato dextrose agar (PDA) 2 % medium amended with streptomycin (1 %). The dishes were incubated at 22 °C with cycles of 12 h light-darkness for 3 to 8 days. Recovered fungi and bacteria were transferred to PDA and nutrient agar (NA), respectively and stored at 2–4 °C. Colonies data were converted to colony-forming units (cfu) per g leaf. Data were square root transformed to reduce variance heterogeneity and Kruskal-Wallis and Mann Whitney Wilcoxon tests (Conover 1980) were then applied. Nonparametric methods were used throughout because of their relatively simple nature and general applicability with limited distribution assumptions.

2.2 Isolation of phytopathogenic fungi

S. tritici, *B. sorokiniana*, *D. tritici-repentis* and *A. triticimaculans* were isolated from wheat leaves in fields that were naturally infected at Experimental Station J. Hirschhorn, Los Hornos, Buenos Aires Province, Argentina. The pathogens were maintained in 2 % PDA at 4 °C until used.

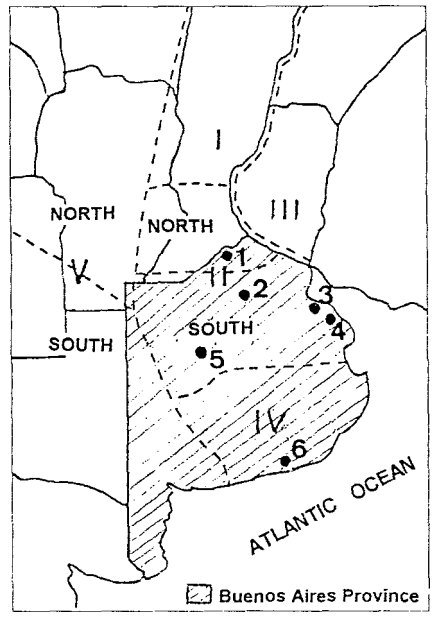

Fig. 1. Map of Argentina wheat area showing isolate collection sites. 1: Pergamino, 2: Alberti, 3: Los Hornos, 4: Bavio, 5: 9 de Julio, 6: Necochea.
Abb. 1. Karte der argentinischen Weizenanbaugebiete, auf der die Probennahmestellen eingezeichnet sind. 1: Pergamino; 2: Alberti; 3: Los Hornos; 4: Bavio; 5: 9 de Julio; 6: Nechochea.

2.3 Antagonistic activity assays

From the 16 saprophytic micro-organisms isolated, 10 were selected due to its effective antagonist behaviour in previous assays. Relative antagonism to the four phytopathogens was assessed macroscopically by growth inhibition on Petri dishes containing 15 ml of PDA 2 %. Disks (6 mm diameter) from colonies margins of each pathogen-antagonist combination were placed at a distance of 35 mm apart. Control consisted in individual cultures of each pathogen. Dual cultures (pathogen-antagonist) had five replications. Observations on the relative growth of the pathogens were made following 2, 4, 6, 8 and 10 days of incubation at 20–22 °C with light alternancy (3500 lux dark cycles of 12 h plus the addition of near UV light (365 nm). The inhibition was evaluated according to the following formula: $IICG = d_1/d_2$, where IICG= inhibition index of colony growth; d_1 = diameter (cm) of colony in the control; d_2 = diameter (cm) of colony in the treatment. Data were analyzed by ANOVA for complete randomised design.

2.4 Colony interaction types

The following phenomena were also considered according to the different types of reaction between the fungi: intermingled growth (Type A); colony surrounding or overlapping the other (Type B); width of the inhibition zone 1–2 mm (Type C) and more than 2 mm (Type D). Changes in colony shape and colour were also considered (PORTER 1924).

3 Results

3.1 Isolation of saprophytic micro-organisms

A total of 15 fungal species and a bacterium were recorded from the wheat phylloplane of the six localities sampled (Table 1). Among fungi, mostly were Hyphomycetes, like *Alternaria alternata* (Fr.) Keissler, *Stemphylium* sp., conidial state of *Pleospora herbarum, Cladosporium herbarum* (Pers.) Link. ex Gray, *Epicoccum nigrum* Link., *Nigrospora sphaerica* (Sacc.) Mason, *Aspergillus niger* van Tiegh., *Fusarium moniliforme* Sheldon var. *anthophilum* (A. Braun) Wollen, *Penicillium* sp., *Penicillium lilacinum* Thom, *Trichoderma harzianum* Pers. ex Fr. and *Chaetomium globosum* Kunze ex Fr. The Coelomycetes were

Table 1. Micro-organisms from wheat leaves in six localities of Buenos Aires Province. Mean colony-forming units (CFU) per gram of fresh weight
* Data represent mean of CFU × 10^{-3}/gr fresh weight in four replicates. For each micro-organism, means followed by the same letter indicate no differences according to Mann-Whitney-Wilcoxon test (P = 0.05). (+) Indicates presence in low quantity (1000 CFU/gr fresh weight
Tab. 1. Mikroorganismen von Weizenblättern aus sechs Regionen in der Provinz Buenos Aires. Durchschnittliche Zahl der Kolonie bildenden Einheiten (CFU) pro Gramm Frischgewicht

MICRO-ORGANISMS	Los Hornos	Necochea	Alberti	9 de Julio	Bavio	Pergamino	CFU
Trichoderma harzianum	9*	3	4	4.5	3	2	0.67 a
Cladosporium herbarum	–	2.5	4	1	–	1	0.83 ab
Bw/97	1	2	0.5 +	–	–	1.5	0.92 ab
Rhizopus sp.	4.5	2.5	3.5	4	5.5	2	1.00 ab
Aspergillus niger	1.5	7.5	3.5	4.5	3	3.5	1.42 abc
Phoma sp.	4.5	1.5	3.5	4.5	4.5	1	1.50 bcd
Fusarium moniliforme var. anthophilum	1.5	1.5	0.5	1	–	1.5	2.25 cde
Nigrospora sphaerica	3	1	3.5	2.5	1.5	2	2.42 bcde
Cryptococcus sp.	6	3	4.5	1.5	3	–	2.83 cdefg
Chaetomium globosum	6	0.5 +	1	4.5	3	–	3.00 defg
Penicillium lilacinum	5.5	–	1.5	0.5 +	1.5	3	3.25 defg
Epicoccum nigrum	–	3	–	1	–	–	3.67 fg
Rhodotorula rubra	3	3.5	3.5	2.5	3	1.5	3.83 fg
Stemphylium sp.	3.5	4.25	3.5	2.5	3	4	3.87 fg
Penicillium sp.	3.5	4	4	3.5	3	5	3.92 fg
Alternaria alternata	0.5 +	–	–	0.5 +	–	4.5	4.25 g
Average of localities	3.31 a	2.48 a	2.56 a	2.40 a	2.12 a	2.03 a	

represented only by *Phoma* sp. and the Zygomycetes by *Rhizopus* sp. A white yeast: *Cryptococcus* sp. and a pink yeast: *Rhodotorula rubra* Harrison were also isolated. The bacterial isolate was identified as *Bacillus* sp. and registered as Bw/97. There were no significant differences between the total of micro-organisms isolated from each locality. However, quantitative and qualitative differences in the fungal population recorded within each locality could be noted. Particularly, *A. alternata* was the dominant genera. The lowest values were found for *T. harzianum.*

3.2 Antagonistic activity

Significant effects of antagonists, evaluation time and the interaction antagonist-time on the growth reduction of the four pathogens were shown (Table 2). Micro-organisms effect on the reduction of growth of *A. triticimaculans, B. sorokiniana, D. tritici-repentis* and *S. tritici* was different according to the pathogen and evaluation time. A general tendency of a better antagonistic effect after a greater

Table 2. Mean square of Inhibition Index of the 10 antagonists against *Alternaria triticimaculans, Bipolaris sorokiniana, Drechslera tritici-repentis* and *Septoria tritici*
, * Significant at P = 0.01 and 0.001, respectively
Tab. 2. Durchschnittliche Quadratzahl des Hemmungsindex der 10 Antagonisten gegen *A. triticimaculans, B. sorokiniana, D. tritici-repentis* und *S. tritici*

SOURCE OF VARIATION	*A. triticimaculans*	*B. sorokiniana*	*D. tritici-repentis*	*S. tritici*
ANTAGONISTS	4.71***	0.50***	3.50***	0.11***
TIME	6.95***	0.94***	5.72***	0.81***
ANTAGONIST × TIME	0.31***	0.38***	0.37***	0.01**
RESIDUAL	0.03	0.00	0.01	0.07

exposure time was performed (Table 3). Except for *R. rubra,* which showed an inhibition index of 1.01, all antagonists appreciably reduced growth of *A. triticimaculans* on PDA (Table 4). Bw/97 was the most pronounced and consistent inhibitor of the growth of the pathogen in all the evaluation times, followed by *N. sphaerica* and *A. niger.* With respect to *B. sorokiniana,* multiple comparisons showed that *P. lilacinum* performed significantly better than the rest of antagonists. At the other extreme, the white yeast and *E. nigrum* presented the less inhibition index. In the case of *D. tritici-repentis, A. niger* performed significantly better than any other antagonist did. On the contrary, Bw/97 showed the worst behaviour. For *S. tritici,* except *E. nigrum,* all the antagonists tested had a significant inhibitory effect over the pathogen growth, although this effect was not very important. Considering the average of the four pathogens, *A. niger, N. sphaerica* and Bw/97 showed the best antagonist effect considering the average of the four pathogens (1.81; 1.60 and 1.57, respectively).

For *A. triticimaculans, A. niger, Ch. globosum, N. sphaerica* and Bw/97 showed the best performance from the beginning of evaluation. On the other hand, *Stemphylium* sp. and *R. rubra* exerted little or not effect (Table 5). With respect to *B. sorokiniana,* the antagonists Bw/97, *Cryptococcus* sp., *E. nigrum* and *R. rubra* showed little or non-significant differences in their behaviour against the pathogen during all the evaluation times tested (Table 6). Regarding *D. tritici-repentis,* a good inhibitory effect of *A. niger* after 4 days of incubation was shown. A group of antagonists manifested later their antagonistic effect,

Table 3. Inhibition Index after 2, 4, 6, 8, 10 and 12 days of incubation of the 10 antagonists and *Alternaria triticimaculans, Bipolaris sorokiniana, Drechslera tritici-repentis* and *Septoria tritici* in dual cultures on agar media.
Means followed by the same letter indicate no differences according to Tukey's test (P = 0.05)

Tab. 3. Hemmungsindex nach 2, 4, 6, 8, 10 und 12 Tagen Inkubation der 10 Antagonisten und *A. triticimaculans, B. sorokiniana, D. tritici-repentis* und *S. tritici* in Doppelkulturen auf Nährboden

	INHIBITION INDEX			
TIME	*A. triticimaculans*	*B. sorokiniana*	*D. tritici-repentis*	*S. tritici*
2	1.11 a	1.12 a	1.11 a	1.02 a
4	1.41 b	1.19 b	1.28 b	0.99 a
6	1.60 c	1.21 b	1.66 c	1.10 b
8	1.85 d	1.29 c	1.88 d	1.15 b
10	2.15 e	1.39 d	1.97 e	1.26 c
12	2.15 e	1.54 e	2.01 e	1.37 d

Table 4. Inhibition Index of each antagonist against *Alternaria triticimaculans, Bipolaris sorokiniana, Drechslera tritici-repentis* and *Septoria tritici* on agar media.
Means followed by the same letter indicate no differences according to Tukey's test (P = 0.05)

Tab. 4. Hemmungsindes eines jeden Antogonisten gegen *A. triticimaculans, B. sorokiniana, D. tritici-repentis* und *S. tritici* auf Nährboden

ANTAGONIST	*A. triticimaculans*	*B. sorokiniana*	*D. tritici-repentis*	*S. tritici*
A. niger	2.12 e	1.23 bc	2.68 f	1.21 ef
Bw/97.	2.51 f	1.27 c	1.25 a	1.25 f
Cryptococcus sp.	1.40 b	1.21 abc	1.45 bc	1.13 bcde
Ch. globosum	1.79 d	1.26 c	1.69 e	1.14 bcde
E. nigrum	1.58 bc	1.14 a	1.44 b	1.00 a
F. moniliforme var. *anthophilum*	1.51 bc	1.21 abc	1.62 de	1.13 bcde
N. sphaerica	2.18 e	1.40 d	1.65 de	1.16 cdef
P. lilacinum	1.61 cd	1.64 e	1.57 d	1.10 b
R. rubra	1.01 a	1.18 ab	1.63 de	1.12 bc
Stemphylium sp.	1.41 b	1.35 d	1.56 cd	1.18 cdef

e. g., *E. nigrum, Ch. globosum, Stemphylium* sp., *P. lilacinum, Cryptococcus sp., N. sphaerica* and *Bacillus sp.* (Table 7). Considering *S. tritici,* it was observed that in general, there were no differences between micro-organisms effect during the first 2–8 days after exposure. On the contrary, from 10–12 days, the micro-organisms exerted the most pronounced antagonistic effects (Table 8).

3.3 Colony interactions

The antagonism types observed on direct opposition plates between the tested phylloplane saprobes against *A. triticimaculans, B. sorokiniana, D. tritici-repentis* and *S. tritici* are shown in Table 9. The antagonistic effects differed according to each particular combination pathogen-antagonist respect to the controls (Fig. 2). Among the interactions tested, only a few were mutually antagonistic and unilateral inhibition capacities were prevalent. A greater variability of the different types of interaction (A, B, C, D) was observed in all combinations that involve *A. triticimaculans, D. tritici-repentis and S. tritici* (Fig. 3, 4, and 5a, b, c, d). There was a more uniform behaviour between *B. sorokiniana* and the

Table 5. Inhibition Index of *Alternaria triticimaculans* after 2, 4, 6, 8, 10 and 12 days of incubation in dual cultures with the 10 antagonists on agar media.
Means followed by the same letter indicate no differences according to Tukey's test (P = 0.05)

Tab. 5. Hemmungsindex von *A. triticimaculans* nach 2, 4, 6, 8, 10 und 12 Tagen Inkubation in Doppelkulturen mit den 10 Antagonisten auf Nährboden

EVALUATION TIME	*E. nigrum*	*Ch. globosum*	*F. moniliforme var. anthophilum*	*Stemphylium sp.*	*A. niger*	*P. lilacinum*	*Cryptococcus sp.*	*R. rubra*	*N. sphaerica*	*Bw/97*
2	1.17 a	1.13 a	1.07 a	1.03 a	1.09 a	1.00 a	1.01 a	1.04 a	1.10 a	1.49 a
4	1.25 ab	1.35 b	1.15 ab	1.28 a	1.41 a	1.30 ab	1.21 ab	1.00 a	1.44 a	2.15 b
6	1.38 ab	1.86 bc	1.43 abc	1.07 a	2.04 b	1.56 abc	1.43 ab	1.04 a	2.10 b	2.75 c
8	1.76 b	2.00 c	1.67 bc	1.00 a	2.53 bc	1.81 bc	1.50 b	0.99 a	2.61 bc	2.68 bc
10	1.98 c	2.21 c	1.87 c	2.06 b	2.84 c	2.00 c	1.64 b	1.01 a	2.93 c	3.00 c
12	1.98 c	2.21 c	1.87 c	2.06 b	2.84 c	2.00 c	1.64 b	1.00 a	2.93 c	3.00 c

Table 6. Inhibition Index of *B. sorokiniana* after 2, 4, 6, 8, 10 and 12 days of incubation in dual cultures with the ten antagonists on agar media.
Means followed by the same letter indicate no differences according to Tukey's test (P = 0.05)

Tab. 6. Hemmungsindex von *B. sorokiniana* nach 2, 4, 6, 8, 10 und 12 Tagen Inkubation in Doppelkulturen mit den 10 Antagonisten auf Nährboden

EVALUATION TIME	*E. nigrum*	*Ch. globosum*	*F. moniliforme var. anthophilum*	*Stemphylium sp.*	*A. niger*	*P. lilacinum*	*Cryptococcus sp.*	*R. rubra*	*N. sphaerica*	*Bw/97*
2	1.04 a	1.06 a	1.02 a	1.16 a	1.06 a	1.08 a	1.16 a	1.12 a	1.18 a	1.33 ab
4	1.15 a	1.13 ab	1.14 ab	1.19 ab	1.07 a	1.55 b	1.15 a	1.11 a	1.29 ab	1.16 a
6	1.08 a	1.23 abc	1.17 ab	1.29 ab	1.13 ab	1.57 b	1.15 a	1.05 a	1.32 ab	1.14 a
8	1.11 a	1.37 bc	1.19 abc	1.42 bc	1.25 abc	1.67 bc	1.22 a	1.12 a	1.46 bc	1.15 a
10	1.17 a	1.39 bc	1.32 bc	1.49 bc	1.37 bc	1.83 c	1.25 a	1.25 ab	1.47 bc	1.37 ab
12	1.34 b	1.40 c	1.44 c	1.59 c	1.50 c	2.14 d	1.38 a	1.46 b	1.71 c	1.48 ab

Table 7. Inhibition Index of *D. tritici-repentis* after 2, 4, 6, 8, 10 and 12 days of incubation in dual cultures with the ten antagonists on agar media.
Means followed by the same letter indicate no differences according to Tukey's test (P = 0.05)

Tab. 7. Hemmungsindex von *D. tritici-repentis* nach 2, 4, 6, 8, 10 und 12 Tagen Inkubation in Doppelkulturen mit den 10 Antagonisten auf Nährboden

EVALUATION TIME	*E. nigrum*	*Ch. globosum*	*F. moniliforme var. anthophilum*	*Stemphylium sp.*	*A. niger*	*P. lilacinum*	*Cryptococcus sp.*	*R. rubra*	*N. sphaerica*	*Bw/97*
2	1.00 a	1.47 a	1.22 a	1.08 a	1.09 a	1.02 a	1.05 a	1.20 a	1.00 a	0.98 a
4	0.99 a	1.37 ab	1.13 a	1.19 a	1.72 b	1.19 a	1.16 a	1.30 ab	1.34 ab	1.44 a
6	1.34 b	2.10 ab	1.62 b	1.38 a	2.65 c	1.44 a	1.30 a	1.62 bc	1.53 b	1.65 ab
8	1.69 c	1.66 bc	1.83 b	1.75 b	3.36 d	1.83 b	1.65 b	1.83 c	1.91 c	1.29 ab
10	1.80 c	1.74 bc	1.94 b	1.94 b	3.57 d	1.94 b	1.75 b	1.94 c	2.03 c	1.07 bc
12	1.85 c	1.79 c	2.00 b	2.99 b	3.67 d	1.00 b	1.80 b	1.88 c	2.08 c	1.10 c

Table 8. Inhibition Index of *S. tritici* after 2, 4, 6, 8, 10 and 12 days of incubation in dual cultures with the ten antagonists on agar media.
Means followed by the same letter indicate no differences according to Tukey's test (P = 0.05)

Tab. 8. Hemmungsindex von *S. tritici* nach 2, 4, 6, 8, 10 und 12 Tagen Inkubation in Doppelkulturen mit den 10 Antagonisten auf Nährboden

EVALUATION TIME	*E. nigrum*	*Ch. globosum*	*F. moniliforme var. anthophilum*	*Stemphylium sp.*	*A. niger*	*P. lilacinum*	*Cryptococcus sp.*	*R. rubra*	*N. sphaerica*	*Bw/97*
2	0.91 ab	1.10 ab	1.06 ab	1.04 ab	1.05 a	0.96 a	0.96 a	1.02 a	1.02 a	1.13 a
4	0.88 a	1.00 a	1.00 a	1.99 a	1.04 a	1.02 ab	0.97 a	0.99 a	1.98 a	1.09 a
6	0.97 ab	1.08 ab	1.19 ab	1.15 ab	1.14 ab	1.14 ab	1.06 ab	1.04 a	1.07 a	1.24 a
8	1.05 ab	1.13 abc	1.05 a	1.22 ab	1.19 ab	1.11 ab	1.20 ab	1.15 ab	1.17 ab	1.24 a
10	1.11 ab	1.30 bc	1.19 ab	1.35 bc	1.34 b	1.13 ab	1.23 ab	1.22 ab	1.34 bc	1.40 b
12	1.13 b	1.36 c	1.34 b	1.52 c	1.51 c	1.25 b	1.38 b	1.31 b	1.51 c	1.41 b

Table 9. Interactions observed between adjacent pathogen-antagonist colonies on agar media. Types of antagonism based on the observations of PORTER (1924)

Tab. 9. Interaktionen zwischen benachbarten Kolonien von Pathogen und Antagonisten auf Nährboden. Der Typ des Antagonismus beruht auf Beobachtungen von PORTER (1924)

ANTAGONISTS	*S. tritici*	*B. sorokiniana*	*D. tritici-repentis*	*A. triticimaculans*
E. nigrum	C	D	D	D
Ch. globosum	B	D	D	C
F. moniliforme var. anthophilum	B	C	C	C
Stemphylium sp.	B	D	A	C
A. niger	B	D	B	B
P. lilacinum	B	C	D	D
Cryptococcus sp.	A	D	C	C
R. rubra	A	D	B	B
N. sphaerica	B	B	B	B
Bw/97	D	B	D	C

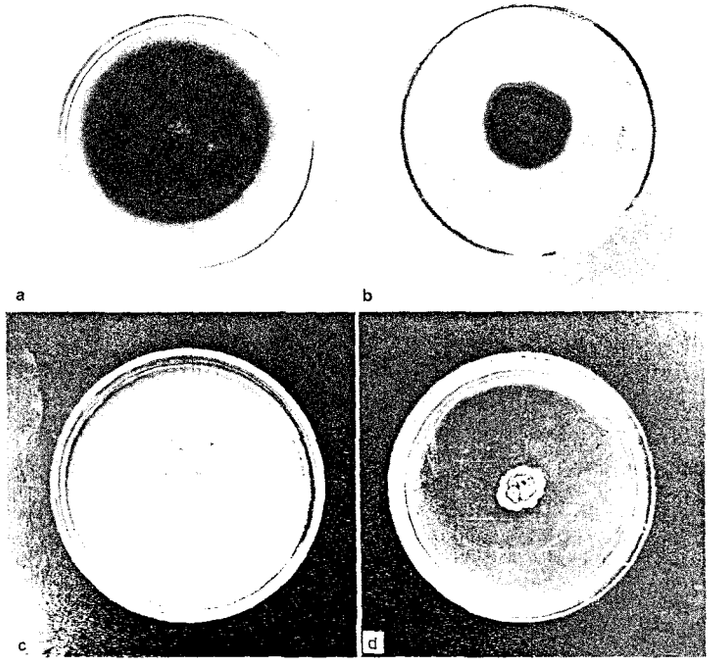

Fig. 2. [illegible] of the pathogens [illegible] on PDA (2 %). a. *A. triticimaculans*; b. *B. sorokiniana*; c. *D. triticirepentis*; d. *S. tritici*.
Abb. 2. [illegible] der Pathogene [illegible] auf Nährboden PDA (2 %). a. *A. triticimaculans*; b. *B. sorokiniana*; c. *D. triticirepentis*; d. *S. tritici*.

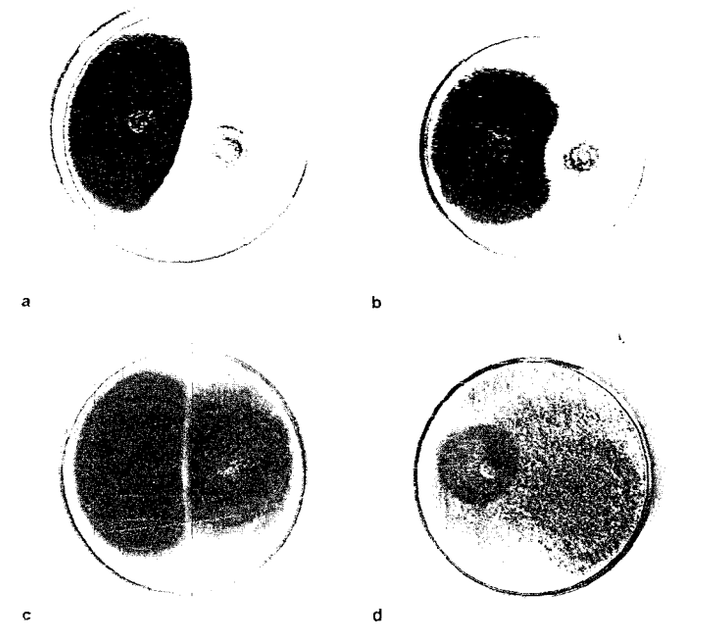

Fig. 3. Dual culture on PDA (2 %) of *Alternaria triticimaculans* (left) and: a. *Penicillium lilacinum* (Type of antagonism D); b. *Epicoccum nigrum* (Type of antagonism D); c. *Stemphylium* sp. (Type of antagonism C); and d. *Nigrospora sphaerica* (Type of antagonism A and B).
Abb. 3. Doppelkultur auf PDA (2 %) von *A. triticimaculans* (links) und: a. *P. lilacinum* (Antagonismus Typ D); b. *E. nigrum* (Antag. Typ D); c. *Stemphylium* sp. (Antag. Typ C) und d. *N. sphaerica* (Antag. Typ A und B).

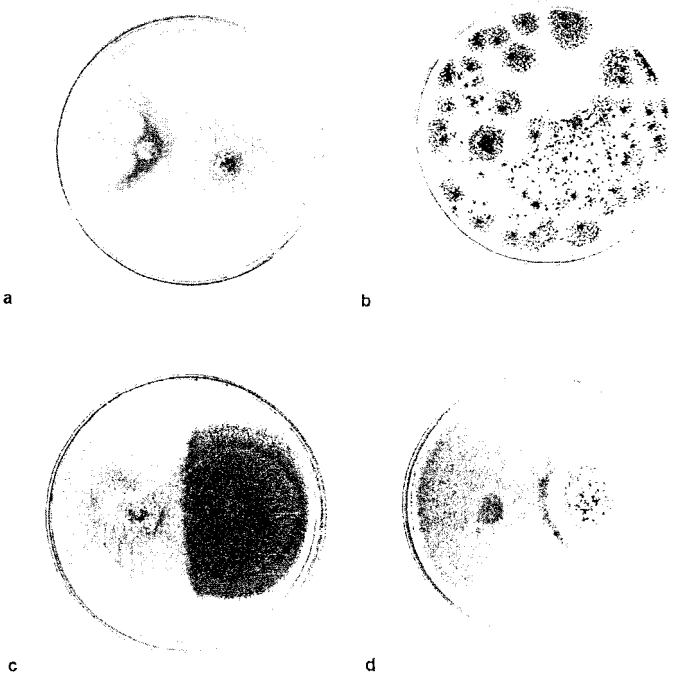

Fig. 4. Dual culture on PDA (2 %) of *Drechslera tritici-repentis* (left) and: a. *Chaetomium globosum* (Type of antagonism C); b. *Aspergillus niger* (Type of antagonism B); c. *Stemphylium* sp. (Type of antagonism A) and d. *Epicoccum nigrum* (Type of antagonism C).
Abb. 4. Doppelkultur auf PDA (2 %) von *D. tritici-repentis* (links) und: a. *C. globosum* (Antagonismus Typ C); b. *A. niger* (Antag. Typ B); c. *Stemphylium* sp. (Antag. Typ A) und d. *E. nigrum* (Antag. Typ C).

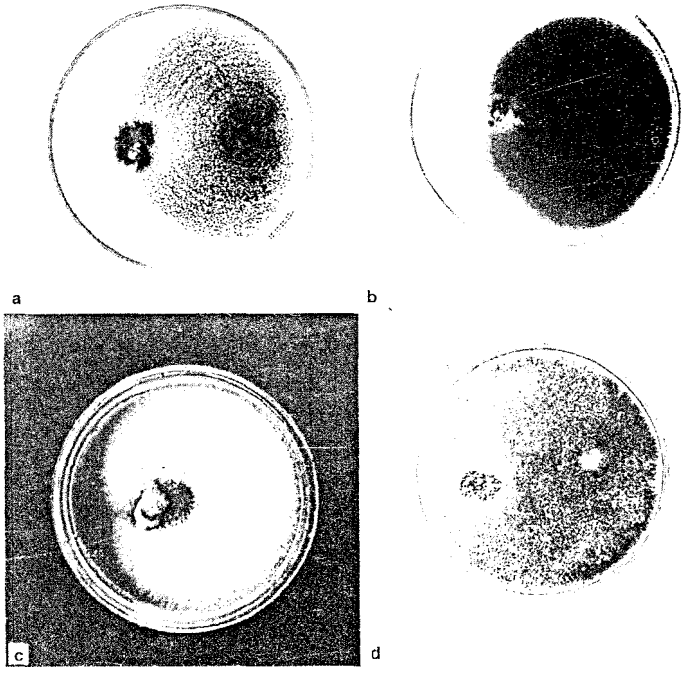

Fig. 5. Dual culture on PDA (2 %) of *Septoria tritici* (left) and: a. *Aspergillus niger* (Type of antagonism B) b. *Stemphylium* sp. (Type of antagonism B) c. *Fusarium moniliforme var. anthophilum* (Type of antagonism B and C) and d. *Nigrospora sphaerica* (Type of antagonism B).
Abb. 5. Doppelkultur auf PDA (2 %) von *S. tritici* (links) und : a. *A. niger* (Antagonismus Typ B); b. *Stemphylium* sp. (Antag. Typ B); c. *F. moniliforme* var. *anthophilum* (Antag. Typ B und C) und d. *N. sphaerica* (Antag. Typ B).

leaf surface micro-organisms tested. In this case, inhibition zones of 0.1 mm or more (types C and D) were registered (Fig. 6a, b, c, d). A strong inhibition of *S. tritici* occurred in direct opposition of many of the saprophytes inhabiting the phylloplane, being type B the dominant. Of the saprophytes tested, *A. niger* and *N. sphaerica* generally induced antagonism type B, while *F. moniliforme* var. *anthophilum* induced antagonism type C or D, against most of the pathogen tested. Only in the combination *D. tritici-repentis-R. rubra* the fungal pathogen grew across the colony of the candidate micro-organism and overpassed them (Fig. 7).

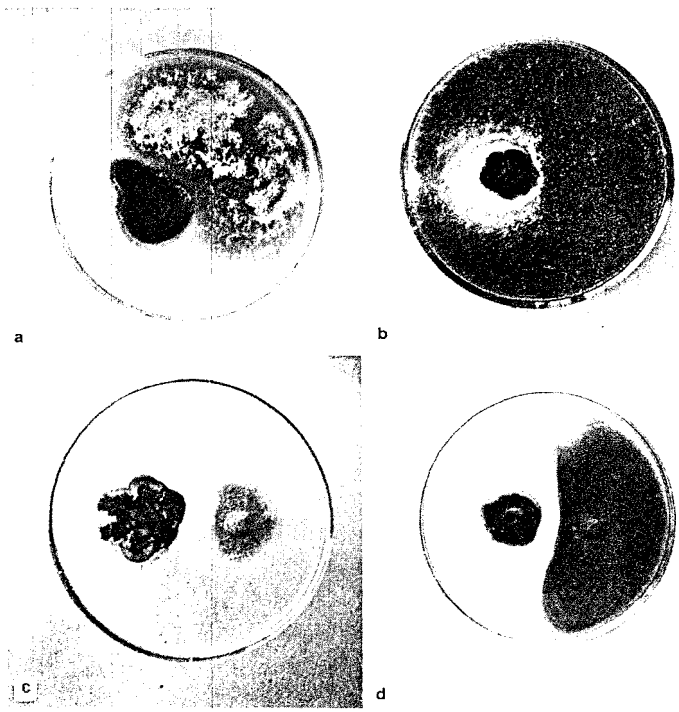

Fig. 6. Dual culture on PDA (2 %) of *Bipolaris sorokiniana* (left) and: a. *Fusarium moniliforme* var. *anthophilum*. Type of antagonism C; b. *Nigrospora sphaerica*. Type of antagonism B and D); c. *Epicoccum nigrum* (Type of antagonism D and d. *Stemphylium* sp. Type of antagonism D).
Abb. 6. Doppelkultur auf PDA (2 %) von *B. sorokiniana* (links) und: a. *F. moniliforme* var. *anthophilum* (Antagonismus Typ C); b. *N. sphaerica* Antag. Typ B und D; c. *E. nigrum* (Antag. Typ D) und d. *Stemphylium* sp. Antag. Typ D).

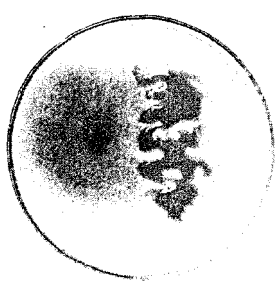

Fig. 7. Dual culture of *Drechslera tritici-repentis* (left) and *R. rubra*. The pathogen overpassed the antagonist colony (Type of antagonism B).
Abb. 7. Doppelkultur von *D. tritici-repentis* (links) und *R. rubra*. Das Pathogen überwächst die Kolonie des Antagonisten (Typ B).

4 Discussion

Drawing some conclusions from our research on the wheat phylloplane, it can be said that mostly common saprobes were recovered. Also two potential pathogens: *Alternaria alternata* and *Cladosporium herbarum* were found.

Field collection of micro-organisms reported herein might be considered for screening as a first step in detecting potential control agents of wheat diseases. Relevant publications have demonstrated that the saprophytic microflora plays an important role acting as competitor and reducing the incidence of several foliar diseases (ANDREWS 1985, 1990, 1992a, b; BAKER and COOK 1974; BETTIOL 1991; BLAKEMAN 1985; BLAKEMAN and FOKKEMA 1982; COOK 1993; COOK and BAKER 1983; DUBOS 1987; DUBOS and BULIT 1981; FOKKEMA 1993; LEBEN 1965; WINDELS and LINDOW 1985).

DICKINSON and SKIDMORE (1976) and MANGIAROTTI et al. (1987) showed that wheat leaves surface are covered by a rich microflora native composed mainly by bacteria, yeast and filamentous fungi. In this work, a microflora composed mainly of Hyphomycetes was determined from wheat leaf isolates. As far as filamentous fungi are concerned, *Cladosporium* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp. and *Epicoccum* spp. are some of the most common registered genera (LUZ 1991). As in this work, these fungi were abundant on leaves of many others plants, and grouped by HUDSON (1968) as common primary saprophytes. Some of the fungi isolated could be considered as residents of wheat phylloplane according to LEBEN (1965) and DICKINSON (1976); others, as casuals (e. g., *Trichoderma* sp., *Gliocladium* sp.), invaders or exotones (e. g., *Cryptococcus* sp.) according to PARK classification (1957). Moreover, we can note that some of them, e. g., *A. niger, E. purpurascens, F. moniliforme* var. *anthophilum* and *Penicillium* sp., were previously recognised as antagonists of a great potential against other phytopathogenic fungi of cereals (FOKKEMA 1973; RAI and SINGH 1980). In many cases, antagonism is likely to be due to nutrient competition restricting superficial hyphal development and consequently reducing infection (BASHI and FOKKEMA 1977; FOKKEMA 1973).

With respect to yeast fungi, it is known that *Sporobolomyces* sp. and *Rhodotorula* sp. were efficient in the control of *Bipolaris sorokiniana* and *Leptosphaeria nodorum* (LUZ 1982a, b). Results of FOKKEMA et al. (1979) indicated that infection of necrotrophic leaf spotting pathogens of wheat could be reduced by yeast by about 50 %. In our work, the white and the pink yeasts did not show a good antagonistic behaviour *in vitro*. However, previous experiences on wheat plants under greenhouse conditions showed the contrary (PERELLÓ 1998; PERELLÓ et al. 1998b).

Attempts to use bacteria to control fungal diseases of the wheat phylloplane *in vitro* and under field conditions, was pointed out by several authors (BETTIOL 1991; LEVY and EYAL 1988; LEVY et al. 1989, 1992; LI, 1991; LI and SUTTON 1995; MEHDIZADEGAN and GOUGTH 1987; D'ERCOLE et al. 1983; ROBBS 1991; SPURR and KNUDSEN 1985). *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 the application of chemical products (LEVY et al. 1989; LUZ 1991). The results of this experiment, and our previous studies in Argentina about the potential of *Bacillus, Paenibacillus* and *Brevibacillus* in the biocontrol of wheat foliar diseases are in agreement with the stressed (PERELLÓ 1998; ALIPPI et al. 1999).

The preliminary results obtained *in vitro* showed a great potential for the development of biological control of wheat diseases in our country. *In vitro* assays do not necessarily reflect the relative importance of events *in vivo*. However, information from plate trials provide a starting point for testing hypotheses as to how antagonistic organisms may function on the wheat phylloplane. Several methods are available to control wheat foliar pathogens, but it is well known that no single control method can solve the problems, and that integrated management is required (LECUONA 1990; COOK and VESETH 1991; COSCIA 1991; JACOBSEN and BLAKEMAN 1993).

A sustained long-term programme of integrated control could benefit with the inclusion of biological control as a suitable method for decreasing incidence and severity of several foliar diseases of wheat in Argentina. Greenhouse experiments are in progress.

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