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Biological control of *Fusarium graminearum sensu stricto*, causal agent of Fusarium head blight of wheat, using formulated antagonists under field conditions in Argentina

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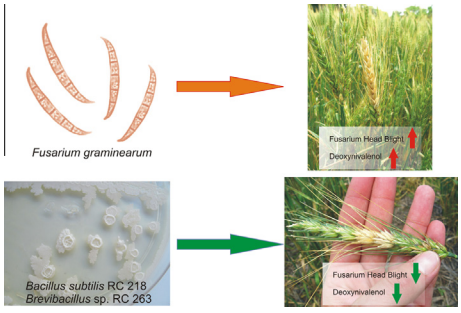
HIGHLIGHTS

- Biological control of Fusarium head blight in wheat.
- Two bacterial strains as biocontrol agents against *Fusarium graminearum*.
- Biocontrol agents reduced disease severity by 42–76%.
- Biocontrol agents reduced deoxynivalenol on spikes to undetectable levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Fusarium head blight (FHB) mainly caused by *Fusarium graminearum* is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. The biocontrol effect of two bacterial strains on FHB incidence, severity and deoxynivalenol (DON) accumulation in wheat was evaluated in field trials during 2010 and 2011 at Marcos Juarez, Córdoba province, Argentina. *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 applied at anthesis period were evaluated through several combinations of cell type, strains, inoculum density (10^4 and 10^6 cfu/ml) and physiological modification. A significant and consistent biocontrol effect on FHB severity and DON contamination was observed in all the evaluated treatments during both 2010 and 2011 field trials. Reduction in FHB severity ranged 62–76% and 42–58% for 2010 and 2011 field trials, respectively. When evaluating the effect of the combined strains ($10^4 + 10^4$ and $10^6 + 10^6$ cfu/ml), a better biocontrol effect was observed in 2010 field trial. After biocontrol treatments, no DON accumulation was observed in wheat heads; meanwhile in control plots an average of 1372 μg/kg DON was detected during the two trials. FHB incidence was significantly reduced by biocontrol treatments during the 2010 field trial but not during the 2011 field trial. The results showed the effectiveness of the two formulated biological control agents in reducing both FHB severity and DON accumulation by *F. graminearum* under semi controlled field conditions.

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1. Introduction

Fusarium head blight (FHB) mainly caused by *Fusarium graminearum sensu stricto* is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. Besides the economic losses due to reduction in grain yield, the main problem is the potential mycotoxin contamination of wheat mainly with deoxynivalenol (DON) (McMullen et al., 2012). During the last 50 years, several epidemics of FHB of varying degrees of severity have occurred in Argentina and *F. graminearum* "*sensu stricto*" was isolated as the main pathogen associated with FHB (Dalcero et al., 1997). In 1993, during a severe FHB outbreak, the highest estimated losses reached 50% in areas with no-tillage after maize crops. The extent of the damage was magnified by a considerable loss in grain trading value resulting from low grain weight, the presence of scabby grains, and DON contamination (Kikot et al., 2011; Ramirez et al., 2007).

Different strategies are used to reduce the impact of FHB including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars. Among these strategies, fungicide control seems to be the most effective (Homdork et al., 2000; Mesterházy et al., 2011), although it was observed that certain fungicides could increase DON content on grains (Ramirez et al., 2004) and pathogens can generate fungicide resistance (Yuan and Zhou, 2005). Genetic resistance is also a viable option, but at present no successful results have been achieved (Bai et al., 2000, 2001; Buerstmayr et al., 2009; Miedaner and Korzun, 2012; Talas et al., 2012). None of these strategies by themselves are able to reduce the impact of FHB (Dill-Macky and Jones, 2000; Hollins et al. 2003). Biological control offers an additional strategy and can be used as part of an integrated management of FHB.

Anthesis is the stage of greatest susceptibility for *F. graminearum* infection. It is presumed that anthers are the common pathogen entry route into the plant (McMullen et al., 2012). Thus, antagonists with high ecological competence in this niche may prevent infection during anthesis when conditions for the pathogen and antagonists, temperature and humidity, are adequate (Khan et al, 2001). In fact, biocontrol agents (BCA) against the pathogen causing FHB have been evaluated using this application strategy. Nevertheless, formulation of a BCA applied during anthesis has not been fully developed since applications were done with bacterial broths and culture supernatants (da Luz et al., 2003; Khan et al., 2004; Khan and Doohan, 2009; Palazzini et al., 2007; Schisler et al., 2006).

Spray-drying technology for BCAs after their mass production in liquid fermentation systems allows a high processing rate with almost continuous production at low operation costs and short operation time so that production costs are 30–50 fold lower compared to freeze drying technologies (Lian et al., 2002; Silva et al., 2005; Xueyong et al., 2008). However, the production process can drastically affect the viability of biocontrol agents, especially bacteria and yeasts (Abadias et al., 2005; Silva et al., 2005). In order to conserve viability during the drying process, several attempts of physiological improvement have been done to increase desiccation tolerance during this process (Cañamas et al., 2007; Montazeri and Greaves, 2002; Teixidó et al., 2006). In some studies a better FHB control was achieved after improving the BCA quality by changing carbon and nitrogen ratios during fermentation (Zhang et al., 2005), including additives such as chitosan (Khan and Doohan, 2009) and enhancing bioformulated survival by nutrient amendments (Schisler et al., 2004). In previous studies, we have demonstrated that physiologically modified strains of *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 were effective biocontrol agents to control FHB under greenhouse conditions (Palazzini et al., 2009).

The objectives of the present study were to evaluate the effect of two formulated antagonists (*B. subtilis* RC 218 and *Brevibacillus* sp. RC 263) applied alone or in combination, at two level doses (10^4 and 10^6 cfu ml^{-1}) on: - Fusarium head blight incidence and severity and DON accumulation on wheat spikes under field conditions.

2. Materials and methods

2.1. Biocontrol strains, biomass production and formulation

B. subtilis RC 218 and *Brevibacillus* sp. RC 263 strains used in this study were originally isolated from wheat anthers as potential biocontrol agents against *F. graminearum* in Argentina (Palazzini et al., 2007, 2009). These strains are maintained in the culture collection Department of Microbiology and Immunology at Universidad Nacional de Río Cuarto; Río Cuarto, Córdoba, Argentina). Biomass of *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 was produced in liquid basic medium (sucrose 10 g/l, yeast extract 5 g/l) described by Costa et al. (2001) with an incubation of 48 h at 28 °C in a rotatory shaker (150 rpm). Additionally, liquid media was modified with NaCl (a_W 0.97) for *B. subtilis* RC 218 biomass production in order to obtain a physiological improvement of the strain by intracellular accumulation of betaine (Palazzini et al., 2009). After biomass production, cells were centrifuged at 10,000 rpm for 5 min, washed with sterile distilled water, centrifuged again and, finally, resuspended in sterile skimmed milk (20% w/v), as a protective agent during spray drying as the final formulation step (Palazzini et al., 2010).

Biomass of *B. subtilis* RC 218 was also produced in a 50 l fermentor by Bio-ferm GmbH (Tulln, Austria) in order to obtain bacterial spores. These bacterial spores were freeze dried and also tested in the field trials.

2.2. Pathogen inoculum production

Two strains of *F. graminearum*, RC276 and KRC7, were used in the field trials. These strains were isolated from head blight infected ears from commercial fields located in Pergamino, Buenos Aires, Argentina. Toxigenic profiles were determined in a previous study (Palazzini et al., 2007). *F. graminearum* conidia were produced in Mung bean broth (Rosewich Gale et al., 2002). After 7–10 days of incubation at 25 °C and 200 rpm on a rotatory shaker, cultures were centrifuged (7000 rpm; 5 min), resuspended in sterile distilled water plus Tween 80 (0.05%) and filtered through sterile gauze to obtain a conidia suspension. Macroconidia concentration was determined using a haemocytometer and conidia concentration was adjusted to 5×10^5 conidia/ml (1:1 mixture of RC276 and KRC7 strains).

2.3. Field trials

Two field trials were conducted in Marcos Juarez, Córdoba province, Argentina, during the 2010 and 2011 harvest seasons. The brad wheat (*Triticum aestivum* L.) cultivar BioInta 1005 (susceptible to *F. graminearum*) was sown at the end of July during both trials. During the 2010 harvest season, the experimental plots consisted of two rows (1 m/row, 0.2 m between rows; 80 heads per plot) with three replicates per treatment. During 2011 field trial, the experimental plots consisted of 3 rows (2 m/row, 0.2 m between rows; 250 heads per plot) with three replicates per treatment. The experiments were done in a random block design with 1 m separation between plots. Temperature in the field plots was monitored by an Agro-climatic station located in the experimental fields.

2.3.1. Treatments and application time

During the field trials, the application of the biocontrol agents was done at the anthesis stage with co-inoculation of *B. subtilis* RC 218 and/or *Brevibacillus* sp. RC 263 and *F. graminearum* strain mixture. The anthesis stage was considered at the period where 50% of the heads in the plots were at flowering stage (Feekes stage 10.5.2–10.5.3; Wiese, 1987). Before applications of the BCA, wheat heads were always misted with water for 2 min in order to increase the humidity in the heads. The formulated biocontrol agents were resuspended in sterile distilled water + Tween 80 (0.05%) and allowed to stabilize for 30 min before application. The viability of the biocontrol agents was evaluated by plate counting. Control negative plots were treated with sterile distilled water + Tween 80 (0.05%). Treatments evaluated during field trials consisted in single or mixed bacteria at two level doses (10^4 and 10^6 cfu/ml). In the case of *B. subtilis* RC 218, additional treatments consisted in physiologically improved cells (vegetative cells) and also a spore treatment. All treatments evaluated are described in Table 1.

2.3.2. Inoculum application

Bacterial and *F. graminearum* suspensions were applied using a commercial sprayer consisting of 5 linear sprinklers and a CO_2 pressure source. The sprayer was adjusted to 30 mbar and flow to 15 ml per second. Application was done at a rate of 15 ml per linear meter for all treatments.

2.3.3. Humidity control in the field trials

During the field trials, wheat plots were misted with water for 5 min every 30 min from 8:00 am to 18:00 pm for six days after inoculation. Water sprinklers (fine misting) were located between the plots and also surrounding them.

2.3.4. FHB evaluation

FHB incidence and severity were evaluated 21 days after inoculation with *F. graminearum*. FHB incidence was determined by counting infected heads and divided from the total spikes of the plot (treatment replicate); FHB severity was evaluated by observing symptomatic spikelets (decoloured, browny) and visually compared with a 0–100% scale proposed by Stack and McMullen (1995).

2.3.5. Evaluation of deoxynivalenol accumulation in wheat heads

At harvest, wheat heads were collected to determine DON concentration in entire heads. Toxin extraction was done using the methodology originally proposed by Cooney et al. (2001) with small modifications. Briefly, a sub-sample (15 g) was extracted by mixing with acetonitrile/methanol (14:1; 40 ml), shaken for 2 h and then filtered through filter paper (Whatman N°1). Additionally, blank wheat samples were used as negative control. A syringe was plugged with glass wool and dry-packed with alumina/carbon (20:1; 500 mg) to form a mini-clean up column. A 2 ml aliquot of extract was applied to the column and allowed to drain under gravity and the eluant collected. The column was washed with acetonitrile/methanol/water (80:5:15; 500 µl), and the combined eluant was evaporated to dryness (N_2, 50 °C). The cleaned-up residue was dissolved in methanol/water (5:95; 500 µl). DON concentration was determined by liquid chromatography using the methodology described by Palazzini et al. (2007).

Table 1
Treatments evaluated at 2010 and 2011 field trials.

Antagonist treatment	Concentration (CFU ml^{-1})
B. subtilis RC 218	10^6
Brevibacillus sp. RC 263	10^6
B. subtilis RC 218 (NaCl a_W = 0,97)	10^6
B. subtilis RC 218 (spores)	10^6
B. subtilis RC 218	10^4
Brevibacillus sp. RC 263	10^4
B. subtilis RC 218 (NaCl a_W = 0,97)	10^4
B. subtilis RC 218 (spores)	10^4
B. subtilis RC 218 (NaCl a_W = 0,97) + *Brevibacillus* sp. RC 263	$10^6 + 10^6$
B. subtilis RC 218 (NaCl a_W = 0,97) + *Brevibacillus* sp. RC 263	$10^4 + 10^4$
Positive control (*F. graminearum*)Negative control (water + Tween)	–*

* All plots were spray-inoculated with *F. graminearum* at anthesis (5×10^5 conidia ml^{-1}). In an additional treatment, plots were not inoculated with *F. graminearum* (negative control).

2.4. Statistical analyses

FHB incidence data from individual years was subjected to a one way ANOVA and means were separated by Holm-Sidak's method. Additionally, incidence data was subjected to a two way ANOVA considering treatment and year interaction. Disease severity data were subjected to Kruskal–Wallis one way analysis of variance on ranks and means were separated with the multiple comparison Dunn's test. Deoxynivalenol accumulation was expressed as the plot means ± standard deviation. All statistical analyses were performed using SigmaStat for Windows version 3.5 (SPSS Inc.).

3. Results

The biocontrol effect of *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 in simple or combined applications was evaluated against a mixture of *F. graminearum* inoculum during two consecutive years in order to evaluate reduction on FHB incidence and severity, and DON accumulation on grains. Temperature in the field during the 21 days-trial averaged 20.1 and 18.9 °C for 2010 and 2011, respectively. Temperature during the first 6 days during inoculation averaged 19.7 and 18.7 °C for 2010 and 2011, respectively. During 2010 field trials, *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 treatments caused significant reductions in FHB incidence (mean 10.76%) in comparison with the control treatments (mean 22%) ($P \leqslant 0.001$; Fig. 1); but when comparing FHB incidence between treatments, no statistically significant differences were observed ($P \leqslant 0.001$). During the 2011 field trial, FHB incidences were not statistically different between the biocontrol treatments and the positive control (mean FHB incidence 6.02 and 8%, respectively, Fig. 1). Negative control plots (inoculated with sterile distilled water plus Tween 80) showed a FHB incidence of 2%. In general, lower FHB incidences were observed during the 2011 trial in comparison with the 2010 field trial. Data on incidence was subjected to a two way ANOVA analysis considering treatment and year variables. Statistical differences on either treatments or years were observed (Table 2) but no statistically significant interaction was observed in FHB incidence comparing treatment x year ($P = 0.106$).

FHB severity was reduced by the different biocontrol treatments applied during both 2010 and 2011 field trials. *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 treatments showed significant reductions in FHB severity with values ranging 62–76% and 42–58% for 2010 and 2011 field trials, respectively (Fig. 2). Statistical significant differences were observed between the different biocontrol treatments applied during both 2010 and 2011 trials by data analysis using a multiple comparison Kruskal–Wallis ANOVA on ranks (Dunn's test, $P \leqslant 0.05$). Regarding *Brevibacillus* sp. RC 263 strain, no differences were observed in the dose level applied during both year trials, but when applied together with *B. subtilis*, better biocontrol effect was observed during 2010 field trial.

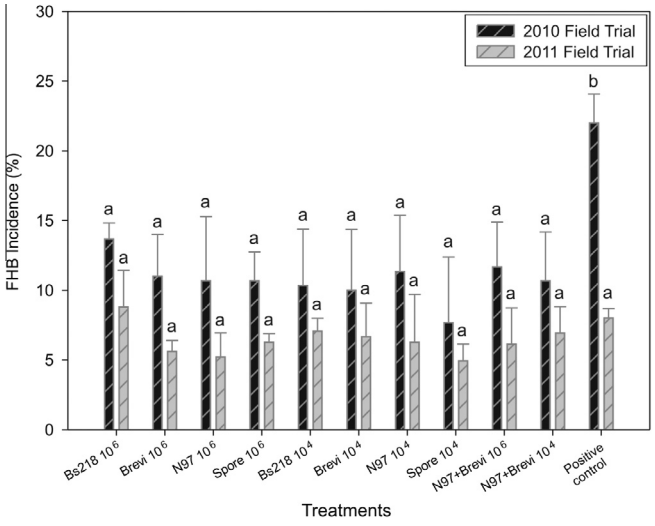

Fig. 1. FHB incidence at Marcos Juarez 2010 and 2011 field trials. Columns with different letters indicate significant differences according to Holm-Sidak's test ($P \leqslant 0.001$). Bars on the columns indicate standard deviation (SD) of replicates. TREATMENTS: *B. subtilis* RC 218 at a concentration of 10^6 cfu/ml; Brevi 10^6: *Brevibacillus* sp. RC 263 at a concentration of 10^6 cfu/ml; N97 10^6: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^6 cfu/ml; Spore 106: *B. subtilis* RC 218 spores at a concentration of 10^6 cfu/ml; Bs218 10^4: *B. subtilis* RC 218 at a concentration of 10^4 cfu/ml; Brevi 10^4: *Brevibacillus* sp. RC 263 at a concentration of 10^4 cfu/ml; N97 10^4: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^4 cfu/ml; Spore 104: *B. subtilis* RC 218 spores at a concentration of 10^4 cfu/ml; Spore 10^4: *B. subtilis* RC 218 spores at a concentration of 10^4 cfu/ml; N97 + Brevi 10^6: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^6 cfu/ml + *Brevibacillus* sp. RC 263 at a concentration of 10^6 cfu/ml; N97 + Brevi 10^4: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^4 cfu/ml + *Brevibacillus* sp. RC 263 at a concentration of 10^4 cfu/ml; Positive control: *F. graminearum* mixture of strains applied at anthesis stage at a concentration of 5×10^5 spores/ml.

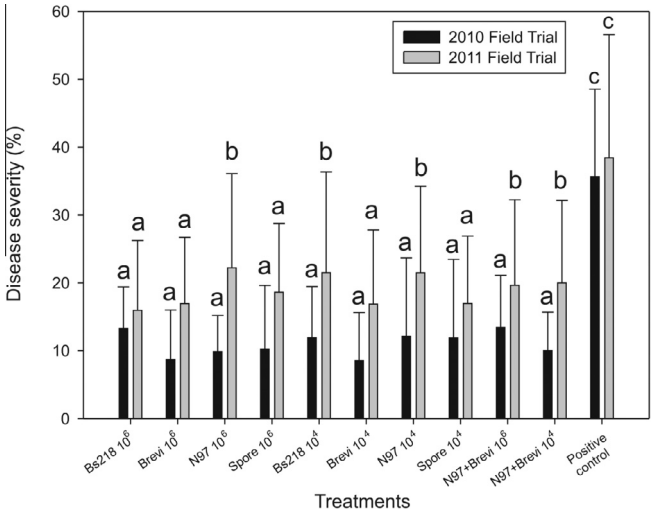

Fig. 2. FHB severity at Marcos Juarez 2010 and 2011 field trials. Columns of the same trial (year) with different letters indicate significant differences according to Dunn's test ($P \leqslant 0.05$). Bars on the columns indicate standard deviation (SD) of replicates. TREATMENTS: Bs218 10^6: *B. subtilis* RC 218 at a concentration of 10^6 cfu/ml; Brevi 10^6: *Brevibacillus* sp. RC 263 at a concentration of 10^6 cfu/ml; N97 10^6: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^6 cfu/ml; Spore 106: *B. subtilis* RC 218 spores at a concentration of 10^6 cfu/ml; Bs218 10^4: *B. subtilis* RC 218 at a concentration of 10^4 cfu/ml; Brevi 10^4: *Brevibacillus* sp. RC 263 at a concentration of 10^4 cfu/ml; N97 10^4: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^4 cfu/ml; Spore 104: *B. subtilis* RC 218 spores at a concentration of 10^4 cfu/ml; Bs218 Spore 10^4: *B. subtilis* RC 218 spores at a concentration of 10^4 cfu/ml; N97 + Brevi 10^6: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^6 cfu/ml + *Brevibacillus* sp. RC 263 at a concentration of 10^6 cfu/ml; N97 + Brevi 10^4: *B. subtilis* RC 218 grown under NaCl modified medium at a concentration of 10^4 cfu/ml + *Brevibacillus* sp. RC 263 at a concentration of 10^4 cfu/ml; Positive control: *F. graminearum* mixture of strains applied at anthesis stage at a concentration of 5×10^5 spores/ml.

Table 2
Two-way Analyses of variance on FHB incidence of Biocontrol treatments applied during two field trials in 2010 and 2011.

Source of variation	df [a]	MS[b]	*F* [c]	*P*
Treatments [d]	10	31.024	3.743	0.001
Year [e]	1	472.539	57.016	<0.001
Treatments × year	10	14.280	1.723	0.106

[a] Degrees of freedom.
[b] Mean square.
[c] Snedecor *F*.
[d] Treatments: different biocontrol combinations applied in the field trials.
[e] Year: Years (2010 and 2011) in which the field trials were carried out.

Treatments involving spores, vegetative cells and physiologically improved *B. subtilis* RC 218 cells (betaine accumulation) showed better effectiveness during 2010 field trial.

Application of the BCA at anthesis stage resulted in reduced DON accumulation to undetectable levels during both 2010 and 2011 field trials. Heads inoculated with the pathogens (positive controls) showed a DON mean level of 1140 ± 280 μg/kg during the 2010 field trial. During the 2011 field trial, the mean of DON in the control plot (positive) was 1605 ± 157 μg/kg. No DON was detected under biocontrol treatments neither in 2010 or 2011 field trials (detection limit: 50 μg/kg).

4. Discussion

From the point of view of wheat quality and safety, reducing the impact of FHB in wheat remains a major challenge. The present study showed the effect of *B. subtilis* RC 218 and *Brevibacillus sp.* RC 263, used as a formulated dry product, in diminishing both FHB severity and DON accumulation under semi-controlled field conditions. Previously, we have shown the selection and effectiveness of these bacteria under greenhouse trials (Palazzini et al., 2007, 2009). The results obtained agree with previous studies, under greenhouse and field trials that showed that species within the genera *Bacillus, Pseudomonas* and *Streptomyces* were also able to reduce *F. graminearum* growth and FHB severity (da Luz et al., 2003; Khan et al., 2004; Khan and Doohan, 2009; Schisler et al., 2002; Schisler et al., 2014). The effectiveness of *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 in reducing FHB severity was similar to that observed by Nourozian et al. (2006), who found that the application of *Streptomyces* sp. on wheat heads reduced FHB severity by approximately 50%. Also, Jochum et al. (2006) reported that the bacteria *Lysobacter enzymogenes* strain C3 was able to reduce FHB severity in 5 of 8 wheat cultivars under field conditions. In addition, the effectiveness of *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 was comparable to yeasts belonging to the genera *Cryptococcus*, which have been effective in controlling the FHB both under greenhouse and field conditions (Schisler et al., 2002, 2006, 2014). The reduction of DON production at field level by diverse BCA is not always easily achieved. On one hand, Schisler et al. (2002) showed that no influence was observed on DON production on six BCA evaluated at field level; on the other hand, Zhao et al. (2014) obtained up to 69% of DON inhibition at field level by using *B. subtilis* SG6. The results obtained in the present study showed reduction in DON accumulation to undetectable levels on all treatments evaluated during both 2010 and 2011 field trials; which were consistent with previous findings under greenhouse experiments (Palazzini et al., 2007). Also, it is noticeable that the

present study was carried out with formulated biocontrol agents, which retained at least 80% shelf life after two years of storage at room temperature (data not shown).

The application period of the BCA at anthesis stage were effective in reducing FHB severity and DON accumulation on wheat heads. These results are promising since it was observed that late infection of wheat heads by *F. graminearum* (up to 14 days after anthesis) could contribute to increase heads damage since the pathogen appears to develop more rapidly in plant tissues nearing natural senescence (Scanlan and Dill-Macky, 2010). Also, Cowger et al. (2009) showed that the maintenance of high humidity after anthesis (10, 20 or 30 days) contributes to increase FHB development and DON accumulation. So, despite conditions favouring FHB may not be present during anthesis stage, humidity conditions after anthesis can allow FHB development, resulting in a wider window for biocontrol applications.

The inoculum levels of the BCA used (10^4 and 10^6 cfu/ml) had no differences in the effectiveness of the biocontrol agents. It seems that the low-dose inoculum was enough to control FHB. These results are consistent with those obtained by Khan et al. (2004) who found no differences in the biocontrol effectiveness with several bacteria and yeasts; although the authors used higher inoculum levels than in our experiments (10^9 and 10^7 cfu/ml for bacteria and yeast, respectively). In addition, Jochum et al. (2006) also found no differences in the effectiveness of *Lysobacter enzymogenes* strain C3 to control FHB using 10-fold dilutions in one experiment under greenhouse, although contrasting results were observed in a second experiment where dilutions were less effective than pure culture. Schisler et al. (1997) showed that incremental differences in biological control effect are often seen with increasing biocontrol agent dose. Our findings that low inoculum levels of both *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 were effective in controlling FHB severity and DON accumulation are remarkable from the economic point of view of biomass production of the BCAs, as it was previously suggested by Köhl et al. (2011).

Physiological improvement of the potential BCAs is a common strategy to improve the effectiveness of these agents under field experiments (Bochow et al., 2001; Cañamas et al., 2007; Teixidó et al., 2005). This strategy is used since BCA can be not adapted to fluctuating environmental conditions (mainly water availability) and could render in an ineffective biocontrol activity. Schisler et al. (2002) observed that *B. subtilis* AS 43.3, without physiological improvement, was less effective against FHB in the field than under greenhouse conditions. In a previous study we have demonstrated that physiological modifications by osmotic stress treatments (NaCl, glycerol and glucose) rendered in the accumulation of the compatible solute betaine and maintained the effectiveness of *B. subtilis* RC 218 against *F. graminearum* under greenhouse experiments (Palazzini et al., 2009). Under field conditions, the physiological improvement of *B. subtilis* RC 218 (N97) did not render in better biocontrol effectiveness, as previously observed on greenhouse experiments.

In addition, spore treatments showed similar effectiveness in controlling FHB when comparing with the physiological improved *B. subtilis* RC 218 treatments. Further studies are necessary to elucidate the effect of cell type inoculum on biocontrol effectiveness.

The possible mode of action of both *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 could be antibiosis, as it was demonstrated in previous studies (Edwards and Seddon, 2001), although other modes-of-action such as lipopeptides production (iturins, fengicins, mycosubtilins) or induced resistance in wheat plants is not discarded for these biocontrol agents.

The biocontrol agents can be used alone or in combination with other management tools to reduce risks of FHB and DON accumulation. In no-tillage systems (with higher risks for FHB), the additional application of BCA on crops residues could also be a promising tool (Palazzini et al., 2013).

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