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Biocontrol and population dynamics of *Fusarium* spp. on wheat stubble in Argentina

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The biocontrol effect of *Clonostachys rosea* (strains 016 and 1457) on *Fusarium graminearum*, *F. avenaceum*, *F. verticillioides*, *F. langsethiae*, *F. poae*, *F. sporotrichioides*, *F. culmorum* and *Microdochium nivale* was evaluated on naturally infected wheat stalks exposed to field conditions for 180 days. Experiments were conducted at two locations in Argentina, Marcos Juarez and Río Cuarto. Antagonists were applied as conidial suspensions at two inoculum levels. Pathogens were quantified by TaqMan real-time qPCR. During the first year at Marcos Juarez, biocontrol was observed in one antagonist treatment for *F. graminearum* after 90 days (73% reduction) but after 180 days, the pathogen decreased to undetectable levels. During the second year, biocontrol was observed in three antagonist treatments for *F. graminearum* and *F. avenaceum* (68·3% and 98·9% DNA reduction, respectively, after 90 days). *Fusarium verticillioides* was not controlled at Marcos Juarez. At Río Cuarto, biocontrol effects were observed in several treatments at different intervals, with a mean DNA reduction of 88·7% for *F. graminearum* and *F. avenaceum*, and 100% reduction for *F. verticillioides* in two treatments after 180 days. Populations of *F. avenaceum* and *F. verticillioides* were stable; meanwhile, *F. graminearum* population levels varied during the first 90 days, and low levels were observed after 180 days. The other pathogens were not detected. The study showed that wheat stalks were important reservoirs for *F. avenaceum* and *F. verticillioides* populations but less favourable for *F. graminearum* survival. *Clonostachys rosea* (strain 1457) showed potential to reduce the *Fusarium* spp. on wheat stalks.

Keywords: biocontrol, *Clonostachys rosea*, *Fusarium* spp., real-time qPCR, wheat stubble

Introduction

Fusarium head blight (FHB) is an important fungal disease of small grain cereals such as wheat and barley, causing yield losses and mycotoxin contamination of grains. The principal causal agent of FHB is *Fusarium graminearum* (teleomorph *Gibberella zeae*; lineage 7 within the *F. graminearum* complex; O'Donnell *et al.*, 2004; Starkey *et al.*, 2007), which potentially produces trichothecenes deoxynivalenol (DON) and nivalenol (NIV) (Desjardins, 2006). Other related species such as *F. culmorum*, *F. poae*, *F. proliferatum*, *F. avenaceum* and *Microdochium nivale* can also cause FHB (McMullen *et al.*, 1997; Champeil *et al.*, 2004; Osborne & Stein, 2007). In Argentina, FHB epidemics have occurred in wheat in 17 of the last 50 years, resulting in yield and quality losses (Reynoso *et al.*, 2011).

Fusarium head blight of wheat can be controlled by chemical control or crop rotation strategies (Homdork *et al.*, 2000; Lori *et al.*, 2009; Mesterházy *et al.*, 2011). Biological control has been demonstrated as an alternative strategy both in greenhouse trials and under field conditions (Jochum *et al.*, 2006; Schisler *et al.*, 2006; Khan & Doohan, 2009; Palazzini *et al.*, 2009). Since the 1980s, conservative tillage practices, including no tillage, are increasingly followed in Argentina, so that crop stubble and straw residues are left on field soils. *Fusarium graminearum* and other *Fusarium* species including *F. verticillioides*, *F. avenaceum* and *F. poae* have a saprophytic stage and can survive in residues of crops such as maize, soyabean and wheat (Leslie & Summerell, 2006). Such colonized residues can be the inoculum sources within and outside wheat fields. In many studies, residues of previously infected crops have been found to be the main sources of spores causing head blight of wheat and maize (Dill-Macky & Jones, 2000; Shaner, 2003; Vogelgsang *et al.*, 2011). Antagonistic microorganisms applied to crop stubbles can reduce the survival and multiplication of pathogens present in the residues and thus prevent or delay disease epidemics. Reduction of pathogen populations through biocontrol applied to crop stubble has been evaluated by Luongo *et al.* (2005) by using strains 016 and 1457 of the fungus *Clonostachys rosea* (teleomorph *Bionectria ochroleuca*; Schroers *et al.*, 1999). In this study, the authors demonstrated biocontrol against various *Fusarium* spp. on wheat and maize stub-

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ble under controlled conditions and on stalks and maize ears under field conditions by traditional methodologies.

The objectives of the present work were to study the effects of two *C. rosea* strains on the populations of main pathogenic *Fusarium* spp. and *M. nivale* on wheat stalks and to evaluate the population dynamics of the pathogens on stalks. Three field experiments with naturally infected wheat stalks exposed on field soils were carried out in Argentina over two consecutive years, and the colonization of wheat pathogens was followed by using species-specific TaqMan real-time quantitative PCR (qPCR).

Materials and methods

Biocontrol strains

Clonostachys rosea strains 016 (from Plant Research International (PRI), the Netherlands) and 1457 (from Istituto Sperimentale per la Patologia Vegetale (ISPaVe), Italy) were selected, based on the effectiveness of these strains in previous studies (Luongo *et al.*, 2005). Conidia were produced on oatmeal agar (25°C for 14 days and 12 h dark/light cycle). Conidial suspensions were obtained by flooding the cultures with sterile distilled water containing Tween 80 (0·05%). Concentrations of conidial suspensions were determined by using a haemocytometer chamber and were adjusted to 10^6 and 10^4 conidia mL^{-1}.

Microdochium nivale and *Fusarium* strains

Fusarium graminearum 88/790, *F. poae* 93-1780, *F. langsethiae* PRI 07-01, *F. culmorum* PD 90-283, *F. avenaceum* IPO 92-3, *M. nivale* 766, *F. verticillioides* ITEM 2142 and *F. sporotrichioides* ITEM 3596 were obtained from the collection of PRI. *Fusarium graminearum* KRC6, *F. avenaceum* T187 and *F. verticillioides* RC2096 were isolated from cereal grains from Argentina (Table 1).

Biomass production and DNA extraction

Pathogen species were grown in complete medium (Correll *et al.*, 1987) and incubated on an orbital shaker (150 rpm) for 3–5 days at 25°C. The resulting mycelia were harvested by filtration through non-gauze milk filters (Ken AG). Excess water was removed by blotting mycelia between clean paper towels and dried mycelia were stored at −20°C. DNA was extracted with a cetyltrimethylammonium bromide (CTAB) method (Leslie & Summerell, 2006). Fungal DNA concentrations were determined with a spectrophotometer (NanoDrop 2000, Thermo Scientific).

Table 1 *Fusarium* species and *Microdochium nivale* used in the experiments, host origin and source

Strain/isolate	Host origin	Source
F. avenaceum IPO 92-3	Wheat	Germany
F. avenaceum T187	Wheat	Argentina
F. culmorum PD 90-283	Barley	Finland
F. graminearum 88/790	Carnation	Netherlands
F. graminearum KRC6	Wheat	Argentina
F. langsethiae PRI 07-01	No data	PRI collection
F. poae 93-1780	No data	PRI collection
F. sporotrichioides ITEM 3596	Oat	Norway
F. verticillioides ITEM 2142	Sorghum	Indiana, USA
F. verticillioides RC2096	Maize	Argentina
M. nivale 766	No data	PRI collection

Field experiments

Wheat stalks

Stalks were collected from commercial wheat fields located in Marcos Juarez for the 2010 and 2011 experiments and in Río Cuarto for the 2011 experiment; both areas were located in Córdoba province, Argentina. Stalk pieces 10–12 cm long containing the crown node and first node were used in the study. Commercial wheat was planted in June and harvested in early December, when stalks were collected. Wheat cultivars ProInta Halcon and Klein Escorpión were used at Marcos Juarez and Río Cuarto, respectively. Field rotation at Marcos Juarez was wheat over wheat, and at Río Cuarto the previous crop was soyabean.

Treatments and antagonist applications

Stalks were put in polyethylene net bags (20 × 20 cm; 4 mm^2 mesh), each bag containing 20 stalks in the 2010 experiment and 50 stalks in the 2011 experiments. Each bag with stalks was treated as a single sample unit. Bags containing wheat stalks were submerged for 1 min in suspensions containing conidia of *C. rosea* or sterile distilled water. A total of five treatments were used: two strains of *C. rosea* (016 and 1457) at two inoculum levels (10^6 and 10^4 conidia mL^{-1}) and a control treatment of sterile water. Treated bags were fixed on fallowed field soil with iron nets to prevent disturbance, e.g. by birds. A completely randomized block design with five blocks (replicates), five plots (treatments), each treatment consisting of two bags allowing for two sampling dates, was used in each experiment. Additionally, five bags with water-treated stalks as (controls) were stored at −18°C without exposure to field soil.

Daily mean temperature, mean humidity and total precipitation were obtained from weather stations located in the area of the field trials (INTA Marcos Juarez and Río Cuarto University).

Sampling

Bags with wheat stalks were collected 90 and 180 days after being placed in the field (five control samples and 20 treated samples per collecting date). At Río Cuarto, additional sets of bags were sampled at 15, 30 and 60 days. The Río Cuarto experiment started on 20 January 2010 with sampling dates for 15, 30, 60, 90 and 180 days on 8 and 22 February, 17 March, 19 April and 20 July 2011, respectively. Sampling dates corresponding to 15, 30 and 60 days were evaluated to monitor population dynamics of the *Fusarium* pathogens on wheat stubble without biocontrol treatments. The Marcos Juarez 2010 experiment started on 18 December 2009 and the collecting dates for 90 and 180 days were 16 March and 12 June 2010, respectively. The Marcos Juarez 2011 experiment started on 21 January 2010 and the sampling dates for 90 and 180 days were 22 April and 16 July 2011, respectively.

Sample analysis and qPCR conditions

Each sample (bag), consisting of the residues of 20 stalk pieces, was split. One subsample was dried at 105°C for 24 h to determine the dry matter content and assess stalk decomposition (dry weight loss). The other subsample was freeze-dried and pulverized in a mill with a 1 mm^2 mesh screen (Cyclotech, Foss Tecator). DNA was extracted from approximately 10 mg of the pulverized subsamples using the DNeasy 96 plant kit (QIAGEN) with small modifications: incubation (step 8 in the

manufacturer's protocol) was 30 min instead of 10 min and the DNA elution step was done twice with 50 μL instead of once with 100 μL. qPCR quantifications were done on an ABI Prism 7500 Sequence Detection System (Applied Biosystems). Separate TaqMan reactions were performed in 25 μL, using 12·5 μL of TaqMan 2× universal PCR master mix (Applied Biosystems), 100 nM of FAM-labelled probe and internal control probe, and 400 nM of forward and reverse primer for both the target *Fusarium* species and *M. nivale* (Waalwijk *et al.*, 2004, 2008) as well as the internal positive control (Klerks *et al.*, 2004). qPCR reactions were performed on 2 μL of DNA preparations from stalk samples and pure pathogen DNA. Thermal cycling conditions consisted of 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Standards curves were generated by using tenfold serial dilutions of pure DNA in the range of 0·1 to 1×10^4 pg μL^{-1}, and five replicates of pathogen quantifications were done. When analysing samples, samples of the corresponding pathogen DNA were run in parallel using a tenfold serial dilution ranging from 0·1 to 1×10^4 pg μL^{-1} as reference. From the obtained C_t values of qPCR for the different pathogen DNA dilution series and for DNA extracts from wheat stalk samples, the concentrations of pathogen DNA in the samples were calculated, expressed as pg of DNA of fungal pathogen per mg of plant tissue (dry weight).

To determine the reproducibility of the assays, independent experiments were performed on different days with a selected set of samples consisting of negative samples ($n = 18$), low pathogen DNA content samples (<100 pg DNA mg^{-1} dry wheat stalk, $n = 15$) or high pathogen DNA content samples (>100 pg DNA mg^{-1}, $n = 10$) of *F. avenaceum* and *F. verticillioides*. Robustness of the qPCR quantifications was analysed at two levels. First, quantifications were performed on two independent DNA extractions of this set of samples; and secondly, quantifications of samples were done at three different positions in the 96-well plate. This resulted in a coefficient of variation (CV, in percentage) of the original scale.

Statistical analysis

Experiments were analysed separately. Data were transformed to natural logarithm before analysis of variance (ANOVA). Means of DNA concentrations of the various *Fusarium* spp. for the different treatments and sampling dates were separated with Holm–Sidak method ($P \leq 0{\cdot}001$). All statistical analyses were done using SIGMASTAT for windows v. 3.5 (SPSS Inc.).

Results

Validation of qPCR measurements

In the cases where the quantification of the internal control was underestimated indicating PCR inhibition, qPCR runs were repeated with diluted DNA suspensions. New qPCR runs with diluted DNA samples (maximum dilution was 1/20) resulted in no inhibitions of the internal control and C_t values for the internal control were always similar ($C_t = 30{\cdot}4 \pm 0{\cdot}7$). Quantifications of DNA extracted independently from the selected set of samples ($n = 43$) gave an overall CV of 20·41% on the original scale. For samples with values >100 pg DNA mg^{-1} ($n = 10$), quantified on different days, an averaged CV of 3·66% was observed. For samples with values < 100 pg DNA mg^{-1} ($n = 15$), the CV was higher (35·32%). When analysing quantifications of the same sample in different well positions on the same run, averaged CV values were 8·86% for samples with high DNA content ($n = 10$) and 47·97% for samples with low DNA content ($n = 15$). The absence of pathogen DNA (negative samples, $n = 18$) was always confirmed by the measurement of a second subsample.

Biocontrol effect at Marcos Juarez

At the beginning of the 2010 field trial, high amounts of *F. graminearum*, *F. avenaceum* and *F. verticillioides* DNA were quantified, with mean levels of 424, 224 and 1702 pg DNA mg^{-1}, respectively (backtransformed values). *Fusarium poae* was also detected, but below the level for reliable quantification (quantification limit 0·1 pg DNA mg^{-1}). No amplification of *F. culmorum*, *F. sporotrichioides*, *F. langsethiae* or *M. nivale* DNA was detected. After 90 days, *F. graminearum*, *F. verticillioides* and *F. avenaceum* DNA levels had decreased significantly and remained low until the end of the experiment (180 days; Fig. 1a). Other pathogens detected at the beginning of the experiment remained undetectable after 90 days and were not analysed after 180 days. A biocontrol effect was only observed against *F. graminearum* when *C. rosea* strain 1457 was applied at 1×10^6 spores mL^{-1} (Cr1457–10^6), significantly reducing the concentration of *F. graminearum* DNA from 4·88 (control treatment) to 1·32 pg DNA mg^{-1} (73% reduction) after 90 days (Fig. 2). After 180 days, *F. avenaceum* and *F. verticillioides* were present in control treatments with 5·2 and 52·4 pg DNA (backtransformed values) detected, respectively. No biocontrol effect was observed and no differences were observed either between treatments and controls or between controls at 90 or 180 days for *F. avenaceum* or *F. verticillioides* (Fig. 2b,c). Temperatures were moderate during the first half of the experiment (mean temperature from December 2009 to March 2010 was 23·4°C) and 14·3°C during the second half (April–June 2010). Precipitation during the experiment was low (61·2 mm).

In the Marcos Juarez 2011 experiment, lower amounts of *F. graminearum*, *F. avenaceum* and *F. verticillioides* DNA were quantified at the beginning in comparison with the 2010 field trial (Fig. 1b). *Fusarium poae* was detected in two of five control plots, with a mean DNA level of 108·5 pg at the beginning of the experiment, but after 90 days the mean DNA level was 25 pg and only detected in one of five control plots. No amplification of *F. culmorum*, *F. sporotrichioides*, *F. langsethiae* or *M. nivale* DNA was detected. A similar pattern of decline in DNA was observed for *F. avenaceum* and *F. verticillioides* in 2011 as in 2010. No statistical differences were observed for *F. avenaceum* DNA quantifications between the initial and 90 days samplings. A small but not statistically significant decrease was observed in *F. avenaceum* between 90 and 180 days (Fig. 1b). After 90 days, *F. graminearum* decreased to low DNA levels and was not detected after 180 days. Treatments Cr016–

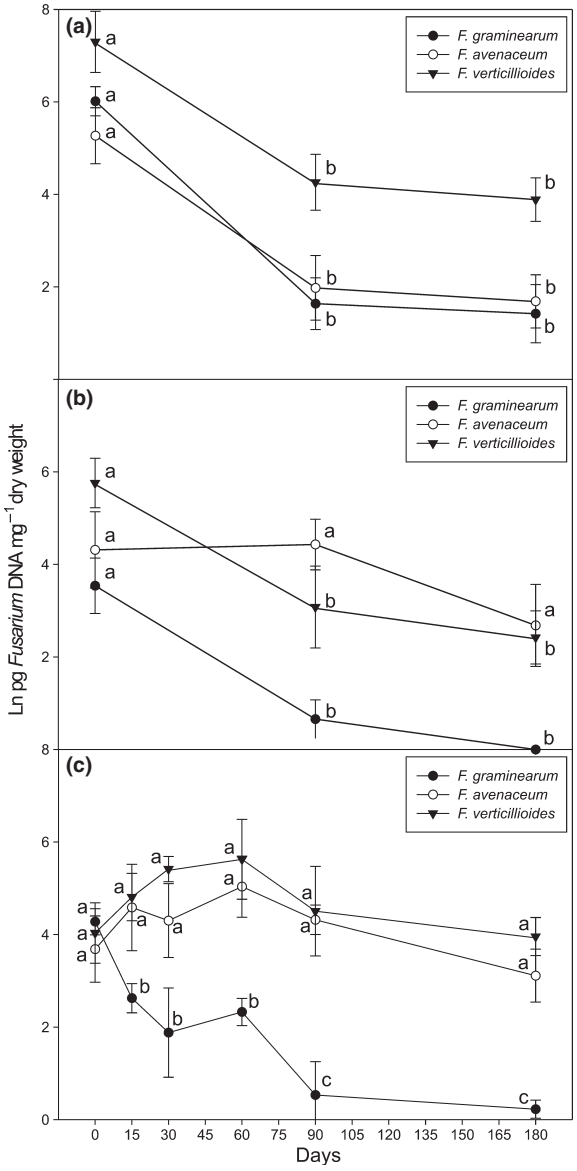

Figure 1 DNA quantification of *Fusarium* species on wheat stubble in different field experiments at Marcos Juarez and Río Cuarto, Argentina. (a) Marcos Juarez 2010; (b) Marcos Juarez 2011; (c) Río Cuarto 2011. For each *Fusarium* species on each graph, different letters indicate significant differences according to Holm–Sidak test ($P = 0{\cdot}006$). Standard deviation is represented by error bars.

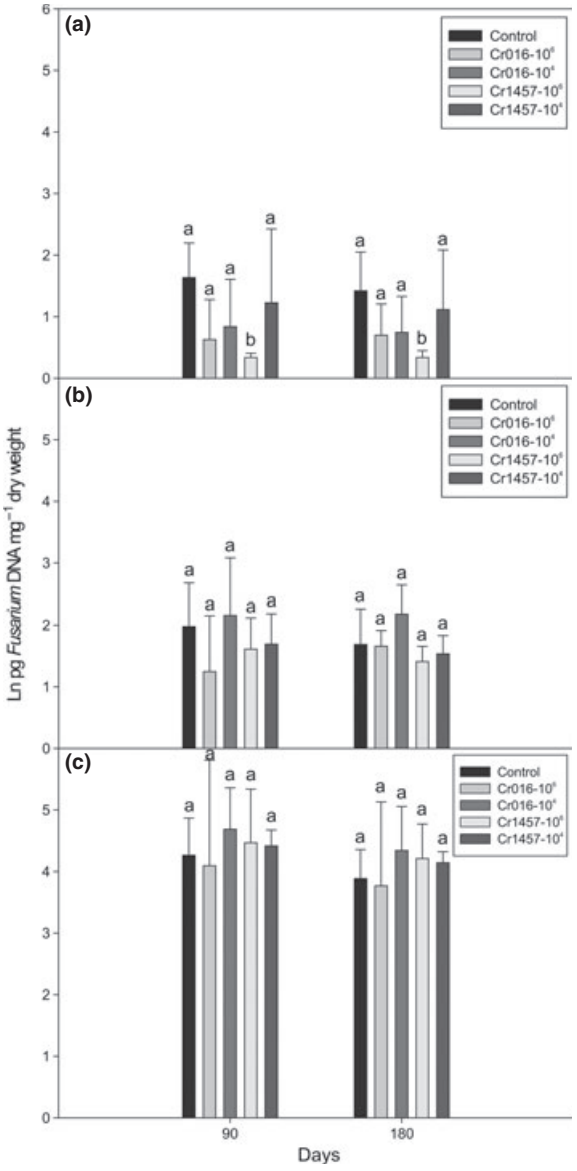

Figure 2 Effects of treatments with *Clonostachys rosea* strains 016 and 1457 on *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* on wheat stalks exposed to field conditions in the Marcos Juarez 2010 experiment. Control: wheat stalks treated with water; Cr016–10^6 and Cr016–10^4: *C. rosea* strain 016 at 10^6 and 10^4 conidia mL^{-1}, respectively; Cr1457–10^6 and Cr1457–10^4: *C. rosea* strain 1457 at 10^6 and 10^4 conidia mL^{-1}, respectively. (a) *Fusarium graminearum*; (b) *F. avenaceum*; (c) *F. verticillioides*. For each sampling date, different letters indicate significant differences according to Holm–Sidak test ($P \leq 0{\cdot}001$). Error bars represent standard deviations.

10^6, Cr1457–10^6 and Cr1457–10^4 statistically reduced *F. graminearum* DNA measured after 90 days (68·3% overall reduction) but it was not possible to evaluate at 180 days because the pathogen DNA had decreased to undetectable levels (Fig. 3a). In contrast to *F. graminearum*, *F. verticillioides* and *F. avenaceum* control treatments maintained high DNA levels until 180 days (18·22 and 11·74 pg DNA mg^{-1}, respectively). A biocontrol effect was observed for *F. avenaceum* in treatments Cr016–10^6, Cr1457–10^6 and Cr1457–10^4 after 90 days (98·9% overall reduction) and in Cr016–10^6 and Cr1457–10^4 after 180 days (96·4% overall reduction; Fig. 3b). No biocontrol effect was observed against *F. verticillioides* at Marcos Juarez during the 2 years of experiments (Figs 2c and 3c). Temperatures during the experiment were similar to 2010 (23·2°C from January to March and 16·8°C from April to June) but total precipitation was 357 mm.

Additionally, stalk decomposition was analysed based on dry weight loss during the experiments but no differences were observed between treatments and controls after 90 or 180 days (data not shown).

Biocontrol effect and population dynamics of *Fusarium* species at Río Cuarto

Initial quantities of *F. graminearum*, *F. avenaceum* and *F. verticillioides* DNA were similar, ranging from 46 to 72 pg DNA mg^{-1} (control treatments; Fig. 1c). *Fusarium poae* was detected in one of five control plots, with a DNA level of 74 pg at the beginning of the experiment, and was not detected after 90 days. No amplification of *F. culmorum*, *F. sporotrichioides*, *F. langsethiae* or *M. nivale* DNA was detected at the beginning of the experiment. After 90 days, *F. graminearum* DNA decreased to 2·4 pg mg^{-1} in the control and a significant biocontrol effect was observed in Cr016–10^6 and Cr1457–10^4 treated plots (85·9% overall reduction). After 180 days, DNA values for *F. graminearum* in treatments and control were close to the quantification limit (0·1 pg DNA mg^{-1}). A biocontrol effect was also observed for *F. avenaceum* in treatments Cr016–10^6 and Cr1457–10^4, with DNA reductions from 91·6 to 100% after 90 or 180 days. *Fusarium verticillioides* was reduced by treatments Cr016–10^6 and Cr1457–10^4 after 180 days, when no pathogen DNA quantification was obtained (Fig. 4).

Populations of *F. verticillioides* and *F. avenaceum* increased during the first 60 days from 47·4 to 206·4 pg DNA mg^{-1} (without significant differences between different sampling dates) and remained stable during the 180 day period of the experiment. However, the *F. graminearum* population decreased significantly between the initiation of the experiment and the 15 day sampling, and between the 60 and 90 day samplings. *Fusarium graminearum* DNA was close to zero at the end of the experiment (Fig. 1c).

Temperatures during the experiment were similar to the Marcos Juarez experiments during the first half of the trial (mean January to March temperature of 19·8°C) and slightly lower during the second part of the experiment (10·9°C from March to June). Total precipitation was 428 mm.

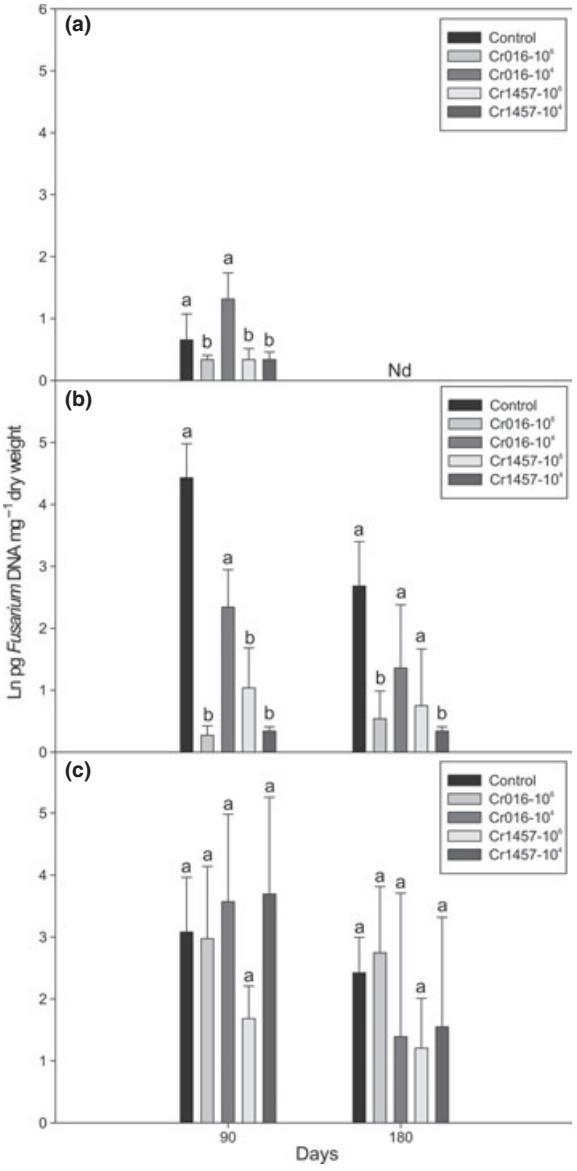

Figure 3 Effects of treatments with *Clonostachys rosea* strains 016 and 1457 on *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* on wheat stalks exposed to field conditions in the Marcos Juarez 2011 experiment. Control: wheat stalks treated with water; Cr016–10^6 and Cr016–10^4: *C. rosea* strain 016 at 10^6 and 10^4 conidia mL^{-1}, respectively; Cr1457–10^6 and Cr1457–10^4: *C. rosea* strain 1457 at 10^6 and 10^4 conidia mL^{-1}, respectively. (a) *Fusarium graminearum*; (b) *F. avenaceum*; (c) *F. verticillioides*. For each sampling date, different letters indicate significant differences according to Holm–Sidak test ($P \leq 0{\cdot}001$). Error bars represent standard deviations. Nd: not detected (no amplification).

Discussion

The biocontrol effect of *C. rosea* strains 016 and 1457 against *Fusarium* spp. pathogens was studied on wheat stalks over 2 years at Marcos Juarez and 1 year at Río Cuarto, Córdoba province, Argentina. Additionally, the population dynamics of three *Fusarium* pathogens was analysed at Río Cuarto over a period of 180 days. In general, higher concentrations of both biocontrol agents were more effective in controlling pathogen populations than lower concentrations. A better biocontrol effect of *C. rosea* strain 1457 was also observed. The effectiveness of *C. rosea* is attributed to its ability to colonize senescent and dead tissue faster than the pathogen and to suppress pathogen sporulation (Morandi *et al.*, 2000, 2001) and, in this study, it is possible that the *C. rosea* strains used could prevent the recolonization of wheat stalks by the *Fusarium* pathogens evaluated. Biocontrol activity of *C. rosea* against several pathogens, mainly *Botrytis cinerea* in rose, rose debris and other crops such as straw-

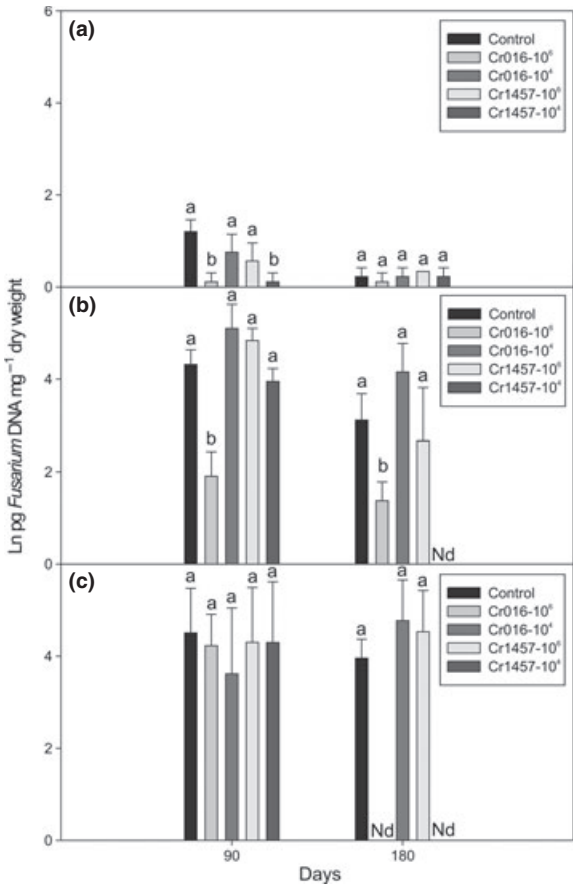

Figure 4 Effects of treatments with *Clonostachys rosea* strains 016 and 1457 on *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* on wheat stalks exposed to field conditions in the Río Cuarto 2011 experiment. Control: wheat stalks treated with water; Cr016–10^6 and Cr016–10^4: *C. rosea* strain 016 at 10^6 and 10^4 conidia mL^{-1}, respectively; Cr1457–10^6 and Cr1457–10^4: *C. rosea* strain 1457 at 10^6 and 10^4 conidia mL^{-1}, respectively. (a) *Fusarium graminearum*; (b) *F. avenaceum*; (c) *F. verticillioides*. For each sampling date, different letters indicate significant differences according to Holm–Sidak test ($P \leq 0{\cdot}001$). Error bars represent standard deviations. Nd: not detected (no amplification).

berry, carrots and wheat seeds have been reported (Tatagiba *et al.*, 1998; Morandi *et al.*, 2000; Nobre *et al.*, 2005; Cota *et al.*, 2008; Roberti *et al.*, 2008). Isolate *C. rosea* strain 016 used in the present study was demonstrated to control *B. cinerea* in cyclamen under commercial growing conditions in an earlier study (cited as *Gliocladium roseum* 1813 by Köhl *et al.*, 1998). *Clonostachys* strains used in the present study have been previously evaluated by Luongo *et al.* (2005) against several *Fusarium* pathogens in experiments under controlled conditions with wheat and maize stalks inoculated artificially after irradiation and in field assays on maize stalks and ears. These strains were effective in reducing pathogen sporulation by 60–90% on stalks in controlled environment experiments. In field experiments on maize stalks pieces, *C. rosea* strain 1457 was effective in diminishing *F. verticillioides* and *F. proliferatum* in different years and *F. graminearum* in both years on maize grains. However, a direct comparison of the results obtained in the two studies is not possible because different parameters, e.g. spore counts versus total DNA concentrations, have been measured to assess biocontrol effects.

At Marcos Juarez, high levels of *F. graminearum*, *F. avenaceum* and *F. verticillioides* DNA were quantified at the beginning of the 2010 experiment, which can be explained by the favourable climatic conditions prior to the sampling, with a mean temperature from October to December 2009 of 21·5°C and an accumulated precipitation of 247 mm. During the experiment, a significant biocontrol effect of *C. rosea* strain 1457 was observed against *F. graminearum* at an inoculum level of 10^6 but no effect was detected against *F. verticillioides* and *F. avenaceum* populations 180 days after placement in the field.

In the Río Cuarto experiment, the most effective treatments were Cr016–10^6 and Cr1457–10^4. However, Cr1457–10^6 (high spore level) was not effective for unknown reasons. *Fusarium graminearum* controls showed the same behaviour observed at Marcos Juarez, and decreased from 72·8 pg DNA mg^{-1} to low levels after 90 days (<2·4 pg DNA mg^{-1}) and 0·27 pg DNA mg^{-1} after 180 days (near quantification limit). *Fusarium verticillioides* remained at high DNA level whereas *F. avenaceum* decreased after 180 days (23·85 pg DNA mg^{-1}, 50% of the initial level).

Populations of *F. avenaceum* and *F. verticillioides* were relatively stable in the Río Cuarto experiment, but *F. graminearum* declined to very low levels within 90 days. During the first 30 days, an accumulated precipitation of 230 mm and a mean temperature of 22·6°C were recorded. These climatic conditions seemed favourable for *F. avenaceum* and *F. verticillioides* maintenance, but not for *F. graminearum*. It is known that *F. graminearum* is not a strong saprophytic colonizer and can be replaced by other more effective decomposers, especially in wheat tissues (Bowen, 1990). The dynamics of *F. graminearum* in residues of wheat exposed to the surface or buried was also observed by Pereyra *et al.* (2004) during a 2-year experiment. After the first year, the authors found 80% survival in nodes exposed to the surface and approximately 60% in buried residues; they concluded that ascospores can be produced in wheat residue and remain active to contribute to new infections for the next crop. In the Marcos Juarez 2010 experiment, *F. graminearum* inoculum (measured as DNA) disappeared after 180 days, but it was present in stubble for the following crop at high levels. It is therefore possible that sufficient amounts of inoculum remained viable until the next year, or wind transported spores arrived at the field, which is strongly supported by several studies (Maldonado-Ramirez *et al.*, 2005; Schmale *et al.*, 2005; Schmale, 2006). The latter option is also valid for the prevalence of *F. graminearum* inoculum observed in previous studies (Pereyra *et al.*, 2004; Schmale, 2006). The dynamics of the FHB pathogens and *M. nivale* was stud-

ied by Köhl *et al.* (2007) in crops and crop residues in winter wheat assessed by TaqMan qPCR. The authors found a significant decrease of the pathogens in internodes and nodes of wheat residues after 10 months, but not in stem base residues. This correlates with the results observed for *F. avenaceum* and *F. verticillioides* in the current study. The authors suggested that the stem base can act as a residue for the long term survival of inoculum of *Fusarium* spp. Wheat stalk segments used in the present study contained the crown node and the first node, as previously analysed by Köhl *et al.* (2007). Pereyra & Dill-Macky (2008) analysed the presence of *Gibberella zeae* in artificially inoculated wheat, maize, barley and sunflower grown in minimum or no tillage systems. They found a high incidence of the pathogen at harvest time in the crops but reported a decline in the crop residues during the following 2 years. Wheat and barley residues produced more ascospores in comparison to maize. Similarly, Lori *et al.* (2009) analysed the occurrence of FHB in wheat grown in different tillage systems under natural infection over 3 years. Climate conditions had a stronger effect on FHB than tillage conditions. During the Marcos Juarez 2010 experiment, mean temperatures were moderate and precipitation was extremely low. It is possible that the prevailing climatic conditions during the field experiment did not support *Fusarium* spp. development. *Fusarium verticillioides* is an important maize pathogen (Pereira *et al.*, 2007), able to produce fumonisins that cause several diseases in animals and are associated with oesophageal and liver cancer in humans (Stankovic *et al.*, 2012). This pathogen was present in wheat stalks in high amounts. As wheat is commonly grown in Argentinean fields in rotation with maize or soyabean (Lori *et al.*, 2009) using minimum or no tillage practices (Edwards, 2004; Lori *et al.*, 2009), wheat stubble can maintain the inoculum of *F. verticillioides* and increase the risk for other crops grown later in rotation in the same field. As maize, wheat and soyabean are the most important grain crops in Argentina (Pereira *et al.*, 2007; Reynoso *et al.*, 2011), special attention must be paid to diminish the pathogen inoculum. Adequate rotation of these crops associated with biocontrol or other management strategies could reduce the risk of crop infection and mycotoxin accumulation in commercial commodities.

This is the first report from Argentina on biocontrol and *Fusarium* species dynamics on wheat stubbles assessed by qPCR. The results showed strong effectiveness (68–100% pathogen DNA reduction) of a single application of the biocontrol agents assayed up to 6 months later. In control treatments, the *F. graminearum* populations naturally decreased and *F. avenaceum* and *F. verticillioides* populations were relatively stable over 6 months exposure in two fields and in both years under simulated no tillage conditions. Further experiments are needed to evaluate the effectiveness of *C. rosea* isolates in different fields under different climatic conditions on wheat stalks and on the development of diseases caused by *Fusarium* spp. in the subsequent crops.

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