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DISEASE CONTROL

Evaluation of chemical and biological seed treatments to control charcoal rot of soybean

Sebastian Reznikov[1] · Gabriel R. Vellicce[1] · Victoria González[1] · Vicente de Lisi[1] · Atilio P. Castagnaro[1] · L. Daniel Ploper[1]

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Abstract Charcoal rot caused by *Macrophomina phaseolina* (Mp) affects economically important crops around the world. In northwestern Argentina, the disease is present in seasons with dry and hot weather. Here, the efficacy of seed treatments with two biological products (*Trichoderma viride* or *Bacillus subtilis*) or one chemical treatment (thiophanate methyl + pyraclostrobin) were evaluated in the field to control Mp on two soybean cultivars in Tucumán, Argentina. In field tests, Mp was added to the soil at planting in the 2010/2011 and 2011/2012 growing seasons. Plant emergence (PE) was reduced for both cultivars: 6.7 and 56.8 % for NA8000 RG and 12.5 and 61.3 % for Munasqa RR in 2010/2011 and 2011/2012, respectively. On a 1–5 disease severity scale, the inoculated control of NA8000 RG scored 2.5 and Munasqa RR 1.8 during the 2010/2011 season, and 3.6 for NA8000 RG, and 2.7 for Munasqa RR during 2011/2012. Likewise, Mp CFU/g values in the inoculated control increased during the 2011/2012 season: from 2100 to 2366 in NA8000 RG and from 300 to 566 in Munasqa RR. Crop yield and 1000-seed weight were also reduced by fungal infection. Although the treatments counteracted PE reduction in both genotypes, disease severity was higher in NA8000 RG than in Munasqa RR, coincident with the highest CFU/g values. The 1000-seed weight of the two cultivars did not differ significantly among treatments. The highest yields were obtained in chemically treated plots, followed by those treated with *Trichoderma* or *Bacillus*.

Keywords *Macrophomina phaseolina* · *Glycine max* · *Trichoderma viride* · *Bacillus subtilis*

✉ L. Daniel Ploper
dt@eeaoc.org.ar

[1] Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA), Estación Experimental Agroindustrial Obispo Colombres (EEAOC)-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. William Cross 3150, C.P. T4101XAC Las Talitas, Tucumán, Argentina

Introduction

Soybean [*Glycine max* (L.) Merr.] plants are susceptible to root and stem base rots caused by soil pathogens at all growth stages. One of these diseases is charcoal rot of soybean, caused by the polyphagous fungus *Macrophomina phaseolina* (Tassi) Goid (Mp). This pathogen infects a wide host range of nearly 500 species in more than 100 families around the world, including other important crops such as cotton (*Gossypium* spp.), chickpea (*Cicer arietinum*), corn (*Zea mays*), and common bean (*Phaseolus vulgaris*) (Srivastava et al. 2001). Morphologically, physiologically, genetically, and pathogenically, the fungus varies widely, enabling it to adapt to different environmental conditions and hence become widely distributed geographically (Su et al. 2001).

Soybean seedlings affected by Mp develop reddish brown lesions on the hypocotyl, which becomes ash-gray and then turns black (Wyllie 1989). The presence of small, black sclerotia in the cortical tissue confers the charcoal appearance that gives the disease its name (Mengistu et al. 2015). In addition to these symptoms and signs, mature plants develop chlorotic lesions on their leaves, which then die but remain attached to the stem, and finally the plants die prematurely (Ploper and Scandiani 2009). A combination of water stress and high temperatures favors disease development (Mihail 1992).

Charcoal rot of soybean is an economically important disease in North and South America, Asia, Australia, Africa and Europe (Wrather et al. 1997). Worldwide in 2006, yield losses caused by charcoal rot reached 4.2 % (Wrather et al. 2010). Yield loss in field experimental plots was estimated to be 30 % (Mengistu et al. 2011). In Argentina, hot, dry weather prevailed in the 2000/2001 growing season, which favored charcoal rot development on soybean. The provinces of Catamarca, Chaco, Córdoba, Entre Rios, Santa Fe, Salta, Santiago del Estero and Tucumán were affected with varying levels of yield losses, including total losses in some fields (Ploper et al. 2001).

Biological agents can provide an alternative to control certain plant diseases, especially when other methods such as chemicals are difficult to use. Cook and Baker (1983) successfully controlled *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Pythium* spp. using antagonistic microorganisms such as *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., and some bacteria. Jeyarajan et al. (1993) reported that a talc-based formulation of *Trichoderma viride*, produced on a commercial scale, was widely used by farmers to treat seeds of sesame (*Sesamum indicum*), groundnut (*Arachis hypogaea*), sunflower (*Helianthus annus*), chickpea and mung bean (*Vigna radiata*) for the biocontrol of root rot disease caused by Mp. Sankar and Jeyarajan (1996) studied *Trichoderma* spp. and *Gliocladium virens* as seed treatment for sesame (*Sesamum indicum*) to control Mp and concluded that treating the seeds with the formulation and storing them was more advantageous than storing the formulation and treating the seeds just before planting. Elad et al. (1986) found that *Trichoderma harzianum* inhibited the in vitro linear growth and microsclerotia production of Mp. When the biocontrol agent was applied to seeds, disease incidence was reduced in bean and melon (*Cucumis melo*) in the greenhouse and in melons and corn in the field.

To control fungal pathogens, chemical products are available for application of soil, seeds and/or foliage. Fungicide seed treatments are intended to control diseases that cause seed rot and damping-off before and after emergence. Mueller et al. (1999) used thiram, fludioxonil, and captan + pentachloronitrobenzene + thiabendazole to control *Sclerotinia sclerotiorum* in field trials and managed to reduce sclerotia formation in infected soybean seed by 98.0 %. Moreover, soybean seeds treated with thiram reduced the incidence of *Phomopsis sojae* and increased seed germination (Hepperly and Sinclair 1978). In studies on the control of *R. solani*, Martin et al. (1984) demonstrated differences in sensitivity to different fungicides among *R. solani* isolates, and Dorrance et al. (2003) assessed five fungicide seed treatments to control *R. solani* on soybean in the greenhouse. However, none of the evaluated seed treatments completely controlled the four tested isolates.

Currently, no chemicals are available to control charcoal rot in soybean. Mengistu et al. (2015) mentioned that seed treatments may be helpful if soybean seeds are infected with Mp, but there is no information on specific active ingredients effective against this pathogen.

Among fungicides tested against Mp in infected cotton seeds, carbendazim, quintozene and benomyl enhanced plant emergence and disease control (Chauhan 1986a, b, 1988; Dwivedi and Ghaube 1985). Monceren, Pencycuron, Carboxin 200, tolclofos-methyl, and Maximum AP also increased the percentage of surviving seedlings from Mp-infected seeds (Aly et al. 2001; Omar 2005). However, whether any seed treatments can efficiently control Mp of soybean in northwestern Argentina is not known. Thus, we analyzed the efficacy of one chemical and two biological products in controlling charcoal rot of soybean and their effects on plant emergence, disease severity, Mp colony forming units (CFU), crop yield and 1000-seed weight in two soybean cultivars maturity group (MG) VIII inoculated with Mp in Tucumán, Argentina.

Materials and methods

Macrophomina phaseolina isolation

During the 2009/2010 season, plants with typical charcoal rot symptoms were collected from commercial fields in Tucumán and taken to the Phytopathology Laboratory of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC) to isolate and characterize *Macrophomina phaseolina* (Mp). Infected samples were rinsed, and 0.5 cm sections were surface sterilized with 70 % ethanol for 30 s, and with a 5 % NaClO solution for 1 min. These sections were later rinsed with sterile water and then dried with sterile air. Samples were cultivated on potato dextrose agar (PDA, Difco, Sparks, Maryland, USA) plates with 0.2 % v/v lactic acid at 28 ± 2°C for 5 days, after which cultures were maintained at 4°C. Single sclerotia were removed using a stereomicroscope and transferred to a new PDA plate acidified with 2 % lactic acid. Then, after 24–48 h, pure cultures of each isolate were obtained, which were reserved for further analysis.

Field trials

The field trials were done at the Monte Redondo substation of the EEAOC research station in Cruz Alta department, Tucumán, Argentina (26°49′S, 64°51′W). On 29 December 2010 and 28 December 2011, seeds of MG VIII soybean cultivars NA8000 RG and Munasqa RR were planted by hand in four replications of randomized blocks, and each treatment comprised four 3-m-long rows, with a planting

Table 1 Mean monthly temperature and rainfall in the 2010/2011 and 2011/2012 seasons in Monte Redondo (Cruz Alta, Tucumán) compared with historical average values for this site (average temperature and precipitation in 1996–2012 and 1981–2010, respectively)

	November		December		January		February		March		April		Total
	T[a]	P[b]	T[a]	P[b]	T[a]	P[b]	T[a]	P[b]	T[a]	P[b]	T[a]	P[b]	P[b]
2010/2011	23.2	50.8	25.6	159.3	25.5	208.3	23.7	162.6	22.2	109.0	20.2	46.0	736
Differences	−0.9	−41.7	+0.1	+4.9	−0.4	+21.2	−0.8	+26.2	−0.7	−29.6	+0.5	−16.2	−35.2
2011/2012	25.5	67.3	25.4	84.1	27.1	84.6	27.3	81.3	23.8	71.4	19.9	93.0	481
Differences	+1.4	−25.2	−0.1	−70.3	+1.2	−102.5	+2.8	−55.1	+0.9	−67.2	+0.2	+30.2	−290
Mean values	24.1	92.5	25.5	154.4	25.9	187.1	24.5	136.4	22.9	138.6	19.7	62.2	771

[a] *T* monthly average temperature

[b] *P* rainfall in mm

density of 23 seeds per m. For inoculum in field trials, we used sterile rice that had been inoculated with Mp isolate number 55 and incubated for 20 days at 30 °C in darkness to promote microsclerotium development. When soybean seeds were planted, 5 g per linear meter of this rice inoculum was added by hand at planting depth. Treatments were as follows: (1) uninoculated and untreated control (neither inoculation, nor chemical or biological treatments); (2) untreated control inoculated with Mp; (3) Mp added to the soil and seed treated with a mixture of strobilurin + benzimidazole (pyraclostrobin 5 % + thiophanate methyl 45 %, 100 mL/100 kg of seed); (4) Mp added to the soil and seed treated with *Trichoderma viride*. (Trichoderma SP®, *Trichoderma viride*, 700 mL/50 kg of seed, Laboratorio San Pablo, Tucumán, Argentina); and (5) Mp added to the soil and seed treated with *Bacillus subtilis* (Bacillus subtilis SP®, *Bacillus subtilis*, 500 mL/50 kg of seed, Laboratorio San Pablo, Tucumán, Argentina).

The effects of each treatment were evaluated considering plant emergence (PE) percentage, disease severity, Mp colony forming units per gram of root (CFU/g), crop yield and 1000-seed weight (W 1000). PE was calculated as the percentage of plants that emerged 7, 14, and 21 days after planting (DAP). Charcoal rot severity was recorded at stage R7 (Fehr and Caviness 1977) using the scale of Paris et al. (2006): 1 = no discoloration and no microsclerotia visible; 2 = no discoloration of vascular tissue, with very few microsclerotia visible in the pith, vascular tissue or under the epidermis; 3 = partially discolored vascular tissue, with microsclerotia partially covering the tissue; 4 = discolored vascular tissue, with numerous microsclerotia visible in the tissue under the outer epidermis, in stem and root sections; 5 = vascular tissue with numerous microsclerotia producing a dark color inside and outside of the stem and root tissue.

Values of CFU were calculated according to the protocol proposed by Mengistu et al. (2007), using 10 plants from each plot. The samples were obtained by cutting 10 cm above and below soil level, ensuring that they included root and stem tissue, then washed and rinsed three times with tap water to remove any soil, dried at room temperature and ground. From each sample, 0.005 g of ground tissue was transferred to a test tube, disinfected with 5 % NaClO for 1 min, and rinsed with sterile distilled water for 1 min, three times. Then 5 mL of sterile PDA was added at 60 °C and poured into sterile Petri dishes, which were incubated at 28 °C for 3–5 days for CFU to be recorded.

Crop yield (kg/ha) and 1000-seed weight (g) were measured at harvest.

Data for crop yield and 1000-seed-weight were analyzed for statistical differences among treatment means using an ANOVA and LSD test at $\alpha = 0.05$ in INFOSTAT software version 2008 (Balzarini et al. 2008). Data on PE, CFU and disease severity were square-root transformed ($\sqrt{x}$) before the analysis.

To evaluate whether prevailing environmental conditions during the growing seasons corresponded to those that favor charcoal rot development, average monthly temperatures and precipitation were compared for the 2010/2011 and 2011/2012 growing seasons (Table 1).

Results

Using the *Macrophomina phaseolina* (Mp) isolate number 55, we were able to reproduce the disease in the field. Inoculation reduced the number of inoculated NA8000 RG control plants that had emerged (PE), for instance, at 21 DAP in the 2010/2011 season to 86.4 %, compared with 92.6 % for the noninoculated control, although without statistically significant differences (Table 2). But in the 2011/2012 season, by 21 DAP, the inoculated control had significantly lower PE values (22.4 %), than the noninoculated control (51.9 %). Similarly, in Munasqa RR by 21 DAP in the 2010/2011 season, the PE of 79.2 % for the inoculated control differed significantly from 90.5 % PE for the noninoculated control (Table 3). Moreover, by 21

Table 2 NA8000 RG emergence by 7, 14 and 21 days after planting in field trials after treatment with chemicals or biological control agents in Monte Redondo (Cruz Alta, Tucumán) in 2010/2011 and 2011/2012 seasons

Treatment	2010/2011			2011/2012		
	7	14	21	7	14	21
1-Untreated and uninoculated control	89.2 a*	92.4 a	92.6 a	30.1 a	51.9 ab	51.9 a
2-Untreated control + Mp	84.6 a	86.2 b	86.4 a	16.4 ab	30.8 c	22.4 c
3-(Pyraclostrobin + thiophanate methyl) + Mp	84.8 a	92.4 a	90.2 a	22.8 ab	62.8 a	58.9 a
4-*Trichoderma viride* + Mp	84.8 a	88.6 ab	86.4 a	19.5 ab	43.5 bc	36.3 b
5-*Bacillus subtilis* + Mp	84.1 a	88.2 ab	86.4 a	13.9 b	36.1 c	26.4 bc

* Means in each column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$)

Table 3 Emergence of Munasqa RR plants by 7, 14 and 21 days after planting in field trials after treatment with chemicals or biological control agents in Monte Redondo (Cruz Alta, Tucumán), 2010/2011 and 2011/2012 seasons

Treatment	2010/2011			2011/2012		
	7	14	21	7	14	21
1-Untreated and uninoculated control	90.7 a*	90.0 a	90.5 a	52.6 ab	73.6 a	65.9 a
2-Untreated control + Mp	60.2 d	82.8 b	79.2 b	32.5 c	30.8 b	25.5 b
3-(Pyraclostrobin + thiophanate methyl) + Mp	87.1 ab	92.0 a	91.1 a	56.0 a	78.6 a	61.5 a
4-*Trichoderma viride* + Mp	75.2 c	88.1 ab	88.8 a	37.8 bc	43.3 b	34.4 b
5-*Bacillus subtilis* + Mp	78.2 bc	87.1 ab	88.1 a	32.7 c	40.6 b	32.2 b

* Means in each column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$)

DAP in 2011/2012, the inoculated control had a PE of 25.5 %, differing significantly from 65.9 % for the non-inoculated control.

The effects of different treatments on PE of seeds inoculated with Mp 7, 14 and 21 DAP in NA8000 RG and Munasqa RR are also shown in Tables 2 and 3, respectively. In NA8000 RG during the 2010/2011 season, only the treatment with pyraclostrobin + thiophanate methyl (PE: 92.4 %) reduced the adverse effect of inoculation with Mp and differed significantly from the inoculated control (86.2 %) at 14 DAP. PE values at 7 and 21 DAP did not differ significantly among treatments (Table 2).

The 2011/2012 season showed environmental conditions favorable for charcoal rot (Table 1). As reflected in Table 2, significant differences were observed between the Mp-inoculated and the noninoculated control of NA8000 RG at 14 and 21 DAP; the inoculated plants had lower PE values (16.4 % by 7 DAP, 30.8 % by 14 DAP and 22.4 % by 21 DAP). At 7 DAP, none of the Mp-inoculated plants treated with either chemicals or biological agents differed significantly in PE from the untreated Mp-inoculated control (Table 2). At 14 DAP, the PE for plants in treatment 3 [(pyraclostrobin + thiophanate methyl) + Mp] was 62.8 % and differed significantly from the rest of the treatments except for the untreated and uninoculated control (51.9 %). At 21 DAP, all treatments yielded statistically significant differences in PE in relation to the inoculated control (22.4 %), except treatment 5 (*B. subtilis* + Mp) (26.4 %). Treatments 1 (untreated and uninoculated control) (51.9 %) and 3 ([pyraclostrobin + thiophanate methyl] + Mp) (58.9 %) had the highest PE values with no differences between them, whereas treatment 4 (*T. viride* + Mp) (36.3 %) and treatment 5 (*B. subtilis* + Mp) (26.4 %) gave the lowest PE values. It is worth mentioning that treatment 5 did not differ from the inoculated control (22.4 %) at this stage.

PE values for Munasqa RR after the different treatments are shown in Table 3 for three dates during the 2010/2011 and 2011/2012 seasons. In 2010/2011, the PE for the inoculated and the noninoculated control differed significantly at all dates. Seven days after planting, all treatments had PE values that contrasted significantly with those of the inoculated control (60.2 %). At 14 DAP, treatments 4 (*T. viride* + Mp) (88.1 %) and 5 (*B. subtilis* + Mp) (87.1 %) did not differ statistically from the inoculated control (82.8 %), but treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) (92.0 %) did show significant differences. At 21 DAP, all treatments differed statistically from the inoculated control (79.2 %), but not among them.

For Munasqa RR in the 2011/2012 season, the PE of plants in the untreated and uninoculated control (52.6 %) and treatment 3 [(pyraclostrobin + thiophanate methyl) + Mp] (56.0 %) differed statistically from the inoculated control (32.5 %) at 7 DAP (Table 3). On the other hand, the PE of plants in treatments 4 (*T. viride* + Mp) (37.8 %) and 5 (*B. subtilis* + Mp) (32.7 %) did not differ statistically from the inoculated control (32.5 %). At 14 DAP, the PE of inoculated control (30.8 %) plants did not differ statistically from treatments 4 (*T. viride* + Mp) (43.3 %) and 5 (*B. subtilis* + Mp) (40.6 %) but differed from treatments 1 (untreated and uninoculated control) (73.6 %) and 3 ([pyraclostrobin + thiophanate methyl] + Mp)

(78.6 %). At 21 DAP, the PE for treatments 4 (*T. viride* + Mp) (34.4 %) and 5 (*B. subtilis* + Mp) (32.2 %) did not differ from the inoculated control (25.5 %), but differed significantly from the PE for treatments 1 (untreated and uninoculated control) (65.9 %) and 3 ([pyraclostrobin + thiophanate methyl] + Mp) (61.5 %).

The decrease in PE values in the 2011/2012 season compared with those in the 2010/2011 season is primarily attributable to the marked differences in environmental conditions, especially with regard to precipitation from December to January, key months for root emergence and plant establishment. According to data in Table 1, in the first season, the combined precipitation of those 2 months amounted to 367.6 mm, whereas in the second season that value dropped to 168.7 mm. This low value of precipitation was accompanied by high temperatures in January 2012 (Table 1), resulting in favorable environmental conditions for charcoal rot development in the 2011/2012 seasons.

Table 4 shows data related to crop yield, 1000-seed weight and charcoal rot severity recorded in NA8000 RG in the 2010/2011 and 2011/2012 seasons. In 2010/2011, although no significant differences were found among treatments in relation to crop yield, crop yield increased with all control treatments. For instance, treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) led to 277.7 kg/ha more soybeans than the inoculated control (4492.4 kg/ha). With respect to 1000-seed weight, no significant differences were observed among treatments. Severity value in the inoculated control was 2.5, and no significant differences were found among treatments (Table 4).

In the 2011/2012 season, treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) (1345.8 kg/ha) and treatment 1 (untreated and uninoculated control) (1445.8 kg/ha) differed statistically from treatment 2 (inoculated control) (504.2 kg/ha), treatment 4 (*T. viride* + Mp) (804.2 kg/ha) and treatment 5 (*B. subtilis* + Mp) (741.7 kg/ha) (Table 4). Treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) resulted in 841.6 kg/ha more in yield than the inoculated control. As for 1000-seed weight, treatment 4 (*T. viride* + Mp) (186.7 g) differed statistically with respect to the untreated and uninoculated (170.0 g) and the inoculated (169.7 g) controls, but not with respect to treatments 3 [(pyraclostrobin + thiophanate methyl) + Mp] (179.9 g) and 5 (*B. subtilis* + Mp) (181.9 g). The inoculated control showed high charcoal rot severity values (3.6) and differed statistically from the non-inoculated control (1.3), treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) (2.6) and treatment 4 (*T. viride* + Mp) (2.7), but not from treatment 5 (*B. subtilis* + Mp) (3.1) (Table 4). Again, the higher charcoal rot values in 2011/2012 compared with 2010/2011 can be accounted for by the favorable environment conditions that prevailed during that season (Table 1).

In Munasqa RR, yield of all treatments differed significantly from the fungus-inoculated control (3456.1 kg/ha) in the 2010/2011 season (Table 5). Treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) yielded 658.3 kg/ha more than the inoculated control, while treatments 4 (*T. viride* + Mp) and 5 (*B. subtilis* + Mp) showed increments of 635.6 kg/ha and 634.3 kg/ha, respectively. No significant differences were observed for 1000-seed weight among different treatments and the inoculated and uninoculated controls. With regard to disease severity, no significant differences were found among treatments and the inoculated control, which showed a rating of 1.8.

In the 2011/2012 season, yields for treatment 3 [(pyraclostrobin + thiophanate methyl) + Mp] (1945.8 kg/ha) and the noninoculated control (1866.7 kg/ha) differed statistically from that of the Mp-inoculated control (870.8 kg/ha) and treatments 4 (*T. viride* + Mp) (1491.7 kg/ha) and 5 (*B. subtilis* + Mp) (1304.2 kg/ha). Treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) yielded 1075.0 kg/ha more than the inoculated control and 79.1 kg/ha more than the noninoculated control. With regards to 1000-seed weight, treatment 3 ([pyraclostrobin + thiophanate methyl] + Mp) (151.4 g) differed statistically from the inoculated control (169.4 g). The Mp-inoculated

Table 4 Mean crop yield (kg/ha), 1000-seed weight (W 1000 in g) and charcoal rot severity (Sev) on a scale from 1 to 5 in NA8000 RG in field trials after treatment with chemicals or biological control agents in Monte Redondo (Cruz Alta, Tucumán), 2010/2011 and 2011/2012 seasons

Treatment	2010/2011			2011/2012		
	Yield	W 1000	Sev	Yield	W 1000	Sev
1-Untreated and uninoculated control	4515.1 a*	145.6 a	1.0 a	1445.8 a	170.0 b	1.3 c
2-Untreated control + Mp	4492.4 a	148.6 a	2.5 a	504.2 b	169.7 b	3.6 a
3-(Pyraclostrobin + thiophanate methyl) + Mp	4770.1 a	145.7 a	2.0 a	1345.8 a	179.9 ab	2.6 b
4-*Trichoderma viride* + Mp	4687.3 a	146.0 a	1.5 a	804.2 b	186.7 a	2.7 b
5-*Bacillus subtilis* + Mp	4631.2 a	144.6 a	1.0 a	741.7 b	181.9 ab	3.1 ab

* Means in each column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$)

Table 5 Mean yield (kg/ha), 1000-seed weight (W 1000 in g) and charcoal rot severity (Sev) on a scale from 1 to 5 in Munasqa RR cultivar in field trials after treatment with chemicals or biological control agents in Monte Redondo (Cruz Alta, Tucumán), 2010/2011 and 2011/2012 seasons

Treatment	2010/2011			2011/2012		
	Yield	W 1000	Sev	Yield	W 1000	Sev
1-Untreated and uninoculated control	4166.5 a*	137.3 a	1.0 a	1866.7 a	163.2 ab	1.2 b
2-Untreated control + Mp	3456.1 b	139.4 a	1.8 a	870.8 c	169.4 a	2.7 a
3-(Pyraclostrobin + thiophanate methyl) + Mp	4114.4 a	140.8 a	1.5 a	1945.8 a	151.4 b	2.5 a
4-*Trichoderma viride* + Mp	4091.7 a	140.5 a	1.0 a	1491.7 b	160.8 ab	2.5 a
5-*Bacillus subtilis* + Mp	4090.4 a	140.0 a	1.5 a	1304.2 b	158.8 ab	2.5 a

* Means in each column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$)

control showed the highest charcoal rot severity values (2.7), but differed statistically only from the noninoculated control (1.2). The fact that higher charcoal rot severity values (2.7) were observed in the 2011/2012 season than in the 2010/2011 season (1.8) can also be accounted for by environmental conditions (Table 1).

With respect to CFU/g of root, the Mp-inoculated control presented higher CFU/g values than in the other treatments for both cultivars and seasons. NA8000 RG presented 2100 CFU/g in 2010/2011 and differed significantly from all the other treatments (Fig. 1). In 2011/2012, Mp-inoculated control (2366 CFU/g) differed significantly from treatments 1 (untreated and uninoculated control) (166 CFU/g), 3 ([pyraclostrobin + thiophanate methyl] + Mp) (834 CFU/g) and 4 (*T. viride* + Mp) (934 CFU/g), but not from treatment 5 (*B. subtilis* + Mp) (2100 CFU/g). On the other hand, in 2010/2011 Munasqa RR inoculated control showed 300 CFU/g and differed statistically from treatments 1 (untreated and uninoculated control) (50 CFU/g) and 4 (*T. viride* + Mp) (100 CFU/g) but did not from treatments 3 ([pyraclostrobin + thiophanate methyl] + Mp) (200 CFU/g) and 5 (*B. subtilis* + Mp) (266 CFU/g) (Fig. 2). In 2011/2012 season, Munasqa RR inoculated control was 566 CFU/g and differed statistically only from the noninoculated control (166 CFU/g). All treatments had lower CFU/g values than the inoculated control in both cultivars and seasons.

Discussion

Field inoculation of soybean with *Macrophomina phaseolina* (Mp) affected all variables studied, as compared with the noninoculated control in NA8000 RG and Munasqa RR. In the 2010/2011 season, Mp inoculation resulted in a PE reduction of 6.7 % and 12.5 % in NA8000 RG and

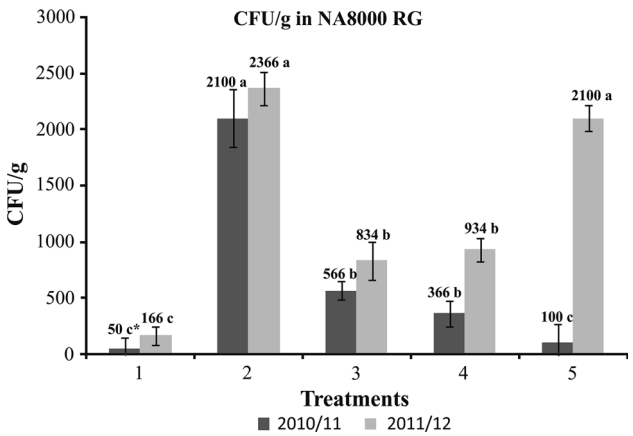

Fig. 1 CFU/g of *Macrophomina phaseolina* in NA8000 RG during the 2010/2011 and 2011/2012 seasons in field trials with different seed treatments. Treatment *1* untreated and uninoculated control, *2* untreated control + Mp, *3* (pyraclostrobin + thiophanate methyl) + Mp, *4 Trichoderma viride* + Mp and *5 Bacillus subtilis* + Mp. * Means in each season followed by the same letter are not significantly different (LSD, $\alpha = 0.05$)

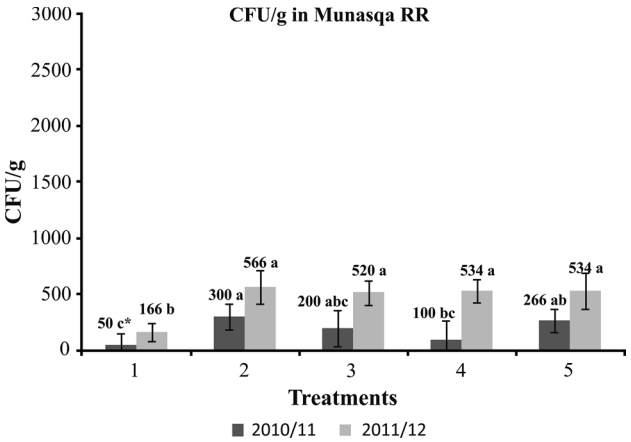

Fig. 2 CFU/g of *Macrophomina phaseolina* in Munasqa RR during the 2010/2011 and 2011/2012 seasons in field trials with different seed treatments. Treatment *1* untreated and uninoculated control, *2* untreated control + Mp, *3* (pyraclostrobin + thiophanate methyl) + Mp, *4 Trichoderma viride* + Mp and *5 Bacillus subtilis* + Mp. * Means in each season followed by the same letter are not significantly different (LSD, $\alpha = 0.05$)

Munasqa RR, respectively. In the 2011/2012 season, the PE reduction was even more conspicuous and amounted to 56.8 % in NA8000 RG and 61.3 % in Munasqa RR. These results are in accordance with Smith and Wyllie (1999), who reported high seedling mortality from charcoal rot in the tropics.

Disease severity values recorded at R7 in the inoculated control were also higher in the 2011/2012 season and in both cultivars: 3.6 for NA8000 RG and 2.7 for Munasqa RR, compared with 2.5 and 1.8, respectively, as recorded in 2010/2011. In addition, values of CFU/g root in the fungus-inoculated control reached 2100 in 2010/2011 and 2366 in 2011/2012 in NA8000 RG (Fig. 1). In Munasqa RR, these values were 300 CFU/g in 2010/2011 and 566 CFU/g in 2011/2012 (Fig. 2).

The higher PE reduction, disease severity and CFU/g values observed in the 2011/2012 season were likely the consequences of climatic conditions, with high temperatures and low rainfall levels (Table 1). Similarly, Mengistu et al. (2013) greater symptoms of charcoal rot in plants stressed by high heat and drought, especially when drought occurred during reproductive growth.

Genotypes also differ in their reaction to charcoal rot. Mengistu et al. (2013) identified sources of charcoal rot resistance in 628 accessions from soybean germoplasm by field screening in five environments. In this study, we found that Mp on Munasqa RR was less severe and developed fewer CFU/g than on NA8000 RG in both seasons.

All treatments evaluated in this research were effective in counteracting PE reduction caused by the pathogen in both seasons and cultivars. In 2011/2012 the fungicide treatment led to higher PE values than the biological treatments in both cultivars. In NA8000 RG, compared with the inoculated control, the fungicide enhanced PE by 70.3 %, *T. viride* by 26.8 % and *B. subtilis* by 7.7 %. As for Munasqa RR, the fungicide increased PE by 54.6 %, *T. viride* by 13.5 % and *B. subtilis* by 6.1 % with respect to the inoculated control. The lower effectiveness showed by the biological treatments could be due to the fact that weather conditions (Table 1) favoring Mp infection (high temperature and drought) could have affected these microorganisms negatively.

Biological and chemical seed treatments have been shown to be effective to control Mp on various crops. For cotton, several fungicides were tested as seed treatments to determine their efficacy in controlling Mp infection. Treatments with carbendazim, quintozene and benomyl enhanced plant emergence and disease control (Chauhan 1986a, b, 1988; Dwivedi and Ghaube 1985). Omar (2005) found that Monceren® 250 FS and tolclofos-methyl were the best performing fungicides in controlling Mp on cotton in the greenhouse. Pineda and Gonnella (1988) also found a reduction in the percentage of dead sesame plants affected by Mp when seeds were treated with biological antagonists such as *Trichoderma* sp. and *Aspergillus* sp.

In NA8000 RG and Munasqa RR, treatments had a similar performance trend: the highest crop yield values were obtained with the pyraclostrobin + thiophanate methyl mixture, followed by those obtained with *T. viride* and *B. subtilis*. Similar yield increases were also observed by Pineda and Avila (1988): an increment of 100 kg was obtained when applying chemical seed treatments (propineb and dicarboximide) in sesame to control Mp, as compared with yield values obtained with the untreated control.

Mp did infect Munasqa RR and NA8000 RG, but severity levels and CFU/g were higher in NA8000 RG in both seasons. The chemical and biological treatments resulted in lower disease severity and CFU/g values than in the inoculated control. These results agree with those reported by Ramezani (2008), who found lower disease incidence in eggplants (*Persea americana* L.) treated with different *Trichoderma* sp. strains.

Results from this study of chemical and biological seed treatments of soybean to control charcoal rot will be useful for farmers in Tucumán to develop more efficient management strategies for this important disease. Additional research should be extended to other regions of Argentina and neighboring soybean-producing countries in South America to further assess this plant–pathogen interaction under different environmental conditions.

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