THE ROLE OF POTENTIAL BIOCONTROL AGENTS IN THE MANAGEMENT OF PEANUT ROOT ROT IN ARGENTINA

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SUMMARY

Cultural practices such as tillage and crop rotation can be used as components of pest-management programs. The appropriate combination of tillage systems and crops may favor the development of beneficial microorganisms, preventing the spread of fungal pathogens. A long-term field study was carried out to analyze the effect of crop management on the abundance of actinomycetes, *Trichoderma* spp., and *Gliocladium* spp., as potential biocontrol agents (PBAs), and their relationship with the incidence of peanut root rot caused by *Fusarium solani*. Soil samples were taken at sowing and harvest, and root rot incidence was evaluated at harvest. There was an inverse relationship between root rot incidence peanut PBA populations under no-till suggesting a possible role of PBAs in the control of *F. solani*, the incidence of root rot being low under no-till and disc harrow, associated with a high concentration of potential antagonists. However, this correlation was not observed when soybean preceded peanut, when the incidence of root rot was low despite relatively lower populations of biocontrol agents present in the soil, in comparison with maize as previous crop.

Key words: biocontrol microorganisms, crop rotation, disease management, peanut root rot, tillage systems.

INTRODUCTION

Soilborne fungal peanut diseases are becoming increasingly widespread in Argentina, causing such losses that they are considered one of the most important factors in the decrease of peanut yield (Marinelli *et al*., 1998). Analysing why producers avoid sowing peanuts in Argentina, Busso *et al*. (2004) found that the principal reason was the increasing losses that soilborne fungi have been causing recently.

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March and Marinelli (2006) stated that in the main producing area of Córdoba province, peanut root rot caused by *Fusarium solani* is among the most important diseases caused by soilborne fungi, with up to 95% disease incidence in some fields during drought seasons (Rojo *et al*., 2006). With the aim of controlling the spread of fungal pathogens, a number of alternative tillage practices, cover and rotational crop schemes, and the use of composts and mulches are being promoted in many production systems (Abawi and Widmer, 2000).

These practices influence not only population densities of major and minor soilborne pathogens but also total crop pests and beneficial microflora and fauna. Actinomycetes, *Trichoderma* spp. and *Gliocladium* spp. are among the most frequently mentioned groups that can interfere with fungal pathogen development in soil (Burke and Miller, 1983; Rojo *et al*., 2006).

Minimum and no-tillage practices have been widely adopted in Argentina to control erosion, increase water use efficiency in summer crops and improve productivity (Buschiazzo *et al*., 1998). Direct seeding of field crops also has some confirmed beneficial side effects on soil microflora, increasing populations of microorganisms (Wander *et al*., 1995; Elliot and Stott, 1997). Giller (1996) reported that soil disturbance by tillage is the major factor affecting microbial communities and may result in reduction in soil microflora due to desiccation, mechanical destruction, soil compaction, reduced pore volume, and disruption of access to food resources. However, soil-dwelling organisms can improve crop production by releasing available forms of nutrients from soil organic and inorganic sources, fixing N within plant roots and increasing P uptake (Carpenter-Boggs *et al*., 2003), thereby sustaining healthier crops with attendant greater resistance to infection by pathogens.

Zaitlin *et al*. (2004) stated that although total number and diversity of actinomycetes were affected by tillage regime, more detailed studies are needed to identify the more subtle effects of such practices. The development and establishment of soil fungal populations (such as *Trichoderma* spp. and *Gliocladium* spp., which are common soil inhabitants) are influenced by tillage regime (Beare *et al*., 1992).

Crop rotation and residue management also regulate

soil microbial biomass, which mediates residue decomposition, nutrient cycling, and organic matter turnover (Doran and Smith, 1987). Microbial communities associated with the rhizosphere vary with different plant species (Grayston *et al.*, 1998) and crop rotation (Lupwayi *et al.*, 1998). Cover crops can affect soil microbial communities and thereby suppress plant diseases (Mazzola, 1999; Smalla *et al.*, 2001). Different crops within a rotation scheme have healthier root systems than plants in monoculture because fewer deleterious rhizosphere microorganisms are present under rotation (Jawson *et al.*, 1993).

The general aim of this work was to show that improvement of soil quality through appropriate cultural practice increases the soil microorganisms that may take part in biological control of soilborne diseases, resulting in enhanced plant root health. The objectives of the present study were: (i) analyze the effect of tillage systems on the incidence of *Fusarium* root rot, (ii) analyze the effect of crop rotation on the incidence of root rot, and (iii) analyze the population dynamics of Actinomycetes, *Trichoderma* spp., and *Gliocladium* spp. as potential biocontrol agents (PBAs) in response to tillage systems and crop rotation and their relationship to root rot incidence. These taxa include strains which have exhibited antagonism toward *F. solani* in peanut (Sivan and Chet, 1993; Sharma *et al.*, 2005; Rojo *et al.*, 2006).

MATERIALS AND METHODS

Study site. A field experiment was conducted over four years (2000-2004), 5 km southeast of General Cabrera, Department of Juarez Celman, Córdoba province, Argentina. The soil at the site is of high lime and sand content and is very susceptible to erosion (Entic Haplustoll of General Cabrera Series, 50.0% sand; 39.5% silt, 10.5% clay; USDA textural class sandy-loam). The site was selected because of historically high *F. solani* inoculum pressure.

Previously the site was used for peanut production, but this was ended because of successive root rot outbreaks. The trial consisted of 50x100 m plots, planted to three different crops: maize (*Zea mays*), soybean RR (*Glycine max*), or peanut (*Arachis hypogaea*). These crops were annually rotated, all being present each year. Care was taken to avoid the presence two or more consecutive peanut crops in the same strip (Table 1). Each crop was under three different tillage systems: no tillage, disc harrow and mouldboard plough.

Table 1. Crop rotation during the trial, over four agricultural cycles.

	Agricultural cycles			
	2000/01	2001/02	2002/03	2003/04
Crops rotated	Maize	Peanut	Maize	Peanut
	Peanut	Soybean	Maize	Peanut
	Peanut	Maize	Maize	Peanut
	Maize	Soybean	Maize	Peanut
	Soybean	Maize	Peanut	Maize

Strips were treated with herbicides suitable for weed control and fertilized when necessary. Crops were harvested at full maturity with appropriate equipment. Plots were established following a split plot design. The main plot corresponded to the rotation treatment between maize, soybean and peanut; the subplot corresponded to the tillage systems.

Disease sampling. Ten days before peanut harvest, root rot incidence was evaluated by establishing 10 sampling points in each peanut plot, regularly distributed along a V-shaped design; each point comprised 50 plants (Delp *et al.*, 1986).

Disease was evaluated once before harvest, according to Burke and Miller (1983). Peanut plants with root rot symptoms were collected from plots, brought to the laboratory, disinfested by placing them in 5% of sodium hypochlorite for 2 min, and plated on Nash and Snyder (1962) medium to confirm the presence of *F. solani*. Plates were incubated for 10 days in the dark, at 25ºC. Root rot incidence was calculated as the cumulative percentage of plants confirmed as infected by *F. solani*, on a total of 500 plants sampled.

Quantification of soil microorganisms. To quantify soil populations of PBAs under various treatments, soil was sampled twice: 20 days after sowing and 10 days before harvest. Six soil samples, each consisting of 10 subsamples, were taken from each plot, using a 3 cm diameter core at a depth of 0-5 cm, near the root. Samples were air-dried, sieved through a screen (2 mm) and stored at 4ºC until further use. To determine soil moisture, a 5-8 g sub-sample was dried at 90ºC and its weight was recorded before and after drying.

For actinomycete quantification, 1 g aliquots of soil were taken from each of the 6 samples per plot, suspended in 100 ml of sterile distilled water, and placed on an orbital shaker for 30 min at 150 rpm. The suspensions were serially diluted and spread on Küster medium modified by the addition of cycloheximide (0.15 g l^{-1}) and sodium propionate (0.4 g l^{-1}). Plates were incubated in the dark at 25ºC and actinomycete colonies were counted after 15 days following the protocol of Vargas Gil *et al.* (2007). *Trichoderma* spp. and *Gliocladium* spp. were quantified similarly, except that a 10 g 100 ml^{-1} soil suspension in sterile distilled water was serially diluted and plated on PDA supplemented with rose bengal (20 mg l^{-1}), streptomycin (100 mg l^{-1}), and chloramphenicol (300 mg l^{-1}) as described previously by Vargas Gil *et al.* (2007). Plates were incubated at 25ºC for 7 days, with 8 h of light.

Chemical analysis of soil. Soil organic matter content was measured in each agricultural cycle. Field samples were sieved at 2 mm and air dried for chemical analysis. Total soil organic C concentration was determined by dry combustion using a LECO CHN-2000 analyzer (St. Joseph, MI, USA).

Statistical analysis. Normality of data was tested using the Shapiro-Wilks test and log 10 transformation of CFU was applied to stabilize variances and improve normality when appropriate. To simplify data presentation throughout the text, CFU is described as log 10 CFU g^{-1} dry soil. The least significant difference test (LSD) was used to test for treatment differences. Statistical analyses were performed using INFOSTAT/Professional 2007 (FCA-Universidad Nacional de Cordoba, Argentina) at $P<0.05$.

RESULTS

Soil populations of Actinomycetes, *Trichoderma* spp., and *Gliocladium* spp.

Tillage systems and rotation crops. There was a statistically significant effect of tillage systems and previous crop on soil populations of PBAs but no significant interaction between the treatments (Table 2). Populations of actinomycetes, *Trichoderma* spp., and *Gliocladium* spp. were most abundant when peanut was under no tillage, followed by peanut under disc harrow, being 19, 13, and 58% lower, respectively, than under no tillage.

The lowest PBA populations were found under mouldboard plough, 46, 36, and 99% lower than peanut under no tillage (Table 3).

The previous crop influenced PBA dynamics. With maize preceding peanut, populations of actinomycetes,

Table 2. Mean squares (MS) and significance levels (P) of LSD test for the effects of tillage system and previous crop and their interactions on soil populations of potential biocontrol agents and peanut root rot incidence.

Source of variation	log 10 CFU* g^{-1} soil of potential biocontrol agents						Peanut root rot incidence (%)	
	Actinomycetes		*Trichoderma* spp.		*Gliocladium* spp.		(*Fusarium solani*)	
	MS	P	MS	P	MS	P	MS	P
Tillage	26.03	0.0001	7.26	0.0003	8.67	0.0001	3.23	0.0045
Previous crop	7.11	0.0001	50.03	0.0001	44.49	0.0001	4.65	0.0032
Tillage x Previous crop	7.65	0.6532	1.68	0.7623	1.59	0.0767	1.12	0.0009

MS: mean square; P: significant level observed at $P<0.05$.
*CFU: colony forming units (fungi expressed as $x10^2$, actinomycetes as $x10^4$).

Table 3. Total colony forming units (CFU) of potential biocontrol agents in peanut under different tillage systems and preceding crops.

Field treatments		log 10 CFU g^{-1} soil of potential biocontrol agents		
		Actinomycetes	*Trichoderma* spp.	*Gliocladium* spp.
Tillage system	No tillage	7.00 a	4.20 a	2.90 a
	Disc harrow	5.90 b	3.70 b	1.90 b
	Mouldboard plough	4.80 c	3.10 c	1.50 c
Previous crop	Maize	7.00 a	5.40 a	4.00 a
	Soybean	6.00 b	2.80 b	1.40 b

For each biocontrol agent, numbers followed by the same letter are not significantly different according to LSD test at $P<0.05$. Each number is the average of samples taken after peanut sowing and before harvest. Actinomycetes are expressed as $x10^4$ and fungi as $x\ 10^2$.

Trichoderma spp. and *Gliocladium* spp. were more abundant (17, 93, and 186%, respectively) than when soybean was the previous crop (Table 3).

Soil organic matter. Tillage treatments and previous crop influenced soil organic matter content. This was significantly higher at 1.22% under no tillage than disc harrow or mouldboard plough, at 1.06 and 1.05%, respectively. When maize preceded peanut, soil organic matter content was almost 20% higher than when soybean preceded peanut (Fig. 1).

Soil organic matter content was significantly affected by the tillage system and the previous crop (P= 0.0001); there was no interaction between the two factors (Table 4).

Table 4. Effect of tillage system and previous crop on soil organic matter content (%).

		Soil organic matter content (%)	
		MS	P
Source of variation	Tillage system	1.55	0.0001
	Previous crop	5.87	0.0001
	Tillage X Previous crop	0.21	0.5426

MS: mean square; P: observed significant level at P <0.05.

Soil organic matter content was directly correlated with PBAs. Significantly higher populations of Actinomycetes, *Trichoderma* spp. and *Gliocladium* spp. were associated with higher percentages of organic matter (Fig. 2). Correlations were measured using Pearson's correlation analysis; all the correlations were statistically significant (data not shown).

Incidence of peanut root rot.

Disease occurrence during the trial. Peanut root rot was recorded in each year, except in 2002/2003 when hail damage caused total loss of the experiment. The highest root rot incidence occurred in 2001/02, whereas the lowest was in 2000/01. In 2003/04 low root rot values were recorded, compared with 2001/02 (Fig. 3).

Cultural practices. There was a statistically significant interaction between tillage system and previous crop on the incidence of root rot (Fig. 3; Table 2). The highest root rot incidence occurred under mouldboard plough, with soybean as the previous crop. However, when the preceding crop was maize, root rot incidence was also higher under mouldboard plough, the lowest values being found under no tillage in 2003/04.

Peanut root rot incidence was also markedly affected by the previous crop: when this was soybean, disease in-

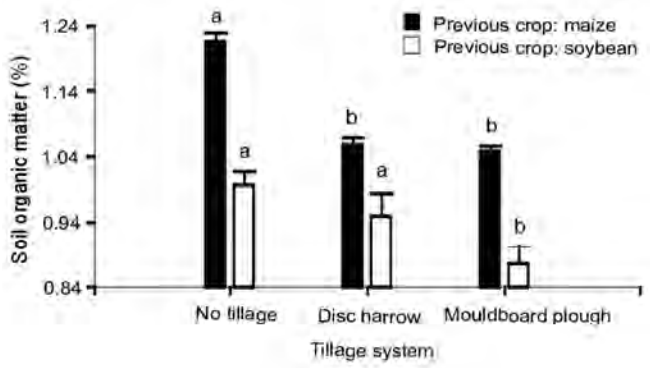

Fig. 1. Effect of tillage systems and previous crop on soil organic matter content. Bars with the same colour followed by the same letter are not significantly different according to LSD test at P <0.05. Each bar is the average of values obtained from plots under the same treatment.

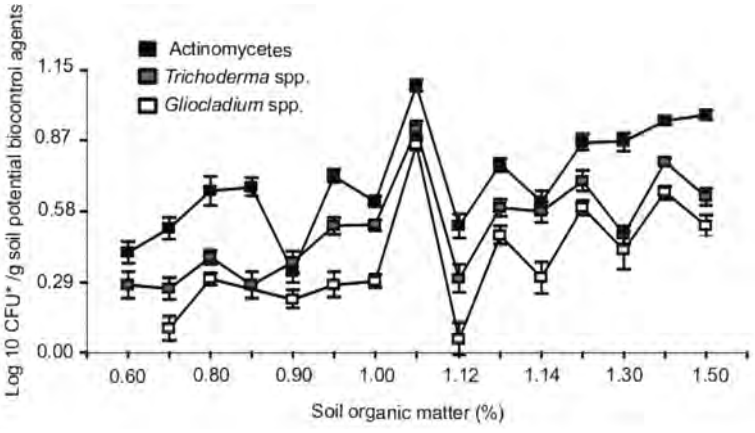

Fig. 2. Soil organic matter content and soil populations of Actinomycetes, *Trichoderma* spp. and *Gliocladium* spp. Each point is the average of samples obtained from plots under the same treatment throughout the four years of the field trial.

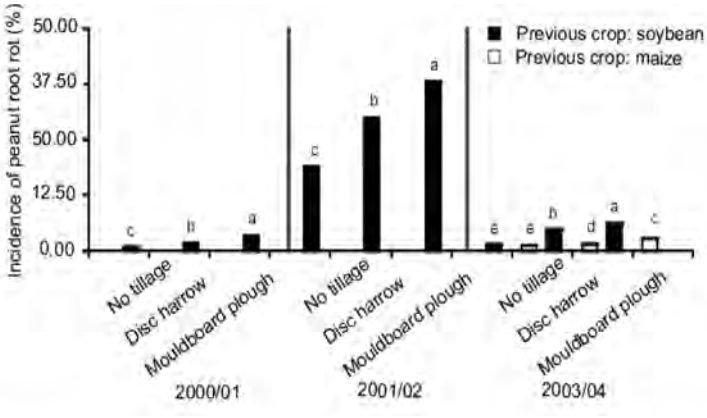

Fig. 3. Disease incidence of peanut root rot during three agricultural years, with peanut under different tillage systems (no tillage, disc harrow, mouldboard plough) and with one of two previous crops (maize, soybean).Values correspond to the mean of the incidence (%) recorded at 10 sampling points in each plot, with 50 plants per point. In a given agricultural cycle, values with the same letter do not differ significantly according to LSD test (P<0.05).

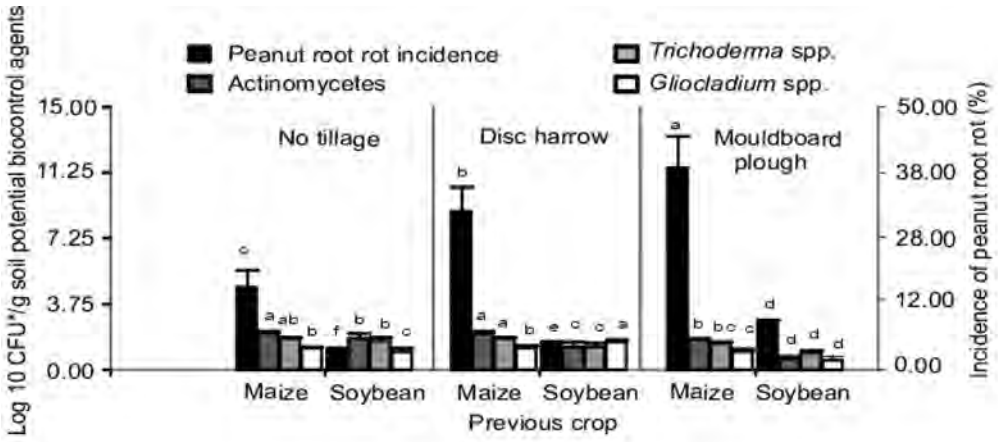

Fig. 4. Root rot incidence and soil populations of potential biocontrol agents in peanut plots under different tillage systems (no tillage, disc harrow, mouldboard plough) and with one of two previous crops (maize, soybean).*CFU: colony forming units (fungi expressed as $\times 10^2$, actinomycetes as $\times 10^4$). Black bars correspond to the mean of the incidence (%) recorded at 10 sampling points in each plot, with 50 plants at each point. Bars with the same colour and the same letter do not differ significantly according to LSD test ($P<0.05$).

cidence was lower than when it was maize, in 2003/04. In 2000/01 and 2001/02 root rot incidence was 0% when maize was the previous crop.

There was a direct relationship between root rot incidence and populations of Actinomycetes, *Trichoderma* spp. and *Gliocladium* spp. (Table 5).

DISCUSSION

We evaluated the tillage treatments and rotation sequences most widely used in Argentina for the last twenty years, and found, in concurrence with others (Steinkellner and Langer, 2004; Al-Kaisi *et al*., 2005), that tillage systems have a clear effect on soil microbial populations. The significantly higher populations of PBAs associated with lower peanut root rot incidence suggests that they may have been involved in reducing root rot. At the same time, organic matter was higher in soil under no tillage and disc harrow. Some authors (Angers *et al*., 1993; Carpenter-Boggs *et al*., 2003) state that biotic soil quality factors may be inversely related to tillage intensity, and that no-till systems increase microbial activity. The distribution of crop residues in soil, determined by the type of tillage system employed, is reported to greatly influence soil organic matter content (Rasmussen and Collins, 1995; Bending *et al*., 2002).

Our study showed that tillage intensity affected organic matter, which in turn was inversely correlated with the abundance of potential biocontrol agents. This finding is in accord with established knowledge that intensive tillage increases organic matter loss by accelerating decomposition, and that organic matter is the main nutrient source for microflora development and exerts a positive influence on microbial biomass (McGill *et al*., 1986).

It has been reported that soil microbial biomass is positively correlated with the amount of crop residues and mediates soil organic matter turnover, nutrient cycling, and soil aggregation (Larkin, 2003). In the present study, maize and soybean residues had a strong effect on populations of Actinomycetes, *Trichoderma* spp. and *Gliocladium* spp., which were higher when maize was the previous crop and lower when it was soybean. This result was correlated with soil organic matter content and can be attributed to the differing respective quality and quantity of carbon residues returned to the soil by maize and soy-

Table 5. Pearson coefficients showing correlation between disease incidence (%) and soil populations of Actinomycetes, *Trichoderma* spp. and *Gliocladium* spp.

Peanut root rot incidence (%) (*Fusarium solani*)		Correlation coefficients ($P<0.05$)		
		Actinomycetes	*Trichoderma* spp.	*Gliocladium* spp.
Tillage	No tillage	0.79*	0.84*	0.65*
	Disc harrow	0.81*	0.75*	0.73*
	Mouldboard plough	0.65*	0.66*	0.54*
Previous crop	Maize	0.36	0.30	0.50
	Soybean	0.15	0.23	0.34

* Significant at $P<0.05$

bean (McGill *et al*., 1986; Biederbeck *et al*., 1997). Our results further agree with those of Martens (2000), who reported that maize residues doubled soil organic matter content, compared with soybean. The fact that maize as the preceding crop can enhance soil organic matter content may be an important reason for the notable increase in PBAs found in this work.

In a similar study, Larkin (2003) found that soil populations of actinomycetes, *Trichoderma* spp., and fluorescent pseudomonads increased when maize and barley were included in the rotation. Moreover, Doran (1980) also described an increase of the communities of soil microorganisms like actinomycetes and fungi following some previous crops.

Root rot was also affected by cultural practice, being higher under conventional tillage (mouldboard plough) and lower under conservative systems (no tillage, disc harrow).

Conversely PBAs were more abundant under conservative tillage and lower under conventional tillage, suggesting that they may be involved in controlling *F. solani*. In agreement, increases in microbial populations and their activities have been described in low-tillage production systems (Angers *et al*., 1993; Carpenter-Boggs *et al*., 2003). These may have increased antagonism toward pathogens.

The possible causes of increased PBA populations, which were inversely related to root rot incidence, probably include an increase in nutrients due to accumulation of crop residues in the top five centimeters of soil, or higher water content under conservative tillage due to presence of crop residues on the soil surface (Stone *et al*., 2004). This not only benefits the antagonistic microflora but is also unfavorable to the development of diseases caused by such pathogens as *F. solani*, which generally occur when the crop is under water stress (Harveson *et al*., 2005; Oddino, 2007). However, the reason for the high disease incidence despite the presence of higher PBAs, as for maize as the preceding crop, may be due to the effect of parameters such as soil texture, silt and sand content, and pH, that may significantly affect the infection capacity of such soilborne pathogens as *F. solani* (Sanogo and Yang, 2000). It could be thought that a high *F. solani* inoculum pressure could be associated with maize residues, causing high disease incidence, but according to some authors (Johnson *et al*., 2001; Estevez de Jensen *et al*., 2004), many fungal pathogens that infect peanut are not sustained on monocot crops.

The activity of microbial communities, given their rapid growth and turnover makes them the component most reactive to external changes in a terrestrial ecosystem (Panikov, 1999). Thus, microbial populations can be employed as indicators of soil condition and crop health, with the aim of developing sustainable crop system, minimizing the use of chemicals and favouring intrinsic soil richness.

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