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Selected Soil-Borne Fungi under Glyphosate Application and Crop Residues from a Long-Term Field Experiment

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ABSTRACT

A study was carried out to determine the short-term effects of glyphosate and crop residues on the population dynamics of soil-borne fungi under field conditions. Field experiments were conducted during two growing seasons. The assay were done in plots that were subjected to a peanut (*Arachis hypogaea*)-corn (*Zea mays*)-soyabean (*Glycine max*) rotation system with two sequences: corn-soyabean-peanut and soyabean-corn-peanut. Soil samples were randomly collected from a peanut-corn-soyabean rotation field located at Córdoba province (Argentina) in order to quantify native populations of *Fusarium*, *Pythium*, *Trichoderma*, *Gliocladium* and culturable total fungi populations. Independently of the present crop, the highest population of *Trichoderma* and *Gliocladium* were recorded in soil with corn residue. *Pythium* populations increased after glyphosate treatment. *Trichoderma*, *Gliocladium* and culturable total fungi populations were not affected by glyphosate applications. Information on the actual time and duration of population responses of various important soil-borne fungi after glyphosate treatment is currently limited since it is dependent on numerous parameters such as soil condition, type of hosts involved and soil microbial interactions. The use of corn residue appeared as an interesting alternative to increase the population of potential antagonistre fungi, and reduce crop diseases; therefore more field research along this line is clearly needed.

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INTRODUCTION

In recent years, the intensive use of herbicides has increasingly become a matter of environmental concern, partially because of the adverse effects of these chemicals on soil microorganisms (Araújo *et al*., 2003). Herbicides can alter soil ecosystems by having a direct effect on various components of the soil microflora (Duncan & Paxton, 1981; Wilcox, 1996). These effects may increase or decrease the inoculum density, for example through promotion or suppression of beneficial microorganims activities (Altman & Rovira, 1989).

Glyphosate is a widely popular herbicide known for its effective control of competing vegetation, rapid inactivation in soil and low mammalian toxicity (Busse *et al*., 2001). The commercial success of glyphosate as a highly effective herbicide has stimulated several studies on its behaviour and persistence in soil (Forlani *et al*., 1999; Jonge & Jonge, 1999). Glyphosate has shown to suppress plant defences and to enhance diseases (Lévesque *et al*., 1993; Haney *et al*., 2000; Sanogo *et al*., 2001). Kawate *et al*. (1997) observed that glyphosate increased populations of *Fusarium solani* and *Pythium ultimum* in soil under greenhouse conditions. However, other authors reported no effect of glyphosate on the number of microorganisms isolated from soil (Stratton & Stewart, 1992). There are also inconsistencies in the effect of glyphosate on soil microorganism conducted under controlated conditions compared with experiments conducted under field conditions (Lévesque & Rahe, 1992).

Corn-peanut-soyabean rotations have been widely used for decades in Argentina. The sequence of plant species in crop rotation affects bacterial populations in soil (Lupwayi *et al*., 1998) and also pathogenic populations of root colonizing fungi like *Pythium* (Watanabe *et al*., 1977), *Phialophora* (Adee *et al*., 1997), *Rhizoctonia* (Ogoshi *et al*., 1990), *Fusarium* (Cook 1968) and other fungi that survive on organic residues in soil. Crop rotation also has been reported to suppress pathogenic fungi populations and to reduce diseases on many hosts (Mathre *et al*., 1999).

It is widely accepted that crop residues can also influence the relative abundance of plant pathogens (Luque *et al*., 2005). Manici *et al*. (2004) demonstrated that the use of green manure caused *Pythium* population to increase concurrent with an increase in soil microbial population. Elliott *et al*. (1978) demonstrated that microbial production of phytotoxic substances may alter crop yield when surface residues are maintained. Other authors studied the effect of organic and synthetic fertility amendments on *Trichoderma* populations (Bulluck III *et al*., 2002).

Little information is avaible in relation to the effect of previous crop residues on potential antagonism fungi. Vargas Gil (2008) studied the effect of rotation crop and tillage systems on potential biocontrol agents and on the incidence of soil-borne diseases. Meriles *et al*. (2006) studied the effect of glyphosate and crop residue on potential deleterious and antagonism soil-borne

fungi. However, these results were not conclusive because they were obtained from experiments under in vitro and controlled conditions. Field experiments are necessary to produce final results, and to further an understanding of this process. Control tactics focused toward the management of potential pathogen population densities may offer solutions to low the risk of yield losses. The objectives of this study were to (i) determine the effect of previous crop residues on *Trichoderma* and *Gliocladium* populations from a peanut-corn-soyabean rotation, and (ii) to determine the effect of glyphosate applied in field conditions with these residues on selected soil-borne fungi.

MATERIALS AND METHODS

Previous crop residue effect on the potential biocontrol agents, *Trichoderma* spp. and *Gliocladium* spp.

Data reported for this experiment were collected from November 2003 to April 2005 on a long-term (6 years) experimental site located at an Agropecuarian Experimental Station (EEA INTA Manfredi), Córdoba Province, Argentina. The assay was conducted in eight plots that were submitted to a peanut (*Arachis hypogaea*)-corn (*Zea mays*)-soyabean (*Glycine max*) rotation system in two sequences: corn-soyabean-peanut and soyabean-corn-peanut. The soil type is characterized as a typical argiudoll soil, clease textural (USDA) silt loam, silt content 12.3%, loam content 68.3% and clay content 19.2%. Details of chemical and physical characteristics of the soil are shown in Table 1. Plots of peanut, corn and soyabean were established in a randomized complete block design with two replicates in 20 × 70 m strips. Crop strips were harvested at full maturity using appropriate equipment to remove grain. After harvest, residues of each crop were left on the soil surface and no tillage was performed. Six bulk soil samples (0–10 cm depth) were randomly taken from each plot at planting and harvest of each crop. All soil samples were sieved through a 2 mm screen and stored at 4ºC until processing.

In the laboratory, each composite sample was mixed again and two subsamples were taken. The first subsample was used to determine soil moisture level and the second one for dilution plating. For the determination of soil moisture, a 5–8 g subsample was weighed before and after 7 days of drying at 90ºC. For the quantification of *Trichoderma* and *Gliocladium*, a 10 g subsample of soil was weighed, transferred into 100 ml of distilled and sterile water, and mixed on a rotary shaking machine during 30 min. Six plates were used for each sample. *Trichoderma* spp. and *Gliocladium* spp. were counted on PDA supplemented with rose bengal (20 mg l^{-1}), streptomycin (100 mg l^{-1}) and chloramphenicol (300 mg l^{-1}) after 7–10 days at 22ºC (Table 2). Data are expressed as number of colony forming units (CFUs) per g of dry soil. According to Elmholt & Laboireau (2005), the dilution plating method is

TABLE 1
Soil physical, chemical, and textural characteristics of soil with peanut and corn residues.

Crop sequence (previous crop at sampling is underlined)	Corn-soyabean-peanut	Soyabean-corn-peanut	Soyabean-corn-peanut
Organic matter (%)	2.59	2.22	2.35
Total N content (%)	0.15	0.15	0.15
C:N ratio	9.70	8.70	9.00
Total P content (ppm)	61.00	55.80	52.90
pH (1:2 soil/water)	6.84	6.28	6.63
Cation exchange capacity (meq 100 g^{-1})			
Ca^{2+}	13.50	13.50	12.50
Mg^{2+}	2.50	3.00	4.50
Na^{+}	0.22	0.20	0.20
K^{+}	3.59	3.36	3.50

TABLE 2
Previous crop residue effects on *Trichoderma* and *Gliocladium* populations from soil with peanut, corn and soyabean residues in a peanut-corn-soyabean rotation field.

Genus of fungi**	Peanut plot		Corn plot		Soyabean plot	
	Corn residue (×100 CFU g^{-1})*	Soyabean residue (×100 CFU g^{-1})*	Peanut residue (×100 CFU g^{-1})*	Soyabean residue (×100 CFU g^{-1})*	Corn residue (×100 CFU g^{-1})*	Peanut residue (×100 CFU g^{-1})*
Trichoderma spp.	688.96 a	464.38 b	207.50 c	193.33 c	456.04 b	233.75 c
Gliocladium spp.	351.46 a	158.02 c	125.42 cd	86.77 d	269.58 b	111.46 cd

Means within the same genus of fungi (rows) followed by the same letter/s are not significantly different according to Fisher's test at $p < 0.05$.
*CFU = Colony forming units.
**Mean values of 2004 and 2005 assessments.

suited to assess the soil contents of *Trichoderma* spp. and *Gliocladium* spp. on agar medium.

Glyphosate and previous crop residue effect on soil-borne fungi population

In this experiment, soil-borne fungi populations were studied as a function of time after glyphosate treatment. In summer 2005, soyabean plots with different preceeding crops (peanut and corn) were selected. Soyabean at V2 growth stage was sprayed with glyphosate (Roundup® ultra) at the recommended field application rate (2.40 kg a.i ha^{-1}). Glyphosate was applied to plants with a tractor-sprayer at a spray volume of 190 l ha^{-1} and a pressure of 207 KPa. Control treatments with no glyphosate application were included for comparison with the herbicide treatment. At 0, 3, 6, 9, 12, 15, 18, 21 days

after glyphosate treatment (DAGT), six bulk soil samples (0–10 cm depth) were randomly taken from each plot. All soil samples were sieved through a 2 mm screen and stored at 4ºC until processing.

Culturable total fungi, *Trichoderma*, *Gliocladium,* *Fusarium* and *Pythium* populations were monitored by serial dilution plating on selective or semiselective media. In the same way as in the previous experiment, *Trichoderma*, *Gliocladium* and culturable total fungi populations were counted on PDA supplemented with rose bengal, streptomycin and chloramphenicol. *Fusarium* spp. was counted on Nash and Snyder medium (Nash & Snyder, 1962) according to Cho *et al*. (2001). *Pythium* spp. was counted on maize meal agar supplemented with the following antifungal agents (per liter of distillated water): benomyl (Benlate, 50% active), 250 mg; PCNB (pentacloronitrobenzene), 100 mg; and with the following antibacterial agents: riphampicin, 10 mg and ampicillin, 250 mg. This is a modification of the medium used by Jeffers & Martin (1986).

Statistical analyses

Statistical analyses were conducted using INFOSTAT/Professional 2005 p.1 (F.C.A.-Universidad Nacional de Córdoba, Argentina) at $p < 0.05$. Normality of data was tested using the Shapiro-Wilks test. In both experiments, square-root transformation of CFU was applicated to stabilize variances and improve normality when appropriate. In the second experiment, this variable was used to determine the area under the population curve (AUPC) as calculated by Euler´s rectangular integration method (Rabbinge *et al*., 1989). Untransformed means are reported. The least significant difference test (LSD) was used to test the treatment differences (Steel *et al*., 1997).

RESULTS

Effect of previous crop residue on *Trichoderma* and *Gliocladium* populations

Previous crop residues affected populations of *Trichoderma* in soil in both years. In general, *Trichoderma* were higher than *Gliocladium* populations. The total CFU of *Trichoderma* ranged from about 0 to 1.85×10^5 in 2004 (Figure 1) and from 0 to 1.20×10^5 in 2005 (Figure 2). In both years, the highest populations of *Trichoderma* were observed when peanut was planted on corn residue. Plots following corn fell into the following ranges: 0–1.85×10^5 CFU soil for peanut following corn in 2004, 0-1.00 x10^5 CFU for soyabean following corn in 2004, 0–1.20×10^5 CFU for peanut following corn in 2005,

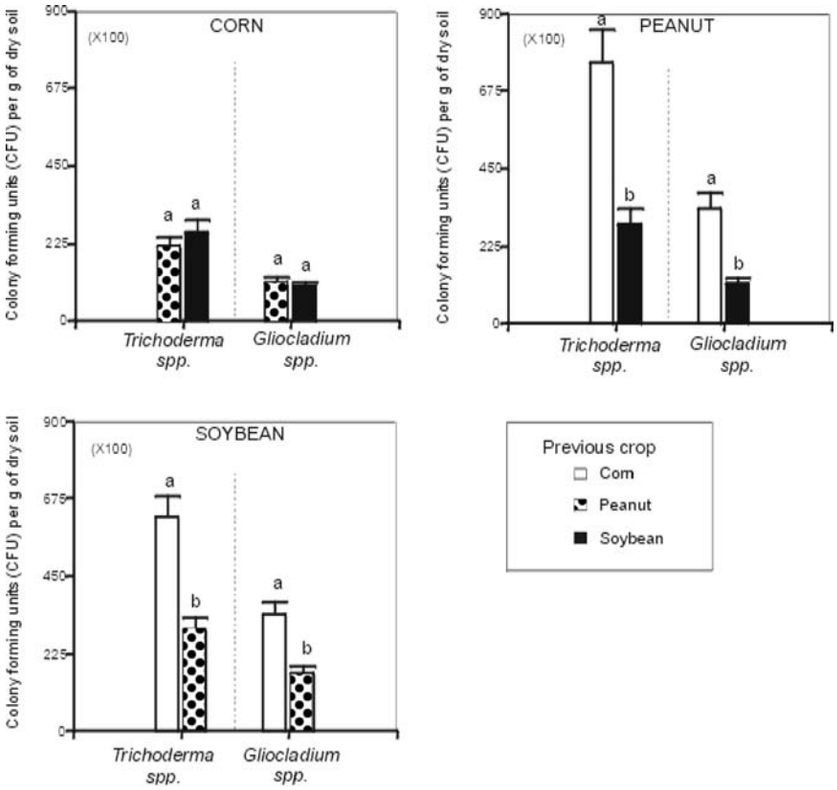

FIGURE 1. Isolation frequency of *Trichoderma* spp. and *Gliocladium* spp. from soil with peanut, corn and soyabean residues in a peanut-corn-soyabean rotation field 2003-2004. Within each genus of fungi, bars with a common letter/s are not significantly different according to Fisher´s test at $p < 0.05$.

and 0–5.8 × 10^4 CFU g^{-1} soil for soyabean following corn in 2005. The lowest mean values of *Trichoderma* were observed in corn following peanut in 2004 and in soyabean following peanut in 2005. Based on the analysis of variance the isolation frequency of *Trichoderma* was highest in peanut following corn (data for 2004 and 2005 are averaged, Table 2).

Previous crop residues also affected *Gliocladium* populations. In a similar way, the plots following corn showed the highest populations of *Gliocladium*. In 2004, *Gliocladium* ranged from about 0 to 8.50 × 10^4 CFU for peanut following corn, and 0 to 6.8 × 10^4 CFU for soyabean following corn. In 2005, *Gliocladium* ranged from 0 to 8.00 × 10^4 CFU for peanut following corn, and 0 to 4.00 × 10^4 CFU for soyabean following corn. In both years the lowest values of *Gliocladium* were observed in corn planted on peanut and soyabean

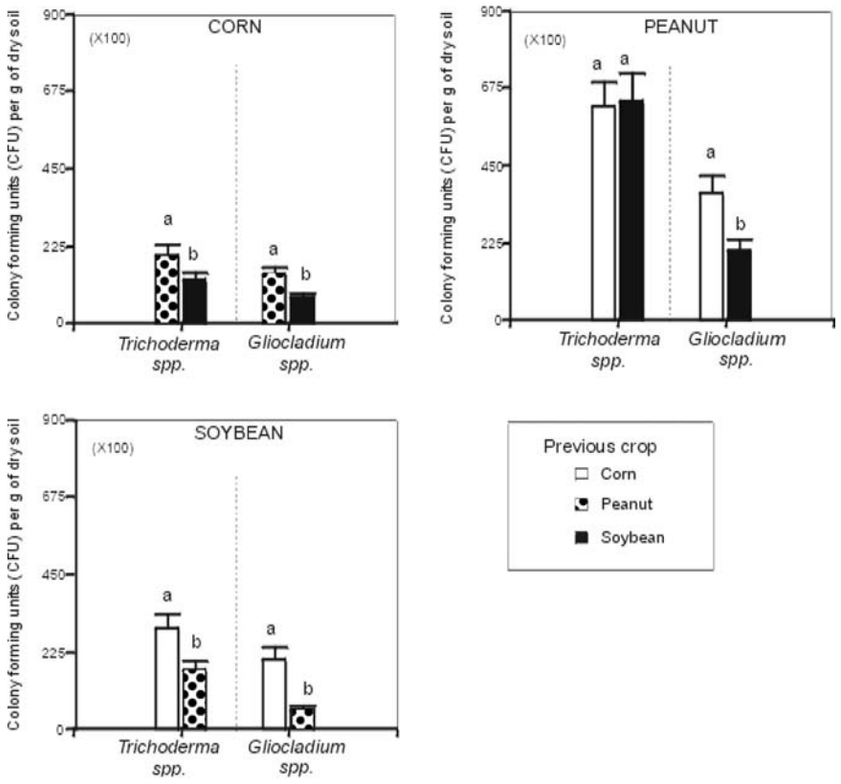

FIGURE 2. Isolation frequency of *Trichoderma* spp. and *Gliocladium* spp. from soil with peanut, corn and soyabean residues in a peanut-corn-soyabean rotation field 2004-2005. Within each genus of fungi, bars with a common letter/s are not significantly different according to Fisher´s test at $p < 0.05$.

residues (Table 2). Significant interaction between previous crop residue and year was observed in both *Trichoderma* and *Gliocladium* populations at $p < 0.05$.

Effect of glyphosate and previous crop residue on soil-borne fungi populations

The highest populations of *Trichoderma* were observed in soil with corn residue at all DAGT (Figure 3). The population level ranged from about 2.70×10^3 to 2.3×10^4 CFU in soil with corn residue, and 0 to 1.40×10^4 CFU in soil with peanut residue. The highest values of CFU soil were found at 9 and 15 DAGT. The AUPC ranged from about 9.60×10^4 to 2.86×10^5

(Table 3). For each crop residue, there were no significant differences between glyphosate treatment and the control.

A similar and uniform response was observed for *Gliocladium* populations (Figure 3). The number of CFU of *Gliocladium* isolated from soil with corn residue was higher than from soil with peanut residue at all DAGT (Figure 3). The population level ranged from about 0 to 4.20×10^4 CFU in soil with corn residue and 0 to 2.50×10^4 CFU in soil with peanut residue. The AUPC ranged from about 6.80×10^4 to 3.23×10^5 (Table 3). For each crop residue, there were no significant differences between glyphosate treatment and the control.

Fusarium populations showed little variation between crop residue and glyphosate treatment (Figure 3). The population level ranged from about 0 to 2.90×10^4 CFU in soil with corn residue, and 0 to 2.40×10^4 CFU in soil with peanut residue. No consistent differences in the responses to the four treatments involving glyphosate treatment and the two crop residues were evident during 21 DAGT, although a distinct population peak in the corn residue + glyphosate was observed at 18 DAGT. However, no significant differences were found between the AUPCs of both crop residues and glyphosate treatments (Table 3).

Glyphosate treatment increased populations of *Pythium* spp. in both corn and peanut residues (Figure 3). The population level ranged between 0 and 7.00×10^3 CFU during 0 to 3 DAGT. *Pythium* increased from 3.00×10^3 immediately after 3 DAGT to 5.30×10^4 by 12 DAGT, and then decline to 1.79×10^4 at 21 DAGT. The population ranged between 0 and 5.30×10^4 CFU for the duration of the sampling period. There were statistical differences

TABLE 3

Glyphosate and previous crop residue effects on *Trichoderma*, *Gliocladium*, *Fusarium*, *Pythium* and culturable total fungi populations from soil with peanut and corn residues in a soyabean plot.

	Peanut residue		Corn residue	
Genus of fungi**	AUPC* Control (×1000)	AUPC Treated (×1000)	AUPC Control (×1000)	AUPC Treated (×1000)
Trichoderma spp.	95.67 a	116.05 a	286.06 b	216.82 b
Gliocladium spp.	103.71 ab	68.15 a	291.63 b	323.28 b
Fusarium spp.	80.94 a	105.50 a	114.89 a	112.86 a
Pythium spp.	94.51 a	293.01 b	85.29 a	262.43 b
Culturable total fungi	1674.57 a	2185.03 a	4156.97 b	4179.28 b

Means within the same genus of fungi (rows) followed by the same letter/s are not significantly different according to Fisher's test at $p < 0.05$.

* AUPC = area under the population curve.

** Monitored during 21 days after glyphosate treatment (DAGT).

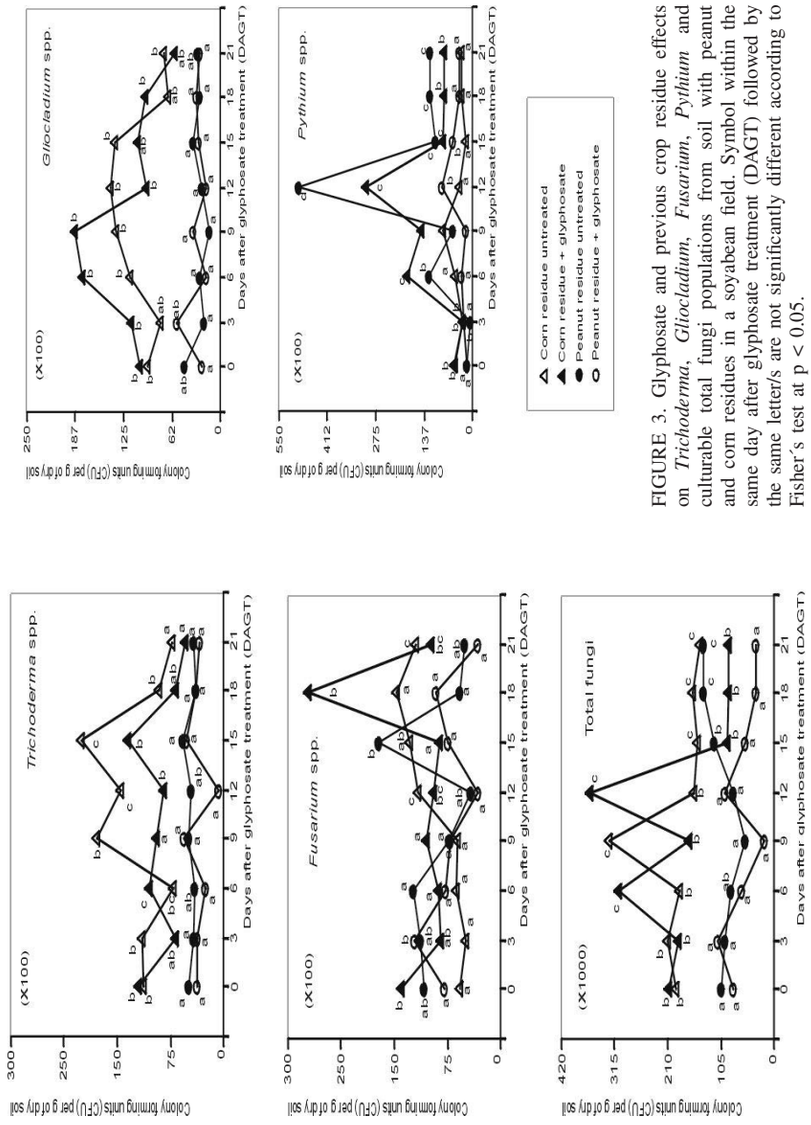

FIGURE 3. Glyphosate and previous crop residue effects on *Trichoderma*, *Gliocladium*, *Fusarium*, *Pythium* and culturable total fungi populations from soil with peanut and corn residues in a soyabean field. Symbol within the same day after glyphosate treatment (DAGT) followed by the same letter/s are not significantly different according to Fisher´s test at $p < 0.05$.

beetween AUPCs from glyphosate treatment and the control in both corn and peanut residue (Table 3).

Except 15, 18, and 21 DAGT, culturable total fungi isolated from soil with corn residue was higher than from soil with peanut residue (Figure 3). The population level ranged from about 5.00×10^4 to 5.00×10^5 CFU in soil with corn residue and 1.00×10^4 to 3.50×10^5 CFU in soil with peanut residue. The AUPC ranged from about 1.67×10^5 to 4.18×10^6 (Table 3). For each crop residue, there were no significant differences between AUPCs from glyphosate treatment and the control.

DISCUSSION AND CONCLUSIONS

Previous crop residues differentially affected *Trichoderma* and *Gliocladium* populations. In both years of this study, the highest populations of *Trichoderma* and *Gliocladium* were found on plots with corn residue. In contrast, plots following soyabean showed the lowest values. These results agree with a recent report on the potential of certain tillage systems and crop sequences to increase populations of biocontrol microorganims (Vargas Gil *et al*., 2008). Soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plant residues in the soil profile (Kandeler *et al*., 1999). Bandick & Dick (1999) demonstrated that each crop has unique qualities that may regulate the activities of soil microorganisms. Galantini & Rosell (2006) also address that the biological component of a system is carbon (C) and nitrogen (N) controlled, which are affected mainly by plant residues, suggesting that the quality of the crop residue should influence soil microflora. According to this, Dolan *et al*. (2006) outline that corn residue, as a C4 plant, produces larger amounts of carbon than soyabean, which may have been one of the reasons for the increase of *Trichoderma* and *Gliocladium* populations. Generally, for each plot/crop residue combination, the population of *Trichoderma* was higher than the population of *Gliocladium*.

Glyphosate also affected soil-borne fungi populations. The data showed that glyphosate increased *Pythium* population from both peanut and corn residues. A similar finding was reported by other investigators, who have observed a strong correlation between *Pythium* population and herbicide application, with special reference to glyphosate (Lévesque & Rahe, 1992; Dissanayaque *et al*., 1998). The fast *Pythium* response to glyphosate application in the open field revealed that this genus poses potential problems during approximately 20 DAGT in soils with *Pythium* as an agent of root rot complex.

The effect of glyphosate on *Fusarium* population is less clear and differences among untreated and treated plots are difficult to interpret. Kawate *et al*. (1997) reported that peas planted in soil where either downy brome (*Bromus tectorum*) or henbit (*Lamium amplexicaule*) had been treated with glyphosate could be

exposed to higher populations of *F. solani* f. sp. *pisi*. However, other authors (Sanogo *et al*., 2001) reported that there was no cultivar-herbicide interaction with respect to the severity of foliar symptoms of the disease and the frequency of isolation of *Fusarium solani* f. sp. *glycines* from roots of soyabean plants. In the present study, an increase of *Fusarium* population was observed on some DAGT, although no statistical differences were found when the AUPCs for both peanut and crop residues were compared. *Trichoderma*, *Gliocladium* and culturable total fungi populations were not affected by glyphosate applications. Information on the actual time and duration of population responses of various important soil-borne fungi after glyphosate treatment is currently limited since it is dependent on numerous parameters such as soil condition, type of hosts involved and soil microbial interactions, therefore more research along this line is clearly needed.

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