

Occurrence and distribution of soil *Fusarium* species under wheat crop in zero tillage

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Abstract

The presence of *Fusarium* species in cultivated soils is commonly associated with plant debris and plant roots. *Fusarium* species are also soil saprophytes. The aim of this study was to examine the occurrence and distribution of soil *Fusarium* spp. at different soil depths in a zero tillage system after the wheat was harvested. Soil samples were obtained at three depths (0-5 cm, 5-10 cm and 10-20 cm) from five crop rotations: I, conservationist agriculture (wheat-sorghum-soybean); II, mixed agriculture/livestock with pastures, without using winter or summer forages (wheat-sorghum-soybean-canola-pastures); III, winter agriculture in depth limited soils (wheat-canola-barley-late soybean); IV, mixed with annual forage (wheat-oat/Vicia-sunflower); V, intensive agriculture (wheat-barley-canola, with alternation of soybean or late soybean). One hundred twenty two isolates of *Fusarium* were obtained and identified as *F. equiseti*, *F. merismoides*, *F. oxysporum*, *F. scirpi* and *F. solani*. The most prevalent species was *F. oxysporum*, which was observed in all sequences and depths. The Tukey's test showed that the relative frequency of *F. oxysporum* under intensive agricultural management was higher than in mixed traditional ones. The first 5 cm of soil showed statistically significant differences ($p = 0.05$) with respect to 5-10 cm and 10-20 cm depths. The ANOVA test for the relative frequency of the other species as *F. equiseti*, *F. merismoides*, *F. scirpi* and *F. solani*, did not show statistically significant differences ($p \leq 0.05$). We did not find significant differences ($p \leq 0.05$) in the effect of crop rotations and depth on Shannon, Simpson indexes and species richness. Therefore we conclude that the different sequences and the sampling depth did not affect the alpha diversity of *Fusarium* community in this system.

Additional key words: crop rotation; cultivated soil; soil depth; species diversity.

Introduction

The *Fusarium* species are widely distributed in different soil types commonly associated with plant debris and roots. *Fusarium* spp. also exhibits a saprophytic activity in the soil, which can be attributed to the capacity of *Fusarium* species to grow on a wide

range of substrates and their efficient dispersion mechanisms (Burgess, 1981). It is also cited the endophyte condition of this fungus (Pitt & Hocking, 1999). In agricultural systems, the importance of this fungus is strongly associated with several diseases that cause losses on crop yields and mycotoxin contamination of grains.

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Abbreviations used: AM (arbuscular mycorrhiza); CLA (carnation leaf agar); Fr (relative frequency); PCR (polymerase chain reaction); PDA (potato dextrose agar); SNA (Spezieller Nährstoffarmer Agar); VAM (vesicular arbuscular mycorrhiza).

† Angélica M. Arambarri passed away after this article was accepted for publication. We dedicate this article to her memory.

In Argentina, over the last 30 years conventional tillage has been replaced by zero-tillage and minimum tillage systems. These systems maintain and/or improve the quality of natural resources in the agricultural production process (Acevedo & Silva, 2003). The zero-tillage system brings important qualitative and quantitative changes in soil environment: the stubble cover, less disturbance with moisture and temperature conditions completely different from those in conventional tillage. The advantages offered by zero-tillage, such as soil conservation, increased efficiency in water use and fuel economy in the machines, are sometimes confronted with disadvantages such as the high nutrient requirements at the time to install the practice, the need to replace planters and the potential threat of necrotrophic pathogens found in the crop stubble conditions that favor its saprophytic phase (Ivancovich, 1992; Fernández *et al.*, 2008).

The interaction of the tillage system with *Fusarium* infection has been a matter of particular interest, especially at wheat and maize growing areas (Fernández *et al.*, 2008). Different researches suggest that the diversity of *Fusarium* community has a strong relationship with crop rotation (Steinkellner & Langer, 2004; Luque *et al.*, 2005; Wakelin *et al.*, 2008).

Within zero tillage systems the top up layer breakdown brings carbon and organic forms of nitrogen on soil surface, producing the increase of bacterial and fungal biomass in the first 10 cm of soil. *Fusarium* spp. is a fungus with the ability to survive in this layer of soil. *Fusarium* spp. can survive as chlamidospore in the soil, therefore the soil may be infected, and when environmental conditions are favorable for the fungus a disease development on plants may occur. Rupe *et al.* (1997) observed that the density of *F. solani* was lower when sorghum [*Sorghum bicolor* (L.) Moench] or wheat (*Triticum aestivum* L.) was incorporated in the soybean [*Glycine max* (L.)] system. The same effect was observed for root rot on green bean (Hall & Philips, 1992). However, some authors considered that the limited soil tillage increases the abundance of *Fusarium* sp. in the soil (Wakelin *et al.*, 2008). Luque *et al.* (2005) observed that the addition of maize stubble increases the diversity of *Fusarium* species. Some reports suggested the high influence of soil depth on the amount of *Fusarium* propagules in the soil (Rodríguez-Molina *et al.*, 2000; Yi *et al.*, 2002; Steinkellner & Langer, 2004).

In Argentina, many reports evaluate the effects of tillage systems on fungal pathogens populations, arbuscular (AM) and vesicular-arbuscular mycorrhiza (VAM) or entomopathogenic fungi (Bonel & Morrás,

2000; Luque *et al.*, 2005; Nesci *et al.*, 2006; Schalamuck *et al.*, 2006, 2007; Gómez *et al.*, 2007; Lori *et al.*, 2009). The studies available in Argentina establish relationships among *Fusarium* spp. populations and stubble crops or plant roots (Luque *et al.*, 1999; Weber *et al.*, 2001; Fernández *et al.*, 2008). To our knowledge, comparative studies about the effects of agricultural managements on soil *Fusarium* community are scarce. Nesci *et al.* (2006) observed that the *Fusarium* community was significantly different comparing conventional with zero tillage. Steinkellner & Langer (2004) observed that the soil *Fusarium* spp. frequency was affected by sampling year, tillage and cultivated crop. Wakelin *et al.* (2008) using molecular tools proved that the community of *Fusarium* spp. is strongly related with the agricultural management.

The aim of this study was to examine the occurrence and distribution of soil *Fusarium* spp. at different sampling depths, when the wheat was harvested under a zero tillage crop system.

Material and methods

Origin of samples

The experiment was established in 1997 in the Barrow Experimental Station (38° 19' 25" S; 60° 14' 33" W), Tres Arroyos, Buenos Aires Province, Argentina, with a long history of zero tillage. The soil is a typical petrocalcic argiudol, Serie Tres Arroyos, depth ranging between 50 and 55 cm, clay loam texture. The agroclimatic conditions of the experimental place and characteristics of soils in the rotation systems are shown in Table 1 and Table 2. The determination of P, NO₃

Table 1. Agro-climatic information of Barrow, Tres Arroyos, Buenos Aires province, Argentina (38° 19' 25" S; 60° 14' 33" W)

	Average	Month
Rainfall (mm)	79.1	61.2
Relative humidity (%)	57	54
T mean (°C)	21	20.8
T maximum (°C)	26.9	27
T minimum (°C)	11.6	12
Frost number	0.1	0
Hours of sun	9.6	10
T 5 cm of soil (°C)	10.3	11

Average: dates from 1938-2008 Month: December 2009. Source: <http://anterior.inta.gov.ar/?url=http://anterior.inta.gov.ar/barrow/info/documentos/agrometeo/indice.htm>

Table 2. Soil characteristics at experimental place (Barrow Experimental Station, Tres Arroyos, Buenos Aires Province)

Sequences	P (ppm)		NO ₃ ⁻ (ppm)		OM (%)			Stubble supply (kg ha ⁻¹)
	0-5 cm	5-20 cm	0-5 cm	5-20 cm	0-5 cm	5-10 cm	10-20 cm	
I. Conservationist agriculture	47.06	27.56	104.43	106.16	4.38	4.1	3.96	4,020
II. Mixed agriculture/livestock with pastures (without using winter or summer forages)	38.63	13.73	165.10	125.96	5.26	4.22	3.94	4,280
III. Winter agriculture in depth limited soils	51.96	20.36	231.23	163.46	5.87	4.34	3.74	3,865
IV. Mixed with annual forage	41.8	14.73	199.96	154.03	4.72	4.00	3.84	3,755
V. Intensive agriculture	42.6	15.93	208.46	160.73	4.72	4.29	4.02	4,180

P: phosphorous; NO₃: nitrate; OM: organic matter.

(CuSO₄ Snedd) (Daniel & Marban, 1989), and % OM (organic matter Walkley and Black) was carried out by Soil Laboratory of Ing. Mariana Porsborg, Moreno N° 420, Tres Arroyos, Argentina. Stubble supply was calculated by Industrial Quality of grains of Experimental Station of Barrow, Tres Arroyos, Argentina.

The plots were arranged in complete blocks and the treatments (rotation systems) randomized, using three replicates and 420 m² plot area (14 m × 30 m). Sequences consisted of five different crop rotations: I, conservationist agriculture (wheat-sorghum-soybean); II, mixed agriculture/livestock with pastures, without using winter or summer forages (wheat-sorghum-soybean-canola-pastures); III, winter agriculture in depth limited soils (wheat-canola-barley-late soybean); IV, mixed with annual forage (wheat-oat/*Vicia sativa*-sunflower); V, intensive agriculture (wheat-barley-canola, with alternation of soybean or late soybean). The cultivars used were barley Quilmes Ayelen; canola SW 2836; soybean A 4613 RG; late soybean A 3726 RG; oat Bonaerense Maja; sorghum DK 61 T; wheat BIOINTA 2001 and sunflower DK 3920.

The sequences reflected what happens in regional production systems. The two mixed production situations (II and IV), both with grazing animals, make a difference in various physical soil parameters compared with agricultural sequences. The three remaining situations (I, III and V) are exclusively agricultural. Sequence I is held by many “traditional” farmers, who generally own the land with one crop per year. Sequence V appeared in recent years by hand of companies that lease the land, with the intention of intensifying the rotation (double cropping). Sequence III has been the most volatile of all. It started with a sequence of one crop per year in the first cycle (regular rotation in the area of

limited soils), but the arrival of soybean made it appropriate to be included as a double crop in those years when soil moisture conditions made soybean planting possible, on the basis of winter crops that are more secure in such soils.

The soil samples were extracted with a hydraulic borer to a depth of 20 cm, and each one was divided in 0-5 cm, 5-10 cm and 10-20 cm fractions, when the wheat cultivar was harvested (December 2009).

Fungal isolation and identification

Each soil sample (n = 45; 5 sequences × 3 replicates × 3 depths) was washed according to the modified methodology of Parkinson & Williams (1961). Fifty soil particles of each soil sample were placed on Petri dishes containing potato dextrose agar (2% PDA) amended with 250 mg chloramphenicol L⁻¹ to suppress bacterial growth. Plates were incubated for 5 days in a controlled chamber at 25°C under 12-h light/dark conditions, fungal colonies morphologically similar to those of *Fusarium* were taken with a sterile claw and subcultured in Petri dishes with carnation leaf agar (CLA) (Fisher *et al.*, 1982) and PDA tubes (16 mm × 150 mm). Plates were incubated for 6 to 15 days in a controlled chamber at 25°C under 12-h light/dark conditions. The *Fusarium* species were morphologically identified according to the taxonomic keys proposed by Nelson *et al.* (1983) and Leslie & Summerell (2006).

The isolates identified as *Fusarium* spp., were cultivated in Spezieller Nährstoffarmer Agar (SNA) (Nirenberg, 1976) and conserved in the fungal collection of the Laboratory of Functional Biology and Biotechno-

logy (BIOLAB), Agronomy Faculty, University of the Center of Buenos Aires Province, Argentina.

To avoid miss-identifications at morphological level, 47 monosporic representative isolates selected at random (43 isolates of *F. oxysporum*, one of *F. equiseti* and three of *F. solani*), were cultured in Petri dishes with 2% PDA for 7 days at 25°C under 12-h light/dark conditions. The mycelia were harvested and the genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) protocol described by Stenglein & Balatti (2006). The DNA quality was examined by electrophoresis in 0.8% (w/v) agarose gels containing GelRed™ (Biotium, Hayward, USA) at 80 V in 1X Trisborate-EDTA buffer for 3 h at room temperature. The DNA was visualized under UV light. DNA concentrations were calculated using a fluorometer (Qubit™-Invitrogen, Buenos Aires, Argentina).

Polymerase chain reaction (PCR) analyses (XP Thermal Cycler, BIOR Technology CO, Hangzhou, China) using available species-specific primers for *F. oxysporum* (Mishra *et al.*, 2003) and *F. equiseti* (Jurado *et al.*, 2005) were made and compared with positive controls. In order to confirm the identity of *F. solani* isolations, the elongation factor 1- α (EF-1 α) was amplified (O'Donnell *et al.*, 1998; Geiser *et al.*, 2004). The fragments were purified by *AccuPrep*® Gel Purification Kit (Bioneer Corporation, California, USA). DNA sequencing, from both the sense and antisense ends of the fragments, were carried out using Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyzer 3130). The sequences were compared with the NCBI database using BLASTN (Altschul *et al.*, 1990).

Products from PCR reactions were examined by electrophoresis in 1.5% (w/v) agarose gels containing GelRed™ (Biotium, Hayward, USA) at 80 V in 1 X Trisborate-EDTA buffer for 1 h at room temperature. Fragments were visualized under UV light. The size of the DNA fragments were estimated by comparing the DNA bands with a 100 bp DNA ladder (Genbiotech SRL, Buenos Aires, Argentina).

Community diversity

The isolation frequency (Fr) was calculated according to Marasas *et al.* (1988) and the alpha-diversity through the Shannon (H'), Simpson (J) indexes and

species richness (S), according to Magurran (1988) using the software PRIMER 5 (PIMER-E Ltd, UK, 2001). To detect the effect of crop rotation and depth on the mean of alpha-diversity parameters a variance analysis (ANOVA) was made using a free version of the INFOSTAT software (Di Rienzo *et al.*, 2010).

Results

Fungal growth was observed on 983 soil particles from 2,250 analyzed ones. One hundred and twenty two isolates of *Fusarium* spp. were obtained. The 35.53% of isolates were obtained from soils with the conservationist agriculture rotation system (I), 27.27% from mixed agriculture/livestock with pastures (without using winter or summer forages) (II), 15.70 % from intensive agriculture (V), 12.40% from winter agriculture in depth limited soils (III), and 9.09% from mixed agriculture with annual forage (IV).

The *Fusarium* spp. species identified were: *F. equiseti* (Corda) Saccardo, *F. merismoides* Corda, *F. oxysporum* Schlechtendahl emend Snyder & Hansen, *F. scirpi* Lambotte & Fautrey and *F. solani* (Martius) Appel & Wollenweber emend Snyder & Hansen [Suppl. Table 1 (pdf)]. A representative sequence of *F. solani* was deposited in the NCBI/GeneBank database under the accession number: JQ793953.

The number of *Fusarium* species isolated per sample varied from 1 to 3. The most prevalent species was *F. oxysporum*. This species was observed in all sequences and sampled depths. ANOVA analyses showed significant differences among sequences and depths for *F. oxysporum* Fr (Table 3). Tukey's test showed that *F. oxysporum* Fr under intensive agriculture (V) was significantly higher than mixed with annual forage (IV) (Fig. 1a). The first 5 cm of soil had a statistically significant higher *F. oxysporum* Fr index with respect to 5-10 cm and 10-20 cm depths (Fig. 1b).

Other *Fusarium* species were observed in lower percentages compared with *F. oxysporum*. The effect of sequences and depths on these species did not show statistically significant differences (Table 3). *F. scirpi* was isolated from soil particles from the first 5 cm of soil in winter agriculture in depth limited soils (III) and intensive agriculture (V) crop rotation systems; *F. equiseti* was obtained from the first 5 cm of soil in sequence V and from 10-20 cm of sequence III; *F. merismoides* was only obtained from 5-10 cm of sequence III and from 10-20 cm in sequence IV. *F. solani* was

Table 3. Mean square values from the ANOVA analysis of the amount of *Fusarium* species

Source of variation	Df	<i>F. equiseti</i>	<i>F. merismoides</i>	<i>F. oxysporum</i>	<i>F. scirpi</i>	<i>F. solani</i>
Replications	2	0.6221 ^{ns}	0.6221 ^{ns}	0.4441 ^{ns}	0.6221 ^{ns}	0.8746 ^{ns}
Sequence ¹	4	0.5828 ^{ns}	0.5828 ^{ns}	0.0186 ^{***}	0.5828 ^{ns}	0.4192 ^{ns}
Depth ²	2	0.6221 ^{ns}	0.6221 ^{ns}	0.0001 ^{***}	0.1638 ^{ns}	0.8746 ^{ns}
Sequence × Depth	8	0.4011 ^{ns}	0.4011 ^{ns}	0.1645 ^{ns}	0.6690 ^{ns}	0.6015 ^{ns}
Error	28					
Total	44					

¹ Sequence: I, II, III, IV and V (see text). ² Depth: 0-5 cm, 5-10 cm, 10-20 cm. ns: non-significant differences. *** F value significant at $p \leq 0.05$.

observed in the first 5 cm II and III, from 5-10 cm in IV and from 10-20 cm of II.

The ANOVA analysis for the H', J indexes and S did not show significant differences between crop rotation systems and sample depth for all species, including *F. oxysporum* (Table 4).

Discussion

It is known that *Fusarium* spp. is a cosmopolitan genus and a natural soil fungus. In agricultural soils, *Fusarium* spp. is a typical genus as *Penicillium* spp. and *Trichoderma* spp. among others. The results of this work agree with previous reports from other authors, who observed that *Fusarium* spp. is one of the most abundant fungi in agricultural soils and grasslands (Thorn, 1997; Nesci *et al.*, 2006; Samaniego-Gaxiola & Madinaveitia-Chew, 2007). The presence of *Fusarium* spp. in agricultural soils could impact on crops health and conse-

quently on the amount of human and animal food. Wakelin *et al.* (2008) suggested that the incorporation of stubble into the soil could produce increase of *Fusarium* spp. populations. *Fusarium* is a cellulolytic fungus and the stubble incorporation could increase fungal development and favour the breakdown of C and N source incorporated into the soil (Collins *et al.*, 1990).

The diversity of *Fusarium* spp. species depended on crop rotations. Wakelin *et al.* (2008) showed that the diversity of *Fusarium* spp. community under stubble-burnt was less than stubble-retained systems. In the same study they observed, under glasshouse conditions, that the origin of stubble-plant affects significantly the *Fusarium* community structure.

In this study, in a long-term zero tillage soil, we identified five *Fusarium* species: *F. equiseti*, *F. merismoides*, *F. oxysporum*, *F. scirpi* and *F. solani*. Bateman & Murray (2001) in the UK met the same *Fusarium* species from wheat-field soils. In the same way, Steinkellner & Langer (2004) identified *F. oxysporum*, *F. equisetii*,

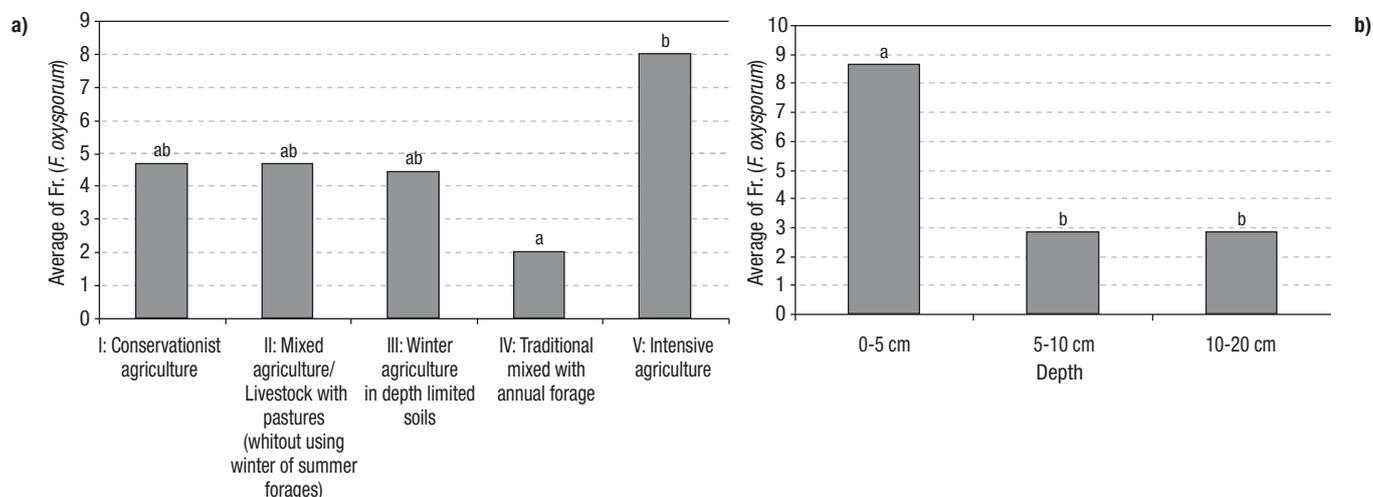


Figure 1. Frequency (Fr) values of *Fusarium oxysporum* populations in crop rotation systems (a) or soil depth (b). Different letters indicate significant differences between groups ($p \leq 0.05$).

Table 4. Mean square values from the ANOVA analysis of H', J indexes and S of *Fusarium* community

Source of variation	Df	H'	J	S
Replications	2	0.6356 ^{ns}	0.7379 ^{ns}	0.7760 ^{ns}
Sequence ¹	4	0.3635 ^{ns}	0.3190 ^{ns}	0.4402 ^{ns}
Depth ²	2	0.6462 ^{ns}	0.7172 ^{ns}	0.1682 ^{ns}
Sequence × Depth	8	0.6375 ^{ns}	0.7410 ^{ns}	0.6043 ^{ns}
Error	28			
Total	44			

^{1,2} See Table 3. ns: non-significant differences.

F. merismoides and *F. solani* as the most frequent species in agricultural soils under different tillages in Ansfelden, Upper Austria. Moreover, Marasas *et al.* (1988) observed that *F. oxysporum*, *F. equisetii* and *F. solani* were the most prevalent species obtained from plant debris in South African agricultural soils.

The distribution of the five *Fusarium* species was different as regards agricultural sequences and depths. However, these differences were statistically significant only for *F. oxysporum* (Table 2). We observed the highest Fr in the first 5 cm of soil and the same result was found in Coronel Suárez (Buenos Aires province, Argentina) by Cabello (1986a). The highest value of Fr (8%) for *F. oxysporum* was observed in the sequence of intensive agriculture, a sequence of crops actually suggested by agricultural companies who rent the land (Forjan & Manso, 2010, 2011). The lowest value of Fr (2%) for *F. oxysporum* was observed in the crop rotation system mixed with annual forage. This result could be explained by the presence of grassing-cattle and the low availability of stubble in this crop rotation compared with other rotation systems. In the same way, Wakelin *et al.* (2008) observed the presence of *F. oxysporum* across rotations. Chehri (2011) detected *F. oxysporum* in wheat soils with higher percentage than in other crops. Moreover, *F. oxysporum* is considered a cosmopolitan species (Edel *et al.*, 1997). Jeschke *et al.* (1990) showed *F. oxysporum* as the principal *Fusarium* species obtained from different altitudinal zones in Southern Africa. The high density of *F. oxysporum* in agricultural soils is a matter of study, because this fungus develops pathogenic and non-pathogenic isolates (Burgess, 1981). This feature could lead to the selection of non-pathogenic isolates of *F. oxysporum* to be used as biocontrol agents (Paulitz *et al.*, 1987; Postma & Rattink, 1992).

In this work, *F. scirpi* was isolated from soil particles of the upper soil layer (0-5 cm) in the crop rotation sys-

tems of soils with limitations (III) and of intensive agriculture (V). Both sequences present a high agricultural use due to double-cropping (late soybean that is usually sowed at the harvesting time of wheat or another winter crop). Chehri (2011) also observed *F. scirpi* in agricultural soils of Iran. *F. scirpi* has been usually/commonly present soils of arid and semiarid environments (Leslie & Summerell, 2006).

The isolates of *F. equiseti* were obtained from winter agriculture in soils with limitations (III) and with intensive agriculture crop rotation systems (V). Similar results were observed by Marasas *et al.* (1988) and Fernández *et al.* (2008) who showed that the presence of *F. acuminatum*, *F. equiseti* and *F. poae* could be related with the incorporation of oilseed crops in the field sequence. *F. equiseti* was isolated from those sequences that presented combination of cereals (wheat/barley) with an oilseed crop. The change in the structure of fungal community could be produced by the presence of oilseed crops in the rotation that increases the N supply (Douglas *et al.*, 1980).

In our study, *F. merismoides* was obtained only from 5-10 cm soil layer from the rotation of winter agriculture in soil with limitations (III) and from 10-20 cm soil layer of the traditional mixed rotation with annual forage (IV). Jeschke *et al.* (1990) observed low frequency of *F. merismoides* in soil samples collected at different altitudes in Southern Africa. Leslie & Summerell (2006) suggested that *F. merismoides* is a fungal saprophyte of soil, but it has the potential to cause some plant diseases if the environmental conditions are appropriate. Bateman & Murray (2001) observed that *F. merismoides* frequency increases in wheat field soil under dry conditions, suggesting that the presence of a mucilaginous matrix produced by this fungal species may favor its survival and ability to compete in dry conditions (Louis & Cooke, 1983).

We also detected isolates of *F. solani* in the 0-5 cm and 10-20 cm soil layers of II and in the 0.5 cm soil layer of III, and in the 10-20 cm soil layer in mixed agriculture with annual forage. Rupe *et al.* (1997) observed that the presence of sorghum and wheat in the sequence decreases the density of *F. solani* compared with the sequence with soybean and/or fescue. However, we observed *F. solani* in the sequences with soybean and wheat-oat/vicia-sunflower. The knowledge about the distribution of *F. solani* in crop rotations is important, because this pathogen is one of the causal agents of Soybean Sudden Death Syndrome (SDS). Cabello (1986a) observed a high frequency of *F. solani* in the

first centimeters of the Natraucol soil in Coronel Suárez, Buenos Aires province, without agricultural practices but with stubble of grasses and other herbaceous dicotyledonous species. Moreover, Marasas *et al.* (1988) found that the frequency of *F. solani* is higher in agricultural than in uncultivated soils. The same authors observed *F. solani* in soil samples collected at different altitudes in Southern Africa.

We observed a different distribution and abundance of *Fusarium* species related to crop rotation systems and soil depth, however the ANOVA did not show significant differences for H⁺, J and S richness indexes (Table 4). Therefore, we could not conclude that the alpha diversity of *Fusarium* community is affected by crop sequences and depths. However, Maina *et al.* (2009) observed significant differences among soil depth for S index in a Kenya soil from Taita Taveta district. Wakelin *et al.* (2008), using PCR analyses and denaturalizing gradient gel electrophoresis (DGGE), observed a strong relationship between the *Fusarium* community structure and crop sequences.

Several aspects (rotation, biodiversity, fertilization, biotechnology and soil nutrient balance) involved in zero tillage can produce a number of changes on fungal populations with particular ecological importance. Understanding the response of soil *Fusarium* spp. community to different agroecosystem managements is crucial to analyze the agricultural practices.

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References

- Acevedo E, Silva P, 2003. Agronomía de la labranza cero. Serie Ciencias Agronómicas N° 10. Universidad de Chile, Santiago, Chile. 132 pp.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DP, 1990. Basic local alignment search tool. *J Mol Evol* 215: 403-410.
- Bateman GL, Murray G, 2001. Seasonal variations in populations of *Fusarium* species in wheat-field soil. *Appl Soil Ecol* 18: 117-128.
- Bonel B, Morrás H, 2000. Estudio de la morfología del horizonte superficial de un Argiudol con diferentes manejos de rastrojos. *Actas XVII Congreso Argentino de la Ciencia del Suelo*. Mar del Plata. [CD Edition].
- Burgess LW, 1981. General ecology of the Fusaria. In: *Fusarium: disease, biology, and taxonomy* (Nelson PE, Toussoun TA, Cook RJ, eds). Penn Univ Press. University Park, PA, USA. pp: 225-235.
- Cabello M, 1986a. Variación vertical y estacional de los hongos del suelo de la región interserrana (Partido de Coronel Suárez, Pcia de Bs As). *Ciencia del Suelo* 2: 147-154.
- Cabello M, 1986b. Análisis de la metodología empleada en el aislamiento de hongos en suelos de la región interserrana (Partido de Coronel Suárez). *Ciencia del Suelo* 2: 226-229.
- Collins HP, Elliott LF, Papendick RI, 1990. Wheat straw decomposition and changes in decomposability during field exposure. *Soil Sci Soc Am J* 54: 1013-1016.
- Chehri K, 2011. Occurrence of *Fusarium* species associated with economically important agricultural crops in Iran. *Afr J Microb Res* 5: 4043-4048.
- Daniel P, Marban L, 1989. Adaptación de un método espectrofotométrico reductivo para la determinación de nitratos. *Bol Asoc C Suelo* 58: 3-8.
- Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW, 2010. InfoStat. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina.
- Douglas Jr CL, Allmaras RR, Rasmussen PE, Ramig RE, Roager Jr NC, 1980. Wheat straw decomposition and placement effects on decomposition in dry-land agriculture of the Pacific Northwest. *Soil Sci Soc Am J* 44: 833-837.
- Edel V, Steinberg C, Gautheron N, Alabouvette C, 1997. Populations of nonpathogenic *Fusarium oxysporum* associated with roots of four plant species compared to soil-borne populations. *Phytopathology* 87: 693-697.
- Fernández MR, Huber D, Basnyat P, Zentner RP, 2008. Impact of agronomic practices on populations of *Fusarium* and other fungi in cereal and noncereal crop residues on the Canadian Prairies. *Soil Till Res* 100: 60-71.
- Fisher NL, Burgess LW, Toussoun TA, Nelson PE, 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151-153.
- Forján H, Manso L, 2010. Cambios en las secuencias de cultivos de la región: ¿hacia dónde vamos? *AgroBarrow* N° 46. Publicaciones Regionales INTA-MAA. Tres Arroyos, Argentina. pp: 20-23.
- Forján H, Manso L, 2011. ¿Hasta dónde podemos llegar con la soja? *AgroBarrow* N° 49, Publicaciones Regionales INTA-MAA, Tres Arroyos, Argentina. pp: 1-6.
- Geiser DM, Jiménez-Gasco MM, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K, 2004. *Fusarium-ID* v. 1.0: A DNA sequence database for identifying *Fusarium*. *Eur J Plant Pathol* 110: 473-479.
- Gómez E, Pioli R, Conti M, 2007. Fungal abundance and distribution as influenced clearing and land use in a vertic soil of Argentina. *Biol Fert Soil* 43: 373-377.

- Hall R, Phillips LG, 1992. Effects of crop sequence and rainfall on population dynamics of *Fusarium solani* f.sp. *phaseoli* in soil. *Can J Bot* 70: 2005-2008.
- Ivancovich A, 1992. Manejo cultural de enfermedades. Agricultura sostenible. Agricultura Sostenible N° 16. INTA Pergamino, Argentina. 10 pp.
- Jeschke N, Nelson P, Marasas WFO, 1990. *Fusarium* species isolated from soil samples collected at different altitudes in the Transkei, Southern Africa. *Mycologia* 82: 727-733.
- Jurado M, Vásquez C, Patiño B, González-Jaén MT, 2005. PCR detection assays for the trichotecene producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. *Syst Appl Microbiol* 28: 562-568.
- Leslie JF, Summerell BA, 2006. The *Fusarium* Laboratory Manual, 1st ed. Blackwell. Oxford, UK. 388 pp.
- Lori GA, Sisterna MN, Sarandon SJ, Rizzo I, Chidichimo H, 2009. Fusarium head blight in wheat: impact of tillage and other agronomic practice under natural infection. *Crop Prot* 28: 495-502.
- Louis I, Cooke RC, 1983. Influence of the conidial matrix of *Sphaerellopsis filum* (*Darluca filum*) on spore germination. *Trans Br Mycol Soc* 81: 667-670.
- Luque AG, Pioli RN, Sianca R, Sachi O, 1999. Hongos celulolíticos asociados al rastrojo de trigo y su relación con algunas variables ambientales. *Boletín Micológico de Chile* 14: 67-71.
- Luque AG, Pioli R, Tonel B, Álvarez DP, 2005. Cellulolytic fungi populations in stubble and soil as affected by agricultural management practices. *Biol Agr Hort* 23: 21-142.
- Magurran AE, 1988. Ecological diversity and its measurement. Princeton Univ Press. NJ, USA. 179 pp.
- Maina PK, Okoth SA, Monda E, 2009. Impact of land use on distribution and diversity of *Fusarium* species in Taita Taveta, Kenya. *Trop Subtrop Agroecosyst* 11: 323-335.
- Marasas WFO, Burgess LW, Anelich RY, Lamprecht SC, Van Schalkwyk DJ, 1988. Survey of *Fusarium* species associated with plant debris in South African soils. *S Afr J Bot* 54: 63-71.
- Mishra PK, Fox RTV, Culhan A, 2003. Development of a PCR-based assay for rapid and reliable identification of pathogenic Fusaria. *FEMS Microbiol Lett* 218: 329-332.
- Nelson PE, Tousson TA, Marasas WFO, 1983. *Fusarium* species: an illustrated manual for identification. Penn State Univ Press. University Park, PA, USA. 193 pp.
- Nesci A, Barros G, Castillo C, Etcheverry M, 2006. Soil fungal population in preharvest maize ecosystem in different tillage practices in Argentina. *Soil Till Res* 91: 143-149.
- Nirenberg HI, 1976. Untersuchungen über die Morphologische und Biologische Differenzierung in der *Fusarium* Sektion *Liseola*. Mitteilungen aus der Biologischen Bundesanstalt Für land- und Forstwirtschaft (Berlin-Dahlem) 169: 1-117.
- O'Donnell K, Kistler HC, Cilgeline E, Ploetz RC, 1998. Multiple origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proc Natl Acad Sci USA* 95: 2044-2049.
- Parkinson D, Williams ST, 1961. A method for isolating fungi from soil microhabitats. *Plant Soil* 13: 347-355.
- Paulitz TC, Park CS, Baker R, 1987. Biological control of *Fusarium* wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum*. *Can J Microbiol* 33: 349-353.
- Pitt JI, Hocking AD, 1999. Fungi and food spoilage, 2nd ed. Aspen Publ Inc. Gaithersburg, MD, USA.
- Postma J, Rattink H, 1992. Biological control of *Fusarium* wilt of carnation with a nonpathogenic isolate of *Fusarium oxysporum*. *Can J Bot* 70: 1199-1205.
- Rodríguez-Molina MC, Tello-Marquina JC, Torres-Vila LM, Bielza-Lino P, 2000. Micro-scale systematic sampling of soil: heterogeneity in populations of *Fusarium oxysporum*, *F. solani*, *F. roseum* and *F. moniliforme*. *J Phytopathol* 148: 609-614.
- Rupe JC, Robbins RT, Gbur Jr EE, 1997. Effect crop rotation on soil population densities of *Fusarium solani* and *Heterodea glycines* and on the development of sudden death syndrome of soybean. *Crop Prot* 16: 575-580.
- Samaniego-Gaxiola JA, Madinaveitia-Chew Y, 2007. Diversidad de géneros de hongos de suelo en tres campos con diferente condición agrícola en La Laguna, México. *Revista Mexicana de Biodiversidad* 78: 383-390.
- Schalamuk S, Velázquez S, Chidichimo H, Cabello M, 2006. Fungal spore diversity of arbuscular mycorrhizal associated with spring wheat: effect of tillage. *Mycologia* 1: 22-28.
- Schalamuk S, Chidichimo H, Cabello M, 2007. Variación en la composición de especies de Glomeromycota (Fungi) en cultivos de trigo bajo distintos sistemas de labranza. *Bol de la Soc Argentina de Botánica* 42: 45-53.
- Steinkellner S, Langer I, 2004. Impact of tillage on the incidence of *Fusarium* spp. in soil. *Plant Soil* 267: 13-22.
- Stenglein SA, Balatti PA, 2006. Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by virulence and molecular markers. *Physiol Mol Plant Pathol* 68: 158-167.
- Thorn RG, 1997. The fungi in soil. In: *Modern soil microbiology* (Van Elsas JD, Trevors JT, Wellington EMH, eds). Marcel Dekker, NY. pp: 63-108.
- Wakelin S, Warren R, Kong L, Harvey P, 2008. Management factors affecting size and structure of soil *Fusarium* communities under irrigated maize in Australia. *Appl Soil Ecol* 39: 201-209.
- Weber R, Hrynczuk B, Runowska-Hrynczuk B, Kita W, 2001. Influence of the mode of tillage on diseases of culm base in some winter wheat varieties, oats and spring wheat. *J Phytopathol* 149: 185-188.
- Yi C, Kaul HP, Kuebler F, Aufhammer W, 2002. Populations of *Fusarium graminearum* on crop residues as affected by incorporation depth, nitrogen and fungicide application. *Z Pflanzenk Pflanzen* 109: 252-263.