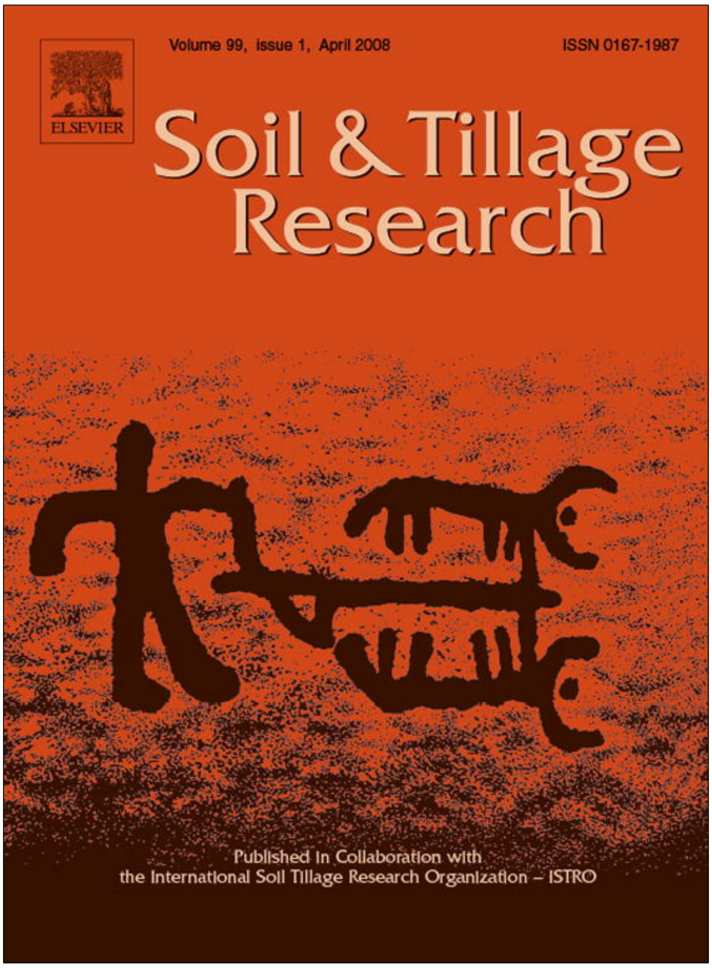
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Effects of no tillage and genetic resistance on sunflower wilt by *Verticillium dahliae*

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Abstract

From 2001 to 2006 crop seasons three field experiments were run to compare the effect of no tillage (NT) vs. conventional tillage (CT) on Verticillium wilt of sunflower. One experiment had sunflower monocropping (SM) and the others the sequence wheat–sunflower (WS) with 6 years of fescue pasture or seven WS cycles as previous crops. All experimental fields have history of the disease. One cultivar with low resistance was used in SM and two genotype-resistance levels (high and low; characterized by six and eight cultivars each, respectively) in the WS sequences. Leaf mottle severity in all environments, *Verticillium dahliae* colony forming units (CFU)/g of soil in 2005 or 2006 trials, density of microsclerotia in the stem pith at 0.5 m above the soil line in WS, and grain yield and oil content in WS with fescue as previous crop were recorded. Every year, disease severity was higher in CT than in NT in all trials. In SM, disease severity increased during the 3 years in CT from 58% to 88%, while in NT disease severity remained around the initial level (49%). The *V. dahliae*-CFU/g of soil after 3 years in SM or three cycles of WS was approximately three times higher in CT than in NT. In WS, density of microsclerotia in stem pith were higher in CT than in NT. In WS with fescue pasture as previous crop, grain yield and oil content tend to increase with NT in relation to CT. The combination of NT and high-resistant genotypes reduced the disease and the production of microsclerotia in stem pith to very low values. NT + high resistance should be viewed as a preventative, not a curative disease management option because the microsclerotia persist in soil for a long time. Therefore, NT + high resistance programs should be initiated early, before inoculum builds up to high levels in the soil. Thus, the combination of NT and high-resistant cultivars promises to be an interesting tool to manage *V. dahliae* and Verticillium wilt in sunflower and would have potential in other crops like alfalfa, cotton or strawberry.

Keywords: No tillage; Sunflower; Crop rotation; Resistance; Soil infestation; Microsclerotia; Yield; Oil

1. Introduction

Argentina is the world's largest exporter of sunflower oil and protein flour, and 90% of the harvest is located in the Pampas region (Casaburi et al., 1998). Verticillium wilt by *Verticillium dahliae* Kleb is the most important sunflower disease in Argentina (Pereyra and Escande, 1994), causing leaf mottle, early dying and stem break. Yield losses by leaf mottle and early dying have reached up to 73% in highly infested fields (Pereyra et al., 1999).

V. dahliae is a natural soil invader that increases in population by mono cropping or rotation with susceptible crops. The pathogen has a wide host range including 350 plant species in more than 160 families, and important crops like alfalfa (*Medicago sativa*),

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cotton (*Gossypium hirsutum*), olive (*Olea europaea*), pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), sunflower (*Helianthus annuus*) and tomato (*Lycopersicon esculentum*) (Pegg, 1974). The sunflower root exudates stimulate germination of hyphae from microsclerotia. To start the invasion, hyphae penetrate at the root hair zone, as shown in canola by Eynck et al. (2005). The pathogen invades the xylem and, close to R9 stage (Schneiter and Miller, 1981), produces microsclerotia in the stem, surrounding or disorganizing the pith. Therefore, most of this new inoculum is produced in sunflower aerial stems.

Resistance to *V. dahliae* had two components: the resistance to the invasion and the tolerance to the effects of the invasion (symptoms or yield reduction). The manifestation of symptoms is related directly to losses of yield and grain oil content (Bertero de Romano and Vázquez, 1985; Pereyra et al., 1999). The resistance to *V. dahliae* invasion in sunflower is also related to the production of inoculum for future epiphytotics. The first source of genetic resistance to Verticillium wilt of sunflower was reported by Putt (1958).

No till (NT) is a conservation cropping system increasingly used around the world (Paulitz, 2006). At least 2 million hectares were cropped with sunflower in Argentina during 2004/2005 cycle, of which 44% were under NT (SAGPyA, 2006). This system affects the plant growing environment and pathosystems, such as soybean–*Phytophthora sojae* (Workneh et al., 1998), rapeseed–*Sclerotinia sclerotiorum* (Wahmhoff et al., 1999), soybean–*Macrophomina phaseolina*, (Wrather et al., 1998; Almeida et al., 2003), sunflower–*Plasmopara halstedii* (Calviño et al., 2003), oilseed rape–*V. dahliae* (Sochting and Verreet, 2004) and wheat–*Rhizoctonia solani* (Schroeder and Paulitz, 2006). It was decided to investigate the effect of NT on the interaction of sunflower–*V. dahliae*.

Verticillium wilt management is based on the use of resistant cultivars and crop rotations. Because the NT system can modify the relative effects of both control strategies, our objective was to quantify the effect of NT by itself or combined with low and high levels of sunflower resistance to Verticillium wilt during 3-successive years of sunflower monocropping or three consecutive cycles of the sequence wheat–sunflower.

2. Materials and methods

2.1. Sunflower monocropping (SM)

A commercial field at González Moreno (S35°35′17″ W63°23′56″, west of Buenos Aires province, Argentina) with history of Verticillium wilt was used. The soil was a typical Hapludol with sandy texture and an average annual precipitation of 800 mm (80% in spring–summer). Tillage systems were conventional tillage (CT) and no tillage (by a Baumer™ planter, Pergamino, Argentina) (NT). CT included one double action disc, 45 days before sowing, and preemergence herbicides at sowing time. The herbicides glyphosate (48% a.i., Glifosato Atanor, Atanor, Munro, Buenos Aires province, Argentina), acetochlor and fluorochloridone (90 and 25% a.i. respectively, Magan, Capital Federal, Argentina) and cypermethrin (25% a.i., Galgotrin, Chemotécnica SA, Carlos Spegazzini, Buenos Aires province, Argentina) were applied at 960, 540, 150 and 25 a.i. g/ha, respectively. No tillage had glyphosate applied between consecutive crops and the same preemergence herbicide treatment described for CT at sowing time. The cultivar ACA 884 (ACA Semillas, Pergamino, Buenos Aires province, Argentina), susceptible to Verticillium wilt, was cropped each year from 2003 to 2005. A complete randomized block design with three replications was used. The experimental unit was a plot of 16 rows 0.7 m width and 50 m long with aproximately 3200 plants. Plot locations were the same during the 3 years. At sunflower-stage R6 (Schneiter and Miller, 1981), severity of leaf mottle was recorded in 60 plants per plot using a six-point scale (ASAGIR, 2002). Six evaluation stations were delimited starting in a randomized initial point and every 20 steps following a W shape pattern. At each station, 10-successive plants in a row were evaluated. During 2005, soil samples to quantify *V. dahliae* colony forming units (CFU) were collected starting at a randomized initial point and every 15 steps, following a W shape pattern. Sampling was during R6 stage (Schneiter and Miller, 1981). Twenty soil cores (20-cm deep and 3-cm diameter) were removed from each experimental unit. To eliminate conidia and mycelium of *Verticillium*, samples in open polyethylene bags were air-dried in the lab for 30 days at 22 ± 6 °C. One hundred milligrams of soil from each bag were spread by hand, following Goud and Termorshuizen (2003), on a 9-cm diameter Petri dish containing soil pectate tergitol (SPT) agar medium (Hawke and Lazarovits, 1994), with P-3889 poligalacturonic acid (Sigma–Aldrich, St. Louis, USA) as carbon source, and biotin (50 mg/L; Fluka Chemie, Buchs, Switzerland). The latter was incorporated to SPT medium following Mpofu and Hall (2003). P-3889 was considered a valid replacement for P-1879 following Kabir et al. (2004), but it was incorporated to SPT medium instead of NP-10 medium (Rojo R.,

Table 1
Cultivars used to characterize high and low resistance levels, seed company and year of inclusion in the wheat–sunflower sequence experiments

	Seed company	Year of inclusion
High-resistant cultivars[a]		
ACA 872	ACA Semillas, Pergamino, Buenos Aires province, Argentina	2001, 2002, 2003, 2004, 2005
ACA 885	ACA Semillas, Pergamino, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
Albisol 2	Riestra semillas S.A., Manuel Ocampo, Buenos Aires province, Argentina	2001, 2002, 2003, 2004, 2005
Paraíso 20	Nidera S.A., Junín, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
Proton R 100	Produsem S.A., Pergamino, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
VDH 488	Advanta Semillas S.A. Junín, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
Low-resistant cultivars[a]		
ACA 884	ACA Semillas, Pergamino, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
Agrobel 967	Seminium S.A., Paraná, Entre Ríos, Argentina	2001, 2002, 2003, 2005
Bagual	KWS Argentina S.A., Balcarce, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
GH 1100	Asociados Don Mario S.A., Chacabuco, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
MG 2	Dow Agrosciences Argentina S.A., Colón, Buenos Aires province, Argentina	2001, 2002, 2003, 2004
PAN 7009	Pannar ®, Venado Tuerto, Santa Fe, Argentina	2002, 2003, 2004, 2005
Cauquén	El Cencerro, Coronel Suárez, Buenos Aires province, Argentina	2001, 2002, 2003, 2004, 2005
Paihuén	El Cencerro, Coronel Suárez, Buenos Aires province, Argentina	2001, 2002, 2003, 2004, 2005

[a] Quiroz et al. (2002).

personal communication, rojorodrigo@yahoo.com.ar). Five dishes were used to estimate the number of CFU per gram of soil. Cultures were incubated at 25 ± 2 °C and darkness for 2 weeks. Plates were washed-off under running tap water. *V. dahliae* colonies were identified and counted following the morphological key proposed by Goud et al. (2003) in a dissecting microscope at 15×; however, 40× was used when needed. Leaf mottle data were analyzed with the repeated measures analysis of variance (Procedure GLM, SAS vs. 6.12, SAS Institute, Cary, NC), selecting the *F* test according to the sphericity test of orthogonal components, for tillage, year, and tillage × year interaction. ANOVA (SAS, SAS Institute, Cary, NC) was used to open the interaction. For microsclerotia in soil analysis, plates were nested into replications and the procedure ANOVA for tillage systems was run.

2.2. *Wheat–sunflower sequence (WS)*

Materials and methods were as indicated for SM, with the following modifications. Wheat–sunflower, the most common rotation in the main sunflower growing region of Argentina, was used. Two commercial fields in Coronel Suárez (S 37°27′46″ W61°51′56″, south of the province of Buenos Aires) with history of the disease, one with 6 years of fescue pasture and two crops of sunflower in the last 14 years and the other with seven cycles of the same wheat–sunflower sequence as previous crops were included. The study was run during 5 years, from 2001 to 2005, and includes three crops of sunflower in the field coming from fescue and two ones in the other. The soil was a Typic Argiudol with sandy loam texture and an average annual precipitation of 800 mm (70% in spring–autumn). Two levels of genotype resistance were tested: high and low (characterized by six and eight cultivars, respectively) (Table 1). A split-plot design with three complete blocks was used. Tillage was the main plot and cultivars – to characterize levels of resistance – the subplot. Main plots were located in the same place throughout the study, while subplots were randomized each year. The experimental unit was a plot of three rows 0.7 m width and 5 m long with 60 plants. To estimate the number of CFU/g of soil 10 soil cores were removed from each subplot, starting at 30 cm from the head of the central row and taking five samples at each edge of the row (seasons 2005–2006 or 2006–2007 for the fields with fescue or WS as previous crops, respectively). One hundred grams of soil samples of each subplot were mixed to produce a combined sample for each block and tillage system. At physiological maturity (stage R9 according to Schneiter and Miller, 1981) and 0.5 m high from the soil line, the density of microsclerotia in the stem pith of six contiguous plants in the center of each subplot was estimated based on a six-point scale (ASAGIR, 2002). In the experiment with 6 years of fescue pasture as previous crop, grain yield and oil content were evaluated. Grains of the central row of each plot, excluding the border plants were harvested at approximately 11% grain-water content and weighged with 1-g precission. Oil concentration was measured in duplicate samples by nuclear magnetic resonance (Analyzer Magnet Type 10, Newport Oxford Instruments, Buckinghamshire, UK) and averaged. Oil concentration were expressed on a dry weight basis.

For leaf mottle, density of microsclerotia in pith, grain yield and oil content the ANOVA of repeated measures for tillage system, level of resistance, year and their interactions was used (procedure GLM, SAS, SAS Institute, Cary, NC). For the analysis, cultivars were nested into levels of resistance. When a significant

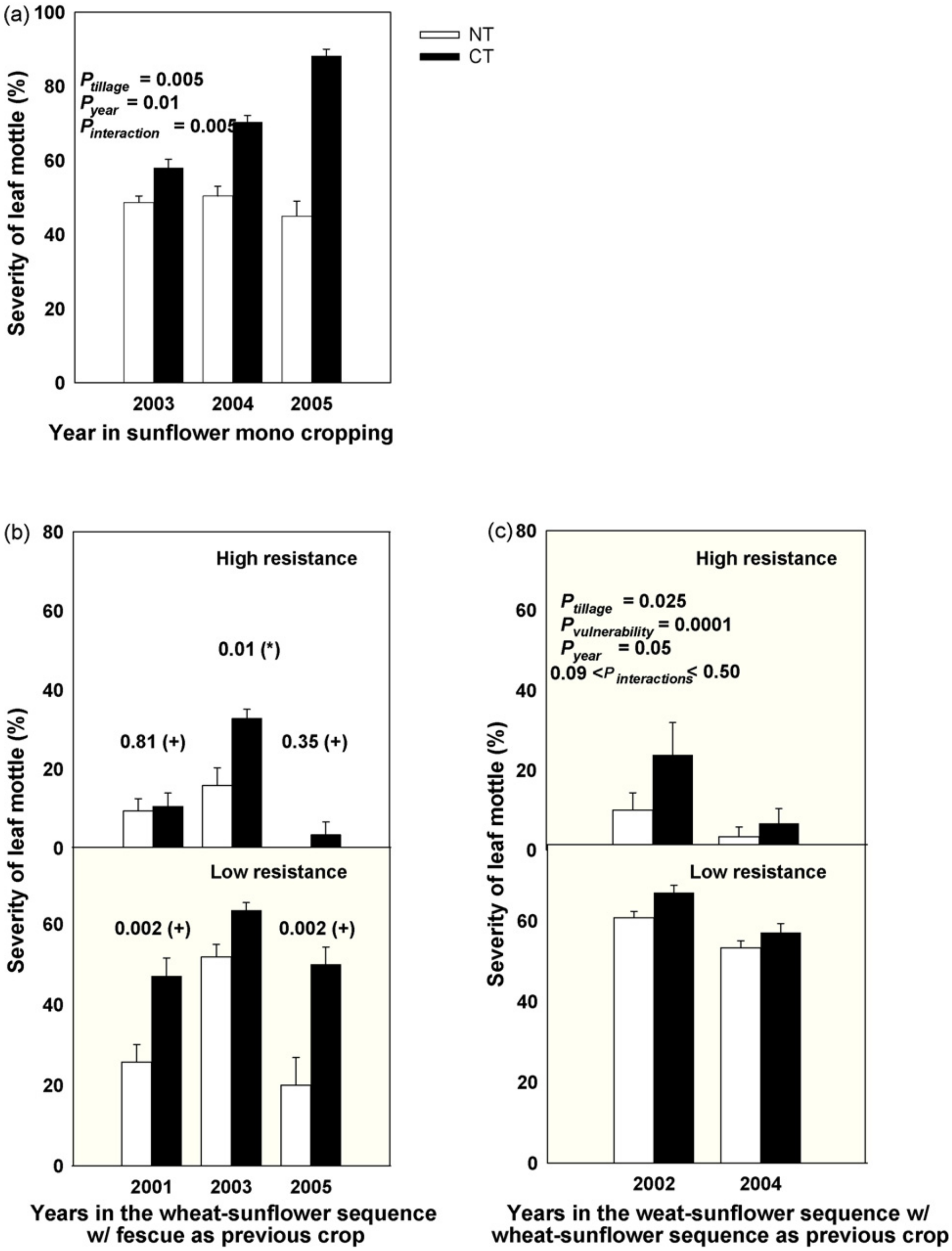

Fig. 1. Severity of sunflower leaf mottle by *V. dahliae* with no tillage (NT) and conventional tillage (CT). The experiments included (a) 3 consecutive years of sunflower monocropping at González Moreno, Buenos Aires province, Argentina, or the sequence wheat–sunflower at Coronel Suárez, Buenos Aires province, Argentina during (b) three cycles of wheat–sunflower after 6 years of fescue as previous crop, or (c) two cycles of wheat–sunflower after seven cycles of the same sequence as previous crop. Results are the average of three replications of a low-resistant cultivar in (a), or three replications of low and high-resistant cultivars, as stated in Table 1, in (b) and (c). Bars indicate one standard error. (*) indicates Pr > F, where F is the F_{tillage}-probability test for both levels of resistance at each year. (+) indicates Pr > F, where F is the F_{tillage}-probability test for each level of resistance at each year. Statistics values were for (a) $R^2 = 0.91$; degrees of freedom of the between subjects effects standard error = 2; degrees of freedom of the within subjects effects standard error = 4; for (b) $0.01 < P_{\text{tillage*resistance}} < 0.29$; $P_{\text{resistance}} = 0.0001$; $0.55 < R^2 < 0.82$; 24 < degrees of freedom of the model standard error <64; and for (c) $R^2 = 0.79$; degrees of freedom of the model standard error = 136.

interaction of tillage with year or level of resistance was detected, the analyses was open by year or eventually by year and resistance level. Data were not transformed and are presented in the figures by level of resistance. Comparison of cultivars is not included in this paper because of annual information to the growers as a decision-making tool (Quiroz et al., 2002). Pearson correlations of leaf mottle and density of microsclerotia in stem pith with grain yield and oil content were run (procedure CORR, SAS, SAS Institute, Cary, NC).

3. Results

3.1. Sunflower monocropping

The interaction between year and tillage system was highly significant ($P = 0.005$) but quantitative (Fig. 1(a)). Higher disease severity was registered in CT and increased every year ($P_{year\ CT} = 0.002$), reaching 88% in the last. In NT, disease severity remained at the same level (approximately 49%) during the 3 years of the study ($P_{year\ NT} = 0.42$).

The tillage system affected soil invasion. After 3 years of sunflower monocropping, NT plots had three times less inoculum of *V. dahliae* in soil than CT plots ($P = 0.0001$) (Fig. 2(a)).

3.2. Wheat–sunflower sequence

Disease severity was higher in CT than in NT when fescue was the previous crop (Fig. 1(b)). Because of a significant interaction of main effects with year ($P = 0.0016$), the analyses were run for each year. The effect of the environment (year) did not permit detection of a tendency of disease over time as in SM. A significant and non-reversal interaction between tillage and resistance level was detected in 2001 and 2005 (Fig. 1(b)). High-resistant cultivars had lower disease severity than low-resistant ones. The reduction of disease by NT was detected only in 1 out of the 3 years

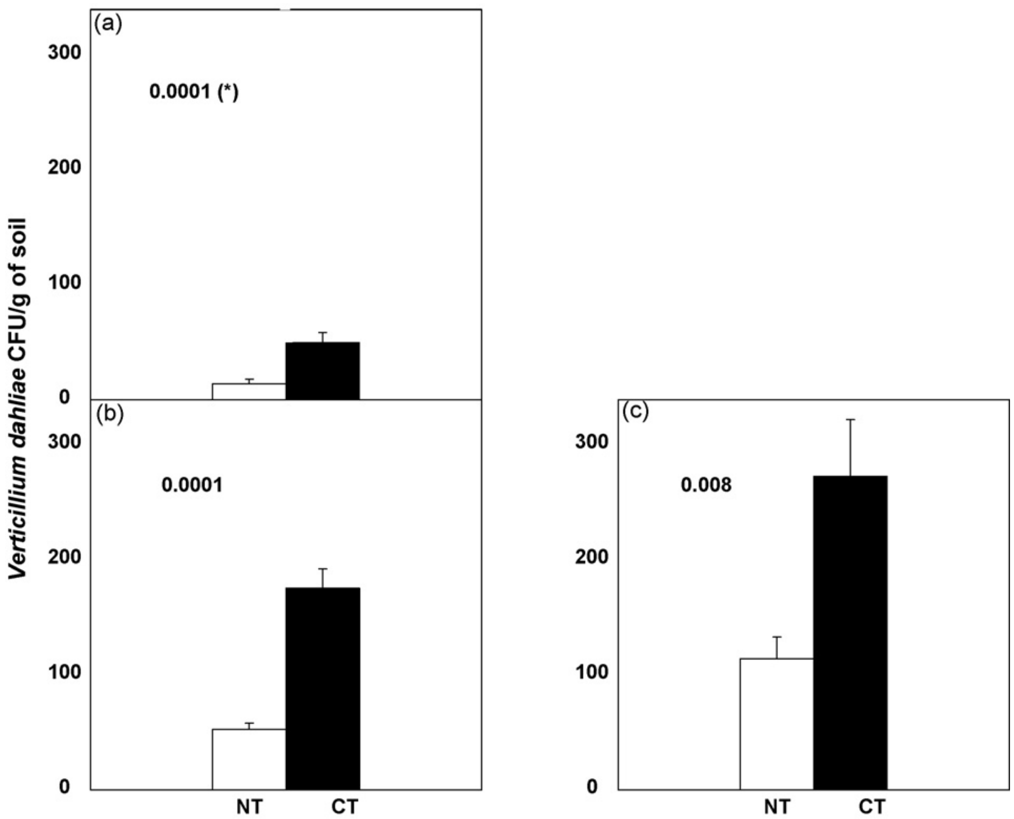

Fig. 2. *Verticillium dahliae* number of colony forming units per gram of soil with no tillage (NT) and conventional tillage (CT) at the third cycle of cropping in (a) sunflower monocropping at González Moreno, Buenos Aires province, Argentina, (b) the sequence wheat–sunflower (WS) after 6 years of fescue as previous crop at Coronel Suárez, Buenos Aires province, Argentina and (c) the sequence wheat–sunflower after seven sequences of WS as previous crops at Coronel Suárez, Buenos Aires province, Argentina. Results are the average of three replications and five plates by replication as indicated in materials and methods. Soils were cultivated with a low-resistant cultivar in (a) or a combination of low and high-resistant cultivars in (b) and (c). Bars indicate one standard error. (*) indicates Pr > *F*, where *F* is the $F_{tillage}$-probability test for each location. Statistics values were for (a) $R^2 = 0.63$; degrees of freedom of the standard error = 23; for (b), $R^2 = 0.77$; degrees of freedom of the standard error = 22; and for (c), $R^2 = 0.58$; degrees of freedom of the standard error = 14.

with high-resistant cultivars (2003), and in all seasons with the low-resistant ones. With seven cycles of wheat–sunflower sequence as previous crop no interaction between year and main effects or between tillage and resistance level were detected. Resistance had the largest effect on leaf mottle (Fig. 1(c)). High-resistant cultivars had less disease severity than the low-resistant ones. Tillage system also caused a minimal but significant reduction of disease severity: from 39% in CT to 32% in NT.

Soil invasion was affected by the tillage system after three cycles of WS. NT plots presented significantly lower CFU of *V. dahliae* in soil than CT plots with fescue or WS as previous crops ($P = 0.0001$ and 0.008 respectively) (Fig. 2(b) and (c)).

The density of microsclerotia in the stem pith was higher in CT than in NT. This effect was detected in all experiments (Fig. 3). With fescue as previous crop and because of a significant interaction of main effects with year ($P = 0.0001$), the analyses were run for each year. In 2001 and 2003, significant but quantitative interactions between tillage and resistance were detected ($P = 0.0025$ and 0.0017, respectively) (Fig. 3(a)), therefore a general conclusion for tillage was proposed. Similar results were obtained in 2004 with seven cycles of WS as previous crops ($P = 0.0012$) (Fig. 3(b)). Independently of the level of resistance, NT gave no production of microsclerotia in stem pith during 2003 and 2005 trials (Fig. 3(a)), and minimal production of microsclerotia in 2004 (Fig. 3(b)). Large effects of resistance on microsclerotia in the stem pith were detected (Fig. 3), with the exception of year 2005 ($P = 0.28$).

NT tends to give more grain yield than CT plots when fescue was the previous crop. Only in 2005, the difference was significant ($P = 0.001$) (Fig. 4(a)). In 2005, associations of grain yield with leaf mottle ($r = -0.74$; $P = 0.03$) and density of microsclerotia in the stem pith ($r = -0.65$; $P = 0.02$) were detected in low resistance genotypes. Also with fescue as previous crop,

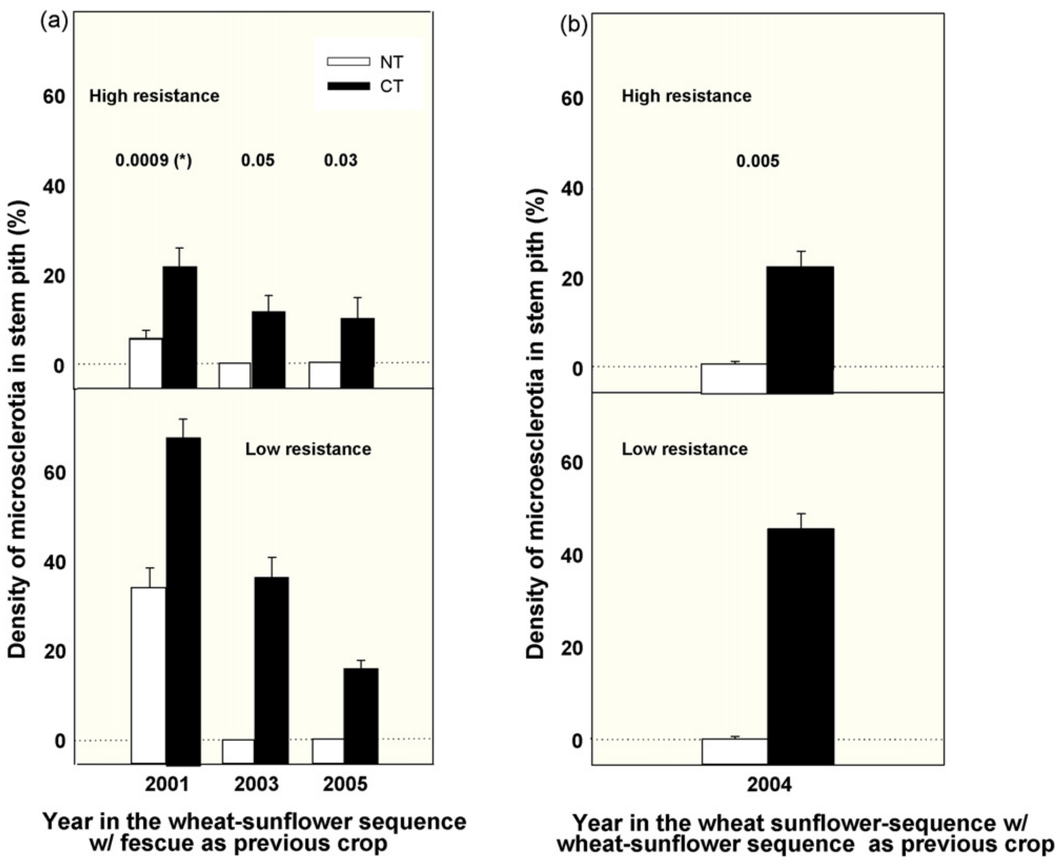

Fig. 3. Density of microsclerotia of *V. dahliae* in sunflower stem pith at 0.5 m high from the soil line with no tillage (NT) and conventional tillage (CT). The experiments included the sequence wheat–sunflower at Coronel Suárez, Buenos Aires province, Argentina, during (a) three cycles of the sequence wheat–sunflower after 6 years of fescue pasture as previous crop, or (b) the year 2004 with seven cycles of the same sequence as previous crop. Results are the average of three replications of low and high-resistant cultivars as stated in Table 1. Bars indicate one standard error. (*) indicates $Pr > F$, where F is the $F_{tillage}$-probability test for both levels of resistance at each year. Statistics values were for (a) $0.002 < P_{tillage*resistance} < 0.28$; $0.0001 < P_{resistance} < 0.28$; $0.62 < R^2 < 0.84$; 24 < degrees of freedom of the model standard error < 64; and for (b) $P_{tillage*resistance} = 0.001$; $P_{resistance} = 0.002$; $R^2 = 0.71$; degrees of freedom of the model standard error = 57.

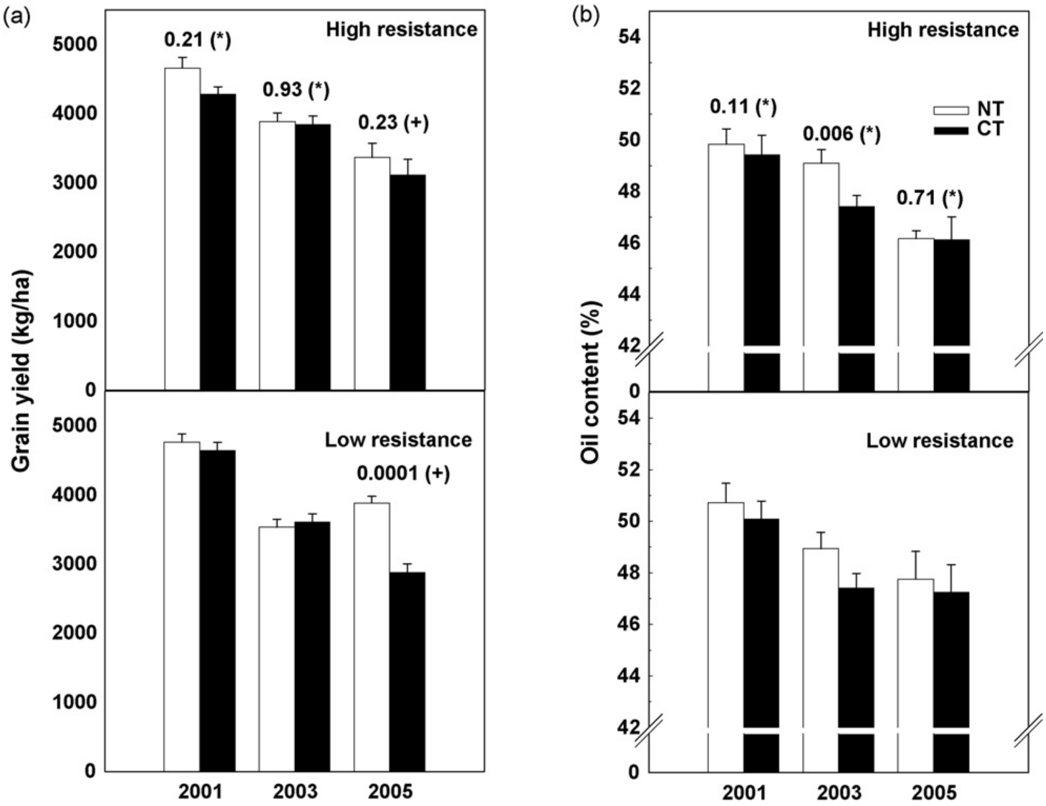

Fig. 4. Grain yield (a) and oil content (b) of sunflower with no tillage (NT) and conventional tillage (CT). The experiments included three cycles of wheat–sunflower after 6 years of fescue as previous crop. Results are the average of three replications of low and high-resistant cultivars, as stated in Table 1. Bars indicate one standard error. (*) indicates $Pr > F$, where F is the $F_{tillage}$-probability test for both levels of resistance at each year. (+) indicates $Pr > F$, where F is the $F_{tillage}$-probability test for each level of resistance at each year. Statistics values were for (a) $0.02 < P_{tillage*resistance} < 0.53$; $0.003 < P_{resistance} < 0.42$; $0.49 < R^2 < 0.69$; 22 < degrees of freedom of the model standard error < 64; and for (b) $0.58 < P_{tillage*resistance} < 0.75$; $0.003 < P_{resistance} < 0.76$; $0.84 < R^2 < 0.91$; 24 < degrees of freedom of the model standard error < 64.

NT plots gave more oil content than CT plots in all cases, but the difference was significant only in 2003 ($P = 0.006$) (Fig. 4(b)). In 2003, association of grain oil content with density of microsclerotia in the stem pith was detected in low ($r = -0.65$; $P = 0.02$) and high resistance genotypes ($r = -0.45$; $P = 0.08$).

4. Discussion

The effect of NT on the level of disease depends on the particular pathosystem and the environment where the crop is grown. Thus, NT does not imply higher disease level than CT. No tillage reduced the effects of *Rhizoctonia solani* in potato (Leach et al., 1993; Gudmested et al., 1978) and in soybean (Sturz and Carter, 1995). Also Sturz et al. (1997) listed disease increases or decreases by NT, but did not mention Verticillium diseases. In most cases of our study, NT decreased significantly Verticillium leaf mottle. In cases of non-significance between tillage treatments, as in high-resistant cultivars of the WS sequence (Fig. 1(b) and (c)), the low disease severity registered could account for these results. High-resistant cultivars were very efficient to reduce the disease, but their combination with NT led to an extra reduction of disease.

According to Khan (1975) and McFadden and Sutton (1975), pathogen inoculum concentrations in NT systems can be several orders of magnitude greater than those of conventionally ploughed soils. Thus, roots confined to or growing near the soil surface may be prone to pathogen attack. However, Doran and Linn (1994) found that conservation tillage (no tillage) increased the level of microbial populations from 0.5 to 2.7 times in the first 15 cm of uppermost soil; and increases in soil microbial activity would provide a highly competitive environment, leading to competition effects between soil microbial residents and resulting in disease suppression (Chen et al., 1988). Also, increased microbial biomass and microbial activity in these soil layers have been associated with higher root density and plant root activity (Lynch and Panting, 1980; Carter and Rennie, 1984). Thus, a more vigorous root system may balance disease damage and associated yield reductions (Sturz et al., 1997). The reduction of disease severity by

NT in the 1st year of our study (Fig. 1(a)–(c)) could be explained for the results and ideas of Lynch and Panting (1980), Carter and Rennie (1984), Chen et al. (1988), Doran and Linn (1994) and Sturz et al. (1997).

During 3 years of NT in SM the disease severity remain at the same level. Populations of the pathogen decline very slowly through mortality over time and microsclerotia of the pathogen persist in soil for a long time, even in the absence of a susceptible host, therefore is normal to expect that NT did not reduce leaf mottle severity in our SM-study. In addition fallowing a field by leaving it uncropped and weed-free during the growing seasons or planting non-susceptible crops for several consecutive seasons do not lead to significant reductions in the populations of microsclerotia (Berlanguer and Powelson, 2000).

The level of resistance could affect the level of inoculum in the soil. Davis and Everson (2005) found that following the continuous cropping of either of two Verticillium-resistant potato clones for 5 consecutive years, *V. dahliae* population remained the same but following the continuous cropping of a susceptible cultivar, *V. dahliae* population increased. In the present study the susceptible genotypes produces much more microsclerotia in stem pith than the resistant ones. Thus, our results are in harmony with those of Davis and Everson (2005).

Large effects of NT on the production of microsclerotia in the stem pith (Fig. 2) and the density of microsclerotia in soil after three sunflower cycles (Fig. 3) were observed. The density of microsclerotia in stem pith is a measure of the resistance of the plant to the pathogen invasion and could be related to the amount of viable inoculum of the pathogen in soil and the level of disease during the succeeding crop. Also Scholte (1989) confirmed that stem infection by *V. dahliae* microesclerotia correlated with wilt incidence in potato. In a colonization study of *V. dahliae* in lettuce, microsclerotia formation was only observed in plants exhibiting severe wilt symptoms or death, immediately after the eruption of mycelia into surrounding non-vascular tissues (Vallad et al., 2005). In sunflower, microsclerotia are also formed surrounding the stem pith when plants reach maturity. Mol (1995), working with potato, field bean and barley, confirmed the fact that removal of post-cropping foliar debris from sequential field crops reduced disease incidence in the succeeding crop and that infection was proportional to soil inoculum density (microsclerotia), as was also observed by Bruni (1970) in sunflower. If stems of diseased plants are not buried, as in NT, the inoculum in the soil will be less than in CT and if so, this can explain the lower number of diseased plants in NT compared to CT in the second and subsequent cycles of our study (Fig. 1).

Markakis et al. (2005) found that even three microsclerotia per gram of soil are enough to infect susceptible olive cultivars. For sunflower, such infection threshold of microsclerotia has not been reported yet in the literature. However, according to Berbegal et al. (2005), artichoke wilt incidence and severity increased with increasing inoculum density at the beginning and the end (autumn–spring) of the first cropping season. Thus, the inoculum concentration is frequently used to predict a disease event. However, soilborne pathogenic populations are dynamic and constantly cycling through dormant, saprophytic, infective and dissemination phases (Mitchell, 1979). As a result, estimates of inoculum concentrations provide a static view of the quantity and capacity of plant pathogenic inoculum in the root zone (Bouhot, 1979), making the prediction of disease events difficult. The severity of disease to compare tillage systems or resistance levels was used in the present study, which provided a non-biased estimation of the effect of these independent variables on disease.

Verticillium wilt affects mainly foliar expansion and senescence (Sadras et al., 2000) and causes reduction of grain yield and oil content (Bertero de Romano and Vázquez, 1985) and Pereyra et al., 1999). In our experiments and after three WS cycles, the larger severity of leaf mottle and the bigger amount of *V. dahliae* inoculum in soil with CT than NT (Figs. 1 (b-low resistance) and 3(b)) could partially explain the effect of NT on grain yield detected in the low resistance level during 2005 (Fig. 4(a)). With high resistance, a significant effect of NT on grain yield was not detected. Same arguments could explain the reduction in oil content detected in 2003 in both resistance levels (Fig. 4(b)).

NT is efficient to manage Verticillium wilt of sunflower. Its competence in the highest infested soil of our study (Fig. 3(c)) is demonstrated for the reduction in the severity of leaf mottle detected in the WS study with seven cycles of WS as previous crop (Fig. 1(c)), but its effect was not as evident as in the other study sites (Fig. 1(a and b)). Also in soils with low levels of inoculum, NT is a good strategy to avoid the build-up of *V. dahliae* inoculum in soils, because of the reduction of inoculum production (Fig. 2) and the lower levels of microsclerotia in soil after three cycles of NT–sunflower cropping in comparison with CT plots (Fig. 3). NT is a promising tool for the management of Verticillium wilt in sunflower and would have potential in other crops like alfalfa, cotton or strawberry.

5. Conclusions

The combination of NT and high-resistant genotypes reduced the disease and the production of microsclerotia in stem pith to very low values. In comparison with CT and after three cycles of cropping in the three experiment sites, NT reduced (i) disease severity between more than three and two times; (ii) density of microsclerotia of *V. dahliae* in sunflower stem pith at 0.5 m high from the soil line between more than 69 and 3 times, reaching always values lower than 1%; and (iii) *V. dahliae* number of colony forming units per gram of soil from 3.6 to 2.3 times. NT also tends to increase grain yield and oil content. The effect of NT on soil population was larger in sunflower monocropping than in the WS sequence. NT should be viewed as a preventative, not a curative disease management option because the microsclerotia persist in soil for a long time. Therefore, NT programs should be initiated early, before inoculum builds up to high levels in the soil. Thus, the combination of NT and high-resistant cultivars promises to be an interesting tool to manage *V. dahliae* and Verticillium wilt in sunflower.

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