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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.,